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

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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 defence mechanisms can be costly to maintain, it is to the advantage of an organism to activate its defence 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 defence 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 (Lürling and van Donk, 1997; Lürling, 1999). Subsequent studies showed that this defensive response is triggered by ‘infochemicals’ released during the grazing process (Lampert *et al.*, 1994; Lürling and van Donk, 1997; Lürling, 1998), although the nature of these infochemicals is still under dispute (Wiltshire and Lampert, 1999; Lürling 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 dimethylsulphoniopropionate, a precursor of the climatically active gas dimethylsulphide (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 prevalence of the colonial form in natural *Phaeocystis* sp. blooms in contrasting water types prompts the ideas that *Phaeocystis* 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 *Scenedesmus* sp., *Phaeocystis* sp. colonies are hollow, balloon-like structures with individual cells lying beneath a thin mucous skin (Van Rijssel *et al.*, 1997). This arrangement allows the colonies to increase their size with a limited number of cells—colonies of up to a few millimetres 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.*, 1998; 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 declined (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 boiled mud added to the medium, indicating 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* cells. 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 characterize 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 $19 \pm 1^\circ\text{C}$ with 60–100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ 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 dominans* was originally isolated from Øresund, Denmark. Another heterotrophic dinoflagellate, *Glenodinium* 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). Protozoan 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 $19 \pm 1^\circ\text{C}$ with $60\text{--}100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ 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 $2 \mu\text{m}$ 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

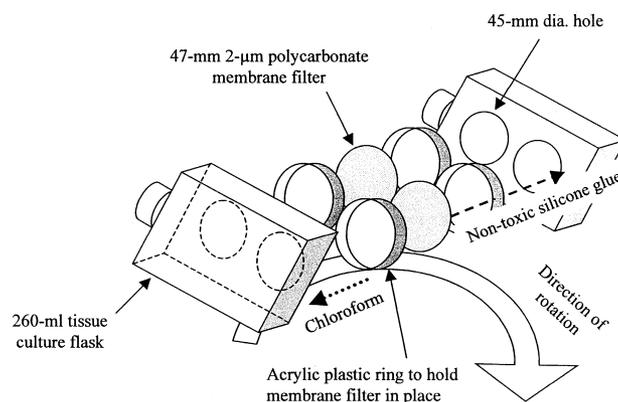


Fig. 1. Diffusion incubator. Each incubator contains two chambers made of polystyrene tissue culture flasks. Two polycarbonate membrane filters are first glued to one flask with non-toxic silicone glue and held in place by acrylic plastic rings. The rings are fused to both flasks by chloroform, which is volatile and leaves no harmful traces. The incubator is rotated in a direction that ensures equal amounts of light for both sides.

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). Changes in cell abundance, *P. globosa* colony abundance

Table I: Phytoplankton and grazer species used in experiments

Phytoplankton	Taxonomic group	Size (μm)	Carbon content (pg cell^{-1})
<i>Phaeocystis globosa</i>	Prymnesiophyte	4.4	9.3
<i>Isochrysis galbana</i>	Prymnesiophyte	4.2	8.2
<i>Dunaliella tertiolecta</i>	Chlorophyte	7.0	30.2
<i>Rhodomonas salina</i>	Cryptophyte	6.9	29.8
<i>Thalassiosira weissflogii</i>	Diatom	15.0	105
Grazers	Taxonomic group	Size (μm)	
<i>Gyrodinium dominans</i>	Naked heterotrophic dinoflagellate	15	
<i>Glenodinium cf. danicum</i>	Thecate heterotrophic dinoflagellate	9.8	
<i>Temora longicornis</i>	Calanoid copepod	1000	

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.

