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Effects of small-scale turbulence on the growth of two diatoms of different size in a phosphorus-limited medium

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Abstract

The effect of turbulence on the nutrient flux towards osmotrophic cells is predicted to be size dependent. This should translate into growth. We experimentally followed and modelled the growth of two marine diatoms of different size (*Thalassiosira pseudonana*, 6 μm in diameter and *Coscinodiscus* sp., ca. 109 μm in diameter) under still water and turbulent conditions, using a shaker table. Experiments were done with phosphorus-limited cultures and lasted for ca. 5 days. Turbulence enhanced the growth of *Coscinodiscus* sp. in agreement with theory but not the growth of *T. pseudonana*, which was actually slightly lower under turbulence. At the end of the experiments there were about 1.7 times as many *Coscinodiscus* sp. cells in the turbulent treatment than in the still treatment, while for *T. pseudonana* almost the same cell concentration was found in both conditions. In addition, the *Coscinodiscus* sp. cells growing under still conditions presented a higher specific alkaline phosphatase activity than those growing in turbulence which indicates a higher need for phosphorus in the still cultures. A simple dynamic model, based on Michaelis–Menten nutrient uptake kinetics, needed nearly no optimisation other than using observed initial conditions of phosphate and cell concentrations. The model showed how an increased nutrient flux towards the cells translates non-linearly into cell growth, most likely by affecting the half-saturation constant (K_M). However, since *Coscinodiscus* sp. experienced significant mortality and cells partially settled to the bottom of the containers, unequivocal support for the size-dependent effect of turbulence on nutrient uptake will require further experiments and more sophisticated modelling. The mechanisms to connect an increased nutrient flux towards cells with population growth and whether this process is size dependent are important in parameterizing the effects of turbulence on marine plankton in coupled physical–biological models.
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Keywords: Turbulence; Diatom growth; Size; Nutrient uptake; Phosphorus affinity; Half-saturation constant; Maximum uptake velocity

1. Introduction

In nutrient-limited ecosystems the interaction between small-scale turbulence, phytoplankton cells and nutrients is important to understanding the dynamics of the whole ecosystem. Nutrients are transported to cells by diffusion or advection. In still water and for non-motile cells, the nutrient supply is by diffusion alone. When the nutrient uptake capacity of these cells is higher than the diffusive flux, the cells become diffusion-limited and a nutrient-depleted region is created around them (Kiørboe, 1993). Relative motion of the cells with respect to the fluid, either by swimming or sinking or by laminar or turbulent movement of the water, will generate an advective transport of nutrients to renew the depleted zone. The relative importance of the two nutrient transport mechanisms is given by the Sherwood

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number (*Sh*), which is the ratio between the total flux of nutrients arriving to the cell surface and the transport of nutrients by diffusion alone. If the advective transport is zero (*Sh* = 1), the nutrient transport is purely diffusive. The Sherwood number increases with cell size and thus, when relative motion between the fluid and the cells is present, large cells experience a larger increase in nutrient flux to their cell surface than small cells (Karp-Boss et al., 1996). These authors concluded that the increase of advective transport owing to small-scale turbulence under normal oceanic conditions is only significant for cells larger than ca. 60 μm.

The different components of a phytoplankton community compete for the limiting nutrient. Under still conditions, the smallest organisms should be better competitors for the limiting nutrient due to their high surface to volume ratio. In addition, motile organisms could have an advantage in calm waters by increasing nutrient flux through swimming. However, under turbulent conditions, the increase in the nutrient flux due to turbulence would mainly benefit the growth of the largest cells. Thus, turbulence could change the competition interactions of the different components of the phytoplankton community. This could have important implications in the trophic ecology of the whole ecosystem, as turbulence would favour the "classical" food web over a more microbial-based food web (Legendre and Rassoulzadegan, 1995) and this is starting to be addressed in ecosystem models (Metcalfe et al., 2004). In nature, the presence of particular life forms of phytoplankton is related to the physico-chemical conditions of the medium (Margalef, 1978). In upwelling areas, with high turbulent mixing and high nutrient concentrations, diatoms are usually the main component of the phytoplankton community and may achieve high biomass. Most diatoms are non-motile and thus may be favoured by the enhancement of nutrient flux into the cells produced by the relative movement of water with respect to them. There are few experimental studies that have assessed the effect of fluid motion on nutrient uptake and growth of diatoms. Savidge (1981) found mixed results in the nutrient uptake of the diatom *Phaeodactylum tricornutum* depending on whether the cultures were phosphate or nitrate limited. This author observed that, under turbulence, the uptake of nitrate was enhanced while that of phosphate was decreased. For *Ditylum brightwellii* growing in a nitrogen-limited medium, Pasciak and Gavis (1975) found that the transport limitation of nitrate and nitrite decreased with turbulence. Although theoretical approaches conclude that turbulence effects are only significant for cell sizes exceeding several tens of micrometers (Mann and Lazier, 1991; Karp-Boss et al., 1996), no experimental work has been done assessing the effect of small-scale turbulence on the growth of different sized diatoms. This knowledge may be key to understand diatom bloom formations in marine ecosystems and a factor to be considered in the parameterizations of phytoplankton growth models.

Changes in Sherwood numbers need to be incorporated into a nutrient uptake model in order to evaluate their effect on cell growth. The most widely used nutrient-uptake model for algae is that of Michaelis–Menten (Fig. 1) where the uptake velocity (V, time^{-1}) for a certain dissolved substrate concentration (S) is characterized by a maximum uptake velocity (V_{max}) and a half-saturation constant (K_M) as:

$$V = V_{max}S/(S + K_M) \quad (1)$$

In this formulation, the increased nutrient flux through turbulence must either increase V_{max} or decrease K_M. It

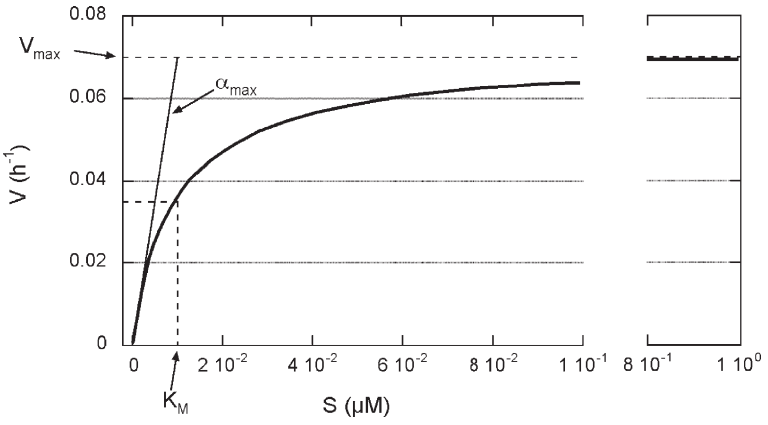

Fig. 1. Nutrient uptake velocity as a function of nutrient (substrate) concentration for a V_{max} of 0.07 h^{-1} and a K_M of 10^{-2} μM.

has been argued that while K_M depends on both biological and physical parameters, V_{max} should only depend on the number of uptake sites on the cell surface, a biological parameter (Aksnes and Egge, 1991). As far as we know, this has not been proven.

The ratio of V_{max} and K_M defines the maximum affinity (α_{max}):

$$\alpha_{max} = V_{max}/K_M \quad (2)$$

which can be calculated from theoretical grounds (Thingstad and Rassoulzadegan, 1999) and serves as a comparison for field measurements.

