Grazing and colony size development in Phaeocystis globosa (Prymnesiophyceae): the role of a chemical signal

K Tang
Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/vimsarticles

Part of the Marine Biology Commons

Recommended Citation
https://scholarworks.wm.edu/vimsarticles/1644

This Article is brought to you for free and open access by W&M ScholarWorks. It has been accepted for inclusion in VIMS Articles by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
Grazing and colony size development in 
Phaeocystis globosa (Prymnesiophyceae):
the role of a chemical signal

KAM W. TANG*1

1PRESENT ADDRESS: VIRGINIA INSTITUTE OF MARINE SCIENCE, COLLEGE OF WILLIAM & MARY, PO BOX 1346, GLOUCESTER POINT, VA 23064, USA
*CORRESPONDING AUTHOR: kamtang@vims.edu

The bloom-forming prymnesiophyte Phaeocystis globosa forms hollow, spherical, mucilaginous colonies that vary from micrometres to millimetres in size. A recent paper gave the first empirical evidence that colony size increase in P. globosa is a defensive response against grazers, and knowing the signalling mechanism(s) behind this response will thus be a key to understanding the trophodynamics in systems dominated by this species. I conducted experiments with specially designed diffusion incubators, each of which consists of a non-grazing chamber (with P. globosa only) and a grazing chamber (grazers + phytoplankton) connected by 2 µm polycarbonate membrane filters. The results showed that physical contact with grazers was not required to initiate the defensive response; instead, P. globosa colony size increase was found to be stimulated by dissolved chemicals generated by ambient grazing activities. This signal was non-species specific, such that various combinations of three species of grazers and four species of phytoplankton in the grazing chambers all resulted in significant, but different extents of colony enlargement in P. globosa in the non-grazing chambers (30–300% larger than the ‘grazer-free’ control). High concentrations of ambient solitary P. globosa cells and other phytoplankton seemed to suppress colony enlargement in P. globosa, and grazers would help reduce this inhibition by removing the ambient solitary P. globosa cells and other phytoplankton. These non-species-specific mechanisms would allow P. globosa to regulate colony size development and defend itself in diverse planktonic systems, which may help to explain the global success of this species.

INTRODUCTION

Organisms of lower trophic levels face the danger of predation, and the ability to defend against predators is therefore a main driving force in their evolutionary histories (Mole, 1994; Tollrian, 1995). Because defense mechanisms can be costly to maintain, it is to the advantage of an organism to activate its defense mechanisms only when predation risk is sensed (Agrawal, 1998). Signals for predation risk may come in visual, audio, physical or chemical forms. Since most lower trophic organisms lack visual or audio perceptions, detecting predators will rely on chemical or physical cues. Research on defensive responses among phytoplankton has been largely limited to freshwater systems. A well-studied example is morphological defense in the colony-forming chlorophyte Scenedesmus sp., which is often abundant in lakes. When solitary Scenedesmus sp. cells are grazed by daphnids, colony formation intensifies such that the particle size increases to beyond the handling capacity of the grazers (Lürリング and van Donk, 1997; Lürリング, 1999). Subsequent studies showed that this defensive response is triggered by ‘infochemicals’ released during the grazing process (Lampert et al., 1994; Lürリング and van Donk, 1997; Lürリング, 1998), although the nature of these infochemicals is still under dispute (Wiltshire and Lampert, 1999; Lürリング and von Elert, 2001).