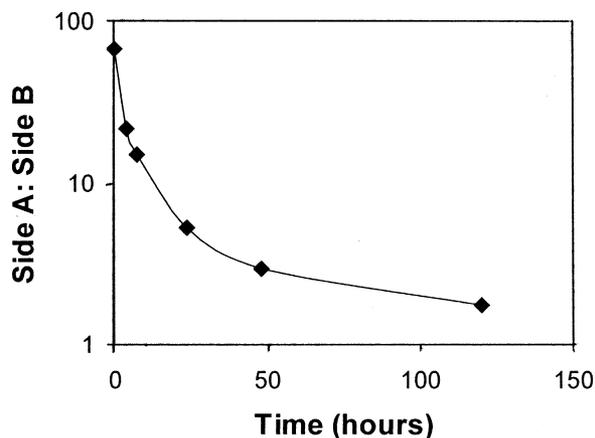


Fig. 2. Diffusion of food dye across membrane filters of a diffusion incubator. Side A of the incubator began with red food dye and side B with distilled water. The incubator was then secured on a rotating plankton wheel, and aliquots were taken through time from both sides for measuring absorbance at 320 nm. Results are expressed as the ratio of absorbance between A and B, and a ratio of 1 indicates equilibrium distribution of food dye between the two sides.

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 II: Initial conditions of diffusion incubators for Experiments 1–4

Expt	Treatment	'Grazing' chamber		'Non-grazing' chamber (cells ml ⁻¹)	n
		Phytoplankton conc. (cells ml ⁻¹)	Grazer abundance ^a (cells ml ⁻¹)		
1	PHA	2 × 10 ⁴ <i>P. globosa</i>	400 <i>G. dominans</i>	2 × 10 ⁴	2
	DUN	2 × 10 ⁴ <i>D. tertiolecta</i>	400 <i>G. dominans</i>	2 × 10 ⁴	3
2	RHO	2 × 10 ⁴ <i>R. salina</i>	200 <i>G. dominans</i>	2 × 10 ⁴	3
3	RHO	2 × 10 ⁴ <i>R. salina</i>	180 <i>G. cf. danicum</i>	10 ⁴	3
4	TW	9000 <i>T. weissflogii</i>	10 <i>T. longicornis</i> (total)	1.3 × 10 ⁴	4

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.

^aGrazer abundance for *T. longicornis* is expressed as the total number of females.

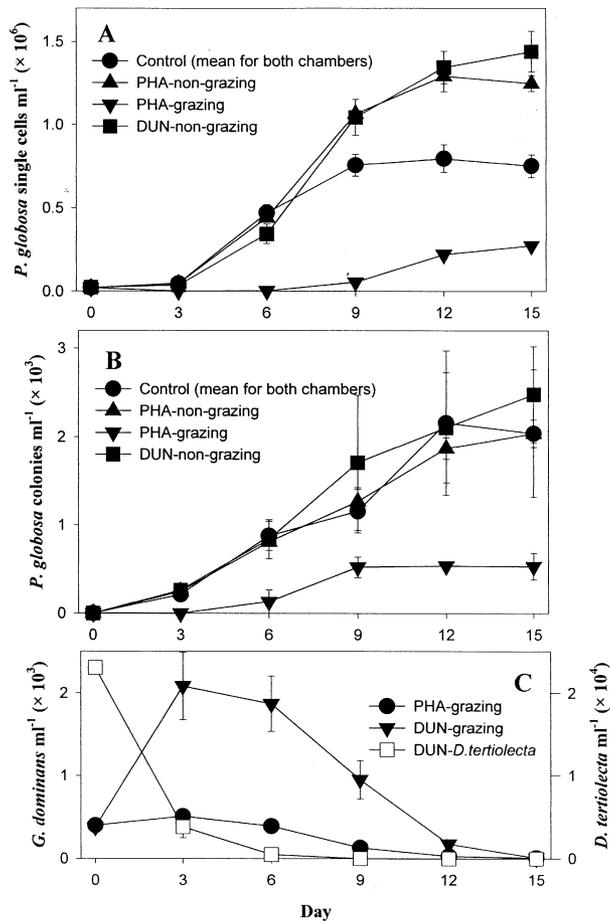


Fig. 3. Diffusion incubator with *G. dominans* as grazers (Experiment 1). PHA, *P. globosa* treatment; DUN, *D. tertiolecta* treatment. (A) *Phaeocystis globosa* solitary cell concentrations. (B) *Phaeocystis globosa* colony concentrations. Results for the control are expressed as average concentrations per chamber. (C) Cell concentrations of *G. dominans* and *D. tertiolecta*. Error bars are standard deviations of 3–4 replicates. Initial experimental conditions are given in Table II.

