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Effect of fluid shear and irradiance on population growth and cellular toxin content of the dinoflagellate *Alexandrium fundyense*

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Abstract

The potential for in situ turbulence to inhibit dinoflagellate population growth has been demonstrated by experimentally exposing dinoflagellate cultures to quantified shear flow. However, despite interest in understanding environmental factors that affect the growth of toxic dinoflagellates, little is known of the effect of shear on the growth of toxin-producing dinoflagellate species. Cultures of the dinoflagellate, *Alexandrium fundyense*, a producer of toxins responsible for paralytic shellfish poisoning, were exposed to quantified laminar shear generated in Couette flow for 1–24 h d^{-1} over 6–8 d. Shear stress in all experiments was 0.003 N m^{-2}, similar to levels expected in near-surface waters on a windy day. Net population growth decreased with shear exposures >1 h d^{-1} and became negative with exposures >12 h d^{-1}. Cellular toxin content at the end of each experiment was measured by a receptor-binding assay that used [^{3}H]saxitoxin. Toxin $cell^{-1}$ of cultures sheared for >1 h d^{-1} increased up to three times that of control cultures. Cellular toxin content increased significantly as growth rate of sheared cultures decreased. However, varying culture growth rate using irradiance had no significant effect on toxin $cell^{-1}$. Because shear stress levels used in this study were plausible for near-surface turbulent flows, oceanic turbulence may inhibit population growth and increase cellular toxin content of *A. fundyense.* However, in natural populations it would be difficult to distinguish the effect of turbulence on toxin content from other influences on toxin variability, particularly if volume- or mass-specific, rather than cell-specific, measures of toxin are used.

Field studies have found negative correlations between red-tide dinoflagellate abundance and physical parameters that relate to turbulence. Tynan (1993) and Rapoport and Latz (1998) both found that the abundance of red-tide dinoflagellates in La Jolla Bay, California, was negatively correlated with the significant wave height of previous days. In Lake Kinneret (Israel), yearly spring blooms of the dinoflagellate *Peridinium gatunense* (=*cinctum*) do not occur until after the yearly onset of stratification (Pollingher and Zemel 1981), and *P. gatunense* cell concentration within blooms is negatively correlated with ε, the rate of turbulent energy dissipation (Berman and Schteinman 1998). Unusually high rates of energy dissipation in 1996 may explain why it was the only year of a 32-yr time series that lacked a spring *P. gatunense* bloom (Berman and Schteinman 1998).

Such field observations are consistent with a number of hypotheses. Dinoflagellates are active swimmers and are known to exhibit diel vertical migrations (Eppley et al. 1968; Kamykowski 1995). One possibility is that these abilities allow dinoflagellates to remain suspended in calm, stratified conditions and may also allow them to assimilate nutrients at depth, whereas less motile phytoplankton may sink out of the euphotic zone or become nutrient limited (e.g., Eppley and Harrison 1975; Margalef 1978). Mixing associated with turbulent conditions may discourage bloom formation because of physical dispersion and reduced, time-integrated light exposure of individual cells. An alternative perspective, which may be complementary, suggests that dinoflagellate population growth is negatively impacted by turbulence through direct effects of the fluid forces on cell physiology. Laboratory experiments that used quantified shear flow have demonstrated that population growth of some dinoflagellate species is inhibited by daily exposure to laminar shear (Thomas and Gibson 1990, 1992; Tynan 1993; Juhl et al. 2000). Because shear levels in these studies were comparable to turbulent shear in near-surface waters, the results demonstrate the potential importance of turbulence in regulating growth of natural dinoflagellate populations.

Laminar shear can be used to simulate the small-scale effects of turbulence on dinoflagellates and other plankton that are smaller than the typical Kolmogorov length scales of in situ turbulence (Lazier and Mann 1989; Thomas and Gibson 1990). In theory, below the Kolmogorov length scale, turbulent flow is manifested as laminar shear because

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viscosity smoothes out velocity fluctuations (Lazier and Mann 1989). Empirically, the response of dinoflagellates to fluid motion has been best characterized by use of flow-stimulated bioluminescence. As expected for organisms smaller than the Kolmogorov scale, in laminar and turbulent flow, dinoflagellate bioluminescence is stimulated at similar values of shear stress (Latz et al. 1994; Latz and Rohr 1999). These findings suggest that dinoflagellates are insensitive to the turbulent or laminar nature of the flow, responding instead to fluid shear.

