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# Growth and grazing response of a ciliate feeding on the red tide dinoflagellate *Gyrodinium aureolum* in monoculture and in mixture with a non-toxic alga

**Per Juel Hansen***

**National Environmental Research Institute, Department of Marine Ecology and Microbiology, Frederiksborgvej 399, DK-4000 Roskilde, Denmark**

ABSTRACT: The effect of the red tide dinoflagellate *Gyrodinium aureolum* on the growth of the tintinnid ciliate *Favella ehrenbergii* was studied. The ciliate is unable to sustain growth with this alga as the only food source, irrespective of concentration. Ciliate survival decreases at very high concentrations of *G. aureolum* probably due to toxic substances exuded from *G. aureolum* to the medium. This assumption is supported by the fact that growth of the ciliate is not affected by even very high concentrations of a non-toxic dinoflagellate, *Heterocapsa triquetra*. However, direct attempts to demonstrate toxic effects of exudates, using filtrates of dense cultures of *G. aureolum*, failed. Growth and grazing experiments were also carried out in which the ciliate was fed mixtures of *G. aureolum* and *H. triquetra* at relatively low algal concentrations. The growth of the ciliate was unaffected until *G. aureolum* accounted for about 70% of the total biomass. In cases where *G. aureolum* accounted for about 90% of the algal biomass, the growth rate of the ciliate was reduced by less than 25%. Grazing experiments demonstrated that *F. ehrenbergii* cannot selectively avoid ingestion of *G. aureolum*.

KEY WORDS: Red-tide dinoflagellate · Ciliate · Growth · Grazing · Toxic algae · *Gyrodinium aureolum*

## INTRODUCTION

*Gyrodinium aureolum* was first described from the northeast coast of the USA (Hulburt 1957), but the first bloom of *G. aureolum* was reported from European waters along the coasts of Norway (Braarud & Heimdal 1970). Since then, the species has regularly formed blooms along the west coasts of Europe and the northeast coast of the USA (e.g. Partensky & Sournia 1986, Mahoney et al. 1990).

The connection between blooms of *Gyrodinium aureolum* and harmful effects on fish and bottom invertebrates is well documented (Braarud & Heimdal 1970, Helm et al. 1974, Tangen 1977, Southgate et al. 1984, Potts & Edwards 1987, Mahoney et al. 1990). Both oxygen depletion and the production of toxins have been suggested as the cause of these effects. Experiments in which fish or mussels were exposed either to natural blooms or to laboratory cultures of *G. aureolum* suggest that the harmful effect is due to the production of toxins rather than to oxygen depletion (Widdows et al. 1979, Jones et al. 1982, Roberts et al. 1983, Turner et al. 1984, Nielsen & Strømgren 1991). Recently, 2 toxins, a glucolipid (1-acyl-3-digalactosylglycerol) and a fatty acid (octadecapentaenoic acid) have been isolated from *G. aureolum* (Yasumoto et al. 1990). These toxins damage membranes of blood cells causing cell lysis, and they are often referred to as hemolysins or ichthyotoxins due to their effect on blood cells and fish (Yasumoto et al. 1987). At present a chemical method for the quantification of these toxins does not exist, and our knowledge regarding the variability of toxin production is restricted.

*Present address: Marine Biological Laboratory (University of Copenhagen), DK-3000 Helsingør, Denmark

Blooms of *Gyrodinium aureolum* often last for a considerable period of time (several weeks), suggesting a mechanism which inhibits grazing. The literature does not provide much information on this subject, but recent laboratory experiments have demonstrated significantly reduced grazing activity and egg production rates of copepods when fed *G. aureolum* in monoculture (Gill & Harris 1987). Bjørnsen & Nielsen (1991) also found a drastic reduction in microzooplankton concentration at a depth coinciding with a subsurface layer of *G. aureolum* in the Kattegat.

The aim of the present work was to study the ability of a ciliate to feed and grow when fed a monoculture of *Gyrodinium aureolum*. In addition, the effect of the presence of an alternative prey on the feeding and growth rates of the ciliate was studied.

