

## Trophic significance of solitary cells of the prymnesiophyte *Phaeocystis globosa* depends on cell type

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### Abstract

With the use of five different isolates of *Phaeocystis globosa* solitary cells from the North Sea, we conducted experiments to reveal whether grazing and development of the nauplii of the calanoid copepod *Temora longicornis* varies in response to the cell type. Two *P. globosa* strains representing nonflagellated cells were ingested at intermediate to high rates and resulted in high survival and development, comparable to the *Rhodomonas* sp. control. In contrast, the response to three mesoflagellate strains was highly variable. Feeding on two of these strains was avoided, whereas the third strain was ingested; however, the mesoflagellates induced poor survival and development regardless of the feeding response. These observations differ from previous results, which generally demonstrate microzooplankton feeding on *Phaeocystis*. The morphological characterization of strains, together with mixture experiments, revealed that neither the production of transparent exopolymer particles and chitinous threads nor toxicity can explain the observed response. The cohesion of the threads into pentagonal stars was observed only in the avoided mesoflagellate and might cause a mechanical hindrance for the ingestion of mesoflagellates. Our results suggest that grazing loss and trophic transfer efficiency might be overestimated when solitary cells are treated as a single functional group with regard to their trophic position.

The prymnesiophyte *Phaeocystis* is an important taxon with a strong effect on marine ecosystem function and biogeochemical cycling of organic carbon and volatile trace gases. The genus has a worldwide distribution in both coastal and open waters, and frequent blooms of the colonial stage of *Phaeocystis* can temporally and spatially dominate temperate and cold-water ecosystems (Rousseau et al. 1994; Schoemann et al. 2005). However, the factors leading to bloom formation and the fate of the large organic production in different environments is not yet completely resolved. Blooms can lyse and contribute to nuisance accumulation of dissolved organic carbon, sink to the seafloor as aggregates, or enter the food web via grazing (Schoemann et al. 2005).

Bloom formation and trophodynamic significance of *Phaeocystis* appear to be strongly related to its life cycle, the food web structure, and complex interactions among potential grazers. Although some investigations report a negative effect of *Phaeocystis* on marine organisms

related to some toxin production (Stabell et al. 1999), most studies so far indicate that grazing on *Phaeocystis* depends largely on its life stage and, thus, on a size-based match between prey and predator communities (Schoemann et al. 2005). The heteromorphic life cycle of *Phaeocystis* involves the transition between solitary cells of a few micrometers and mucilaginous colonies of up to several millimeters in size (Cariou et al. 1994; Rousseau et al. 1994). The few experimental laboratory and field studies performed generally demonstrate a high vulnerability of the solitary cell stage to microzooplankton grazing. Ciliates and heterotrophic dinoflagellates readily ingest solitary cells and show a positive growth response during the formation of a *Phaeocystis* bloom in the field (Weisse and Scheffel-Möser 1990) or in the laboratory (Tang et al. 2001). In contrast, because of their small size, solitary cells are not grazed efficiently by metazoan zooplankton species such as adult copepods (Verity and Smayda 1989; Breton et al. 1999) unless cell concentrations are high (Cottonnec et al. 2001; Turner et al. 2002). Although likely not triggered by a single factor (Peperzak et al. 1998), the transition of single cells to larger colonies is interpreted as a mechanism by which *Phaeocystis* reduces large grazing losses by protozooplankton grazing (e.g., Weisse et al. 1994; Verity 2000), a hypothesis recently supported by the observation of an enlargement of colony size in the presence of protist grazers or a chemical signal produced by potential grazers (Jakobsen and Tang 2002; Tang 2003). In contrast to single cells, colonies suffer only little grazing loss to the zooplankton community in temperate ecosystems (Bautista et al. 1992; Gasparini et al. 2000). Only small-sized colonies were found to be ingested by *Acartia tonsa* (Verity 2000).

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Table 1. Strain characteristics of *Phaeocystis globosa* used in experiments and concentration of transparent exopolymer particles (TEP,  $\mu\text{g GX}_{\text{eq.}} \text{L}^{-1}$ ).

<i>P. globosa</i> strain number	Cell type	Area	Year isolated	Threads/pentagonal star	TEP
Pg20	Nonflagellate	Marsdiep Texel	2001	-/-	9.6±1.3
Pg1	Nonflagellate	German Bight	1999	-/-	14.5±4.6
Pg6	Flagellate	NS-offshore Texel	1994	+/+	12.0±2.3
Pg5	Flagellate	NS-Oyster Grounds	2000	+/+	23.5±2.7
Pg2	Flagellate	Oosterschelde	1991	+/(+)	5.9±1.5

The generally high grazing rate of microzooplankton on *Phaeocystis* solitary cells is a key process in the current understanding of the trophodynamic significance of *Phaeocystis* blooms. Solitary cells precede the bloom phase, co-occur with dominating colonies during the blooms, and are liberated at high rates on termination of the colonial bloom (Rousseau et al. 1994; Schoemann et al. 2005). Microzooplankton grazing on these cells might not only influence bloom formation but also constitute the major pathway for transfer of the high *Phaeocystis* biomass to higher trophic levels (Rousseau et al. 2000; Tang et al. 2001).