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.

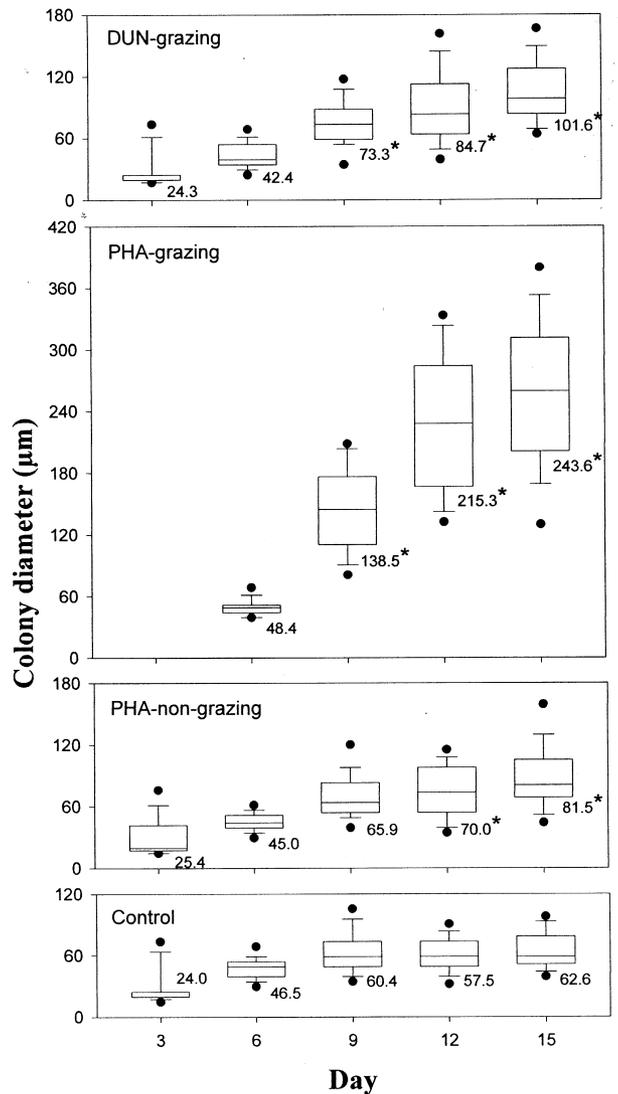


Fig. 4. Box plots showing 5, 10, 25, 50, 75, 90 and 95 percentiles of *P. globosa* colony diameters (Experiment 1). PHA, *P. globosa* treatment; DUN, *D. tertiolecta* treatment. Each box represents 90–120 measurements. Numbers are the geometric mean colony diameters, and asterisks indicate significant difference ($P < 0.05$) from the control of the same sampling day.

