Vol. 53: 105–116, 1989 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published April 27

The red tide dinoflagellate *Alexandrium tamarense:* effects on behaviour and growth of a tintinnid ciliate

Per Juel Hansen

Marine Biological Laboratory, University of Copenhagen, DK-3000 Helsingør, Denmark

ABSTRACT: The effect was studied of the red tide dinoflagellate *Alexandrium tamarense* (= *Gonyaulax excavata, G. tamarensis*) on the behaviour and growth of the tintinnid ciliate *Favella ehrenbergii*. Six clones of *A. tamarense* with different levels of PSP toxin content were used. *A. tamarense* produces substances lethal to *F. ehrenbergii*. The algae are ingested by the ciliate, which is only affected by exudates in the medium. The exudate acts on the cell membrane and induces ciliary reversals resulting in continuous backwards swimming. After some time, ciliates swell and subsequently lyse. The exudate is very labile and the toxic effect of filtrates disappears within a few hours. In batch cultures of *A. tamarense*, the toxicity of the medium depends on the growth phase. The highest level of toxicity is found in the period following exponential growth; this suggests that cells especially exudate toxins when growth becomes limited. Indirect evidence suggests that the toxic effect on ciliates is caused by PSP toxins. The growth response of *F. ehrenbergii* on *A. tamarense* is clone-specific. The ciliate will grow when fed low concentrations of the Wh 7 clone of *A. tamarense* at generation times of 26 to 32 h. Similar rate constants are obtained with similar concentrations (in terms of volumes) of the non-toxic dinoflagellate *Heterocapsa triquetra*. *Favella ehrenbergii* will not grow when fed the Ply 173a clone of *A. tamarense* at any concentration.

INTRODUCTION

A number of marine dinoflagellates are known to produce non-protein toxins. These can act directly to cause fish kills or accumulate in suspension-feeding animals (zooplankton, bivalves), causing illness or death to consumers (fish, birds, man). The toxins, which are either water-soluble or lipid-soluble, can be neurotoxic, hemolytic, or have gastrointestinal activity (Steidinger & Baden 1984). It is unclear why dinoflagellates produce these substances although suggestions have been plentiful: toxins of *Alexandrium tamarense* (Lebour) Balech have been related to nucleic acid metabolism (Mickelson & Yentsch 1979) and suggested to be important compounds involved in the structure and function of the algal genome (Anderson & Cheng 1988). Steidinger & Baden (1984) speculated that since toxins modulate the flow of ions across membranes they might play a similar physiological role in the overall well-being of the organism itself.

Most toxin-producing dinoflagellates are capable of forming monospecific blooms (red tides) suggesting a mechanism that inhibits zooplankton grazing. Recent laboratory experiments on copepods (Huntley et al. 1983, 1986) demonstrated significantly reduced grazing rates on different species of dinoflagellates *(Alexandrium tamarense, Gonyaulax grindleyi, Ptychodiscus brevis* and *Scrippsiella trochoidea)*. Evidence suggesting that the reduced grazing rates are chemically mediated was provided by Ives (1985). He investigated the grazing response of 2 species of copepods to clones of *A. tamarense* with different cell toxin contents. These experiments suggested a decrease in ingestion rates with increasing cell toxin content. Direct observations on the copepod *Calanus pacificus* (Huntley et al. 1986, Sykes & Huntley 1987) show that *G. grindleyi* cells cause reverse peristalsis and regurgitation and that *P. brevis* cells cause elevated heart rate and loss of motor control.

Field studies (Needler 1949, Prakash 1963, White 1979, Watras et al. 1985) show that tintinnids belonging to the genus *Favella* often are found at high concentrations during blooms of *Alexandrium tamarense* and so are believed, in part, to regulate such blooms. Laboratory experiments (Stoecker et al. 1981) showed

0171-8630/89/0053/0105/$ 03.00

that *Favella ehrenbergii* is able to grow on *A. tamarense*. However, the possibility that grazing by tintinnids is affected by toxins produced by dinoflagellates has not been investigated. In this study, the behaviour and growth response of *F. ehrenbergii* when introduced to different clones of the *A. tamarense* complex are investigated.

At present the generic as well as the specific names of this complex are being disputed (Taylor 1984, Balech 1985, Balech & Tangen 1985, Taylor 1985, Moestrup & Hansen 1988). The clones Ply 173a and LF 1 to 4 all possess a pore in the first apical plate (1'). The name *Alexandrium tamarense* is used for these clones, following the nomenclature suggested by Moestrup & Hansen (1988). The clone Wh 7 lacks this pore and *A. fundyense* is suggested by Balech (1985) for this form. As the taxonomic status of this form is still uncertain, *A. tamarense* is also used for this clone.

MATERIALS AND METHODS

Isolation and culture of dinoflagellates. The study is based on 6 clones of *Alexandrium tamarense* and one clone of the non-toxic dinoflagellate *Heterocapsa triquetra*. Four of the *A. tamarense* clones (LF 1 to 4) were isolated from a surface water sample from the Limfjord, Denmark, in May 1987. The other 2 originate from Plymouth, UK (Ply 173a) and the Bay of Fundy, Canada (Wh 7), respectively. *H. triquetra* was isolated from the Øresund, Denmark. Cell sizes and volumes during exponential growth are shown in Table 1. Algae were grown as non-axenic batch cultures in constant light

Table 1. Size and volume of studied dinoflagellate clones

Species/clone	Size[a] (µm)	Volume[b] (µm^3)
Alexandrium tamarense		
Ply 173a	29	12 800
Wh 7	32	17 200
LF 1	30	14 100
LF 2	28	11 500
LF 3	28	11 500
LF 4	33	18 800
Heterocapsa triquetra		
Het	L:27 D:17	2 050

[a] Mean of 30 cells
[b] Volume of *A. tamarense* was calculated as a sphere; that of *H. triquetra* as 2 cones

(about 3000 lux of cool fluorescent light) at 18 ± 1 °C. Cells were counted using a 1 ml Sedgewick-Rafter chamber. Each count was based on at least 400 cells (SD = 5 %). All clones were cultured in B medium. The medium was based on 30 % seawater and made up from the following solutions:

Solution 1: Na_2EDTA, 45 g; $NaNO_3$, 100 g; H_3BO_3, 33.6 g; NaH_2PO_4, 20 g; $MnCl_2 \cdot 4H_2O$, 0.36 g; $FeCl_3 \cdot 6H_2O$, 1.3 g; demineralized H_2O to 1000 ml.

Solution 2: $ZnCl_2$, 2.1 g; $CoCl_2$ $6H_2O$, 2.0 g; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.9 g; $CuSO_4$ $5H_2O$, 2.0 g; demineralized H_2O to 1000 ml; drops of 0.1 *N* HCl until clear solution.

Vitamin stock solution: Thiamin 200 mg; Biotin 1 mg; Cyanocobalamin 1 mg; demineralized H_2O to 1000 ml.

The 3 solutions were autoclaved separately. A stock solution of 1000 ml Solution 1 and 1 ml Solution 2 was made. Aliquots of 1 ml of this stock solution and 1 ml of the vitamin stock solution were added to 1 l seawater.

Isolation and culture of *Favella ehrenbergii*. These were isolated from a surface water sample from Århus Bay, Denmark, in July 1987. 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 containing ca 40 ml B medium. *H. triquetra* (10^4 cells) were regularly added to these dishes. *F. ehrenbergii* was subcultured weekly.

