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To link to this article: https://doi.org/10.1080/09670262.2011.602430

Published online: 26 Aug 2011.

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The role of nitrogen on the growth and colony development of *Phaeocystis globosa* (Prymnesiophyceae)

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The effects of nitrate, ammonium and urea on the growth and colony formation of three strains of *Phaeocystis globosa* were investigated. Although ammonium and urea supported growth, nitrate was the favoured nitrogen source for the growth of solitary cells for all three strains. *Phaeocystis globosa* CCMP 1528 and 629 formed colonies in all cultures where nitrate was the sole nitrogen source, but only a few colonies were observed in ammonium and urea treatments. Ammonium and urea were far less effective in supporting growth, biomass generation and colony formation in all three strains. Once colonies developed, colonial cells accounted for at least 15% of the total cells when grown with nitrate; colonial chlorophyll also contributed up to 60% to the total chlorophyll. The growth rates of colonial cells when using nitrate were greater than solitary cells. Changes in colony size, colonial cell abundance and total *P. globosa* abundance as affected by the nitrogen source may influence the carbon flux within the pelagic food web.

Key words: carbon flux, colony formation, growth, Haptophyta, nitrogen, *Phaeocystis*, solitary cells

Introduction

The genus *Phaeocystis* (Prymnesiophyceae) is globally distributed, has a distribution that ranges from tropical to polar oceans, and plays a significant role in global carbon and sulphur cycles as well as regional food webs (Lancelot *et al.*, 1998; Schoemann *et al.*, 2005). It has an unusual heteromorphic life cycle that includes gelatinous colonies and solitary cells. Individual cells are generally 3–10 μm in diameter, whereas colonies, with a few to thousands of cells embedded in a mucilaginous matrix, can occasionally be up to 3 cm in diameter (Rousseau *et al.*, 1994; Chen *et al.*, 2002). Among the six species that have been identified, *P. globosa*, *P. pouchetii* and *P. antarctica* have been reported to form massive blooms in colonial form (Medlin & Zingone, 2007). The formation of colonies contributes to the success of *Phaeocystis*, as it has been suggested that it may provide protection against grazers, viruses and bacteria (Hamm *et al.*, 1999; Jakobsen & Tang, 2002; Brussaard *et al.*, 2005). There is also evidence that the colonial matrix can supply energy and nutrients when light and nutrients become limiting (Lancelot & Mathot, 1985; Schoemann *et al.*, 2001).

*Phaeocystis* is regarded as a harmful algal genus in coastal waters (Lancelot *et al.*, 1998) because of negative influences on ecosystem structure, function, fisheries and tourism (Lancelot *et al.*, 1987; Peperzak & Poelman, 2008). Dense blooms have been observed off the coasts of the North Sea (Lancelot *et al.*, 1987), North Atlantic (Gieskes *et al.*, 2007), southeast China (Qi *et al.*, 2004), Vietnam (Tang *et al.*, 2004) and Norway (Larsen *et al.*, 2004). Clones isolated from Chinese and Norwegian waters have been shown to have hemolytic properties (Shen *et al.*, 2004; van Rijssel *et al.*, 2007). All of the locations listed above are considered to be eutrophic, due to riverine and anthropogenic nutrient enrichment (Lancelot *et al.*, 1987; Zhang *et al.*, 2000; Cadée & Hegeman, 2002; Thanh *et al.*, 2004). Increased N:Si and P:Si ratios have been proposed as a mechanism for the increased number and duration of blooms of non-siliceous phytoplankton (Jacobsen *et al.*, 1995), and the development of *Phaeocystis* blooms has been associated with increasing nutrient loads, especially NO₃ and PO₄, and subsequent changes in the N:P ratio (Riegman *et al.*, 1992). *Phaeocystis* colony development is controlled mainly by NO₃ supply (Lancelot *et al.*, 2007). Sharp decreases in N:P ratios as a result of high consumption of nitrate have been observed in two
Phaeocystis colonies and support the growth of solitary and colonial cells. Phaeocystis globosa is the most widespread of all known colony-forming Phaeocystis species, and forms extensive blooms that have deleterious impacts on coastal ecosystems. Understanding the species’ use of nitrogen may help in predicting its appearance and concentrations in coastal environments.