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

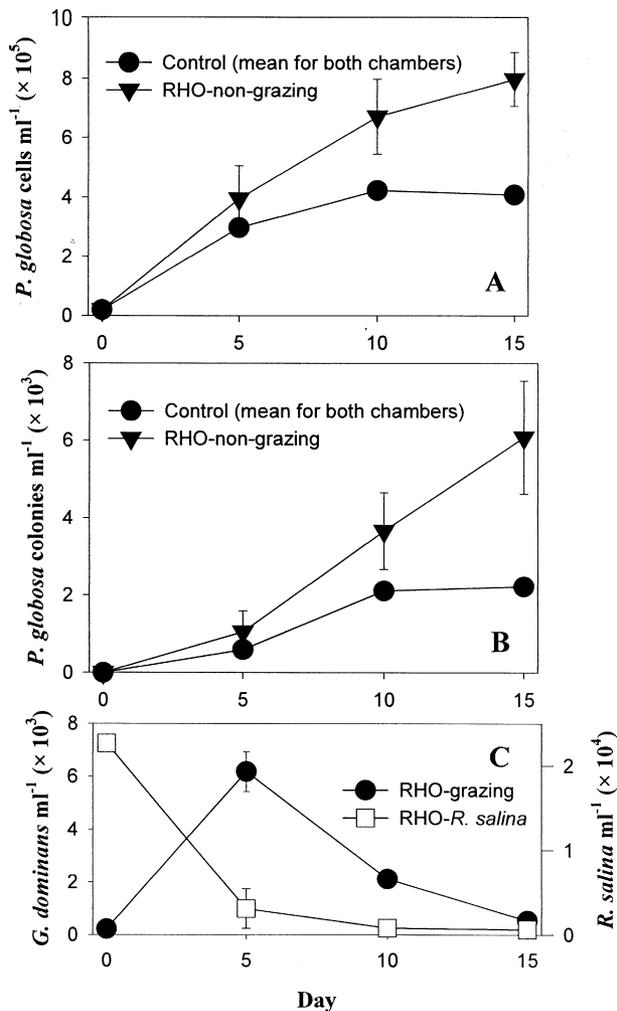


Fig. 5. Diffusion incubator with *G. dominans* as grazers (Experiment 2). RHO, *R. salina* treatment. (A) *Phaeocystis globosa* solitary cell concentrations. (B) *Phaeocystis globosa* colony concentrations. Results for the control are expressed as average concentrations per chamber. (C) Cell concentrations of *G. dominans* and *R. salina*. Error bars are standard deviations of 2–3 replicates. Initial experimental conditions are given in Table II.

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 1.44×10^6 cells ml^{-1} ; thus, the average concentration for the entire incubator (grazing chamber + non-grazing chamber) would be 7.2×10^5 cells ml^{-1} . Similarly, the average solitary *P. globosa* cell concentration in the PHA (*P. globosa*) treatment was 7.6×10^5 cells ml^{-1} for the entire incubator. Both values were not significantly different from the average solitary cell concentration in the control, which was 7.5×10^5 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.

Colony size in the control increased slightly, then remained steady at $\sim 60 \mu\text{m}$ 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 8.0×10^5 cells ml^{-1} ; thus, the final average concentration for the entire incubator was 4.0×10^5 cells ml^{-1} , the same as that in the control (Figure 5). Colony concentration in the RHO treatment increased to $6080 \times 0.5 = 3040$

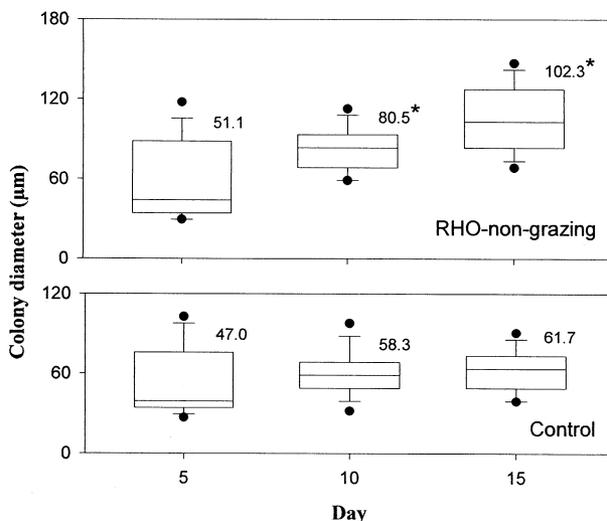


Fig. 6. Box plots of *P. globosa* colony diameters (Experiment 2). RHO, *R. salina* treatment. Each box represents 60–90 measurements. Numbers are the geometric mean colony diameters, and asterisks indicate significant difference from the control of the same sampling day.