## MATERIALS AND METHODS

**The algae.** The potentially toxic dinoflagellate *Gyrodinium aureolum* Hulburt was isolated from Norwegian waters by Karl Tangen in 1977, whereas the non-toxic dinoflagellate *Heterocapsa triquetra* (Ehrenb.) Stein was isolated by Gert Hansen from the Øresund, Denmark, in 1986. Both species were provided by the Scandinavian Culture Collection, Botanical Institute, Department of Fungi and Algae, Copenhagen University. The algae were grown in B medium (Hansen 1989) based on 30‰ seawater and with the omission of silicate as non-axenic batch cultures under constant illumination (60 µmol photons $m^{-2}$ $s^{-1}$) at 15 ± 1°C. Cells were counted using a 1 ml Sedgewick-Rafter chamber, and each count was based on at least 400 cells. The dimensions of the algae were determined by measurements of Lugol fixed cells (n = 40) under the microscope, and volume was estimated assuming the shape of a double cone for *H. triquetra* and of an ellipsoid for *G. aureolum*. The carbon content of the algae was measured by filtering dense algal suspensions (4 to 20 ml) onto 6 mm precombusted (500°C) GF/F filters. Filters were dried at 40°C and stored for 24 h prior to infrared gas analysis. Each disc was combusted at 960°C in a flow of oxygen that carried the produced $CO_2$ to a Hartman-Braun carbon analyzer, equipped with a Hewlett Packard integrator. Each measurement was based on between 3 and 6 replicates and 3 reference spots without sample. Weighted crystals of oxalic acid were used as standards. The dimensions as well as carbon content are given in Table 1.

**The ciliate.** This work was based on several clones of the tintinnid ciliate *Favella ehrenbergii* (Clap. et Lach.) Jörg., isolated from surface water samples from the Limfjord, Denmark, in 1992, 1993 and 1994. The ciliates were initially grown in a Nunclon multidish with 2 ml of *Heterocapsa triquetra* suspension at a concentration of $10^3$ to $10^4$ cells $ml^{-1}$. Subsequently they were transferred to petri dishes or tissue culture flasks containing 40 to 60 ml B medium, to which *H. triquetra* was regularly added (at concentrations of $10^3$ to $10^4$ cells $ml^{-1}$). The cultures were transferred weekly.

**Experimental procedures.** All experiments were carried out at 15 ± 1°C and at a salinity of 30‰ using the B growth medium (Hansen 1989). Experiments were carried out at continuous low-intensity light (between 5 and 15 µmol $m^{-2}$ $s^{-1}$) to ensure no or very low growth of the prey during the experiments. In the growth/survival experiments the changes in algal density during incubation never exceeded 15%. In order to eliminate excessive changes especially at low prey concentrations, only a part of the ciliate population was transferred; the rest was fixed and counted.

**Monoculture experiments.** Experiments were conducted to study the growth response of *Favella ehrenbergii* fed either *Gyrodinium aureolum* or *Heterocapsa triquetra* in monoculture. Growth experiments in which *F. ehrenbergii* was fed *H. triquetra* were conducted at prey concentrations ranging from 35 to 5000 cells $ml^{-1}$. The ciliates were adapted to the experimental suspension for 1 or 2 d prior to the experiments. The experiments were carried out in 61.5 ml tissue culture flasks. Using a micropipette, 25 to 200 ciliates were added to each flask which was mounted on a plankton wheel (2 rpm). The duration of the experiment was ca 24 h, at which time the experiment was terminated with Lugol's iodine with acetic acid. Three replicates were set up for each food concentration. The prey concentration was measured at the beginning as well as at the end of each experiment and the average prey concentration determined: $C = (C_0 - C_t)(\ln C_0 - \ln C_t)^{-1}$, where $C_0$ = the concentration of algae at the beginning of the experiment and $C_t$ = concentration of cells at the end; $t$ = incubation time (h). The instantaneous growth rates of the ciliates were determined as: $\mu = (\ln N_t - \ln N_0)\ t^{-1}$, where $N_0$ = the number of cells at the beginning of the experiment and $N_t$ = the number of cells at the end; $t$ = incubation time. The growth rate ($\mu$) as a function of food

Table 1. *Gyrodinium aureolum* and *Heterocapsa triquetra*. Cell dimensions, volumes and carbon content of the studied dinoflagellates

| Dinoflagellates | Cell dimensions l × b × d (µm) | Volume (µm³) | Carbon content (pg C cell⁻¹) (SE) |
|---|---|---|---|
| *Heterocapsa triquetra* | 25 × 17 × 17 | 1890 | 507 (50) |
| *Gyrodinium aureolum* | 26 × 22.5 × 16 | 5200 | 651 (68.5) |

concentration was fitted to Michaelis-Menten kinetics: $\mu = \mu_m C(K_m + C)^{-1}$, where $\mu$ = growth rate ($h^{-1}$), $\mu_m$ = maximal growth rate, $C$ = average prey concentration, and $K_m$ = half-saturation constant.

Growth/survival experiments in which *Favella ehrenbergii* was fed *Gyrodinium aureolum* were carried out at prey concentrations ranging from 200 to 2500 cells ml$^{-1}$. Algal cultures were inoculated and allowed to grow into late exponential growth phase (cell concentration between 30 000 and 40 000 cells ml$^{-1}$) and maintained at this level by daily dilution. To attain experimental concentrations of between 200 and 2500 cells ml$^{-1}$, the culture was diluted with fresh B medium. The experiments were carried out in 61.5 ml tissue culture flasks. Using a micropipette, 24 ciliates were added to each flask which was mounted on a plankton wheel (2 rpm). The ciliates were transferred daily to a fresh algal suspension and counted. The duration of the experiments was 8 d.

