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Net growth of the bloom-forming dinoflagellate *Heterocapsa triquetra* and pH: why turbulence matters

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ABSTRACT: The effect of turbulence on pH and the proliferation of the phototrophic dinoflagellate *Heterocapsa triquetra* was monitored for 10 d. Four turbulent energy dissipation rates ranging from 0.0001 to 1 $cm^2 s^{-3}$ were employed. The net growth rate of *H. triquetra* was on average 0.42 d^{-1} at all turbulence levels, as long as cell densities were low and pH stayed below 8.9. When cell densities increased and the pH exceeded 9.0, the net growth rate of *H. triquetra* decreased in all cases. However, at high cell densities, the pH and net growth rate of *H. triquetra* depended on the turbulence level. At the highest turbulence level, the net growth rate of *H. triquetra* was higher and the pH lower than at the lowest turbulence level, because turbulence increased the exchange of CO_2 between the medium and the atmospheric air. At the second highest turbulence level, the net growth rate of *H. triquetra* was also higher than the net growth rate at lower turbulence; this was likely to be due to a higher influx of inorganic carbon, even though pH measurements from the middle of the light period did not differ significantly among turbulence levels. However, during the night, pH decreased more at the second highest turbulence level than at the 2 lowest turbulence levels and thereby allowed a higher net growth rate of *H. triquetra*. Our results suggested that when forming blooms in eutrophic waters under conditions of high pH, *H. triquetra* will benefit from turbulent energy dissipation rates between 0.05 and 1 $cm^2 s^{-3}$.

KEY WORDS: Turbulence · pH · Growth · Phytoplankton blooms · Dinoflagellates · *Heterocapsa triquetra*

INTRODUCTION

Marine surface waters contain high concentrations of inorganic carbon (~2.2 mM) that buffer the pH of seawater; consequently, pH is usually constant at around 8.2 in the sea. Nevertheless, a number of biological and physical processes may influence the pH of marine surface waters. Uptake of inorganic carbon by phytoplankton during photosynthesis may increase pH, while release of CO_2 through respiration processes may decrease pH. In the open ocean pH is quite stable because, in general, the biomass of phototrophic and heterotrophic organisms is relatively low. In contrast, in eutrophic estuaries, embayments and in coastal lagoons, where primary productivity is much higher, pH may rise to very high levels during the summer and reach values as high as 9 and even 9.5 (Macedo et al. 2001, Hansen 2002, Hinga 2002). Even in more open waters, e.g. the North Sea, the pH may increase to 8.7 during algal blooms (Brussard et al. 1996). Although the occurrence of high pH in marine waters is not uncommon, pH has generally not been considered an important determinant of pelagic processes, and discussions about the possible effect of pH on the growth and succession of marine phytoplankton are sparse. However, recent studies have indicated that pH can have an effect on phytoplankton growth and that this effect drives species succession (Schmidt & Hansen 2001, Pedersen & Hansen 2003, Lundholm et al. 2005). Some phytoplankton species are quite sensitive to high pH and stop growing as soon as pH reaches 8.5, whereas others can grow even at pH 10.

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Excursions of pH in marine waters are counteracted by the physical exchange of CO_2 between the atmosphere and surface water. The exchange of CO_2 at the air/water transition is highly dependent on vertical mixing of the water column (Hansen 2002, Hinga 2002). Vertical mixing driven by e.g. wind creates turbulence in the water column that may affect (positively or negatively) the growth of marine planktonic algae.

Among planktonic algae, dinoflagellates have shown strong negative net population growth rates in response to turbulence in laboratory experiments (White 1976, Pollingher & Zemel 1981, Estrada & Berdalet 1988). The turbulence levels in these studies were considerably higher than those typically found in the ocean (evaluated by Peters & Marrasé 2000). Typical turbulent energy dissipation rates in the ocean (ε) are between 10^{-6} and 1 cm^2 s^{-3} (Kiørboe & Saiz 1995 and references therein). Later investigations that used natural levels of turbulence ($\varepsilon \leq 1$ cm^2 s^{-3}) also reported negative effects on the net growth of a few dinoflagellate species (Juhl et al. 2000, 2001, Zirbel et al. 2000). Recently, however, the view of dinoflagellates as a turbulence sensitive group was challenged experimentally by Sullivan & Swift (2003), who discovered that the net growth of 7 out of 10 dinoflagellate species was not affected or was even stimulated by turbulence at natural levels ($\varepsilon \leq 1$ cm^2 s^{-3}).

