

## Effect of turbulence on sedimentation and net population growth of the dinoflagellate *Ceratium tripos* and interactions with its predator, *Fragilidium subglobosum*

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### Abstract

We investigated the effect of natural levels of turbulence on the vertical distribution and net population growth of the dinoflagellate *Ceratium tripos* and on the interaction with its predator, the mixotrophic dinoflagellate *Fragilidium subglobosum*. Unialgal cultures of each species were exposed to four kinetic energy dissipation rates,  $\varepsilon$ : 0.0001, 0.01, 0.05, and  $1 \text{ cm}^2 \text{ s}^{-3}$ , generated by vertically oscillating grids in 2-liter cylindrical containers. Autotrophic growth of *F. subglobosum* was not affected by any level of turbulence tested. In contrast, at  $\varepsilon \geq 0.05 \text{ cm}^2 \text{ s}^{-3}$  (this value is generated in the upper 10 m of the ocean by a moderate gale or at 0.5 m in depth by a gentle breeze), *C. tripos* decreased its net population growth, and the vertical distribution of the cells was affected. At the highest turbulence level, *C. tripos* stopped swimming, settled, and accumulated at the bottom. Mixotrophic growth of *F. subglobosum*, when fed *C. tripos* at high densities (i.e.,  $\gg 10$  prey cells  $\text{mL}^{-1}$ ), was not affected by turbulence. However, at low prey cell densities (i.e., 5 to 8 *C. tripos* cells  $\text{mL}^{-1}$ ), growth and ingestion rates of *F. subglobosum* were significantly higher at the highest turbulence level compared to the rates at other turbulence levels and were close to the rates measured at high prey cell densities, presumably because sedimentation of *C. tripos* cells resulted in patches where the cell densities were not food-limiting for *F. subglobosum*.

Plankton dynamics are affected by turbulence (Mann and Lazier 1991; Kiørboe 1993). The intensity of turbulence is expressed by the turbulent kinetic energy dissipation rate  $\varepsilon$  ( $\text{cm}^2 \text{ s}^{-3}$ ) (i.e., the reduction of the turbulent energy over time). Typical dissipation rates in the ocean are between 0.000001 and  $1 \text{ cm}^2 \text{ s}^{-3}$  (Kiørboe and Saiz 1995). At small spatial scales, turbulence may affect predator–prey contact rates (and hence grazing rates) (Rothschild and Osborn 1988), nutrient uptake of planktonic osmotrophs (Karp-Boss et al. 1996), cell integrity (Thomas et al. 1997, and references therein), cell morphology (Zirbel et al. 2000), cell physiology (Berdalet 1992), and swimming behavior of cells (Karp-Boss et al. 2000). Among phytoplankton, dinoflagellates have shown particularly strong negative responses of net population growth to turbulence in laboratory experiments (Estrada and Berdalet 1998), and dinoflagellate red tides often appear after a period of calm weather and water

column stability (Wyatt 1975; Margalef et al. 1979). The idea of negative effects of small-scale turbulence on growth of dinoflagellates in general was questioned, however, by Smayda (2002), who pointed out that many dinoflagellates display maximum abundances in frontal areas associated with upwelling and mixing, and therefore tolerate turbulence levels associated with these events (Smayda 2002).

Many earlier laboratory studies on the effect of small-scale turbulence on dinoflagellates reported negative effects on cell integrity, cell division, and net growth rates (e.g., White 1976; Pollinger and Zemel 1981; Berdalet 1992). The turbulence levels in these studies were, however, considerably higher than those typically found in the ocean (Peters and Redondo 1997; Peters and Marrasé 2000). More recent investigations have also reported negative effects at turbulent dissipation rates  $\leq 1 \text{ cm}^2 \text{ s}^{-3}$  (e.g., Juhl et al. 2000; Zirbel et al. 2000), but, also, no effects or even positive effects on net growth have been observed (Sullivan and Swift 2003). These investigations included both thecate and atecate dinoflagellate species belonging to different families, but no general trends emerged, even for species belonging to the same genus (Sullivan and Swift 2003).

Most available data were obtained from species growing autotrophically. The effect of turbulence on growth of heterotrophic dinoflagellates has only been studied for two species, *Cryptothecodinium cohnii* (Yeung and Wong 2003) and *Oxyrrhis marina* (Havskum 2003). *C. cohnii* displayed a transient cell cycle arrest at G<sub>1</sub> phase (growth phase in which the chromosomes are prepared for replication) and an increased cell size at dissipation rates in the range of 0.1 to  $1 \text{ cm}^2 \text{ s}^{-3}$  (calculated in an identical system by Zirbel et al. 2000). Net growth rate of *O. marina* decreased by 20% at  $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ . Effects of turbulence on growth of mixotrophic species have not been reported before.

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### Acknowledgments

The oscillating grid device was designed and produced by F. Peters (Institut de Ciències del Mar, Spain). We thank A. S. Bisgaard (Marine Biological Laboratory, Denmark) for technical assistance and B. Vismann (Marine Biological Laboratory, Denmark) for setting up the computer model used in the calculation of feeding rates. The article profited from valuable comments of two anonymous reviewers and the editor. This study was performed as part of the European research project NTAP (Nutrient dynamics mediated through Turbulence And Plankton interactions, contract EVK3-CT-2000-00022) within the framework of the EU RTD Programme “Environment and Sustainable Development” that forms part of the ELOISE projects cluster. It is ELOISE contribution 513/40. P.J.H. was supported by the Danish Natural Research Council, project 21-03-0449.

