Phaeocystis blooming enhanced by copepod predation on protozoa: evidence from incubation experiments

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ABSTRACT: Laboratory experiments were carried out to investigate the effect of protozoan, copepod and combined grazing on Phaeocystis biomass. Phaeocystis cf. globosa single cells were offered to 3 different protozoan species, to the calanoid copepod Temora longicornis, as well as to mixtures of both grazer types. The heterotrophic dinoflagellate Oxyrrhis marina and the oligotrich ciliate Strombidinopsis acuminatum ingested Phaeocystis at much higher rates than did the copepod. Nevertheless, protozoan growth and ingestion rates were submaximal, indicating Phaeocystis to be suboptimal food. The oligotrich ciliate Strombidium elegans did not feed on Phaeocystis. In grazing experiments with mixtures of both predator types, the decline of Phaeocystis single cells could be explained by protozoan grazing alone, implying no grazing by the copepods on Phaeocystis. Instead, copepods ingested the protozoans at high rates. Predation on O. marina and S. acuminatum by T. longicornis resulted in a reduction of the total grazing pressure on Phaeocystis of 21 and 67 % respectively. We conclude that mesozooplankton predation on herbivorous ciliates and heterotrophic dinoflagellates, which consumed Phaeocystis cells, can considerably reduce the overall grazing pressure and may enhance Phaeocystis blooming.

KEY WORDS: Ciliates - Copepods - Dinoflagellates - Grazing - Oxyrrhis marina - Phaeocystis - Predation - Selective feeding - Strombidinopsis acuminatum - Strombidium elegans - Temora longicornis

INTRODUCTION

The prymnesiophyte alga Phaeocystis spp. is distributed worldwide (Sournia 1988 and references therein) and has received considerable attention in the past decade due to the broad environmental impact attributed to its intense blooms. They are thought not only to affect pelagic and benthic ecosystems, but also to influence fishery and tourism negatively, and to contribute to acid rain (e.g. Lancelot et al. 1987, Keller 1988, Wassmann et al. 1990). In the Southern Bight of the North Sea, Phaeocystis spring blooms have increased in intensity and duration during the past 3 decades (e.g. Cadée & Hegeman 1986). This trend coincides with increased riverine nutrient inputs (Lancelot et al. 1987) and a shift in nutrient composition (Riegman et al. 1992).

Several mechanisms have been proposed as important loss factors in controlling Phaeocystis blooms: lysis following nutrient depletion (van Boekel et al. 1992), mass sedimentation (Wassmann et al. 1990) and grazing (Tande & Bämstedt 1987, Hansen et al. 1990). The potential significance of herbivorous metazoan grazing, however, is controversial (see review by Weisse et al. 1993). Several authors report Phaeocystis to be unsuitable food for copepods (e.g. Verity & Smayda 1989, Hansen & van Boekel 1991), which may be related to certain metabolites released by the cells (Sieburth 1960, Estep et al. 1990). Two investigations of the 1990 Phaeocystis spring bloom (Davies et al. 1992 off Plymouth in the English Channel and van Boekel et al. 1992 in Dutch inshore waters) show that metazoan grazing indeed is a negligible loss factor concerning Phaeocystis bloom dynamics.

On the other hand, some studies on protozoa indicate high abundances (Admiraal & Venekamp 1986).
and high grazing pressure on Phaeocystis single cells (Weisse & Scheffel-Möser 1990) during Phaeocystis blooms. A ciliate peak was also observed during the 1990 Phaeocystis spring bloom (van Boekel et al. 1992). During that period, grazing by the dominant copepod Temora longicornis on Phaeocystis was very low, although copepod biomass increased (Hansen & van Boekel 1991). These authors therefore assumed that the copepods switched to ciliates as food source. If the protozoa are indeed important consumers of Phaeocystis cells, the predation of copepods on these grazers might have a positive influence on the Phaeocystis population and thus stimulate the development of a bloom. The experiments presented here were designed to test this hypothesis.