Experimental procedures. In order to examine swimming patterns of *Favella ehrenbergii*, cells were placed in a Sedgewick-Rafter cell and recorded with a video recorder (Sony video camera DXC 101 P and Panasonic recorder AG-6200) fitted to a dissection microscope. Recordings were analyzed by plotting the position of individual ciliates, frame by frame, on a plastic film covering the monitor screen. Swimming behaviour of ciliates added to a suspension of *Heterocapsa triquetra* (4000 cells ml^{-1}) or *Alexandrium tamarense* (clone Wh 7, 3000 cells ml^{-1}) were studied.

All other experiments were carried out in Nunclon multidishes at 18 ± 1 °C under constant light (3000 lux) unless otherwise stated. In experiments on ciliate behaviour, subsamples of the dinoflagellates (Wh 7 and Ply 173a) were taken at specified stages of the batch cultures. One ml of suspension was pipetted into 4 replicate chambers. Using a micropipette, 12 or 13 ciliates were added to each chamber and the experiment was run for 120 min in the light. The behaviour of individual ciliates at time 1 to 15, 20, 30, 40, 60 and 120 min was observed at a magnification of 12 ×

In experiments on the response to dinoflagellate exudates, algal suspensions were centrifuged and 1 ml of the supernatant was pipetted into 2 replicate chambers. Again, 12 or 13 ciliates were added with a micropipette. The whole procedure took exactly 3 min whereafter ciliate behaviour was observed as described above. In order to test the lability of exudates, the supernatant was aged for 15 or 30 min.

The term CBS 50 is defined as the Concentration of cells required to induce 50 % Backwards Swimming of the tested ciliates within 10 min. To test whether the toxic effect of dinoflagellates on the ciliates was caused by PSP toxins, the relationship between the toxin content of the dinoflagellates and CBS 50 was studied.

For the 6 clones of *Alexandrium tamarense* chosen, the range in toxin content was from undetectable to 27.5 pg cell^{-1} (as estimated from mouse bioassays). To compare the ability of cells in exponential and stationary phase to induce backwards swimming of ciliates, algal cultures were inoculated and the culture tested for its ability to induce backwards swimming until the cell concentration had reached the CBS 50 value. The culture was then allowed to grow into the stationary phase, at which time dilutions of the culture were made until the cell concentration had reached the CBS 50 level.

Particle uptake by ciliates was measured simultaneously with the behaviour experiments. Two ml of algal suspension was pipetted into 4 replicate chambers, and 20 to 25 ciliates were added to each chamber. They were allowed to graze for 2, 4, 6, 8, 10, 15, 20, 30, 40, 60 and 120 min and then fixed by adding 50 µl 30 % formaldehyde. Particles inside food vacuoles, which could clearly be identified as *Alexandrium*, were then counted under the microscope.

Experiments were conducted to determine the growth of *Favella ehrenbergii* on algal concentrations within the range of 250 to 5000 cells ml^{-1}. Clones Ply 173a and Wh 7 were used; among the studied clones they represent the least and the most toxic ones, respectively. Both were inoculated and allowed to grow to 3000 cells ml^{-1} and were then maintained at this level by daily dilution. To attain experimental concentrations of from 250 to 3000 cells ml^{-1}, the culture was diluted with fresh B medium. A subculture of the Ply 173a clone was allowed to grow to 5000 cells ml^{-1} and maintained at this level. Growth experiments were also conducted with *Heterocapsa triquetra* (concentration range: 500 to 10 000 cells ml^{-1}) for comparison with a non-toxic form. Each growth experiment was initiated with one *F. ehrenbergii* cell. For each algal concentration, 24 replicates were made. Every day the ciliates were transferred to a fresh algal suspension and counted. The experiments were run in the dark as well as in the light.

Toxin extraction. Simultaneously with all the experiments described above at least 10^6 algae cells were set aside for measurements of toxin content. The algae suspension was gently centrifuged and the supernatant decanted. Toxins were extracted by adding 15 ml 0.1 *N* HCl to the pellet. The tube containing algae and acid was placed in boiling water for 8 min (in an autoclave). The sample was cooled on ice and subsequently filtered through a 1 µm filter. The filtrate was adjusted to pH 3 to 3.5 with drops of 5 *N* and 1 *N* HCl. Toxin content was determined by injection (i.p.) of 1 ml in mice, using the standard mouse biomassay (Horwitz 1984). Cell toxin content is expressed in units of saxitoxin equivalents because the mouse bioassay was standardized with pure saxitoxin. Toxicities of the individual clones are shown in Table 2.

Table 2. Toxin content of *Alexandrium* clones

Clone	Mouse bioassay[a] (pg cell^{-1})	HPLC[b] (pg cell^{-1})
Ply 173a	Undetectable (<0.6)	0.12
Wh 7	12.8–27.5	11.4
LF 1	10.6–16.2	–
LF 2	8.3–15.0	14.1
LF 3	4.1– 6.5	–
LF 4	1.1– 1.3	–

[a] Range (Ply 173a and Wh 7, n = 6; LF 1–4, n = 3)
[b] For conversion of HPLC data see Boyer et al. (1986), n = 1

The toxicity (Table 2) and toxin composition (Fig. 1) of 3 clones were determined by HPLC. At least 10^6 cells were harvested from late exponential phase cultures and concentrated by centrifugation. The toxins were extracted by adding 2 ml 0.05 *N* acetic acid to the pellet followed by disruption of cells by sonication for 5 min in an ice bath. The toxins were separated on a polystyrene divinylbenzene resin column in the reverse mode, using hexane and heptane as ion-pair reagents. The toxins were detected by fluorescence after postcolumn oxidation with alkaline periodate. For a more detailed description of the method see Boyer et al. (1986).

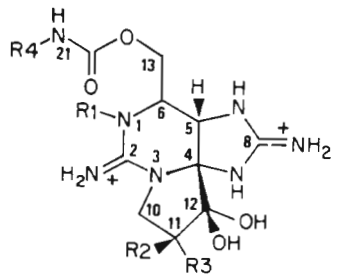
RESULTS

Behaviour of *Favella* in contact with *Alexandrium*

The normal mode of motility in *Favella ehrenbergii* is swimming in a forward, smooth, helical path interrupted by ciliary reversals (tumbles) during which the cell for a short period of time swims backwards to continue in a new direction (Fig. 2A).

When added to 3000 cells ml^{-1} of the highly toxic Wh 7 clone *Alexandrium tamarense* (late exponential phase cultures) ciliates begin to tumble more frequently and periods of backwards swimming are prolonged. After longer exposure (3 to 10 min) the ciliates are unable to swim forward at all. Often they swim in an irregular and jerky manner (Fig. 2B). The swimming velocity of the ciliates gradually decreases and, subsequently, a number of ciliates can be found

A



	R1	R2	R3	R4
STX	H	H	H	H
Neo	OH	H	H	H
GTX_1	OH	H	OSO_3^-	H
GTX_2	H	H	OSO_3^-	H
GTX_3	H	OSO_3^-	H	H
GTX_4	OH	OSO_3^-	H	H
B_1	H	H	H	SO_3^-
B_2	OH	H	H	SO_3^-
C_1	H	H	OSO_3^-	SO_3^-
C_2	H	OSO_3^-	H	SO_3^-
C_3	OH	H	OSO_3^-	SO_3^-
C_4	OH	OSO_3^-	H	SO_3^-