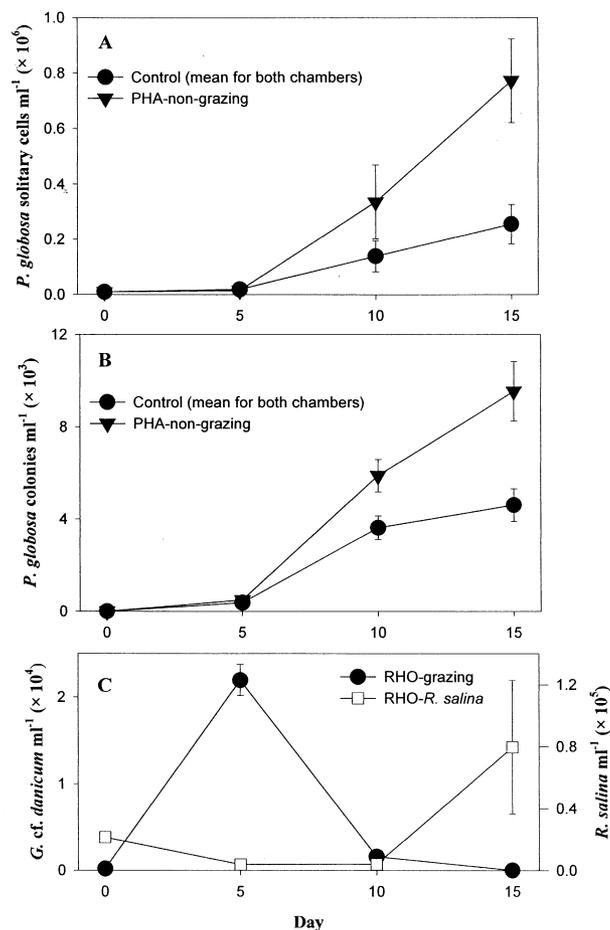


Fig. 7. Diffusion incubator with *G. cf. danicum* as grazers (Experiment 3). RHO, *R. salina* treatment. (A) *Phaeocystis globosa* solitary cell concentrations. (B) *Phaeocystis globosa* colony concentrations. Results for the control are expressed as average concentrations per chamber. (C) Cell concentrations of *G. cf. danicum* and *R. salina*. Error bars are standard deviations of 3–6 replicates. Initial experimental conditions are given in Table II.

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. danicum*

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

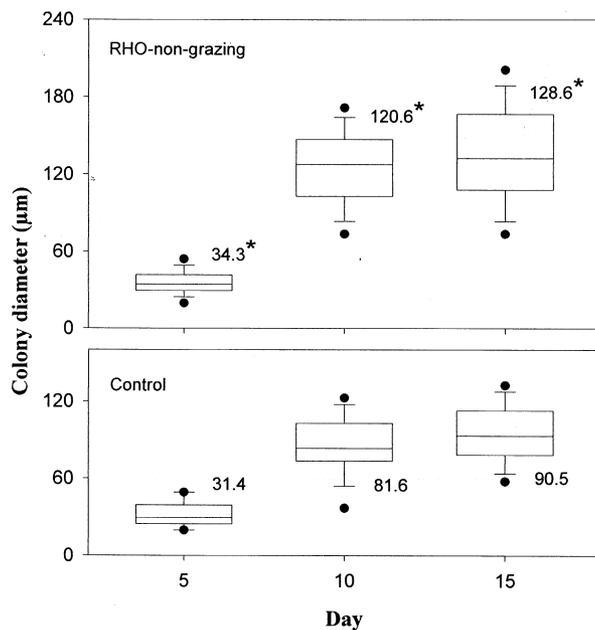


Fig. 8. Box plots of *P. globosa* colony diameters (Experiment 3). RHO, *R. salina* treatment. Each box represents 90–180 measurements. Numbers are the geometric mean colony diameters, and asterisks indicate significant difference from the control of the same sampling day.