In contrast to the general conclusion of a high grazing loss of solitary cells, the different *Phaeocystis* cell types have been so far inadequately considered in trophodynamic studies. In nature, solitary cells of *Phaeocystis globosa* are not represented by a single cell type (Kornmann 1955; Peperzak et al. 2000). In addition to colonial cells lacking flagella, at least three flagellated cell types (micro-, meso-, and macroflagellates) differing in size, ploidy, and morphology have been described to occur as free-living solitary cells (Kornmann 1955; Peperzak et al. 2000). Their position in the life cycle is poorly known. The diploid macroflagellate is similar in size to the nonflagellated cell, can form new colonies, and has only occasionally been observed in the field. The smaller micro- and mesoflagellates are haploid and are presumably formed by meiosis from the macroflagellate (Peperzak et al. 2000). In the field, both cell types are difficult to separate by light microscopy but occur simultaneously and often peak after a colonial bloom (Veldhuis et al. 1986; Peperzak et al. 2000). Unlike microflagellates, mesoflagellates produce chitinous threads known to form pentagonal stars.

We studied the feeding and growth response of nauplii of the calanoid copepod *Temora longicornis* to five different strains of *P. globosa*, to reveal whether grazing by *T. longicornis* nauplii is strain specific and potentially related to the cell type. These questions have not been addressed in previous studies concerning microzooplankton grazing on *Phaeocystis*. The strains consisted either of the solitary colonial cell type or of mesoflagellates, which predominantly occur in different phases of a *Phaeocystis* bloom. The grazing and growth experiments were accompanied by measurements of transparent exopolymeric particles (TEP), which recently have been found to inhibit grazing of nauplii (Dutz et al. 2005). In contrast to previous studies, our results reveal that high grazing vulnerability is not a general feature of solitary cells of *P. globosa*.

## Material and methods

**Cultures**—Algal cultures: Five different strains of *P. globosa* were used in experiments, whereas *Rhodomonas* sp. or *Isochrysis* sp. served as a control for good food. The *Phaeocystis* strains originated from different areas of the Southern Bight of the North Sea and generally co-occur with *T. longicornis* (Table 1). *Phaeocystis* strains were grown in 1-liter batch cultures at 15°C with a 16 : 8 h light : dark (LD) cycle at an irradiance of 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in F/2 media (-Si). The strains were used in experiments in exponential growth phase approximately 1 week after the inoculation of each culture. *Rhodomonas* sp. was grown in continuous cultures with a dilution rate of 0.2  $\text{d}^{-1}$ , and *Isochrysis galbana* was grown in 0.5-liter batch cultures; both species were kept under similar conditions as the *Phaeocystis* strains. The cell concentration and average cell volume of all algae were measured at the start of each experiment with an ELZONE electronic particle counter (Particle Data Inc.). The carbon content of algae was estimated on the basis of a volume to carbon conversion of 0.11  $\text{pg C } \mu\text{m}^{-3}$  (Edler 1979). The cell type and the presence or absence of threadlike filaments were examined by light microscopy (Zeiss Axioscope,  $\times 1000$  magnification, phase contrast) from subsamples fixed in acidic Lugol (final concentration 2%) or glutaraldehyde (final concentration 1%). To identify filaments and five-rayed stars, cultures were stained with Alcian Blue (0.02%, Merck) on the microscope slide.

**Copepod culture**: The calanoid copepod *T. longicornis* originated from a laboratory culture isolated from the Dutch Wadden Sea >10 generations earlier. The copepods were kept at 15°C, with a 12 : 12 LD cycle of dim light. The cultures were fed an excess mixture of *Rhodomonas* sp., *I. galbana*, and *Oxyrrhis marina* (Klein Breteler et al. 1999). Nauplii used in grazing experiments had hatched in cultures approximately 5 d before the start of the experiments.

**Experiments**—Grazing: Grazing of nauplii was measured with all five *Phaeocystis* strains, offered as a single-species diet, and in 1 : 1 mixtures of two *Phaeocystis* strains (Pg2 and Pg5) and *Rhodomonas* sp. The food concentration of each food species was approximately 150  $\mu\text{g C L}^{-1}$ ; *Rhodomonas* sp. and *Isochrysis* sp. controls were run with approximately 150 or 300  $\mu\text{g C L}^{-1}$ . At the start of the

experiments, 50–150 nauplius III–V stages were isolated from the culture and placed into 625-mL acclimation bottles containing the intended food solution. The number of nauplii was chosen to guarantee sufficient feeding response during the experiments (>10% compared with grazing controls without copepods), without seriously reducing the food concentration.