A comparable example in the marine systems is the cosmopolitan phytoplankton genus Phaeocystis (Prymnesiophyceae). The life cycle of Phaeocystis sp. involves an alternation between solitary cells and colonies (Rousseau et al., 1994). Massive blooms of Phaeocystis sp. frequently cause environmental and economic damage to coastal areas (Weisse et al., 1994) review). Phaeocystis sp. also plays important roles in the global sulphur cycle and climate
regulation owing to its high production of dimethyl- 
"methylsulphide (Liss et al., 1994). Much remains 
unknown about the biological roles of different life stages 
and the regulation of transition between stages (Rousseau 
et al., 1994; Lancelot et al., 1998); Nevertheless, the preva-
ence of the colonial form in natural Phaeocystis sp. blooms 
contrasting water types prompts the ideas that Phaeo-
cystis sp. colony development is regulated by common 
factors (Lancelot and Rousseau, 1994); and contributes to 
the success of the genus in marine systems (Rousseau 
et al., 1994; Lancelot et al., 1998). Unlike the chain-
forming Cososomonas sp., Phaeocystis sp. colonies are hollow, 
balloon-like structures with individual cells lying beneath 
a thin mucous skin (Van Rijssel et al., 1997). This arrange-
ment allows the colonies to increase their size with a 
limited number of cells—colonies of up to a few milli-
metres in size with only a few thousand individual cells 
are common in natural Phaeocystis sp. populations 
(Rousseau et al., 1990), and colonies of 2 cm in diameter 
have been recorded in the laboratory (Riegman and van 
Boekel, 1996). A long-held belief is that Phaeocystis sp. 
colony formation and growth is a defence strategy 
because large colonies create a size-mismatch problem for 
small grazers (Weisse et al., 1994; Lancelot et al., 1996; 
Hamm et al., 1999; Ploug et al., 1999), but empirical 
evidence had been missing until recently. Jakobsen and 
Tang (Jakobsen and Tang, 2002) showed that Phaeocystis 
globosa formed colonies in the absence of grazers, but the 
presence of grazers induced the colonies to increase in 
size significantly. Mean colony diameter in the grazing 
treatments was up to 3-fold larger than that in the control; 
the overall range of colony diameter was also higher in 
the grazing treatments, indicating that the change in 
colony diameter was not due to selective grazing on small 
colonies (figure 5 in [Jakobsen and Tang, 2002]). Colony 
size increase provided protection for individual P. globosa 
cells such that the grazers subsequently starved and 
decided (Jakobsen and Tang, 2002). While the phenotypic 
response of P. globosa to grazing is clear, the 
signalling mechanism(s) behind such a response remains 
unclear. Earlier work by Kornmann showed that colony 
size development was a function of the concentration of 
an extract from horseshad mud added to the medium, indi-
cating that an unspecified chemical substance favours 
colony enlargement (Kornmann, 1955). In the present 
study, several possibilities will be considered.

(i) Physical signal: colony size increase is induced by 
physical contact between P. globosa cells and grazers. 
If this is the case, it requires that P.globosa cells escape 
or survive ingestion before the defence mechanism can 
be initiated. (ii) Chemical signal caused by non-specific grazing: colony size increase is induced by chemicals released by grazers independent of their feeding on P. globosa. In this case, the chemicals indicate the presence of potential grazers, but the resultant defensive 
response is non-specific to grazing mortality of 
P.globosa cells. (iii) Chemical signal caused by specific grazing: chemicals are released when P.globosa cells are grazed upon. In this case, colony size increases only when P.globosa cells are affected. (iv) Indirect grazing effect: grazing activities alter the 
constituents of the ambient water, which then 
induces P. globosa colony size to increase. Examples are alteration of ambient nutrient composition and 
availability due to grazers’ excretion, and removal of 
co-existing phytoplankton cells.

The formation of large mucilaginous colonies in 
Phaeocystis sp. is one of the most intriguing examples of 
morphological defence among phytoplankton. This study 
was part of a continuing effort to investigate how grazers affect P. globosa colony development. The goal was to char-
acterize the signalling mechanism(s) behind P. globosa 
colony size increase as a defensive response to grazing, 
which will help us better understand the trophodynamics 
in systems dominated by P. globosa and explain its success 
in the world’s oceans.

METHOD

Phytoplankton and grazers

The present study used P. globosa, which forms nearly 
perfect spherical colonies and is one of the most widely 
distributed Phaeocystis species in the world’s oceans 
(Lancelot et al., 1998). Non-axenic P. globosa (CCMP 1528; 
which originated from the Pacific coast of South America) 
and other phytoplankton cultures (Table I) were grown in 
L-medium (Guillard and Hargraves, 1993) at 19 ± 1°C 
with 60–100 µmol photons m⁻² s⁻¹ in a 12 h light:12 h 
dark cycle. The cultures were maintained in exponential 
growth phase by regular dilution with fresh medium.