Additional experiments were carried out in which *Favella ehrenbergii* was fed *Gyrodinium aureolum* at high concentrations (range 2500 to 30 000 cells ml$^{-1}$). These experiments were carried out in multidishes (2 ml algal suspension). Each experiment was initiated with 1 *F. ehrenbergii* cell per chamber, and 24 replicates were made for each algal concentration. The ciliates were transferred daily to a fresh algal suspension and counted. Growth experiments were conducted with *Heterocapsa triquetra* (concentration range: 5000 to 60 000 cells ml$^{-1}$) for comparison with a non-toxic form.

**Exudate experiments.** In order to study toxic effects on the growth of *Favella ehrenbergii* due to toxic substances exuded by *Gyrodinium aureolum* into the medium, 2 kinds of experiments were carried out. In the first kind of experiments, cylindrical chambers (dimensions: 20 × 30 mm) were made, in which 2 equally large parts were separated from the each other by plankton gauze (mesh size 5 µm). In this way, cells of *G. aureolum* were separated from cells of *Heterocapsa triquetra*, allowing an exchange of dissolved substances. Experiments were carried out by applying a dense suspension of *G. aureolum* (40 000 or 80 000 cells ml$^{-1}$) on 1 side of the gauze and a suspension of *H. triquetra* (10 000 cells ml$^{-1}$) on the other. The growth/survival response of *F. ehrenbergii* was studied by inoculating 15 ciliates on both sides of the plankton gaze. The ciliates were transferred daily to fresh suspensions and counted. The duration of the experiment was 5 d. Between 3 and 4 replicates were made. In another experiment, a dense suspension of *G. aureolum* (about 60 000 cells ml$^{-1}$) was centrifuged (100 × *g*) and the supernatant (2 ml) added to multidishes prior to the inoculation of ciliates. Filtered B medium served as a blank.

**Mixture experiments.** Experiments were conducted to study the growth and grazing of *Favella ehrenbergii* exposed to mixtures of *Gyrodinium aureolum* and *Heterocapsa triquetra*. In the first set of experiments the ratio between the 2 algae was kept constant. The following mixtures were used: 200/200, 500/500, 1000/1000, 2000/2000, 4000/4000 cells ml$^{-1}$. In another experiment, the concentration of *G. aureolum* was kept constant (2000 cells ml$^{-1}$), while different concentrations of *H. triquetra* were used (200, 600, 2000, 6000 and 20 000 cells ml$^{-1}$). All experiments were carried out in tissue culture flasks (61.5 ml) and mounted on a plankton wheel. The ciliates were transferred daily to fresh suspensions and counted. The duration of the experiments was between 6 and 8 d.

Grazing experiments were also carried out in which ciliates were fed mixtures of *Gyrodinium aureolum* and *Heterocapsa triquetra*. The concentration of cells was kept constant, while the ratio between the 2 algae was changed. The following mixtures of *H. triquetra*/*G. aureolum* were used: 250/2250, 750/1750, 1250/1250, 1750/750, 2250/250. Feeding rates were measured by adding between 100 ciliates to 61.5 ml algal suspension, using Nunclon flasks. The ciliates were allowed to graze for ca 40 h, whereafter they were fixed with Lugol (final concentration 1%). The grazing rates were determined as the reduction in particle concentration according to Frost (1972).

## RESULTS

The growth rate of *Favella ehrenbergii* was satiated at a prey concentration of ca 1000 *Heterocapsa triquetra* cells ml$^{-1}$ reaching a maximum of 0.034 h$^{-1}$, which corresponds to a doubling time of 20 h (Fig. 1, Table 2). The growth remained positive down to a prey concentration of 35 cells ml$^{-1}$ (= 20 µg C l$^{-1}$). Accepting a threshold prey concentration of 10 cells ml$^{-1}$, these data fit Michaelis-Menten kinetics closely ($R^2$ = 0.91, Statgraphics® 2.0).