B

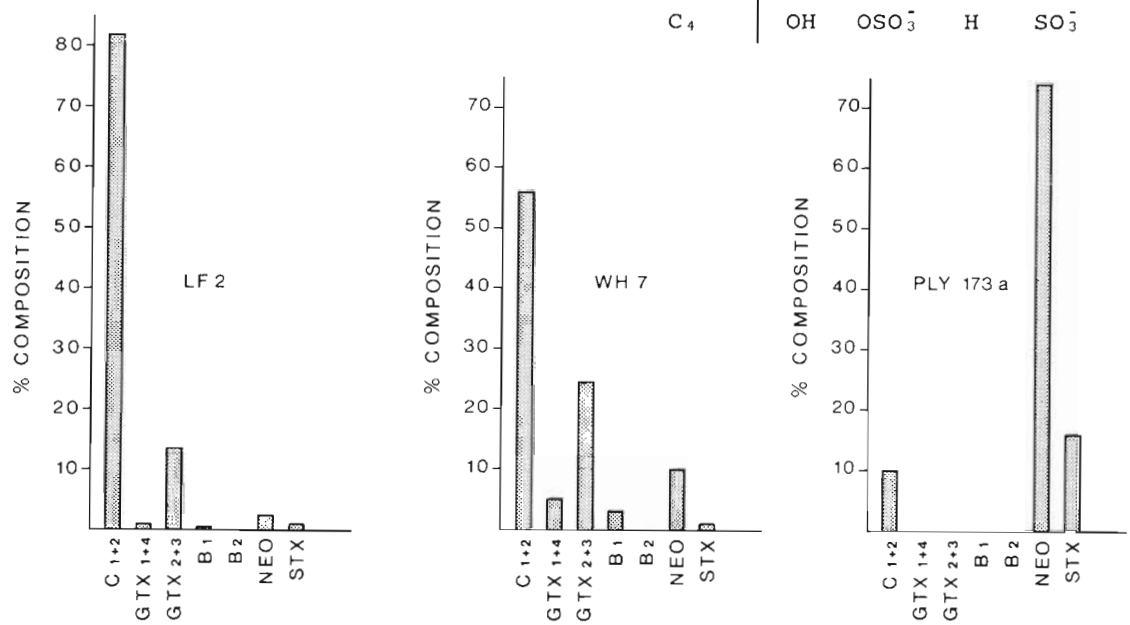


Fig. 1 Toxin composition of *Alexandrium tamarense* isolates. (A) Structures and abbreviations for saxitoxin (STX) and 11 other PSP toxins. GTX: gonyautoxins; B, C: carbamyl-N-sulfo compounds. (B) Toxin composition measured by HPLC (as % total toxin, $n = 1$) for 3 isolates during late exponential growth phase

immobile and swollen on the bottom; the cilia still beat although in an uncoordinated manner. After some hours these ciliates lyse. Figs. 3 and 4 distinguish between 3 types of behaviour: normal swimming, backwards swimming and immobility. At a concentration of 500 cells ml^{-1} of the Wh 7 clone all the tested ciliates swim normally.

The less toxic clone (Ply 173a) does not induce backwards swimming at concentrations of 500 or 3000 cells ml^{-1}. A concentration of 5000 cells ml^{-1}, however, does induce backwards swimming although the effect is not as strong as with the Wh 7 clone using 3000 cells ml^{-1}.

Filtrate experiments

These experiments were carried out to reveal if the medium in which *Alexandrium tamarense* has grown has any effect on ciliates. Results for late exponential cultures are shown in Figs. 3B and 4B, respectively. With respect to clone Wh 7, the behavioural response of the ciliates to the medium (derived from a 3000 cells ml^{-1} suspension) is nearly identical to the one in which the dinoflagellates are present. The only difference is a somewhat delayed response and that, even after 2 h of exposure, some ciliates are still swimming backwards.

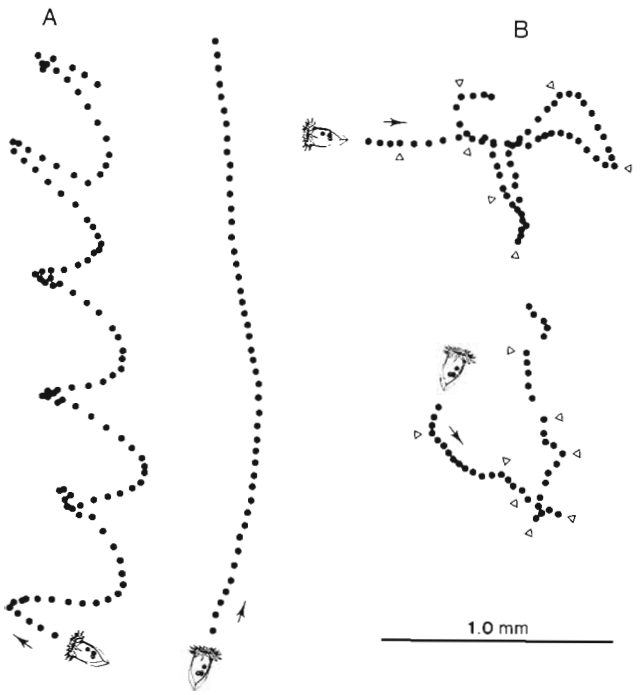


Fig. 2. *Favella ehrenbergii*. Paths plotted from video recordings of swimming individuals. Time interval between dots: 80 ms. (A) Paths characteristic of normal swimming cells. (B) Paths characteristic of backwards-swimming cells induced by a suspension of *Alexandrium tamarense* (Wh 7, 3000 cells ml^{-1}). (△) Periods (up to 1 s) where cells are immobile. Dots corresponding to these periods are omitted

The response of ciliates to the medium (based on a 5000 cells ml^{-1} suspension) in which Ply 173a has been grown is much weaker than when cells are present. Furthermore, cells that swim backwards after 1 h exposure to the algae medium eventually recover (after 2 h). This indicates that the exudate is labile. To study this, aged exudates were tested. Results are shown in Fig. 3 B to D and 4 B to D.

In Wh 7 medium, the time required to induce backwards swimming increases with the age of the medium and the number of ciliates swimming backwards or becoming immobilized decreases. After about 4 to 8 h (not shown in Fig. 3) the ciliates that previously swam backwards recover to display a normal swimming pattern. In the experiment with Ply 173a medium, the number of ciliates swimming backwards also gradually decreases with age of the medium. After 30 min the toxic effect of Ply 173a medium disappears.

The growth curves of the different clones of *Alexandrium* are shown in Fig. 5. The doubling time in the log phase ranges from 1.0 to 2.7 d. Arrows indicate at what concentration the CBS 50 values is reached. In all cases, the culture has passed exponential growth phase before the CBS 50 value is reached. The relationship between the toxin contents measured by the bioassay and the CBS 50 is presented in Fig. 6. Even though the toxin content of the cells decreases after the culture has reached the stationary phase, the toxic effect of the medium increases. This indicates that the toxins are exudated to the medium at a higher rate during this phase.

The results of particle uptake experiments are presented in Fig. 7. In all experiments *A. tamarense* was ingested by *Favella ehrenbergii*. When backwards swimming is induced no particle uptake takes place (as found with Ply 173a: 5000 cells ml^{-1}). When *F. ehrenbergii* is added to a suspension of 500 cells ml^{-1} of *A. tamarense* (Ply 173a, Wh 7), the particle uptake (mean number of cells counted in food vacuoles) increases with time, giving a hyperbolic relation. The particle uptake rate can be estimated from a linear relation between uptake and time from the start of the experiment (0 to 10 min). This shows a higher uptake rate by *F. ehrenbergii* of the Ply 173a clone than of the Wh 7 clone, (3 and 1.5 particles h^{-1}, respectively).