Materials and methods

Stock maintenance

Three strains of Phaeocystis globosa isolated from two geographical areas were used. P. globosa CCMP 627 and CCMP 629 were originally isolated from the North Atlantic, whereas P. globosa CCMP 1528 was isolated from the South Pacific. The three strains were maintained in f/2 medium in coastal seawater (Guillard & Ryther, 1962) at 20 °C, with a salinity of 32 PSU under an irradiance of 50 μmol photons m⁻² s⁻¹ on a 12 : 12 light : dark cycle. The stocks were maintained in exponential growth by regular dilutions with fresh media.

The cultures were non-axenic, and no attempt was made to reduce bacterial growth.

Experimental cultures

Experiments were conducted in a walk-in growth chamber in the same conditions as for stock maintenance. Prior to the experiments, solitary cells of the three strains of P. globosa were isolated by passing the culture through a 10-μm nylon sieve twice under gravity (Tang, 2003), and experiments were initiated with an initial solitary cell density of 10⁴ cells ml⁻¹. Triplicate one-litre batch cultures of each strain were grown in f/2 medium with a salinity of 32 PSU, where NH₄⁺, NO₃⁻ or urea were added as the nitrogen source with an initial N concentration of 8.82 x 10⁻⁴ M. All cultures were bubbled with filtered air to maintain a uniform distribution (Wang et al., 2010).

Microscopy and chlorophyll determinations

Samples for biomass assessment were collected every other day. Aliquots of known volume were filtered through Whatman GF/F filters to obtain total chlorophyll a, and through 20-μm polycarbonate membranes (Poretics) to obtain the >20-μm fraction, which was assumed to be representative of colonial chlorophyll (Tang et al., 2008; Wang et al., 2010). All chlorophyll a samples were extracted in darkness at 0 °C for 24 h in 7 ml of 90% acetone; fluorescence of extracted chlorophyll a was determined on a TD-700 fluorometer (Turner Designs) before and after acidification (Parsons et al., 1984). Samples for microscopical enumeration of solitary cell and colony abundances were preserved in Lugol’s solution (final concentration 2%). Solitary cell concentrations were measured using 1-ml Sedgwick–Rafter chambers. Colony concentration, colony size and cells per colony were measured in 24 multi-plates using a Nikon inverted microscope.
with a calibrated micro-ruler (Tang, 2003; Wang et al., 2010). Colonies were counted within 24 h of collection to limit colony disruption in the preserved state. Net growth rates (divisions d\(^{-1}\)/C\(_0\)) of triplicate samples of each strain growing on each substrate were determined from the slope of a regression between ln (N) (cells ml\(^{-1}\)/C\(_0\)) vs time (days).

Statistical analysis

SigmaStat (v. 3.50 SPSS) was used for statistical analyses. Statistical comparisons of the effects of nitrogen source on cell abundance with time were made by 2-way RM ANOVA. Comparisons of changes in colony concentrations, colony size, colonial cell abundance, partitioning of cell and chlorophyll with time, as well as comparison of growth rates between colonial and solitary cells, were conducted by 1-way RM ANOVA and \(t\)-tests. Prior to ANOVA, percentage data were normalized by an arcsine transformation (Zar, 1984). Linear regressions were fit to log cells colony\(^{-1}\) vs. log colony diameter, and comparison of the slopes of regressions between nitrogen was done by 1-way analysis of covariance (ANCOVA). The significance level for all statistical tests was set \(a\) priori at a critical \(P\) value of 0.05.

