The wax and wane of Phaeocystis globosa blooms
Peperzak, Louis

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Chapter 8

Inorganic carbon availability influences cell numbers in colonies of *Phaeocystis globosa* (Prymnesiophyceae)

“Despite a centennial of investigations, factors influencing the inception and regulation of *Phaeocystis* blooms are poorly understood”

P.G. Verity, T.J. Smayda and E. Sakshaug (1991)
ABSTRACT. The phenomenon that *Phaeocystis globosa* colonies gradually disappear in f/2, a culture medium in which inorganic carbon (C), and CO\(_2\) in particular, is a potentially limiting nutrient was investigated in three steps. First, it was demonstrated by measuring pH and alkalinity changes during batch culture growth, that *P. globosa* cells take up CO\(_2\) and not the major C species HCO\(_3^-\). Second, the effect of C availability on colony formation was investigated by aerating one litre batch cultures with 0, 8 and 80 ml air minute\(^{-1}\). Increased aeration did not lead to higher colony concentrations or larger colonies. However, it did lead to significant increases in the numbers of cells per colony: from 0.32 ± 0.10 to 0.87 ± 0.18 and 1.33 ± 0.13 cells per 1000 µm\(^2\) colony surface while in the non- and medium-aerated cultures organic carbon δ\(^{13}\)C, an indicator of CO\(_2\)-stress, was significantly less negative (-24.8 ± 0.3 ‰) than in the high-aerated culture (-26.3 ± 0.2 ‰). Third, a C\(_i\) uptake model for *P. globosa* was developed on the argument that by raising the interior pH to 9 growing colonies establish CO\(_2\), OH\(^-\) and HCO\(_3^-\) gradients with the bulk sea water (pH 8), which lead to fluxes of CO\(_2\) and HCO\(_3^-\) into, and of OH\(^-\) out of the colony. These fluxes were calculated as a function of colony diameter and turbulence (both influencing diffusion from and towards the colony surface) and the pH difference (ΔpH) between the colony and the bulk seawater. It is shown that despite high turbulence, relatively large (> 500 µm diameter) colonies are prone to C\(_i\) limitation when bulk seawater pH rises to 8.8 (ΔpH = 0.2), which may well occur during batch culture growth. Under such circumstances the number of cells in colonies decreases, the percentage of solitary cells will increase and eventually the number of colonies in subsequent cultures declines.

Introduction

*Phaeocystis* colonies are found world-wide in nutrient-rich seas where they can dominate the phytoplankton assemblage in terms of cell numbers and organic carbon (Kashkin 1963, Gieskes and Kraay 1975, Davidson and Marchant 1992, Peperzak *et al.* 1998) which makes *Phaeocystis* a prominent genus in the global carbon cycle (Smith *et al.* 1991, Verity and Smetacek 1996, Arrigo *et al.* 1999). Over the years the importance of *Phaeocystis* has lead to a variety of culture studies. Unfortunately, the amount of colonies that *P. globosa* and *P. pouchetii* produce declines in nutrient-rich culture media such as f/2 (Stein 1975) and repetitive transfer in ‘nutrient-poor’ media (f/20 - f/50) is necessary to maintain colonial growth (Guillard and Hellebust 1971, Verity *et al.* 1991). Understanding how nutrient concentrations influence colony development in cultures will not only lead to improved culture methods but it can also provide new insights on *Phaeocystis* growth in the sea.

One reason for the reduction in colony abundance appears to be the high concentration of inorganic phosphorus (P\(_i\) ≥ 36 µmol l\(^{-1}\)) at f/2 nutrient levels. Cariou *et al.* (1994) found that high initial P\(_i\) concentrations (5 µmol l\(^{-1}\)) delayed
the appearance of *P. globosa* colonies and Veldhuis and Admiraal (1987) measured less than 20% colonial *P. globosa* cells in cultures with P$_i$ over 10 µmol l$^{-1}$.

A second reason for the decline of *Phaeocystis* colonies may be related to the relatively low inorganic carbon (C$_i$) concentration in f/2. The total amount of C$_i$ in seawater is 2200 µmol l$^{-1}$ so that in f/2 medium the C$_i$ : N$_i$ : P$_i$ ratio is 2200 : 880 : 36, or 61 : 24 : 1. The general C : N : P ratio of phytoplankton cells is 106 : 16 : 1 (Redfield *et al.* 1963) which means a potential C$_i$ shortage in f/2 by a factor of 2 relative to N$_i$ and P$_i$.

C$_i$ limitation may even be more severe if the form of inorganic carbon that the phytoplankton take up from the medium is CO$_2$, as is the case for certain marine diatoms (Riebesell *et al.* 1993). CO$_2$ is the substrate for RUBISCO, the primary inorganic carbon fixing enzyme in marine phytoplankton (Raven and Johnston 1991). At a normal seawater pH of 8, the CO$_2$ fraction of C$_i$ is less than 1% (Stumm and Morgan 1996) and, therefore, the CO$_2$ : N$_i$ : P$_i$ ratio in f/2 medium is approximately 1 : 24 : 1.