Our study focuses on the effect of natural levels of turbulence on the dinoflagellate *Ceratium tripos*, an important primary producer in temperate seas (Smetacek 1984). Extensive blooms of *C. tripos* during late summer/early autumn are known from many areas (e.g., the Kiel Bay, in Germany [Elbrächter 1973], the New York Bay, in the United States [Malone 1978], the coastal waters off Southern California, in the United States [Eppley et al. 1984], and the Kattegat, in Denmark [Nielsen 1991]). Given the importance of *C. tripos* as a primary producer, it is important to study how this organism is affected by turbulence and what effect turbulence has on interactions between *C. tripos* and a predator. Because both growth and grazing rates may be affected by a patchy prey distribution (Leising 2001), we began by studying the effect of turbulence on the distribution of *C. tripos* in the water column. When looking at a predator, we chose the mixotrophic dinoflagellate *Fragilidium subglobosum*, because *C. tripos* is one of its preferred food sources (Hansen and Nielsen 1997). We also studied the autotrophic growth of *F. subglobosum* in relation to turbulence.

## Material and methods

**Cultures**—The dinoflagellates *C. tripos* (O. F. Müller) Nitzsch 1817 and *F. subglobosum* (von Stosch) Loeblich 1982 were provided by the culture collection of the Marine Biological Laboratory (Helsingør, Denmark). Both species had originally been isolated from the Kattegat (Denmark) and were grown in f/2 medium (Guillard 1983) based on seawater with a salinity of 32. The cultures were grown at  $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$  at an irradiance of  $45 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and a light:dark cycle of 16:8 h. The same conditions were used in the experiments described below. Prior to the experiments, *C. tripos* (cell volume  $110,000 \mu\text{m}^3$ ; Hansen and Nielsen 1997) and *F. subglobosum* (cell volume  $39,600 \mu\text{m}^3$ ; Skovgaard et al. 2000) were kept in batch cultures that were reinoculated every second week into f/2 medium (dilution 1:10) to keep them in an active growth phase.

**Generation of turbulence**—The oscillating grid device used to generate different levels of turbulence is described by Dolan et al. (2003). The grids were made of stainless steel coated with a plastic polyamide and had a diameter of 12.9 cm and a mesh size of 1.42 cm. Vertically oscillating movements in eight 2-liter containers made of Plexiglas were provided by four independently controlled motors, each attached to two grids. The oscillating grids changed direction approximately 1 mm above the bottom of the containers. The frequencies were 0.5, 1.5, 3.5, and 15 rpm, and the stroke radii were 2, 5, 7, and 7 cm, resulting in four different turbulence levels with average kinetic energy dissipation rates ( $\varepsilon$ ) of 0.0001, 0.005, 0.05, and  $1 \text{ cm}^2 \text{ s}^{-3}$ , respectively (calculated according to Peters and Gross 1994), which correspond to naturally occurring turbulence levels in the upper 10 m of the ocean that can be generated by, for example, almost no wind, moderate breeze, moderate gale, and storm, respectively (MacKenzie and Leggett 1993; Kiørboe and Saiz 1995). The calculated average turbulent energy dissipation rates reflect transient high values just after the grid passage and decreasingly smaller values until the next grid

passage. Peters and Gross (1994) measured one order of magnitude of variation of  $\varepsilon$  in their system. The present oscillating grid device was built in the same way, but with a motor running more smoothly, resulting in a variation of  $\varepsilon$  that is similar or less (Peters pers. comm.).

**Microscopical observations, enumerations, and measurements**—Unless indicated differently, samplings were performed in the middle of the light period, after we mixed the water column by gently turning the containers upside down 10 times. The organisms were fixed with Lugol's solution (final concentration 10%) and settled in Utermöhl chambers (HydroBios). At least 50 cells were counted in every sample, and densities were always determined in triplicate, using an Olympus inverted microscope with  $\times 40$  and  $\times 100$  magnification. Aggregates of *C. tripos* cells found at the bottom of high-turbulence containers were gently taken up using a pipette and were inspected using an Olympus light microscope with  $\times 100$  magnification. Average swimming velocities of *C. tripos* and *F. subglobosum* in still conditions were determined by measuring 20 swimming tracks of each species, recorded by a JAI Protec video camera fitted to an Olympus BX50 microscope at  $\times 20$  magnification.

**Experiments on the effect of turbulence on the distribution of *Ceratium tripos* cells in the water column**—This study was based on previous observations indicating that *C. tripos*, when exposed to high turbulence, tended to accumulate at the bottom of an experimental container. In duplicate experiments (A and B) *C. tripos* was exposed to the four turbulence levels for 48 h, with initial cell densities between 12 and  $15 \text{ C. tripos cells mL}^{-1}$  (corresponding to a bloom; Elbrächter 1973). In a third experiment, three replicate cultures with initial cell density of 1 *C. tripos* cell  $\text{mL}^{-1}$  (corresponding to a common cell density found in nature; Elbrächter 1973) were exposed to the highest turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) for 48 h. From each experimental container, six samples were taken from the top (4 to 6 cm below the surface) and six samples from the bottom (1 to 2 cm above the bottom) without mixing the water column. Subsequently, the water column was carefully mixed by gently turning the containers upside down 10 times. Then six samples were taken from the middle of each container. A fourth experiment was performed to resolve whether the cells aggregated in the water column. A Minitron charge-coupled device video camera (lens: 105 mm, 1:2.5) was placed on the side of a container and recorded a  $0.56\text{-cm}^2$  visual field at a depth 2 cm above the bottom. The initial density in the container was 32 cells  $\text{mL}^{-1}$ . Cells were recorded 0.5 h before turbulence was applied and when they were exposed to the highest level of turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) for 23 h. Exposure to turbulence started 1 h before the middle of the light period. In order to obtain replicate counts at a given time, cells were counted on five pictures taken 3 s apart. At the end of the experiment, the camera was placed below the bottom, recording the fate of the aggregated cells for a few hours when turbulence had stopped.