In the present study, we investigate the effect of small-scale turbulence on the growth of selected marine diatoms. Our approach mixes laboratory experiments in batch cultures with dynamic modelling. Our main interest is to test whether the turbulence effect depends on cell size. Two diatoms, *T. pseudonana* and *Coscinodiscus* sp., were chosen because they are similar in shape and other characteristics (both are centric diatoms and belong to the Order Biddulphiales, suborder Coscinodiscineae), but are very different in size. *T. pseudonana* has a diameter of around 6 μm and *Coscinodiscus* sp. is approximately twenty times larger (ca. 109 μm in diameter). The growth of both algae was monitored in a phosphorus-limited medium under still and turbulent conditions.

2. Material and methods

2.1. Experimental setup

We used batch cultures of the diatoms *T. pseudonana* (original strain from Instituto de Ciencias del Mar de Andalucía–Puerto Real–Cádiz) and *Coscinodiscus* sp. (CCMP1584 strain from Provasoli-Guillard National Center for Culture of Marine Phytoplankton–Bigelow Laboratory for Ocean Sciences). These diatoms are regularly maintained in the culture collection of our laboratory growing in f/2 or f/20 media with silicate (Guillard, 1975). Small *Thalassiosira* species and *Coscinodiscus* sp. are normally found in NW Mediterranean waters (Estrada, 1991; Gómez and Gorsky, 2003).

For this study, we used a phosphorus-limited medium for both stock cultures and experiments. It was prepared by adding known quantities of sterile stocks of inorganic nutrients (as nitrate, phosphate, silicate, metals and vitamins) and TRIS buffer to nutrient-depleted, aged, sea water, after filtering through Whatman GF/F and sterilizing at 121 °C for 50 min. Nutrient-depleted water was obtained from open ocean Mediterranean surface water. The medium was adjusted to have a phosphorus concentration of 1 μM and an N/P ratio of 88.2. Metals and vitamins were added in the same proportion with respect to nitrate as in the f/2 medium. The choice of phosphorus as the limiting nutrient was based on studies in the Mediterranean (Thingstad et al., 1998; Sala et al., 2002; Krom et al., 2004) indicating a phosphorus-limitation at least for surface waters and/or for some times of the year.

Two experiments were carried out (Experiment 1 and Experiment 2) with independent stock cultures. At the beginning of an experiment, known volumes of either *T. pseudonana* or *Coscinodiscus* sp. were inoculated into four 2.5 l Nalgene polycarbonate bottles to achieve starting concentrations of 0.037 μM P in algal biomass. This biomass was calculated from algal cell volumes (Table 1) and average literature values of cell carbon content (Mullin et al., 1966, Blasco et al., 1982, Brzezinski, 1985; Moal et al., 1987; Montagnes et al., 1994) and Redfield C/P ratio (Redfield et al., 1963). Theoretical initial concentrations resulted in 3200 cells ml^{-1} for *T. pseudonana* and 3.35 cells ml^{-1} for *Coscinodiscus* sp. Measured initial cell numbers resulted, on average, in 3118 cells ml^{-1} for *T. pseudonana* and 7.0 cells ml^{-1} for *Coscinodiscus* sp. Experiments were run in an environmental chamber at a temperature of 22 ± 1 °C and under a 12:12 h light/dark period with a light intensity between 180 and 220 μmol photons m^{-2} s^{-1}. Two bottles of each species were grown under still conditions and the other two bottles were grown under turbulent conditions. Turbulence was

Table 1
Parameter values in model runs

Parameter	Units	*T. pseudonana*	*Coscinodiscus* sp.
Cell size (diameter)	μm	5.87	109
Cell volume	μm^3	106	509 871
P δ	cm^2 s^{-1}	6.12×10^{-6}	6.12×10^{-6}
Viscosity	cm^2 s^{-1}	10^{-2}	10^{-2}
Turbulent kinetic energy dissipation rate	cm^2 s^{-3}	0 and 10 [a]	0 and 10 [a]
Sh (under turbulence)	–	1.17	2.94
V_{max} [a]	h^{-1}	0.0806	0.0112 (0.025) [b]
K_M [a]	μM P	3.9×10^{-3}	2.5×10^{-1}
P α_{max} [a]	l μmol P^{-1} h^{-1}	20.6	0.045 (0.1) [b]
C/P	mol C mol P^{-1}	253	106
Carbon content	fg C μm^{-3}	113	63.5

[a] Nominal values under still water conditions; turbulence may affect V_{max} or K_M and consequently α_{max}.
[b] Values in parenthesis are optimized parameters.

generated with an orbital shaker at 130 rpm. We estimated turbulence from the power spectrum of 5 min. time series of particle velocities (5 μm hollow glass spheres). Three-dimensional particle velocities were measured using acoustic Doppler velocimetry with a custom-made side-looking probe. The turbulent kinetic energy dissipation rate was computed from averaging all three dimensions, giving an estimate of 10 $cm^2\ s^{-3}$. This value was used in model runs.

2.2. Measurements

Samples for algal and bacterial cell numbers and inorganic nutrient concentration were taken at the beginning of the experiment and four or five times more during the experiment (which lasted for ca. 5 days). Cell numbers and biovolume of *T. pseudonana* were monitored with a Multisizer Coulter Counter. *Coscinodiscus* sp. cells were fixed with Lugol's solution and counted each day using 10-ml settling chambers in an inverted microscope (Utermöhl, 1958). The mean volume of these cells was obtained by measuring the diameter and height of twenty live cells and applying the formula of a cylinder. The specific growth rate (μ) was obtained from non-linear regression (quasi-Newton algorithm) of the logistic equation for *T. pseudonana* ($D=KD_0/(D_0+(K-D_0)e^{-\mu t})$), where D_0 is initial cell concentration and K is the carrying capacity. Since stationary phase was not reached for *Coscinodiscus* sp., μ was estimated from the linear regression of logarithmic transformed cell concentrations ($D=D_0e^{\mu t}$).

Bacterial concentration and cell volume was estimated by flow cytometry following a methodology described in Gasol and del Giorgio (2000). Samples of 1.2 ml were fixed with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration), left in the dark at room temperature for 10 min and then stored frozen at −70 °C. At a later date, samples were unfrozen and run through a FACSCalibur (Becton and Dickinson) flow cytometer with a laser emitting at 488 nm. A subsample of 200 μl was stained with Syto13 (Molecular Probes) at 1.6 μM (diluted in DMS), left in the dark for 15 min, and then run at low speed (approx. 12 $\mu l\ min^{-1}$). Data were acquired in log mode until 10 000 events had been processed. As an internal standard, 10 μl of a $10^6\ ml^{-1}$ solution of yellow-green 0.92 μm Polyscience latex beads were added to subsamples. Bacteria were detected by their signature in a plot of side scatter (SSC) vs. FL1 (green fluorescence).

Bacterial phosphate consumption during the period of bacterial exponential growth was calculated dividing the bacterial increase in this period in terms of phosphorus (using a carbon content of 0.35 pg C μm^{-3} (Bjørnsen, 1986) and a C/P ratio = 50 (Faggerbakke et al., 1996)) by the decrease in phosphate concentration during the same period.

Inorganic nutrients (phosphate, nitrate and silicate) were analyzed in an Evolution II (Alliance Instruments) autoanalyzer, using the methods of Grasshoff et al. (1983).

