ELSEVIER

Journal of Experimental Marine Biology and Ecology 335 (2006) 27–38

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Effects of small-scale turbulence on *Phaeocystis globosa* (Prymnesiophyceae) growth and life cycle

Mathilde Schapira [a,*], Laurent Seuront [a,b], Valérie Gentilhomme [a]

[a] *FRE CNRS ELICO 2816, Station Marine de Wimereux, Université des Sciences et Technologies de Lille 1, 28 Avenue Foch BP-80,62930 Wimereux, France*

[b] *School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide 5001, Australia*

Received 16 August 2005; received in revised form 3 February 2006; accepted 25 February 2006

Abstract

The response of *Phaeocystis globosa* to small-scale turbulence was studied in 5 l microcosms. Turbulence was generated by oscillating grids. The effect of small-scale turbulence was examined under 3 turbulence levels representative of the *P. globosa* natural environment, and in non-turbulent control cultures. Single cell numbers, nitrogen concentrations and colony formation (number and diameter) were followed over 13 days in each experimental culture. Small-scale turbulence decreased single cell growth and also influenced colony formation. More colonies were formed when turbulence increased to a given threshold, but above this turbulence level, fewer and smaller colonies were observed in *P. globosa* cultures. The ecological significance of these results, particularly, the potential influence of small-scale turbulence on competition mechanisms between *P. globosa* and diatoms are finally discussed and suggested as a key factor to understand phytoplankton successions in the Eastern English Channel.

Keywords: Colony formation; Growth; Microcosms; Nutrient uptake; *Phaeocystis globosa*; Turbulence

1. Introduction

Water motion occurs in the marine environment over wide spatio-temporal scales (Denman and Gargett, 1983) and a large variety of energetic input is capable of causing these motions (e.g. wind, tide, internal waves). The kinetic energy generated at large scale cascades without dissipation through a hierarchy of eddies of decreasing size to the viscous Kolmogorov scale (typically ranging between a few mm to a few cm) where it is dissipated into heat (Kolmogorov, 1941). Planktonic organisms are thus constantly subjected to a complex physical and chemical environment imposed by these small-scale phenomena.

The importance of turbulence as a source of auxiliary or external energy to the pelagic environment has been widely acknowledged (Margalef, 1978; Legendre et al., 1986). In particular, small-scale turbulence could be an important factor determining which phytoplanktonic taxon dominates the ecosystem (Smayda and Reynolds, 2000). Phytoplankton in the ocean are continually subjected to small-scale turbulence which may have a positive or negative effect on their cell physiology (Sullivan et al., 2003). Small-scale turbulence has been reported to be beneficial for phytoplankton by increasing the diffusion rate of nutrients to the cell surface (Savidge, 1981; Lazier and Mann, 1989; Karp-Boss et

* Corresponding author. Tel.: +33 3 21 99 64 30; fax: +33 3 21 99 64 01.
E-mail address: mathilde.schapira@univ-lille1.fr (M. Schapira).

0022-0981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2006.02.018

al., 1996). However, negative effects of small-scale turbulence on phytoplankton have also been shown to occur through a variety of mechanisms, including behavioural alteration (Karp-Boss et al., 2000), inhibition or reduction of cell division rates, modification of cell sizes, morphology, motility and physiological impairment (Thomas et al., 1995; Estrada and Berdalet, 1997; Juhl and Latz, 2002; Sullivan and Swift, 2003; Sullivan et al., 2003). Experimental data have shown that sensitivity and responses to turbulence differ among taxa (Sullivan and Swift, 2003), and certain phytoplankton species may show specific adaptive responses to turbulence. Colony formation or the presence of appendages such as horns or spines could represent mechanisms to change the relative size in order to take advantage of turbulent flow fields (Margalef, 1978, 1997; Karp-Boss et al., 1996), and the production of mucilage could play a role in controlling diffusion coefficients (Jenkinson, 1986).

Phaeocystis spp., are a common Prymnesiophyte genera in temperate and polar seas, occurring as solitary cells or colonies and producing large amounts of mucus, predominantly made of carbohydrates (Lancelot and Mathot, 1985; Van Boekel, 1992). In the Eastern English Channel, intense spring blooms are recurrently composed of *Phaeocystis* and diatoms. The *Phaeocystis* bloom consists of a high spring peak, usually appearing a few weeks after the spring diatom peak (Gieskes and Kraay, 1975; Cadée and Hegeman, 1986). Proposed hypotheses to explain the *Phaeocystis*/diatoms succession include differential competition for light and/or nutrients (Matrai et al., 1995; Peperzak et al., 1998; Meyer et al., 2000) but specific causes remain uncertain. Despite the megatidal regime and the related high turbulent conditions with values of ε ranging between 10^{-6} and 10^{-4} m^2 s^{-3} encountered in the Eastern English Channel (Seuront et al., 2002), no attention has been given to the effect of microscale turbulence on *Phaeocystis* spp. dynamics and seasonal phytoplankton succession.

To our knowledge, no experiments have been conducted using quantified turbulence levels to test the potential effect of small-scale turbulence on the growth and life cycle of *P. globosa*. Only observations have been made on the effect of unquantified shaking on *P. globosa* life cycle and inorganic carbon acquisition (Kornmann, 1955; Cariou et al., 1994; Peperzak, 2002). In this study we investigate the potential effect of small-scale turbulence on the growth and life cycle of *P. globosa*, using quantified turbulence levels fully representative of those found in the Eastern English Channel. We followed single cell growth, nitrogen acquisition, and colony formation over time under 3 realistic turbulence levels, generated in microcosms. While significant advances in the study of the ecological effects of turbulence on plankton have been mainly achieved by the use of microcosms (Alcaraz et al., 2002), the present work is the first to investigate the interaction between small-scale turbulence and the *P. globosa* spring bloom in the Eastern English Channel.

2. Material and methods

2.1. Organisms

P. globosa (Scherffel, 1900) clone Ph 91 was maintained in f/2-Si (Guillard, 1975) enriched medium in a constant temperature chamber at 13 °C ± 1 °C. Seawater used for cultures media was collected in the Eastern English Channel during summer (low nutrients concentrations) and filtered through GF/C filters (0.45 μm porosity). Cultures were illuminated with five 58 W fluorescent lights in a 12/12 light–dark cycle at an irradiance of 60–70 μmol m^{-2} s^{-1} measured closed to cultures with a quantum sensor Li-192 SA connected to a Li-cor Data Logger LI 1400 (Li-Cor, Lincoln, NB, USA).