The nauplii were first acclimated for 24 h, after which the experiment was run for 6 h; all incubations were performed in 135-mL bottles, rolled at the speed of 1 rpm. At the end of each experiment, the number and life stages of the nauplii were checked. The number of algal cells in three replicate experimental bottles and three replicate controls was measured at the start and end of the experiment with an ELZONE electronic particle counter; the size distributions of the two *Phaeocystis* strains used in mixture experiments did not overlap with *Rhodomonas* sp., allowing all algal counts to be made with the particle counter. The food concentration during experiments never decreased more than 10%. The clearance and carbon-specific ingestion rates were calculated according to Frost (1972) after transformation of the stage composition into carbon weights according to  $C (\mu\text{g}) = \exp(-1.3398 + 0.1899X \text{ stage}; \text{Koski unpubl. data})$ . The calculation of selectivity was based on the selection coefficient  $\alpha$  of Chesson (1983).

**Development and mortality:** The development rate of nauplii was determined with four *Phaeocystis* strains (Pg1, Pg2, Pg5, and Pg20), at a concentration of  $150 \mu\text{g C L}^{-1}$ , with *Rhodomonas* sp. controls at  $150 \mu\text{g C L}^{-1}$  and with 1 : 1 mixtures of Pg2 and Pg5 and *Rhodomonas* sp. (total food concentration of  $300 \mu\text{g C L}^{-1}$ ). Duplicate experiments were started with approximately 200 early nauplius stages (NI–II) and were incubated in bottles of 330 mL on a rolling apparatus at 1 rpm for 6 d. At days 2, 4, and 6 of the experiment, approximately 100, 150, and 330 mL, respectively, of the bottle was sampled for nauplius abundance and stage distribution (generally at least 30 individuals), and about 80% of the food suspension was renewed. Development was expressed as an increase in the mean stage over time, whereas mortality was calculated from a linear model of mortality, assuming constant mortality during the experiment and after correcting for sampling mortality.

**Measurements of TEP**—TEP concentrations in food suspensions from single-species diet experiments were measured spectrophotometrically in six replicates according to the method of Passow and Alldredge (1995). The food suspensions (20 mL) were filtered onto 0.4- $\mu\text{m}$  pore size polycarbonate membrane filters (Poretics, Osmomics) under low vacuum ( $<10^4$  Pa). TEP were stained on damp filters for approximately 2 s with 500  $\mu\text{L}$  of 0.02% Alcian Blue dissolved in aqueous acetic acid (0.06%). The stained filters were washed twice with distilled water, transferred to glass test tubes, and soaked in 6 mL of 80% sulfuric acid for at least 6 h. The test tubes were agitated regularly. Finally, light extinction of the solution in a 1-cm cuvette was measured spectrophotometrically at 787 nm against

80% sulfuric acid as a reference. Three to four filter blanks were measured simultaneously. TEP concentrations were calculated as  $C_{\text{TEP}} = (E_{787} - C_{787}) \times f_x / V$ . In this formula,  $E_{787}$  is the absorption of the sample,  $C_{787}$  is the absorption of the blank,  $V$  the filtered volume, and  $f_x$  a calibration factor (Passow and Alldredge 1995). Gum Xanthan was used as a standard to calibrate the dye. The calibration factor  $f_x$  was  $76.6 \mu\text{g}$ . TEP concentrations are expressed as micrograms of Gum Xanthan staining equivalent per liter ( $\mu\text{g GX}_{\text{equiv. L}^{-1}}$ ).

**Statistics**—All data were tested for homogeneity of slopes and equality of variances; if assumptions were not met, the data were log transformed. The differences in clearance and ingestion rates between the algae and between mixture and single-species experiments were tested with a one-way analysis of variance, followed by a Tukey's honestly significant difference (HSD) test for pairwise comparisons. The significance of selection was formally tested against  $\alpha = 0.5$  (no selection) by a one-sample  $t$ -test. The differences in development between different treatments were tested by running a one-way analysis of variance and Tukey HSD for the final life stage reached in the 6-d experiment.

## Results

**Strain characteristics**—The five *P. globosa* strains differed in size (Fig. 1). The two largest strains, Pg1 and Pg20, had an equivalent spherical diameter (ESD) of 5.6 and 5.7  $\mu\text{m}$ , respectively. These strains possess neither flagella, threadlike filaments, nor pentagonal stars. In contrast, the three smaller strains Pg2, Pg5, and Pg6 with an ESD of 3.6, 3.7, and 4.2  $\mu\text{m}$ , respectively, exhibited the two characteristic flagella, filaments, and pentagonal stars. These features characterize the three smaller strains as mesoflagellates (sensu Peperzak et al. 2000), whereas the two larger strains represent the nonflagellated cell type. Significant differences were observed in the number of filaments present per cell, with on average  $6.7 \pm 2.7$ ,  $5.0 \pm 2.5$ , and  $3.4 \pm 2.4$  in strains Pg6, Pg5, and Pg2, respectively (one-way ANOVA,  $p < 0.001$ ;  $F_{31,2} = 11.6$ ). However, only Pg6 differed significantly from the other strains (Tukey HSD;  $p < 0.05$ ). The formation of pentagonal stars was rarely seen in strain Pg2, in which the filaments appeared much thinner than in the other mesoflagellates (Table 1). Although *Rhodomonas* sp. (ESD = 7.1  $\mu\text{m}$ ) was larger than the *Phaeocystis* strains, *Isochrysis* sp. (ESD = 4.1  $\mu\text{m}$ ) lay in between the mesoflagellates Pg2 and Pg6 (Fig. 1).