Alkaline phosphatase activity (APA) was determined by using a fluorogenic substrate (MUF) following the procedure described in Sala et al. (2001). In brief, 1-ml samples were incubated in replicates with the substrate 4-MUF-P-phosphate (100 μM final concentration). Fluorescence was measured at 365 nm excitation and 446 nm emission wavelengths before and after an incubation of 1 h in the dark at room temperature. The increase of fluorescence with time was converted to activity units using a standard curve prepared with the end product of the reaction. Specific activity, i.e. activity per cell, was calculated dividing activity by the number of algal cells in the culture. Bacterial contribution to APA was considered unimportant since the experiments were designed to maximize algal biomass and minimize bacterial biomass. The limitation on phosphate uptake was estimated by the increase of the specific alkaline phosphatase activity. The activity of this ectoenzyme has been used in different environments as an indicator of phosphorus limitation (Gage and Gorham, 1985; Li et al., 1998; Sala et al., 2001). A higher alkaline phosphatase activity in the cells is expected when the cells are phosphorus-limited.

2.3. Dynamic model

We set up a numerical model of diatom growth based on phosphorus limitation to simulate population growth in the experimental containers. The model was developed with the computer package Stella (Stella Research 6.0, High Performance Systems, Inc.). It consists of only two state variables, diatoms (D) and phosphorus (P), that were tracked over time with units of μM P. The instantaneous uptake of phosphorus by diatoms (F_{PD}) was computed as:

$$F_{PD} = D \cdot V_{max} \cdot P/(P + K_M). \quad (3)$$

Then the diatom mass at time t is

$$D_t = D_{t-1} + F_{PD} \cdot dt$$

and the phosphorus mass is

$$P_t = P_{t-1} - F_{PD} \cdot dt.$$

The model was run with the Euler integration method and a time step (dt) of 0.1 h. No attempt was made to reach some equilibrium state since we wanted to simulate the transient batch culture. Diatom cells ml^{-1} were calculated from diatom P concentration using the estimates for cell volume, carbon content per unit volume, the molar C/P ratio (see Table 1) and a value of 12 g C mol C^{-1}.

Two different models for the effects of turbulence were considered. In the first, turbulence was assumed to change the maximum uptake rate $V'_{max} = ShV_{max}$ where V_{max} under still water was obtained from Moloney and Field (1989). In the second, turbulence was assumed to change the half-saturation constant $K'_M = K_M/Sh$. The still water value for K_M was determined from (Eq. 2) where V_{max} was from Moloney and Field (1989) and $\alpha_{max} = 3 \cdot \delta \cdot \sigma^{-1} \cdot r^{-2}$, being σ the volume specific P-content and r the cell radius (Thingstad and Rassoulzadegan, 1999). In model runs where turbulence was considered to affect uptake, we used either V'_{max} or K'_M but not both together.

The Sherwood number was calculated from estimates of the Péclet number (Pe) for spherical cells, as seen in Table 2. Pe is defined as:

$$Pe = E \cdot r^2 / \delta \quad (4)$$

where E (s^{-1}) is the shear rate, r is the radius of the cell (cm) and δ is the diffusivity of solute (cm^2 s^{-1}). In turn, E is calculated from the kinematic viscosity (v, cm^2 s^{-1}) of seawater and the turbulent kinetic energy dissipation rate (ε, cm^2 s^{-3}).

$$E = (\varepsilon / v)^{1/2}. \quad (5)$$

Comparison between model output and experimental data was done with regression analysis. Both the slope and the percentage of variance explained by the regression (R^2) for cell numbers and inorganic phosphorus were used to evaluate the goodness of fit of the model. We used these statistics to objectively optimize parameter values in a systematic way, and make a few very minor parameter adjustments (see Results).

Table 2
Equations utilized (from Karp-Boss et al., 1996) in order to calculate the Sh number according to the Pe number

Pe range	Equation for Sherwood number	Original source in Karp-Boss et al., 1996
$Pe \leq 0.01$	$1 + 0.29\ Pe^{1/2}$	Eq. (48)
$Pe \geq 100$	$0.55\ Pe^{1/3}$	Eq. (49)
$0.01 < Pe < 100$	Mean of $1.014 + 0.150\ Pe^{1/2}$ and $0.955 + 0.344\ Pe^{1/3}$	Fig. 6

3. Results

3.1. Experimental results

In both still and turbulent conditions, exponential growth of *T. pseudonana* was observed during the first 90 h of the experiment (Fig. 2); then, the population started to reach stationary phase (Exp. 1) or a lower growth rate phase (Exp. 2). Final concentrations averaged 3.24×10^5 cells ml^{-1}. Slightly faster growth rates of *T. pseudonana* have been calculated in the still than in the turbulent conditions ($\mu = 1.74 \pm 0.131$ day^{-1} (still) and 1.52 ± 0.044 day^{-1} (turbulence) in Exp. 1 (mean ± S.E). and 1.69 ± 0.003 day^{-1} (still) and 1.35 ± 0.169 day^{-1} (turbulence) in Exp. 2. In all containers, the inorganic phosphorus (with an initial concentration between 1 and 2 μM) was consumed during the first 70 h. The measured initial phosphate concentration was higher than foreseen, probably due to phosphate added as carryover with the inocula, although the inocula were from a phosphorus-limited medium as well. Nitrate and silicate decreased during the experiment until approximately half of their initial concentration (both nutrients in Exp. 2 and nitrate in Exp. 1) or until their total depletion (silicate in Exp. 1 for the turbulence treatment) (data in the Appendix). In this case, the Si/P ratio dropped from 250 to 6 in the last experiment sampling point. However, P concentrations also reached non-detection levels and diatoms seemed to almost reach a plateau. Probably Si became co-limiting with P by the very end of the Exp. 1 under turbulence.

Exponential growth of *Coscinodiscus* sp. was observed during the experiment (Fig. 2) for the still and turbulent cultures. The calculated growth rates were 0.37 ± 0.052 day^{-1} (still) and 0.42 ± 0.008 day^{-1} (turbulence) in Exp. 1 and 0.27 ± 0.069 day^{-1} (still) and 0.32 ± 0.025 day^{-1} (turbulence) in Exp. 2 (mean ± S.E.). In the last 50 h of both experiments, higher growth rates in turbulent than in still conditions were observed. Final cell concentrations reached 33 cells ml^{-1} (still) and 55 cells ml^{-1} (turbulence) in Exp. 1 and 22 cells ml^{-1} (still) and 38 cells ml^{-1} (turbulence) in Exp. 2. In both conditions, phosphate concentration decreased during the first 70 h of the experiment and then remained at low levels (<0.5 μM) until the end.