Growth experiments

Results of these experiments are presented in Fig. 8. *Favella ehrenbergii* is able to grow when fed the Wh 7 clone of *Alexandrium tamarense* at concentrations between 250 and 500 cells ml^{-1} at doubling times within the range 26 to 32 h. This corresponds to growth rates obtained with similar concentrations (in terms of volume) of the non-toxic dinoflagellate *Heterocapsa triquetra* (not shown). However, at concentrations exceeding 1500 cells ml^{-1} of WH 7, ciliates die at least as quickly as they would when starved. At a concentration of 1000 cells ml^{-1}, the ciliates grow during the first 2 d, but then die off at a slow rate. *F. ehrenbergii* is not able to grow when fed the Ply 173a clone of *A. tamarense* at any concentration and survival decreases as the dinoflagellate concentration increases. Nevertheless, ciliates do not show altered swimming (except at 5000 cells ml^{-1}), although they eventually die, possibly from starvation.

DISCUSSION

Physiological basis of toxicity

Alexandrium tamarense produces substances which are lethal to the marine ciliate *Favella ehrenbergii* when released to the medium. The exudate acts on the cell membrane of the ciliate to induce continuous ciliary reversal. After some time, the ciliate swells and subsequently lyses. That *A. tamarense* exudates substances which affect animal cells has been shown by

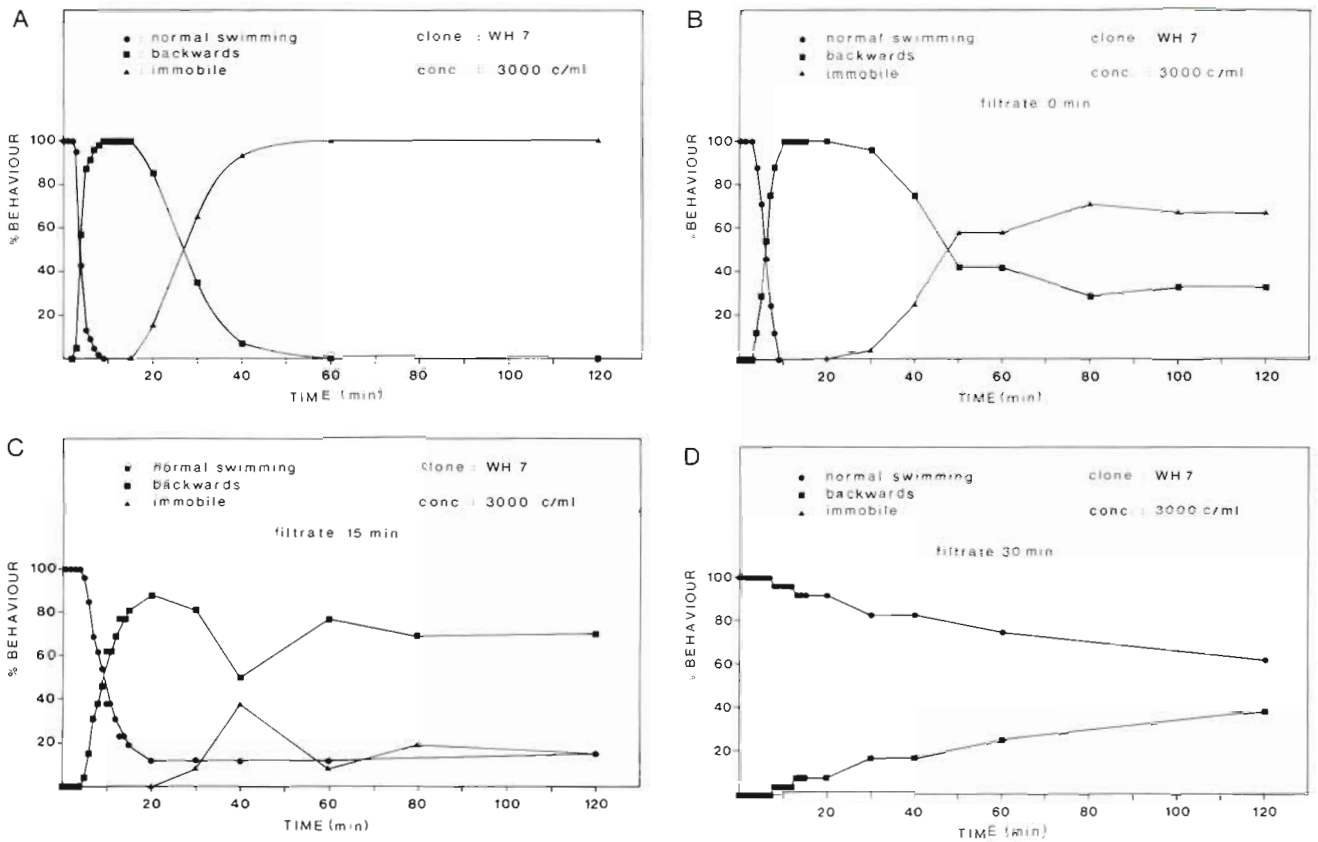


Fig. 3. *Favella ehrenbergii*. Effect on motility following exposure to *Alexandrium tamarense*, clone Wh 7. (A) 3000 cells ml^{-1} (B to D) Filtrate from 3000 cells ml^{-1}. (B) not aged before addition of ciliates; (C) aged 15 min; (D) aged 30 min

Ogata & Kodama (1986). They found that the culture medium induces lysis of erythrocytes, and histological observations of exposed fish (rainbow trout) show swollen epithelial cells that are exfoliated from the pillar cells.

All toxins known to be produced by *Alexandrium spp.* are water-soluble and collectively known as PSP toxins. At least 12 closely related compounds exist of which saxitoxin was the first to be purified and characterized (Steidinger & Baden 1984). Early experiments using frog nerve showed that saxitoxin blocks action potentials without depolarization (Kao & Nishiyama 1965). Voltage clamp studies (Hille 1968, Narahashi 1975) revealed that saxitoxin selectively inhibits the inward sodium flux. The bioelectric control of locomotion in ciliates was studied by Eckert & Naitoh (1972), primarily on the freshwater ciliate *Paramecium caudatum*. They showed that ciliary reversal (backwards swimming) is due to influx of Ca^{2+}. The present observations suggest that the *A. tamarense* toxins lead to an increased influx of Ca^{2+} or repeated depolarizations of the ciliate cell membrane. This is however not easily reconciled with the previous observations on frog nerves.

Several workers (Prakash 1967, White & Maranda 1978, Boyer et al. 1986) have found for different *Alexandrium* clones that the toxin content of the cells decreases rapidly as the culture leaves the log phase to reach a constant level during the stationary phase of culture growth. Prakash (1967) showed that PSP toxins could not be detected in the medium until after the end of the exponential growth phase but stressed that it cannot be excluded that the toxins are, to some degree, excreted by cells during the exponential growth phase because of the sensitivity limit of the bioassay (0.2 µg). Lacking a reliable method for the estimation of toxin content of seawater, the relationship between toxin content per cell and the CBS 50 is used here as an indicator of the possible relationship of altered ciliate swimming pattern and the presence of PSP toxins.

