Growth and grazing of a naked heterotrophic dinoflagellate, *Gyrodinium dominans*

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ABSTRACT: Growth and grazing rates of a naked heterotrophic dinoflagellate, *Gyrodinium dominans* [equivalent spherical diameter (ESD) = ca 20 μm], were examined in batch cultures. Strains isolated from the Seto Inland Sea (Harima strain) and Tokyo Bay (Tokyo strain) in Japan were used for the experiments. Both strains grew well when fed amorphous materials (such as bacterial flocs with attached nanoflagellates and aggregates of a small diatom) and plankton species with ESD of 15 to 26 μm. The Tokyo strain also grew rapidly when fed small (ca 5 μm) phytoplankton species at levels higher than 1×10^5 ml^{-1}. However, rates of ingestion of *Nephroselmis* aff. *rotunda* (ESD = 4.5 μm) by the Tokyo strain were too low to sustain rapid growth of *G. dominans* at natural levels of small (2 to 8 μm) flagellate abundance ($<3 \times 10^4$ ml^{-1}). Ingestion of *Heterocapsa triquetra* (ESD = 15.3 μm) followed Michaelis-Menten kinetics with half-saturation constants of 820 and 180 ml^{-1} and maximum ingestion rates of 0.82 and 0.59 prey predator^{-1} h^{-1} for the Harima and Tokyo strains, respectively. The clearance rates of both strains feeding on *H. triquetra* were about 2 orders of magnitude higher than those for *N.* aff. *rotunda*. These results indicate that *G. dominans* can play an important role in the decline of red tides caused by large (> 15 μm) nanoflagellates such as *Gymnodinium mikimotoi*.

KEY WORDS: Grazing · Growth · Heterotrophic dinoflagellate · Microzooplankton · Red tide

INTRODUCTION

The importance of grazing by microzooplankton (20 to 200 μm in size) on autotrophic and heterotrophic production has been discussed recently (e.g. Anderson 1988, Capriulo 1990). In some systems, microzooplankton herbivory could be more than 1 order of magnitude greater than copepod herbivory (Burkill et al. 1993). The major components of microzooplankton populations are planktonic ciliates and heterotrophic dinoflagellates. The trophic roles of ciliates have been studied extensively with special emphasis on their roles in the 'microbial loop' (e.g. Bernard & Rassoulzadegan 1990, Verity 1991). However, although ecological information about heterotrophic dinoflagellates is now increasing (e.g. Lessard & Swift 1985, Goldman et al. 1989, Hansen 1991, 1992, Strom 1991, Nakamura et al. 1992, Jacobson & Anderson 1993, Strom & Buskey 1993, Verity et al. 1993), studies of this group with respect to abundance, succession, grazing and growth are still sparse in comparison with those on ciliates. Since the biomass of heterotrophic dinoflagellates is comparable to or in some cases exceeds that of ciliates in many planktonic ecosystems (Lessard 1991), trophic roles of this group should be evaluated in greater detail for a more complete understanding of carbon flow through microzooplankton populations.

Gyrodinium dominans Hulburt [equivalent spherical diameter (ESD) = ca 20 μm] is one of the most abundant heterotrophic dinoflagellates in the Seto Inland Sea (eutrophic) and Tokyo Bay (hypertrophic), Japan (H. Takayama & M. Sato pers. comm.). The abundance of this species sometimes exceeds 100 ml^{-1} just after red tides of *Gymnodinium mikimotoi* (ESD = ca 15 μm) in the Seto Inland Sea (H. Takayama pers. comm.). Furthermore, during the period when abundance of small (2 to 8 μm) heterotrophic nanoflagellates (HNF) was rapidly decreasing in the Seto Inland Sea, '*G.*

dominans-like particles' were very often observed with epifluorescent microscopy of DAPI-stained seawater samples (Nakamura et al. 1994; not enumerated). These observations suggest that G. dominans is a potential predator for small (<8 μm) as well as large (>15 μm) nanoflagellates. Thus, there is a clear need to examine what kind of food G. dominans prefers and how fast it ingests prey and grows when supplied with different food concentrations. To these ends, we have established clonal cultures of G. dominans from the Seto Inland Sea and Tokyo Bay, and report here their growth and grazing rates under controlled conditions.