Little is known about the effect of shear on growth of toxic dinoflagellates, although much of the interest in understanding how environmental factors affect dinoflagellate population dynamics stems from the impacts of bloom forming and toxin-producing species. The growth of several toxin-producing species has been noted to decrease because of shaking or bubbling of culture medium (White 1976; Anderson et al. 1990; Parkhill and Cembella 1999), but such flows cannot be directly compared with in situ turbulence. Chen et al. (1998) demonstrated shear-induced growth inhibition in *Alexandrium minutum.* However, because the flow field used was unstable, the shear stress values that cells actually experienced could have deviated substantially from the bulk values that were presented. The first objective of the present study was to determine whether daily exposure to quantified laminar shear, at plausible levels for oceanic turbulence, would inhibit net population growth of the dinoflagellate *Alexandrium fundyense,* a producer of the toxins responsible for paralytic shellfish poisoning (PSP).

Saxitoxin (STX) and its derivatives are the causative agents of PSP. Deaths, illnesses, and economic costs associated with PSP (ECOHAB 1995) motivate interest in understanding the environmental factors influencing toxin production or toxin content in dinoflagellates such as *Alexandrium* spp. Previous studies have shown that environmental factors that affect the growth of *Alexandrium* spp. can influence cellular toxin content. These factors include illumination (Ogata et al. 1987), temperature (Anderson et al. 1990; Procter et al. 1975), salinity (Parkhill and Cembella 1999), and nutrient concentration (Anderson et al. 1990; Flynn et al. 1994). The relationship between growth rate and toxin content is not consistent for all factors. Nevertheless, if exposure to shear affected *A. fundyense* growth, it might also affect its toxin content. Therefore, the second objective of the present study was to determine whether toxin content of *A. fundyense* was influenced by shear-induced growth inhibition.

The present study used Couette flow chambers to expose cultures of *A. fundyense* to quantified laminar shear from 1 to 24 h d^{-1}. The effect of increasing daily shear duration on net population growth rate and cellular toxin content was determined. Results were compared with cultures in still Couette chambers and with still cultures whose growth rates were manipulated by changing illumination intensity. Because the shear exposures used were environmentally relevant, the results demonstrate the potential for turbulent shear to affect growth and toxin content of natural *Alexandrium* populations. However, actual effects of turbulent shear on natural populations may be dependent on additional environmental factors (Juhl et al. 2000).

Methods

All experiments used nonaxenic, unialgal cultures of *A. fundyense* (CCMP 1719) grown in f/4 medium minus silicate (i.e., seawater enriched with 1/4 the added nutrients of F medium; Guillard and Ryther 1962) in an environmental chamber at a temperature of 15°C with a 12 : 12 h light : dark (LD) cycle. Experimental design was similar to that of Juhl et al. (2000). Cultures were exposed to quantified shear flow in Couette flow chambers, consisting of two concentric cylinders enclosing a seawater-filled, annular gap. The chambers used in this study were constructed entirely of plexiglas and were slightly larger (inner and outer cylinder radii of 41 and 48 mm, respectively, inner cylinder height of 200 mm) than those described in Juhl et al. (2000) but were otherwise similar in design. The bottoms of the two cylinders met as a bushing surface with no dead space between them. Thus, the entire fluid volume (265 ml) was within the gap. Previous work has shown that, under some conditions, dinoflagellate growth is unaffected by shear exposure in such flow chambers—for example, when shear exposure occurs only during the light phase of the LD cycle (Juhl et al. 2000). Therefore, potential artifacts of cylinder rotation, such as grinding cells between the bushing surfaces, were assumed to be negligible.