2.2. Turbulence generation and quantification

Experiments were conducted at three turbulence intensities: high ($\varepsilon \sim 10^{-4}$ m^2 s^{-3}; HT), medium ($\varepsilon \sim 10^{-5}$ m^2 s^{-3}; MT) and low ($\varepsilon \sim 10^{-6}$ m^2 s^{-3}; LT), representative of the conditions encountered in the Eastern English Channel over several tidal cycles (Seuront et al., 2002). Duplicate chambers for each turbulence level were glass tanks with an internal length, width, and height measuring 17 × 17 × 23 cm, and were filled with seawater to a height of 17 cm (~4.9 l) to ensure the three-dimensional, isotropic character of the generated turbulence (Fig. 1). Turbulence in each chamber was generated by a horizontally oscillating grid, with a stroke of approximately 13 cm centred in the middle of the tank, and driven by an electric motor. The free space between the grid and the side walls of the chambers was 0.4 (± 0.1) cm, and the open to the total area of the grid was 0.48 (holes were 16 cm^2, i.e. 4 × 4 cm, and bars were 0.9 cm thick). As the grids moved through the water, turbulent vortices were generated that interacted and decayed. Changing the horizontal velocity of the grids provided different intensities of small-scale turbulence. The turbulence intensity in each chamber was quantified through particle image velocimetry (PIV; Raffel et al., 1998). In the PIV, the fluid was seeded with polyamide particles (50 μm diameter) tracer particles and a cross section of the flow was illuminated using a laser light sheet. Successive positions of the tracers were recorded

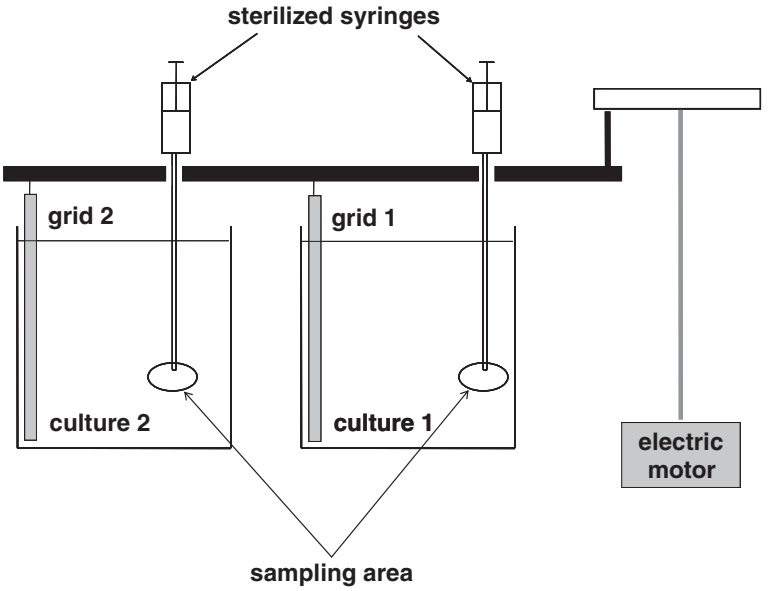

Fig. 1. Diagrammatic side view of turbulence mechanism.

using a digital camera (Sony Handycam, Japan) at a rate of 30 frames s^{-1}. Velocity measurements were made in small (2×2 cm) observation windows parallel to the oscillation of the grid, at 4 different vertical positions (2, 6, 10, and 14 cm from the surface) and 5 horizontal positions located at the centre of the stroke and at 5 and 10 cm on each side of the centre of the stroke. The local horizontal and vertical fluid velocities,v_x,v_y and v_z, we then inferred from the displacements d_x, d_y and d_z between two successive exposures of the tracer particle in the observation window as:

$$v_x = \frac{1}{N}\sum_{i=l}^{N}(x_{t_i} - x_{t_{i+1}})$$

$$v_y = \frac{1}{N}\sum_{i=l}^{N}(y_{t_i} - y_{t_{i+1}})$$

$$v_z = \frac{1}{N}\sum_{i=l}^{N}(z_{t_i} - z_{t_{i+1}}) \quad (1)$$

where N is the number of measured successive displacements. As no significant differences were found between the velocities v_x , v_y and v_z , the related root-mean-square turbulent velocity w was estimated as:

$$w = (v_x^2 + v_y^2 + v_z^2)^{1/2} \quad (2)$$

The energy dissipation rate ε was subsequently estimated as:

$$\varepsilon = Dw^3/L \quad (3)$$

where D is a universal constant (D=1; Stiansen and Sundby, 2001; Seuront et al., 2004), and L is the integral length scale of turbulence, i.e. a characteristic length scale representing largest turbulent vortices. The increase in L with distance from the grid was taken into account as (Thomson and Turner, 1975):

$$L = kd \quad (4)$$

where d is the distance from the centre of the grid oscillation to the location of measurement, and k is a proportionality constant (k=1;). Using 10, 20 and 30 strokes min^{-1}, we obtained dissipation rates $\varepsilon \sim 10^{-4}$ m^2 s^{-3}, $\varepsilon \sim 10^{-5}$ m^2 s^{-3} and $\varepsilon \sim 10^{-6}$ m^2 s^{-3} that were not significantly different between the duplicate turbulent chambers (p>0.05). These dissipation rates were fully congruent with previous estimates derived from an acoustically measured 3D velocity fluctuations behind the same grid in a 1-m long turbulent chamber (Seuront et al., 2004).

Two non-turbulent control cultures, grown in 4 l spherical glass tanks were simultaneously examined, hereafter referred as NT (Table 1).

2.3. Turbulence experiments

Environmental conditions were held constant between each turbulence experiment. *P. globosa* cultures in exponential growth phase were gently filtered through 5 μm nylon mesh in order to exclude colonies and were inoculated into treatments at a

Table 1
Turbulence levels, replicate number, corresponding turbulent energy kinetic dissipation rates; ε (m^2 s^{-3}), experimental culture volume; $V_{microcosm}$ and inoculated *P. globosa* volume; $V_{P.globosa}$ ml

Turbulence levels	Replicates	ε(m^2s^{-3})	$V_{batch\ culture}$ (l)	$V_{P.globosa}$ (ml)
NT	2	0	4	4
LT	2	10^{-6}	5	5
MT	2	10^{-5}	5	5
HT	2	10^{-4}	5	5

For each experiment $V_{P.globosa}$ represents 0.1% of $V_{microcosm}$.

volume equalling 1% of the experimental tank volume (Table 1).

Treatments were followed over 13 days (312 h). Sampling and subsequent measurements were conducted every 12 h during the first 48 h (4 after light initiation and 4 h after dark) and every 24 h (4 h after light initiation) afterwards. The grids were switched off for approximately 3.5 min for sampling and measurements in both microcosms and sampling was conducted with sterilized syringes combined with plastic tubes (Fig. 1).

The pH was directly recorded with a multi-parameter analyser (CONSORT C533). Dissolved oxygen, $[O_2]_d$, was measured in 50 ml sub-samples minutes after sampling, with an INOLAB 2 analyser (WTW, France). Ten ml sub-samples were fixed with Lugol's iodine solution (Throndsen, 1978). Single cells and colony numbers were determined by the Utermöhl technique (Utermöhl, 1958). Counts were made on an inverted microscope (model Ortholux Leitz Wetzlar, Germany) and average colony dimensions were determined using an ocular micrometer. Nitrate concentrations, $[NO_3^-]$, were determined with an Auto-Analyzer (Alliance Instruments, France) from 10 ml sub-samples. Ammonium concentrations, $[NH_4^+]$, were measured by the Koroleff colorimetric method (Koroleff, 1969) from 10 ml sub-samples.

2.4. Statistical analysis

Due to low sample numbers, non-parametric statistics were used (Zar, 1996). The pH and $[O_2]_d$ in cultures were compared using the Kruskal–Wallis non-parametric analysis of variance. When the Kruskal–Wallis test identified a significance difference ($p<0.05$), a post-hoc comparison was used (Tukey least squares comparison) to identify significant differences. The same statistical procedures were used to examine the role of turbulence level on maximum single cell growth rate, apparent NH_4^+ and NO_3^- uptake rates, colony number and colony diameter.

3. Results

3.1. Culture parameters

Average pH values are reported in Table 2 and significant differences were observed ($p<0.05$) between experiments. In NT cultures average pH value was significantly higher than in cultures grown under turbulent conditions ($p<0.05$). Average pH values were significantly higher in LT than those measured in cultures grown under higher turbulent conditions, MT and HT cultures ($p<0.05$). No significant differences in dissolved oxygen concentrations, $[O_2]_d$ (Table 2) have been observed between turbulent and non-turbulent treatments ($p>0.05$).

