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Role of TEP in the microbial food web structure. I. Grazing behavior of a bacterivorous pelagic ciliate

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ABSTRACT: We studied the ingestion of picoplankton-size particles by the oligotrich *Strombidium sulcatum* as a function of transparent exopolymeric particle (TEP) concentration. Fluorescent microspheres of 1 µm (FMS) were used as prey-item analogs. TEP-FMS aggregates were formed by bubbling seawater solutions containing different TEP concentrations in the presence of FMS. After bubbling, concentrations of TEP-FMS aggregates ranged from 0.5 to 1.7 ppm and from 11.9 to 17.9 ppm in the low and high TEP treatments, respectively. When the ciliates were exposed to similar FMS concentrations, but different TEP concentrations, ingestion rates were higher for the low TEP treatments (14.1 FMS ciliate^{-1} h^{-1}) than for the high TEP treatments (5.2 FMS ciliate^{-1} h^{-1}). The number of FMS ingested in the low TEP treatments was 2.7 times higher than in the high TEP treatments and ingestion rates were inversely correlated to TEP volume concentration. Our results suggest that the formation of TEP-FMS reduces FMS availability for micro-grazers by modifying the food size spectra and redistributing FMS to larger size classes, and that at a critical TEP concentration picoplankton-size prey items may become unavailable for the ciliate *S. sulcatum*, while concomitantly they may become available for large-particle grazers. Therefore, one role of TEP in the microbial food web would be to act as a trophic elevator, thus creating a direct lift for picoplankton-size prey items to higher trophic levels.

KEY WORDS: Transparent exopolymeric particles · Fluorescent microspheres · Aggregation · Grazing · Food selection · Food size spectra · *Strombidium sulcatum*

INTRODUCTION

While the importance of the microbial loop for the transfer of energy from the dissolved phase back to the particulate has been shown (Azam et al. 1983) and the significance of protozoans within the microbial loop acknowledged (Fenchel 1984, Sieburth 1984, Porter et al. 1985, Rassoulzadegan & Sheldon 1986), several aspects of microbial loop function are still poorly understood.

One of the uncertainties concerns mechanisms controlling protozoa food selection. Food size is recognized as one of the main limiting factors regarding food selection, and changes in food size spectra (i.e. bacteria) have been attributed to size-selective grazing by protists (Rassoulzadegan & Sheldon 1986, Rassoulzadegan et al. 1988, Epstein & Shiaris 1992, Sherr et al. 1992, Šimek & Chrzanowski 1992, Šimek et al. 1994, 1995, 1997, Pernthaler et al. 1996). As an example, bacteria that are ingested preferentially by flagellates (Andersson et al. 1986, Chrzanowski & Šimek 1990, Gonzàlez et al. 1990) and ciliates (Rivier et al. 1985, Turley et al. 1986, Šimek et al. 1994) can either be free or associated with organic aggregates. Any modification of the ratio between free versus attached bacteria could have implications on the microbial trophic web, as it may unbalance the equilibrium between prey and predator abundances by modifying prey size spectra. Assuming that the fraction of attached bacteria increases due to an increase in aggregation processes, bacterivorous ciliates that graze upon micrometer-size particles will be favored less than other predacious protozoans able to feed on larger particles. It has been shown that most bacterivorous ciliates (e.g. *Strombidium* spp.) prefer to feed on small, suspended food par-

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ticles (Rivier et al. 1985, Bernard & Rassoulzadegan 1990) (e.g. free bacteria) rather than on particle-attached bacteria (Albright et al. 1987). Therefore, it can be hypothesized that any mechanism promoting bacterial aggregation may in turn diminish ciliates grazing on free-living bacteria.

Aggregation of particles is a key mechanism for particle dynamics (McCave 1984) in which exopolymer production plays a central role. High transparent exopolymeric particle (TEP) abundance enhances aggregation of particles (Jackson 1995) by increasing collision frequency (Passow et al. 1994, Logan et al. 1995) and the stickiness of the particles (Kiørboe & Hansen 1993, Dam & Drapeau 1995, Engel 2000).

TEP harbor a rich bacterial community. According to estimates, from 0.5 up to 90% of the total bacterial community is attached to TEP (Passow & Alldredge 1994, Schuster & Herndl 1995, Mari & Kiørboe 1996, Worm & Søndergaard 1998). However, the process by which bacteria become attached to aggregates is still unclear. Some studies suggest a passive aggregation consistent with coagulation theory (Mari & Kiørboe 1996), while others have shown that bacteria could actively colonize sinking marine snow aggregates (Kiørboe & Jackson 2001). Assuming that passive coagulation rules the relationship between bacteria and TEP, it could be hypothesized that, for a given bacterial concentration, an increase in TEP concentration would lead to a diminution of the free bacteria fraction. Under the above assumptions, an increase in TEP concentration should modify the prey/predator relationship for ciliates feeding on free-living bacteria.