This raises the question of how turbulence affects the growth of bloom-forming dinoflagellates, which tend to dominate the phytoplankton in some eutrophic coastal waters. Will turbulence negatively affect the proliferation of these dinoflagellates in these environments because they are sensitive to turbulence? Or will turbulence promote the proliferation of these dinoflagellates when their growth is limited by pH, because turbulence will decrease the pH of the water due to draw down of CO_2?

The aim of the present study was to test whether (1) a common, bloom-forming dinoflagellate was sensitive to natural levels of turbulence when its growth was not limited by pH, and (2) natural levels of turbulence could counteract the effect of high pH on the net growth rate at high cell densities, by decreasing pH in the seawater and thereby allowing the dinoflagellate to grow faster. The photosynthetic dinoflagellate *Heterocapsa triquetra* was selected because it is a common bloom-forming species in eutrophic fjords characterized by high pH during summer (Fenchel et al. 1995, Hansen 2002).

MATERIALS AND METHODS

Cultures. A unialgal culture of the phototrophic dinoflagellate *Heterocasa triquetra* (Ehrenberg) Stein 1883 (clone K-0481) was provided by the culture collection of the Marine Biological Laboratory, Helsingør, Denmark. The species originates from the Kattegat, Denmark, and was isolated in 1988 by G. Hansen. Cultures were grown in f/2 medium (Guillard 1983) at 15 ± 1°C, at an irradiance of 90 µmol photons m^{-2} s^{-1}, and followed a light:dark cycle of 16:8 h. *H. triquetra* was kept in batch cultures that were reinoculated every week into f/2 medium (dilution 1:10) to keep them in an active growth phase prior to experiments.

Generation of turbulence. The oscillating grid device used to generate different levels of turbulence was an exact copy of the one described by Dolan et al. (2003). The grids were made of stainless steel coated with a plastic polyamide, had a diameter of 12.9 cm, and a mesh size of 1.42 cm. Vertically oscillating movements in eight 2 l containers made of Plexiglas were provided by 4 independently controlled motors, each attached to 2 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 4 different turbulence levels (T1, T2, T3, and T4) with average kinetic energy dissipation rates (ε) of 0.0001, 0.005, 0.05, and 1 cm^2 s^{-3}, respectively (calculated according to Peters & Gross 1994). These correspond to naturally occurring turbulence levels in the upper 10 m of the ocean that can be generated by e.g. almost no wind, moderate breezes, moderate gales, and storms, respectively (MacKenzie & Leggett 1993, Kiørboe & Saiz 1995).

Determination of growth and pH. Samples for enumeration were taken every day in the middle of the light period. During the diurnal investigation, samples were taken every hour. Samples were taken after mixing the water column by gently turning the containers upside down 10 times. Organisms were fixed with Lugol's solution (final concentration 10%) and settled in 1 ml chambers (Sedgewick). At least 100 cells were counted in every sample, and densities were always determined in triplicates using an Olympus inverted microscope with 40× magnification (Utermohl 1958). Instantaneous growth rates were determined in steps of 24 h as $\mu = [\ln(y_{t_1} \times y_{t_0}^{-1})]t^{-1}$, where y_{t_0} = densities of cells at Day t_0 (cells ml^{-1}), y_{t_1} = densities of cells at Day t_1 (cells ml^{-1}), and t = duration of each experiment (d). The pH of the medium was monitored every day when the samples for enumeration were taken using a Senton 2001 pH system equipped with an ISFET pH electrode.

Expt 1. Growth of *Heterocapsa triquetra* (initial density 3.5×10^3 cells ml^{-1}) and pH were measured in 4 containers exposed to high (T4) and 4 containers exposed to low (T1) turbulence (ε = 1 and 0.0001 cm^2 s^{-3}, respectively) over 10 d. Cell dimensions of 100 *H.*

triquetra cells were measured on Day 3 and Day 7, and cell volume was calculated.