**MATERIAL AND METHODS**

**Cultures.** The heterotrophic dinoflagellate Oxyrrhis marina Dujardin (cell length: 17 to 35 μm; cell width: 10 to 15 μm) was obtained from A. Whiteley (Plymouth Marine Laboratories, UK) and grown in the prymnesiophycean phytoflagellate Isochrysis galbana Parke (diameter 5 μm). Strombidinopsis acuminatum Feuré Fremiet, an oligotrich ciliate (length: 68 to 117 μm; width: 48 to 58 μm), was isolated from the inside of Phaeocystis cf. globosa colonies originating from the Dutch tidal inlet Marsdiep, and also grown on I. galbana. The oligotrich ciliate Strombidium elegans Florentin (length: 50 to 65 μm; width: 31 to 44 μm) was isolated from Marsdiep water and grown on Photobacterium sp. I. galbana and Phaeocystis cf. globosa were obtained from W. van Boekel (University of Groningen, The Netherlands). Protozoa and algae were grown in batch cultures in autoclaved f/2 medium (Guillard & Ryther 1962) at 15°C and illuminated 16 h per day with 100 μE m−2 s−1. Under these conditions, no colony formation in the Phaeocystis cultures was observed. Temora longicornis copepods were obtained from a continuous culture system (Klein Breteler et al. 1990).

**Experimental design.** Single grazer incubation experiments were carried out with either Temora longicornis or with one of the various protozoan species to determine the grazing pressure by each of them on Phaeocystis. In addition, mixtures of copepods and protozoans were used to determine copepod grazing on protozoans and the overall effect on the density of Phaeocystis. A control without grazers was used to correct for growth of the algae. Each experiment was carried out in triplicate.

Prior to the experiments, protozoans and copepods were adapted to their food and experimental conditions for 24 h. For each experiment, 12 glass incubation bottles (289 ml) were mounted on a slowly vertically rotating wheel (1 rpm) and incubated for 18 or 24 h at 12°C. The bottles were illuminated for 12 h per day with 40 μE m−2 s−1. For the experiments with copepods, 5 adult females were added per bottle. Temperature, light and protozoan biomass (ca 200 μg C l−1) were adjusted to approach the in situ conditions of the Phaeocystis bloom in the Marsdiep in April 1990. Algal concentrations were chosen to approximate the carbon concentration of the protozoans.

**Measurements.** Cell densities and cell volumes were determined at the beginning and at the end of each experiment. Phaeocystis was counted with an electronic particle counter (Particle Data, Inc.) or a haemocytometer, Oxyrrhis marina with the particle counter, and the ciliates were enumerated in Utermöhl settling chambers using an inverted microscope. Cell volumes were determined with the particle counter using unpreserved samples, since preservation resulted in considerable cell shrinkage of up to 22%. Samples for cell counts were preserved in acid Lugol's solution (2% final concentration) and counted within 1 wk. The counts were corrected for cell losses due to fixation, ranging between 0 and 4%, depending on the species as determined from independent measurements.

**Calculations.** Average prey concentrations, growth and grazing coefficients, total filtration and ingestion rates were calculated according to Frost (1972). This method assumes prey growth rates to be independent of grazer presence. Cell volumes were converted to carbon content applying a conversion factor of 0.11 pg C μm−3 (Edler 1979). Copepod carbon content was calculated from individual prosome length measurements which were converted to ash free dry weights (AFDW) (Klein Breteler & Gonzalez 1988) and further to carbon applying a factor of 40% C AFDW−1 (Omori 1969). In the combined grazer experiments, the potential contribution of the protozoans (Ipot) was calculated by multiplying the specific ingestion rates measured in the single grazer experiments with the protozoan biomass in the combined grazer experiments, assuming that protozoan ingestion was not affected by the presence of the copepods. The contribution of the copepods in the combined grazer experiments was calculated from the difference between the total measured ingestion (Itot) and the calculated potential contribution by the protozoans (Ipot).

**RESULTS**

During all single grazer experiments, the Phaeocystis biomass in the bottles containing Temora longicornis as sole grazer increased almost as much as in
the controls (Fig. 1a to c). Also, the ciliate *Strombidium elegans* did not reduce the biomass of *Phaeocystis* compared to the controls (Fig. 1c). In contrast, the *Phaeocystis* biomass did not increase in the bottles containing *Strombidinopsis acuminatum* or *Oxyrrhis marina* as sole grazer (Fig. 1a, b).

Derived specific daily rations of *Phaeocystis* were very low for the copepod *Temora longicornis* (2 to 18% body C d⁻¹). For the dinoflagellate *Oxyrrhis marina* and for the ciliate *Strombidinopsis acuminatum* these rates were much higher, amounting to 34 and 66% body C d⁻¹, respectively (see Table 1).