of the experiment. In the RHO treatment, the final concentration of solitary *P. globosa* cells averaged $7.7 \times 10^5 \times 0.5 = 3.9 \times 10^5$ cells ml^{-1} , significantly higher than that in the control (2.6×10^5 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 *Thalassiosira weissflogii* and *T. longicornis*. 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 (7.3×10^4 cells ml^{-1}). In the non-grazing chamber, solitary *P. globosa* cells increased to a high concentration on Day 10, then decreased to 8.5×10^5 cells ml^{-1} on Day 15. Thus, the average cell concentration

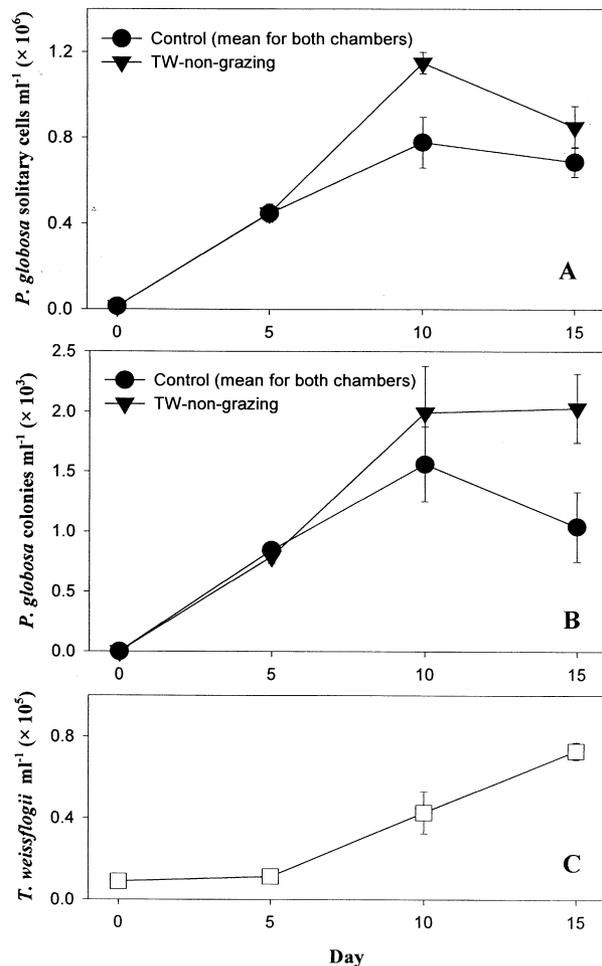


Fig. 9. Diffusion incubator with *T. longicornis* as grazers (Experiment 4). TW, *T. weissflogii* treatment. (A) *Phaeocystis globosa* solitary cell concentrations. (B) *Phaeocystis globosa* colony concentrations. Results for the control are expressed as average concentrations per chamber. (C) Cell concentrations of *T. weissflogii*. Error bars are standard deviations of 4–6 replicates. Initial experimental conditions are given in Table II.

for the incubator would be 3.6×10^4 *T. weissflogii* + 4.3×10^5 *P. globosa* cells ml^{-1} , which was equivalent to $7.8 \mu\text{g C ml}^{-1}$. The final average solitary *P. globosa* cell concentration in the control was 6.9×10^5 cells ml^{-1} , or $6.4 \mu\text{g C ml}^{-1}$, 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 $57 \mu\text{m}$ (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.