Solitary P. globosa cells for the experiments were collected 
by passing the culture twice through an 11 µm sieve under 
gravity (Jakobsen and Tang, 2002). Two protozoan and 
one copepod species were used as grazers (Table I). The 
heterotrophic dinoflagellate, Gyrodinium dicoum, was 
originally isolated from Øresund, Denmark. Another 
heterotrophic dinoflagellate, Gymnodinium cf. danicum, which 
originated from the North Sea, was generously provided 
by Dr. Suzanne Stromm (Western Washington University, 
USA). Both are common coastal protozoan species, and
grazing by *G. dominans* has been shown to induce colony enlargement in *P. globosa* (Jakobsen and Tang, 2002). Protosporan cultures were grown in L-medium on the experimental diets for at least five generations before experiments. *Temora longicornis* is a common calanoid copepod species that co-exists with *Phaeocystis* sp. in temperate waters (Weisse, 1983; Hansen, 1995; Cotonnec et al., 2001). Mature females of *T. longicornis* collected from the North Sea were allowed to produce eggs in the laboratory, which were then used to establish a cohort. A new generation of *T. longicornis* was raised to adults on a diet of *Rhodomonas salina*. Before the experiments, new mature female *T. longicornis* were acclimated to the experimental diets for at least 2 days. All experiments were carried out on a rotating plankton wheel (0.4 r.p.m.) at 19 ± 1°C with 60–100 µmol photons m–2 s–1 in a 12 h light:12 h dark cycle. Aliquots were removed from experimental containers and preserved in 4% acid Lugol’s solution. Protozoan cells, solitary *P. globosa* cells and *P. globosa* colonies were enumerated and sized according to the method of Jakobsen and Tang (Jakobsen and Tang, 2002).

**Test for a chemical signal (Experiments 1–4)**

To test for the presence of a chemical signal, experiments were carried out with specially designed diffusion incubators. Each incubator was made of two polystyrene chambers connected by two 2 µm polycarbonate membrane filters (Figure 1); the ‘grazing chamber’ contained grazer and phytoplankton cells, whereas the ‘non-grazing chamber’ contained only *P. globosa* solitary cells. Thus, grazers on one side and *P. globosa* cells on the other side of the incubator could exchange dissolved chemicals across the membrane filters without physical contact. Unlike dialysis bags commonly used for similar experiments, the diffusion incubators can be fastened onto a rotating plankton wheel to keep particles in suspension. The incubators were arranged on the plankton wheel such that both chambers received equal amounts of light. Preliminary tests with food dye showed that diffusion across the membrane filters reached ~50 and 90% of equilibrium after 3 and 5 days, respectively (Figure 2). For controls, both chambers were filled with *P. globosa* solitary cells. The species specificity of the signal was tested with different combinations of grazers and phytoplankton in the grazing chamber (Table II).

<table>
<thead>
<tr>
<th>Phytoplankton</th>
<th>Taxonomic group</th>
<th>Size (µm)</th>
<th>Carbon content (pg cell–1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phaeocystis globosa</em></td>
<td>Prymnesiophyte</td>
<td>4.4</td>
<td>9.3</td>
</tr>
<tr>
<td><em>Isochrysis galbana</em></td>
<td>Prymnesiophyte</td>
<td>4.2</td>
<td>8.2</td>
</tr>
<tr>
<td><em>Dunaliella tertiolecta</em></td>
<td>Chlorophyte</td>
<td>7.0</td>
<td>30.2</td>
</tr>
<tr>
<td><em>Rhadomorpha saxia</em></td>
<td>Cryptophyte</td>
<td>6.9</td>
<td>29.8</td>
</tr>
<tr>
<td><em>Thalassiosira weissflogii</em></td>
<td>Diatom</td>
<td>15.0</td>
<td>105</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grazers</th>
<th>Taxonomic group</th>
<th>Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gyrodinium dominans</em></td>
<td>Naked heterotrophic dinoflagellate</td>
<td>15</td>
</tr>
<tr>
<td><em>Glenodinium cf. danicum</em></td>
<td>Thecate heterotrophic dinoflagellate</td>
<td>9.8</td>
</tr>
<tr>
<td><em>Temora longicornis</em></td>
<td>Calanoid copepod</td>
<td>1000</td>
</tr>
</tbody>
</table>

All phytoplankton cultures were grown in L-medium (with silica for diatoms). Carbon content was calculated from cell size according to Strathmann (Strathmann, 1967). Phytoplankton cell size was measured on a particle counter. The size of heterotrophic dinoflagellates was measured by inverted microscopy. Copepod size was measured as approximate body length.