Growth calculations were done with all counted *Coscinodiscus* sp. shells as living cells. Empty shells of *Coscinodiscus* sp. were found in the experiments (Fig. 3) as is normal in cultures of this alga. If only living shells were compared, turbulence would have had an even larger effect of population dynamics. The growth phase in still water would be reduced to the

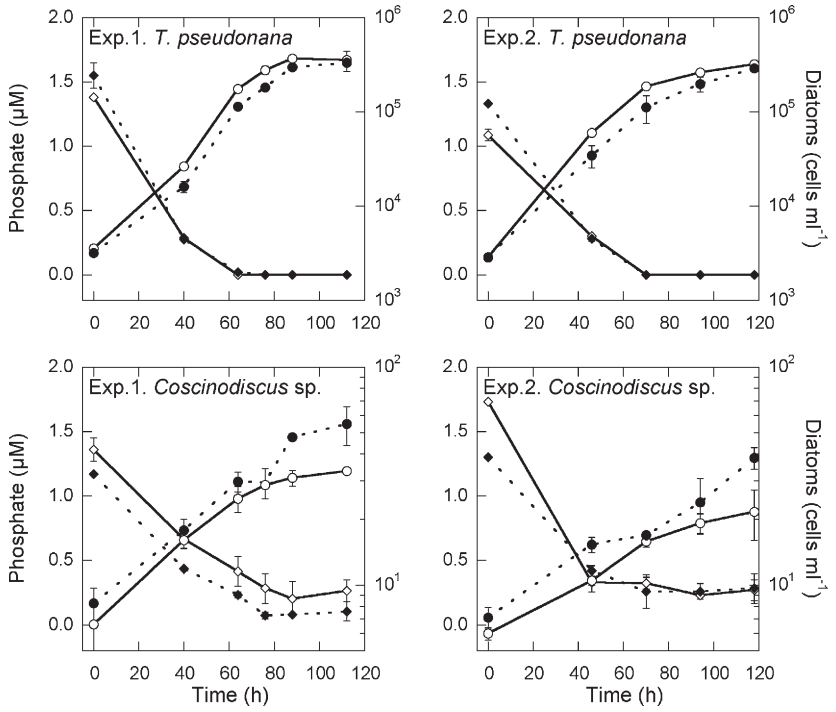

Fig. 2. Time course of phosphate (diamonds) and diatom concentration (circles) under still water (white symbols, continuous lines) and turbulence (black symbols, dashed lines) conditions. Symbols are means of two replicates and error bars are ±1 S.E. The different experiments and diatoms are labeled on the plots.

first 40 h, declining thereafter, while it continued to the end of the experiments under turbulent conditions. Because of the difficulty in determining true growth rate for the living cells as mortality was also present and varying in time, we decided to compute growth rates with the total number of shells. This underestimates true growth rate, but is of minor concern in this study as the emphasis is not on the absolute rates of growth but on the relative growth between turbulent and still water treatments. In any case, both the number of living cells and the total number of shells produced during the experiments was larger under turbulence conditions.

Although we used sterile techniques throughout the experiments, the algal cultures were not axenic. We took care in reducing bacteria carryover in the inocula for the experiments, achieving initial bacterial concentrations from 2.3×10^3 to 3.0×10^5 cells ml^{-1} (Table 3), depending on the experiment. This resulted in particulate-P contribution from bacteria of 10.38%±0.16% (mean±S.E., Exp. 1) and 0.87%±0.03% (Exp. 2) of the P-biomass in the *T. pseudonana* cultures and of 5.72%± 0.10% (Exp. 1) and 1.95%±0.11% (Exp. 2) in the *Coscinodiscus* sp. cultures. There were no significant differences in bacterial abundance between the still and turbulent conditions for *T. pseudonana* and *Coscinodiscus* sp. cultures (ANCOVA with time as a covariate, $N=87$, $P>0.1$ for the turbulence treatment). In general, an increase in bacterial numbers was observed during the first 40 or 75 h of the experiment; thereafter, they decreased (Exp. 1) or remained at a more or less constant concentration (Exp. 2, Table 3). Estimated phosphate consumption by bacteria was between 4.8% and 17.5% (on average 9.1%).

The ratios of specific-APA-still : specific-APA-turbulence ($APA_s : APA_t$) for both experiments are shown in Fig. 4. In *T. pseudonana* cultures, these ratios were always near unity or slightly higher ($P=0.055$ for a *t*-test of the mean being equal to 1; $N=8$) suggesting an almost equal need for phosphorus when growing in still and turbulent conditions. However, at the end of the *Coscinodiscus* sp. experiments, the ratio $APA_s : APA_t$ reached values between 2.3 or 4.5 times higher than at the 40 or 46 h time point ($P=0.008$ for a *t*-test of the mean being equal to 1; $N=8$). The possible contribution of bacterial biomass to the ratio of $APA_s : APA_t$ was estimated to be less than 1% in all cases except at the end of Exp. 2 for *Coscinodiscus* sp. where the ratio could be overestimated by 10%. We think that the lower activity under turbulence is

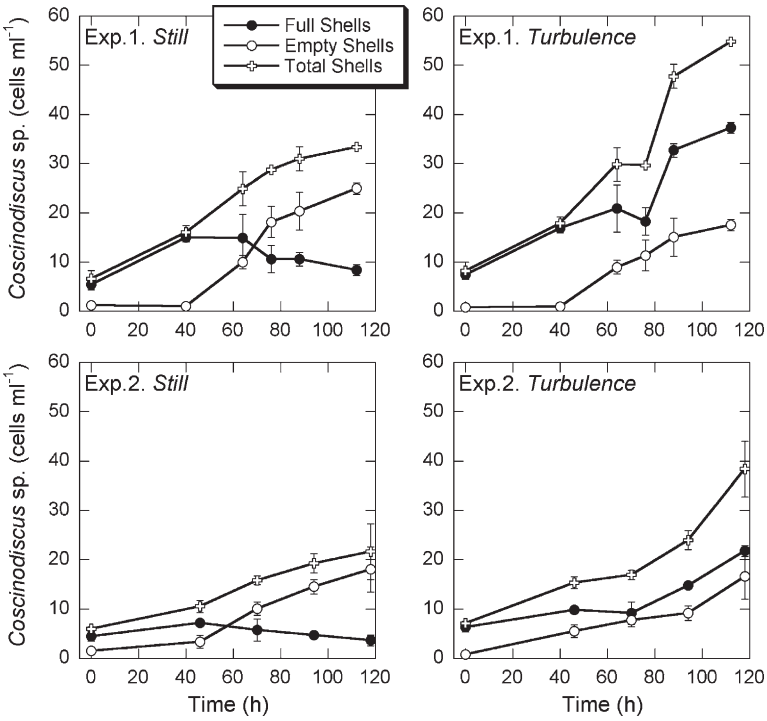

Fig. 3. Time course of *Coscinodiscus* sp. shells. Living cells (black circles), empty shells (white circles) and total shells (plus symbol). Symbols are means of two replicates and error bars are ±1 S.E.

because the cells experiment a higher nutrient flux and in their surroundings the "apparent" concentration of phosphorus is higher signaling less need to synthesize extracellular enzymes.

3.2. Model predictions and parametererizations

The calculation of $V_{max}(d^{-1}) = 3.6M(pgC)^{-0.25}$ (Moloney and Field, 1989) requires an estimate of carbon content per cell (M). Using the maximum observed cell yield, we assumed that all P in the experiments wound up in diatom cells and we adjusted the molar ratio of C/P so that we would get an estimate of C content per unit cell volume in the range of reported literature values. A value of C/P of 253 was used for *T. pseudonana*, which is not unrealistically high since Perry (1976) reported values up to 664 for this alga in a phosphorus-limited continuous culture. In the case of

Table 3
Bacterial concentration (mean±S.E. of two replicates) at the beginning and end of the experiments and at the end of the bacterial exponential phase, with time in parenthesis

Experimental condition	Bacterial concentration cells ml^{-1} ($\times 10^6$)		
	Initial	End exp. phase (time: h)	Final
Exp. 1			
T. pseudonana-still	0.03±0.002	2.28±0.75 (40)	0.14±0.009
T. pseudonana-turb.	0.03±0.001	2.25±0.31 (40)	0.11±0.002
Coscinodiscus sp.-still	0.22±0.02	2.06±0.83 (76)	0.65±0.23
Coscinodiscus sp.-turb.	0.29±0.02	3.28±0.87 (76)	0.65±0.23
Exp. 2			
T. pseudonana-still	0.02±0.0002	0.97±0.04 (70)	0.77±0.02
T. pseudonana-turb.	0.03±0.0001	1.57±0.55 (70)	0.99±0.32
Coscinodiscus sp.-still	0.06±0.007	2.99±0.13 (70)	5.74±2.08
Coscinodiscus sp.-turb.	0.06	5.07±1.21 (70)	5.13±2.05

No replicate for *Coscinodiscus* sp.-turb. was available on the initial day of Exp. 2.