Simple Couette flow is generated by rotating the outer cylinder while the inner cylinder is held in place. A nearly linear velocity gradient is produced within the fluid-filled gap between the cylinders. Fluid shear stress (τ) was calculated as

$$\tau = \mu[2\omega_o r_o r_i/(r_o^2 - r_i^2)],$$

where μ is the dynamic viscosity, ω is the angular velocity ($\omega = 2\pi N/60$), N is rotational speed in rpm), and r_o and r_i are the inner and outer cylinder radii, respectively (Latz et al. 1994). Shear stresses corresponding to the entire range of typical oceanic values can be generated in the gap by changing the rotation speed of the outer cylinder. The above calculations and future mention of shear stress refer to the bulk fluid shear stress, which can be compared with the shear stress in other flows. However, shear stress at any point on the surface of a cell is a function of the bulk shear stress, local inhomogenieties in the flow, cell rotation, and cell deformation (Cherry and Kwon 1990). Couette flow is experimentally convenient because all relevant hydrodynamic aspects of the flow within the gap can be calculated a priori and are constant throughout the fluid volume for the duration of treatment. Design considerations and details of calculating the relevant hydrodynamic parameters can be found in previous studies of quantified shear on dinoflagellate growth (Thomas and Gibson 1990; Tynan 1993; Juhl et al. 2000).

The rotation rate of the outer cylinder used in all experiments was 4.5 rpm, giving a shear stress of 0.003 $N\ m^{-2}$, approximately equivalent to that produced during isotropic turbulence with an energy dissipation rate $\varepsilon = 10^{-5}\ m^2\ s^{-3}$ (Thomas and Gibson 1990). Daily shear exposure duration for treated cultures ranged from 1 to 24 h d^{-1} in different experiments. The 1–12-h daily exposures all ended at the end of the dark period, the time of greatest shear sensitivity in the dinoflagellates *Lingulodinium polyedrum* and *P. ga-*

tunense (Pollingher and Zemel 1981; Juhl et al. 2000). The 16-h daily exposure included the first and last 2 h of each light period. The 24-h exposures were continuous. Illumination level in all shear experiments was ~30 μmol m^{-2} s^{-1}. Low irradiance was selected to maintain low growth rates, even in the controls. Low growth rates ensured that cultures were in the exponential phase of growth for the duration of each experiment. Low irradiance may also maximize shear sensitivity (Juhl et al. 2000), increasing the relative effect of shear on population growth.

At the beginning of each shear experiment, *A. fundyense* cells were inoculated into fresh f/4 medium minus silicate at an approximate concentration of 750 cells ml^{-1} and then poured into paired Couette flow chambers. Each experiment consisted of initial and treatment periods. During the initial period of 3–5 d, daily monitoring ensured that both cultures were healthy and growing at similar rates. During the 6–8-d treatment period, one randomly selected chamber was sheared each day for the specified duration, whereas the other remained still throughout the experiment as a control. The exponential population growth rate was calculated from cell counts made every 1–2 d. Triplicate 0.5-ml samples were taken from each Couette flow chamber after removing the inner cylinder and gently mixing the culture. Samples were always collected during the middle of the light phase, when cell division does not occur and shear sensitivity is minimal (Juhl et al. 2000). From each 0.5-ml sample, two subsamples were taken for counting. Subsample volumes were adjusted so that each generally contained 20–30 cells. Subsamples were briefly chilled, to minimize swimming during counting. A minimum of five consecutive cell counts during the treatment period were used to calculate the mean daily population growth rate within each Couette flow chamber. Counts, expressed as cells ml^{-1}, were log-normal transformed and fitted with a least-squares linear regression of log normal (cell concentration) versus time (d). The regression slope was taken as the best estimate of the net daily population growth rate. For each experiment, treatment and control growth rates were calculated from data collected on the same days.

At the end of the treatment period, samples were also collected for analysis of cellular PSP toxin content. Three to four samples of 30–70 ml (depending on cell concentration) were removed from each Couette flow chamber. The cell concentration within each sample was determined from triplicate 0.5-ml subsamples, as described above. For each sample, a volumetric subsample of 20–60 ml was then filtered through a 47-mm diameter Whatman type HA filter (0.45 μm pore diameter) to collect the *A. fundyense* cells. Filters were frozen immediately in liquid N_2 and stored at −80°C in 15-ml plastic centrifuge tubes. Before analysis, filters were thawed, and 5 ml of distilled water was added to each tube. Samples were sonicated for 1 min at 1 pulse s^{-1} and 100 W by use of a Branson 450 sonifier with a 1 cm-diameter probe. Microscopic inspection after sonication ensured complete cellular fragmentation. Tubes were centrifuged at 1,000 × *g* for 15 min, to pellet debris. Supernatents were analyzed in duplicate for toxin content by use of a receptor-binding technique (Doucette et al. 1997; Trainer and Poli 2000). A rat brain homogenate containing neuronal membranes with the specific sodium channel receptors to which the PSP toxins bind was incubated with the dinoflagellate cell lysate and a final concentration of 1 nM [^{3}H]STX (Amersham). After 0.5 h of incubation, the bound material was collected by filtration. Filters were washed to remove unbound [^{3}H]STX. Bound radioactivity was measured by scintillation counting of the radioactivity remaining on the filters. Because PSP toxins in the cell lysate competed with labeled STX for receptor-binding sites, radioactivity was inversely related to toxin content of the cell lysate. Standard curves were constructed with known concentrations of unlabeled STX for each batch of samples analyzed. To calculate toxin $cell^{-1}$, total toxin was normalized to the volume of culture filtered and the sample cell concentration. The receptor-binding assay measured the biological activity of PSP toxins in the cell lysate. Because the specific activity of PSP toxins varies, the assay could not identify which toxins were present. For this reason, quantitative reference to cellular toxin content will be described as STX equivalents. Only toxin in the particulate phase of the cultures was measured; toxin that was exuded or released by cells that lysed before sample collection was not quantified.