---

**Table I: Phytoplankton and grazer species used in experiments**
and size were followed for up to 15 days. While positive results (i.e. *P. globosa* colony size increased relative to the control) would indicate the involvement of a chemical signal, additional experiments were required to further characterize the chemical signal (see below).

### Test of cell concentration effects (Experiment 5)

Experiments were conducted to test whether the initial solitary *P. globosa* cell concentration affected their response to the chemical signal. Four diffusion incubators were filled with *G. dominans* (~1000 cells ml⁻¹) and *Dunaliella tertiolecta* (~45,000 cells ml⁻¹) on one side and L-medium on the opposite side. After 3 days, *D. tertiolecta* was grazed to low concentration and grazing-related dissolved chemicals would have diffused and accumulated in the non-grazing chambers of the incubators. Medium from the non-grazing chambers was then harvested and used to incubate various concentrations of *P. globosa* solitary cells (initial concentration 50, 10 and 2 × 10³ cells ml⁻¹, in triplicates) for 13 days. Untreated L-medium was used for controls. If the increase in colony size (relative to the controls) was inversely proportional to the initial cell concentrations, it would suggest that the ambient chemical signal perhaps behaved similarly to nutrients and was consumed by the cells during the colony development process.

### Test of interspecific inhibition (Experiment 6)

Natural populations of *Phaeocystis* sp. co-exist with other phytoplankton species (Riegman et al., 1992; Hegarty and Villareal, 1998). If the presence of competing phytoplankton species inhibits *Phaeocystis* sp. bloom development and colony development, grazers may reduce such inhibition by selectively removing the co-existing phytoplankton species. To test the possibility of interspecific inhibition, duplicate diffusion incubators were set up such that one side was filled with *P. globosa* solitary cells while the opposite side was filled with a different phytoplankton species. Four phytoplankton species were tested (Table I). For controls, both sides were filled with *P. globosa* solitary cells. All cell concentrations were adjusted to ~1–1.5 µg C ml⁻¹ based on cell volume-to-carbon conversions (Strathmann, 1967). Cell abundance, colony abundance and size were measured after 10 days.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Treatment</th>
<th>'Grazing' chamber</th>
<th>'Non-grazing' chamber</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phytoplankton conc.</td>
<td>Grazer abundance*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cells ml⁻¹)</td>
<td>(cells ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>PHA</td>
<td>2 × 10⁷ <em>P. globosa</em></td>
<td>400 <em>G. dominans</em></td>
<td>2 × 10⁶</td>
</tr>
<tr>
<td>2</td>
<td>DUN</td>
<td>2 × 10⁷ <em>D. tertiolecta</em></td>
<td>400 <em>G. dominans</em></td>
<td>2 × 10⁶</td>
</tr>
<tr>
<td>3</td>
<td>RHO</td>
<td>2 × 10⁷ <em>R. salina</em></td>
<td>200 <em>G. dominans</em></td>
<td>2 × 10⁶</td>
</tr>
<tr>
<td>4</td>
<td>TW</td>
<td>9000 <em>T. weissflogii</em></td>
<td>10 <em>T. longicornis</em> (total)</td>
<td>1.3 × 10⁶</td>
</tr>
</tbody>
</table>

All ‘non-grazing’ chambers were filled with solitary *P. globosa* cells. For controls, both sides of the diffusion incubators were filled with solitary *P. globosa* cells (2–6 replicates).

n: number of replicates.