MATERIALS AND METHODS

Isolation and culture of Gyrodinium dominans. Clonal cultures of G. dominans were established by isolating a single cell from the surface seawater of Harima-nada, the Seto Inland Sea (Harima strain) and Tokyo Bay (Tokyo strain). The water temperatures of these sites during sampling were 25°C for the Harima strain and 23°C for the Tokyo strain. The Tokyo strain was kept in f/2 medium (Guillard & Ryther 1962) with Thalassiosira sp. (see Table 1) as food. The Harima strain was initially maintained in 10 ml of N,P-reduced f/2 medium ($NO_3^- = 100$ μM, $PO_4^{3-} = 7$ μM; henceforth f* medium) plus 0.5 ml of STP medium (= dissolved organic C/N source; Provasoli et al. 1957) with Nephroselmis aff. rotunda (>10^5 ml^{-1}; see Table 1), HNF (>10^4 ml^{-1}; species not identified, see Table 1) and bacteria (rod, >10^7 ml^{-1}). The Harima strain fed on the bacterial flocs with attached algae and HNF which were formed in this 'medium' (henceforth P medium). Later, the Harima strain was kept in f/2 medium with Thalassiosira sp. as the Tokyo strain. Potential growth rate and food source experiments (below) for the Harima strain were conducted using cells grown in P medium. In other experiments, cells grown in f/2 with Thalassiosira sp. were used. All cultures for both maintenance and experiments were kept in borosilicate test tubes (18 × 180 mm) at 24 ± 1°C with ca 100 μE m^{-2} s^{-1} under a 12 h light : 12 h dark cycle. Ingestion experiments (see below) were conducted during the light period. Cultures were rotated on a plankton wheel at 1 rpm during the experiments of ingestion and growth dynamics as a function of prey concentration (see below) Otherwise, the cultures were not agitated.

Basic characteristics of Gyrodinium dominans. Species identifications of the Harima and Tokyo strains was conducted by H. Takayama under light microscope and SEM observations. Cells of the Harima and Tokyo strains in late exponential phase were 33.4 ± 4.2 (n = 50) and 31.0 ± 2.5 μm (n = 50) in length and 4.8 × 10^3 and $4.0 × 10^3$ μm^3 in volume, respectively. Epifluo-

rescent microscope observations of the 2 strains clearly ruled out the presence of chloroplasts. In addition, intact cells of the Harima strain fluoresced bright green under blue light excitation while the Tokyo strain did not.

Both strains captured prey using a tow filament and prey was ingested through the flexible sulcas as Hansen (1992) observed in 3 Gyrodinium species.

In a previous study (Nakamura et al. 1992), we examined the growth and grazing of Gyrodinium 'dominans' (strain Gyro) feeding on the large prey Chattonella antiqua (see Table 1). Although the shape of strain Gyro was identical to the original description of G. dominans (H. Inoue pers. comm.), Gyro (ca 60 μm) was much longer than the original description (18 to 43 μm). Unfortunately, the strain Gyro culture died 2 yr ago for uncertain reasons and SEM observations were not made. It is plausible that strain Gyro belonged to another species (H. Takayama pers. comm.).

Potential growth rate determination. Thirty-five tubes with P medium (11.6 ml) or f/2 plus Thalassiosira sp. (11 ml, ca $1 × 10^5$ ml^{-1}) were prepared. A 0.5 ml volume of the maintenance culture of the Harima or Tokyo strain was inoculated into the tubes of P medium or f/2 plus Thalassiosira sp., respectively. Five tubes were taken daily and cell concentrations of Gyrodinium dominans were counted: samples were taken from each tube and 50 μl of polyethylene oxide in filtered seawater (1%) was added to the sample (2 ml) to slow down the movement of G. dominans (Spoon et al. 1977). Then 1 ml of the sample was counted in a Sedgewick-Rafter chamber under a light microscope. Growth rates were estimated as the slope of the linear portion of ln(Gyrodinium ml^{-1}) regressed against time.

Growth experiments using different food sources. A set of experiments was conducted to determine the range of plankton species that would support growth of Gyrodinium dominans. Eighteen species of phytoplankton in 7 classes, 1 strain of bacteria and 1 strain of HNF with bacteria (see Table 1) were offered. The linear dimensions (length and width) of the prey cells were measured under microscopes, and cell volumes were calculated assuming simple geometric forms. For bacteria, Synechococcus sp. and HNF, cells were fixed, stained with DAPI (except for Synechococcus sp.), and observed under an epifluorescent microscope. For other prey, the linear dimensions of intact cells were measured under a light microscope. When phytoplankton species were the prey, 1 ml of a dense culture of each species grown in f/2 was added to 10 ml of f* medium in test tubes. Ten cells of G. dominans washed 5 times in f* were then introduced to each tube by micropipet and incubated for 4 or 5 d. Our preliminary experiments showed that washing and isolation of the

dinoflagellates did not affect their viability. After each incubation, *G. dominans* cells were counted as described above and the growth rate (μ) calculated as $\mu = \ln (N_f/N_i)/T$, where T is the incubation time and N_i ($= 0.91$ ml^{-1}) and N_f the initial and final concentrations of *G. dominans*, respectively. When bacteria was offered as prey, 'STP+f•' medium was prepared by adding 0.5 ml of STP medium to 10 ml of f• medium, and 0.2 ml of stock bacterial cultures was added. Then 10 cells of pre-washed *G. dominans* were inoculated to each tube, incubated and counted as above. In cases where HNF plus bacteria were the prey, HNF were precultured in STP+f• medium for 2 d (HNF concentrations reached over 10^5 ml^{-1}). Then 10 cells of pre-washed *G. dominans* were introduced and their growth was followed as above. We also checked for the possibility of osmotrophic growth: 2 cells of *G. dominans* washed 7 times in f• were introduced to 10.5 ml of STP+f• medium and incubated for 7 d. For each experiment, initial and final prey concentration were also determined.