To test whether cellular toxin content was a direct function of growth rate, cultures of *A. fundyense* were grown in unagitated glass Erlenmeyer flasks (250 ml of culture in 500-ml flasks) under irradiance levels ranging from 15 to 130 μmol m^{-2} s^{-1}. Cell counts and toxin analyses were conducted as described above, to determine net population growth rates and toxin $cell^{-1}$.

Relative cell sizes in sheared and control cultures were compared on the basis of the forward light scatter (FSC) of cells measured by use of a FACSort flow cytometer (Becton Dickinson) with an Ar laser (488 nm). FSC is related to cross-sectional area of a particle and should increase with target size (Given 1992). For the purposes of this study, investigating the potential for relative changes in cell size did not require absolute calibration of FSC. FSC of cultures from the 12, 16, and 1 of the 24-h daily shear experiments were measured. For each culture tested, 500–1,000 live cells were run through the flow cytometer approximately midway through the daily light period. Cells were distinguished from other particles on the basis of their light scattering properties and chlorophyll fluorescence.

All mean values presented in the text are given with the SE and the number of replicates. Significance of linear regressions between measured variables was tested by use of the analysis of variance (ANOVA). Test results are given with the *F* values, *P* values, and degrees of freedom of the *F*-test.

Results

During experiments exposing *A. fundyense* cells to Couette shear, cell abundance in the sheared chambers typically began to diverge from those of the corresponding still controls after initiation of the treatment period. Data from two representative experiments are shown in Fig. 1. With 4 h of daily shear, growth rate of the sheared culture (0.05 d^{-1}) was lower than the control growth rate but remained positive.

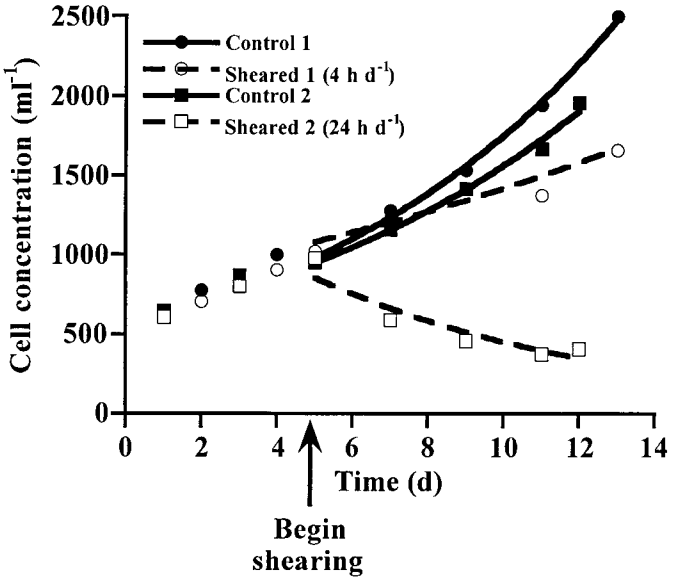

Fig. 1. Cell count data from two representative experiments. Symbols represent the mean of triplicate cell counts from each Couette flow chamber. Error bars were omitted. In both experiments shear exposure began on day 5. In experiment 1, the sheared culture was exposed to 0.003 N m^{-2} shear stress for 4 h d^{-1} during the 4 h preceding the daily light period. Experiment 2 used the same shear stress, with continuous exposure. Curves represent the exponential regression of cell concentration vs. time. Net population growth rates were calculated by use of mean cell counts from days 5–13 for experiment 1 and from days 5–12 for experiment 2.