**Experiments on net growth and grazing rates**—In the first set of experiments, the effect of turbulence on autotrophic

net growth of *C. tripos* and *F. subglobosum* was studied. Cultures with initial cell densities of between 4 and 8 *F. subglobosum* mL<sup>-1</sup>, and between 5 and 15 *C. tripos* mL<sup>-1</sup>, were exposed to four different turbulence levels for 2 weeks. These initial cell densities were chosen to ensure that pH had no effect on the growth of the two species (*see* section on pH measurements). The second set of experiments focused on the effect of turbulence on the growth and ingestion of *F. subglobosum* at high and low prey cell densities. *F. subglobosum* (initial cell density 0.3 to 1.9 cells mL<sup>-1</sup>) was mixed with *C. tripos* at high ( $\gg 10$  *C. tripos* cells mL<sup>-1</sup>) and low (5 to 8 *C. tripos* cells mL<sup>-1</sup>) cell densities, representing food saturation and food limitation, respectively, in still containers (Hansen and Nielsen 1997) and was incubated at the four different turbulence levels for 10 d. All experiments were replicated three times. For each of the three replicates at each of the four turbulence levels, the average value of growth and ingestion was calculated over time. Only growth and ingestion rates of *F. subglobosum* measured at prey densities  $\gg 10$  and 5 to 8 cells mL<sup>-1</sup> were used to calculate average rates at high and low prey cell densities, respectively. In all experiments, samples for enumeration were taken every second day in the middle of the light period.

Instantaneous net population growth rates were determined in steps of 48 h as  $\mu = [\ln(y_{t_1}/y_{t_0})]t^{-1}$ , where  $y_{t_0}$  = densities of cells at day  $t_0$  (cells mL<sup>-1</sup>);  $y_{t_1}$  = densities of cells at day  $t_1$  (cells mL<sup>-1</sup>); and  $t$  = duration of each experiment (d). Ingestion rates of *F. subglobosum* were determined from the reduction in prey density over 48-h periods, using the average net growth rate of *C. tripos* from the same level of turbulence but without predators. Ingestion rate,  $U$ , was estimated using the following two equations:

$$dx/dt = \mu_x x - Uy \quad (1)$$

$$dx/dt = \mu_y y \quad (2)$$

Prey ( $x$ ) is ingested by predator ( $y$ ). It is assumed that prey and predator grow exponentially with rate constants  $\mu_x$  and  $\mu_y$ , respectively. The prey mortality due to predation is  $Uy$ , where  $U$  (dimension: prey cells ingested predator<sup>-1</sup> time<sup>-1</sup>) is the per capita ingestion rate. The ingestion rate ( $U$ ) was iteratively calculated with time on a computer, allowing steps of 1 h (Hansen and Nielsen 1997).

*pH measurements*—Above pH 8.24, the growth of *C. tripos* is negatively affected (Schmidt and Hansen 2001). To check that the pH of the medium had no influence on the outcome of the different experiments, it was monitored every second day using a Senton 2001 pH system equipped with an Ion-selective Field Effect Transistor (ISFET) pH electrode.

*Statistical analyses*—Whether cell densities of *C. tripos* in samples taken from the bottom and the top of the containers without mixing the water column and in samples taken after mixing the water column were significantly different at a given turbulence level was tested by paired  $t$ -tests. The effect of the level of turbulence on autotrophic net growth of *C. tripos* and *F. subglobosum* and on mixotrophic net growth and ingestion of *F. subglobosum* at high and low

prey cell densities was tested using one-way analyses of variance by comparing the three average values over time at one turbulence level with those at other turbulence levels. When there was a significant difference in cell density, net growth, or ingestion, an all pairwise comparison procedure (Tukey test) was run. All statistical tests were performed with the SigmaStat software package.

## Results

*Effect of turbulence on the distribution of C. tripos cells in the water column*—*C. tripos* cells were homogeneously distributed in the water column after an exposure time of 48 h at the two lowest turbulence levels ( $\epsilon = 0.0001$  and  $0.005$  cm<sup>2</sup> s<sup>-3</sup>). No significant differences in cell densities were found between samples taken from the top, the bottom, and after mixing the water column (Fig. 1). No accumulation of cells was observed at the bottom of the containers. At the second highest turbulence level,  $\epsilon = 0.05$  cm<sup>2</sup> s<sup>-3</sup> cell densities measured at the bottom of the containers after 48 h were higher than and significantly different from those measured at the top (Fig. 1). In addition, more than 100 loose aggregates were observed at the bottom (data not shown). At the highest turbulence level,  $\epsilon = 1$  cm<sup>2</sup> s<sup>-3</sup>, 15 to 20 dense aggregates containing hundreds of cells were observed at the bottom next to the wall and in the middle of the bottom (Fig. 2). Inspection of these dense aggregates under the microscope showed that the *C. tripos* cells were entangled in each other with their horns and that the cells were alive. Samples taken from the top and the bottom of the containers before mixing were not significantly different (Fig. 1). After mixing, cell densities were 5 to 10 times higher than those measured in the samples taken from the bottom before mixing, indicating that most of the aggregated cells at the bottom had not been sampled at the highest turbulence level before mixing (Fig. 1).

A similar experiment was carried out at the highest turbulence level ( $\epsilon = 1$  cm<sup>2</sup> s<sup>-3</sup>) using a considerably lower initial cell density (i.e., 1 *C. tripos* cell mL<sup>-1</sup>; data not shown). The data from the three replicates were pooled. After 48 h, the average cell densities were  $0.197 (\pm 0.042)$  cells mL<sup>-1</sup> in samples taken from the top and  $0.173 (\pm 0.052)$  cells mL<sup>-1</sup> in samples taken from the bottom. Significantly higher cell densities ( $1.134 [\pm 0.113]$  cells mL<sup>-1</sup>; data not shown) were found after mixing the water column. As in the previous experiment, where higher initial cell densities were used, 80% to 90% of all *C. tripos* cells must have been in aggregates at the bottom of the containers at the highest turbulence level, because they were only sampled after mixing the water columns (Fig. 1).

