

# Growth and grazing of a heterotrophic dinoflagellate, *Gyrodinium dominans*, feeding on a red tide flagellate, *Chattonella antiqua*

Yasuo Nakamura<sup>1</sup>, Yuichiro Yamazaki<sup>2</sup>, Juro Hiromi<sup>2</sup>

<sup>1</sup> National Institute for Environmental Studies, Tsukuba, Ibaraki 305, Japan

<sup>2</sup> College of Agriculture and Veterinary Medicine, Nihon University, Setagaya, Tokyo 154, Japan

**ABSTRACT:** Growth and grazing of a heterotrophic dinoflagellate, *Gyrodinium dominans*, were examined in laboratory batch cultures. When *Chattonella antiqua* was used as prey, *G. dominans* grew at a rate of ca 0.9 d<sup>-1</sup> (at 25°C). Cell division of *G. dominans* was not affected by the light:dark period. Growth rate of *G. dominans* was significantly affected by temperature and was maximal within the range 25 to ≥ 27.5°C. Rate of ingestion of *C. antiqua* (at ca 1 × 10<sup>3</sup> to 2 × 10<sup>3</sup> cells ml<sup>-1</sup>) by *G. dominans* was 3.8 prey cell<sup>-1</sup> d<sup>-1</sup> and growth efficiencies on the basis of carbon and nitrogen were 0.31 and 0.33, respectively. Results suggest the ecological importance of *G. dominans* in the disappearance of *C. antiqua* red tides.

## INTRODUCTION

The ecological importance of heterotrophic dinoflagellates as microheterotrophic grazers has been highlighted recently (Lessard & Swift 1985, Bjørnsen & Kuparinen 1991, Hansen 1991, Strom 1991). However, there is still very little quantitative information from which the ecological roles of heterotrophic dinoflagellates in plankton communities can be assessed (see Lessard 1991). In summer 1989, we encountered a small red tide due to a raphidophycean flagellate, *Chattonella antiqua* (Hada) Ono, around the Ie-shima Islands (Seto Inland Sea, Japan). In parallel with the bloom decline, the cell concentration of a heterotrophic dinoflagellate, *Gyrodinium dominans* Hulburt, increased to a level of 10 to 10<sup>2</sup> cells ml<sup>-1</sup>. Furthermore, food vacuoles apparently due to *C. antiqua* cells were observed in *G. dominans* cells (see Nakamura & Umemori 1991). In the present paper, data on the maximum growth and grazing rates of *G. dominans* fed *C. antiqua* are described.

## MATERIALS AND METHODS

During a bloom of *Gyrodinium dominans* (ca 50 to 60 µm long and 30 µm wide, 2 to 3 × 10<sup>4</sup> µm<sup>3</sup> volume) around the Ie-shima Islands (12 August 1989; see

Nakamura & Umemori 1991), surface seawater (ca 10 ml) was introduced into a *Chattonella antiqua* culture (ca 100 ml) grown in *f/2* medium (Guillard & Ryther 1962), and then brought back to our laboratory. From the crude culture, *G. dominans* cells were micropipetted and washed with sterile *f/2* medium several times. Then 20 cells were introduced into a *C. antiqua* culture (*f/2*; 10 ml) and incubated at 25°C with 150 µE m<sup>-2</sup> s<sup>-1</sup> illumination under a 12 h light:12 h dark photoperiod (lights on at 08:00 and off at 20:00 h). The *G. dominans* culture established was not axenic, and was maintained by regular transfer (ca 0.2 ml, 1 wk interval) to fresh cultures of *C. antiqua* (10 ml) grown in *f/2* medium. Although 8 strains of *C. antiqua* were arbitrarily used for the *G. dominans* maintenance culture, Strain 89-1 (volume = ca 2 × 10<sup>4</sup> µm<sup>3</sup>; isolated by Y. Nakamura) was used as prey throughout the experiments. Except where otherwise stated, culture conditions (for *G. dominans* and pure culture of *C. antiqua*) were the same as described above.

Throughout the experiments, prey cultures were grown in a seawater medium [Whatman GF/F filtered seawater from the Kuroshio area enriched with 200 µM NO<sub>3</sub><sup>-</sup>, 20 µM PO<sub>4</sub><sup>3-</sup>, 100 ng l<sup>-1</sup> vitamin B<sub>12</sub> and 1.5 ml l<sup>-1</sup> N1-metals (Nakamura & Umemori 1991)]. Sterilization of the medium was not carried out. The *f/2* medium used for the maintenance culture of *Gyrodinium dominans*

(see above) sometimes causes sticky accumulation of *Chattonella antiqua* cells on the bottom of the culture vessel and is therefore not suitable for grazing experiments.