Expt 2. Growth of *Heterocapsa triquetra* and pH were measured in containers exposed to 3 different turbulence levels (T1: $\varepsilon = 0.0001$ cm^2 s^{-3}, T2: $\varepsilon = 0.005$ cm^2 s^{-3}, T3: $\varepsilon = 0.05$ cm^2 s^{-3}) over 10 d. Cultures in 6 containers (2 for each turbulence level) were investigated simultaneously (Expt 2A); this experiment was subsequently repeated (Expt 2B). Expts 2A and 2B were both initiated with 3.3×10^3 cells ml^{-1}. In Expt 2B, the diurnal variation in pH was studied by measuring pH every hour between Day 8 and Day 9.

At the end of Expt 2B culture samples were taken for cell carbon analyses. For each turbulence level, 4 replicates of 50 ml from 1 container were filtered onto precombusted GF/C glass fibre filters to determine the cellular carbon content of *Heterocapsa triquetra*. Because the cultures of *H. triquetra* were not bacteria-free, we also measured the carbon content of a similar quantity of cultures that had passed a nitrocellulose filter with a pore size of 8 µm. Microscopic examination confirmed that no *H. triquetra* cells passed through such a filter. Non-algal carbon was subtracted from total carbon, and cell carbon content of *H. triquetra* was calculated from the average cell density of *H. triquetra* in each sample. Organic carbon was measured using a CHN-analyser (Fisons Instrument NA1500NC).

Statistical analyses. The effect of the level of turbulence on growth of *Heterocapsa triquetra* above and below pH 9, on cell carbon content above pH 9, and on pH during the diurnal investigation was tested using a 1-way ANOVA. When there was a significant difference in growth, an all pairwise comparison procedure (Tukey test) was run on data from Expt 2. The effect of high and low turbulence in Expt 1 on cell volume of *H. triquetera* above and below pH 9 was tested using a Kruskal-Wallis 1-way ANOVA on ranks. For these statistical tests the software package SigmaStat was used. In addition, 1-way ANCOVA tested whether the pH at a given cell density of *Heterocapsa triquetra* depended on the level of turbulence, using turbulence as the independent variable and cell density as the covariate (STATISTICA software). For each turbulence level the 4 replicates had equal variances and were pooled.

RESULTS

Expt 1

The net growth of *Heterocapsa triquetra* was on average 0.42 d^{-1} until Day 5, without significant differences between high (T4: $\varepsilon = 1$ cm^2 s^{-3}) and low turbulence (T1: $\varepsilon = 0.0001$ cm^2 s^{-3}) ($p = 0.914$, Fig. 1). Up until Day 4, pH stayed below 8.9 at both turbulence levels, and on Day 5, pH reached 8.9 to 9.0 (Fig. 1). When cell densities exceeded 3×10^4 ml^{-1}, between Day 6 and Day 10, the net growth of *H. triquetra* was on average 0.17 d^{-1} when exposed to high turbulence. This net growth rate was significantly higher than the average net growth at low turbulence, which was on average