Video recordings of a culture with an initial density of 32 *C. tripos* cells mL<sup>-1</sup> showed cells with visible longitudinal flagella in still conditions. After exposure to high turbulence ( $\epsilon = 1$  cm<sup>2</sup> s<sup>-3</sup>), no cells with longitudinal flagella were observed, indicating that the cells had retracted their longitudinal flagella and stopped swimming. The number of *C. tripos* cells counted per visual field increased within 10 min from 17 to 28 and reached a maximum of 35 cells per field after 0.5 h (Fig. 3), indicating that cells sinking from above

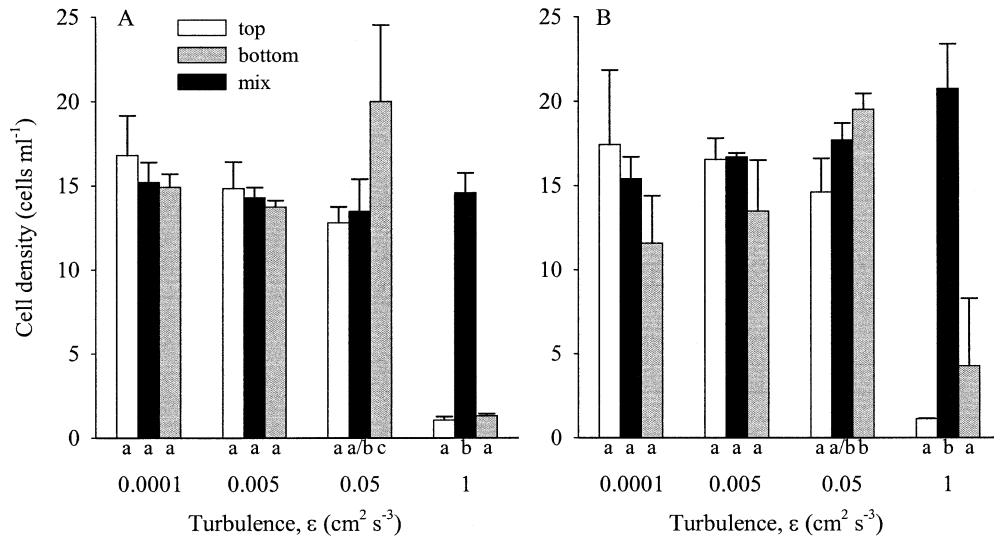


Fig. 1. Cell distribution of *Ceratium tripos* after 48 h exposed to four different levels of turbulence in duplicate experiments (A and B). Initial cell density 12 to 15 cells  $\text{mL}^{-1}$  (bloom density in nature). Top = cell density in samples taken 4 to 6 cm below the surface—without mixing the water column; bottom = cell density in samples taken 1 to 2 cm above the bottom—without mixing the water column; mix = cell density in samples taken after mixing the water column. At a given turbulence level, cell densities with dissimilar letters (a, b, c) below the columns are significantly different ( $p < 0.05$ ). Error bars = standard deviation between six samples.

were increasing the cell density 2 cm above the bottom. Only single cells and dividing cells were observed, which showed that no cell aggregates were formed in the water column. After 5 h, the number of *C. tripos* per field had decreased to below initial values, indicating that the cells were sinking out of the water column (Fig. 3). At the bottom of the container, especially next to the wall, cell aggregates appeared after 5 h.

A few minutes after turbulence had been switched off, the longitudinal flagella of the aggregated cells emerged from the flagella cavities and became visible again, the cells started swimming, and the diameter of the aggregate observed doubled within 1 h. Subsequently, the cell aggregate became increasingly loose and eventually disappeared. The average

swimming velocities in still conditions were  $240 \pm 39 \mu\text{m s}^{-1}$  for *C. tripos* and  $340 \pm 53 \mu\text{m s}^{-1}$  for *F. subglobosum*.

*Effect of turbulence on autotrophic net growth of C. tripos and F. subglobosum*—At the two lowest turbulence levels ( $\epsilon = 0.0001$  and  $0.005 \text{ cm}^2 \text{ s}^{-3}$ ), the autotrophic net growth rates of *C. tripos* were not significantly different from each other, averaging  $0.102 (\pm 0.008) \text{ d}^{-1}$  (Fig. 4). At turbulent dissipation rates higher than  $0.005 \text{ cm}^2 \text{ s}^{-3}$ , net growth decreased significantly with increasing turbulence: at  $\epsilon = 0.05 \text{ cm}^2 \text{ s}^{-3}$  and  $\epsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ , net growth rates were, on average,  $0.060 (\pm 0.011)$  and  $0.023 (\pm 0.008) \text{ d}^{-1}$ , respectively. These rates were both significantly different from each other and from the growth at the two lowest turbulence levels (Fig.

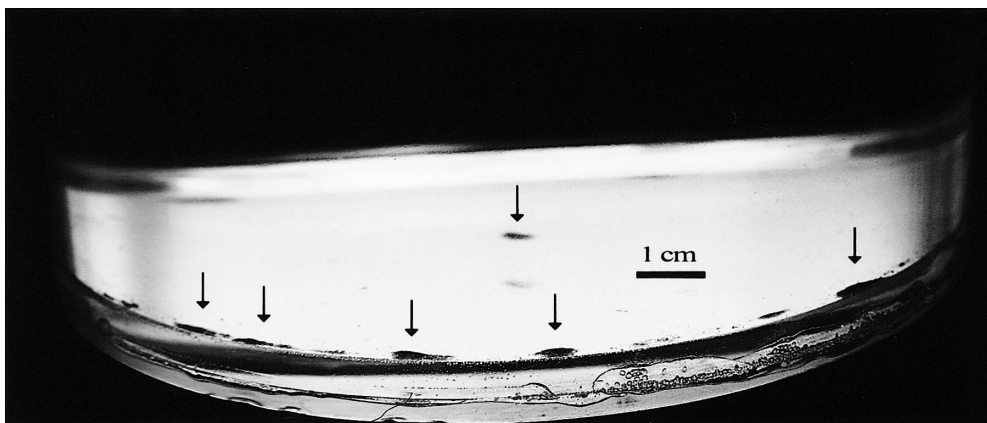


Fig. 2. Aggregates of hundreds of cells (arrows) at the bottom of a container where *Ceratium tripos* was exposed to high turbulence ( $\epsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) for 48 h. Experiment shown in Fig. 1B.