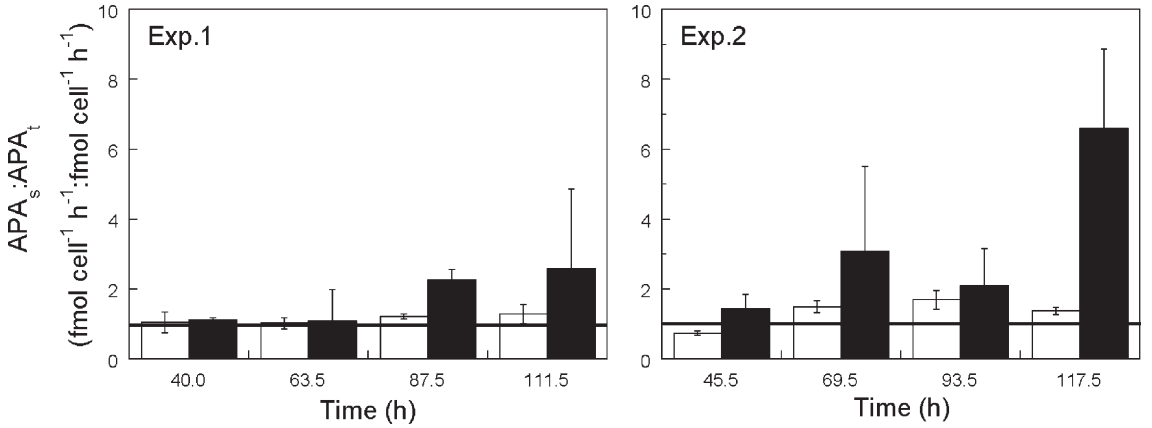

Fig. 4. Ratios of cell-specific alkaline phosphatase activity (APA) between still and turbulent conditions ($APA_s : APA_t$) for *T. pseudonana* (white bars) and *Coscinodiscus* sp. (black bars) cultures in Experiments 1 and 2. Horizontal line shows no difference between turbulence and still water treatments. Errors were calculated using error propagation theory from the variability of the two replicates per condition.

Coscinodiscus sp. a C/P of 106 was used since a larger C/P would have meant a C content per unit cell volume beyond those reported in the literature. A C/P of 106 does not necessarily imply that phosphorus is not limiting since this is just an average value for plankton and each species should have its own ratio under balanced growth. We decided to use a constant C/P in the model although C/P probably changes over time. In order to model a changing C/P, internal nutrient pools need to be tracked and both carbon fixation and nutrient uptake need to be modelled independently. This would have increased tremendously the number of parameters in the model and we did not have the experimental data to constrain the parameters.

In order to assess whether to pursue turbulence effects on V_{max} or K_M, preliminary model runs were done with an initial P concentration of 1 μM and initial diatom concentrations were 3200 (*T. pseudonana*) and

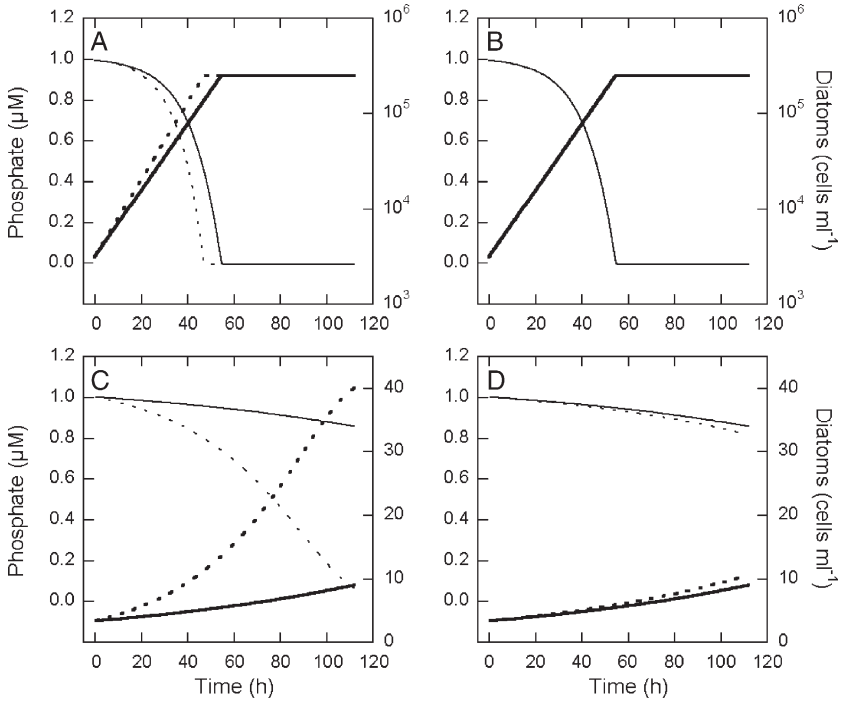

Fig. 5. Baseline model output for *T. pseudonana* (A and B) and *Coscinodiscus* sp. (C and D). Turbulence was affecting V_{max} in A and C and K_M in B and D. Phosphate is depicted by thin lines and diatom concentration by thick lines. Still water is shown as continuous lines and turbulence treatments as dashed lines.

3.35 (*Coscinodiscus* sp.) cells ml^{-1}. These were the planned initial concentrations for the experiments. Turbulence did not have a major effect in the case of *T. pseudonana* (Fig. 5). Diatoms reached a maximum concentration after about 55 h, when P was exhausted. The model suggests an increase in growth rate when turbulence affects V_{max} but the effect is small. *Coscinodiscus* sp. showed much faster growth when turbulence affected V_{max} compared to K_M or to still water. P decrease also showed this accelerated trend. In further model runs, we decided to use a turbulence effect on K_M rather than on V_{max} since the latter was giving a much too large response to turbulence in terms of cell yield.

When using measured initial concentrations of P and diatoms from Exp. 1, model and experimental data matched quite well (Table 4). No parameter adjustments were made for *T. pseudonana* although model output always showed somewhat faster growth rates than experimental data. An attempt to reduce the growth rate by lowering V_{max} below the estimated value from Moloney and Field (1989) for *T. pseudonana*, increased the phosphate mismatch further. Lowering the C/P ratio gave better matches in the P concentration but the C content per unit cell volume fell below reported literature values. Thus, no parameter adjustments were made. In the case of *Coscinodiscus* sp., the model underestimated growth. We optimized it by approximately doubling V_{max} from the value estimated by the relationship between V_{max} and cell size reported by Moloney and Field (1989). The value finally used, $V_{max}=0.025\ h^{-1}$, is within the range of experimentally observed values. Enhancement of *Coscinodiscus* sp. growth was observed in the experiments and could be reproduced by the model (Figs. 2, 5 and 6). This gave almost perfect matches for diatom growth, especially for the turbulence treatment. However, experimentally, the enhancement of *Coscinodiscus* sp. growth was only observed in the last days of the experiment. In the case of *T. pseudonana*, the experiments showed slightly higher cell concentrations in still water during the exponential growth phase while this trend was barely noticeable in the model.