For each prey, 3 to 10 tubes were used in each experiment and 2 to 4 independent experiments were conducted. In all experiments, growth of *Gyrodinium dominans* in P medium for the Harima strain or in f• with *Thalassiosira* sp. for the Tokyo strain was examined as a control.

Ingestion experiments. Rates of ingestion of *Nephroselmis* aff. *rotunda* and *Heterocapsa triquetra* by *Gyrodinium dominans* were estimated by counting the number of ingested prey cells inside the predator cells after a known period of time (cf. Hansen 1992). Before determining the ingestion rates as a function of prey concentrations, we monitored the time course of ingestion. Twice-washed *G. dominans* were introduced by micropipet into 10 test tubes with f• medium (10 ml) to a final concentration of ca 30 ml^{-1}. *N.* aff. *rotunda* or *H. triquetra* were then added to each tube at concentrations of 1.5×10^5 or 3.5×10^3 ml^{-1}, respectively. The heterotrophic dinoflagellates were allowed to ingest *N.* aff. *rotunda* for 0.5, 1 and 2 h or *H. triquetra* for 1, 2 and 3 h and were then fixed in glutaraldehyde (EM grade, final concentration 1%). For each sampling period, 3 tubes were taken and 1 tube was used as a '0-time' sample. The fixed samples were stained with DAPI (final concentration 1 μg ml^{-1}) and then filtered (vacuum pressure <100 mm Hg) onto 0.8 μm pore-size black Nuclepore filters (25 mm diameter). These filters were observed with a Nikon epifluorescent microscope under UV or blue-light excitation (Nakamura et al. 1994) at a magnification of ×500. The number of vacuoles originating from *N.* aff. *rotunda* or *H. triquetra* inside each *G. dominans* cell was enumerated by monitoring the red autofluorescence of the vacuoles under blue-light excitation. At least 50 *G. dominans*
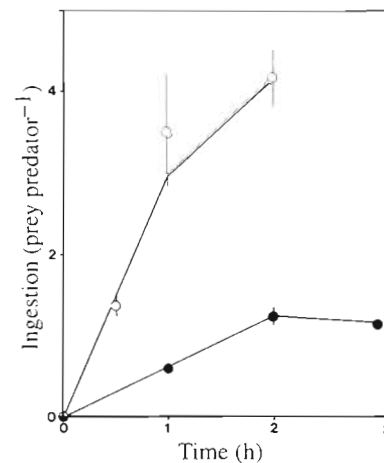

Fig. 1. *Gyrodinium dominans* (Tokyo strain). Number of particles inside predator cell clearly resembling (○) *Nephroselmis* aff. *rotunda* and (●) *Heterocapsa triquetra* as a function of incubation time. Error bars = ±1 SD

cells were observed per sample. Although Hansen (1992) stated that algae lose their autofluorescence very quickly upon ingestion, this was not the case for *N.* aff. *rotunda* and *H. triquetra* ingested by *G. dominans*. The rates of ingestion of *N.* aff. *rotunda* and *H. triquetra* were constant during the first 1 or 2 h of incubation, respectively (Fig. 1). Therefore, an incubation time of 1 h was subsequently used in the experiments to estimate ingestion rates as a function of prey cell concentrations (see below).

Twice-washed *Gyrodinium dominans* were introduced to 15 test tubes with f• (10 ml) at a concentration of ca 40 ml^{-1}. *Nephroselmis* aff. *rotunda* or *Heterocapsa triquetra* were added at 5 different concentrations (3 replicate tubes at each concentration). The ranges of the prey concentrations tested were $(0.1$ to $11) \times 10^4$ ml^{-1} for *N.* aff. *rotunda* and $(0.04$ to $6.2) \times 10^3$ ml^{-1} for *H. triquetra*. The heterotrophic dinoflagellates were allowed to ingest the prey for 1 h, then fixed, stained, filtered and observed under the epifluorescent microscope as described above. These experiments were repeated 3 times for each prey.

Rates of ingestion (I) of *Heterocapsa triquetra* by the 2 *Gyrodinium* strains were fitted to the Michaelis-Menten equation:

$$I = I_{max} \cdot N_{prey}/(K + N_{prey}) \tag{1}$$

where I_{max} is the maximum ingestion rate, K is the half-saturation constant, and N_{prey} is the prey concentration.