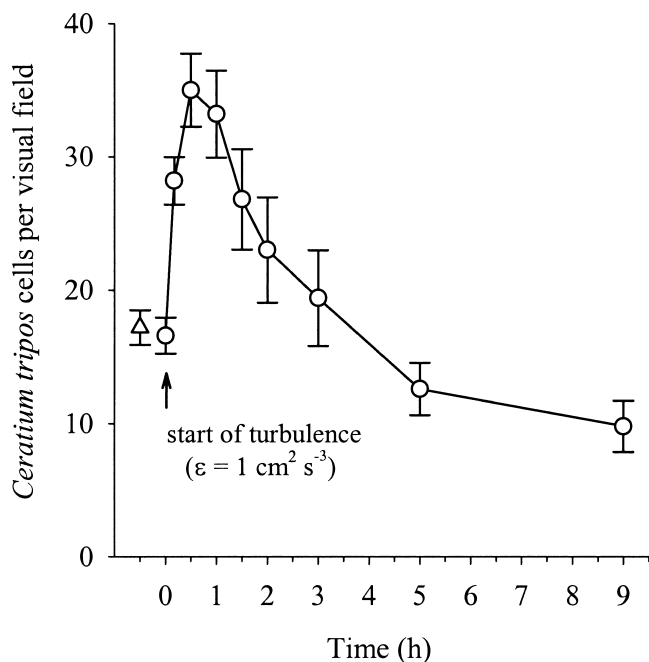


Fig. 3. Number of *Ceratium tripos* cells per visual field ( $0.56 \text{ cm}^2$ ) recorded at a depth 2 cm above the bottom of a container using a video camera. Triangle = in still condition; circle = 0 to 9 h after turbulence ( $\epsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) was applied. Error bars = standard deviation between counts on five frames taken 3 s apart.

4). In contrast, the autotrophic growth of *F. subglobosum* averaged  $0.131 (\pm 0.012) \text{ d}^{-1}$  and did not depend on the level of turbulence (Fig. 5A). Throughout the experiments, pH never exceeded 8.2.

*Effect of turbulence on mixotrophic net growth and ingestion of F. subglobosum at high and low prey densities—*

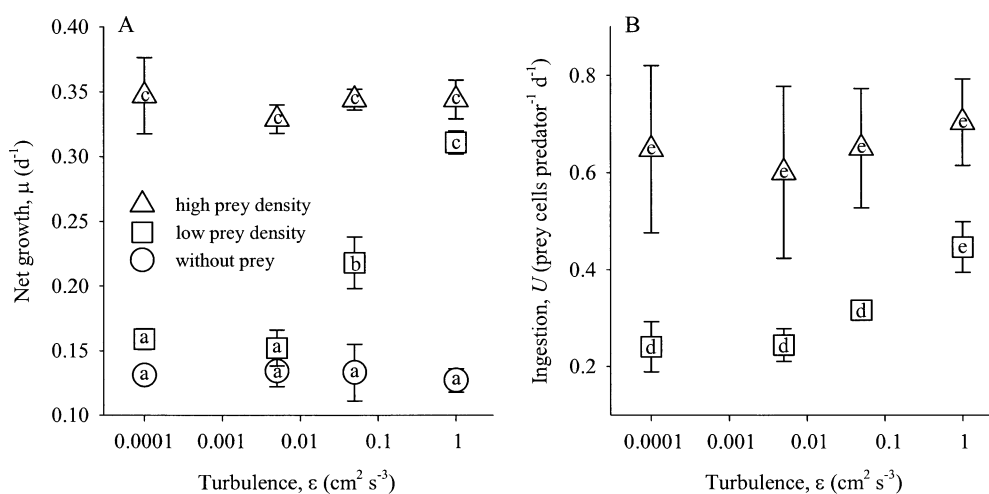


Fig. 5. (A) Autotrophic and mixotrophic net growth and (B) ingestion of *Fragilidium subglobosum* as a function of turbulence at an irradiance of  $45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Triangle = high prey cell densities ( $\gg 10$  *Ceratium tripos* cells  $\text{mL}^{-1}$ ). Square = low prey cell densities (5 to 8 *C. tripos* cells  $\text{mL}^{-1}$ ). Circle = without prey (autotrophic net growth). Error bars = standard deviation of three replicated experiments. Values with dissimilar letters are significantly different ( $p < 0.05$ ). No significant difference between “a” values ( $p = 0.056$ ), “c” values ( $p = 0.096$ ), “d” values ( $p = 0.055$ ), and “e” values ( $p = 0.234$ ).