Table 4
Regression analysis of model output against observed values in phosphate and cell number concentration

		[P]		Cell number	
		Slope	R^2	Slope	R^2
T. pseudonana	S	1.00	0.70	0.91	0.76
	T	0.99	0.62	1.09	0.59
Coscinodiscus sp.	S	0.80	0.58	1.53	0.83
	T	1.00	0.77	1.00	0.88

Experimental data is the independent variable and model output the dependent variable; $n=6$.

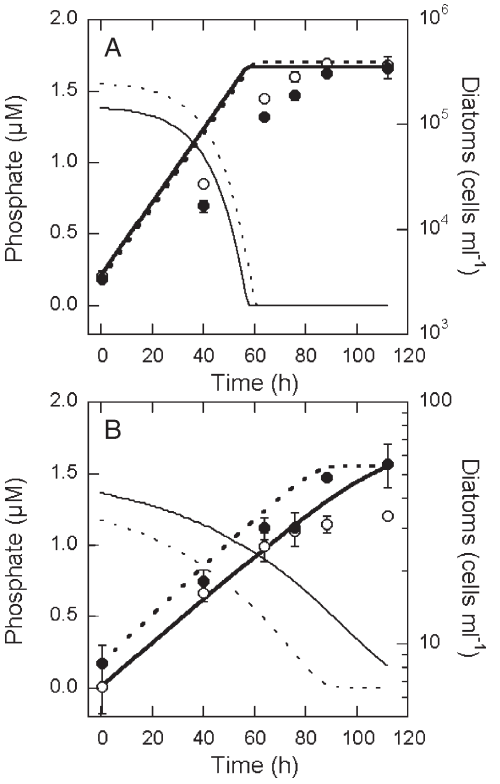

Fig. 6. Model output using experimental initial conditions for *T. pseudonana* (A) and *Coscinodiscus* sp. (B). Lines as in Fig. 5. Symbols are data from Exp. 1 for the turbulence treatment (black circles) and the still water (white circles) treatment.

Phosphate depletion in *T. pseudonana* cultures was the reason for the decrease in their growth rates at the end of the experiment. In the *Coscinodiscus* sp. cultures, the experiments were cut somewhat short of stationary phase resulting in some leftover phosphate by the end of the experiments. Had the experiments been run longer, *Coscinodiscus* sp. would have continued to grow until phosphate exhaustion. Alternatively, or maybe in addition, a certain phosphorus recycling level by bacteria could keep the diatoms growing with a background phosphorus level.

Phosphate consumption was slower in the model than in the experiments. Bacteria were present in the cultures and the fast increase of bacteria observed in the first hours of the experiment (Table 3) means that part of the phosphate in the medium was consumed by these organisms. This could explain a faster decrease of phosphate in the experiments compared to the model output (where bacteria were not considered). However,

we estimated that no more than 17.5% (average of 9.1%) of the phosphate would have been consumed by bacteria. Moreover, the fact that bacteria did not continue growing to achieve much higher numbers implies that they became C-limited which reduces their influence on the dynamics of P in the cultures. Since there were no statistical differences in the concentration of bacteria between turbulence and still treatments in any of the experiments, we assumed that the differences observed for the diatoms were due to turbulence. This agrees with theoretical expectations since the phosphate transport to a bacterial cell would increase only by 0.6% due to turbulence (Karp-Boss et al., 1996). Therefore, as the bacterial nutrient consumption would be almost the same with and without turbulence, the differences observed in the phosphate concentration in the still and turbulent conditions would fundamentally be due to differences in the nutrient consumption of the algae. Thus, we did not consider including bacteria in the model as it would have increased unnecessarily the number of uncertain parameter values.

The model tracked only living cells while empty shells of *Coscinodiscus* sp. were found during the experiments (Fig. 3). Thus, cell mortality was a factor in the experiments that affected population dynamics. After considering the possibility of including an algal mortality term in the model, we decided against it. The limited knowledge on non-predatory algal mortality does not allow for a trivial parameterization based on first principles and we would only be fitting the model to the data ad hoc.

4. Discussion

4.1. Experimental observation and model output agreement

Both the experimental data and the results of a simple model of nutrient uptake point to a differential effect of turbulence on population dynamics for different sized diatoms. While minor or no effects are observed for the small *T. pseudonana*, turbulence seems to favor the growth of large *Coscinodiscus* sp. cells. This is in accordance with the hypothesis of Karp-Boss et al. (1996) of an increased nutrient flux towards cells for organisms larger than ca. 60 μm owing to the relative water motion with respect to the particles. An experimental observation, for which we do not have an explanation, is that *T. pseudonana* shows a slightly larger growth in still water. The 20% irradiance variance among treatments could in principle explain the differences in growth for *T. pseudonana*. But the results are consistent for two independent experiments, and in each case the experimental containers and their replicates were placed at random making consistent irradiance differences between treatments highly unlikely.

When osmotrophic plankton cells become phosphorus limited, the alkaline phosphatase activity increases in an attempt to scavenge as much P as possible from the surrounding environment. The ratio of APA for still water with respect to turbulence (Fig. 4) shows that the effect of turbulence is minor for *T. pseudonana* since it fluctuates around a value of 1. On the contrary, *Coscinodiscus* sp. shows higher APA values for still water, with ratios $APA_s : APA_t$ higher than 1, especially after ca. 40 to 60 h, indicating that P-limitation is not as strong under turbulence. The data, with minor variations, is consistent across both experiments. It indirectly links nutrient uptake to cell population dynamics and is further evidence that turbulence increased the flux of phosphorus towards cells, alleviating limitation.

4.2. Shortcomings and confounding factors

Two issues weaken the conclusiveness of the results. These are, the observed cell mortality for *Coscinodiscus* sp. and the sedimentation of cells to the bottom of the containers.

Empty shells of *Coscinodiscus* sp. were observed throughout the experiments and we may be concerned about mortality dominating population dynamics. One could argue that there are less *Coscinodiscus* sp. cells in still water because of a higher mortality rate. This is possible but we do see that the specific APA values were higher in still than in turbulent conditions for *Coscinodiscus* sp. indicating an effect of turbulence unrelated to mortality. Secondly, crude estimates of particle C/P ratios at the end of the *Coscinodiscus* sp. experiments give higher values for the turbulence treatment (computed from living cells). This may seem to contradict the higher P need under still water observed with the APA but it does not. Under still water, the flux of nutrients towards the cells is slower and cells have a higher need for P, because what matters is the very surroundings of the cell. On the contrary, under turbulence, this flux is increased and cells do not appear to need so much P. As a consequence nutrient concentrations are drawn down even further. Since division and C fixation are separated processes, cells keep fixing C beyond the possibility to divide further and the C/P increases. In still water, the processes are slower and division and growth are not so out of phase. This has been observed previously with community level experiments (Maar et al., 2002). This

could not be explained from mortality since there would be no reason for a different C/P composition, an evidence that the uptake part is important. Thirdly, *Coscinodiscus* sp. living cells do increase over the first 40 h in the still water treatment, meaning that cells were actively growing and physiologically healthy. It is only after this time that mortality is high, probably because nutrients are declining. We have to take into account that, normally, the diatom cultures are maintained at very high nutrient concentrations (f/2 or f/20 medium; 14 to 140 μM P) and that cells may not do as well at ca. 1 μM P. Turbulence seemed to alleviate this condition. Finally, another argument for the nutrient uptake increase for large cells is that it agrees with theoretical predictions.