Growth rate dependence on prey concentration. Fifteen tubes of f• (10 ml) were prepared and 40 cells of *Gyrodinium dominans* which had been washed 4 times were introduced into each tube. *Heterocapsa tri-*

quetra were then added as prey at 5 different concentrations [(0.04 to 3.9) × 10³ ml⁻¹] with 3 replicate tubes at each prey concentration. The tubes were incubated for 2 d and the final concentrations of *G. dominans* and *H. triquetra* were enumerated as described in 'potential growth rate determination'. The growth rates of *G. dominans* were calculated assuming exponential growth during incubation (see 'Growth experiments using different food sources').

Ingestion (*I*) and growth rate (*μ*) data were interrelated by:

$$\mu = GGE \cdot I\,(v_{prey}/v_{pred}) \tag{2}$$

where *GGE* is the gross growth efficiency, and v_{prey} and v_{pred} are cell volumes of prey and predator, respectively.

Fig. 2. *Gyrodinium dominans*. Growth curves for (O) the Harima strain in P medium and (●) the Tokyo strain fed *Thalassiosira* sp. Error bars = ±1 SD

RESULTS

Potential growth rates

Growth rates (with 95% confidence limits) of *Gyrodinium dominans* in P medium and f/2 with *Thalassiosira* sp. were 1.24 (1.17, 1.31) and 1.36 (1.28, 1.44)
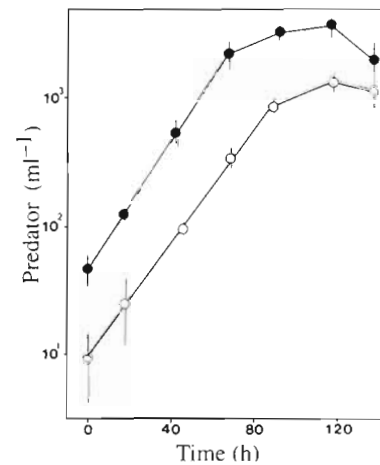
d⁻¹ for the Harima and Tokyo strains, respectively (Fig. 2). Changes in *G. dominans* cell size were not apparent during the exponential growth period but small cells of ca 20 µm in length appeared during the stationary and senescent period. However, the fraction

Table 1. *Gyrodinium dominans*. Growth using different food sources. ESD: equivalent spherical diameter; IN_{prey}: initial prey concentration; *μ*: growth rate; n: total number of cultures examined. Classes: Cy, Cyanophyceae; Ch, Chlorophyceae; Pry, Prymnesiophyceae; Pra, Prasinophyceae; D, Dinophyceae; R, Raphidophyceae; B, Bacillariophyceae. If final cell concentration of *G. dominans* was below 1 ml⁻¹, 0.0 d⁻¹ was assumed for the growth rate

Food (Class)	ESD (µm)	Remarks	IN_{prey}	Harima strain n	$\mu \pm SD$ (d⁻¹)	Tokyo strain n	$\mu \pm SD$ (d⁻¹)
STP medium		DOC and DON		19	0.00 ± 0.00	8	0.00 ± 0.00
STP + bacteria	1.2	Rod	(3–5) × 10⁷	16	0.00 ± 0.00	8	0.00 ± 0.00
Synechococcus sp.[a] (Cy)	1.3		(0.6–1) × 10⁷	6	0.00 ± 0.00	8	0.00 ± 0.00
Chlorella capsulata[a] (Ch)	4.2		(2–4) × 10⁵	6	0.00 ± 0.00	7	0.59 ± 0.10
Isochrysis galbana[a] (Pry)	4.2		(2–5) × 10⁵	11	0.14 ± 0.14	7	0.70 ± 0.09
Nephroselmis aff. *rotunda* (Pra)	4.5		(1–2) × 10⁵	18	0.19 ± 0.22	7	0.79 ± 0.08
Gephyrocapsa oceanica[a] (Pry)	4.7		2 × 10⁵	5	0.03 ± 0.07	7	0.64 ± 0.10
Amphidinium carterae[a] (D)	8.4	Naked	(3–7) × 10³	10	0.05 ± 0.08	9	0.45 ± 0.20
Pyramimonas parkeae[a] (Pra)	10.5		(1–2) × 10⁴	7	0.03 ± 0.07	8	0.10 ± 0.10
Heterosigma akashiwo[a] (R)	10.6		(1–3) × 10⁴	16	0.05 ± 0.09	9	0.23 ± 0.19
Heterocapsa triquetra[a] (D)	15.3	Thecate	(4–9) × 10³	8	0.62 ± 0.20	7	0.81 ± 0.08
Scrippsiella trochoidea[a] (D)	16.3	Thecate	(1–2) × 10³	10	0.04 ± 0.10	7	0.55 ± 0.10
Gymnodinium mikimotoi[a] (D)	16.8	Naked	(1–2) × 10³	5	0.40 ± 0.16	9	0.73 ± 0.14
Prorocentrum micans[a] (D)	24.3	Thecate	(1–2) × 10³	10	0.00 ± 0.00	7	0.11 ± 0.14
Chattonella marina (R)	26.3		(2–3) × 10³	7	0.39 ± 0.12	7	0.69 ± 0.10
Chattonella antiqua[a] (R)	35.3		(1–2) × 10³	8	0.12 ± 0.18	8	0.05 ± 0.09
Thalassiosira sp. (B)	5.4	1–10 cells colony⁻¹	(1–4) × 10⁵	25	0.98 ± 0.30	23	1.10 ± 0.13
Skeletonema costatum[a] (B)	5.6	4–10 cells chain⁻¹	(1–4) × 10⁵	8	0.00 ± 0.00	8	0.37 ± 0.13
Asterionella glacialis (B)	7.8	2–4 cells colony⁻¹	(1–2) × 10⁴	8	0.00 ± 0.00	8	0.00 ± 0.00
Chaetoceros sociale (B)	8.3	2–8 cells chain⁻¹	(1–2) × 10⁴	8	0.00 ± 0.00	8	0.00 ± 0.00
STP + bacteria + HNF	2.0	Bacteria floc	(1–2) × 10⁵	21	0.00 ± 0.00	8	0.00 ± 0.00
P medium		Bacteria floc		63	0.91 ± 0.16	9	0.82 ± 0.10