By shifts in the seawater carbonate system, CO$_2$ can be formed by dehydration of HCO$_3^-$: However, the rates of such uncatalysed dehydration and of CO$_2$ diffusion to the cell surface are small compared to potential CO$_2$ fixation rates (Gavis and Ferguson 1975, Riebesell *et al.* 1993). Furthermore, HCO$_3^-$ dehydration also leads to OH$^-$ production, an increase in pH and hence a further decrease of CO$_2$ concentration if the seawater is not in equilibrium with the atmosphere.

In view of the important role attributed to *Phaeocystis* blooms in the global carbon cycle, it is surprising that the acquisition of inorganic carbon has not been studied extensively. In this chapter several aspects of carbon acquisition by *P. globosa* are investigated: (i) the species of C$_i$ taken up, (ii) the effect of C$_i$ availability on colonial growth (colony concentration and the number of cells per colony) and, (iii) the effect of pH and turbulence on inorganic carbon uptake by growing, differently sized colonies.

**Method**

**C$_i$ uptake assay**

Cultures of non-flagellated colonial-type cells and mesoflagellates of *Phaeocystis globosa* clone Ph91 (Peperzak *et al.* 2000c), and calcifying (clone Ch24-90) and non-calcifying (clone LN) *Emiliania huxleyi* (van Bleijswijk *et al.* 1994) were grown in 100 ml PEP-Si (Peperzak *et al.* 2000a) with a salinity of 32.3 psu, at 15°C, 36 W m$^{-2}$ irradiance in a 12:12 L:D cycle. The calcifying (CO$_2$ and HCO$_3^-$ using) and non-calcifying (CO$_2$ using) *E. huxleyi* clones were used as controls for the assay's ability to detect decreases in alkalinity (HCO$_3^-$ uptake)
and increases in pH (CO₂ uptake). All cultures and experiments were done in an orbital incubator set at 90 rotations per minute. Both types of *P. globosa* grew as solitary cells. Calcification of *E. huxleyi* clone Ch24-90 was confirmed microscopically. Biomass was measured as in vivo fluorescence with a Hitachi F-2000 (λex = 435 nm, λem = 683 nm). Cultures were transferred to fresh culture medium when the stationary growth phase had been reached.

The measurement of inorganic carbon (Cᵢ)-uptake was a slight modification of the method described by Sikes *et al.* (1980). All cultures were diluted with PEP-Si medium one day before the experiments to ensure continued exponential growth. Cᵢ-uptake measurements were performed in the light period and were started by adding 25 ml culture to air-equilibrated 100 ml PEP-Si medium. Dark control flasks were covered with foil. Both light and dark incubations were made in duplicate. pH and alkalinity samples were taken after 1 and 6 hours incubation and processed directly (see below). The remaining culture was fixed with acid Lugol (0.4% v/v) or paraformaldehyde (0.1% w/v) and the cell concentrations were measured (see below). The pH and alkalinity changes during the five hour incubation were calculated as the difference between dark and light flasks. All cultures were assayed twice, on different days, bringing the total number of measurements for each clone at four. Because the assimilation of either NO₃⁻ or NH₄⁺ will lead to an increase or decrease of alkalinity respectively (Stumm and Morgan 1996), control Cᵢ-uptake measurements were made with non-flagellated *P. globosa* cells cultured in PEP-Si with the NO₃⁻ replaced by NH₄⁺.

**Cᵢ availability and colonial growth**

*Phaeocystis globosa* strain 677-3 was inoculated at a concentration of 10⁶ cells l⁻¹ in 1 litre PEP-Si culture medium in airtight 1.14 litre Schott bottles. The medium had a salinity of 31.9 psu, and NO₃⁻ and PO₄³⁻ were ten times regular strength (1000 and 63 µmol l⁻¹ respectively), i.e. at approximately f/2 levels (Stein 1975). Temperature was 23 ± 1°C. Irradiance was 12 W m⁻² and was provided 24 h day⁻¹ in order to prevent an effect of diel changes on the measurements (Flynn and Davidson 1993). Air from a well ventilated laboratory, i.e. with a constant CO₂ and δ¹³C content, was supplied at 0 ml min⁻¹, 8 ml min⁻¹ and 80 ml min⁻¹ through tubes with a fritted glass end; the three cultures will be referred to in the text as non-, medium- and high-aerated cultures.