Results

Solitary cell abundances

The maximum solitary cell abundance of \(P.\) globosa CCMP 627 cultures grown on nitrate was significantly greater than those grown using ammonium or urea (\(P < 0.05\)), but there was no significant difference in maximum abundance using urea and ammonium as the N source (\(P > 0.05\); Fig. 1). The response of \(P.\) globosa CCMP 1528 was similar to that of CCMP 627, indicating that nitrate was the favoured nitrogen source for the growth of solitary cells in both strains. However, for \(P.\) globosa CCMP 629 the solitary cell abundance grown on nitrate was higher only on days 12 and 14 (\(P < 0.05\)) when compared with cultures using ammonium and urea, and the absolute difference was far less than observed with CCMP 627. Regardless of nitrogen source, however, the solitary cell abundance of \(P.\) globosa CCMP 627 was significantly higher than for the other two strains. For example, even when grown on urea, the maximum mean solitary cell abundance was \(2.85 \pm 1.10 \times 10^6\) cells ml\(^{-1}\) (mean \pm standard deviation), four orders of magnitude higher than in other strains. All three strains showed similar response to urea and ammonium, in that reduced nitrogen was far less effective in supporting growth and biomass generation (Table 1).

Fig. 1. \(Phaeocystis\) globosa solitary cell abundance (mean \pm standard deviation; \(n = 3\)) in nitrate (open circles), ammonium (solid circles) and urea (open triangles) cultures.

Colony abundances

Unlike in nature, colony development did not occur in our \(P.\) globosa CCMP 627 culture, regardless of the nitrogen source. \(Phaeocystis\) globosa CCMP 1528 and CCMP 629 formed colonies in all experiments when nitrate was provided as the nitrogen source, whereas only a few colonies were observed when ammonium and urea were provided as nitrogen sources (Table 1 and Fig. 2).
Furthermore, even these few colonies disappeared soon after being formed (Fig. 2). With nitrate, colony concentrations of CCMP 1528 increased from 95 to 2309 colonies ml$^{-1}$ by day 16; colony abundance then decreased slightly toward the end of the experiment. The highest colony numbers for CCMP 629 were also observed on day 16. However, in CCMP 629 colony abundance was significantly less than in CCMP 1528 ($P < 0.01$).

**Partitioning of cells and chlorophyll**

Nitrogen source strongly influenced the partitioning of cells between solitary and colonial forms. CCMP 627 existed exclusively as solitary cells in all nitrogen treatments (Table 1). At the beginning of the experiments (that is, on day 4), <1% of the total cells were in colonial form within non-nitrate treatments for CCMP 1528 and CCMP 629, whereas colonial cells accounted for at least 15% of the total cells when grown with nitrate (Fig. 3). This percentage increased to 50% for both CCMP 1528 and CCMP 629, and then decreased again to c. 15% of the total cells at the end of the experiments.

Size-fractioned chlorophyll also differed significantly among nitrogen treatments. Because there were relatively few colonial cells in non-nitrate treatments, the >20 μm chlorophyll fraction was not detectable for CCMP 1528 and CCMP 629 (confirming the use of this fraction of chlorophyll as a proxy for colonies). With nitrate as the nitrogen source, the percentage of colonial chlorophyll remained unchanged over time ($P > 0.05$), contributing up to 60% to the total chlorophyll for both CCMP 1528 and CCMP 629 (Fig. 3). In addition, nitrogen source did not influence the chlorophyll $a$ per cell in colonies of both CCMP 1528 and CCMP 629 ($P > 0.05$, data not shown).

![Fig. 2. Colony abundances (mean ± standard deviation; $n = 3$) of Phaeocystis globosa grown with nitrate (open circles), ammonium (solid circles) and urea (open triangles) as a nitrogen source. Colony formation of CCMP 1528 and 629 within non-nitrate treatments were only observed at the beginning of the experiment.](image1)

![Fig. 3. The percentage of colonial cells of Phaeocystis globosa relative to the number of total cells (top) and the percentage of chlorophyll in the >20 μm fraction relative to total chlorophyll $a$ (bottom) during supply of nitrate (open circles), ammonium (solid circles) and urea (open triangles). Due to the relatively small number of colonial cells within non-nitrate treatments, the >20 μm chlorophyll fraction was not detectable for CCMP 1528 and CCMP 629.](image2)
**Colony size and colonial cell**

With nitrate as the nitrogen source, mean colony size increased from 12.5 to 58.4 μm by day 10 for CCMP 1528, and then decreased slightly but significantly towards the end of the experiment (Fig. 4). Similarly, changes in colony diameter also occurred in the CCMP 629 culture. The colony diameters of CCMP 1528 and 629 were less than 40 μm in the ammonium and urea treatments when colonies developed, significantly less than those in the nitrate-replete cultures after the same period of growth.