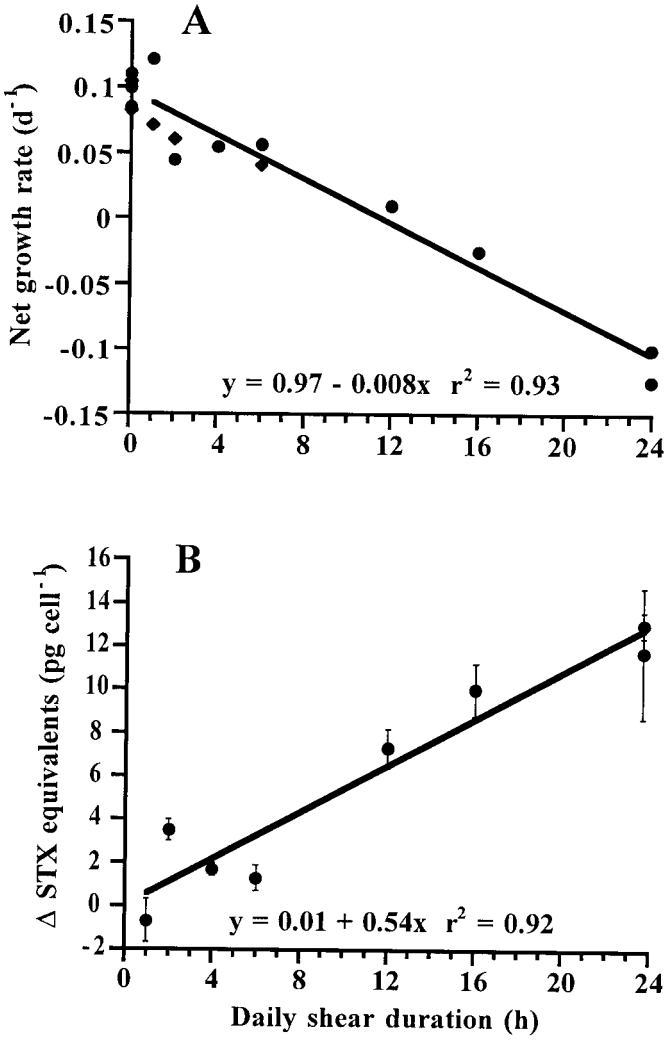

Fig. 2. Effects of increasing daily shear duration on net growth rate and cellular toxin content. (A) Effect on net growth rate. Each point represents the growth rate of a single culture in a still or sheared (0.003 N m^{-2}) Couette flow chamber. Experiments represented by circles had samples collected for toxin analysis, and diamonds represent experiments without toxin samples. The line represents the least-squares linear regression of net growth rate vs. daily shear duration; control growth rates (at 0 h daily shear duration) were not included in the regression. (B) Effect on Δ toxin cell^{-1}. Each point represents mean Δ toxin cell^{-1} (±1 SE) of triplicate subsamples from a single sheared Couette flow chamber. The line represents the least-squares linear regression of mean Δ toxin cell^{-1} vs. daily shear duration.

When shear was continuous, the sheared culture growth rate became negative (−0.13 d^{-1}). Control growth rates were similar in both experiments (0.11 d^{-1} and 0.10 d^{-1}).

Net population growth rate of sheared cultures decreased with increasing daily shear duration (Fig. 2A). Mean control growth rate (at 0 h daily shear) was 0.10 ± 0.004 d^{-1} (n = 8). The effect of a 1-h daily shear treatment was variable. In one case, there was a slight decrease in growth rate, but when the treatment was repeated, growth rate of the sheared culture was higher than in the control. For 2–6 h of daily shear, net growth was positive but decreased to approximately one half the control growth rate. Twelve hours of daily shear decreased growth essentially to 0. In the 16- and 24-h treatments, net growth was negative. The regression slope of daily shear duration versus growth rate of sheared cultures was highly significant ($F_{1,9}$ = 120.0, $P < 0.0001$).