The main objectives of this study were: (1) to investigate the grazing behavior of *Strombidium sulcatum* towards micrometer-size artificial food (i.e. fluorescent microspheres) as a function of TEP concentration, and (2) to discuss the implications of variations in the TEP pool on the structure and functioning of the microbial loop.

MATERIALS AND METHODS

Formation of TEP-microsphere aggregates. Fluorescent microspheres (FMS) of micrometer size (1 µm, Fluoresbrite Bright Blue Carboxylate Microspheres, Polysciences) were used to form mixed aggregates of TEP-FMS. TEP precursors originated from natural seawater samples collected with 5 l Niskin bottles at the layer of maximum fluorescence (determined by CTD casts on each sampling occasion) during a spring cruise in 3 sub-arctic Norwegian fjords (Malangen fjord, Balsfjord, Ullsfjord). This experiment was conducted once for each of the 3 stations. Since seawater collected during this spring cruise was only used to provide colloidal TEP precursors, this experiment has to be considered as a case study and, thus, no attempt was made to link this study to environmental features.

Seawater samples were filtered at low and constant vacuum pressure (<100 mbar) through 47 mm diameter polycarbonate filters of 0.1 and 1.2 µm pore size, for the low TEP concentration treatments (called LTCT hereafter) and the high TEP concentration treatments (called HTCT hereafter), respectively. The filtrates were transferred into two 2.5 l bubble adsorption columns (Mari & Dam 2004). FMS were added to yield a final concentration in the columns (C_f) of 5×10^5 FMS ml^{-1} (ca. 50% of bacterial concentration). The initial volume of FMS solution (V_i) to add was calculated as:

$$V_i = C_f V_f / C_i$$

where C_i is the initial concentration of FMS and V_f is the volume of the bubbling column. The C_i was calculated as follows:

$$C_i = 6W10^{12}/\rho\pi d^3$$

where W is the mass of polymer ml^{-1} of solution (0.027 g ml^{-1} for 2.7% solid volume according to manufacturer), ρ is the density of polymer in g ml^{-1} (1.05 for polystyrene) and d is the FMS diameter in µm (average diameter 0.93 ± 0.02 µm). In order to avoid clumping prior to bubbling, the FMS suspension was diluted in 10 ml of 0.1 µm filtered seawater (FSW), and sonicated for 5 min. Bubbles were produced by a 10 to 20 µm pore size glass frit. Each solution was bubbled with air at a constant gas flow rate of 100 ml min^{-1} (Kepkay 1991) for 1 h. The air for bubbling was passed through a 0.1 µm air filter (Polycap TF, Whatman). Between each experiment, the column and the glass frit were soaked for 2 h with 10% HCl and then rinsed with Milli-Q water.

TEP determination. After 1 h bubbling, the concentration of TEP in each column was determined. TEP were stained with Alcian Blue and slides prepared following Passow et al. (1994). TEP size spectra were determined by counting and sizing TEP at successive magnifications (Mari & Burd 1998). Ten images were taken per slide and for each magnification, and the TEP size spectra were compiled by combining the size distributions obtained at each magnification. Images were analyzed using an image analyzing system (ImagePro Plus, MediaCybernetics). TEP size distributions were described using a power relationship.

TEP size versus number of attached FMS. A relationship between TEP size and numbers of attached FMS was obtained by sizing individual TEP and enumerating the associated FMS, switching between UV and visible light. The entire volume of each TEP-FMS aggregate was examined by changing the focus of the microscope in order to count all associated FMS. As a result, during the 6 experiments conducted, a total of 350 TEP were sized and their attached FMS counted.

The number of attached FMS (n) per TEP was fitted to a power-law relationship:

$$n = ad_p^b$$

where d_p is the equivalent spherical TEP diameter (ESD; µm), and a and b are constants for a given sample. Numbers of associated FMS and TEP diameters were plotted in log-log coordinates to obtain a and b (Mari & Kiørboe 1996). The fraction of attached FMS as a function of TEP size spectra was calculated by combining (1) the above relationship, (2) the TEP size distribution and (3) the total concentration of FMS in seawater. The characteristics of the TEP pool and the relationship TEP size versus number of attached FMS were determined only at the end of the bubbling period, i.e. the starting point for the incubations.