The concentration of TEP in *Phaeocystis* food suspensions was low and varied from 5.9 to 23.5  $\mu\text{g GX}_{\text{eq. L}^{-1}}$  (Table 1). Observations by microscopy revealed that most of the TEP was present as discrete particles. In strains Pg20 and Pg1, part of the staining was, however, associated with the cells and, thus, represents exopolymer secretions. No relationship between cell type and TEP concentration was found.

**Grazing**—Filtration and ingestion rates differed significantly between different algal species and strains (one-way

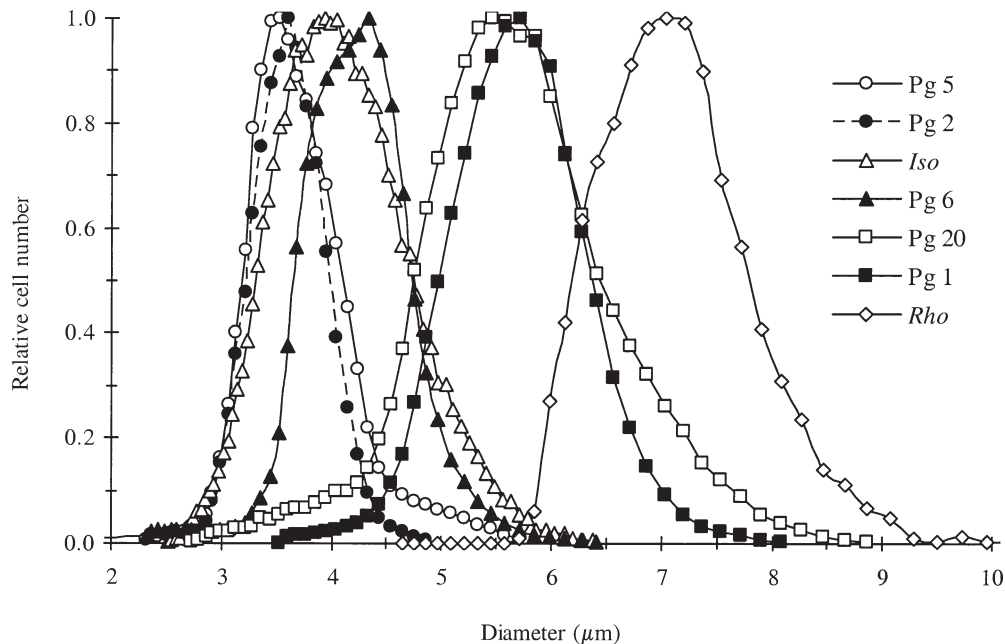


Fig. 1. Size distribution of the relative cell number in five strains of *Phaeocystis globosa* (Pg), *Rhodomonas* sp. (Rho), and *Isochrysis galbana* (Iso).

ANOVA;  $F_5 = 28$  and  $23$  for filtration and ingestion, respectively;  $p < 0.0001$ ). Filtration and ingestion rates on Pg1, Pg5, and Pg6 were significantly lower than on *Rhodomonas* sp. or *Isochrysis* sp. at the same concentration (Tukey HSD;  $p < 0.05$ ), whereas grazing on Pg20, Pg2, *Rhodomonas* sp. and *Isochrysis* sp. were not significantly different (Fig. 2a,b). Grazing on *Phaeocystis* was thus strain specific and varied by a factor  $>10$  (Fig. 2a). While the feeding rate on strain Pg1 was intermediate, strains 5 and 6 were apparently avoided. The order of preference—*Rhodomonas* sp.  $>$  *I. galbana*  $>$  Pg2 and Pg20  $>$  Pg1  $>$  Pg5 and Pg6—did not correspond to the size of the algae (cf. Fig. 1). Furthermore, the feeding response and amount of TEP in food suspensions were not related.

In mixtures with *Rhodomonas* sp. and Pg2 or Pg5, *T. longicornis* nauplii always significantly selected for *Rhodomonas* sp. ( $t$ -test;  $p < 0.01$ ), although, in accordance with single-species experiments, selection was much stronger against Pg5 than Pg2 (Fig. 2b,c). Offering two species in a mixture only affected clearance and ingestion of Pg2, which was significantly lower when *Rhodomonas* sp. was present ( $t$ -test;  $p < 0.05$ ), whereas clearance or ingestion of Pg5 or *Rhodomonas* sp. were not different, irrespective of whether they were offered as a single-species diet or in mixtures ( $t$ -test;  $p > 0.05$ ). Similarly, the total ingestion in 1 : 1 mixtures with Pg2 or Pg5 was not significantly different from ingestion in either of the *Rhodomonas* sp. concentrations (1-way ANOVA;  $p > 0.05$ ), indicating that the two *Phaeocystis* strains did not disturb nauplii in feeding on *Rhodomonas* sp. (vs. *Rhodomonas* sp. at  $150 \mu\text{g C L}^{-1}$ ), but rather contributed to the total food intake (vs. *Rhodomonas* sp. at  $300 \mu\text{g C L}^{-1}$ ) (Fig. 2b).