Except where otherwise stated, experimental cultures were only swirled just before sampling. For cell counts, samples (3 ml) were taken from the culture flasks and prey cell concentrations were measured by observing 1 ml of intact sample (after vortex mixing for ca 20 s) in a Sedgewick-Rafter chamber using a microscope. For enumeration of *Gyrodinium dominans*, 50  $\mu$ l of polyethylene oxide in filtered seawater (1%) was added to the sample (2 ml) to stop the movement of *G. dominans* (Spoon et al. 1977) and then its concentration was measured as described above.

**Monitoring of growth.** Approximately 1 ml of *Gyrodinium dominans* maintenance culture was inoculated into each of five 120 ml *Chattonella antiqua* cultures [cell concentrations of *C. antiqua* ( $N_c$ ) = ca  $5 \times 10^2$  cells ml $^{-1}$ ]. Concentrations of *G. dominans* ( $N_g$ ) and  $N_c$  were monitored almost daily for 7 d and the growth rate of *G. dominans* was calculated during exponential growth.

**Cell division under a light:dark regime.**  $N_g$  was monitored at 3 h intervals for 24 h under a 12 h light:12 h dark regime.

**Effects of temperature.** One liter of *Chattonella antiqua* culture in exponential phase ( $N_c$  = ca  $1 \times 10^3$  cells ml $^{-1}$ , grown at 25°C) was divided into 8 subcultures (100 ml). The maintenance culture of *Gyrodinium dominans* was then inoculated (ca 2 ml) into each subculture and incubated at 4 different temperatures (2 flasks at each temperature, range 20 to 27.5°C).  $N_g$  was monitored daily for 4 d and converted to the growth rate. Throughout the experimental period,  $N_c$  always exceeded 1000 cells ml $^{-1}$ . Experiments were conducted 3 times.

**Grazing experiments.** Using 2 cultures (500 ml each) of exponentially growing *Gyrodinium dominans* ( $N_g$  and  $N_c$  at the beginning of the experiment were  $6 \times 10^2$  and  $1.8 \times 10^3$  cells ml $^{-1}$ , respectively),  $N_c$  and  $N_g$  were monitored at 1 h intervals from 08:30 to 17:30 h. Since the growth of *Chattonella antiqua* is synchronized under the 12 h light:12 h dark photoperiod and no cell division occurred during the experimental period (Nakamura et al. 1990), the ingestion rate ( $I$ , prey cell $^{-1}$  h $^{-1}$ ) was calculated as:

$$I = (N_c^S - N_c^E) / (\bar{N}_g \cdot T) \quad (1)$$

$$\text{and } \bar{N}_g = (N_g^E - N_g^S) / (\ln N_g^E - \ln N_g^S) \quad (2)$$

where  $N_c^S$  and  $N_g^S$  = cell concentrations at the start of the experiment (08:30 h);  $N_c^E$  and  $N_g^E$  = cell concentrations at the end of the experiment (17:30 h);  $T$  = length of experiment (9.0 h); and  $\bar{N}_g$  = average of  $N_g$  (see Grover 1990).

Effects of shaking on the ingestion rate were also studied. One liter of the exponentially growing culture of *Gyrodinium dominans* ( $N_g$  and  $N_c$  were 880 and 1940 cells ml $^{-1}$ , respectively) was divided into 8 subcultures. Four of these were placed in a water bath (at 25°C with illumination of ca 150  $\mu$ E m $^{-2}$  s $^{-1}$ ) with a shaker (8 rpm; the lowest setting) and the others were used as controls.  $N_c$  and  $N_g$  were monitored at 1 to 4 h intervals from 09:00 to 17:00 h.

**C and N contents and growth efficiency.** One liter of the *Chattonella antiqua* culture growing at the maximum rate (2850 cells ml $^{-1}$ ; 0.7 d $^{-1}$ ) was filtered (500 ml  $\times$  2; < 10 cm Hg) through pre-combusted (400°C for 4 h) Whatman GF/D filters at 13:00 h. The filters were used for determining particulate carbon and nitrogen (PC, PN) by an elemental analyzer (MT-3, Yanaco, Japan). PC and PN measured were divided by the total cell number of *C. antiqua* filtered to obtain the C and N contents of *C. antiqua* cell $^{-1}$  ( $Q_c^i$  where  $i$  = C or N respectively).