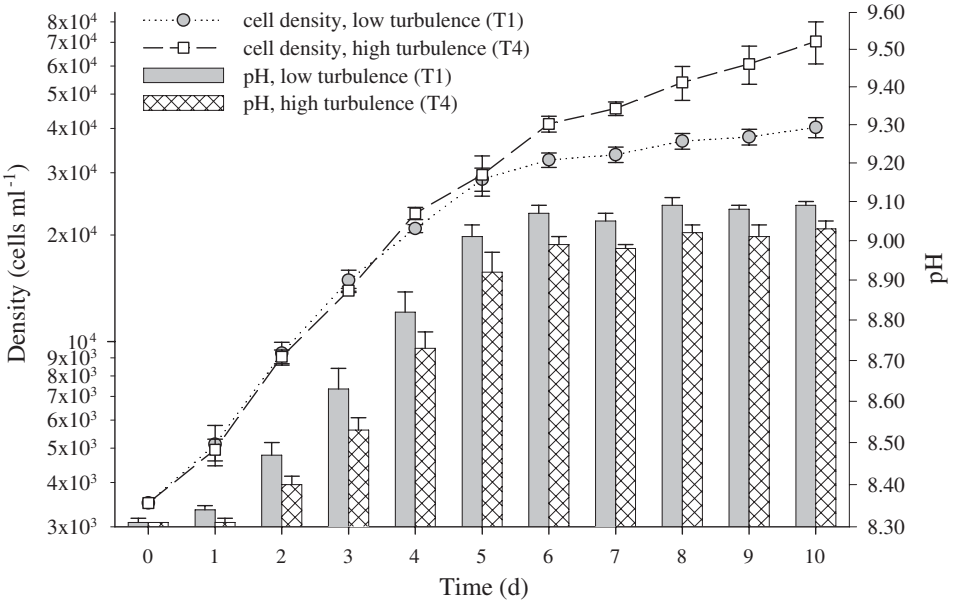

Fig. 1. *Heterocapsa triquetra*. Net growth (cell density) and pH over time at high (T4) and low (T1) turbulence ($\varepsilon = 1$ and 0.0001 cm^2 s^{-3}, respectively). Error bars = SD among 4 replicates

0.07 d^{-1} ($p = 0.006$, Fig. 1). Judged from the coloration of the cultures at cell densities $>3 \times 10^4$ cells ml^{-1}, cells were homogeneously distributed at both turbulence levels and did not settle in response to high turbulence ($\varepsilon = 1$ cm^2 s^{-3}) as observed for the dinoflagellate *Ceratium tripos* (Havskum et al. 2005). Throughout the experiment the pH at high turbulence was significantly lower than at low turbulence ($p = 0.041$, Fig. 1). No significant difference in cell volume (approximately 3000 μm^3) was observed between cultures exposed to high or low turbulence, or to pH values in the range 8.53 to 8.63 or 8.98 to 9.05 ($p = 0.147$, Fig. 2).

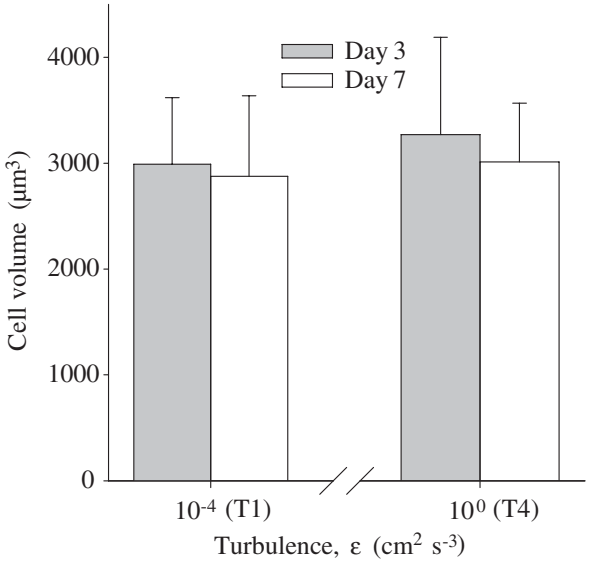

Fig. 2. *Heterocapsa triquetra.* Cell volume when exposed to high (T4) and low (T1) turbulence ($\varepsilon = 1$ and 0.0001 cm^2 s^{-3}, respectively). Error bars = SD among 100 measurements