*Grazer abundance for *T. longicornis* is expressed as the total number of females.
Data analysis

Cell abundance and colony abundance among treatments were compared by parametric tests (Student’s t-test, or one-way ANOVA followed by Tukey test). For colony diameters, data from within treatments were pooled and presented in box plots showing 5, 10, 25, 50, 75, 90 and 95 percentiles. Differences in colony diameters among treatments were tested by non-parametric tests (Mann–Whitney rank sum test, or Kruskal–Wallis test followed by Dunn’s pairwise comparisons). Colony enlargement was indicated by significantly larger colony size relative to the control on the corresponding days. The significance level for all statistical tests was set at $P = 0.05$.

To simplify the subsequent text, colony size is discussed in terms of the geometric mean colony diameters.

RESULTS

Diffusion incubator experiments: G. dominans

In Experiment 1, grazing by G. dominans reduced solitary P. globosa cells and D. tertiolecta to low concentrations in the grazing chambers initially (Figure 3). Solitary P. globosa cell concentration increased again after 3 days when G. dominans began to decline. However, D. tertiolecta was unable to recover, and disappeared after 9 days. In the
non-grazing chambers, solitary *P. globosa* cell concentrations increased exponentially and levelled off by Day 12. The final concentration of solitary *P. globosa* cells in the non-grazing chamber for the DUN (*D. tertiolecta*) treatment was $1.44 \times 10^6$ cells ml$^{-1}$; thus, the average concentration for the entire incubator (grazing chamber + non-grazing chamber) would be $7.2 \times 10^5$ cells ml$^{-1}$. Similarly, the average solitary *P. globosa* cell concentration in the PHA (*P. globosa*) treatment was $7.6 \times 10^5$ cells ml$^{-1}$ for the entire incubator. Both values were not significantly different from the average solitary cell concentration in the control, which was $7.5 \times 10^5$ cells ml$^{-1}$ (Figure 3). The abundance of *P. globosa* colonies gradually increased in all non-grazing chambers and the control throughout the experimental period. In the grazing chamber of the PHA treatment, colonies also increased with time, but to a lower abundance than the opposite side of the incubator (Figure 3). The average colony abundance for the entire incubator was not significantly different among all treatments.

Colonies in the control increased slightly, then remained steady at ~60 µm after 9 days (Figure 4). Colony size development was markedly different in the other treatments. In the PHA treatment, colonies appeared in the grazing chamber only after 6 days, and rapidly increased in size; by Day 15, the colonies were on average about three times larger than those in the control. In the non-grazing chamber of the PHA treatment, colony size also increased significantly, by 30% relative to the control after 12 days. Significant colony enlargement was also observed in the non-grazing chamber of the DUN treatment from Day 9 to Day 15, and the final colony size was 60% larger than the control. The final colony size in the DUN treatment was also significantly larger than that in the PHA non-grazing chamber, but smaller than that in the PHA grazing chamber.

In Experiment 2, grazing by *G. dominans* reduced *R. salina* concentration to near zero by Day 15 (Figure 5); during this period, *G. dominans* increased initially, then decreased as food became limiting. The solitary *P. globosa* cell concentration in the non-grazing chamber of the RHO (*R. salina*) treatment increased steadily and reached $8.0 \times 10^5$ cells ml$^{-1}$; thus, the final average concentration for the entire incubator was $4.0 \times 10^5$ cells ml$^{-1}$, the same as that in the control (Figure 5). Colony concentration in the RHO treatment increased to $6080 \pm 0.5 = 3040$...
colonies ml–1 after 15 days, not significantly different from the control (average 2200 colonies ml–1). Colony development in the control was similar to that in Experiment 1, and the final colony size was 62 µm (Figure 6). Significant enlargement of \( P. \) globosa colonies was observed in the non-grazing chamber of the RHO treatment from Day 10 to Day 15, and the final colony size was 66% larger than the control.

Diffusion incubator experiments: \( G. \) cf. \( d \) anicum

In Experiment 3, \( R. \) salina was reduced by grazing to low concentration and did not recover until Day 15 (Figure 7). Glenodinium cf. danicum increased to high concentration within 5 days, before decreasing to near zero at the end of the experiment. In the RHO treatment, the final concentration of solitary \( P. \) globosa cells averaged \( 7.7 \times 10^4 \) to \( 3.9 \times 10^5 \) cells ml–1, significantly higher than that in the control (2.6 \( \times 10^5 \) cells ml–1); however, the final colony concentration (average 4800 ml–1) was not different from the control (4600 ml–1). Colony size was significantly larger in the RHO treatment, and by Day 15 the RHO treatment resulted in a 42% colony enlargement relative to the control (Figure 8).