**Development and mortality**—*T. longicornis* nauplii were unable to develop past the second nauplius stage when

feeding on either Pg2 or Pg5 (Fig. 3), suffering high mortality (Table 2). In contrast, high survival and development up to a mean stage of 4.5 and 4.2 were observed in experiments with Pg1 and Pg20, respectively. Development and mortality with these two strains, as well as in mixtures, corresponded to the development in  $150 \mu\text{g C L}^{-1}$  *Rhodomonas* sp., there being no significant differences between Pg1, Pg20, mixtures, and *Rhodomonas* sp. controls in mean development stage at day 6 (Tukey HSD;  $p > 0.05$ ). In contrast, the mean development stage in strains Pg2 and Pg5 at day 6 was significantly lower than either with *Rhodomonas* sp., Pg1 and Pg20, or mixtures (Tukey HSD;  $p < 0.001$ ; Fig. 3; Table 2).

## Discussion

**Grazing on different *Phaeocystis* strains and potential mechanisms behind the variation**—The feeding experiments conducted with nauplii of *T. longicornis* revealed a remarkable variation in the susceptibility of different *P. globosa* strains to copepod grazing. In terms of carbon, two of the strains were ingested at relatively high rates. Ingestion of 9–11% of body C per day (Pg2 and Pg20) was not significantly different from nauplii feeding on *Rhodomonas* sp. or *Isochrysis* sp., which proved to be excellent food for nauplii grazing and growth in this and previous investigations (Klein Breteler et al. 1999). In contrast, two of the remaining strains (Pg5 and Pg6) were nearly avoided by nauplii, and Pg1 was ingested at intermediate rates. These strain-specific differences contrast with previous results, which generally confirm microzooplankton feeding on *Phaeocystis*. High grazing rates were observed during two field studies in the Southern Bight of the North Sea with the dilution technique (Weisse and Scheffel-Möser 1990; Stelfox-Widdicombe et al. 2004). The authors concluded