Expt 2

The net growth of *Heterocapsa triquetra* was on average 0.41 to 0.42 d^{-1} until Day 5, without significant differences among turbulence level T1 ($\varepsilon = 0.0001$ cm^2 s^{-3}), T2 ($\varepsilon = 0.005$ cm^2 s^{-3}), or T3 ($\varepsilon = 0.05$ cm^2 s^{-3}) ($p = 0.909$, Fig. 3). No significant difference in pH at a given cell density was found among the 3 turbulence levels (Days 0 to 5: $p = 0.163$, Fig. 3). When cell densities exceeded 3×10^4 ml^{-1}, the net growth of *H. triquetra* from Day 6 to Day 10 at T1 and T2 was on average 0.07 d^{-1} and did not differ significantly between these low turbulence levels ($p = 0.992$, Fig. 3). The net growth rate at T3 was on average 0.13 d^{-1}, significantly higher than at the 2 lower turbulence levels ($p = 0.008$, Fig. 3). Compared to the net growth rate at T4 in Expt 1 (0.17 d^{-1}), no significant difference between T3 and T4 was found ($p = 0.149$). In the middle of the light period, when daily samples were taken, no difference was found in pH among cultures exposed to the 3 turbulence levels (Days 6 to 10: $p = 0.759$, Fig. 3).

Hourly measurements of pH during a 24 h period showed that pH at T3 decreased by 0.20 pH units

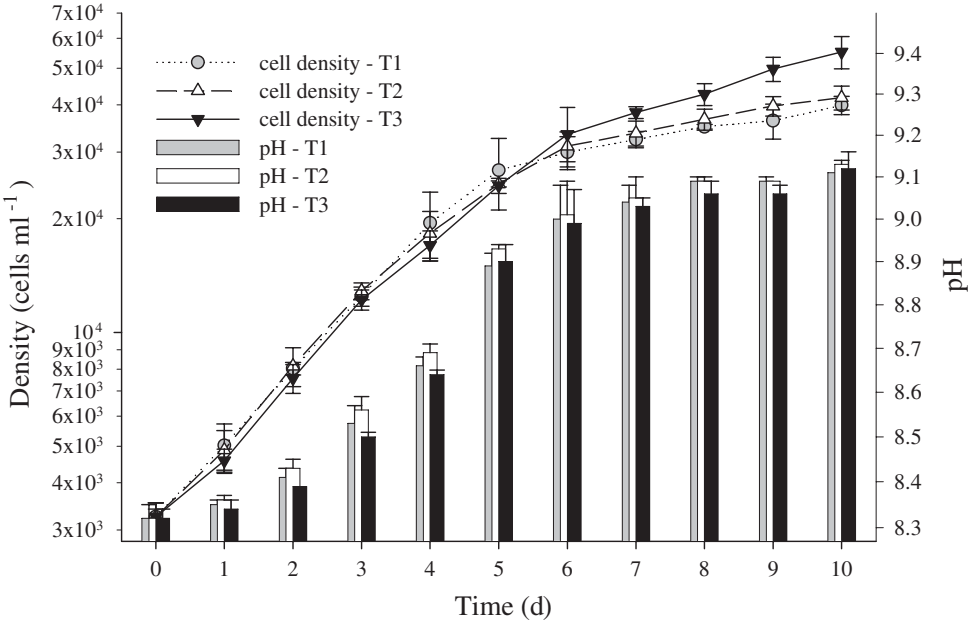

Fig. 3. *Heterocapsa triquetra.* Growth and pH at 3 turbulence levels (T1: $\varepsilon = 0.0001$ cm^2 s^{-3}, T2: $\varepsilon = 0.005$ cm^2 s^{-3}, T3: $\varepsilon = 0.05$ cm^2 s^{-3}). Error bars = SD among 4 replicates