[a] Axenic strain

of small cells differed from tube to tube (20 to 80%). Prey cell concentrations during the stationary phase (120 h after inoculation; Fig. 2) were 2×10^7 ml^{-1} (bacteria), 3×10^5 ml^{-1} (HNF) and 2×10^5 ml^{-1} (*Nephroselmis* aff. *rotunda*) for the Harima strain and 1×10^6 ml^{-1} (*Thalassiosira* sp.) for the Tokyo strain.

Growth experiments using different food sources

Experiments on the growth of *Gyrodinium dominans* supplied with different food sources revealed some tendencies with respect to food utilization in the Harima and Tokyo strains (Table 1). First, both strains prefer amorphous materials such as bacterial flocs with attached flagellates formed in P medium and loose colonies of a small diatom (*Thalassiosira* sp.). Second, they are not osmotrophic and could not utilize pico-sized particles such as heterotrophic bacteria or *Synechococcus* sp. Third, chain forming diatoms (*Skeletonema costatum*, *Chaetoceros sociale*) and some dinoflagellates (*Scrippsiella trochoidea*, *Prorocentrum micans*) were not suitable food sources. Fourth, particles with ESD comparable to that of *G. dominans*

(i.e. *Heterocapsa triquetra*, *Gymnodinium mikimotoi*, *Chattonella marina*; ESD = 15 to 26 µm) served as good food sources. Fifth, *G. dominans* could not effectively utilize large particles with ESD 1.7 times larger than itself. These characteristics are common for both the Harima and Tokyo strains. However, significant strain differences were observed with respect to the utilization of particles less than 12 µm. When particles with ESD less than 12 µm were used as prey, the growth of the Harima strain was very poor. On the contrary, the growth rates of the Tokyo strain fed with particles with ESD of ca 5 µm were high and comparable to those for particles with ESD of ca 20 µm.

Ingestion

Rates of ingestion of *Nephroselmis* aff. *rotunda* (ESD = 4.5 µm) by the Tokyo strain increased linearly with prey concentration (Fig. 3A) and reached ca 3 prey predator^{-1} h^{-1} at a prey level of 1×10^5 ml^{-1}. Volume specific clearance rate (*VSC*) is defined as:

$$VSC = I/(N_{prey} \cdot v_{pred}) \tag{3}$$

where I is the ingestion rate, N_{prey} is the prey concentration and v_{pred} is the cell volume of predator *VSC* was independent of prey cell concentration and was about 6×10^3 h^{-1} (Fig. 3B).

Rates of ingestion of *Heterocapsa triquetra* (ESD = 15.3 µm) by the Tokyo and Harima strains increased hyperbolically with prey cell concentrations (Figs. 4A & 5A) and were fitted to Eq. (1). The calculated I_{max} and K values were 0.59 prey predator^{-1} h^{-1} and 1.8×10^2 ml^{-1}, respectively, for the Tokyo strain and 0.82 prey predator^{-1} h^{-1} and 8.2×10^2 ml^{-1}, respectively for the Harima strain. *VSC* decreased with prey concentrations (Figs. 4B & 5B) and was on the order of 10^5 h^{-1}.