**growth rate and biomass measurements**

Growth rates were computed from in vivo fluorescence values with a logistic model (Peperzak *et al.* 2000a) using SYSTAT software (Wilkinson 1990). The colony concentrations (detection limit: 5 to 15 colonies ml⁻¹) were measured in non-fixed 2 ml subsamples on a Zeiss inverted microscope (magnification 63x).
In early stationary growth phase (day 15, 16 and 17) the colony diameter was measured from 12 colonies per sample (accuracy = ½ eyepiece graticule unit = 3.3 µm). The number of cells per colony was counted on day 16 and 17 in ca. 12 colonies per sample. After pH-alkalinity measurements on day 15, 16 and 17, the samples were fixed with Lugol’s iodine, colonial cells were released by shaking and cell counts were made with a Coulter Counter equipped with a 50 µm aperture tube and a model II Multisizer. The mean spherical equivalent diameter (s.e.d.) and standard deviation of ca. 1000 cells was calculated. The percentage of colonial cells on days 16 and 17 was calculated as \((\text{colony concentration} \times \text{mean number of cells per colony} / \text{total cell concentration}) \times 100\%\).

**pH, alkalinity, \(C_i\) and \(CO_2\) determinations**

pH was measured with a Schott H61 glass-combi electrode and a Schott CG 811 pH meter, calibrated with pH 4 and pH 7 buffers (Baker), in 25 ml sample. The precision was ± 0.01 pH unit. The sample was titrated with 0.01 N HCl (Baker) to pH 4.5 (a ml HCl) and pH 4.2 (b ml HCl). The total alkalinity in milliequivalents (meq) per litre was calculated as: \((2 \times a - b) \times 0.01 \times 1000/25\) (Standard methods for the examination of water and waste water 1975) and had a precision of ± 0.005 meq l⁻¹. The total inorganic carbon (\(C_i\)) and \(CO_2\) concentrations were calculated according to Strickland and Parsons (1972) using a computer program that extended their tabled values of the parameters \(A, F_T\) and \(F_P\) at pH > 8.6 by linear or polynomial extrapolation.

**measurements of \(\delta^{13}C\) and particulate constituents**

The \(^{13}C/^{12}C\) ratio of the particulate carbon was measured as a \(CO_2\)-stress indicator. RUBISCO discriminates against the naturally occurring \(^{12}C\) isotope and, expressed as \(\delta^{13}C\), less negative \(\delta^{13}C\) values indicate \(CO_2\)-stress (Johnston and Raven 1992, Beardall et al. 1998). On day 15, 16 and 17, 50 ml samples for \(\delta^{13}C\) and simultaneous PON and POC analysis were filtered over pre-combusted GF/C glass fibre filters under positive pressure. The filters were dried overnight at 60°C, treated with HCl fume, and combusted in a Carlo-Erba CHN model EA 1180 analyser, yielding PON and POC concentrations. The carbon dioxide was collected in a liquid nitrogen coldtrap and analysed with a Sira II mass spectrometer. The \(^{12}C/^{13}C\) ratio of the seawater was measured after adding 5 ml 2.5 M H₂SO₄ to 50 mL GF/C filtered culture; the carbon dioxide was collected and analysed as described. The \(^{12}C/^{13}C\) ratio was measured relative to Peedee belemnite (PDB). Deviations from this standard are expressed as:

\[
\delta^{13}C = ((^{13}C/^{12}C \text{ sample} / ^{13}C/^{12}C \text{ PDB}) - 1) \times 1000 \text{ [‰]} \quad [8.1]
\]
The precision in the δ^{13}C measurement was ± 0.5‰. In addition 10 ml samples for Chl a analysis were filtered over GF/C filters and stored directly at -70°C. Chl a was extracted according to Gieskes and Kraay (1984) and analysed by HPLC as described by Escaravage et al. (1995).

**statistics**

The overall significance of aeration on the culture variables was calculated in one-way ANOVA’s, followed by Tukey post hoc tests for individual differences among the treatments (Wilkinson 1990). When variance was high, as in the colonial data, a non-parametric (Kruskal-Wallis) test was carried out (Wilkinson 1990). Secondly, the 95% confidence interval (c.i.) around the means of cell measurements and particulate constituents was calculated as:

\[
\text{c.i.} = t_{0.1}(\text{df}) \cdot \text{stdev} / \sqrt{n}
\]

in which \(n\) = number of measurements, \(\text{stdev}\) = the standard deviation from the mean and \(t_{0.1} = \) the value of \(t\) from a \(t\)-distribution at degrees of freedom (df) = \(n - 1\) and \(\alpha = 0.1\) (one-sided test). If the confidence intervals of two means do not overlap, than the means are significantly different at \(P < 0.05\). The benefit of calculating confidence intervals is that the means of each variable can be compared for significant differences individually, while Tukey post hoc tests provide two matrices, one of the absolute differences between means, the second of the levels of their significance. Comparisons of both methods, ANOVA + Tukey and 95% confidence intervals, with data obtained in the CO\(_2\) supply experiment did not lead to different conclusions on the significance of differences between individual means at \(P < 0.05\).