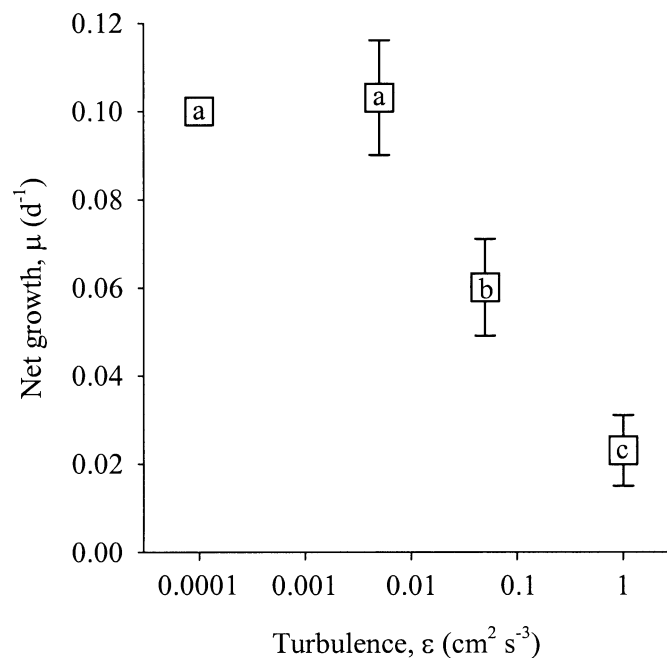


Fig. 4. Autotrophic net growth of *Ceratium tripos* versus turbulence. Irradiance  $45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Error bars = standard deviation of three replicated experiments. Values with dissimilar letters are significantly different ( $p < 0.05$ ). No significant difference between the two values with the letter “a” ( $p = 0.769$ ).

At high prey cell densities ( $\gg 10$  *C. tripos* cells  $\text{mL}^{-1}$ ; food saturation), mixotrophic net growth and ingestion of *F. subglobosum* did not depend on the level of turbulence (Fig. 5). The average net growth was  $0.34 \text{ d}^{-1}$  (Fig. 5A) and the average ingestion  $0.65$  prey cells predator $^{-1} \text{ d}^{-1}$  (Fig. 5B). At low prey cell densities (5 to 8 *C. tripos* cells  $\text{mL}^{-1}$ ), mixotrophic net growth of *F. subglobosum* was lower and

significantly different from the net growth at high prey cell densities when exposed to the three lowest turbulence levels tested ( $\varepsilon = 0.0001, 0.005, \text{ and } 0.05 \text{ cm}^2 \text{ s}^{-3}$ ; Fig. 5A). At  $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ , however, mixotrophic net growth averaged  $0.31 \text{ d}^{-1}$ , which is not significantly different from the net growth at high prey cell densities (Fig. 5A). At the two lowest turbulence levels ( $\varepsilon = 0.0001 \text{ and } 0.005 \text{ cm}^2 \text{ s}^{-3}$ ), mixotrophic net growth averaged  $0.16 \text{ d}^{-1}$ . Mixotrophic growth rates at these turbulence levels were not significantly different from autotrophic net growth (Fig. 5A). At turbulent dissipation rates exceeding  $0.005 \text{ cm}^2 \text{ s}^{-3}$ , the net growth at low prey cell densities increased significantly with increasing turbulence. Net growth rates at  $\varepsilon = 0.05 \text{ and } 1 \text{ cm}^2 \text{ s}^{-3}$  averaged  $0.22 \text{ and } 0.31 \text{ d}^{-1}$ , respectively, and were significantly different from each other and from the net growth at the two lowest turbulence levels (Fig. 5A). Ingestion at low prey cell densities ( $5 \text{ to } 8 \text{ prey cells mL}^{-1}$ ) when exposed to the highest turbulence level ( $1 \text{ cm}^2 \text{ s}^{-3}$ ) averaged  $0.45 \text{ prey cells predator}^{-1} \text{ d}^{-1}$ , which is not significantly different from the average ingestion at high prey cell densities ( $0.65 \text{ prey cells predator}^{-1} \text{ d}^{-1}$ ; Fig. 5B). At the three lowest turbulence levels, ingestion at low prey cell densities was lower (on average  $0.27 \text{ prey cells predator}^{-1} \text{ d}^{-1}$ ) and not significantly different from each other, but significantly different from the ingestion at high prey cell densities (Fig. 5B). Throughout the experiments, pH stayed below 8.2.

## Discussion

*Vertical distribution of C. tripos cells in response to turbulence*—At the highest turbulence level tested,  $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ , *C. tripos* stopped swimming, leading to sedimentation of cells to the bottom of the containers. Sedimentation occurred at high and low cell densities and was not a result of aggregate formation in the water column, because only single cells and dividing cells were observed sinking. Dense aggregates were observed at the bottom after 5 h, and after 48 h, between 80% and 90% of the cells had accumulated into aggregates, mostly next to the wall of the containers. When samples were taken 1 to 2 cm above the bottom without mixing, the aggregated cells were not sampled. When turbulence was stopped, the aggregated cells resumed swimming, showing that the loss of swimming due to turbulence was temporary.

Our experiments showed that some cells sank at  $\varepsilon = 0.05 \text{ cm}^2 \text{ s}^{-3}$ , resulting in a significantly higher density at the bottom than at the top. In contrast, at  $\varepsilon \leq 0.005 \text{ cm}^2 \text{ s}^{-3}$ , *C. tripos* was homogeneously distributed in the water column. The threshold for sedimentation is thus an average dissipation rate of  $0.05 \text{ cm}^2 \text{ s}^{-3}$ , a value that is generated by a wind speed of  $15 \text{ m s}^{-1}$  in the upper 10 m of the ocean, corresponding to a moderate gale (Kiørboe and Saiz 1995). Average dissipation rates are higher in the surface-wave zone. At a depth of 0.5 m, Veron and Melville (1999) found that  $\varepsilon = 0.1 \text{ cm}^2 \text{ s}^{-3}$  for a wind speed of  $6.7 \text{ m s}^{-1}$ . Sedimentation of *C. tripos* from near-surface waters may therefore occur already in response to a gentle breeze, and a subsurface patch of *C. tripos* may not only be a result of cell growth

in this layer, as was previously suggested (Eppley et al. 1984).

*Effects of turbulence on net growth of C. tripos*—*C. tripos* decreased its net growth at  $\varepsilon \geq 0.05 \text{ cm}^2 \text{ s}^{-3}$  compared to the two lowest turbulence levels tested, but it remained positive. The effect of small-scale turbulence on autotrophic net growth of *C. tripos* was also investigated by Sullivan and Swift (2003). In their experiments, turbulence was created by vertically oscillating cylindrical rods in 20-liter containers at an irradiance of  $200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  and a temperature of  $20^\circ\text{C}$ .