C and N contents of *Gyrodinium dominans* cell $^{-1}$  ( $Q_g^i$ ) were determined as follows. One liter of exponentially growing *G. dominans* ( $N_g$  and  $N_c$  were 1230 and 1290 cells ml $^{-1}$ , respectively) was filtered (500 ml  $\times$  2; < 10 cm Hg) through GF/D filters at 14:30 and PC and PN were measured as described above. The PC and PN attributable to *C. antiqua*, calculated by multiplying  $Q_c^i$  by the total number of *C. antiqua* filtered, was subtracted from the original values. Then the corrected PC and PN were divided by the total number of *G. dominans* cells filtered to obtain  $Q_g^i$  (see Caron et al. 1985).

Growth efficiency ( $E$ ) of *G. dominans* was calculated as:

$$E = (I/\mu) (Q_g^i/Q_c^i) \quad (3)$$

where  $I$  = ingestion rate (see Eq. 1), and  $\mu$  = growth rate of *G. dominans* at 25°C.

## RESULTS AND DISCUSSION

*Gyrodinium dominans*, which ingests prey through engulfment (Hansen 1991), grew at a rate of 0.88 d $^{-1}$  (SE 0.08,  $n$  = 5) and ingested *Chattonella antiqua* until  $N_c$  became less than 100 cells ml $^{-1}$  (in some cases  $N_c$  reached below 10 cells ml $^{-1}$ ; Fig. 1). Small starving cells of *G. dominans* (ca 30  $\mu$ m long  $\times$  15  $\mu$ m wide) without food vacuoles appeared (ca 20 to 70% of the total count) at Day 6 (Fig. 1). *G. dominans* can also utilize much smaller species [e.g. *Heterosigma akashiwo* (Raphidophyceae), volume =  $7 \times 10^2$   $\mu$ m $^3$ ] as prey, although it cannot sustain growth in bacterial suspensions (Y. Nakamura unpubl.).

Under a 12 h light:12 h dark photoperiod, the cell concentration of *Gyrodinium dominans* increased steadily (Fig. 2; growth rate = 0.035 h $^{-1}$  or 0.84 d $^{-1}$ ),

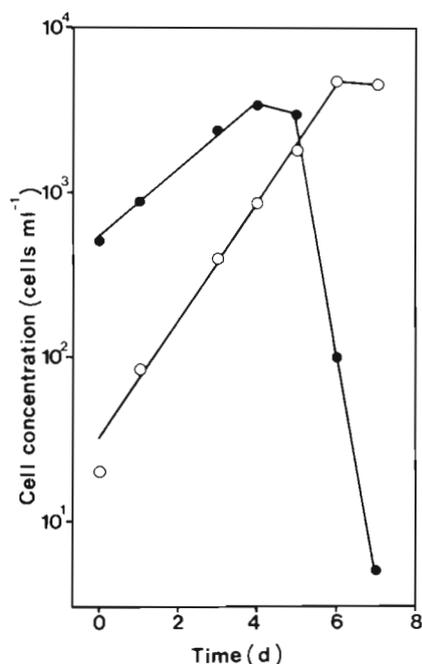


Fig. 1. *Gyrodinium dominans*, *Chattonella antiqua*. Growth curves showing cell concentrations of (○) *G. dominans* and (●) *C. antiqua*

and dividing cells were observed at each sampling period. This indicates that cell division occurs irrespective of light/dark period. Changes in cell size were not apparent throughout the experiments.