**Results**

**C\(_i\) uptake assay**

All clones of *E. huxleyi* and *P. globosa* took up CO\(_2\) during incubation in the light, leading to a rise in pH of the medium (Figure 8.1). Net changes in alkalinity could not be measured in cultures of the non-calcifying *E. huxleyi* and the flagellates of *P. globosa*. NO\(_3^-\) assimilating non-flagellated *P. globosa* cells increased alkalinity of the medium while the same cell type decreased alkalinity when assimilating NH\(_4^+\). In contrast, calcifying *E. huxleyi* cells in a NO\(_3^-\) containing medium decreased alkalinity.
Ci availability and colonial growth

After a lag period of 1 to 2 days *P. globosa* biomass began to increase exponentially (Figure 8.2a). The growth rate was lowest in the high-aerated culture (Table 8.1). Flagellated cells were seen in the high-aerated culture regularly with an abundance of approximately 5%. These flagellates had the same size as non-flagellated cells. Flagellates were observed once in the medium-aerated, but never in the non-aerated culture.

The medium-aerated culture reached the stationary growth phase on day 14 (Figure 8.2a), and it attained the highest biomass yields (Table 8.1). The differences between the cultures in biomass yield (PON, Chlorophyll *a* and cell concentration) were significant (Table 8.1, ANOVA *P* < 0.05). However, there were no significant differences in C:N and C:Chl *a* ratios, nor in C and Chl *a* on a per cell basis between the three aeration treatments (Table 8.2, ANOVA *P* > 0.05). The concentration of colonies in the three cultures followed the trend in biomass development (Figure 8.2b). Colony development in the high-aerated culture lagged behind that in the other two, but eventually a mean of 1500 colonies l⁻¹ was reached in all cultures (Figure 8.2b). The mean colony concentration (Figure 8.2b, ANOVA *P* > 0.05) and colony diameter (Figure 8.3a, Kruskal-Wallis test *P* > 0.05) were not significantly different among aeration regimes.

![Figure 8.1. Rates of pH and alkalinity changes by cells of *Emiliania huxleyi* clone Ch24-90 (calcifying, Ehux+), clone LN (non-calcifying, Ehux-), and of *Phaeocystis globosa* clone Ph91 flagellates (Phfl) and non-flagellated cells (PhNO3) in medium with 100 µmol l⁻¹ NO₃⁻. *P. globosa* clone Ph91 non-flagellated cells were also grown in medium with 100 µmol l⁻¹ NH₄⁺ (PhNH₄). Bars are 95% confidence intervals.](image-url)
On day 15 a remarkable feature was discovered in the sample from the non-aerated culture during microscopic observation. The number of cells per colony appeared to be very low and this was corroborated by colonial cell counts that were made in all cultures on day 16 and 17. The number of cells per colony increased significantly (Kruskal-Wallis test, P < 0.0001) with aeration rate (Figure 8.3b) as did the mean percentage of colonial cells (Figure 8.3c, ANOVA P < 0.05).

During the growth of *P. globosa* the concentration of C\textsubscript{i} declined and pH rose in all cultures. The smallest changes took place in the high-aerated culture while the largest differences were measured in the non-aerated culture (Table 8.1). The C\textsubscript{i} concentration in the high-aerated culture was never lower than 2.0 mmol l\textsuperscript{-1} (Figure 8.2c) and the CO\textsubscript{2} concentration remained above 10 µmol l\textsuperscript{-1} (Figure 8.2d). The δ\textsuperscript{13}C value in this culture was -26.3 ± 0.2 ‰ (Figure 8.3d). In contrast, both the non- and medium-aerated cultures reached concentrations < 2.0 mmol C\textsubscript{i} l\textsuperscript{-1} and < 10 µmol CO\textsubscript{2} l\textsuperscript{-1}, and both had a significantly less negative δ\textsuperscript{13}C value of -24.8 ± 0.3 ‰ (Figure 8.3d; ANOVA, P < 0.001).
### Table 8.1. Growth rate (µ), mean (day 15 - 17) concentrations ± 95% confidence interval of particulate organic carbon (POC), particulate organic nitrogen (PON), chlorophyll a (chl a), cell concentration, and changes in inorganic carbon (C\textsubscript{i}) and pH, in three differently aerated (0, 8 and 80 mL min\textsuperscript{-1}) P. globosa strain 677-3 cultures. n.s. = not significant.