Diffusion incubator experiments: \( T. \) longicornis

In Experiment 4, the non-grazing chamber was filled with \( P. \) globosa, whereas the grazing chamber contained \( T. \) longicornis and \( T. \) weissflogii. The phytoplankton cell dynamics in this experiment were very different from the other experiments (Figure 9). Grazing activity was confirmed by the accumulation of copepod faecal pellets (average 11 pellets ml–1 on Day 15) and the presence of newly hatched nauplii in the grazing chamber; however, no female copepods survived at the end of the experiments. The concentration of \( T. \) weissflogii in the grazing chamber remained low initially, then increased to a high concentration (7.3 \( \times 10^4 \) cells ml–1). In the non-grazing chamber, solitary \( P. \) globosa cells increased to a high concentration on Day 10, then decreased to 8.5 \( \times 10^3 \) cells ml–1 on Day 15. Thus, the average cell concentration
for the incubator would be $3.6 \times 10^4 \ T. \ weissflogii + 4.3 \times 10^5 \ P. \ globosa$ cells ml$^{-1}$, which was equivalent to 7.8 µg C ml$^{-1}$. The final average solitary $P. \ globosa$ cell concentration in the control was $6.9 \times 10^5$ cells ml$^{-1}$, or 6.4 µg C ml$^{-1}$, comparable to the TW ($T. \ weissflogii$) treatment. Colonies in the control were of similar size as in the other experiments, and the final colony size was 57 µm (Figure 10). In the non-grazing chamber of the TW treatment, colonies were significantly enlarged between Day 10 and Day 15, and the final colony size was on average 52% larger than the control.

Effects of initial cell concentrations
Prior to Experiment 5, $D. \ tertiolecta$ was grazed from $4.6 \times 10^4$ ml$^{-1}$ down to 1870 ml$^{-1}$, and $G. \ dominans$ increased from 1235 to 3900 ml$^{-1}$ within 3 days. Medium containing possible dissolved chemicals resulting from the grazing activities was collected and used to incubate various initial concentrations of solitary $P. \ globosa$ cells. Lower initial cell concentration resulted in significantly larger colonies after 13 days (Figure 11). However, the same trend was also observed in the control where untreated L-medium was used for the incubation (Figure 11). For the same initial cell concentration, the colony size did not differ between the dissolved chemical treatment and the control.

Effects of interspecific interaction
Four phytoplankton species were tested in Experiment 7 for their effects on $P. \ globosa$ colony size development. The initial carbon concentration was approximately the same for all phytoplankton species. After 10 days, the concentrations of solitary $P. \ globosa$ cells in all treatments were significantly lower than the control, but the effects on colony abundance were less clear (Figure 12). Colony size ranged from 63 to 78 µm across all treatments, with no significant differences.
DISCUSSION

Role of a chemical signal in *P. globosa* colony size development

In Experiment 1, solitary *P. globosa* cells in the control and all non-grazing chambers increased exponentially, then levelled off by Day 15. The maximum cell concentrations were possibly limited not by major nutrients (based on cell concentrations, <10% of the total nitrogen and phosphorus would have been consumed), but by the alkalinity of the medium (final pH = 9.0). Colony development in the grazing chamber of the PHA treatment was very similar to the results from conventional glass bottle incubation (Jakobsen and Tang, 2002). For example, similar initial cell concentrations of *G. dominans* and *P. globosa* resulted in almost identical final *P. globosa* colony concentration and size distribution (Figures 3 and 4 of this study; figure 5 ‘High grazing’ in Jakobsen and Tang (Jakobsen and Tang, 2002)]. In both studies, grazing by *G. dominans* induced the same degree of colony enlargement (~3-fold increase) relative to the control after 14–15 days. Such comparison shows that the diffusion incubators worked equally well as traditional glass bottles for studying *P. globosa* colony size development. However, the diffusion incubators prevent physical contact between the grazers in the grazing chambers and the *P. globosa* cells in the non-grazing chambers; therefore, colony size increase in the non-grazing chamber could only be induced by a chemical signal that resulted from grazing activities on the
opposite side. In Experiments 1–4, colonies in all non-grazing chambers were significantly enlarged, indicating that physical contact with grazers was not required to trigger the defensive response by *P. globosa*.