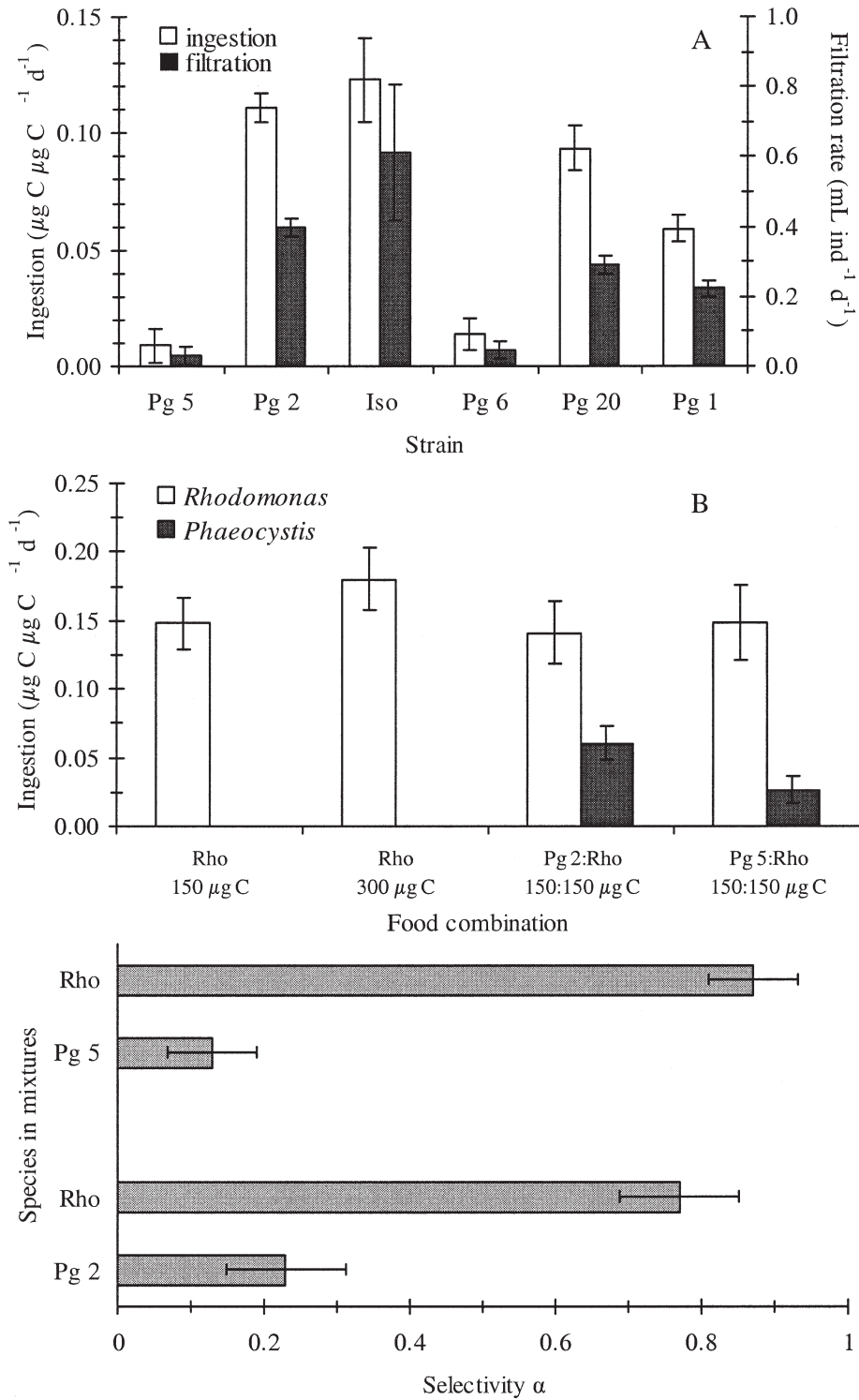


Fig. 2. Filtration and ingestion rates of *Temora longicornis* nauplii on (A) single-species diet of different *Phaeocystis globosa* strains and *Isochrysis galbana*, (B) 1 : 1 mixtures of *Rhodomonas* sp. and strains Pg2/Pg5 and *Rhodomonas* sp. controls (150 and 300  $\mu\text{g C L}^{-1}$ ), and (C) selection in mixtures of *Rhodomonas* sp. and strains Pg2/Pg5 (mean  $\pm$  SD). *Rhodomonas* sp. (Rho), *I. galbana* (Iso). Other abbreviations as in Table 1.

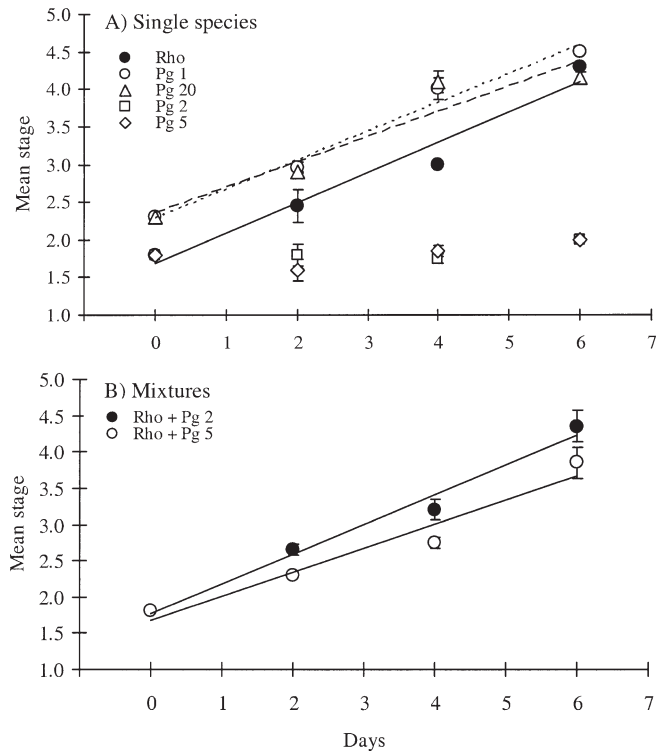


Fig. 3. Development of *Temora longicornis* nauplii with (A) single-species diet of *Rhodomonas* sp., Pg1, Pg2, Pg5, and Pg20 and (B) in 1 : 1 mixtures of *Rhodomonas* sp. and Pg2/Pg5. Solid lines indicate significant linear regressions of the development stage against time with *Rhodomonas* sp. and mixtures, the dotted line with Pg20, and the dashed line with Pg1. Abbreviations as in Fig. 2 and Table 1.

that the community consisting of ciliates, heterotrophic dinoflagellates, and metazooplankton (including nauplii) could potentially control the bloom development. Laboratory studies have generally confirmed these results, although not all protists tested were found to ingest solitary cells. Direct measurements demonstrated that both heterotrophic dinoflagellates, like *O. marina* or *Gyrodinium dominans*, and a variety of ciliates, such as *Strombidinopsis acuminatum* or *Strombidium vestitum*, fed on *P. globosa* solitary cells, but the ciliates *Strombidium elegans* or *Mesodinium pulex* did not (Hansen et al. 1993; Tang et al. 2001). High grazing rates by *Strombidium* sp. were observed by Verity (2000).

Table 2. Final development stage and daily specific mortality of *Temora longicornis* nauplii with four *Phaeocystis* strains in single-species diet, in 1 : 1 mixtures with *Rhodomonas* sp. (Rh), and in *Rhodomonas* sp. control (mean  $\pm$  SD). Parameters of linear model of mortality relating  $\ln(\text{number of individuals})$  to time are shown ( $n = 8\text{--}12$ ). The mortality rates based on insignificant correlations ( $p > 0.05$ ) are in parentheses.