Growth rates as a function of prey cell concentrations

The growth rate of the Tokyo strain increased with prey concentration (Fig. 6). Negative growth was observed when the initial prey concentration (IN_{prey}) was 36 ml^{-1}. The growth rate was ca 0.5 d^{-1} at IN_{prey} of 1×10^2 ml^{-1} and reached a plateau (ca 0.8 d^{-1}) at IN_{prey} higher than 4×10^2 ml^{-1}.

DISCUSSION

The growth rates of *Gyrodinium dominans* feeding on bacterial flocs in P medium or amorphous aggregates of *Thalassiosira* sp. (Fig. 2) far exceeded those of autotrophic dinoflagellates (<0.69 d^{-1}; Loeblich 1967)
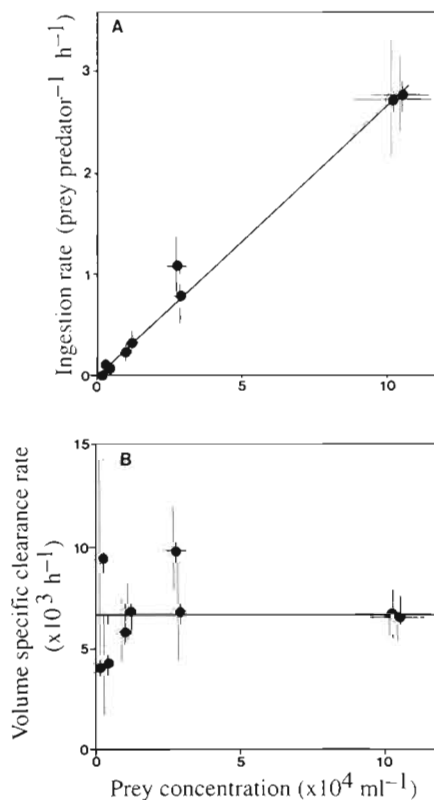

Fig. 3. *Gyrodinium dominans* (Tokyo strain) fed *Nephroselmis* aff. *rotunda*. (A) Ingestion rate and (B) volume-specific clearance rate as functions of prey concentration. Error bars = ±1 SD
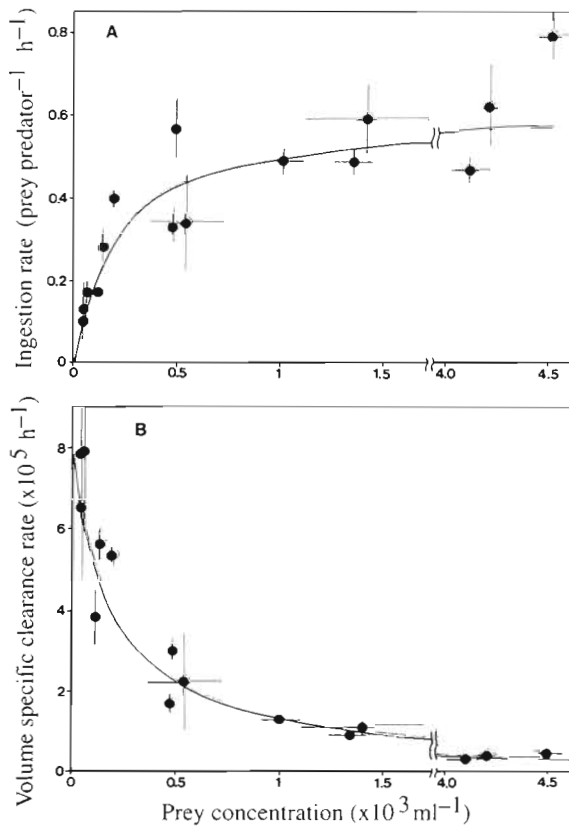
Fig. 4. *Gyrodinium dominans* (Tokyo strain) fed *Heterocapsa triquetra*. (A) Ingestion rate and (B) volume-specific clearance rate as functions of prey concentration. Curves numerically fitted to Michaelis-Menten kinetics. Error bars = ±1 SD
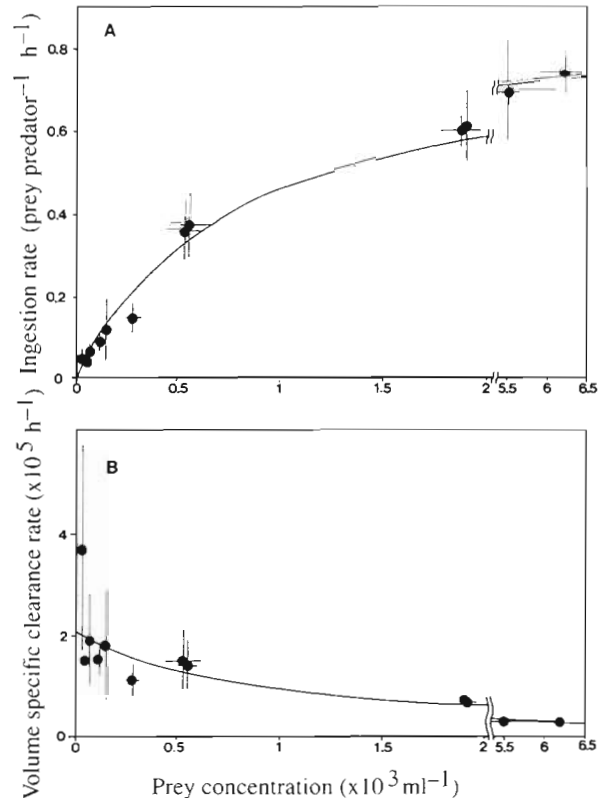