An important observation is that the grazing effect was not species specific: three grazer and four phytoplankton species in various combinations all resulted in *P. globosa* colony enlargement relative to the controls, although the extent of enlargement varied. Thus, *P. globosa* responded to chemicals characteristic of ambient grazing activities in general, and not just to physical contact or damage. This non-species-specific response would allow *P. globosa* lead time to activate the defence mechanism, especially against grazers that selectively feed on an alternatively available food source. For example, many copepods can graze on a monospecific diet of solitary Phaeocystis sp. cells [summarized in Tang et al. (Tang et al., 2001)], but prefer alternative food if Phaeocystis sp. cells are offered in a mixture with other food particles (Hansen et al., 1993; Tang et al., 2001). Thus, grazing on co-existing phytoplankton by copepods may not only reduce competition, but may also initiate the defence mechanism in *P. globosa* in advance (Experiment 4, Figure 10).

**Nature of the chemical signal**

In this discussion, ‘chemical signal’ is broadly defined as a change in the ambient chemical composition that leads to *P. globosa* colony enlargement. Such a change could be due to *de novo* synthesis of chemical compounds, or alteration of existing constituents (e.g. nutrient ratios) of the medium. While Experiments 1–4 confirm the role of a chemical signal in promoting *P. globosa* colony enlargement, Experiments 5 and 6 were designed to further characterize this chemical signal. Two possible working mechanisms need to be considered: a positive signal results from grazing activities that promotes colony size increase; a negative signal suppresses colony size increase and is reduced by grazing activities.

In Experiment 1, the DUN non-grazing chamber and the PHA non-grazing chamber had the same concentrations of *P. globosa* solitary cells and colonies (Figure 3), yet the final colony size was significantly larger in the DUN non-grazing chamber (Figure 4). Such a difference can be explained by a positive signal resulting from the *G. dominans* grazing activities in the corresponding grazing chambers. Colonies in the PHA grazing chamber were the largest, perhaps because the solitary *P. globosa* cells would have received the strongest chemical signal from the immediate surrounding grazing activities. Colony enlargement in the DUN and PHA treatments continued between Day 9 and 15, even though grazing activities were negligible (indicated by the declining grazer concentrations), suggesting that the grazing-related chemical signal lasted at least 6 days, enough time to reach within 90% of equilibrium distribution across the membrane filters (Figure 2). Therefore, the less pronounced colony enlargement in the PHA non-grazing chamber relative to the PHA grazing chamber suggests that the signal was partly lost before diffusing across the membrane filters.

In Experiment 5, the response of *P. globosa* to the chemical signal was inversely proportional to the initial solitary cell concentration. At first glance, these observations seem to be consistent with the hypothesis that the chemical signal behaved as nutrients, such that for an equal amount of the signal, a lower initial cell concentration would respond more strongly (larger colonies). However, this cell concentration-dependent response was also found in the control, where the grazing-related chemicals were replaced by regular L-medium. Thus, results from Experiment 5 led to the hypothesis that a second mechanism might be at work in regulating *P. globosa* colony size: high solitary *P. globosa* cell concentration appeared to suppress colony development, and grazing of *P. globosa* cells decreased such suppression and allowed the colonies to increase in size. This hypothetical suppression also appears to come from other phytoplankton species: interacting with other phytoplankton species reduced the growth of solitary *P. globosa* cells, yet the colonies were still restricted to small size (Experiment 7). Grazing on these phytoplankton species would
Adaptive significance of the chemical signalling mechanism

Our previous study showed that grazing induced a colony size increase in Phaeocystis (Jakobsen and Tang, 2002). The goal of the present study was to characterize the signalling mechanism that induces such a defensive response. Several conclusions can be drawn from the experimental results. Colony enlargement in Phaeocystis could be induced by a chemical signal, which has, compared with a mechanical signal, the following advantages: because P. globosa solitary cells are readily ingested by many protozoan and copepod species, sometimes at high rates (Tang et al., 2001), it would be highly advantageous for P. globosa to initiate the defence mechanism prior to physical contact with the grazers. Thus, the ability of P. globosa to detect and respond to a grazing-related chemical signal by increasing the colony size will enhance the chance of its survival.