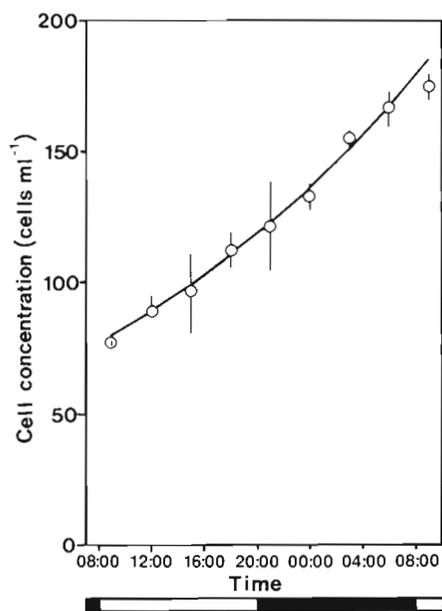


Fig. 2. *Gyrodinium dominans*. Changes in cell concentrations under a 12 h light:12 h dark photoperiod. Solid line was calculated using growth rate = 0.035 h<sup>-1</sup>. Vertical bars show the range of duplicate counts. Cell concentration of *Chattonella antiqua* was ca.  $2.4 \times 10^3$  cells ml<sup>-1</sup>

Within the temperature range examined (20 to 27.5°C), the growth rate of *Gyrodinium dominans* increased with temperature from 20 to 25°C and reached a maximum at or above 25°C (Fig. 3). During the blooming period of *G. dominans* around the Ieshima Islands in summer 1989, the water temperature

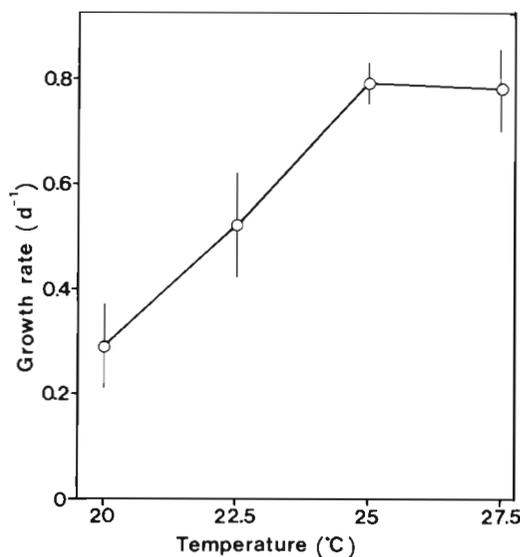


Fig. 3. *Gyrodinium dominans*. Growth rate as a function of temperature. Vertical error bars show the SE of 6 experiments

(at 0 to 5 m; the most abundant layer of *G. dominans*) was within the range 25.2 to 26.4°C (Nakamura & Umemori 1991). Furthermore, the surface water temperature during the bloom of *G. dominans* (followed by a red tide due to *Gymnodinium nagasakiense*) in summer 1991 in the Seto Inland Sea was within the range 25 to 28°C (S. Yoshimatsu pers. comm.). These observations indicate that the blooms of *G. dominans* in the Seto Inland Sea occurred when the water temperature was optimal for growth (25 to  $\geq 27.5$ °C; Fig. 3). However, it should be noted that the water temperatures during the blooming period of *G. dominans* in the Kattegat (Denmark) and Tokyo Bay (Japan) were 5 to 6°C and 16 to 21°C, respectively (P. J. Hansen pers. comm., M. Sato pers. comm.), apparently lower than the optimum temperature for our *G. dominans* culture.

We estimated the ingestion rates of *Chattonella antiqua* by *Gyrodinium dominans* from changes of  $N_c$  in the daytime period when *C. antiqua* does not divide (Nakamura et al. 1990). During these experiments,  $N_c$  decreased constantly with time (Fig. 4A), and the estimated ingestion rates were 0.15 and 0.20 prey cell<sup>-1</sup> h<sup>-1</sup> (3.6 and 4.8 prey cell<sup>-1</sup> d<sup>-1</sup>) in duplicate experiments.

We also examined the effects of shaking on the ingestion rate (Fig. 4B). As controls, the ingestion rate