3.2. Growth of single cells

After the first 72 h, single cell numbers increased in each experimental culture and reached their maximum values between 144 to 268 h (Fig. 2). Maximum cell densities were observed in LT cultures (Table 3). Under higher turbulent conditions, single cell densities were lower and we observed a decrease in single cell number as turbulence intensity increased. Fewer single cells were formed in NT cultures compared to LT cultures. *P. globosa* cultures grown under different turbulence levels showed significantly ($p<0.05$) different maximum single cell growth rates (Table 3). NT, LT and MT cultures had significantly higher growth than HT treatments ($p<0.1$).

3.3. Colony formation

Cells formed globular and regularly distributed colonies in NT cultures and at each turbulence level tested. The first colonies were observed after 48 h, 72 h and 120 h in NT, LT, and both MT and HT conditions, respectively (Fig. 3). Colony number increased gradually until a maximum was reached and then decreased until the end of the experiment (Fig. 3). Colony numbers differed significantly between treatments ($p<0.05$). Colony number was significantly higher under MT

Table 2
Dissolved oxygen concentrations $[O_2]$ (mg l^{-1}) and the pH values during experiments inside each microcosm

	$[O_2]$ (mg l^{-1})	pH
NT	10.60±3.69	8.64±0.43
LT	9.50±1.13	8.45±0.47
MT	8.57±0.70	8.10±0.35
HT	8.63±0.82	7.96±0.22

$[O_2]$ and the pH mean values (± S.D.).

conditions ($p<0.05$), suggesting that more colonies were formed below this turbulence intensity (Table 3). It appeared that a minimum turbulence intensity was required to observe larger colonies. Maximum colony diameter was higher in LT cultures (0.1638 ± 0.0159 mm) than in NT cultures (0.1121 ± 0.0058 mm). No significant differences in colony diameter were observed between turbulence treatments ($p>0.05$; Table 3).

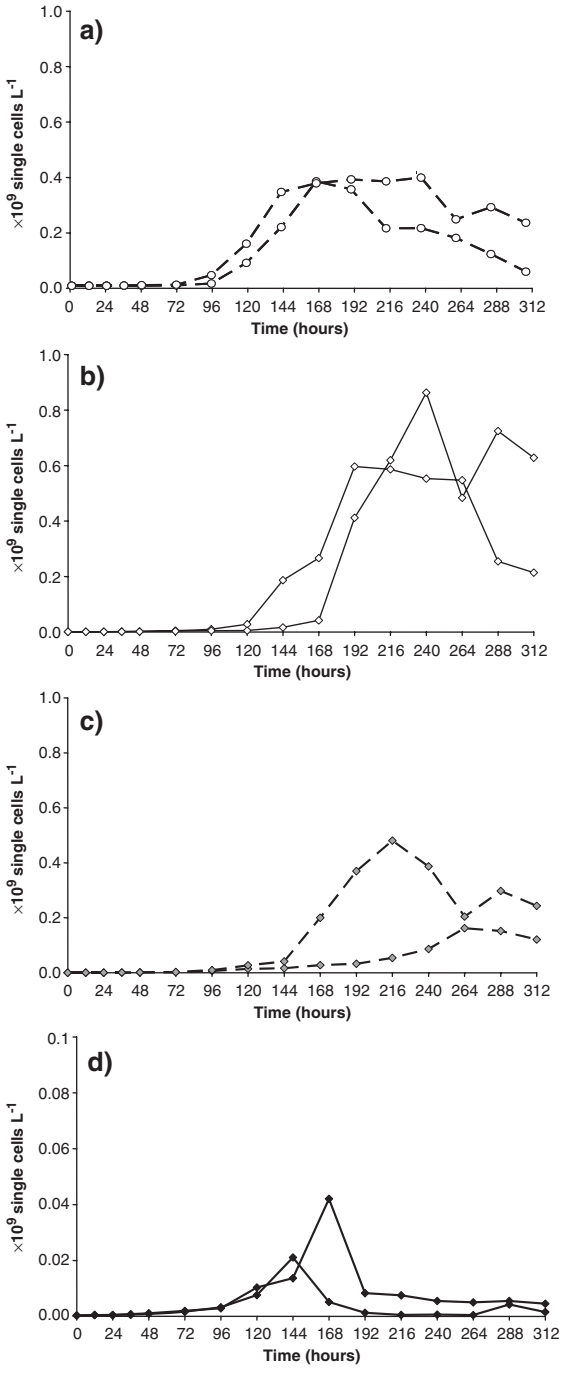

Fig. 2. Time course of single cell numbers (cells l^{-1}) for each turbulence level a) HT, b) MT, c) LT and d) NT.

3.4. Inorganic nutrients

The time course of ammonia concentrations, for each treatment, is shown in Fig. 4. In NT cultures, initial concentrations (1.45 and 1.98 μM) were lower than in turbulent microcosms (2.69 at 6.95 μM) and remained at constant level during the experiments. Under the three turbulence treatments we observed conversely a decrease in $[NH_4^+]$ during the first 72 h. Despite a small increase in $[NH_4^+]$ during the latest hours concentrations remained low. A comparative analysis revealed that there was no significant difference in the maximum apparent uptake rates, μ^*_{max} NH_4^+, between turbulence treatments (Table 3; $p>0.05$). When $[NH_4^+]$ decreased to 1 μM, we observed a regular decline in nitrate concentrations, $[NO_3^-]$ until its disappearance in the media, excepted for HT cultures (Fig. 5). The decrease in $[NO_3^-]$ appeared later when turbulent conditions were higher. Maximum apparent nitrate uptake rates, μ^*_{max} NO_3^-, was significantly higher in HT cultures, than under lower turbulent levels, i.e. NT, LT and MT cultures (Table 3; $p>0.05$). These highest values were nevertheless measured at the end of the experiment when cell concentrations were very low in HT cultures.

4. Discussion

4.1. Initial nitrogen condition

Previous laboratory studies of phytoplankton responses to different levels of turbulence have shown that the effect of turbulence was dependant on initial nutrient conditions (Petersen et al., 1998; Arin et al., 2002). In our microcosms, there were strong variations in initial $[NH_4^+]$ between duplicate experiments (2.69 at 6.95 μmol l^{-1}). However we did not observe any significant correlation between initial $[NH_4^+]$ and different growth parameters described in this study (Fig. 6). Differences in single cell growth rates, nitrate uptake, and colony formation appeared to be linked to turbulence conditions rather than initial $[NH_4^+]$.

4.2. Turbulence and nitrogen uptake rates

Small-scale turbulence is known to enhance the diffusive transport of nutrients toward phytoplankton cells (Karp-Boss et al., 1996; Alcaraz et al., 2002) by breaking boundary layers and increasing chemical

Table 3
Maximum single cell numbers, $cell_{max}$ (cells l^{-1}) ; maximum growth rates, μ_{max} ($1\ h^{-1}$); maximum colony number, $colo_{max}$ (colonies l^{-1}) ; maximum colony diameter, d_{max} (mm) ; maximum apparent ammonia uptake rate, $\rho^*_{max}\ NH_4^+$($\mu M\ cell^{-1}\ h^{-1}$) maximum apparent nitrate uptake rate, $\rho^*_{max}\ NO_3^-$ ($\mu M\ cell^{-1}\ h^{-1}$) under each turbulent level