*Favella ehrenbergii* was unable to sustain growth when *Gyrodinium aureolum* was offered as the only food source, irrespective of the algal concentration (Figs. 2 & 3, Table 3). The growth/survival response was similar within algal concentrations of 500 to 10 000 cells ml$^{-1}$: the ciliates divided up to 3 times within the first 3 d, whereafter they gradually died. After 8 d the number of ciliates was heavily reduced. At lower concentrations (200 cell ml$^{-1}$) no initial increase in ciliate numbers was found, and the cell number declined slowly throughout the experiment. At higher concentrations of *G. aureolum* (20 000 to 30 000 cells ml$^{-1}$), a quicker response was observed, and no ciliates were left after 6 d of exposure. At the highest concentration

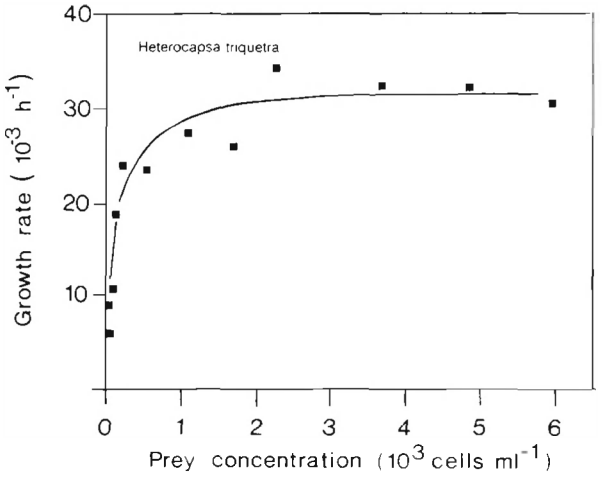


Fig. 1. *Favella ehrenbergii*. Growth rate as a function of cell concentration of the non-toxic dinoflagellate *Heterocapsa triquetra*. Data points represents treatment means ($n = 4$). Curve is numerically fitted to Michaelis-Menten kinetics: $\mu = 0.032 (C_p - 10)[(118 + (C_p - 10)]^{-1}$. For mean values ± 1 SE, see Table 2

the ciliates died within 2 d, which was as quickly as if they had not been fed at all. In contrast to this, *F. ehrenbergii* grew well even at extremely high concentrations of *Heterocapsa triquetra* (Fig. 4). Experiments in which a dense suspension of *G. aureolum* was separated from a suspension of *H. triquetra* using plankton gauze failed to prove any toxic effects. Likewise, filtrates from dense cultures of *G. aureolum* failed to have any negative effect on the growth or survival response of the ciliate (Fig. 5).

Experiments in which *Favella ehrenbergii* was exposed to mixtures *Gyrodinium aureolum* and *Heterocapsa triquetra* at a fixed cell concentration ratio

Table 2. *Favella ehrenbergi*. Growth rate as a function of cell concentration of the non-toxic dinoflagellate *Heterocapsa triquetra*. Growth rate values given as mean values ± 1 SE, n: number of replicates

| Mean prey concentration (cells $ml^{-1}$) | Mean growth rate ± 1 SE ($h^{-1}$) | n |
|---|---|---|
| 37.6 | 0.0090 ± 0.0031 | 4 |
| 59 | 0.0060 ± 0.0021 | 4 |
| 99 | 0.0108 ± 0.0046 | 4 |
| 131 | 0.0187 ± 0.0055 | 4 |
| 210 | 0.0239 ± 0.0020 | 4 |
| 539 | 0.0235 ± 0.0015 | 4 |
| 1088 | 0.0274 ± 0.0012 | 4 |
| 1679 | 0.0261 ± 0.0034 | 3 |
| 2251 | 0.0343 ± 0.0006 | 4 |
| 3660 | 0.0324 ± 0.0008 | 4 |
| 4834 | 0.0323 ± 0.0011 | 4 |
| 5941 | 0.0305 ± 0.0004 | 4 |

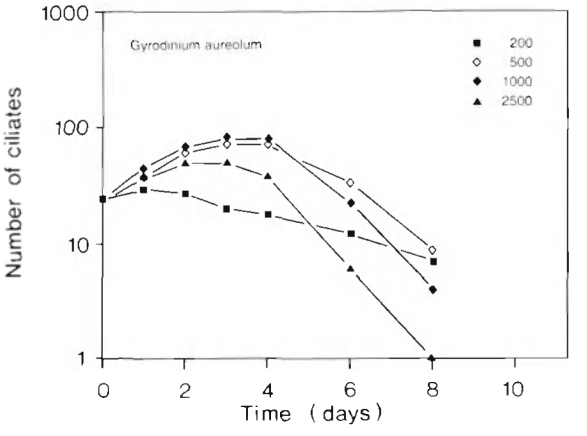


Fig. 2. *Favella ehrenbergii*. Growth/survival response to low concentrations of *Gyrodinium aureolum*. Numbers refer to cells $ml^{-1}$. Late exponential cultures were used. Ciliates were transferred daily to a fresh suspension of dinoflagellates

(50% of each) showed that the growth rate of *F. ehrenbergii* was unaffected within the concentration range of 1000 to 8000 cells $ml^{-1}$. At 400 cells $ml^{-1}$ the growth rate of the ciliate was reduced due to food limitation (see Fig. 1). Experiments in which *F. ehrenbergii* was exposed to a fixed cell concentration of *G. aureolum* (2000 cells $ml^{-1}$) and different concentrations of *H. triquetra* showed that the growth rate of *F. ehrenbergii* was unaffected until *G. aureolum* accounted for about 70% of the total biomass (Figs. 6 & 7). Even in cases where *G. aureolum* accounted for about 90% of the algal biomass, the growth rate of the ciliate was only slightly reduced (reduction less than 25%, Fig. 7).