In all combined grazer experiments, the presence of *Temora longicornis* considerably reduced protozoan biomass (Fig. 2). Copepod predation on protozoa was high (58 to 275% body C d⁻¹) and far exceeded copepod grazing on *Phaeocystis* (see Table 1). The presence of copepods in the combined grazer experiments with the 2 protozoans which fed on *Phaeocystis* led in both cases to higher algal biomass increase (Fig. 1a, b) and consequently to a lower overall grazing pressure compared with the experiments with the protozoans as sole grazers (Table 1). The copepod-induced reduction in grazing pressure on *Phaeocystis* amounted to 21 and 67% in the experiments with *Oxyrrhis marina* and *Strombidinopsis acuminatum*, respectively.

Grazing by *Strombidinopsis acuminatum* seemed to be hampered in the presence of copepods. This is evident from the lower total ingestion by *S. acuminatum* plus copepods in the combined grazer experiment (Iₜot) in comparison with the potential consumption of the ciliates alone, Iₚot (Table 2). Thus, it can be assumed that the reduction of grazing pressure on

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**Fig. 1.** *Phaeocystis* cf. *globosa* with (a) *Oxyrrhis marina*, (b) *Strombidinopsis acuminatum* and (c) *Strombidium elegans*. *Phaeocystis* biomass is shown before (t = 0 h) and after incubation (t = 18 h or 24 h) without grazers (Control), with the copepod *Temora longicornis* (Temora), with the respective protozoan, and with copepod and respective protozoan species (Combined). Mean ± SD, n = 3

**Fig. 2.** *Oxyrrhis marina*, *Strombidinopsis acuminatum* and *Strombidium elegans*. Protozoan biomass after 24 h incubation (18 h for *O. marina*) with (Grazing) and without (Control) the copepod *Temora longicornis* (17 ind. 1⁻¹). Initial biomass were 239, 265 and 116 μg C 1⁻¹ for *O. marina*, *S. acuminatum* and *S. elegans* respectively.
High consumption of protozoa by pelagic 37.1 58.1 -21.0 -1.3 Strombidinopsis acuminatum gested by at a high rate. Temora longicornis 122.0 127.5 -5.5 -0.01 Oxyrrhis marina In all experiments, protozoa were in-

Copepod predation on protozoa Copepod grazing on Phaeocystis Laboratory grazing studies have shown that Phaeocystis can be ingested by several copepod species and thus forms a potential food source for copepods (e.g. Huntley et al. 1987, Hansen 1992). Ingestion rates measured were comparable to or lower than rates found in grazing studies with other phytoplankton food; such inconsistencies may be attributed to differences in Phaeocystis size-spectrum and quality, copepod species and methods applied. Phaeocystis colonies were ingested at higher rates than single cells (Huntley et al. 1987), the size of which is at the lower end of the size-range of particles efficiently retainable by a variety of copepods (Nival & Nival 1976, O’Connors et al. 1980). Field studies in the southern North Sea, however, indicate that Phaeocystis is avoided by copepods (Daro 1986, Hansen & van Boekel 1991, Bautista et al. 1992), which may be dependent on the presence of an alternative food source (Hansen & van Boekel 1991). This view is supported by the experimental results presented here: Temora longicornis grazed on Phaeocystis, although at a low rate, if no protozoans were available. In the presence of both food sources copepods selected for the protozoans, and likely did not feed on Phaeocystis.

Copepod predation on protozoa In all 3 experiments, protozoa were ingested by Temora longicornis at a high rate. High consumption of protozoa by pelagic Phaeocystis in the presence of copepods is a combination of 2 effects: the reduced number of protozoan grazers due to copepod predation and the reduced specific ingestion rate of S. acuminatum. In the experiment with Oxyrrhis marina, the specific protozoan ingestion rate was hardly affected by the copepods.

**DISCUSSION**

**Conclusions**

Although the evidence from our experiments is somewhat restricted by the few organisms tested. 3 conclusions can be drawn. Firstly, herbivorous protozoa can consume Phaeocystis at a much higher rate than the copepod Temora longicornis. Secondly, protozoans are ingested by the copepod T. longicornis at a much higher rate than Phaeocystis single cells. Thirdly, the presence of copepods can relieve the grazing pressure on Phaeocystis single cells by predation on herbivorous protozoans.