In each shear experiment that resulted in growth inhibition, cellular toxin content of the sheared culture was higher than that of the corresponding control. The change of each sample from the mean cellular toxin content of the corresponding control (Δ toxin cell^{-1}) increased with increasing daily shear duration (Fig. 2B). The relationship between daily shear duration and Δ toxin cell $^{-1}$ was highly significant ($F_{1,6}$ = 69.7, P = 0.0002). The slope of absolute cellular toxin content versus net growth rate for all cultures grown in Couette flow chambers was also highly significant (Fig. 3A; $F_{1,14}$ = 39.0, $P < 0.0001$).

In experiments that modified illumination level to vary the growth rate of still cultures, growth rates ranged over almost an order of magnitude, from 0.03 to 0.22 d^{-1} (Fig. 3B). Although the regression slope of growth versus cellular toxin content was negative, the relationship was not significant ($F_{1,6}$ = 1.61, P = 0.25).

Cell size (as measured by FSC) overlapped substantially in control and sheared cultures. Mean relative FSC of sheared and control cultures differed by <2.5 FSC units (<1% of the range) in the three experiments from which data were collected. This difference between sheared and control cultures was not significant (paired t-test; t_2 = 1.02, P = 0.42).

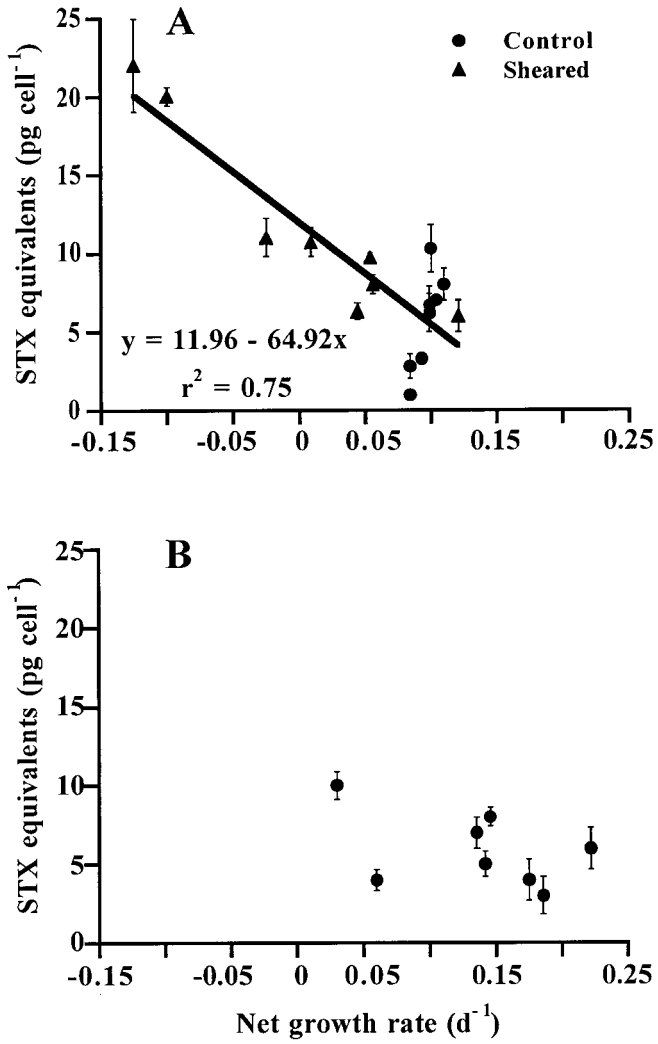

Fig. 3. Change in cellular toxin content as a function of net growth rate. Each point represents mean toxin cell^{-1} ($\pm$1 SE) of triplicate subsamples from a single culture. (A) Growth rate controlled by shear. The line represents the least-squares linear regression of mean toxin cell^{-1} vs. net growth rate. (B) Growth rate controlled by irradiance. The regression slope of net growth rate versus toxin cell^{-1} was not significant.

Discussion

Given the social and economic impacts of toxic dinoflagellates within the *Alexandrium* group, it is important to understand the environmental factors that influence their growth and toxin content (ECOHAB 1995). Exposure to fluid shear has the potential to inhibit the growth of *A. fundyense* and can even cause mortality. Simultaneously, exposure to shear appears to markedly increase cellular toxin content. Because the shear stress levels used in this study were plausible for oceanic turbulence on a windy day, similar effects may occur in natural *Alexandrium* populations.