Grazing experiments, in which *Favella ehrenbergii* was fed mixtures of *Gyrodinium aureolum* and *Hetero-*

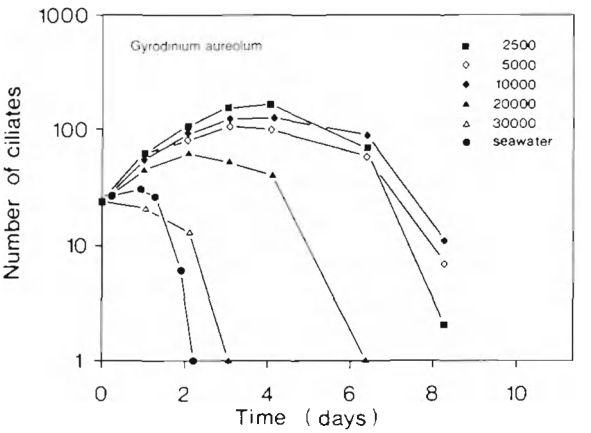


Fig. 3. *Favella ehrenbergii*. Growth/survival response to high concentrations of *Gyrodinium aureolum*. Seawater refers to starving ciliates; otherwise as stated in Fig. 2. For mean values ± 1 SE, see Table 3

Table 3. *Favella ehrenbergii*. Growth/survival response to different concentrations of *Gyrodinium aureolum*. Values refer to average number of ciliates in each well ± 1 SE (n = 24)

| Time (h) | Algal concentration (cells ml$^{-1}$) | | | | | Seawater |
|---|---|---|---|---|---|---|
| | 2500 | 5000 | 10 000 | 20 000 | 30 000 | |
| 5.5 | | | | | | 1.13 ± 0.09 |
| 23 | 2.25 ± 0.19 | 2.63 ± 0.18 | 2.38 ± 0.15 | 1.92 ± 0.13 | 0.88 ± 0.12 | 1.29 ± 0.11 |
| 29.5 | | | | | | 1.13 ± 0.11 |
| 46 | | | | | | 0.25 |
| 49 | 4.50 ± 0.36 | 3.54 ± 0.26 | 3.83 ± 0.29 | 2.58 ± 0.20 | 0.14 ± 0.14 | 0.00 |
| 71 | 6.42 ± 0.62 | 4.42 ± 0.50 | 5.17 ± 0.41 | 2.25 ± 0.24 | | |
| 105 | 7.08 ± 0.87 | 4.33 ± 0.60 | 5.25 ± 0.52 | 1.67 ± 0.28 | | |
| 153 | 2.88 ± 0.73 | 2.50 ± 0.59 | 3.71 ± 0.45 | 0.04 ± 0.04 | | |
| 198 | 0.08 ± 0.06 | 0.29 ± 0.12 | 0.46 ± 0.18 | 0.00 | | |

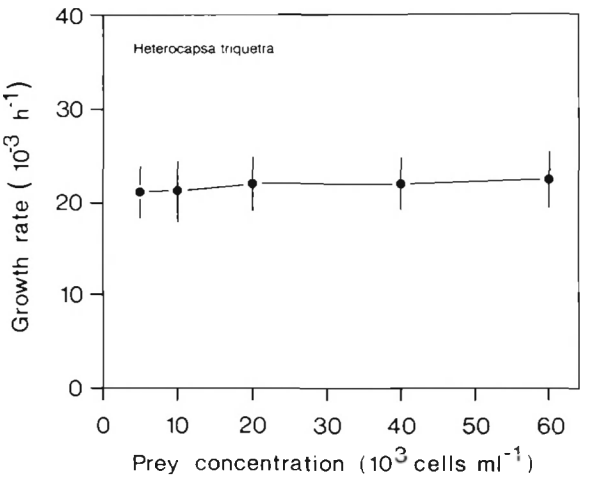


Fig. 4. *Favella ehrenbergii*. Growth rate as a function of cell concentration of the non-toxic dinoflagellate *Heterocapsa triquetra* at high cell concentrations. Data points represent treatment means ± 1 SE (n = 6), otherwise as stated in Fig. 2

*capsa triquetra* at different ratios, keeping the total prey concentration constant, demonstrated that the ciliate ingests *G. aureolum* with a slightly lower efficiency (Fig. 8).