Species	Final stage	Mortality (% d <sup>-1</sup> )	Intercept	R	p
Rho	4.5 $\pm$ 0.3	0.17 $\pm$ 0.07	5.8	0.61	0.04
Pg1	4.5 $\pm$ 0	0.16 $\pm$ 0.05	5.9	0.77	0.02
Pg2	2 $\pm$ 0	0.98 $\pm$ 0.2	5.7	0.89	0.003
Pg5	2 $\pm$ 0	1.08 $\pm$ 0.2	6.0	0.94	0.005
Pg20	4.2 $\pm$ 0.1	0.13 $\pm$ 0.05	5.8	0.75	0.03
Pg2 + Rh	4.4 $\pm$ 0.2	(0.02 $\pm$ 0.02)	5.7	0.38	0.3
Pg5 + Rh	3.9 $\pm$ 0.2	(0.27 $\pm$ 0.13)	5.7	0.84	0.08

Apart from Hansen et al. (1993), who reported that the *Phaeocystis* cf. *globosa* used in their experiments consisted of a mixture of flagellated and nonflagellated cells, solitary strains used in experiments have rarely been characterized. The strains used in our experiments represent two different cell types of *P. globosa* known to occur in nature: the mesoflagellate and solitary nonflagellated cells (cf. Rousseau et al. 1994; Peperzak et al. 2000). The nonflagellated strains suffered intermediate to high grazing losses, whereas ingestion of two out of three flagellated strains was avoided. It is, therefore, tempting to speculate that some factor associated with each of these cell types might explain the observed variation in the grazing rates of *Temora* nauplii. However, we did not find conclusive evidence for a single-factor relationship.

Consistent with previous morphological investigations (e.g., Peperzak et al. 2000), the two largest strains (Pg1 and Pg20) were colonial cells lacking flagella and chitinous filaments, and the three smaller strains were mesoflagellates possessing the threadlike filaments. However, neither size nor cell morphology could explain the observed variation in grazing. Although the feeding of copepods on phytoplankton is limited by a lower size threshold (Berggreen et al. 1988), the smallest strain (Pg2), with an ESD of 3.7  $\mu\text{m}$ , and *Isochrysis* sp., with an ESD of 4.1  $\mu\text{m}$ , were ingested at high rates. Additionally, Pg2 possessed filaments. These are known to be ejected from vesicles of *Phaeocystis* mesoflagellates only and produce a pentagonal starlike structure (Chr etiennot-Dinet et al. 1997; Peperzak et al. 2000). The function of the filaments is unknown, but a role in grazer defense similar to the chitinous threads of diatoms has been suggested (Peperzak et al. 2000). Although close examination of the strains by microscopy revealed that, in the avoided strains Pg5 and Pg6 the filaments per cell were more numerous than in strain 2, the differences were significant for Pg6 only. However, in contrast to Pg5 and Pg6, the filamentous threads did not form pentagonal stars in strain Pg2. Cohesion of the filaments within this structure might therefore constitute a prerequisite for a mechanical hindrance of ingestion of mesoflagellates. The function of the filament and pentagonal star production as the mechanism behind rejection of the mesoflagellates remains to be examined in more detail.

Biochemical characteristics, such as the excretion of polymer substances or toxins by *Phaeocystis*, could not explain the observed variation. High amounts of TEP

derived from the breakdown of a colonial *Phaeocystis* bloom have recently been found to strongly inhibit feeding of nauplii and females of *T. longicornis* (Dutz et al. 2005). However, the concentration of TEP in food suspensions prepared from solitary cell cultures was clearly below the  $50 \mu\text{g GX}_{\text{eq. L}^{-1}}$  demonstrated to be the threshold for negative effects. Because the mesoflagellate-derived TEP consisted mainly of free particles, differences in exopolymer secretions, generally associated with cells and known to affect grazers (Liu and Buskey 2000), can also be excluded as a mechanism for the avoidance of some strains.

Similarly, we did not find any evidence for toxin excretion by *P. globosa*. Light-induced toxic properties against cod larvae and the excretion of an anesthetic toxin with antimetabolic activity have been described for *Phaeocystis pouchetii* (Stabell et al. 1999). If *P. globosa* would excrete such a toxin, a decrease in the ingestion of *Rhodomonas* sp. by nauplii or decreased survival and development rates would be expected in our mixture experiments with the strain Pg5. Because this was not the case, *P. globosa* apparently does not excrete toxins that would affect copepod grazing. Nevertheless, our results do not allow us to exclude the production of inducible defenses or cell-bound predator defense mechanisms. Although the exact mechanism is poorly understood, a combination of high dimethylsulfoniopropionate (DMSP) concentration with a high DMSP lyase activity among Prymnesiophytes is considered to form the basis for such an inducible defense against grazing (Strom et al. 2003). *P. globosa* is characterized by both a high cellular DMSP concentration and a high lyase activity (Stefels 2000), and variation in the lyase activity among strains might cause variable feeding responses. However, dimethyl sulfide (DMS) production in the field was clearly associated with the  $>100 \mu\text{m}$  fraction during a bloom and, thus, with the colonial cell type (Stefels et al. 1995), whereas we did not find any evidence that grazing on colonial cells would have been inhibited. The mechanism behind the avoidance of the two mesoflagellate strains, therefore, awaits further investigations.