Turbulence levels	$cell_{max}$ (cells l^{-1})	μ_{max} (j^{-1})	$colo_{max}$ (colonies l^{-1})	d_{max} (mm)	$\rho^*_{max}\ NH_4^+$($\mu M\ cell^{-1}\ h^{-1}$)	$\rho^*_{max}\ NO_3^-$($\mu M\ cell^{-1}\ h^{-1}$)
NT	4.13×10^8	1.05	2.36×10^4	0.1163	2.48×10^{-7}	4.57×10^{-7}
	4.28×10^8	1.21	3.37×10^4	0.1080	0.58×10^{-7}	2.73×10^{-7}
LT	5.97×10^8	0.76	6.06×10^4	0.1750	2.52×10^{-7}	1.98×10^{-7}
	8.63×10^8	0.92	2.69×10^4	0.1525	6.49×10^{-7}	7.59×10^{-7}
MT	1.62×10^8	0.79	1.41×10^5	0.1586	1.34×10^{-7}	2.64×10^{-7}
	4.81×10^8	0.93	8.08×10^4	0.0842	2.46×10^{-7}	3.40×10^{-7}
HT	2.11×10^7	0.67	1.35×10^4	0.0383	1.35×10^{-7}	1.68×10^{-6}
	4.20×10^7	0.66	2.36×10^4	0.0300	1.00×10^{-7}	

gradients around the cells (Estrada and Berdalet, 1997). However, our results suggest that turbulence did not have any significant effect on the apparent nutrient uptake rates by *P. globosa* cells (Table 3). We stress that this may be related to our calculation of apparent nutrient uptake rates only based on the number of single cells. As a consequence, the method used here to measure the ability of *P. globosa* cells to absorb nutrients under different turbulent conditions did not allow us to determine the proportion of uptake linked to each of the different cell forms present in the medium. In addition, at the beginning of the culture, only single cells were present, with a size range (3–5 μm) well below the Kolmogorov scale, regardless of the level of turbulence applied. At this scale, nutrient transport towards the cells was only driven by molecular diffusion. The increase in turbulence could thus not be responsible for an increase in diffusion rates. This may also explain why no significant difference was observed in the apparent uptake rates of NH_4^+at the start of the experiment in the different turbulent cultures. On the other hand, the colonies that subsequently appeared in the cultures were more subjected to a turbulent environment than in single cells. The existence of a diffusive boundary layer around *P. globosa* colonies was demonstrated by Ploug et al. (1999a) and an increase in turbulence could decrease the thickness of the diffusive boundary layer. It is thus likely to increase nutrient diffusion rates, and thus affect the development of the colonial phase of *P. globosa*.

4.3. Colony formation and P. globosa life cycle

P. globosa cells were able to form globular colonies at any turbulence level tested. However, more colonies were formed with increasing turbulence intensities until a threshold turbulence intensity above which the colony number decreased significantly in cultures. The success of colony formation thus appeared to be affected by the level of turbulence experienced by *P. globosa* cells.

Under conditions of low turbulence the diffusion-limit layer surrounding the colonies is thicker, and as a result, the diffusion of nutrients towards the colonial cells is lower. The weak development of the colonial phase, in terms of duration and density, as well as the formation of large sized colonies observed under these low turbulence conditions (LT cultures) may be the consequences of slow diffusion processes. Indeed, a limitation of the diffusive fluxes may accentuate mucus secretion by the *P. globosa* cells (Myklestad and Haug, 1972) leading to a relative increase in the colony size with respect to the number of cells making up these colonies. This hypothesis is congruent with previous results (Ploug et al., 1999b) who demonstrated that there was a significant correlation between the number of cells inside *P. globosa* colonies and the limitation of diffusive fluxes. Furthermore, within cultures subjected to low levels of turbulence, the number of single cells formed during the colonial phase was very high. The limitation of the diffusion rates of the different nitrogen forms towards colonial cells may cause single cells to be released, the latter being more competitive under nutrient-limiting conditions (Ploug et al., 1999b). The growth of single cells would therefore be sustained by the massive use of NO_3^-. Under stronger conditions of turbulence (MT cultures), the diffusive fluxes were greater (decreased in the thickness of the diffusive boundary layer) leading (i) to the development of a more intense colonial phase, in terms of the number and duration of colonies, and (ii) to the subsequent formation of smaller colonies, which have a greater cellular density in the colonial matrix. In these cultures, the development of the colonial phase, concomitant with that of the single cells of *P. globosa*, was accompanied by a significant decrease in NO_3^- concentrations, until the depletion of reserves between day 9 and 10. However, in the cultures that are subjected to maximal turbulence intensities (HT cultures), although the diffusive fluxes are supposedly greater, the colonial phase was not very developed and NO_3^- consumption remained very low. Under these conditions, the

physicochemical environment of the cells was markedly modified and other mechanisms came into play to limit the growth of single cells and the formation of *P. globosa* colonies. First experimental studies conducted on dinoflagellate cells suggested an inhibitory effect of turbulence on cell division (Berdalet, 1992; Thomas et al., 1995; Juhl and Latz, 2002). Inhibition of cell division by agitation could be due to physical disturbance of the microtubule assemblage (Karentz, 1987; Berdalet, 1992). Such inhibitory effect may occur during mitosis in *Phaeocystis*

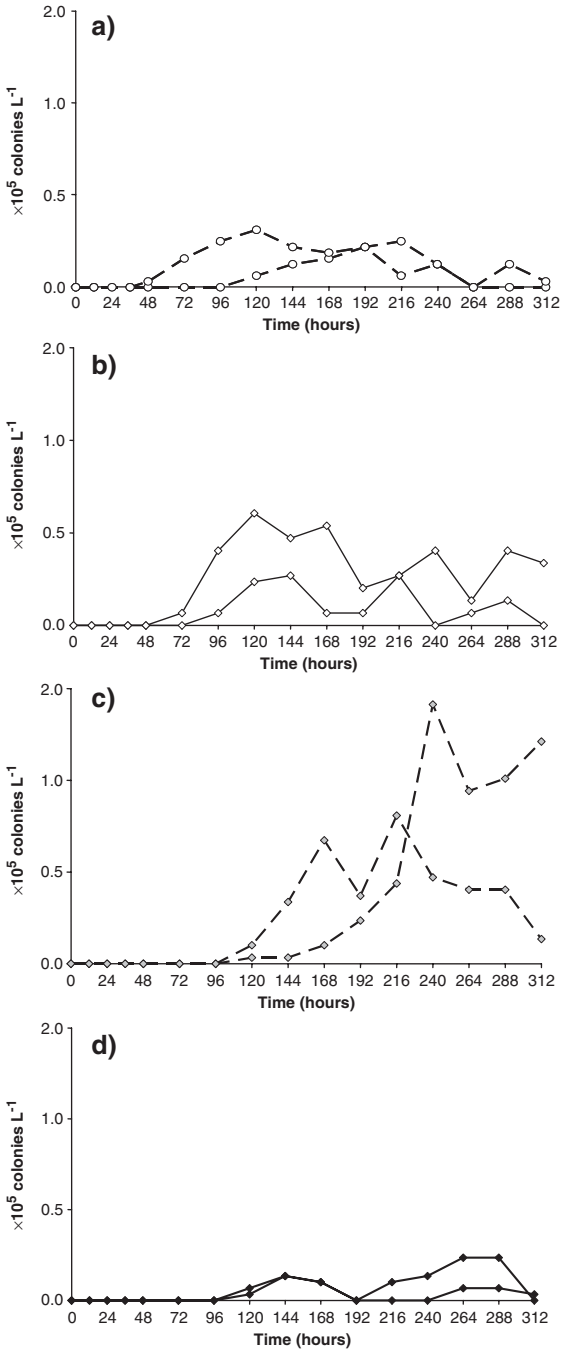

Fig. 3. Time course of colony numbers (colonies l^{-1}) for each turbulence level; a) HT, b) MT, c) LT and d) NT.