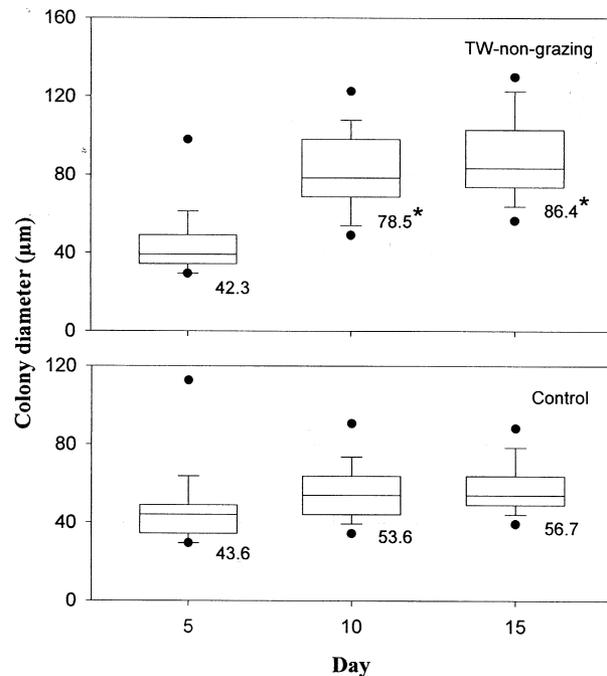


Fig. 10. Box plots of *P. globosa* colony diameters (Experiment 4). TW, *T. weissflogii* treatment. Each box represents 120–180 measurements. Numbers are the geometric mean colony diameters, and asterisks indicate significant difference from the control of the same sampling day.

Effects of initial cell concentrations

Prior to Experiment 5, *D. tertiolecta* was grazed from $4.6 \times 10^4 \text{ ml}^{-1}$ 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 $78 \mu\text{m}$ across all treatments, with no significant differences.

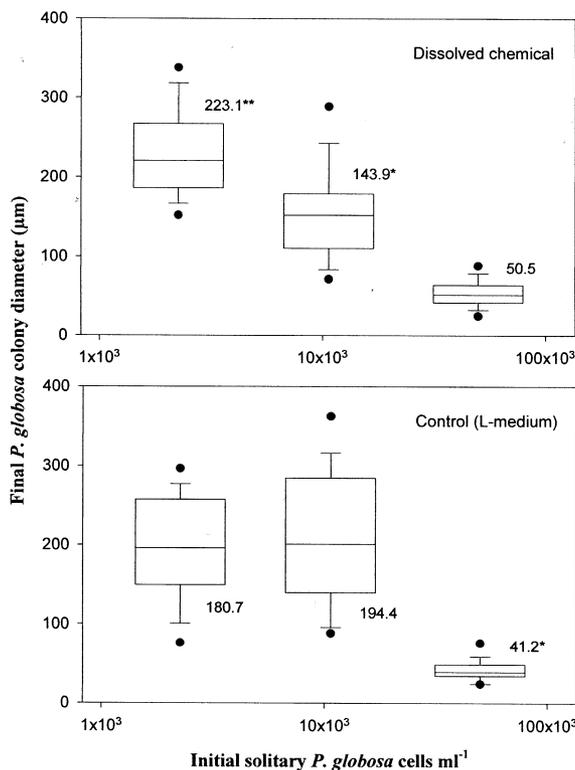


Fig. 11. Response to grazing-related dissolved chemicals as a function of initial solitary *P. globosa* cell concentrations (Experiment 5). Untreated L-medium was used as a control. Each box represents final colony diameters (60 measurements) after 13 days. Numbers are the geometric mean colony diameters; asterisks indicate significant difference between treatments of different initial cell concentrations. For the same initial cell concentrations, there was no significant difference between the dissolved chemical treatment and control in terms of final colony size. Initial experimental conditions are explained in the text.

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;