<table>
<thead>
<tr>
<th></th>
<th>non</th>
<th>medium</th>
<th>high</th>
<th>unit</th>
<th>ANOVA</th>
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<tr>
<td>µ</td>
<td>0.63 ± 0.15</td>
<td>0.65 ± 0.13</td>
<td>0.49 ± 0.08</td>
<td>day\textsuperscript{-1}</td>
<td>-</td>
</tr>
<tr>
<td>POC</td>
<td>288 ± 20</td>
<td>307\textsuperscript{a} ± 39</td>
<td>236 ± 19</td>
<td>µmol l\textsuperscript{-1}</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>PON</td>
<td>49.5 ± 8.5</td>
<td>50.7\textsuperscript{a} ± 8.8</td>
<td>41.0 ± 4.5</td>
<td>µmol l\textsuperscript{-1}</td>
<td>n.s.</td>
</tr>
<tr>
<td>chl a</td>
<td>46.8 ± 2.2</td>
<td>55.2 ± 1.8</td>
<td>44.5 ± 4.9</td>
<td>µg l\textsuperscript{-1}</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>cells</td>
<td>196 ± 42</td>
<td>207 ± 35</td>
<td>157 ± 16</td>
<td>10\textsuperscript{6} l\textsuperscript{-1}</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>∆C\textsubscript{i}</td>
<td>-302\textsuperscript{b}</td>
<td>-270\textsuperscript{c}</td>
<td>-34\textsuperscript{c}</td>
<td>µmol l\textsuperscript{-1}</td>
<td>-</td>
</tr>
<tr>
<td>∆pH</td>
<td>+0.77\textsuperscript{b}</td>
<td>+0.44\textsuperscript{c}</td>
<td>+0.10\textsuperscript{c}</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

\textsuperscript{a} day 16 and 17 only \textsuperscript{b} between day 1 and 17 \textsuperscript{c} between day 1 and 13

### Discussion

**C\textsubscript{i} uptake**

In a first step to investigate the inorganic carbon acquisition of P. globosa, the uptake of CO\textsubscript{2} or HCO\textsubscript{3}\textsuperscript{-}, resulting in a rise in pH or a decline in alkalinity respectively (Sikes \textit{et al.} 1980), was measured in cultures of flagellate and non-flagellate Phaeocystis cells. The observed increase in pH clearly shows that both Phaeocystis cell types take up CO\textsubscript{2} (Figure 8.1). The measurement of HCO\textsubscript{3}\textsuperscript{-} uptake is influenced by the simultaneous uptake of NO\textsubscript{3}\textsuperscript{-} or NH\textsubscript{4}\textsuperscript{+} (alkalinity increase or decrease respectively, Stumm and Morgan 1996). Despite that NO\textsubscript{3}\textsuperscript{-} was the nitrogen source in the control culture with calcifying E. huxleyi, a decrease in alkalinity could be measured (Figure 8.1) which is a clear indication that these cells were taking up HCO\textsubscript{3}\textsuperscript{-} (Sikes \textit{et al.} 1980).

The non-flagellated Phaeocystis cells grew rapidly enough to measure the alkalinity increase in NO\textsubscript{3}\textsuperscript{-} medium, and the decrease in NH\textsubscript{4}\textsuperscript{+} medium (Figure 8.1). Although the possibility that some HCO\textsubscript{3}\textsuperscript{-} was taken up cannot be rejected, it is concluded that the major C\textsubscript{i} species taken up by Phaeocystis is CO\textsubscript{2}. This conclusion is corroborated by Elzenga \textit{et al.} (2000) who found that cells of P. globosa convert HCO\textsubscript{3}\textsuperscript{-} to CO\textsubscript{2} extracellularly by the enzyme carbonic anhydrase (see also chapter 10). The concomitant production of OH\textsuperscript{-} does not change alkalinity; but it does increase pH.
Figure 8.3. Means of Phaeocystis colony diameter (n = 36) (a), colonial cell concentration expressed as numbers of cells per 1000 µm² colony surface (n ≥ 20) (b), the percentage of cells in colonies (n = 2) (c), δ¹³C in ‰ (n = 3) of the particulate organic carbon in the early stationary growth phase (d) in no-, medium- and high-aerated cultures. Bars indicate the 95% confidence interval (a, b, d) or 1 sd (c).

C. availability and colonial growth

Increased aeration of the P. globosa cultures was accompanied by significant increases in the mean number of cells in colonies and in the percentage of colonial cells. In the high-aerated culture the CO₂ concentration remained above 10 µmol l⁻¹ and the δ¹³C value of the particulate organic matter was significantly more negative than in the less aerated cultures (Figure 8.3). A more negative δ¹³C as a result of aeration has also been observed in cultures of Skeletonema costatum (Degens et al. 1968) and Phaeodactylum tricornutum and indicates a relief of CO₂ stress (Johnston and Raven 1992). Low CO₂ concentrations in non-aerated culture media with high nutrient concentrations may therefore contribute to the gradual decline of the percentage of colonial Phaeocystis cells. Furthermore, CO₂ limitation may have been the cause of the relatively low (= 50%) and variable percentages of colonial cells in the f/2, non-aerated Phaeocystis cultures in chapter 2 (Figure 2.3).
inorganic carbon

Table 8.2. Mean particulate carbon, nitrogen and chlorophyll a characteristics and s.e.d. (spherical equivalent diameter of the cells) in three differently aerated (0, 8 and 80 mL min⁻¹) *P. globosa* strain 677-3 cultures (days 15 - 17). If 1.18 pmol C cell⁻¹ from the literature (Rousseau *et al.* 1990) is used, the amount of extracellular organic carbon is 20% of POC. Means ± 95% confidence interval. n.s. = not significant.