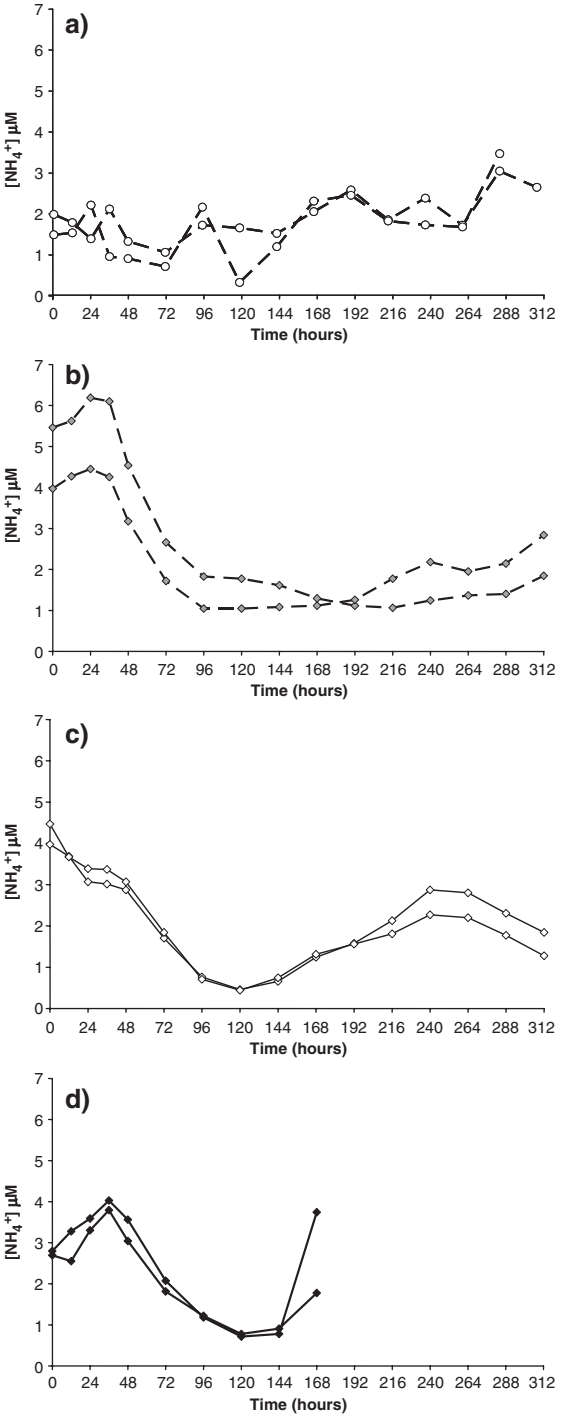

Fig. 4. Time course of ammonia concentrations [NH_4^+] (µM) for each turbulence level, a) HT, b) MT, c) LT and d) NT.

single cells exposed to high turbulent conditions, limiting cell division and thus population growth. The prolonged exposure of *P. globosa* cells to such intensities of turbulence, for example during a storm, could thus have irreversible negative effects on cell growth and the development of the colonial phase. Highly turbulent conditions therefore appear to be a factor that may limit the development of *P. globosa*.

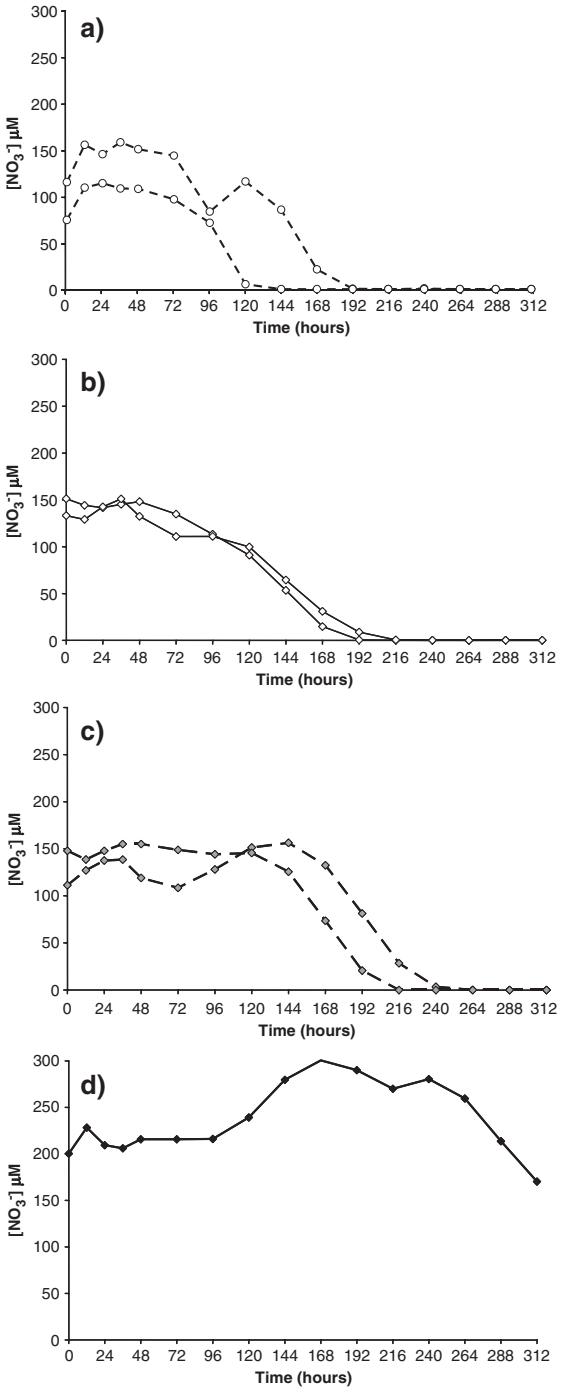

Fig. 5. Time course of nitrate concentrations [NO_3^-] (µM) for each turbulence level, a) HT, b) MT, c) LT and d) NT.

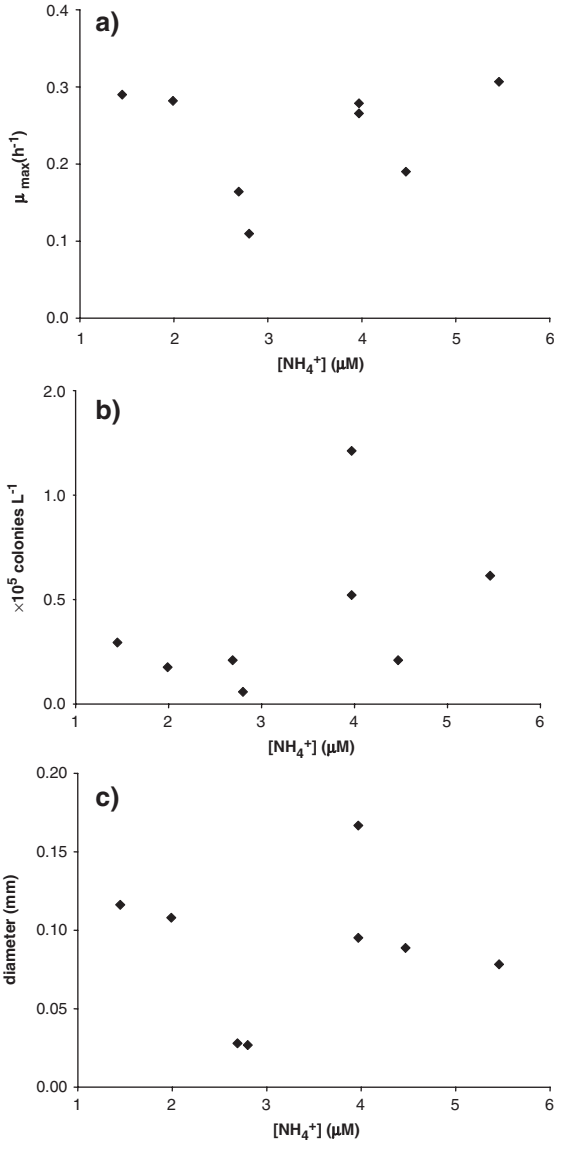

Fig. 6. a) Maximum single cell growth rates, μ_{max} (cell l^{-1} h^{-1}), b) maximum colony numbers (colo l^{-1}), c) maximum colony diameter (mm) as a function of initial [NH_4^+]i (µM) for each turbulent condition tested.