## DISCUSSION

*Gyrodinium aureolum* has been reported to have a maximum growth rate of between 0.012 and 0.015 h$^{-1}$ at 20°C (Paasche et al. 1984, Garcia & Purdie 1992). The obtained maximum growth rate of the ciliate *Favella ehrenbergii* was 0.034 h$^{-1}$, thus exceeding that of *G. aureolum* by a factor of 2. In combination with the fact that *F. ehrenbergii* is capable of growth at low prey concentrations, this suggests that the ciliate potentially may be an important grazer of *G. aureolum* in nature. However, the present study suggests that *F. ehrenbergii* cannot sustain growth when feeding on *G. aureolum* as the only food source, irrespective of the prey concentration (Figs. 2 & 3). If a population of *F. ehrenbergii* is subjected to starvation, a few individu-

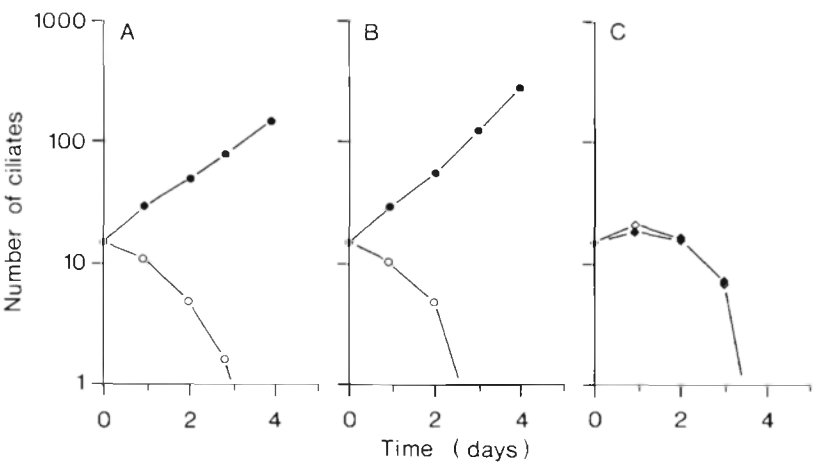


Fig. 5. *Favella ehrenbergii*. Growth/survival response to exudates from *Gyrodinium aureolum*. Data points refer to treatment means of between 3 and 4 replicates. (A, B) Growth experiments in which 2 chambers have been separated by plankton gaze (mesh size 5 µm), allowing passage of dissolved substances, but not of algae. (A) In one chamber, *Heterocapsa triquetra* were added at a concentration of 10 000 cells ml$^{-1}$ (●), while a suspension of 40 000 *G. aureolum* ml$^{-1}$ was added to the other chamber (○). (B) Same as in (A), but a higher concentration of *Gyrodinium aureolum* was added to the second chamber (80 000 cells ml$^{-1}$). (C) Survival response to filtrate from a dense (60 000 cells ml$^{-1}$) culture of *G. aureolum* (◇) and to filtered B medium (◆)

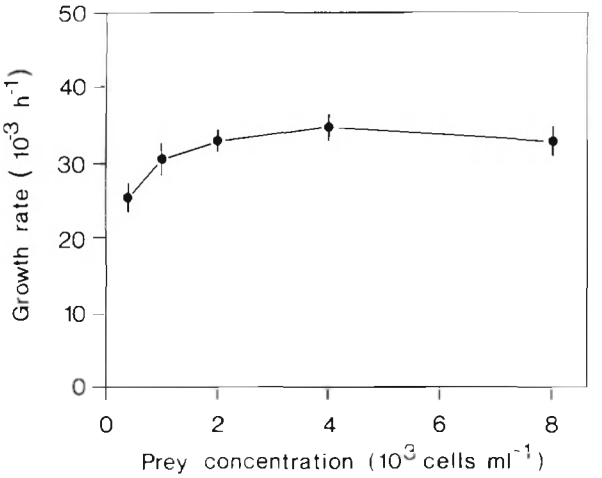


Fig. 6. *Favella ehrenbergii*. Growth rate as a function of total cell concentration of *Gyrodinium aureolum* and *Heterocapsa triquetra* at a fixed relative proportion (50% in numbers). Data points refer to treatments means ± 1 SE