The number of cells per colony of CCMP 1528 and 629 as a function of nitrogen were similar (Fig. 4). There were significant increases in colonial cell concentration over time for both strains within nitrate treatments \( (P < 0.01) \), and the highest mean cell densities were 58.4 and 157 cells colony\(^{-1}\) for CCMP 1528 and CCMP 629, respectively. However, when grown with ammonium or urea, there were less than 10 cells colony\(^{-1}\).

In all cases where colonies developed, there was a significant linear log–log relationship between the number of cells per colony and colony diameter \( (P < 0.01) \) with a slope <2, indicating that the number of cells per unit of colony surface area decreased as the colonies increased in size (Fig. 5). For CCMP 1528, the slope of the regression was significantly smaller when ammonium was the N source when compared to the nitrate treatment \( (P < 0.01) \). Nitrogen source, however, did not significantly affect cell distribution within colonies of CCMP 629 \( (P > 0.05) \). Additionally, within nitrate treatments there was no difference in the slope of the regression lines between CCMP 1528 and 629 \( (P > 0.05) \).

**Growth rates of solitary and colonial cells**

In the cultures supplied with nitrate, the maximum growth rate of solitary cells for CCMP 1528 was 0.28 ± 0.02 d\(^{-1}\), which was significantly less than that of colonial cells \( (0.47 ± 0.04\ d\^{-1}; \ P < 0.001) \). The difference was even greater for CCMP 629; the maximum growth rate of colonial cells was twice as rapid as that of solitary cells \( (0.64 ± 0.18 \text{ vs } 0.33 ± 0.07\ d\^{-1}; \ Table\ 1) \).

**Discussion**

**Nitrogen limitation in the medium**

The objective of this study was to understand how different nitrogen sources influence the growth and biomass generation of *Phaeocystis globosa*, especially in view of the increased nutrient enrichment occurring in many coastal waters. Conversely, studying the nitrogen uptake dynamics of *P. globosa* was secondary to the objective of investigating the role of nitrogen source in colony development, and nitrogen data were not collected during the time-course measurements. However, estimates from rates of particulate matter generation suggest that N-limitation is unlikely. For example, using the ratio of chlorophyll \( a : \text{PON} \) determined by Wang *et al*. (2010), 2.19 μmol N is removed for every 1 μg chlorophyll \( a \) generated.

![Fig. 4](image-url)
Therefore, in f/2 medium with an initial N concentration of 882 \( \mu \text{M} \), we would expect that 402 \( \mu \text{gl} / \text{C0} \) chlorophyll \( a \) would be generated. Only in the cultures of CCMP 627 growing with nitrate did we observe chlorophyll concentrations of 400 \( \mu \text{gl} / \text{C0} \), suggesting that nitrogen limitation occurred infrequently. Therefore, we attribute the different response in growth and colony formation to the form of nitrogen supplied, rather than of N-limitation per se.