During the life cycle of *Phaeocystis*, non flagellates and flagellate cell types alternate (Pouchet, 1892; Scherffel, 1900; Kornmann, 1955; Parke et al., 1971; Moestrup, 1979; Rousseau et al., 1994; Vaulot et al., 1994). A colony starts with one non-flagellate cell, formed from a single macroflagellate (Kornmann, 1955; Cariou et al., 1994; Rousseau et al., 1994). Macroflagellates settle on solid substrates, abiotic (e.g., particles) or biotic (e.g. diatoms)

and secrete polysaccharides (Peperzak, 2002). Several physical mechanisms may bring suspended particles to collide but turbulent fluid rate is the major mechanism able to enhance the encounter rate between particles (Kiorboe, 1997). Small-scale turbulence may thus have increased the collision rate between suspended particles and macroflagellates in our experiments and thus may have enhanced colony formation. But when turbulence reached a given threshold, diffusion forces induced by turbulence may have overcome cell adhesivity, leading to a decrease in colony formation despite an increase in the encounter rate. In addition, unlike mature colonies the single cell stage lacks a rigid colony membrane (Kornmann, 1955; Cariou et al., 1994) and under high turbulent conditions, secreted polysaccharides would be diluted by enhanced diffusion before a colony matrix can be formed (Peperzak, 2002). The lack of colonies formation under high turbulence might be a consequence of these physical mechanisms.

Previous works on *P. globosa* showed that high turbulence could reverse the macroflagellates-to-colony transition (Kornmann, 1955; Cariou et al., 1994; Peperzak et al., 2000). In accordance with these observations, we observed flagellate cells after colony appearance in cultures grown under medium and high turbulent conditions (Fig. 7). Under low turbulent conditions, these flagellates could give rise to a multitude of new colonies (Kornmann, 1955). Under high turbulent condition macroflagellates-to-colony transition may then be an adaptive advantage if these flagellates are able to produce numerous new colonies when calmer conditions return.

4.4. Turbulence and inorganic carbon acquisition

Theoretically, the carbonate system is completely defined by alkalinity, the total inorganic (C_i) concentration and the pH (Stumm and Morgan, 1996). The diffusion of CO_2 from the atmosphere to the seawater is generally slower than the rate of photosynthesis (Stumm and Morgan, 1996). This implies that the bulk seawater pH will not be significantly influenced by CO_2 diffusion, even if turbulence increases in experimental microcosms.

Increased turbulence in *P. globosa* cultures led to significant decreases in mean seawater pH. Since phytoplankton CO_2 uptake can result in a rise in the pH (Sikes et al., 1980), higher pH observed under low turbulent conditions could have been a result of increased CO_2 uptake by *P. globosa* cells. This suggests a negative effect of microscale turbulence on CO_2 acquisition by *P. globosa* cells. Phytoplankton cells smaller then 1 mm are known to be surrounded by a boundary layer in which the transport of nutrients and wastes is governed by molecular diffusion (Lazier and Mann, 1989). Turbulence decreases

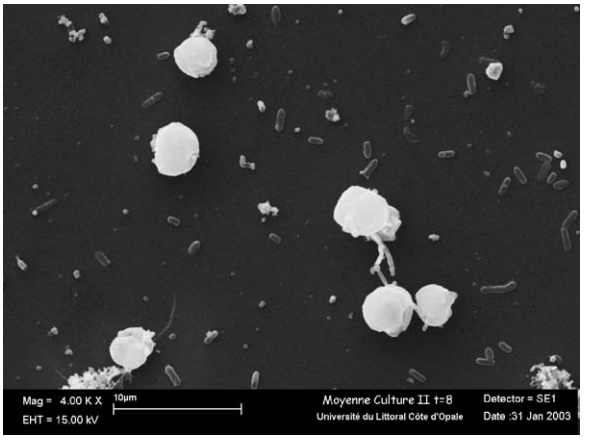

Fig. 7. Flagellated cells of *P. globosa* observed under moderate turbulent conditions during the colonial phase.

the size of this boundary layer and thus the diffusion path length and enhances the flux of inorganic carbon towards phytoplanktonic cells. Perperzak (2002) concluded that an increase of fluxes occurs when colonies grow beyond 500 and 1000 µm in diameter under high (1 s^{-1}) and low (0.01 s^{-1}) shear rates respectively. In our cultures, colonies were smaller than 500 µm diameter under all turbulent conditions, and in accordance with Peperzak (2002) we did not observe any increase in CO_2 acquisition with increasing turbulence. *P. globosa* cells convert HCO_3^- to CO_2 extracellularly by the enzyme carbonic anhydrase and the concomitant production of OH^- does increase the pH (Elzenga et al., 2000). The increase of the pH in the medium causes a modification in the carbonate equilibrium and thus contributes to the decrease in CO_2 concentrations. Extracellular carbonic anhydrase is expressed by many but not all autotrophic species of aquatic unicellular protists (Hobson et al., 2001). The diatoms and other species of phytoplankton that coexist with *P. globosa* in the medium and who cannot compensate for this deficit in CO_2 by using HCO_3^-, will see their growth rates decline due to the limitation of C_i (Peperzak, 2002). The growth rates of several species of diatoms are reduced at a high pH (Hinga, 1992; Riebesell et al., 1993; Chen and Durbin, 1994). Under low turbulence conditions, CO_2 and the pH induced limitation may thus be important clues to understanding the seasonal dominance of *Phaeocystis* over diatoms.

4.5. Turbulence in microcosms

Generation of ecologically relevant turbulence in the laboratory is challenging due to the complexity of turbulent forcing mechanisms in nature and the requirement for a large range of turbulent eddy scales (Sullivan et

al., 2003). Most methods create unrealistically high turbulence intensities (Peters and Redondo, 1997). The apparatus used in this study allowed us to generate three realistic intensities of three-dimensional and isotropic small-scale turbulence.

Although seldom investigated, and as suggested above, small-scale turbulence could be a critical factor in determining which taxon dominates the ecosystem. The effect of small-scale turbulence should be treated much like the effects of light, nutrients, or temperature on different phytoplankton species, as a potential physiological factor in the ecology of many phytoplankton (Sullivan and Swift, 2003). The present laboratory experiments are the first step toward understanding the mechanisms of interaction between small-scale turbulence and *P. globosa*. At a cellular level, turbulence is capable of influencing nitrogen, inorganic carbon uptakes, and single cells division rates. These phenomena could have significant consequences on population growth and on the development of the colonial phase, and thus at larger scale on seasonal phytoplankton succession.