At the lowest turbulence levels tested in our study ( $\varepsilon = 0.005 \text{ and } 0.0001 \text{ cm}^2 \text{ s}^{-3}$ ), the net growth of *C. tripos* was  $0.10 \text{ d}^{-1}$ , considerably lower than the  $0.3 \text{ d}^{-1}$  observed at  $\varepsilon = 0.0001 \text{ cm}^2 \text{ s}^{-3}$  by Sullivan and Swift (2003). This may be explained by the lower irradiance and temperature in our experiments ( $45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ,  $15^\circ\text{C}$ ) or by the use of different strains. At  $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ , Sullivan and Swift (2003) recorded a negative net growth of *C. tripos*. In contrast, we measured a low, but positive, net growth ( $0.023 \text{ d}^{-1}$ ) in our study at the same average turbulence intensity. The different experimental conditions used in the two studies may not only have affected the net growth, but also the magnitude of growth inhibition due to turbulence. Juhl and Latz (2002) reported that the negative effect of turbulence on net growth was more pronounced in late, rather than early, exponential phase cultures of the dinoflagellate *Lingulodinium polyedrum*. The slower growing culture of *C. tripos* in our experiments, however, was less affected by turbulence than was the faster growing culture in the experiments by Sullivan and Swift (2003). Despite similar average turbulence levels in both studies, the  $\varepsilon$  values in the experiments by Sullivan and Swift (2003) varied over two orders of magnitude, with maximum values being one order of magnitude higher than in our experiments, which may have caused cell mortality, thus explaining the negative net growth in their study.

If we, however, had sampled from the top and the bottom of the containers without previous mixing, we would have concluded, as did Sullivan and Swift (2003), that (1) the cells were homogeneously distributed and that (2) the net growth after 48 h of exposure to high turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) was negative. The experimental volume (2 liter) in our study, however, allowed a careful mixing prior to sampling. This procedure ensured that the cells in the aggregates at the bottom of the containers were appropriately sampled. Both studies showed, however, that high turbulence reduced the net growth of *C. tripos*, which may have been caused by direct effects on cell division (through cell cycle arrest), cell mortality, or both, as found in studies with other dinoflagellates (Juhl and Latz 2002; Havskum 2003; Yeung and Wong 2003).

*Effect of turbulence on net growth and ingestion rates of F. subglobosum*—Autotrophic net growth of *F. subglobosum* was not affected by the turbulence levels tested. In contrast, net population growth of *C. tripos* decreased at the two highest turbulence levels ( $\varepsilon = 0.05 \text{ and } 1 \text{ cm}^2 \text{ s}^{-3}$ ). Contrasting effects of turbulence on net growth of different dinoflagellate

Table 1. Calculated percentages of maximum ingestion rates for the predator, *Fragilidium subglobosum*, fed different densities of its prey, *Ceratium tripos*, without and with turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ )\*

	Prey density, C (cells mL <sup>-1</sup> )		
	10	6.5	1
No turbulence			
Encounter rate kernel due to behavior (cm <sup>3</sup> s <sup>-1</sup> ); $\beta_{\text{behavior}} = \pi (r_1 + r_2)^2 (\nu_2^2 + 3\nu_1^2)(3\nu_1)^{-1}$	3.23 × 10 <sup>-6</sup>	3.23 × 10 <sup>-6</sup>	3.23 × 10 <sup>-6</sup>
Encounter rate, e (prey cells predator <sup>-1</sup> d <sup>-1</sup> ); e = $\beta_{\text{behavior}} C$	2.79	1.81	0.279
Ingestion rate (prey cells predator <sup>-1</sup> d <sup>-1</sup> ) (22% of encounter rate)	0.6 (measured)	0.398	0.0614
Percentage of maximum ingestion rate	100	66	10
Turbulence ( $\varepsilon = \text{cm}^2 \text{ s}^{-3}$ )			
Encounter rate kernel due to turbulence (cm <sup>3</sup> s <sup>-1</sup> ) $\beta_{\text{turbulence}} = \pi (r_1 + r_2)^2 \omega$ for $r_1 + r_2 <$ the Kolmogorov length scale: $\omega = 0.42 (r_1 + r_2) \varepsilon^{0.5} \nu^{-0.5}$ $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ (the highest level tested) $\nu = 10^{-2} \text{ cm}^2 \text{ s}^{-1}$ (the kinematic viscosity of seawater)	1.74 × 10 <sup>-6</sup>	1.74 × 10 <sup>-6</sup>	1.74 × 10 <sup>-6</sup>
Encounter rate, e (prey cells predator <sup>-1</sup> d <sup>-1</sup> ); e = $\beta_{\text{behavior} + \text{turbulence}} C$	4.29	2.79	0.429
Ingestion rate (prey cells predator <sup>-1</sup> d <sup>-1</sup> ) (maximum or 22% of encounter rate)	0.6 (maximum)	0.6 (maximum)	0.094
Percentage of maximum ingestion rate	100	100	16

\* Equations regarding the encounter rate per predator from Kiørboe and Saiz (1995).  $r_1 = 21.2 \mu\text{m}$ , the perceptive distance of the predator, set to half body length, because it feeds by direct engulfment (Skovgaard 1996).  $r_2 = 29.7 \mu\text{m}$ , the radius of the prey.  $r_1$  and  $r_2$  were calculated as half equivalent spherical diameters from the cell volumes given in the materials and methods section. The swimming velocities of the predator and the prey were  $\nu_1 = 340 \mu\text{m s}^{-1}$  and  $\nu_2 = 240 \mu\text{m s}^{-1}$ , respectively.

species at the same experimental conditions were also reported by Sullivan and Swift (2003), and show that the effect of turbulence on net growth of dinoflagellates is species specific.