We observed some sedimentation of cells in the experimental containers. The effects of turbulence on the sedimentation rate of phytoplankton are poorly studied. Although turbulence should not affect sedimentation in principle, it is generally accepted that turbulence prevents the loss of phytoplankton cells from the mixed layer (Kiørboe, 1993). However, there are some recent findings (Ruiz et al., 2004) pointing towards an increase of sinking velocity under turbulence. Ruiz et al. (1996) observed that the sedimentation rate of phytoplankton cells could be affected by changes in turbulence levels only when their settling velocity was higher than 1 m day^{-1}. This was the case for *Coscinodiscus* sp. but not for *T. pseudonana*. In addition, diatom cells may modify their sinking velocity by physiological control (e.g. fat accumulation, gas vacuoles or thicker silicate frustules) or by changes in their growth rates (Smayda, 1970). Cells with low growth rate or senescent cells have higher sinking velocities than actively growing cells. What probably happens in a system with a solid bottom boundary is that, as grounded leaves on a windy day, settled cells are resuspended back into the fluid.

If we use the data of Fig. 1 in Smayda (1970) to obtain a regression of sinking rate versus average cell diameter and then introduce the dimensions of *Coscinodiscus* sp. and *T. pseudonana*, we obtain sinking velocities of 2.36×10^{-3} and 1.12×10^{-4} cm s^{-1}, respectively. These sinking velocities are lower than the eddy velocities derived from the turbulence level introduced (5.6×10^{-1} cm s^{-1}) and even for still water (10^{-2} cm s^{-1}) if we consider a background ε of 10^{-6} cm^2 s^{-3} produced simply by convection. This means that, in principle, even if cells are sinking they would also be resuspended, so we would not expect cell accumulation at the bottom of the containers. But we did observe part of the population at the bottom of the containers, which only goes to show how little we know of cells settling in a turbulent field.

Sedimentation and cell mortality could be related in two ways. One possibility is that cells settle out faster in still water, and some of the cells that reach the bottom of the container die. Why they would die is an open question. Maybe settled cells would not be able to take up as much phosphate as cells in suspension and thus would not meet energy requirements. However, the data in Fig. 3 shows that differential settling was not an issue during the first 40 h, as cells in all treatments increased, and thus purely physical mechanisms should be discarded. Another way could be that these large cells in suspension cannot efficiently take up enough nutrients under still water conditions. Some suspended cells could die or become physiologically or energetically challenged to maintain a buoyant position. The true mechanism is probably a combination of a sedimentation-mortality link and the direct effect of turbulence on nutrient uptake. The importance of each mechanism can not be conclusively determined from the present experimental data. The details of these issues could be explored with more complex modelling. Currently, there is no clear and straightforward knowledge of phytoplankton mortality and of settling of cells in general, and even less under turbulent

Table 5
Affinity constants for phosphate from different literature sources and organisms

Source and organism	Affinity (l μmol P^{-1} h^{-1})
Parameterizations	
Thingstad et al. (1997)	
Bacteria	10
Algae	5
Thingstad et al. (1999a)	
Bacteria	5
Phytoplankton	4
Thingstad et al. (1999b)	
Bacteria	100
Cyanobacteria	45
Autotrophic flagellates	36
Thingstad (2000)	
Bacteria	50–210
Algae	300
Measurements	
Moutin et al. (2002)	
0.2–0.6 μm	2.02–224
0.6–2.0 μm	7.71–246
>2.0 μm	0.50–29.4
Tanaka et al. (2003)	
0.2–0.6 μm	0.68–27.2
0.6–2.0 μm	42.1–104
>2.0 μm	1.91–32.5

conditions, and these issues are left for future model improvements.

4.3. Natural conditions

It was not the purpose of this study to mimic natural conditions. Rather we used laboratory experiments to look at the physiological growth response of two diatoms of different size to turbulence under limiting P conditions, and a simple model to infer mechanisms of action.

In terms of the kinetic parameters, *Coscinodiscus* sp. has a much larger nominal K_M than *T. pseudonana*, closer to initial P concentrations. Thus decreasing K_M through turbulence increases the affinity for P. The resulting P α_{max} used in the model for *T. pseudonana* was in the range of reported literature values (Tables 1 and 5). On the contrary, the calculation of α_{max} for *Coscinodiscus* sp. was lower than reported/used values in the literature. Table 5 does not contemplate such large cells, which theoretically have lower α_{max}. On the other hand, large diatoms could have larger α_{max} than theory predicts if the thickness of the cytoplasm (small because of the vacuole) rather than the cell diameter is taken as the characteristic cell dimension.

The shaker table system to generate turbulence has been used in studies of phytoplankton growth (e.g.: White, 1976; Berdalet and Estrada, 1993; Juhl et al., 2000; Yeung and Wong, 2003) and we deemed it appropriate for this study. A turbulence intensity of 10 $cm^2\ s^{-3}$ is rather high for a natural system and can only be found near the surface ocean, in breaking waves or near the shore (MacKenzie and Leggett, 1993; Peters and Marrase, 2000). At lower turbulence intensities modelling shows that the effect on diatom growth should be qualitatively similar (Fig. 7). Thus, our results suggest that turbulence has the potential to favour larger diatoms in natural conditions because of increased nutrient flux towards the cells. However, the outcome of competition between large and small diatoms in the field may be modified by other factors such as predatory and non-predatory mortality, different light requirements, or by the redistribution of cells in the water column by turbulence. Additionally, phytoplankton cells in the open

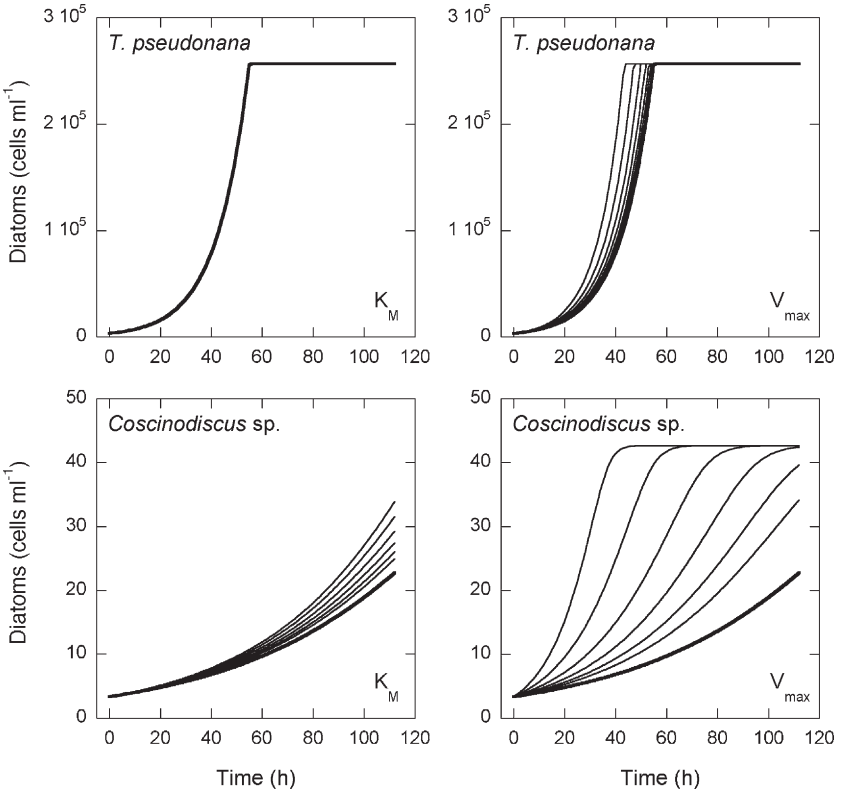

Fig. 7. Model output of diatom cell concentration for a range of turbulence intensities: 10^2, 10^1, 10^0, 10^{-1}, 10^{-2}, 10^{-3} and 0 (thick line) $cm^2\ s^{-3}$. V_{max} and K_M denote whether turbulence is modelled to affect one or the other parameter. *T. pseudonana*: nominal V_{max} = 0.081 h^{-1}; nominal $K_M = 3.91 \times 10^{-3}$ μM P; initial P concentration = 1 μM P; initial cell concentration: 3200 cells ml^{-1}. *Coscinodiscus* sp.: nominal V_{max} = 0.0225 h^{-1}; nominal $K_M = 2.51 \times 10^{-1}$ μM P; initial P concentration = 1 μM P; initial cell concentration: 3.35 cells ml^{-1}.

sea are subject to time-varying levels of turbulence and their growth conditions, in terms of turbulence, may be more complex than what we can currently generate in the laboratory.