Nitrate uptake and colony formation

Our studies suggest strongly that nitrate favours the growth of both solitary cells and colonies of \( P. \ globosa \). Furthermore, reduced forms of nitrogen (ammonium, urea) failed to support extensive growth in this species. Our results are consistent with previous studies that have reported \( Phaeocystis \) blooms in environments where nitrate was the major N source (Riegman et al., 1992; Smith, 1993; Arrigo et al., 1999; Lancelot et al., 2007). Nitrate is the dominant form of nitrogen in the North Sea (Lancelot et al., 2007), contributing more than 50% of total nitrogen. This region has been dominated by massive \( Phaeocystis \) blooms each spring, which are tightly coupled to nitrate loading in both space and time (Lancelot et al., 2007). \( Phaeocystis \) \( \text{antarctica} \) blooms in the relatively deeply mixed (and nitrate replete) waters of the Ross Sea, where the \( \text{NO}_3 : \text{PO}_4 \) removal ratio for \( P. \text{antarctica} \) blooms was markedly higher than the Redfield N : P ratio of 16 (Arrigo et al., 2000, 2002). Results from both of these locations suggest that \( Phaeocystis \) can grow well and reach high abundance using nitrate as a nitrogen source.

The investigations by Hai et al. (2010) presented alternative evidence that high ammonium concentrations (4.9 \( \mu \text{mol l}^{-1} \)) favour the development of \( P. \ globosa \) blooms in the upwelling waters of the south-central coast of Vietnam. The \( Phaeocystis \) blooms are induced and supported by offshore upwelling that brings nutrients from the deep ocean to the surface (Tang et al., 2004). The upwelled waters are also in close contact with sediments, potentially providing a significant ammonium source. Hai et al. (2010) found that nitrate concentrations were still high (2.1 \( \mu \text{mol l}^{-1} \)) and above saturation levels during the \( Phaeocystis \) bloom, but that ammonium was even more elevated. It is possible that nitrate simulated colony development close to the upwelling centre and was reduced in concentration during the shoreward advection of the water; then, following significant ammonium regeneration in shallow water, nitrate uptake was inhibited by high concentrations of ammonium (Tungaraza et al., 2003). In addition, the observed differences may be due to the different strains studied. In Vietnam coastal waters, the diameters of colonies of \( P. \ globosa \) reached up to 1 cm, and thousands of cells were embedded within the colony matrix (W.O.S., unpublished observations). These giant colonies may represent an entirely different biological response to the variable nitrogen sources than was observed in our cultures.

![Graph](https://via.placeholder.com/150)

**Fig. 5.** Relationship between cell abundance per colony and colony diameter of \( Phaeocystis \) \( \text{globosa} \) CCMP 1528 (top) and 629 (bottom) when grown using different nitrogen sources (left, ammonium; right nitrate). Solid lines are linear regressions, and dotted lines are the 95% confidence intervals. \( N \) = number of colonies measured.
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Differences in uptake strategies between solitary and colonial cells

Comparatively few colonies developed when ammonium or urea were provided as N sources, whereas solitary cell abundances increased with time for all three strains, irrespective of nitrogen source. Furthermore, the final biomass of CCMP 627 grown in urea was greater than that grown in nitrate, strongly suggesting that the growth of P. globosa solitary cells was largely independent of nitrogen source. Phaeocystis can remove ammonium when ammonium concentrations exceed certain concentrations (Smith, 1993; Cochlan & Bronk, 2003; Tungaraza et al., 2003). Unfortunately, none of these studies differentiated between solitary and colonial cells and the nitrogen source of each form, but given P. globosa’s failure to form colonies in cultures supplied with reduced nitrogen, we would suggest that ammonium probably was removed by solitary and not colonial cells.

Nitrogen and colonial architecture

The slope of the linear log–log relationship between colony number and colony diameter that we observed is comparable to previous results in Rousseau et al. (1990) and Jakobsen & Tang (2002). In their investigations nitrate was also used as a nitrogen source. The significant difference between the slopes of the regression lines between nitrate and ammonium treatments in our experiments suggests that the cellular density in colonies (the number of cells per unit colony surface) was reduced by ammonium. It is generally considered that ammonium takes less energy to assimilate than nitrate (Syrett, 1981; Thompson et al., 1989), and it is possible that the ‘excess’ energy of NH₄-grown cells may have been used to produce more carbon-rich mucus. However, we did not find increased numbers of colonies in ammonium-based cultures; indeed, colonial growth using NH₄ was extremely limited. Given the large increased partitioning of carbon into the mucus, it is possible that the colonies rapidly became structurally unstable and could not maintain colonial integrity. Our data cannot explain the differences in colony architecture, and further work is necessary to provide the underlying physiological or mechanical explanation.