The toxicity level of the medium depends on the growth phase of *Alexandrium tamarense*. The highest levels of toxicity are obtained in stationary phase cultures. This applies to all studied clones (Fig. 6). For the Limfjord clones, there is a positive correlation between the CBS 50 level and the PSP-toxin content when the cultures are in stationary phase. However, data for the Wh 7 clone do not fit into this pattern. This

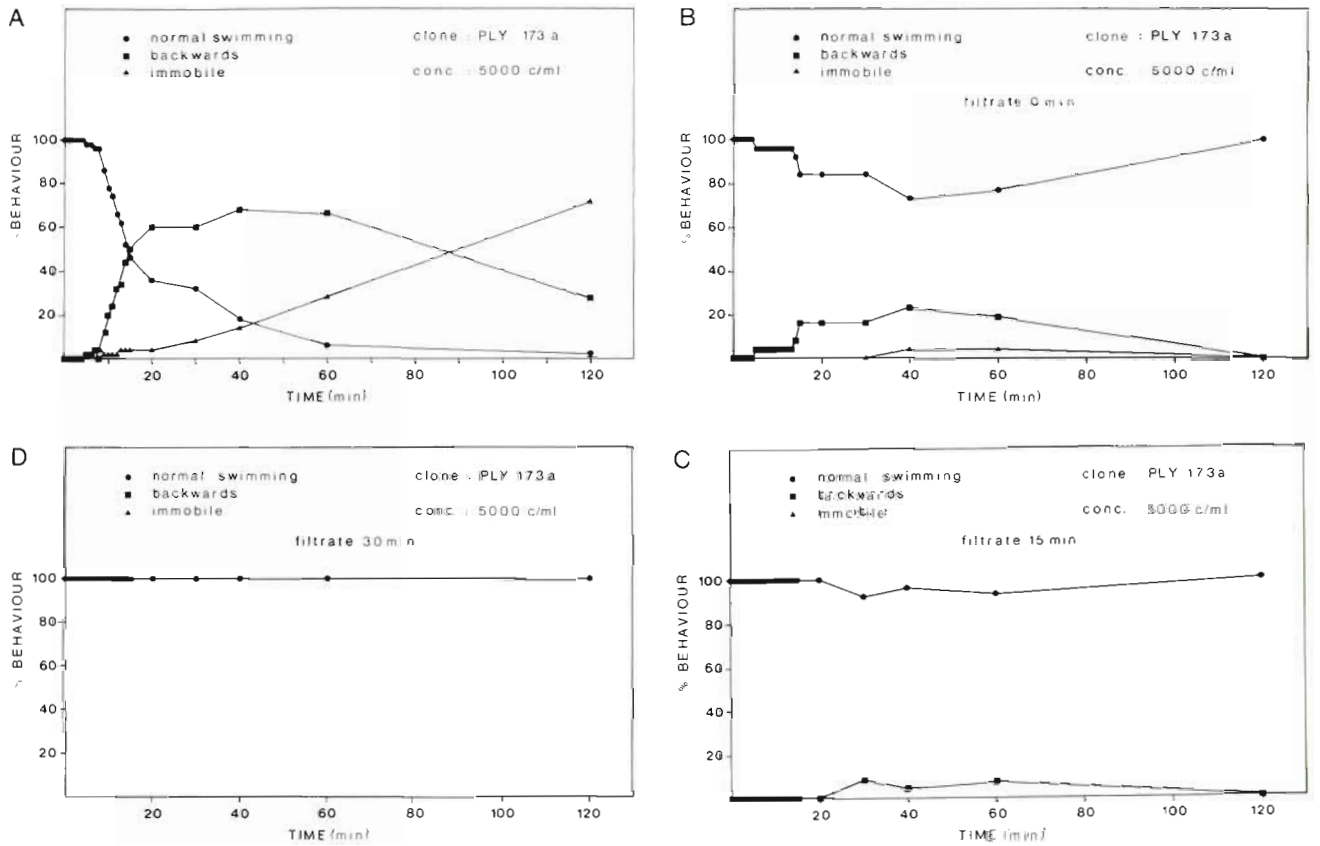


Fig. 4. *Favella ehrenbergii*. Effect on motility following exposure to *Alexandrium tamarense*, clone Ply 173a. (A) 5000 cells ml^{-1}. (B to D) Filtrate from 5000 cells ml^{-1}; (B) not aged before addition of ciliates; (C) aged 15 min; (D) aged 30 min

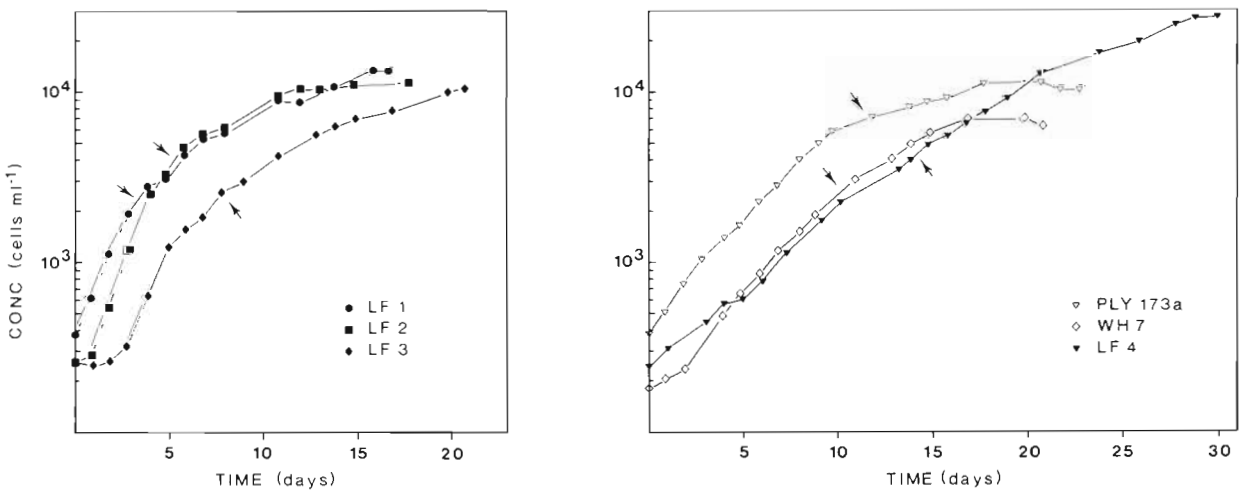


Fig. 5. *Alexandrium tamarense*. Growth of individual clones in batch cultures. Arrows indicate at what concentration the CBS 50 value is reached

clone contains 3 times more toxin than the LF 2 but yields a higher CBS 50 level (a lower toxic effect). It is possible that the Wh 7 clone releases toxins at a lower rate than the Limfjord clones as almost no difference exits in HPLC spectra between the 2 clones (Fig. 1). The relationship between the CBS 50 and PSP toxin content is not so clear cut for cultures that have just passed the exponential growth phase. This is probably due to significant changes in exudation rate taking place during this relatively short and ill-defined phase.