5. Conclusions

Experimental and modelling results are consistent with the idea that the size of osmotrophic cells is indeed important to understand the effect of turbulence on nutrient uptake and growth. Large diatoms are favored presumably by an increased nutrient flux towards the cells which affects growth in a non-linear manner. From this study, it seems that the half-saturation constant of the classical Michaelis–Menten nutrient uptake model would translate the increased nutrient flux into growth as K_M/Sh. Modifications to the uptake velocity of the form ShV_{max} caused changes in growth, owing to turbulence, much larger than observed. However, more sophisticated combinations may be possible.

Although a simple model of diatom growth using Michaelis–Menten dynamics with parameter values estimated from the literature provides a start for interpreting the results from the experiments, the simulations do not completely match the details of the experiments. In particular, the growth rates for *T. pseudonana* are systematically too high and, for *Coscinodiscus* sp., the increased growth in the turbulence experiments was observed only over the second half of the experiment whereas the model showed increased growth over the whole experiment. In addition, the observed non-predatory mortality and the settling rate of *Coscinodiscus* sp. confound the results of the present study. Clearly, further experiments assessing the effects of turbulence on diatoms need to address explicitly the sinking of cells. This is intrinsically difficult since stirring, aerating or otherwise trying to keep cells in suspension also introduces turbulence, and no still water control is possible. All these issues require more complex modelling before a conclusive interpretation can be made on the effect of turbulence on nutrient uptake and growth. This type of studies aim to be building blocks for inclusion into full scale physical–biological coupled models, which are considering ever growing detail of marine plankton and its interactions with turbulence.

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Appendix A

The measured inorganic nutrient concentrations are shown for all experiments. Nutrient concentrations are in μM. Phosphate is depicted as P, silicate as Si and dissolved inorganic nitrogen as N. Data are averages of measurements in two replicate experimental vessels and the number in parenthesis corresponds to the standard error of the mean. When no data is shown, measurements were below detection limits. In addition, ratios of Si/P and N/P are also shown.

Time (h)	Still					Turbulence				
	P	Si	N	Si/P	N/P	P	Si	N	Si/P	N/P
Exp. 1, T. pseudonana										
0.0	1.38 (0.01)	39.06 (9.30)	77.14 (2.67)	28.30	55.90	1.55 (0.10)	33.33 (1.98)	85.80 (0.88)	21.50	55.35
40.0	0.29 (0.00)	47.63 (0.44)	76.54 (0.19)	167.12	268.56	0.28 (0.00)	48.93 (0.14)	78.66 (0.71)	177.91	286.02
63.5	–	32.51 (0.33)	64.00 (2.25)	–	–	0.02 (0.00)	40.26 (0.22)	70.38 (0.74)	2013.00	3518.75
75.5	–	20.05 (1.35)	49.33 (2.22)	–	–	–	30.28 (0.58)	57.34 (0.80)	–	–
87.5	–	1.75 (1.05)	38.66 (1.54)	–	–	–	11.83 (0.61)	45.83 (0.82)	–	–
111.5	–	–	40.93 (1.21)	–	–	–	–	41.26 (0.71)	–	–
Exp. 1, Coscinodiscus sp.										
0.0	1.36 (0.09)	38.26 (6.73)	91.75 (6.10)	28.13	67.46	117 (0.00)	31.33 (9.49)	80.97 (15.35)	26.77	69.20
40.0	0.67 (0.02)	49.75 (0.74)	88.67 (0.11)	74.81	133.34	0.44 (0.00)	45.43 (0.55)	84.90 (1.29)	104.44	195.16

Appendix A (*continued*)

Time (h)	Still					Turbulence				
	P	Si	N	Si/P	N/P	P	Si	N	Si/P	N/P
Exp. 1, Coscinodiscus sp.										
63.5	0.42 (0.12)	49.64 (0.58)	81.73 (0.22)	119.61	196.94	0.23 (0.02)	40.43 (0.11)	81.72 (0.73)	173.71	351.11
75.5	0.29 (0.12)	57.07 (2.78)	82.63 (1.09)	200.23	289.93	0.08 (0.03)	35.09 (1.43)	77.43 (0.24)	467.87	1032.33
87.5	0.21 (0.14)	43.18 (0.05)	75.90 (1.12)	210.61	370.24	0.08 (0.01)	20.08 (2.15)	71.70 (1.57)	250.94	896.25
111.5	0.27 (0.08)	36.33 (0.85)	80.88 (0.52)	137.09	305.21	0.11 (0.08)	0.66 (0.66)	76.54 (0.57)	6.29	728.90
Exp. 2, T. pseudonana										
0.0	1.09 (0.04)	61.28 (6.11)	73.46 (3.00)	56.47	67.70	1.33 (0.00)	82.62 (7.75)	90.10 (0.27)	62.12	67.74
45.5	0.30 (0.00)	74.23 (7.68)	76.40 (0.77)	247.42	254.67	0.28 (0.02)	100.46 (14.49)	80.75 (2.48)	358.79	288.38
69.5	–	50.91 (6.68)	58.03 (1.21)	–	–	–	87.67 (11.66)	67.61 (0.83)	–	–
93.5	–	39.72 (6.65)	46.95 (0.19)	–	–	–	67.05 (13.97)	50.11 (0.60)	–	–
117.5	–	31.44 (5.97)	46.10 (0.72)	–	–	–	49.18 (19.31)	40.07 (6.85)	–	–
Exp. 2, Coscinodiscus sp.										
0.0	1.73 (0.00)	106.68 (15.82)	93.84 (0.16)	61.66	54.24	1.30 (0.00)	98.13 (26.24)	92.35 (0.60)	75.48	71.03
45.5	0.33 (0.02)	110.89 (16.23)	83.99 (0.88)	336.02	254.52	0.42 (0.04)	97.22 (27.70)	81.24 (0.94)	231.46	193.43
69.5	0.33 (0.04)	108.22 (16.31)	79.54 (0.39)	332.97	244.72	0.26 (0.13)	92.05 (29.18)	77.20 (2.45)	354.02	296.92
93.5	0.23 (0.00)	106.93 (14.80)	79.29 (0.69)	464.89	344.72	0.26 (0.06)	83.63 (29.73)	73.87 (2.42)	321.65	284.12
117.5	0.27 (0.08)	100.79 (14.71)	101.09 (14.77)	373.30	374.41	0.29 (0.12)	65.76 (31.00)	71.84 (3.03)	230.72	252.05

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