Fig. 5. *Gyrodinium dominans* (Harima strain) fed *Heterocapsa triquetra*. (A) Ingestion rate and (B) volume-specific clearance rate as functions of prey concentration. Curves numerically fitted to Michaelis-Menten kinetics. Error bars = ±1 SD

and are, in fact, the highest rates ever recorded for heterotrophic dinoflagellates in culture (0.3 to 1.2 d^{-1}; Hansen 1992, Jacobson & Anderson 1993). However, it should be noted that most of the previous heterotrophic dinoflagellate growth experiments were conducted at or below 20°C, while our results were obtained at 24°C. If we assume a Q_{10} of 2.5 (Fenchel & Finlay 1983), then the growth rate of *Gyrodinium spirale* (Hansen 1992) adjusted to 24°C would be 1.2 d^{-1}, comparable to that for *G. dominans*.

In the growth experiments using different food sources, the culture tubes were not rotated and even distribution of prey was not attained. Under non-agitated conditions, chain forming diatoms such as *Skeletonema costatum* and *Chaetoceros sociale* fell to the bottom of the experimental tubes and this might have made it impossible for *G. dominans* to catch the prey, leading to a poor growth of the predator (Table 1).

Both strains of *Gyrodinium dominans* grew well when fed bacterial flocs with attached algae and HNF formed in P medium. In contrast, no growth was observed with 'HNF + bacteria' (Table 1) where bacter-

ial flocs with attached HNF were formed. This is probably because NH_4^+ regenerated by bacterivory of HNF caused toxic effects on *G. dominans* in the 'HNF + bacteria' treatment while NH_4^+ was taken up by the algae in the P medium.

Large strain differences between the Harima and Tokyo strains of *Gyrodinium dominans* were observed with respect to suitable food sources: the former prefers particles with ESD of 15 to 26 µm while the latter grows well when feeding on particles with ESD of 5 µm as well as 15 to 26 µm (Table 1). However, the clearance rates of the Tokyo strain feeding on *Nephroselmis* aff. *rotunda* (ESD = 4.5 µm; Fig 3B) are about 2 orders of magnitude lower than those for *Heterocapsa triquetra* (ESD = 15.3 µm; Fig. 4B), indicating that the Tokyo strain utilizes the larger prey more effectively than the smaller one.

In Tokyo Bay, the abundance of small (2 to 8 µm) flagellates sometimes reaches 3×10^4 ml^{-1} (Nakamura unpubl.). At this level the calculated growth rate of the Tokyo strain by Eq. (2) assuming GGE of 0.6 (the highest value reported for heterotrophic dinoflagellates; Strom & Buskey 1993) would be 0.14 d^{-1}, making it dif-

Table 2. Ingestion parameters for various species of heterotrophic dinoflagellates. K_C: carbon-based half-saturation constant; I^*_{max}: volume-specific maximum ingestion rate; VSC_{max}: volume-specific maximum clearance rate. A carbon/volume conversion factor of 0.14 pg C μm^{-3} was assumed (Lessard 1991)

Predator	Temp. (°C)	Prey	K_C (μg C l^{-1})	I^*_{max} (h^{-1})	VSC_{max} (h^{-1})	Source
Gyrodinium dominans						
Harima strain	24	*Heterocapsa triquetra*	220	0.32	2.1×10^5	This study
Tokyo strain	24	*Heterocapsa triquetra*	50	0.28	8.0×10^5	This study
Gyrodinium spirale	15	*Heterocapsa triquetra*	560	0.14	3.4×10^4	Hansen (1992)
Oblea rotunda	20	*Ditylum brightwellii*	250	0.07	1.1×10^5	Strom & Buskey (1993)
Protoperidinium hirobis	20	*Leptocylindrus danicus*	160	0.38	7.8×10^4	Jacobson & Anderson (1993)

ficult for *Gyrodinium dominans* to sustain its population in the field by ingesting small flagellates alone. The impact of *G. dominans* grazing on small flagellates (d^{-1}) is calculated by:

$$I \cdot N_{pred}/N_{prey} = 6.3 \times 10^{-4} N_{pred} \qquad (4)$$

At N_{pred} of 100 ml^{-1}, close to the maximum observed in Tokyo Bay (M. Sato pers. comm.), only 6.3% of small flagellate population would be grazed per day. Therefore, we conclude that *G. dominans* does not play an important role in controlling the natural populations of small flagellates there, and further that natural levels of the small flagellates are insufficient to support the rapid growth of *G. dominans*.