Mixotrophic growth of *F. subglobosum* at high prey densities ( $\gg 10$  *C. tripos* cells mL<sup>-1</sup>) was not affected by the turbulence levels tested, whereas at low prey cell densities (5 to 8 *C. tripos* cells mL<sup>-1</sup>), the net growth rate increased significantly at turbulent dissipation rates of  $\geq 0.05 \text{ cm}^2 \text{ s}^{-3}$ . At  $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ , the net growth and ingestion of *F. subglobosum* at low prey cell densities were not different from those measured at high prey cell densities at all turbulence levels tested. Increased grazing and growth rates at high turbulence can potentially be explained by increased encounter rates (Kiørboe and Saiz 1995). To evaluate this possibility, we first calculated the predator's encounter rate without turbulence and subsequently calculated the encounter rate under high turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) at different prey cell densities (Table 1).

At a prey density of 10 *C. tripos* cells mL<sup>-1</sup>, without turbulence, *F. subglobosum* theoretically encounters 2.79 *C. tripos* cells d<sup>-1</sup> (Table 1) but displays its maximum ingestion of 0.6 cells predator<sup>-1</sup> d<sup>-1</sup>; below this prey density, the ingestion declines linearly (Hansen and Nielsen 1997). This implies that only 22% of the encountered cells are ingested at prey densities below 10 cells mL<sup>-1</sup>.

At a lower prey cell density (6.5 *C. tripos* cells mL<sup>-1</sup>) without turbulence, *F. subglobosum* theoretically encounters 1.81 prey cells d<sup>-1</sup>, of which it will ingest 22% (i.e., 0.398 prey cells predator<sup>-1</sup> d<sup>-1</sup>), corresponding to 66% of the prey cells needed for maximum ingestion (Table 1). At high turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ), the predator's encounter rate at a prey density of 6.5 *C. tripos* cells mL<sup>-1</sup> theoretically increases to 2.79 prey cells predator<sup>-1</sup> d<sup>-1</sup>. Assuming that the per-

centage of encountered prey cells ingested is the same as under nonturbulent conditions (i.e., 22%), its ingestion rate increases to 0.6 prey cells predator<sup>-1</sup> d<sup>-1</sup> (i.e., 100% of the prey cells needed for maximum ingestion) (Table 1). The observed maximum growth and ingestion at low prey densities (5 to 8 *C. tripos* cells mL<sup>-1</sup>) and high turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) is therefore apparently supported by the theory on increased encounter rates and, hence, grazing rates. The theory, however, implies a homogeneous prey distribution, which was not the case.

As a response to high turbulence ( $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ), the cells stopped swimming, settled, and increased cell density at the bottom. Eventually 80% to 90% of the *C. tripos* cells were aggregated at the bottom at  $\varepsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ . At low prey densities (5 to 8 *C. tripos* cells mL<sup>-1</sup>), on average only 15% remained in the water column, resulting in a cell density of 1 cell mL<sup>-1</sup>. At this prey cell density at high turbulence, *F. subglobosum* encounters theoretically 0.429 *C. tripos* cells d<sup>-1</sup> and ingests 0.094 *C. tripos* cells d<sup>-1</sup> (i.e., only 16% of the cells needed for maximum ingestion) (Table 1), which cannot explain the observed maximum growth and ingestion rates.

The calculations made in Table 1 are quite rough because they are based on homogeneously distributed spherical particles that move in straight lines (Kiørboe and Saiz 1995). The shape of the predator, *F. subglobosum*, is close to a sphere, but it swims in a helix. The prey, *C. tripos*, swims in straight lines, but it stops swimming when exposed to high turbulence. When the swimming velocity of the prey in the calculations in Table 1 is replaced by the sinking velocity, the predator's encounter rate of the prey will be lower. In contrast, inserting a larger prey radius, because *C. tripos* has horns, increases the encounter rate but decreases the percentage of the encountered cells ingested. A doubling of the

prey radius would result in an increase of the percentage of maximum ingestion, but only from 16% to 19% at a prey density of 1 cell mL<sup>-1</sup>. More decisive for the percentage of the maximum ingestion is the prey cell density. A decline in prey cell density from 6.5 cells mL<sup>-1</sup> to 1 cell mL<sup>-1</sup>, which is observed in the water column in response to high turbulence ( $\epsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ), decreases the percentage of maximum ingestion from 100% to 16% (Table 1). Our calculations and observations therefore indicate that only a minor part of the observed maximum ingestion and growth of *F. subglobosum* at low prey cell densities and high turbulence ( $\epsilon = 1 \text{ cm}^2 \text{ s}^{-3}$ ) can be explained by an increased predator:prey encounter rate.

The sedimentation of *C. tripos* cells in response to high turbulence results in patches with high cell densities at the bottom of the containers. A predator that finds and stays in a dense patch of prey cells will not be food-limited, and this may be the main reason for the high growth and ingestion rate of *F. subglobosum* at low prey cell densities.

Sedimentation of prey organisms has to be considered when grazing is measured in response to turbulence in the laboratory, even for swimming cells. If the distribution of prey cells in the containers had not been taken into account, an increased predator:prey encounter rate with increasing turbulence would have been the most likely explanation.

In nature, sinking cells will resume swimming rather than aggregating upon reaching a depth where turbulence is low. Sedimentation, however, will alter the vertical distribution of cells and can lead to increased densities of *C. tripos* cells in a subsurface layer. In such a layer, a predator may display increased ingestion and growth rates. It would be interesting, in the future, to study the vertical distribution of *Ceratium* spp. before and after a storm event and to measure the grazing and production rates of various protist and metazoan grazers at different depth strata.

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Received: 7 July 2004

Accepted: 3 April 2005

Amended: 4 June 2005