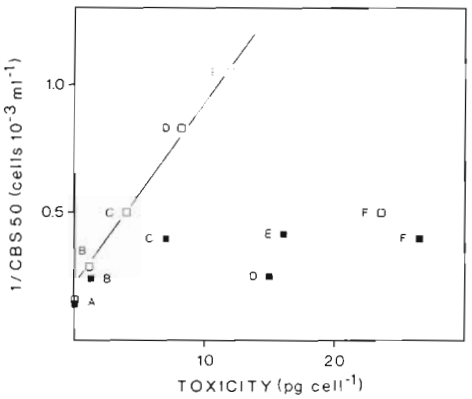


Fig. 6. Concentration of *Alexandrium tamarense* required to induce 50 % of the *Favella ehrenbergii* cells to swim backwards within 10 min, as a function of cell toxin content. (■) Cells from late exponential phase cultures; (□) cells from stationary phase cultures. A: Ply 173a; B: LF1; C: LF2; D: LF3; E: LF4; F: Wh 7

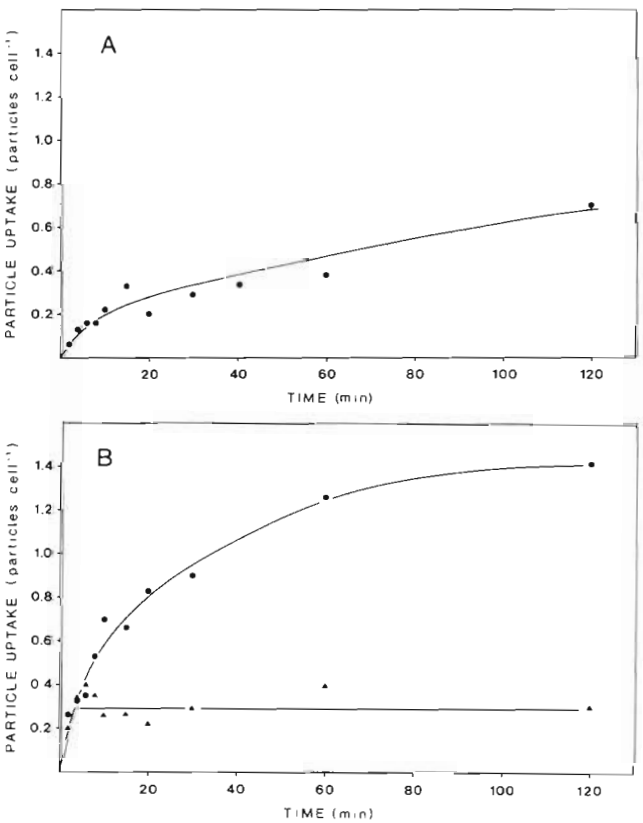


Fig. 7. *Favella ehrenbergii*. Particle uptake as a function of time and concentration of *Alexandrium tamarense*. (●) 500 cells ml^{-1}; (▲) 5000 cells ml^{-1} (A) Clone Wh 7. (B) Clone 173a

Most evidence supports the hypothesis that the toxic effects observed on ciliates exposed to *Alexandrium tamarense* are caused by PSP toxins: the substances are water-soluble, very labile in culture media (pH 8) and are liberated as the culture leaves the exponential growth phase. Furthermore, there is fairly good correlation between CBS 50 and cell toxin content during stationary growth phase.

It is interesting that although *Alexandrium tamarense* is ingested by *Favella ehrenbergii* when presented to the ciliate in low concentrations, this does not induce ciliary reversal. From studies on nerve membranes it is known that saxitoxins only act on the outside of the axons (Hille 1975). This may also apply to protozoa. Another explanation for this observation could be that the toxins are degraded by hydrolytic enzymes in food vacuoles. Finally, it is possible that a high rate of production and exudation of the toxins under certain growth conditions may lead to concentrations in the water which exceed or are comparable to the intracellular concentration.

Particle uptake experiments revealed that *Favella ehrenbergii* ingests cells of the Ply 173a clone at a higher rate than cells of the Wh 7 clone. Studies by Spittler (1973) and Heinbokel (1978) show that tintinnids only ingest particles with a diameter of less than 50 % of the oral lorica diameter. The mean diameter of cells of the Ply 173a and Wh 7 clones, measured during exponential growth phase, is 29 and 32 µm, respectively. As the oral lorica diameter of *F. ehrenbergii* is about 60 µm, a larger proportion of the Ply 173a population is subject to grazing. It seems likely that this can explain the difference in particle uptake rate.

Effect on growth

Favella ehrenbergii is able to grow when fed the Wh 7 clone of *Alexandrium tamarense* at low concentrations (250 to 500 cells ml^{-1}) (Fig. 8). At higher concentrations the ciliates are affected by exudates as described above. Growth rates of the ciliate when fed Wh 7 clone (doubling times 26 to 32 h) were similar to those obtained with similar concentrations (in terms of volume) of the non-toxic *Heterocapsa triquetra*. This suggests that the Wh 7 clone is an appropiate food source for the ciliate. However *F. ehrenbergii* is not able to grow when fed the less toxic Ply 173a at any concentration (Fig. 8), although this algae is ingested by the ciliate. In the presence of low concentrations of the Ply 173a, the ciliates show no abnormal behavioural response, but the survival of the ciliates decreases with increasing algal concentration, which may suggest a toxic effect. At very high concentrations of Ply 173a ($\geq$ 5000 cells ml^{-1}), the ciliates show ciliary reversal. For both the Wh 7 and the Ply 173a clones there were only slight differences in growth/survival response according to whether they were kept in the light or in the dark. The differences which were