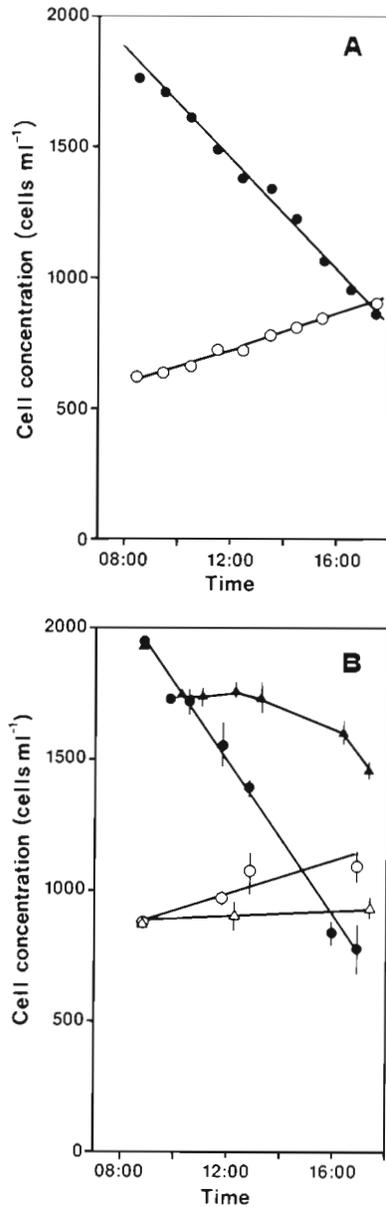


Fig. 4. *Gyrodinium dominans*, *Chattonella antiqua*. (A) Estimation of ingestion rates. Changes in cell concentrations of (○) *G. dominans* and (●) *C. antiqua*. (B) Effects of shaking on grazing. Cell concentrations of *G. dominans* (△) with and (○) without shaking and of *C. antiqua* (▲) with and (●) without shaking. At each sampling period, 2 of the 4 flasks were sampled and vertical error bars show the range of duplicate counts

without shaking was measured, and reproduced the results shown in Fig. 4A ( $I = 0.16$  prey cell<sup>-1</sup> h<sup>-1</sup>, SD = 0.01,  $n = 4$ ). During the first 1.5 h of the experiment (08:30 to 10:00 h),  $N_c$  exposed to shaking decreased comparable to control, but the rate of  $N_c$  decrease with shaking was retarded significantly after that (Fig. 4B).

Parameters used to estimate the growth efficiency of *Gyrodinium dominans* ( $E$ ) are listed in Table 1. In the

calculation of C and N contents of *G. dominans*, we assumed that the C and N contents of *Chattonella antiqua* were equal in both the *G. dominans* culture and the pure culture. This assumption can be rationalized because for both cultures, the growth conditions were same, optimum for the growth of *C. antiqua*, and PC and PN samples were obtained at nearly

Table 1. *Chattonella antiqua*, *Gyrodinium dominans*. Growth efficiency of *G. dominans* ( $E$ ) and parameters for the estimation of  $E$

	<i>Chattonella antiqua</i>	<i>Gyrodinium dominans</i>
Carbon contents (pmol cell <sup>-1</sup> )	83	117
Nitrogen contents (pmol cell <sup>-1</sup> )	15	23
C/N	5.5	5.1
Growth rate (d <sup>-1</sup> )	—	0.83 <sup>a</sup>
Ingestion rate (prey cell <sup>-1</sup> d <sup>-1</sup> )	—	3.8 <sup>b</sup>
$E$ (on the basis of C)	—	0.31
$E$ (on the basis of N)	—	0.33

<sup>a</sup> Average of 11 measurements shown in Figs 1 & 3  
<sup>b</sup> Average of 6 measurements shown in Fig. 4

the same time under a 12 h light:12 h dark photoperiod.  $E$  values on the basis of carbon and nitrogen were 0.31 and 0.33, respectively, in accord with the reported values for herbivorous and bacterivorous flagellates (0.2 to 0.6; Fenchel 1982, Sherr et al. 1983, Caron et al. 1985). Although small particles (less than 10 μm), probably debris of *C. antiqua* egested by *G. dominans*, were present in the culture and would cause overestimation of  $E$  values (see Caron et al. 1985), the contribution of these particles to total particle volume (or PC and PN measurements) appeared to be considerably less than 10% based on microscopic inspection. If we assume that the contribution of small particles on PC and PN measurements was 10%, the re-calculated  $E$  values are 0.25 and 0.28 on the basis of C and N, respectively.