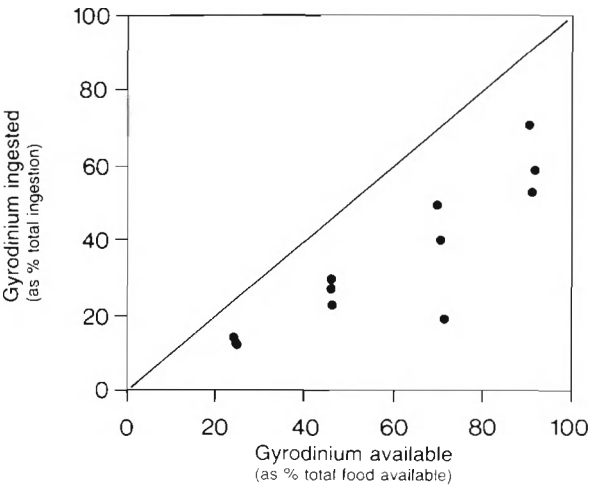


Fig. 8. *Favella ehrenbergii*. Particle uptake at a fixed total concentration (2500 cells $ml^{-1}$) of a mixture of *Heterocapsa triquetra* and *Gyrodinium aureolum* expressed as % uptake of *G. aureolum* as a function of % *G. aureolum* present in the algal suspension. Data points refer to individual values

als will divide once, before they eventually die within 3 d. Initial ingestion of *G. aureolum* is indicated by the fact that *F. ehrenbergii* divides up to 3 times during the first 2 to 3 d, when fed this alga at concentrations of 500 to 10 000 cells $ml^{-1}$ (Figs. 2 & 3). It has previously been shown that *G. aureolum* is a poor food source for copepods. Copepods fed monocultures of *G. aureolum* at low concentrations have virtually no egg production and suffer mortality rates comparable to those obtained in filtered seawater (Gill & Harris 1987). Reduced filtration rates have also been demonstrated in rotifers and copepods fed monocultures of the

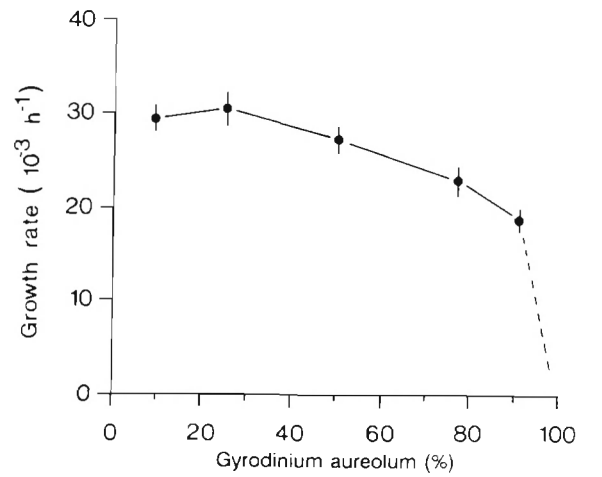


Fig. 7. *Favella ehrenbergii*. Growth rate as a function of the fraction of the total cell cencentration made up by *Gyrodinium aureolum*. Concentration of *G. aureolum* was kept constant (2000 cells $ml^{-1}$), while the concentrations of *Heterocapsa triquetra* were increased (200 to 6000 cells $ml^{-1}$). Data points refer to treatments means ± 1 SE

closely related *Gymnodinium mikimotoi* Mikye and Kominani (= *G. nagasakiense*; Abé & Hirayama 1979, Uye & Takamatsu 1990).

In the present study, the negative effect of *Gyrodinium aureolum* on the growth and survival of *Favella ehrenbergii* at algal concentrations less than 10 000 cells $ml^{-1}$ is without doubt caused by ingestion of the alga. This is clearly demonstrated in the mixture experiments, in which *F. ehrenbergii* was exposed to increasing concentrations of *G. aureolum* and *Heterocapsa triquetra* at a fixed cell concentration ratio (compare Figs. 2, 3 & 6). If dissolved substances were toxic at low concentrations, the growth rate of *F. ehrenbergii* would decrease with increasing concentrations of *G. aureolum*. In fact, the ciliate grows at a rate which is similar to that obtained on a pure diet of *H. triquetra* (compare Figs. 1 & 6).

Substances excreted into the medium by *Gyrodinium aureolum* have previously been reported to suppress the growth of other algae (e.g. diatoms; Gentien & Arzul 1990, Arzul et al. 1993) and it has also been suggested that these substances were responsible for fish kills (Jones et al. 1982). In the latter case, the toxic substances appear to affect the gills of the fish, causing necrosis of the gill filaments (Jones et al. 1982, Turner et al. 1984). The negative effect on the growth rate of *Favella ehrenbergii* increased at high concentrations of *G. aureolum* (above 10 000 cells $ml^{-1}$) indicating toxic effects of exudates. That this is not an unspecific response due to leakages of substances in general is supported by the fact that *F. ehrenbergii* grows well even at extremely high concentrations of *Heterocapsa triquetra* (Fig. 4). However,

experiments with filtrates from dense cultures of *G. aureolum* failed to produce any toxic effect on *F. ehrenbergii* (Fig. 5). The lack of toxic effects of filtrate originating from dense *G. aureolum* cultures in the present study may be due to a rapid turnover of the toxin(s). In a previous paper, it was demonstrated that the toxic effects of filtrates from another red tide dinoflagellate, *Alexandrium tamarense* (Lebour) Balech, disappeared within hours (Hansen 1989).

