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

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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⁻¹ (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 × 10³ to 2 × 10³ cells ml⁻¹) by *G. dominans* was 3.8 prey cell⁻¹ d⁻¹ 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 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 10² cells ml⁻¹. 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 × 10⁴ µm³ 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⁻² s⁻¹ 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 × 10⁴ µm³; 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 NO₃⁻, 20 µM PO₄³⁻, 100 ng l⁻¹ vitamin B₁₂ and 1.5 ml l⁻¹ Ni-metals (Nakamura & Umemori 1991)]. Sterilization of the medium was not carried out. The f/2 medium used for the maintenance culture of *Gyrodinium dominans*
steadily (Fig. 2; growth rate (see Grover 1990). Gyrodinium dominans average of Ng = concentration of concentrations at the end of the experiment (17:30 h); T = cell concentrations at the start of the experiment (08:30 h); NcE and N,~ suspensions (Y. Nakamura unpubl.). Effects of temperature. One liter of the Chattonella antiqua culture growing at the maximum rate (2850 cells ml^-1; 0.7 d^-1) was filtered (500 ml x 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, where i = C or N respectively). C and N contents of Gyrodinium dominans cell^-1 (Q_g) 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 x 2; < 10 cm Hg) through GF/D filters at 14:30 and and PN were measured and divided by the total cell number of C. antiqua filtered to obtain the C and N contents of C. antiqua cell^-1 (Q_c, where i = C or N respectively).

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 μm long x 15 μm wide) without food vacuoles appeared (ca 20 to 70 cells ml^-1) as (Raphidophyceae), volume = 7 x 10^2 μm^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),
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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.

![Growth curves](image)

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 Ie-shima Islands in summer 1989, the water temperature (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 nagasakiiense*) 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 ≥ 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 *Nc* in the daytime period when *C. antiqua* does not divide (Nakamura et al. 1990). During these experiments, *Nc* decreased constantly with time (Fig. 4A), and the estimated ingestion rates were 0.15 and 0.20 prey cell⁻¹ h⁻¹ (3.6 and 4.8 prey cell⁻¹ d⁻¹) in duplicate experiments.

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

![Growth rate](image)
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 ($f = 0.16$ prey cell$^{-1}$ h$^{-1}$, 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 the same time under a 12 h light:12 h dark photo-period. $E$ 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$^{-1}$ (typical cell concentration in a C. antiqua red tide; Nakamura et al. 1989) range from 0.4 to 4.3 % d$^{-1}$ (mean: 1.8 % d$^{-1}$). We also conducted the same calculation for Gyrodinium dominans. We assumed that the ingestion rate of G. dominans ($I$)
at \( N_c = 500 \text{ cells ml}^{-1} \) is the same as that obtained in the present study \((I = 3.8 \text{ prey cell}^{-1} \text{ d}^{-1} \text{ at } N_c = 1 \times 10^3 \text{ to } 2 \times 10^3 \text{ cells ml}^{-1} \); 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 \( G. \text{ dommains} \) is not affected by \( N_c \) at or above 500 cells ml\(^{-1} \) (Fig. 1) and that \( G. \text{ dominans} \) grows at a constant rate under a 12 h light:12 h dark photoperiod without a significant change of cell size (Fig. 2). Using \( N_0 = 10^1 \) to \( 10^2 \text{ cells ml}^{-1} \) (observed during the bloom decline of a \( C. \text{ antiqua} \) red tide in 1989; Nakamura & Umemori 1991) and based on the above assumptions, the rate of daily removal by \( G. \text{ dominans} \) is within the range 8 to 76% 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 \( N_0 \) doubles the following day (Fig. 1) and the daily removal rate reaches ca 20% if \( C. \text{ antiqua} \) does not divide due to nutrient depletion (Nakamura et al. 1989). Thus we conclude that \( G. \text{ dominans} \) has a potential ability to control the population of \( C. \text{ antiqua} \).

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LITERATURE CITED


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