*Nutritional quality of solitary cells for naupliar survival and development*—Low survival in conjunction with a low fatty acid content has previously been observed for *A. tonsa* nauplii by Tang et al. (2001) but contrasts with the high survival and development of *Acartia* nauplii in another study (Verity and Smayda 1989). Reduced egg production rates in two copepod species have also been attributed to a low food quality of *Phaeocystis* (Turner et al. 2002). In all these studies, the cell type was not specified. Our results demonstrate a fundamental difference of the two cell types with regard to the nutritional significance of *P. globosa*. The two nonflagellated strains tested in the development experiments represented an excellent food for development of *Temora* nauplii, suggesting a high nutritional quality as concluded by Verity and Smayda (1989). In contrast, both flagellated strains proved to be a poor food for *Temora* nauplii. As shown by the results from the grazing experiments, this can be attributed to either starvation from a lack of food uptake by nauplii (strain Pg5) or to low nutritional quality, because ingestion rates were generally

high (strain Pg2). Although strain Pg2 was also consumed at a considerable rate in mixture with *Rhodomonas* sp., the ingested biomass was not converted into additional growth compared with the nauplii raised on a single-species diet of *Rhodomonas* sp. These results indicate a lack of some nutritional compounds essential for copepod growth in this strain but exclude potential toxic activity, as pointed out earlier.

Our results have some important implications for the current understanding of the trophodynamic significance and bloom formation of *P. globosa*. The grazing loss in solitary cells of this species is generally thought to be high (e.g., Rousseau et al. 2000). According to this view, the formation of colonial blooms depends mainly on relative differences in the replication rate of prey and potential predators and on the transfer rate of single cells to colonies. Although our results confirm a high grazing loss of solitary nonflagellated cells, this is not a general feature of solitary biflagellated cells. At present, too few strains used in grazing studies have been sufficiently characterized to extend our observations to *Phaeocystis*–microzooplankton interactions in general. Nevertheless, current *Phaeocystis* food web models do not distinguish the different cell types that differ fundamentally in their nutritional quality and trophic significance. Our results suggest that grazing loss and transfer efficiency might be overestimated when solitary cells are treated as a single functional group with regard to their trophic position.

Grazing loss and trophic significance could depend on the relative composition of single cells during the development of *Phaeocystis* blooms. Unfortunately, data on the abundance of the different cell types in the field is scarce. Furthermore, in addition to the mesoflagellate, two other biflagellated cell types have been described in the complex *Phaeocystis* life cycle—macroflagellate and microflagellate (Peperzak et al. 2000)—which could differ greatly in their vulnerability to grazing. Although the macroflagellate is larger and has been observed to occur during a short time period on colony disruption, similar-sized coexisting meso- and microflagellates are difficult to identify routinely in field samples (Peperzak et al. 2000).

Apart from these restrictions, flagellated and nonflagellated cell types co-occur during *Phaeocystis* blooms (Cadee and Hegeman 1992; Rousseau et al. 1994), whereas solitary flagellates appear to dominate the cell composition preceding and following the bloom (Veldhuis et al. 1986; Rousseau et al. 1994). At the initiation and the development of blooms, when the colonial cell type is abundant, the grazing pressure on solitary colonial cells is probably high (Weisse and Scheffel-Möser 1990). The transition of single cells to larger colonies, presumably triggered by a chemical signal produced by potential grazers (Tang 2003), together with rapid growth, reduces large losses by microzooplankton grazing (e.g., Weisse et al. 1994; Verity 2000). When the conditions for colonial growth become unfavorable, emigration of cells from the colonies and transformation into flagellates occurs (Rousseau et al. 1994). Apart from the transformation, cell lysis or grazing by microzooplankton could represent substantial loss factors for the liberated cells (Rousseau et al.

1994, 2000). However, despite the high grazing pressure on termination of the bloom, an accumulation of *Phaeocystis* micro-/mesoflagellates has been observed in field and mesocosm studies (Veldhuis et al. 1986; Escaravage et al. 1995; Peperzak et al. 2000). The haploid micro-/mesoflagellates probably function as gametes or spores to survive unfavorable conditions during the warm summer months (Veldhuis et al. 1986; Peperzak et al. 2000). Our results suggest that reduced vulnerability of these cells to microzooplankton grazing could be part of this life cycle strategy, increase survival, and foster bloom formation when favorable conditions prevail. In future studies, the avoidance of flagellates by various microzooplankters, as well as the mechanism and cause of the variation in the grazing response, need to be addressed.

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