The impact of copepod grazing on *Chattonella antiqua* has been reported by Uye (1986), who measured the ingestion rates of several copepod species on *C. antiqua* as a function of  $N_c$  and estimated the daily removal rate of the *C. antiqua* population based on copepod biomass in the Seto Inland Sea. The estimated values at  $N_c = 500$  cells ml<sup>-1</sup> (typical cell concentration in a *C. antiqua* red tide; Nakamura et al. 1989) range from 0.4 to 4.3% d<sup>-1</sup> (mean: 1.8% d<sup>-1</sup>). We also conducted the same calculation for *Gyrodinium dominans*. We assumed that the ingestion rate of *G. dominans* ( $I$ )

at  $N_c = 500$  cells  $\text{ml}^{-1}$  is the same as that obtained in the present study ( $I = 3.8$  prey  $\text{cell}^{-1} \text{d}^{-1}$  at  $N_c = 1 \times 10^3$  to  $2 \times 10^3$  cells  $\text{ml}^{-1}$ ; Fig. 4) and that the ingestion rate at night is comparable to that in the daytime. These assumptions may be rationalized (although not directly) based on the results that growth rate of *G. dominans* is not affected by  $N_c$  at or above 500 cells  $\text{ml}^{-1}$  (Fig. 1) and that *G. dominans* grows at a constant rate under a 12 h light: 12 h dark photoperiod without a significant change of cell size (Fig. 2). Using  $N_g = 10^1$  to  $10^2$  cells  $\text{ml}^{-1}$  (observed during the bloom decline of a *C. antiqua* red tide in 1989; Nakamura & Umemori 1991) and based on the above assumptions, the rate of daily removal by *G. dominans* is within the range 8 to 76%  $\text{d}^{-1}$ , significantly higher than that by copepods. One might consider that the daily removal rate of 8% is too low for the disappearance of red tides. However, it should be noted that  $N_g$  doubles the following day (Fig. 1) and the daily removal rate reaches ca 20% if *C. antiqua* does not divide due to nutrient depletion (Nakamura et al. 1989). Thus we conclude that *G. dominans* has a potential ability to control the population of *C. antiqua*.

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#### LITERATURE CITED

- Bjørnsen, P. K., Kuparinen, J. (1991). Growth and herbivory by heterotrophic dinoflagellates in the Southern Ocean, studied by microcosm experiments. *Mar Biol.* 109: 397–405
- Caron, D. A., Goldman, J. C., Andersen, O. K., Dennett, M. R. (1985). Nutrient cycling in a microflagellate food chain. II. Population dynamics and carbon cycling. *Mar Ecol. Prog. Ser.* 24: 243–254
- Fenchel, T. (1982). Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Mar Ecol. Prog. Ser.* 8: 225–231
- Grover, J. P. (1990). Grazing by a heterotrophic microflagellate on two diatoms: functional and numerical responses in laboratory cultures. *Arch. Hydrobiol.* 119: 197–214
- Guillard, R. R. L., Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gram. *Can. J. Microbiol.* 8: 229–239
- Hansen, P. J. (1991). Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web. *Mar. Ecol. Prog. Ser.* 73: 253–261
- Lessard, E. J. (1991). The trophic role of heterotrophic dinoflagellates in diverse marine environments. *Mar. Microb. Fd Webs* 5: 49–58
- Lessard, E. J., Swift, E. (1985). Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with a dual-label radioisotope technique. *Mar Biol.* 87: 289–296
- Nakamura, Y., Umemori, T. (1991). Encystment of the red tide flagellate *Chattonella antiqua* (Raphidophyceae): cyst yield in batch cultures and cyst flux in the field. *Mar. Ecol. Prog. Ser.* 78: 273–284
- Nakamura, Y., Umemori, T., Watanabe, M. (1989). Chemical environment for red tides due to *Chattonella antiqua* Part 2. Daily monitoring of the marine environment throughout the outbreak period. *J. oceanogr. Soc. Japan* 45: 116–128
- Nakamura, Y., Umemori, T., Watanabe, M., Kulis, D. M., Anderson, D. M. (1990). Encystment of *Chattonella antiqua* in laboratory cultures. *J. oceanogr. Soc. Japan* 46: 35–43
- Sherr, B. F., Sherr, E. B., Berman, T. (1983). Grazing, growth and ammonium excretion rates of a heterotrophic microflagellate fed four species of bacteria. *Appl. Environ. Microbiol.* 45: 1196–1201
- Spoon, D. M., Feise, C. O. II, Youn, R. S. (1977). Poly(ethylene oxide), a new slowing agent for Protozoa. *J. Protozool.* 24: 471–474
- Strom, S. L. (1991). Growth and grazing rates of the herbivorous dinoflagellate *Gymnodinium* sp. from subarctic Pacific Ocean. *Mar Ecol. Prog. Ser.* 78: 103–113
- Uye, S. (1986). Impact of copepod grazing on the red-tide flagellate *Chattonella antiqua*. *Mar Biol.* 92: 35–43

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