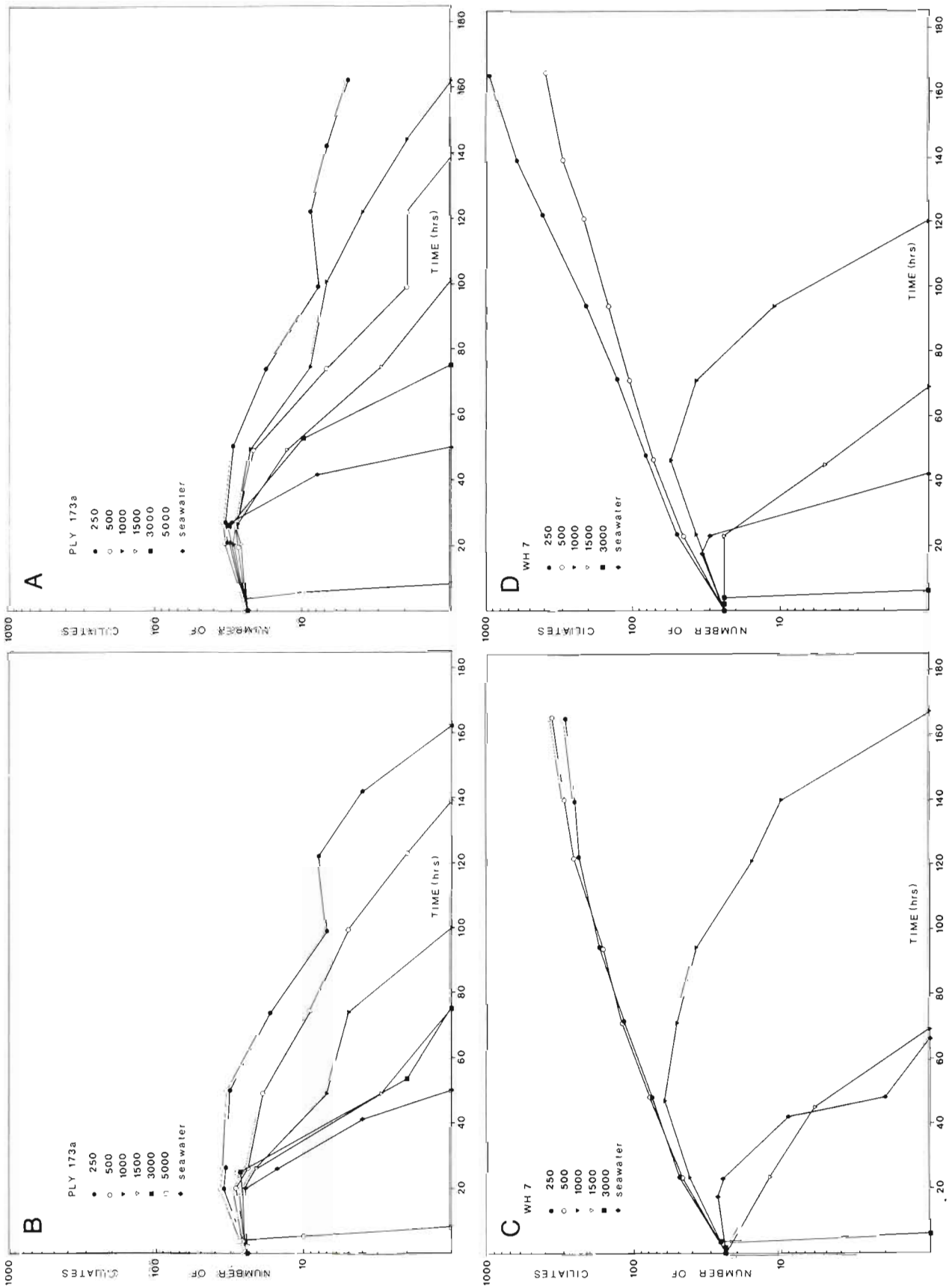


Fig. 8. *Favella ehrenbergii*. Growth/survival response to different concentrations of 2 clones of *Alexandrium tamarense*. Late exponential cultures of *A. tamarense* were used. The ciliates were transferred daily to a fresh suspension of flagellates. (Numbers are cells ml^{-1}; 'seawater' refers to starving ciliates). (A) Ply 173a, dark. (B) Ply 173a, light. (C) Wh 7, dark. (D) Wh 7, light

observed could be explained by the fact that algae kept in light increase in numbers while the ones kept in dark do not.

Ecological significance

Field studies (Needler 1949, Prakash 1963, White 1979) suggest that the ciliate *Favella ehrenbergii* can be an important grazer on populations of *Alexandrium tamarense*. Studies on metazoan zooplankton show that a number of different groups (copepods, cladocerans, barnacle nauplii, polychaete larvae and pteropods) do graze on *A. tamarense* (White 1977, 1979, 1981, Turner & Anderson 1983). However studies of the importance of zooplankton grazing on the development and persistence of *A. tamarense* blooms are scarce.

Turner & Anderson (1983) studied the ingestion rates of the copepod *Acartia hudsonica* and larvae of the polychaete *Polydora* sp. when fed natural particle assemblages spiked with laboratory cultures of C^{14} labelled *Alexandrium tamarense*. The filtration rates obtained were used to estimate the impact of grazing during an *A. tamarense* bloom (< 160 cells ml^{-1}). The maximum grazing loss due to *A. hudsonica* was estimated to be 1 % d^{-1} of the *A. tamarense* population. Both the size and the filtration rate of the copepod population were too low to have a significant impact. This was not the case for the polychaete larvae. During the early stage of the algae bloom, the estimated grazing loss was 3 to 16 % d^{-1}. At the peak of the *A. tamarense* bloom (160 cells ml^{-1}) the population of *Polydora* sp. were sufficiently dense (855 l^{-1}) to represent a potential grazing loss exceeding 100 % of the *A. tamarense* d^{-1}.

Studies by Watras et al. (1985) show that, in addition to the *Polydora* sp., the ciliate *Favella ehrenbergii* can be an important grazer on *A. tamarense* populations. During the start of a bloom of *A. tamarense,* the grazing loss due to *F. ehrenbergii* was low (1 % d^{-1}). At the peak concentration of *A. tamarense* (120 cells ml^{-1}), the estimated loss due to grazing by *F. ehrenbergii* was 25 % d^{-1}, clearly exceeded the estimated growth rate of *A. tamarense* (0.15 d^{-1}).

Carreto et al. (1986) studied a dense bloom (> 10^3 cells ml^{-1}) of *Alexandrium tamarense (Gonyaulax excavata)* in the Argentine Sea. The abundance of macrozooplankton in the patch was low; 147 and 93 ind. m^{-3} in the core and along the periphery of the patch, respectively However, the phagotrophic dinoflagellate *Polykrikos schwartzii* occurred in large numbers and microscopic examination revealed that it may constitute an active predator of *A. tamarense*. The highest predation was observed along the periphery of the patch where both species reached identical concentrations while the grazing pressure in the core of the patch was low (predator : prey concentrations, 1 : 400).

Laboratory experiments show that copepods respond differently to the presence of *Alexandrium tamarense* than do ciliates. Ives (1985) found that, although the dinoflagellates are ingested by copepods, the ingestion rate decreases as the toxin content of the dinoflagellate increases. Additional studies showed that the copepods initially ingest highly toxic cells but subsequently suffer from incapacitation (Ives 1987).

The conclusion to be drawn from these field and laboratory experiments is that when *Alexandrium tamarense* occurs in low concentrations in nature (e.g. at the beginning of a bloom), grazing by zooplankton can be of significance in controlling the bloom if the concentration of predators is sufficiently high. At higher algae concentrations (10^3 to 10^4 cells ml^{-1}), a bloom cannot be subject to any substantial zooplankton grazing The present results clearly show this in the case of ciliates. The concentration limit for grazing depends on the cell toxin content and the physiological state of the dinoflagellates. This inhibition of zooplankton grazing, in part, explains why such blooms can develop and persist for months as observed by Mortensen (1985), Carreto (1986) and Therriault et al. (1985).

Although *Alexandrium tamarense* is probably the most studied toxin-producing dinoflagellate, it is certainly not the only one. Taylor (1985) listed a total of 34 species belonging to 14 genera as toxin producers. Among the planktonic forms, the ability to produce monospecific blooms in estuarine or neritic environments is characteristic (see references in Steidinger 1983, Steidinger & Baden 1984 and Taylor 1985).

From terrestial ecosystems, it is well known that plants have evolved secondary metabolites which discourage herbivores; the production of toxins by algae may be a parallel in aquatic ecosystems. It is, however, a priori difficult to understand how this type of predator defense could evolve in a planktonic species. This is because the excretion of a toxin by one cell would not increase the individual's fitness but rather decrease the level of predation on competing, non-toxic cells as well. If, however, it is assumed that each bloom consists of a clone of cells originating from one individual through asexual fissions and can thus be considered a multicellular individual, the excretion of toxins will increase the inclusive fitness of the founder cell. It would be worthwhile investigating whether this holds true: that blooms of toxin-producing algae consist of individual clones of cells.

Acknowledgements. I thank Prof. Tom Fenchel for constructive criticism and discussion. I am indebted to Jeanne Johansen and Dr Grete Møller Christensen for assistence with

experimental work. I also thank Dr Palle Krogh, Dept of Microbiology, Royal Dental School and Dr Karsten Skydsgaard, Scantox biological laboratory, who did the mouse bioassays tests, and Dr James. M. Hungerford, Dept of Health and Human Services, Food and Drug Administration, Seattle, who did the HPLC analysis. I am grateful to Dr Øjvind Moestrup, Institut for sporeplanter, who kindly provided the inoculum of the clones Ply 173a and Wh 7 Finally I thank Drs Katherine Richardson, Øjvind Moestrup and Palle Krogh for comments on the manusript. The study was supported by the Danish Natural Science Research Council grant nos. 11-6480 and 11-6600.