4.6. Role of turbulence on seasonal phytoplankton successions

Under high turbulent conditions *P. globosa* population growth appeared highly limited. On the other hand, numerous microcosms studies have demonstrated the positive effect of turbulence on the growth of large sized diatoms namely by increasing nutrient uptake rates (Estrada et al., 1998; Arin et al., 2002; Alcaraz et al., 2002; Delaney and Knoechel, 2004; Metcalfe et al., 2004). These observations are in agreement with Margalef's Mandala (1978), associating the development of diatoms with highly turbulent zones. High turbulence levels appear to favour the development of diatoms over *P. globosa*. Below a given threshold (between high and moderate), turbulent conditions seem to control the development (intensity and quality) of the colonial phase. This differential development, depending on the level of turbulence experienced by *P. globosa* cells, can differently modify phytoplankton cells' environment, and thus the growth of co-occurring microalgae. We thus hypothesise that small-scale turbulence can play a critical role on the *P. globosa*-diatoms successions and the amplitude of the spring bloom of *P. globosa*. In particular the high frequency occurrence of strong turbulence events ($\varepsilon > 10^{-5}$ m^2 s^{-3}) in the Eastern English Channel, when compared to other areas where *P. globosa* is also massively blooming (e.g. the southern Bight of North Sea), can provide some phenomenological explanation of the observed differences in bloom amplitude (e.g. Lancelot et al., 2005).

5. Conclusions

Investigations of the effects of realistic levels of small-scale turbulence on *P. globosa* growth rates and colony formation showed differential results suggesting adaptive responses that may lead to a competitive advantage. Such factors may be critically important to understand the dominance of *P. globosa* over diatoms in the coastal waters of the Eastern English Channel. As turbulence increased to a threshold ($\varepsilon = 10^{-5}$ m^2 s^{-3}), *P. globosa* single cells exhibited slower growth rates, an *a priori* negative effect, thought here to be an adaptive response leading to an enhanced colony formation. Above this turbulent threshold the prolonged exposure of *P. globosa* cells to such intensities of turbulence, could have irreversible negative effects on cell growth and on the development of the colonial phase. However, the potential fragmentation of large colonies, and possible formation of flagellated cells under high turbulent conditions might be competitive advantages for *P. globosa* when calmer conditions again prevail in natural waters. This study clearly showed significant effects of small-scale turbulence on critical processes of *P. globosa* growth and life cycle, indicating that small-scale turbulence may be an important factor controlling the seasonal phytoplankton succession in the Eastern English Channel.

Acknowledgements

The authors thank Dr. Louis Peperzak (University of Groningen) for providing the *P. globosa* strain, and two anonymous reviewers for their helpful comments. The help of Dr. Lucie Courcot (Université du Littoral Côte d'Opale) in making the EM pictures is gratefully acknowledged. Dr. R.L. Waters is greatly acknowledged for for her constructive comments and discussion, and for improving the language. This study was financially and infrastructurally supported by the Université des Sciences et Technologies de Lille, the Centre National de la Recherche Scientifique (CNRS), the Contrat de Plan Etat-Région (CPER) '*Phaeocystis*' and the Programme National d'Environnement Côtier (PNEC), Chantier '*Manche Orientale-sud Mer du Nord*'. **[SS]**

References

Alcaraz, M., Marrasé, C., Peters, F., Arin, L., Malits, A., 2002. Effects of turbulence conditions on the balance between production and

respiration in marine planktonic communities. Mar. Ecol. Prog. Ser. 242, 63–71.
Arin, L., Marrasé, C., Maar, M., Peters, F., Sala, M.-M., Alcaraz, M., 2002. Combined effects of nutrients and small-scale turbulence in a microcosm experiment. I. Dynamics and size distribution of osmotrophic plankton. Aquat. Microb. Ecol. 29, 51–61.
Berdalet, E., 1992. Effect of turbulence on the marine dinoflagellate *Gymnodinium nelsonii*. J. Phycol. 28, 267–272.
Cadée, G.C., Hegeman, J., 1986. Seasonal and annual variation in *Phaeocystis pouchetii* (Haptophyceae) in the westernmost inlet of the Wadden Sea during the 1973 to 1985 period. Neth. J. Sea Res. 20, 29–36.
Cariou, V., Casotti, R., Birrien, J.L., Vaulot, D., 1994. The initiation of *Phaeocystis* colonies. J. Plankton Res. 16, 457–470.
Chen, C.Y., Durbin, E.G., 1994. Effects of pH on the growth and carbon uptake of marine phytoplankton. Mar. Ecol. Prog. Ser. 109, 83–94.
Denman, K.L., Gargett, A.E., 1983. Vertical mixing and advection of phytoplankton in the upper ocean. Limnol. Oceanogr. 28, 801–815.
Delaney, M.P., Knoechel, R., 2004. Turbulence effects on cold microbial communities: an enclosure study. J. Mar. Syst. 49, 123–131.
Elzenga, J.T.M., Prins, H.B.A., Stefels, J., 2000. The role of extracellular carbonic anhydrase activity in inorganic carbon utilization of *Phaeocystis globosa* (Prymnesiophyceae): a comparison with other algae using the isotopic disequilibrium technique. Limnol. Oceanogr. 45, 372–380.
Estrada, M., Berdalet, E., 1997. Phytoplankton in a turbulent world. Sci. Mar. 61, 125–140.
Estrada, M., Marrasé, C., Alcaraz, M., 1998. Phytoplankton response to intermittent stirring and nutrient addition in marine microcosms. Mar. Ecol. Prog. Ser. 48, 225–234.
Gieskes, W.W.C., Kraay, G.W., 1975. The phytoplankton spring bloom in Dutch coastal waters of the North Sea. Neth. J. Sea. Res. 9, 166–196.
Guillard, R.R.L., 1975. Culture of marine invertebrate animals. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), pp. 30–60. New York and London.
Hinga, K.R., 1992. Co-occurence of dinoflagellate bloom and high pH in marine enclosures. Mar. Ecol. Prog. Ser. 86, 181–187.
Hobson, L.A., Hanson, C.E., Holeton, C., 2001. An ecological basis for extracellular carbonic anhydrase in marine unicellular algae. J. Phycol. 37 (5), 717–723.
Jenkinson, I.R., 1986. Oceanographic implications of non-Newtonian properties found in phytoplankton cultures. Nature 323, 435–437.
Juhl, A.R., Latz, M.I., 2002. Mechanisms of fluid shear-inducted inhibition of population growth in a red-tide dinoflagellate. J. Phycol. 38, 683–694.
Karentz, D., 1987. In: Kumar, D.H. (Ed.), Dinoflagellate Cell Cycles, *Phycotalk*. Print House, India, pp. 377–397.
Karp-Boss, L., Boss, E., Jumars, P.A., 1996. Nutrients fluxes to planktonic osmotrophs in the presence of fluid motion. Oceanogr. Mar. Biol. Annual Revue 34, 71–107.
Karp-Boss, L., Boss, E., Jumars, P.A., 2000. Motion of dinoflagellates in a simple shear flow. Limnol. Oceanogr. 45, 1594–1602.
Kiorboe, T., 1997. Small-scale turbulence, marine snow formation, and planktivorous feeding. Sci. Mar. 61, 141–158.
Kolmogorov, A.N., 1941. The local structure of turbulence in incompressible viscous fluid for very large Reynold's numbers. Dokl. Acad. Nauk. SSSR 30, 299–303.
Kornmann, P., 1955. Beobachtungen an *Phaeocystis*-Kulturen. Helgoänder wiss. Meersunters 5, 218–233.
Koroleff, F., 1969. Direct determination of ammonia in natural waters as indophenol blue. Int. Cons. Explor. Sea 9, 1–6.
Lancelot, C., Mathot, S., 1985. Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short- and long-term incubations with ^{14}C bicarbonate. II. *Phaeocystis pouchetti* colonial population. Mar. Biol. 86, 227–232.
Lancelot, C., Spitz, Y., Gypens, N., Ruddick, K., Becquevort, S., Rousseau, V., Lacroix, G., Billen, G., 2005. Modelling diatom and *Phaeocystis* blooms and nutrient cycles in the Southern Bight of the North Sea: the MIRO model. Mar. Ecol. Prog. Ser. 289, 63–78.
Lazier, J.R.N., Mann, K.H., 1989. Turbulence and the diffusive layers around small organisms. Deep Sea Res. 36, 1721–1733.
Legendre, L., Demers, S., LeFaivre, D., 1986. Biological production at marine ergoclines. In: J.C.J.N. (Ed.), Marine Interfaces Ecohydrodynamics. Elsevier, Amsterdam, pp. 1–54.
Margalef, R., 1997. Turbulence and marine life. Sci. Mar. 61, 109–123.
Margalef, R., 1978. Life forms of phytoplankton as survival alternatives in an unstable environment. Oceanol. Acta 1, 493–509.
Matrai, P.A., Vernet, M., Hood, R., Jennings, A., 1995. Light-dependence of carbon and sulfur production by polar clones of the genus *Phaeocystis*. Mar. Biol. 124, 157–167.
Metcalfe, A.M., Pedley, T.J., Thingstad, T.F., 2004. Incorporating turbulence into a plankton foodweb model. J. Mar. Syst. 49, 105–122.
Meyer, A.A., Tackx, M., Daro, N., 2000. Xanthophyll cycling in *Phaeocystis globosa* and *Thalassiosira* sp.: a possible mechanism for species succession. J. Sea Res. 43, 373–384.
Moestrup, O., 1979. Identification by electron microscopy of marine nanoplankton from New Zealand, including the description of four new species. New Zeal. J. Bot. 17, 61–95.
Myklestad, S., Haug, A., 1972. Production of carbohydrates by the marine diatom Chaetoceros affinis var. willei (Gran) Hustedt. I. Effects of the concentration of nutrients in the culture medium. J. Exp. Mar. Biol. Ecol. 9, 125–136.
Parke, M., Green, J.C., Manton, I., 1971. Observations on the fine structure of zoids of the genus *Phaeocystis* (Haptophyceae). J. Mar. Biol. Assoc. UK 51, 927–941.
Peperzak, L., 2002. The wax and the wane of *Phaeocystis globosa* blooms. Rijksuniversiteit Groningen, Pays Bas, Groningen, p. 254.
Peperzak, L., Colijn, F., Gieskes, W.W.C., Peeters, J.C.H., 1998. Development of the diatom—*Phaeocystis* spring bloom in the Dutch coastal zone of the North Sea: the silicon depletion versus the daily irradiance threshold hypothesis. J. Plankton Res. 20, 517–537.
Peperzak, L., Colijn, F., Vrieling, E.G., Gieskes, W.W.C., Peeters, J.C.H., 2000. Observations of flagellates in colonies of *Phaeocystis globosa* (Prymnesiophyceae); a hypothesis for their position in the life cycle. J. Plankton Res. 22, 2181–2203.
Peters, F., Redondo, J.M., 1997. Turbulence generation and measurement : application to studies on plankton. Sci. Mar. 61, 205–228.
Petersen, J.E., Sanford, L.P., Kemp, W.M., 1998. Coastal plankton responses to turbulent mixing in experimental ecosystems. Mar. Ecol. Prog. Ser. 171, 23–41.
Ploug, H., Stolte, W., Epping, H.G., Jorgensen, B.B., 1992a. Diffusive boundary layers, photosynthesis, and respiration of the colony-forming plankton algae, Phaeocystis sp. Limnol. Oceanogr. 44 (8), 1949–1958.
Ploug, H., Stolte, W., Jorgensen, B.B., 1992b. Diffusive boundary layers of the colony-forming plankton alga Phaeocystis sp.-implications for nutrient uptake and cellular growth. Limnol. Oceanogr. 44 (8), 1959–1967.
Pouchet, M.G., 1892. Sur une algue pélagique nouvelle. Compt. Rend. Hebd. Séances Mém. Soc. Biol. 44, 34–36.