<table>
<thead>
<tr>
<th>Grazers</th>
<th>Prey</th>
<th>$k'$ (d$^{-1}$)</th>
<th>$g$ (d$^{-1}$)</th>
<th>$F$ (ml ind$^{-1}$ d$^{-1}$)</th>
<th>$I$ (ng C ind$^{-1}$ d$^{-1}$)</th>
<th>SDR (% body C d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.m.</td>
<td>Phae.</td>
<td>0.36 ± 0.08</td>
<td>0.77 ± 0.05</td>
<td>0.001 ± 0.000</td>
<td>0.16 ± 0.01</td>
<td>66.4 ± 2.8</td>
</tr>
<tr>
<td>O.m. + T.I.</td>
<td>Phae.</td>
<td>0.61 ± 0.05</td>
<td>0.001 ± 0.000</td>
<td>0.15 ± 0.00</td>
<td>64.5 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>T.I.</td>
<td>Phae.</td>
<td>0.01 ± 0.03</td>
<td>0.44 ± 1.93</td>
<td>113 ± 502</td>
<td>2.1 ± 13.9</td>
<td></td>
</tr>
<tr>
<td>T.I.</td>
<td>O.m.</td>
<td>0.39 ± 0.05</td>
<td>20.66 ± 2.92</td>
<td>4884 ± 533</td>
<td>113.1 ± 15.5</td>
<td></td>
</tr>
<tr>
<td>S.a.</td>
<td>Phae.</td>
<td>0.18 ± 0.03</td>
<td>0.42 ± 0.07</td>
<td>0.02 ± 0.01</td>
<td>3.63 ± 0.55</td>
<td>34.3 ± 5.2</td>
</tr>
<tr>
<td>S.e. + T.I.</td>
<td>Phae.</td>
<td>0.13 ± 0.11</td>
<td>0.11 ± 0.01</td>
<td>2.19 ± 1.18</td>
<td>20.7 ± 1.2</td>
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</tr>
<tr>
<td>T.I.</td>
<td>Phae.</td>
<td>0.07 ± 0.09</td>
<td>3.66 ± 5.30</td>
<td>1053 ± 1519</td>
<td>18.4 ± 26.9</td>
<td></td>
</tr>
<tr>
<td>T.I.</td>
<td>S.a.</td>
<td>1.30 ± 0.71</td>
<td>92.44 ± 21.56</td>
<td>14970 ± 3724</td>
<td>274.6 ± 61.4</td>
<td></td>
</tr>
<tr>
<td>S.e.</td>
<td>Phae.</td>
<td>-0.22 ± 0.03</td>
<td>-0.20 ± 0.06</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>S.e. + T.I.</td>
<td>Phae.</td>
<td>-0.20 ± 0.02</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.I.</td>
<td>Phae.</td>
<td>0.09 ± 0.07</td>
<td>2.22 ± 0.82</td>
<td>745 ± 275</td>
<td>16.1 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>T.I.</td>
<td>S.e.</td>
<td>0.46 ± 0.24</td>
<td>33.04 ± 17.00</td>
<td>2.71 ± 1.09</td>
<td>57.5 ± 32.0</td>
<td></td>
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</table>

**Table 1.** Total ingestion rates and related parameters (means ± SD, N = 3) for the 3 protozoans Oxyrrhis marina (O.m.), Strombidinopsis acuminatum (S.a.) and Strombidium elegans (S.e.), and the copepod Temora longicornis (T.I.) in grazing experiments with Phaeocystis single cells (Phae). $k'$: protozoan growth rate, $g$: grazing coefficient; $F$: filtration rate, $I$: ingestion rate, SDR: weight-specific daily ration of the grazer; + T.I.: copepod present with negligible contribution to total consumption (Table 2), not considered in calculation of protozoan SDR.
copepods has been reported both in the field (Antia 1991, Dolan 1991) and in the laboratory (e.g. Stoecker & Egloff 1987, Klein Breteler et al. 1990). Oxyrrhis marina has been used as the dominant food source for 4 different copepod species in continuous cultures by Klein Breteler et al. (1990). They measured weight-specific ingestion rates of O. marina by adult female T longicornis of 103% d⁻¹ which is within the range observed in this study. Sherr et al. (1986) call predation on protozoa the 'missing link' between pico/nanoplanktonic production and higher trophic levels (e.g. copepods), for the majority of primary producers (especially under oligotrophic conditions) are too small to be retained efficiently by copepods. Many investigations have focused on protozoan herbivory and bacterivory (e.g. Sherr & Sherr 1987, Caron et al. 1991, Verity 1991) or on predation of copepods on protozoa (e.g. Sherr et al. 1986, Ayukai 1987, Gifford & Dagg 1991). These studies generally support the notion that the grazing hierarchy 'pico/nanoplankton (auto- and heterotrophic) - microzooplankton - copepods' forms a linear chain within the microbial food web. Our data, however, indicate a positive feedback mechanism within the pelagic food web: the stimulation of pico/nanoplanktonic biomass development due to mesozooplankton predation on their main grazers, the protozoans. Dolan (1991) deduces the same mechanism from observations in Chesapeake Bay: vertical maxima of copepods coincided with maxima of microflagellates (and/or chlorophyll a), whereas ciliate maxima coincided with flagellate minima and copepod maxima coincided with ciliate minima.