LITERATURE CITED

Anderson, D. M., Cheng, T. P. (1988). Intracellular localization of saxitoxins in the dinoflagellate *Gonyaulax tamarensis*. J. Phycol. 24: 17–22

Balech, E. (1985). The genus *Alexandrium* or *Gonyaulax* of the tamarensis group. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 33–38

Balech, E., Tangen, K. (1985). Morphology and taxonomy of the species in the tamarensis group (Dinophyceae): *Alexandrium excavatum* (Braarud) comb. nov. and *Alexandrium ostenfeldii* (Paulsen) comb. nov. Sarsia 70: 333–343

Boyer, G. L., Sullivan, J. J., Andersen, R. J., Taylor, F. J. R., Harrison, P. J., Cembella, A. D. (1986). Use of high-performance liquid chromatography to investigate the production of paralytic shellfish toxins by *Protogonyaulax* spp. in culture. Mar. Biol. 93: 361–369

Carreto, J. I., Benavides, H. R., Negri, R. M., Glorioso, P. D. (1986). Toxic red-tide in the Argentine Sea. Phytoplankton distribution and survival of the toxic dinoflagellate *Gonyaulax excavata* in a frontal area. J. Plankton Res. 8: 15–28

Eckert, R., Naitoh, Y (1972). Bioelectric control of locomotion in the ciliates. J. Protozool. 19: 237–243

Heinbokel, J. F. (1978). Studies on the functional role of tintinnids in the Southern California Bight. II. Grazing rates of field populations. Mar. Biol. 47: 191–197

Hille, B. (1968). Pharmacological modifications of the sodium channels of frog nerves. J. gen. Physiol. 51. 199–219

Hille, B. (1975). The receptor for tetrodotoxin and saxitoxin. Biophys. J: 615–619

Horwitz, W (ed.) (1984). Official methods of analysis, 14th edn, Procedure 18.086–18.092. Association of Official Analytical Chemists, Washington D.C.

Huntley, M. E., Barthel, K.-G., Star, J. L. (1983). Particle rejection by *Calanus pacificus:* discrimination between similarly sized particles. Mar. Biol. 74: 151–160

Huntley, M., Sykes, P., Rohan, S., Marin, V. (1986). Chemically-mediated rejection of dinoflagellate prey by the copepods *Calanus pacificus* and *Paracalanus parvus:* mechanism, occurrence and significance. Mar Ecol. Prog. Ser 28: 105–120

Ives, J. D. (1985). The relationship between *Gonyaulax tamarensis* cell toxin levels and copepod ingestion rates. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 413–418

Ives, J. D. (1987). Possible mechanisms underlying copepod grazing responses to levels of toxicity in red tide dinoflagellates. J. exp. mar. Biol. Ecol. 112: 131–145

Kao, C. Y., Nishiyama, A. (1965). Actions of saxitoxin on peripheral neuromuscular systems. J. Physiol. (Lond.) 180: 50–66

Mickelson, C., Yentsch, C. (1979). Toxicity and nucleic acid content of *Gonyaulax excavata*. In: Taylor, D. L., Seliger, H. H. (eds.) Toxic dinoflagellate blooms. Elsevier/North Holland, New York, p. 131–134

Moestrup, Ø., Hansen, P. J. (1988). On the occurrence of the potentially toxic dinoflagellates *Alexandrium tamarense* (= *Gonyaulax excavata*) and *A. ostenfeldii* in Danish and Faroese waters. Ophelia 28: 195–213

Mortensen, A. M. (1985). Massive fish mortalities in the Faroe Islands caused by a *Gonyaulax excavata* red tide. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 165–170

Narahaski, T (1975). Mode of action of dinoflagellate toxins on nerve membranes. In: LoCicero (ed.) Toxic dinoflagellate blooms. Proc. 1st Int. Conf., Mass. Sci. Technol. Foundn., Wakefield, p. 395–402

Needler, A. B. (1949). Paralytic shellfish poisoning and *Goniaulax tamarensis*. J. Fish. Res. Bd Can. 7: 490–504

Ogata, T., Kodama, M. (1986). Ichthyotoxicity found in cultured media of *Protogonyaulax* spp. Mar. Biol. 92: 31–34

Prakash, A. (1963). Source of paralytic shellfish toxin in the Bay of Fundy. J. Fish. Res. Bd Can. 20: 983–996

Prakash, A. (1967). Growth and toxicity of a marine dinoflagellate *Gonyaulax tamarensis*. J. Fish. Res. Bd Can. 24: 1589–1606

Spittler, P. (1973). Feeding experiments with tintinnids. Oikos Suppl. 15: 128–132

Steidinger, K. A. (1983). A re-evaluation of toxic dinoflagellate biology and ecology. Prog. Phycol. Res. 2: 147–188

Steidinger, K. A., Baden, D. G. (1984). Toxic marine dinoflagellates. In: Spector, D. L. (ed.) Dinoflagellates. Academic Press, New York, p. 201–261

Stoecker, D., Guillard, R. R. L., Kavee, R. M. (1981). Selective predation by *Favella ehrenbergii* (Tintinnia) on and among dinoflagellates. Biol. Bull. mar. biol. Lab., Woods Hole 160: 136–145

Sykes, P. F., Huntley, M. E. (1987). Acute physiological reactions of *Calanus pacificus* to selected dinoflagellates: direct observations. Mar. Biol. 94: 19–24

Taylor, F. J. R. (1984). Toxic dinoflagellates: taxonomic and biogeographic aspects with emphasis on *Protogonyaulax*. In: Ragelis, E. P. (ed.) Seafood toxins. Amer Chem. Soc. Symp. Ser 262: 77–97, Washington D. C.

Taylor, F. J. R. (1985). The taxonomy and relationships of red tide flagellates. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 11–26

Therriault, J. C., Painchaud, J., Levasseur, M. (1985). Factors controlling the occurrence of *Protogonyaulax tamarensis* and shellfish toxicity in the St. Lawrence Estuary: freshwater runoff and the stability of the water column. In: Anderson, D. M., White, A. W., Baden, D. G. (eds.) Toxic dinoflagellates. Elsevier/North Holland, New York, p. 141–146

Turner, J. T., Anderson, D. M. (1983). Zooplankton grazing during dinoflagellate blooms in a Cape Cod Embayment, with observations of predation upon tintinnids by copepods. P.S.Z.N.I.: Mar. Ecol. 4: 359–374

Watras, C. J., Garcon, V C., Olson, R. J., Chrisholm, S. W., Anderson, D. M. (1985). The effect of zooplankton grazing on estuarine blooms of the toxic dinoflagellate *Gonyaulax tamarensis*. J. Plankton Res. 7: 891–908

White, A. W. (1977). Dinoflagellate toxins as probable cause of an Atlantic herring *(Clupea harengus harengus)* kill, and

pteropods as apparent vector. J. Fish. Res. Bd Can. 34: 2421–2424

White, A. W. (1979). Dinoflagellate toxins in phytoplankton and zooplankton fractions during a bloom of *Gonyaulax excavata*. In: Taylor, D. L., Seliger, H. H. (eds.) Toxic dinoflagellate blooms. Elsevier/North Holland, New York, p. 381–384

White, A. W. (1981). Marine zooplankton can accumulate and retain dinoflagellate toxins and cause fish kills. Limnol. Oceanogr. 26: 103–109

White, A. W., Maranda, L. (1978). Paralytic toxins in the dinoflagellate *Gonyaulax excavata* and in shellfish. J. Fish. Res. Bd Can. 35: 397–402

This article was presented by Professor T Fenchel, Helsingør, Denmark

Manuscript first received: November 11, 1988
Revised version accepted: January 27, 1989