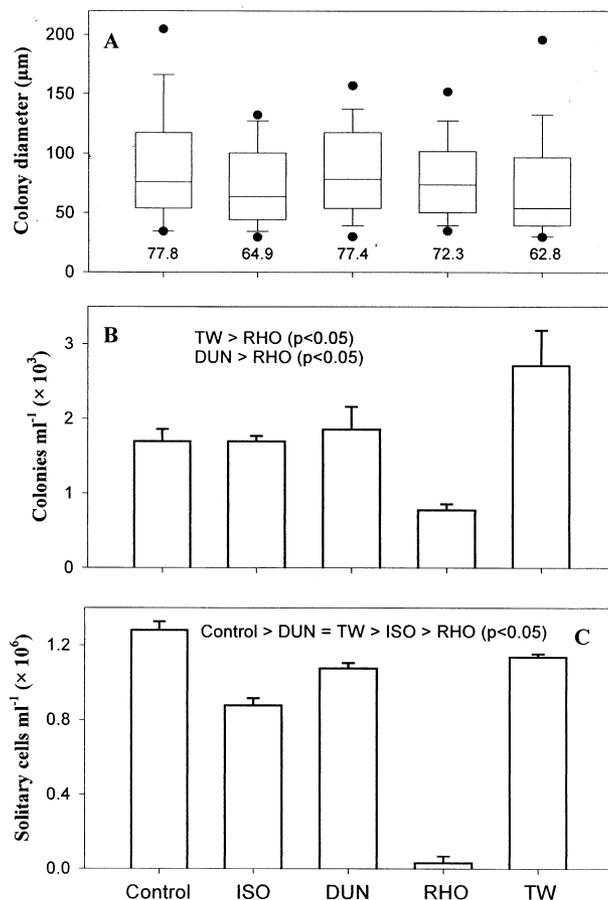


Fig. 12. Effects of interspecific interactions on *P. globosa* colony development (Experiment 7). Diffusion incubators were filled on one side with *P. globosa* solitary cells and on the opposite side with equal carbon concentrations of a different phytoplankton species (Control, *P. globosa*; ISO, *I. galbana*; DUN, *D. tertiolecta*; RHO, *R. salina*; TW, *T. weissflogii*). (A) Box plots of colony diameters after 10 days. Each box represents 60 measurements and numbers are the geometric mean diameters. (B) *Phaeocystis globosa* colony concentrations. (C) *Phaeocystis globosa* solitary cell concentrations. Significant difference among treatments is also shown. Error bars are standard deviations of duplicates. Initial experimental conditions are explained in the text.

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

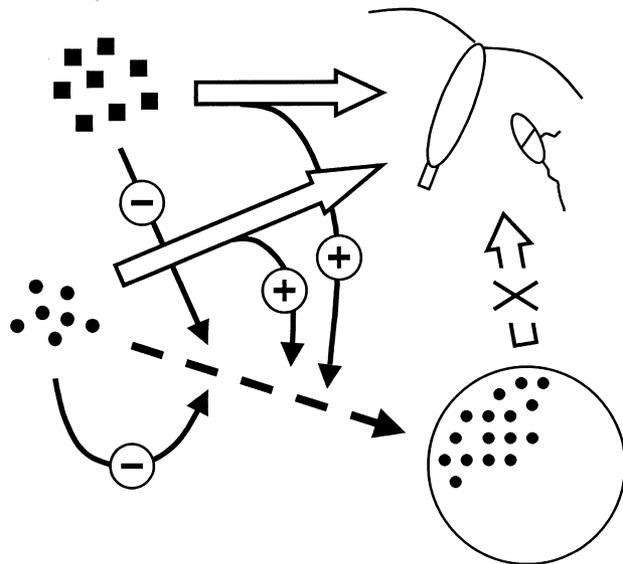


Fig. 13. Dual mechanism for regulating *P. globosa* colony size development. High concentrations of solitary *P. globosa* cells (black circles) and other phytoplankton (squares) suppress (-) colony enlargement. Grazing (block arrows) by zooplankton (e.g. copepods and heterotrophic dinoflagellates) reduces this inhibition and produces a positive signal (+) that stimulates colony size increase, which helps protect *P. globosa* cells.

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

therefore relieve the inhibition on colony enlargement, as shown in Experiments 1–4 (Figure 13).

Adaptive significance of the chemical signalling mechanism

Our previous study showed that grazing induced a colony size increase in *P. globosa* (Jakobsen and Tang, 2002). The goal of the present study was to characterize the signalling mechanism(s) that induces such a defensive response. Several conclusions can be drawn from the experimental results.

Colony enlargement in *P. globosa* could be induced by a chemical signal, which has, compared with a mechanical signal, the following advantage: because *P. globosa* solitary cells are readily ingested by many protozoan and copepod species, sometimes at high rates [(Tang *et al.*, 2001) and references therein], 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 mucus production (Ploug *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 invariant 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-species 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, larger scale experiments and field observations.

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