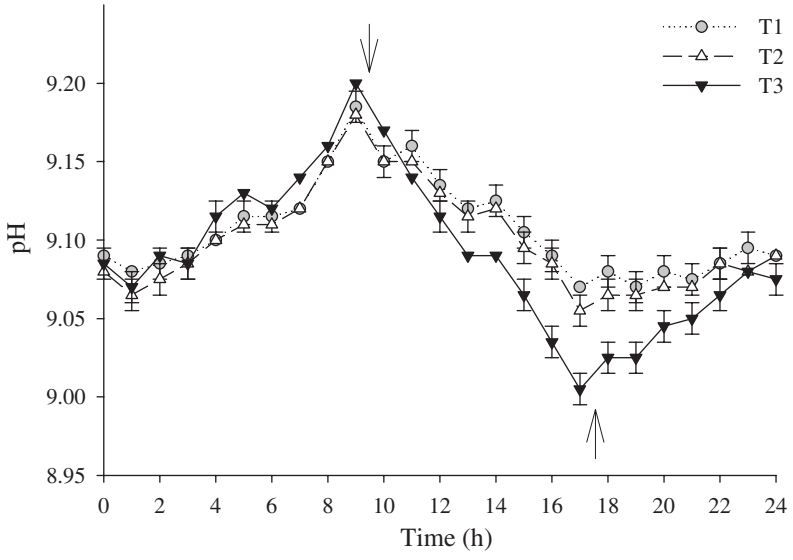

Fig. 4. *Heterocapsa triquetra.* Diurnal response of pH when dinoflagellates were exposed to 3 turbulence levels (T1: ε = 0.0001 cm^2 s^{-3}, T2: ε = 0.005 cm^2 s^{-3}, T3: ε = 0.05 cm^2 s^{-3}); measurements lasted 24 h from Day 8 to Day 9. Arrows represent start (left) and end (right) point of dark period. Error bars = SD between duplicates

during the night, whereas it only decreased by 0.12 pH units at the 2 lower turbulence levels (Fig. 4). At 0.5 to 1.5 h before the light was switched on, pH was significantly lower at T3 than at T1 and T2 ($p = 0.002$), whereas no significant difference in pH was found between T1 and T2 ($p = 0.694$, Fig. 4). During the first 5 h after the light was switched on, pH remained significantly lower at T3 than at T1 and T2 ($p = 0.001$), whereas no significant difference in pH was observed between T1 and T2 ($p = 0.612$, Fig. 4). Cell carbon measurements made at the end of the experimental period showed that the cell carbon content was approximately 400 pg C cell^{-1} at the 3 turbulence levels, with no significant differences observed ($p = 0.097$, Fig. 5).

DISCUSSION

Our study showed that growth of the phototrophic dinoflagellate *Heterocapsa triquetra* was not influenced by average turbulent energy dissipation rates of up to ε = 1 cm^2 s^{-3}, as long as cell densities stayed below 3×10^4 cells ml^{-1} and where pH was too low to affect growth (Figs. 1 & 3). We also found that neither the cell volume nor the carbon content of *H. triquetra* was affected by average turbulent levels up to ε = 1 cm^2 s^{-3}, as created by vertically oscillating grids (Figs. 2 & 5). In contrast, Yeung & Wong (2003) found a transient cell cycle arrest and an increase in cell volume when *H. triquetra* was exposed to 150 rpm for 72 h in an orbital shaker. Yeung & Wong (2003) referred to the study of Zirbel (2000), who estimated that a similar setup at 120 rpm produced an average dissipation rate between 0.1 and 1 cm^2 s^{-3}. Estimated intensities in these setups are, however, average values. In containers with oscillating grids, values are highest just after the grid passage and decrease until the next grid passage. In an orbital shaker, Zirbel (2000) measured lowest values to be between the center and the wall, with highest values along the wall. Despite similar average values, variation in turbulent energy dissipation rates may be different in different setups and cause contrasting results.

Contrasting results for the same species in different setups have been reported previously. For example, White (1976) reported that cell division was completely inhibited in the dinoflagellate *Alexandrium tamarense* when incubated in an orbital shaker at 125 rpm, whereas Sullivan & Swift (2003) reported that *A. tamarense* was unaffected by high turbulence (ε = 1 cm^2 s^{-3}) created by vertically oscillating rods. Thomas et al. (1997) and Peters & Marrasé (2000) estimated that the orbital shaker employed by White (1976) at 125 rpm produced much higher levels of tur-