<table>
<thead>
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<th>medium</th>
<th>high</th>
<th>unit</th>
<th>ANOVA</th>
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<tr>
<td>C:N</td>
<td>5.8 ± 0.8</td>
<td>6.1⁺ ± 0.3</td>
<td>5.8 ± 0.2</td>
<td>mol mol⁻¹</td>
<td>n.s.</td>
</tr>
<tr>
<td>C:Chla</td>
<td>74 ± 2</td>
<td>66⁺ ± 15</td>
<td>64 ± 14</td>
<td>µg µg⁻¹</td>
<td>n.s.</td>
</tr>
<tr>
<td>C cell⁻¹</td>
<td>1.5 ± 0.2</td>
<td>1.4⁺ ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>pmol cell⁻¹</td>
<td>n.s.</td>
</tr>
<tr>
<td>Chla cell⁻¹</td>
<td>241 ± 37</td>
<td>269 ± 38</td>
<td>284 ± 45</td>
<td>µg µg⁻¹</td>
<td>n.s.</td>
</tr>
<tr>
<td>s.e.d.</td>
<td>4.20 ± 0.09</td>
<td>4.06 ± 0.11</td>
<td>4.56 ± 0.14</td>
<td>µm³</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

⁺ day 16 and 17 only  
⁺⁺ including 20% extracellular carbon

The decrease in the number of cells per colony due to CO₂ stress, will reduce the organic carbon content of the colony because cells contain 300x more carbon then the equivalent volume of colony matrix (Rousseau *et al.* 1990). Statistical models have been made (e.g. Davidson and Marchant 1987, Rousseau *et al.* 1990) that relate *Phaeocystis* colony size to intracolonial cell concentration and hence to carbon content. When such a model would be applied to colonies that have developed under CO₂ stress, i.e. with a reduced cell content, the organic carbon content will be overestimated. This overestimation can be calculated by assuming: (i) 1.5 pmol C cell⁻¹ (Table 8.2), (ii) 27.9 amol extracellular C µm⁻³ colony volume (Rousseau *et al.* 1990), and (iii) constant cell numbers per 1000 µm² for each aeration rate (Figure 8.3b: 0.32, 0.87 and 1.33). Then, medium- and non-aerated 100 µm colonies would have 28 to 62%, and 1000 µm colonies 10 to 23% less colonial carbon relative to the high-aerated colonies. The difference becomes less at increasing colony diameter because of the decrease of cell carbon (which is related to colony surface area: Figure 8.3b) relative to extracellular carbon (which is related to colony volume: Rousseau *et al.* 1990).

*a model of C₅ uptake*

Aeration not only tends to keep the pH and CO₂ concentration in the culture medium constant, the simultaneously created turbulence may also decrease the size of the diffusive boundary layer around plankton cells and colonies. The consequences for the growth of *Phaeocystis* colonies are difficult to calculate directly, because the CO₂ flux towards the colony surface is a function of the pH difference between the colony and the bulk seawater (affecting CO₂
concentrations), and of colony size and turbulence (both affecting the diffusive boundary layer thickness).

In general, the phytoplankton, which usually is smaller than 1 mm, is surrounded by a layer of water in which the transport of nutrients and wastes is governed by molecular diffusion (Lazier and Mann 1989). In stagnant water, the thickness of this diffusive boundary layer is equal to the particle radius. In practice this means that large Phaeocystis colonies have such thick diffusive boundary layers, that these layers can be measured with an oxygen electrode (Ploug et al. 1999a).

The flux of a nutrient from high to low concentrations is, according to Fick’s first law, proportional to the concentration gradient (Atkins 1999). This gradient is the difference in concentration divided by the diffusion path length. Turbulence decreases the size of the diffusive boundary layer and hence the diffusion path length. Therefore, an increase in turbulence will increase the concentration gradient, hence the nutrient or waste flux towards or from the colony surface. However, theory predicts that whatever high the turbulence may be, on the small scale size of plankton a diffusive boundary layer of some size will always persist (Mann and Lazier 1991).