Thus, laboratory studies conducted so far suggest that both proto- and metazooplankton are unable to sustain growth when fed *Gyrodinium aureolum* in monoculture. This may be due either to the lack of substances which are essential for the growth of zooplankton or to the production of toxic substances by the alga. The fact that neither proto- nor metazooplankton which are exposed to low concentrations of *G. aureolum* suffer from acute death suggests that toxins are not involved. However, it is worthwhile to note that the dinoflagellate *Alexandrium tamarense*, which produces neurotoxins, does not elicite acute death in copepods which have been exposed. In the case of *Favella ehrenbergii*, the present study suggests that toxic substances exuded by the alga into the medium may partly be responsible for the observed mortality at extremely high concentrations of *G. aureolum*.

*Gyrodinium aureolum* is only one among several phytoflagellates which has been shown to produce hemolysins or ichthyotoxins. Verity & Stoecker (1982) studied the ability of tintinnids to feed and grow on the raphidophycean *Heterosigma carteri* (Hulburt) Taylor (often referred to as *H. akashiwo* or *Olistodiscus luteus*), an organism which also has been implicated in fish kills (Chang et al. 1990, Black et al. 1991). They demonstrated that ciliates were unable to sustain growth when fed a monoculture of this algae, irrespective of algal concentration. Lethal effects were observed sooner at concentrations exceeding ca $10^3$ cells $ml^{-1}$. As in the present study, the authors failed to document toxic effects of filtrates.

### Ecological significance

No data exist on the significance of zooplankton grazing during blooms of *Gyrodinium aureolum* in nature. Nevertheless, the present study suggests that monospecific blooms of *G. aureolum* are not substantially grazed by protozooplankton. This is supported by Bjørnsen & Nielsen (1991), who found a dramatic reduction of protozooplankton numbers in a subsurface layer of algae heavily dominated by *G. aureolum* in the Kattegat, Denmark.

In an initial bloom phase, *Gyrodinium aureolum* will occur together with other phytoplankton species. Will zooplankton survive/grow under such conditions? Experiments in which *Favella ehrenbergii* was fed mixtures of *G. aureolum* and the non-toxic algae *Heterocapsa triquetra* indicate that ciliates are able to maintain positive growth until *G. aureolum* make up a substantial fraction of the available algal biomass. In their study of the negative effects of *Heterosigma carteri* on ciliate growth, Verity & Stoecker (1982) examined the ability of ciliates to sustain growth in mixtures of this toxic alga and an alternative prey. In experiments in which the ciliates were subjected to a fixed cell concentration of non-toxic prey and increasing cell concentrations numbers of the toxic *H. carteri*, they found that the ciliates were able to sustain maximum growth rate until *H. carteri* exceeded 12 to 14% of the available prey (in terms of biomass). The ciliates were unable to sustain growth when the concentration of *H. carteri* accounted for more than between 65 and 80% of the available food. At this concentration of *H. carteri*, the toxic effect was most probably due to toxic exudates in combination with ingestion of the alga. Thus, the experiments done so far suggest that ciliates can thrive among algae producing hemolysins/ichthyotoxins, as long as the concentration of these algae is low and the algae do not dominate the phytoplankton biomass.

Do the ciliates selectively avoid ingestion of *Gyrodinium aureolum*? It is well documented that the efficiency of particle capture in ciliates depends on the size of the particles (Heinbokel 1978, Fenchel 1980, Jonsson 1986). Some studies have found that ciliates take up artificial particles at a lower rate compared to natural particles of a similar size (Stoecker et al. 1986, Stoecker 1988), although this is not a general phenomenon (Fenchel 1986). In addition, a few studies (Heinbokel 1978, Stoecker et al. 1981) have suggested that ciliates can select between algae of comparable sizes, e.g. toxic algae from non-toxic ones (Stoecker et al. 1981). The present studies on the grazing by *Favella ehrenbergii* on *G. aureolum*/*Heterocapsa triquetra* suggest that the ciliate *F. ehrenbergii* cannot selectively avoid feeding on the non-nutritious *G. aureolum*. Thus, *G. aureolum* cannot escape grazing from ciliates until they completely dominate the phytoplankton community.

In conclusion, the available data suggest that monospecific blooms of *Gyrodinium aureolum* inhibit the growth and, subsequently, grazing of the proto- and mesozooplankton. This may be one of the reasons why these blooms can persist for several weeks. However, it is interesting that at least the protozooplankton may grow well when exposed to mixtures of *G. aureolum* and a non-toxic prey. A consequence of this is that *G. aureolum* cannot escape a potentially significant grazing loss in nature, unless the alga dominates the phytoplankton community.

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