Inhibition of population growth as a result of exposure to fluid shear has now been demonstrated for several dinoflagellate species (Thomas and Gibson 1990, 1992; Tynan 1993; Chen et al 1998; Juhl et al. 2000; Zirbel et al. 2000). There are differences between the response of *A. fundyense* and the nontoxic red tide species, *L. polyedrum,* tested under nearly identical conditions (Juhl et al. 2000). A 1-h daily shear exposure at shear stress levels comparable to the present study strongly inhibited growth of *L. polyedrum,* whereas *A. fundyense* was not affected. However, during the exponential phase of growth, *L. polyedrum* growth rate remained positive even with continuous shear exposure (Juhl 2000), whereas net growth of *A. fundyense* became negative with daily shear exposures >12 h. These observations demonstrate species specificity in the dinoflagellate response to shear.

Shear-induced growth inhibition in dinoflagellates has been attributed to failure of the mitotic process (Pollingher and Zemel 1981; Berdalet 1992) leading to decreased cell division rates. Although this could play a role in the decreased growth rates measured in this study, the observation of negative net growth rates in the longer daily exposures demonstrate that mortality can also be important. Several other studies have demonstrated shear-induced dinoflagellate mortality during shaking in flasks (White 1976; Berdalet 1992; Juhl et al. 2000) and exposure to laminar Couette shear (Thomas and Gibson 1990; Juhl 2000; Juhl et al. 2000).

In the continuous shear treatments, toxin cell^{-1} was approximately three times greater than that in controls. This change in toxin content is comparable to proportional changes in toxin content caused by other environmental factors. Cellular toxin content of *A. fundyense* increases by two to six times because of low temperature or phosphate stress (Procter et al. 1975; Anderson et al. 1990). On the other hand, toxin cell^{-1} of *A. tamarense* declines ~50% when growth decreases because of low salinity (Parkhill and Cembella 1999). Although the proportional changes in cellular toxin content are similar for different environmental factors, the changes are probably mediated by different physiological pathways.

Several hypotheses can explain the observed changes in cellular toxin content following shear exposure. There are >20 STX derivatives (Oshima et al. 1993) that vary in toxicity over two orders of magnitude (Hall et al. 1990). Because the receptor-binding assay used in the present study does not distinguish between STX derivatives, changes in measured toxin content could be due to changes in toxin composition. Future studies using high-performance liquid chromatography–based toxin identification should examine changes in toxin composition in response to shear exposure. If toxin composition did not change, increases in toxin content may have been due to increases in toxin production. The largest increases in toxin content coincided with high mortality. Unusual metabolic pathways leading to higher toxin production may have been initiated when the cells experienced a lethal shear environment. More likely, because toxin content reflects the balance between production and loss rates, it is also possible that increased toxin content was due to decreases in toxin loss terms, such as the 50% loss that occurs with each cell division. If toxin production does not change, a decrease in cell division frequency due to shear exposure should increase mean toxin cell^{-1}. Moreover, reduced cell division in eukaryotes is often due to a length-

ening of the G1 phase of the cell cycle (Guiguet et al. 1984). For example, light or nutrient limitation prolongs the G1 phase of the dinoflagellate *Amphidinium carterae* (Olson and Chisholm 1986), and exposure to moderate shear stress prolongs the G1 phase of *L. polyedrum* (Juhl 2000). In *A. fundyense,* PSP toxins are only produced during G1 phase (Taroncher-Oldenburg et al. 1997). If shear exposure decreased cell division frequency by lengthening G1, not only would the division loss of toxin decrease, but the average cell would also have more time to produce and accumulate toxin between divisions.

Mortality could also lead to an increase of mean toxin cell^{-1} if death occurred preferentially in cells with low toxin content. For example, newly divided cells could be more prone to shear-induced mortality. Any combination of the above considerations might have played a role in the observed changes in cellular toxin content. However, the increase in cellular toxin content of sheared cultures was not due to changes in cell size.

Several studies have explicitly considered a direct relationship between growth rate and *Alexandrium* toxin content (e.g., Anderson et al. 1990; Parkhill and Cembella 1999). In the present study, the strong relationship between shear-regulated growth rate and toxin content supports such a relationship. However, no significant relationship between growth and toxin content was found when growth was modified by illumination level. As described above, environmental factors that influence growth can have different effects on toxin content. The relationship between cellular toxin content and growth rate in *Alexandrium* spp. may be indirect, reflecting changes in physiological pathways that respond to the environmental factors controlling growth, rather than to growth rate per se.