In order to compare the ingestion data for *Gyrodinium dominans* with those for other heterotrophic dinoflagellates, carbon-based half-saturation constants (K_C), volume-specific maximum ingestion rates [$I^*_{max} = I_{max} \cdot (v_{prey}/v_{pred})$], and volume-specific maximum clearance rates [$VSC_{max} = I_{max}/(K \cdot v_{pred})$] were calculated (Table 2). The K_C values for the *G. dominans* strains fed *Heterocapsa triquetra* are lower or comparable to and the I^*_{max} and VSC_{max} values are higher or comparable to those for other species, indicating that *G. dominas* is a potential predator for large (>15 μm) nanoflagellates.

The growth rate of a predator as a function of prey (phytoplankton) concentration is usually determined under dim light and at low predator concentration to minimize the changes in prey concentration during the experiment. We tried repeatedly to establish the above relationship under dim light (ca 5 μE m^{-2} s^{-1}, 24 h light:0 h dark). However, the growth rates obtained were unusually low (<0.2 d^{-1}), the prey (*Heterocapsa triquetra*) looked unhealthy and accumulated at the bottom of culture tubes. We also tried to culture under high-light (100 μE m^{-2} s^{-1}, 24 h light:0 h dark) conditions but these experiments also failed: negative growth and unhealthy prey at the bottom. So we examined the relationship under 'usual' conditions (100 μE m^{-2} s^{-1}, 12 h light:12 h dark). Under these

conditions, the prey concentrations changed significantly (Fig. 6), the prey were not distributed uniformly at night and thus the results obtained are somewhat qualitative. However, it should be noted that the Tokyo strain of *Gyrodinium dominans* grew at a rate of 0.5 d^{-1} (ca 60% of the maximum) when the initial prey concentration was 1×10^2 ml^{-1} (Fig. 6). On the contrary, *G. spirale* fed *H. triquetra* could not sustain growth when the prey concentration was below 1×10^3 ml^{-1} (Hansen 1992). Thus *G. dominans* seems to be adapted to lower prey concentration than *G. spirale*.

In the Seto Inland Sea, red tides of *Gymnodinium mikimotoi* (ESD = 16.8 μm) occur during the warm season and the decline of these red tides is often coupled to increases in the abundance of *Gyrodinium dominans* (Nakamura et al. 1995, H. Takayama pers. comm.). Assuming that the rate of ingestion of *G. mikimotoi* by *G. dominans* is comparable to that of *Heterocapsa triquetra* (ESD = 15.3 μm) by the Harima strain, the grazing impact (d^{-1}) on the red tide population is
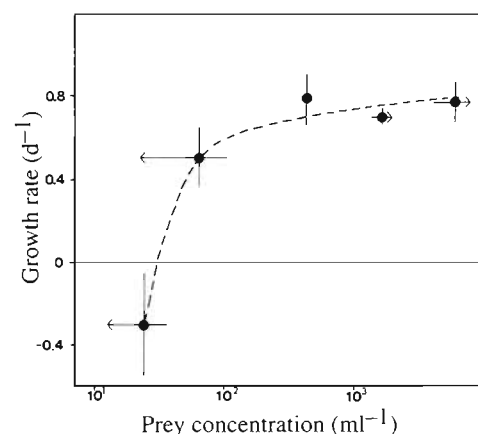

Fig. 6. *Gyrodinium dominans* (Tokyo strain) fed *Heterocapsa triquetra*. Growth rate as a function of prey concentration. Horizontal arrows indicate prey concentrations (from initial to final). Error bars = ±1 SD

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calculated as:

$$I \cdot N_{pred}/N_{prey} = 20\,N_{pred}/(820 + N_{prey}) \qquad (5)$$

At N_{prey} of 5×10^2 ml^{-1} and N_{pred} of 10 to 30 ml^{-1} (typical cell concentrations during the red tide in 1994; Nakamura et al. 1995), 15 to 45% of the *G. mikimotoi* standing crop is grazed per day, indicating that *G. dominans* plays important role in the disappearance of *G. mikimotoi* red tides. In contrast, before the outbreak of *G. mikimotoi*, the abundance of particles with ESD around 20 µm (mainly composed of *G. mikimotoi* and *Chattonella marina*) was ca 10 ml^{-1} (Nakamura et al. 1995) and *G. dominans* does not seem to sustain growth using such particles alone. Perhaps *G. dominans* ingests amorphous materials such as bacterial flocs and organic aggregates to survive under unfavorable conditions.

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