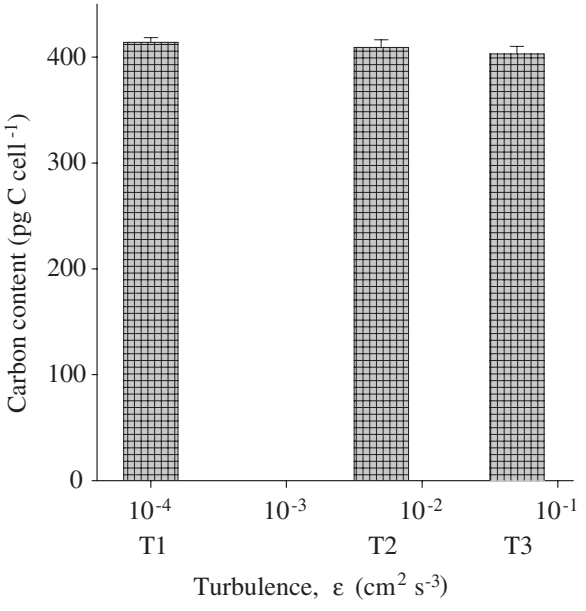

Fig. 5. *Heterocapsa triquetra.* Cell carbon content after exposure to 3 turbulence levels for 10 d (T1: ε = 0.0001 cm^2 s^{-3}, T2: ε = 0.005 cm^2 s^{-3}, T3: ε = 0.05 cm^2 s^{-3}). Error bars = SD among 4 replicate measurements

bulence than $\varepsilon = 1$ cm^2 s^{-3}. Although no negative effects of turbulence on net growth of *Heterocapsa triquetra* were observed in our study at low cell densities ($<3 \times 10^4$ cells ml^{-1}), the 2 highest average turbulence levels ($\varepsilon = 0.05$ cm^2 s^{-3} and $\varepsilon = 1$ cm^2 s^{-3}) were high enough to reduce pH at high cell densities ($>3 \times 10^4$ cells ml^{-1}) and thereby affected the net growth rate (see below).

When planktonic algae are grown in a nutrient rich medium like the f/2-medium that we used, the growth of the algae will become limited by pH and not by nutrients, trace metals or vitamins (see Hansen [2002] and Lundholm et al. [2004] for a discussion on the subject). In our study, the net growth of *Heterocapsa triquetra* started to deviate from the exponential growth phase when pH exceeded approximately 8.9 to 9.0, independent of the turbulence level (Figs. 1 & 3). This result was in agreement with the results obtained by Hansen (2002), who found that the exponential net growth of *H. triquetra* was affected (by more than 20%) when pH exceeded 9.0, and that net growth stopped when pH reached 9.45. *Heterocapsa triquetra* is mainly a HCO_3^- user, with HCO_3^- uptake contributing 80 to 90% to inorganic carbon uptake at both pH 8 and at pH 9.1 (Rost et al. 2005). Seawater normally contains 2.2 mM dissolved inorganic carbon (Mann & Lazier 1991, Thomas & Schneider 1999). At pH 9.3 half of the available inorganic carbon pool is in the form of HCO_3^-. Such high concentrations of HCO_3^- are most likely not limiting for the growth of *H. triquetra*, because *H. triquetra* at high pH increases its uptake rate of HCO_3^- (Rost et al. 2005). Only below 0.2 mM HCO_3^- is the photosynthetic rate affected (Rost et al. 2005). It therefore seems likely that *H. triquetra* is mainly limited by pH itself, both in our experiments and in nature.

The first of our set of experiments, in which *Heterocapsa triquetra* was exposed to high (T4, $\varepsilon = 1$ cm^2 s^{-3}) and low (T1, $\varepsilon = 0.0001$ cm^2 s^{-3}) turbulence levels, showed that pH increased at both levels when cell densities increased (Fig. 1). However, the pH was consistently lower at all sampling dates at the high turbulence level compared to pH at the low turbulence level, even though cell densities were equal to each other on Day 0 to 5 or even twice as high on Day 10 at the high turbulence level. As expected, this indicated an increased influx of CO_2 from the air at the high turbulence level. The higher growth rate observed at the high turbulence level from Day 6 to Day 10 was potentially due to the lower pH found at this level.