In the case of CO$_2$, its flux towards the Phaeocystis colony will be proportional to the concentration difference between the bulk seawater and the colony interior. The colony interior has a pH of 9 (Lubbers et al. 1990, Ploug et al. 1999a), is not in equilibrium with the atmosphere, and hence has a much lower CO$_2$ concentration than the bulk seawater at pH 8 (Stumm and Morgan 1996, chapter 10). In other words, by increasing its interior pH, the Phaeocystis colony steepens the CO$_2$ concentration gradient, thereby increasing the CO$_2$ flux into the colony.

However, if the bulk seawater pH increases too, and if the seawater is not in equilibrium with the atmosphere (chapter 10), the CO$_2$ gradient will become less steep. Eventually, when bulk seawater and colony pH are equal, the net flux of CO$_2$ will be zero. In the non-aerated culture the bulk seawater pH increase was nearly 0.8 pH units (Table 8.1). Such a high pH increase (low CO$_2$ concentration) in combination with low turbulence (thick diffusive boundary layer) will lead to a decrease of the CO$_2$ flux towards and into the colonies. If this reduced CO$_2$ flux leads to inorganic carbon limited growth, an explanation is found for the low colonial cell numbers (Figure 8.3), because the amount of carbon needed to construct the colonial matrix is much lower than the amount needed for cell replication (Rousseau et al. 1990).

For a model calculation of C$_i$ uptake by Phaeocystis it is assumed that the cells in a spherical colony are growing at $\mu = 0.6$ day$^{-1}$ (Table 8.1) in continuous light at 23°C in 32 psu seawater (i.e. present conditions). The increase in the colony diameter was calculated per day using the equation of Rousseau et al. (1990). Under these conditions an inward C$_i$ flux is necessary to sustain growth because the amount of intracolonial C$_i$ in colonies < 1000 µm is < 3% the amount necessary for organic carbon production.

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Turbulence in the sea, expressed as energy dissipation rate, ranges from $10^{-6}$ to $10^{-10}$ m$^2$ s$^{-3}$ (Ploug et al. 1999b). Below the Kolmogorov length scale viscosity dominates over inertia, and turbulent flow results in a laminar shear (Karp-Boss et al. 1996). The shear rates calculated from energy dissipation rates range from 0.01 s$^{-1}$ to 1 s$^{-1}$; these values were taken as minimum and maximum estimates for the non- and well-aerated cultures respectively.

Increases in turbulence lead to increases in the Reynolds number ($Re$, the ratio of inertial and viscous forces), and in the Péclet number ($Pe$, the ratio of convective bulk flow and diffusion). For both the low and the high shear rate, the Sherwood number ($Sh$, the ratio of turbulent and diffusion mass flow) was calculated from $Re$ and $Pe$ (Ploug et al. 1999b), using $D_{CO_2} = 1.58 \times 10^{-9}$ m$^2$ s$^{-1}$ and a kinematic viscosity of $1.03 \times 10^{-6}$ m$^2$ s$^{-1}$ (Vogel 1996).

Because $Re$ and $Pe$ increase with colony size, the calculation of $Sh$ was performed on colony diameters ranging from 10 µm to $\approx 2000$ µm (Karp-Boss et al. 1996, Ploug et al. 1999b). For each diameter the flux of CO$_2$ towards the colony surface was calculated as:

$$Q = 4 \pi r Sh D_{CO_2} ( [CO_2]_{bulk} - [CO_2]_{colony} ) \tag{8.3}$$

With $Q$ is mol CO$_2$ s$^{-1}$, $r$ = colony radius [m], $Sh$ = Sherwood number [dimensionless], $D_{CO_2} = CO_2$ diffusion coefficient [m$^2$ s$^{-1}$] and $[CO_2]$ in mol m$^{-3}$ calculated with the equations in Stumm and Morgan (1996).

The CO$_2$ flux is positive, into the colony, due to the difference between $[CO_2]_{bulk} = 1.4 \times 10^{-2}$ and $[CO_2]_{colony} = 5.6 \times 10^{-4}$ mol m$^{-3}$. However, at an intracolonial pH of 9 the HCO$_3^-$ concentration ($7.4 \times 10^{-1}$ mol m$^{-3}$) is also reduced significantly compared to the bulk seawater (1.9 mol m$^{-3}$). This concentration difference will also lead to a flux of HCO$_3^-$ into the colony. Because the HCO$_3^-$ ion is negatively charged, an exchange with another negative ion is necessary in order to maintain a neutral charge balance. Assuming no differences in major anion concentrations (Cl$^-$, SO$_4^{2-}$) between the bulk seawater and the colony interior, it is most likely that the exchange is made with an OH$^-$ ion because the intracolonial pH of 9 (pOH = 5) implies a 10-fold higher OH$^-$ concentration then in the bulk seawater (pOH = 6). A more detailed description of this C$_i$ uptake model is presented in chapter 10 (Figure 10.7).