In this study, colony size development appeared to be suppressed when the growth of solitary cells is favoured, or when there is strong competition from other phytoplankton. Grazing will not only reduce this inhibition, but also release a positive signal that induces colony enlargement. Large P. globosa colonies may suffer from fast sinking, reduced nutrient uptake and additional metabolic cost for mucous production (Peng et al., 1999; van Rijssel et al., 2000); thus, it would frequently be beneficial for P. globosa to remain as solitary cells and small colonies when grazing pressure is low. This grazing-related dual mechanism (decreased inhibition plus positive signal) would effectively regulate colony size development according to the ambient conditions.

For prey with a single defence mechanism, its chance against diverse predators could be enhanced by a non-specific warning system. In the present case, the globally distributed P. globosa is required to interact and defend against wide-ranging grazers. The limitation of its invariable defence mechanism (i.e., colony formation and enlargement) can be partly compensated for by a non-specific signalling mechanism that responds to general grazing activities in the ambient environment. This study showed that the signal(s) that regulated colony enlargement was not specific to the ambient phytoplankton or the grazers. Thus, this non-species-specific response would allow P. globosa to defend itself in diverse plankton communities.

The ecology of colony development in Phaeocystis

Traditionally, colony development and grazing are treated as separate processes in Phaeocystis research; however, this and our earlier studies (Jakobsen and Tang, 2002) showed that the two processes are linked, leading to new understanding of Phaeocystis sp. bloom dynamics. For instance, field observations show that diatom blooms regularly precede P. globosa blooms in the North Sea (Peperzak et al., 1998; Frangoulis et al., 2001). The conventional view is that this bloom succession is regulated by changes in ambient nutrient composition and concentration, and light intensity (Riegman et al., 1992; Peperzak et al., 1998); yet, the role of grazers could be equally important. Results from the present study suggest that grazing by copepods will not only reduce the diatom population, but also induce P. globosa colony enlargement such that subsequent size mismatch between the colonies and the grazers would further favour P. globosa bloom development.

The application of defence theory has led to new insights into the ecological and evolutionary aspects of P. globosa colony development (Jakobsen and Tang, 2002; this study); nevertheless, several questions await further study. (i) While the involvement of a chemical signal in P. globosa colony enlargement is evident, the chemical nature of the signal remains unknown. Because the signal appeared to be non-specific, it is likely to be a common metabolite(s), such as excretory or cell lysis products. (ii) Although colony formation is a common feature in P. globosa, the relative dominance between the solitary form and colonial form, and colony size distributions, can vary significantly between populations (Lancelot et al., 1998). This geographical variation may reflect the differences in the ambient phytoplankton composition, growth and grazing pressure. (iii) Phaeocystis globosa colony formation in nature is probably regulated by a combination of physical, chemical and biological factors. Different populations of P. globosa are also likely to show different responses to these factors, and evolve differently as a function of such factors plus hydrological conditions. While small-scale laboratory experiments can isolate and test individual factors, study of the synergistic effects of various factors would require more comprehensive, large scale experiments and field observations.

ACKNOWLEDGEMENTS

The author thanks S. Stromm for providing protozoan cultures, Hans Jakobsen for helping with protozoan species identification, Elith Pedersen and Kristine Hansen for technical assistance, T. Kierboe, the editor and two reviewers for constructive comments. This study was supported by a Carlsberg Foundation (Denmark) research fellowship awarded to K.W.T., and Danish Natural Science Research Council Grant # 5801393 awarded to Thomas Kierboe.
REFERENCES


Received on August 28, 2001; accepted on April 3, 2003.