How then to explain the differences observed in the second experiment? Here, we observed a higher net growth rate at T3 ($\varepsilon = 0.05$ cm^2 s^{-3}) compared to those obtained at lower turbulence levels (T1 and T2) when pH exceeded 9, even though pH measurements from the middle of the light period did not differ significantly among turbulence levels. (Fig. 3). Since the cell volume and cellular carbon contents of *Heterocapsa triquetra* were independent of the level of turbulence (Figs. 2 & 5), the influx of CO_2 must have been higher at T3 compared to the influx at the 2 lower turbulence levels. This was supported by results obtained from the experiment on diurnal variations in pH at different turbulence levels (Fig. 4). Here, the pH decreased during the dark period by 0.20 pH units at T3, whereas it only decreased 0.12 pH units at the 2 lower turbulence levels (T1 and T2). At the onset of light, the pH in the *H. triquetra* culture at T3 was significantly lower than at the lower turbulence levels during the first 5 h, which enabled *H. triquetra* to grow faster during this period.

When pH reached an almost constant level (Days 6 to 10 in our experiments), a rough calculation of the net influx of inorganic carbon at the different turbulence levels could be made using the net growth rates and cellular carbon contents at the individual turbulence levels. The net growth rate of *Heterocapsa triquetra* decreased from 0.42 d^{-1} to 0.07 d^{-1} at T1 and T2, and to 0.13 and 0.17 d^{-1} at T3 and T4, respectively (Figs. 1 & 3); however, the difference between T3 and T4 was not significant. Since the average cell carbon was 400 pg C cell^{-1} at all turbulence levels (Fig. 5), the carbon made available for growth by influx of inorganic carbon from the air was 1.16 ng C ml^{-1} d^{-1} at T1 and T2, 2.22 ng C ml^{-1} d^{-1}at T3, and 2.96 ng C ml^{-1} d^{-1} at T4 (data not shown) at a cell density of 4×10^4 cells ml^{-1}. Since the growth rate remained constant when pH reached an almost constant level (Figs. 1 & 3), our data indicated that the draw down of CO_2 at a given turbulence level increased with increasing cell densities. Therefore, the draw down of CO_2 seemed to be dependent on both turbulence level and cell density.

Juhl et al. (2000) studied the effect of turbulence on net growth of the dinoflagellate *Lingulodinium polyedrum*. Late exponential phase cultures of this species exhibited much greater reductions in net growth than early exponential phase cultures when compared to still controls. In our experiments, late exponential growth cultures of *Heterocapsa triquetra* displayed reduced net growth rates at all turbulence levels tested because of the growth limiting effect of high pH. However, at the 2 highest turbulence levels ($\varepsilon = 0.05$ cm^2 s^{-3} and $\varepsilon = 1$ cm^2 s^{-3}), the reduction was lower than at the 2 lowest turbulence levels because of the counteracting effect of turbulence on pH.

In our study, the threshold for an effect of turbulence on pH at high cell densities and consequently on net growth of *Heterocapsa triquetra* was an average dissipation rate of 0.05 cm^2 s^{-3}. This average value is generated in the upper 10 m of the ocean by a wind speed of 15 m s^{-1} (moderate gale) (Kiørboe & Saiz 1995). Aver-

age dissipation rates are higher in the surface-wave zone. At a depth of 0.5 m, Veron & Melville (1999) observed a dissipation rate of $\varepsilon = 0.1$ cm^2 s^{-3} at a wind speed of 6.7 m s^{-1} (moderate breeze).

In nature, dinoflagellate blooms often occur in enclosed eutrophic waters characterized by high nutrient input and a long turnover time of the euphotic surface layer. During these bloom events, pH may become quite high (up to pH 9.75) and potentially be a factor that causes species succession and reduced production (Hansen 2002, Hinga 2002). *Heterocapsa triquetra* is a common bloom-forming dinoflagellate in eutrophic coastal waters (Fenchel et al. 1995, Lindholm & Nummelin 1999, Litaker et al. 2002). It is fast-growing (up to 1 doubling d^{-1}) and can sustain growth up to quite high pH values (pH 9.45), even though growth becomes limited at pH values above 8.9 or 9. The finding that average turbulent energy dissipation rates up to 1 cm^2 s^{-3} do not negatively affect the net growth of *H. triquetra* is important. During blooms under high pH conditions (above 9), *H. triquetra* potentially benefits from turbulent periods through increased draw down of CO_2 from the air to the surface water, which may lead to increased net growth.

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