Generally growth of nano-phytoplankton in the ocean is thought to be based largely on ammonium, with nitrate being relatively more important for net phytoplankton (Malone, 1980; Probyn & Painting, 1985). In our study solitary cells with diameters of a few micrometres were able to remove either nitrate or ammonium, whereas colonies utilized nitrate. This may partly explain why colonies were present only at the beginning of experiments in ammonium-based cultures. Given that individual cells are embedded in the mucoid matrix, it is possible that ammonium diffusion through the mucus was too slow to support growth (perhaps due to the negative charge of the ion). However, Ploug et al. (1999) suggested that the mucus was highly permeable to the inorganic substances they tested, which would argue against the possible ammonium limitation via diffusion. Similarly, in the ammonium-replete cultures reported by Riegmam et al. (1992), cells of P. globosa were solitary, whereas they were colonial when supplied nitrate. Although urea concentrations decreased rapidly during both diatom and Phaeocystis blooms (Tungaraza et al., 2003), there was no direct evidence that urea was directly used as an N substrate by Phaeocystis (Sanderson et al., 2008, Bradley et al., 2010). Only solitary cells of P. globosa removed urea in our cultures, strongly suggesting that solitary cells were responsible for the decrease in urea concentration during blooms of Phaeocystis.

Nitrate load within marine systems dominated by Phaeocystis

Generally colony formation is assumed to represent a potential energy loss, which inevitably results in reduced growth rates of the colonial form (Jakobsen & Tang, 2002). However, colonial and solitary cells have been shown in other studies to have similar growth rates (Hamm, 2000; Jakobsen & Tang, 2002), suggesting that colonial mucus production does not represent a significant energy drain for P. globosa nor result in a reduced growth rate of colonial cells. In our cultures, the growth rates of colonial cells were even higher than those of solitary cells, similar to the finding of Shields & Smith (2009) and Veldhuis et al. (2005). Because the different morphotypes of Phaeocystis have very different trophic roles (e.g. Hamm et al., 2001; Smith et al., 2007), nitrate inputs that induce changes in altered partitioning between solitary and colonial forms will inevitably affect many of the ecological processes in Phaeocystis-dominated systems. Colony formation may protect colonial cells against viral infection, bacterial degradation and zooplankton grazing; thus, colony formation and larger size can be considered to be a defence mechanism (Hamm, 2000; Tang, 2003; Nejstgaard et al., 2007). Increases in nitrate concentrations would enhance magnitude and composition of P. globosa blooms and result in decreased grazing pressure and, combined with the presumed higher sinking rates of colonies, increased inputs to the benthos (Hamm &
Rousseau, 2003; Reigstad & Wassmann, 2007) and decreased POC retention within the pelagic food web (Hamm, 2000).

Finally, it should be noted that the variations we found among the three strains were substantial, both with respect to colony formation and nitrogen assimilation. While this is not completely unexpected, given that clonal variations have been previously observed (van Rijssel et al., 2000; Long et al., 2007; Mills et al., 2010), the magnitude of these variations was surprising large (e.g. the differences in final standing stocks that were independent of the nitrogen source). While the clones appear morphologically similar, it is possible that significant genetic differences exist among clones that result in substantial ecological and geographic separation. Similar findings for oceanic prochlorophytes have been observed (Johnson et al., 2006) and we know of no reason why similar niche differentiation should not occur in haptophytes. Further detailed molecular analysis and linkage to ecological responses in this critical species would allow a greater understanding of the local responses to nitrogen inputs.

Acknowledgements
We thank Dr Kam Tang for technical assistance and constructive comments. The authors are also grateful to three anonymous reviewers whose invaluable comments and advice greatly improved the final form of this manuscript. This study was supported by the National Basic Research Program of China 2011CB403603 (X.W.), 2010CB428702 (Y.W.) and U.S. National Science Foundation OCE-0850910 (W.O.S.).

References


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