The flux of OH$^-$ out of the colony was calculated using equation 8.3 with the appropriate OH$^-$ concentrations and $D_{OH^=} = 5.25 \times 10^{-9}$ m$^2$ s$^{-1}$. $D_{OH^=}$ at 25°C was calculated from ionic mobility (Atkins 1999). The flux of HCO$_3^-$ into the colony was set equal to the slower OH$^-$ flux out of the colony.
Figure 8.4. Calculated inorganic carbon fluxes as CO$_2$ only and as CO$_2$+HCO$_3^-$ into a *Phaeocystis* colony as a function of colony diameter and turbulence at a constant pH difference between bulk seawater and colony interior of $\Delta$pH = 0.2. Both fluxes will increase when this pH difference is larger. The arrows on the right indicate the higher C$_i$ flux as a result of a $10^4$ increase in turbulence. The thick line is the calculated inorganic carbon demand for a colony with cells growing at $\mu = 0.6$ day$^{-1}$ in 24 h day$^{-1}$ light. The carbon demand will increase when the light period decreases ($L < 24$ h day$^{-1}$ while $\mu$ remains 0.6 day$^{-1}$) or when growth rate increases ($\mu > 0.6$ day$^{-1}$).

The efflux of OH$^-$ will increase bulk seawater pH and hence reduce the seawater CO$_2$ concentration (assuming no atmospheric equilibrium) as was actually measured in the non-aerated culture: the pH increased with 0.8 units (Table 8.1). Therefore, the CO$_2$ and C$_i$ (= CO$_2$ + HCO$_3^-$) fluxes into the colony were calculated for a pH difference of 0.2, assuming that the colonial pH was constant at 9.0.

The flux of inorganic carbon into the colony was then compared to the carbon needed by the colony for growth by calculating the organic carbon difference between day $i$ and day $(i+1)$ using the colony size - carbon relations of Rousseau *et al.* (1990). The results of these calculations are shown in Figure 8.4.

Several conclusions can be drawn from the model calculations (Figure 8.4). First, the fluxes of inorganic carbon are proportional to the size of the *Phaeocystis* colony, as is apparent from equation 8.3. The flux of HCO$_3^-$ is an
order of a magnitude higher than the CO$_2$ flux. At the $\Delta$pH = 0.2 used in the calculation, only colonies with a diameter < 30 µm obtain sufficient inorganic carbon by CO$_2$ diffusion alone.

Second, the effect of turbulence on inorganic carbon fluxes is relatively small. Only when the colonies grow beyond 500 and 1000 µm in diameter a doubling of the CO$_2$ and C$_i$ flux respectively, is gained at high compared to low turbulence. In the present experiment the mean colony diameter was =100 µm (Figure 8.3a), therefore, the turbulence differences between the aeration treatments will have had little effect on a potential C$_i$ limitation.

Third, any decrease in daylength or increase in growth rate must lead to an increase in inorganic carbon demand. On the other hand, any increase in $\Delta$pH will enhance CO$_2$ and HCO$_3^-$ fluxes. Daylength, colony diameter and growth rate can be obtained relatively easy but this is less so for the exact value of $\Delta$pH because the pH inside the Phaeocystis colony is difficult to measure. The colony interiors of temperate P. globosa strains reach pH 9.0 to 9.1 in the light (Lubbers et al. 1990, Ploug et al. 1999a) but this crucial variable has not been measured yet in warm water strain 677-3, used in the second experiment. Therefore, in future experiments, a temperate strain of P. globosa should be used or both the extra- and intracolonial pH should be measured.

Furthermore, the present model suggests that colonial growth will stop when the pH of the bulk seawater has reached the intracolonial pH. Then, the bulk seawater CO$_2$ concentration and hence its influx will be very low, and because the OH$^-$ efflux will have stopped the influx of HCO$_3^-$ stopped as well. If so, the pH = 8.8 reached in the non-aerated culture (Table 8.1) is an indication of the maximum intracolonial pH of strain 677-3. This is 0.2 to 0.3 pH units below the values measured in temperate strains (Lubbers et al. 1990, Plough et al. 1999a) and may account for the fact that relatively small (100 µm) colonies of strain 677-3 are already prone to C$_i$ limitation.

It is quite remarkable that the mode of C$_i$ acquisition in Phaeocystis has not been studied extensively. When $\Delta$pH can be measured accurately, the model of C$_i$ uptake presented here can be tested and used in further studies of the role and effect of C$_i$ availability on colony development. When the concentrations of N$_i$ and P$_i$ are high it is probable that the growth of Phaeocystis colonies becomes limited by C$_i$. Therefore, the number of cells in colonies growing in f/2 cultures will decrease, the percentage of solitary cells will increase and the number of colonies will decline. The prevalence of C$_i$ limited growth in the sea and its possible consequences, such as changes in the relative abundance of both colonial and solitary cells, and consequently grazing and sedimentation losses, remain interesting topics for further investigations into Phaeocystis’ role in plankton ecology and biogeochemical cycles.

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