Protozoan grazing on Phaeocystis

Weisse & Scheffel-Möser (1990) identified pelagic ciliates and heterotrophic dinoflagellates as major consumers of Phaeocystis single cells in a bloom situation. In serial dilution experiments, they found high Phaeocystis growth and grazing loss rates, indicating a highly dynamic turnover of Phaeocystis biomass within the microbial loop. Protozoan grazing on Phaeocystis is not restricted to the single cell stage. The ciliate Strombidinopsis acuminatum was isolated from the inside of Phaeocystis colonies and observed to ingest their cells. S. acuminatum could also be cultured with Phaeocystis colonies as food. Grazing on colonial Phaeocystis cells has been described for tintinnids as well (Admiraal & Venekamp 1986).

Strombidium elegans did not feed on Phaeocystis under experimental conditions, but grew well in cultures containing decaying colonies or added bacteria. Apparently, this ciliate is specialized in bacterial food.

In the other protozoans, specific ingestion rates on Phaeocystis were much higher than those by the copepods, but ingestion rates as well as growth rates were still low. While feeding on Isochrysis galbana in batch cultures, Strombidium acuminatum showed a much higher growth rate (0.64 d⁻¹) than while feeding on Phaeocystis flagellates during the experiment (0.18 d⁻¹). The observed growth rate of Oxyrrhis marina (0.37 d⁻¹) while feeding on Phaeocystis was also lower than growth rates found on a variety of other algal food (Antia 1991, Tarran 1992). Our results indicate that Phaeocystis flagellates are less suitable for protozoa to feed on than other algae of the same size class. Phaeocystis has also been found to be an inferior food source for copepods (Verity & Smayda 1989); this is supported by a biochemical analysis (Claustre et al. 1990).

Implications for Phaeocystis blooms

This work was intended to test the hypothesis that copepods can stimulate the growth of Phaeocystis single cells by preying on protozoa. In addition to 2 endemic ciliates from the Meso-Scip, Oxyrrhis marina was chosen as a model organism for the group of heterotrophic dinoflagellates, which is increasingly believed to form a ubiquitous and ecologically important group in various ecosystems (Lessard 1991).

In their description of the 1990 spring bloom, van Boekel et al. (1992) showed that during the onset and early stage of the Phaeocystis bloom, while colonies predominated, grazer biomass was low. They suggested that the influence of grazing was negligible and that the microbial food-chain was probably carbon ('bottom-up') controlled. During the late and decline stages of the spring bloom, dominated by Phaeocystis microflagellates, increased biomass of potential micro- and mesozooplankton grazers (ciliates, copepods) may have led to a grazing ('top-down') controlled system. In this period, grazing pressure on Phaeocystis by the dominant copepod Temora longicornis is low, as measured in the field (Hansen & van Boekel 1991) and indicated experimentally (this study). Assuming a simple linear food-chain from Phaeocystis flagellates via ciliates to copepods with respective biomasses of 460, 200 and 50 μg C ¹⁻¹ (van Boekel et al. 1992), ciliate grazing (34 % body C d⁻¹) could remove 15% of the Phaeocystis standing stock daily. This figure does not include grazing by heterotrophic flagellates and might therefore be an underestimation of total grazing pressure. On the other hand we do not know what fraction of the food of the ciliates did not consist of algae. Copepod predation on ciliates (275% d⁻¹) would amount to 69% of ciliate biomass and reduce the gra-
ing pressure on Phaeocystis to 5% d⁻¹. This simplified estimation indicates that T. longicornis may exert considerable control of an important grazer on Phaeocystis but suggests that other factors may govern Phaeocystis spring bloom dynamics. In contrast, summer blooms of Phaeocystis are likely to be affected more severely by grazers due to their higher biomass and grazing activity and due to lower phytoplankton biomass and growth rates than can be found during spring blooms.

The positive feedback between copepod predation on herbivorous protozoa and pico- and nanoplanckton standing stocks may be a general mechanism favouring small autotrophs, which dominate oligotrophic systems (Li et al. 1983, Lenz 1992 and references therein). Our findings support the concept that plant populations are positively affected by carnivore predation on herbivores in 'trophic cascades' as proposed by Hairston et al. (1960) and recently discussed in articles on theoretical ecology (Power 1992, Strong 1992).

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