Raffel, M., Willert, H., Meier, G.E.A., 1998. Particle image velocimetry. Springer, Berlin.

Riebesell, U., Wolf-Gladrow, D.A., Smetacek, V., 1993. Carbon dioxide limitation of marine phytoplankton growth rates. Nature 361, 249–251.

Rousseau, V., Vaulot, D., Casotti, R., Cariou, V., Lenz, J., Gunkel, J., Baumann, M.E.M., 1994. The life cycle of *Phaeocystis* (Prymnesiophyceae): evidence and hypotheses. J. Mar. Syst. 5, 23–39.

Savidge, G., 1981. Studies of the effect of small-scale turbulence on phytoplankton. J. Mar. Biol. Assoc. UK 61, 477–488.

Scherffel, A., 1900. *Phaeocystis globosa* nov. spec. nebst einigen Betrachtungen Über die phylogenie niederer, indbesondere brauner Organismen. Wiss. Meer. Abteilung Helgoland N. F. Bd. 4, 1–29.

Seuront, L., Gentilhomme, V., Lagadeuc, L., 2002. Small-scale nutrient patches in tidally mixed coastal waters. Mar. Ecol. Prog. Ser. 232, 29–44.

Seuront, L., Yamazaki, H., Souissi, S., 2004. Hydrodynamic disturbance and zooplankton swimming behavior. Zool. Studi. 43, 376–387.

Sikes, C.S., Roer, R.D., Wilbur, K.M., 1980. Photosynthesis and coccolith formation: Inorganic carbon sources and net inorganic reaction of deposition. Limnol. Oceanogr. 25, 248–261.

Smayda, T.J., Reynolds, C.S., 2000. Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellates blooms. J. Plankton Res. 23, 447–461.

Stiansen, J.E., Sundby, S., 2001. Improved methods for generating and estimating turbulence in tanks suitable for fish larvae experiments. Sci. Mar. 65, 151–167.

Stumm, W., Morgan, J.J., 1996. Aquatic Chemistry. Wiley J. and Sons, New-York.

Sullivan, J.M., Swift, E., 2003. Effects of small-scale turbulence on net growth rate and size of ten species of marine dinoflagellates. J. Phycol. 39, 83–94.

Sullivan, J.M., Swift, E., Donaghay, P.L., Rines, J.E.B., 2003. Small-scale turbulence affects the division rate and morphology of two red-tide dinoflagellates. Harmful Algae 2, 183–199.

Thomas, W.T., Vernet, M., Gibson, C.H., 1995. Effects on small-scale turbulence on photosynthesis, pigmentation, cell division, and cell size in the marine dinoflagellate *Gonyaulax polyedra* (dinophyceae). J. Phycol. 31, 50–59.

Thomson, S.M., Turner, J.S., 1975. Mixing across an interface due to turbulence generated by an oscillating grid. J. Fluid Mech. 67, 349–368.

Throndsen, J., 1978. Preservation and storage. In: Sournia, A. (Ed.), Phytoplankton manual. UNESCO, Paris, pp. 69–74.

Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt. int. Verein. Limnol. 9, 1–39.

Van Boekel, W., 1992. Phaeocystis colony mucus components and the importance of calcium ions for colony stability. Mar. Ecol. Prog. Ser. 87, 301–305.

Vaulot, D., Birrien, J.L., Casotti, D., Veldhuis, R., Kraaij, M.J.W., Chrétiennot-Dinet, G.W., 1994. Morphology, ploidy, pigment composition, and genome size of cultured strains of *Phaeocystis* (Prymnesiophyceae). J. Phycol. 30, 1022–1035.

Zar, J.H., 1996. Biostatistical analysis. Prentice Hall International Editions. 662 pp.