The present study was not designed to fully simulate in situ conditions but rather to investigate the potential for oceanic turbulence to affect *Alexandrium* growth and toxin content. Although these potential effects of turbulence were demonstrated, many questions remain to be explored. Several caveats to the present study require discussion. When toxin data from control and sheared cultures were analyzed in a pairwise fashion (as in Fig. 2B), the effect of shear exposure was detectable in all exposures >1 h d^{-1}. However, there was substantial variation in control values of toxin cell^{-1} (Fig. 3A). The only values of toxin cell^{-1} from sheared cultures that substantially exceeded the range of control values resulted from continuous shear treatment. However, continuous shear exposure may produce unrealistic results even though the shear stress levels used were physiologically relevant. Oceanic turbulence generates intermittent shear (Baker and Gibson 1987; Wijesekera et al. 1993; Jimenez 1997), whereas Couette shear is constant as long as the outer cylinder is rotated. The importance of intermittency on the dinoflagellate response to shear is not known.

The effect of irradiance should also be considered. When field populations of *Alexandrium* are exposed to light levels higher than those used in this study, the relative effects of shear on growth rate and toxin content may be less than were measured here (Juhl et al. 2000). In fact, the near-surface depths that generally have the highest levels of turbulence are also those with the highest light levels. However, vertical motions associated with high turbulence generally lower the time-integrated light exposure of natural phytoplankton populations. Thus, turbulent mixing could contribute to creating the conditions under which turbulent shear would have the greatest effects on cell physiology.

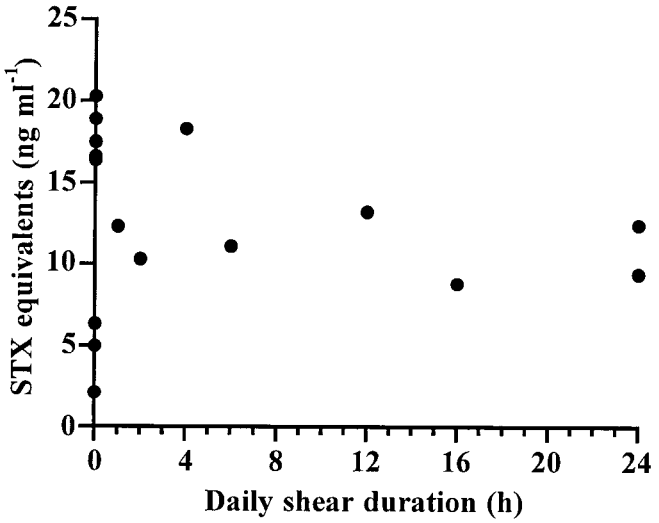

Fig. 4. Effect of shear duration on volume-specific toxin content. Total toxin ml^{-1} was calculated as the mean cellular toxin content of each culture multiplied by the cell concentration (ml^{-1}) on the final day of the treatment period. Toxin ml^{-1} of sheared cultures remained relatively constant with increasing daily shear duration despite decreasing cell concentrations.

The consequences of shear exposure on toxin content can be viewed from another perspective by calculating the volume-specific toxin content (toxin ml^{-1}) at the end of each experiment (Fig. 4). Toxin ml^{-1} of sheared cultures was quite consistent, despite a range of final cell concentrations from 400 to 2,000 ml^{-1}. For a manager interested in shellfish toxicity, for example, instead of toxin cell^{-1}, equivalent toxin assimilation by shellfish might occur despite changes in cell concentration. However, given the range of volume-specific toxin content in control samples, it appears unlikely that increases in toxin ml^{-1} due to turbulent shear in the environment could be distinguished from background variation in natural samples.

Despite the above considerations, this study is unique in demonstrating growth inhibition of a toxic dinoflagellate in quantified flow conditions. This is also the first study to suggest turbulent shear as an environmental parameter affecting cellular toxin content. The results suggest that oceanic turbulence can be an important determinant of growth rate and toxin content in natural *Alexandrium* populations. However, the physiological pathways underlying the effect of shear on growth and toxin content have yet to be understood.

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