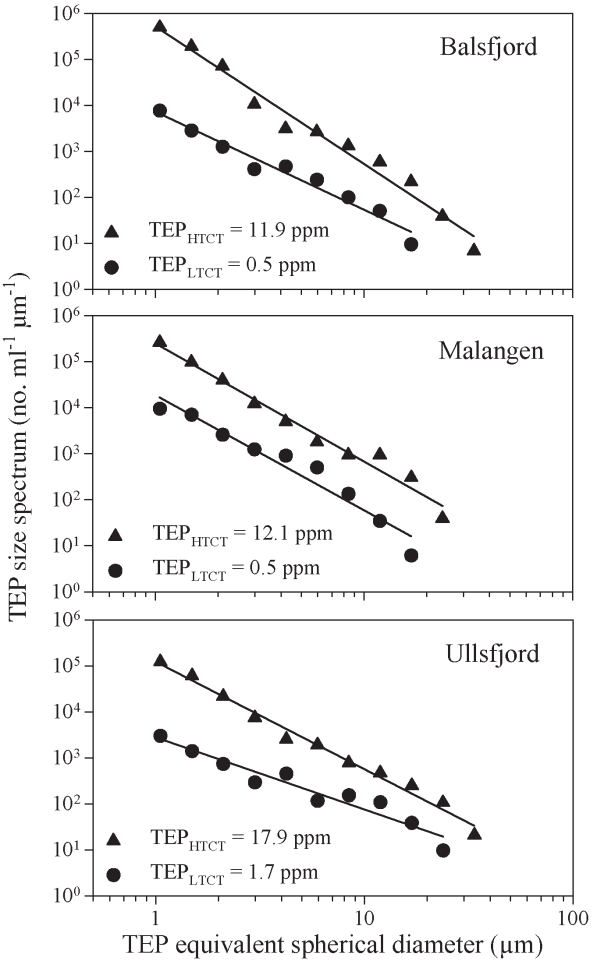

Fig. 1. Size distribution of the transparent exopolymeric particle-fluorescent microsphere (TEP-FMS) aggregates for (▲) high TEP concentration treatments (HTCT) and (●) low TEP concentration treatments (LTCT) described using a power relation of the type $dN/d(d_p) = kd_p^{\delta}$, where dN is the number of particles per unit volume and per size class (no. ml^{-1} µm^{-1}) in the size range d_p to $[d_p + d(d_p)]$ (µm), where d_p = equivalent spherical TEP diameter and k is a constant that depends on the concentration of particles

Incubations. Two sets of incubations were conducted in 250 ml polycarbonate bottles, according to initial TEP concentration (i.e. HTCT and LTCT). After 1 h bubbling, 6 sub-samples of 250 ml were collected in each bubbling column and placed in 250 ml flasks. *Strombidium sulcatum* were added to each 250 ml sub-sample to yield a final concentration of ca. 10 ciliates ml^{-1}. *S. sulcatum*, originally isolated from the Bay of Villefranche-sur-Mer, France, were maintained in stationary-phase on a wheat-grain media at 17°C prior to inoculation (Rivier et al. 1985). Sub-samples of 250 ml were removed at 15 min intervals for 90 min. Incubations were conducted at room temperature (ca. 20°C). For counting *S. sulcatum*, 100 ml samples were fixed with alkaline Lugol's solution (final conc. 2%) and refrigerated until analysis.

Ciliate abundance and number of ingested microspheres. Aliquots were allowed to settle in 100 ml Hydrobios settling chambers. After sedimentation and prior to counting, samples were de-stained by adding 2 drops of 3% sodium thiosulfate. For a given sample, the total surface area of the chamber was examined at 200× magnification with an inverted microscope equipped with epifluorescence and all the ciliates were recorded. For each time-course sample, FMS inside individual ciliates (50 to 200 ciliates per sample) were counted at 400× magnification in order to calculate ingestion and clearance rates in HTCT and LTCT.

RESULTS

Concentration and size distribution of TEP produced by bubbling

The abundance of TEP-FMS aggregates was >10^5 ml^{-1} in HTCT, while they occurred in much lower concentrations in LTCT (i.e. <2×10^4 ml^{-1}) (Fig. 1). TEP-FMS concentrations in HTCT were on average 30 times higher than in LTCT.

Initial TEP volume concentration ranged between 0.5 and 1.7 ppm (average 0.9 ± 0.7 ppm) and between 11.9 and 17.9 ppm (average 14.0 ± 3.4 ppm), in LTCT and HTCT, respectively. For all experiments, larger mixed aggregates of TEP-FMS occurred in HTCT (>20 µm ESD) compared to LTCT.

Relationship between TEP size and number of attached FMS

All TEP were associated with FMS (Fig. 2). The fluorescent microspheres were observed both attached to the surface of and embedded inside TEP. The number

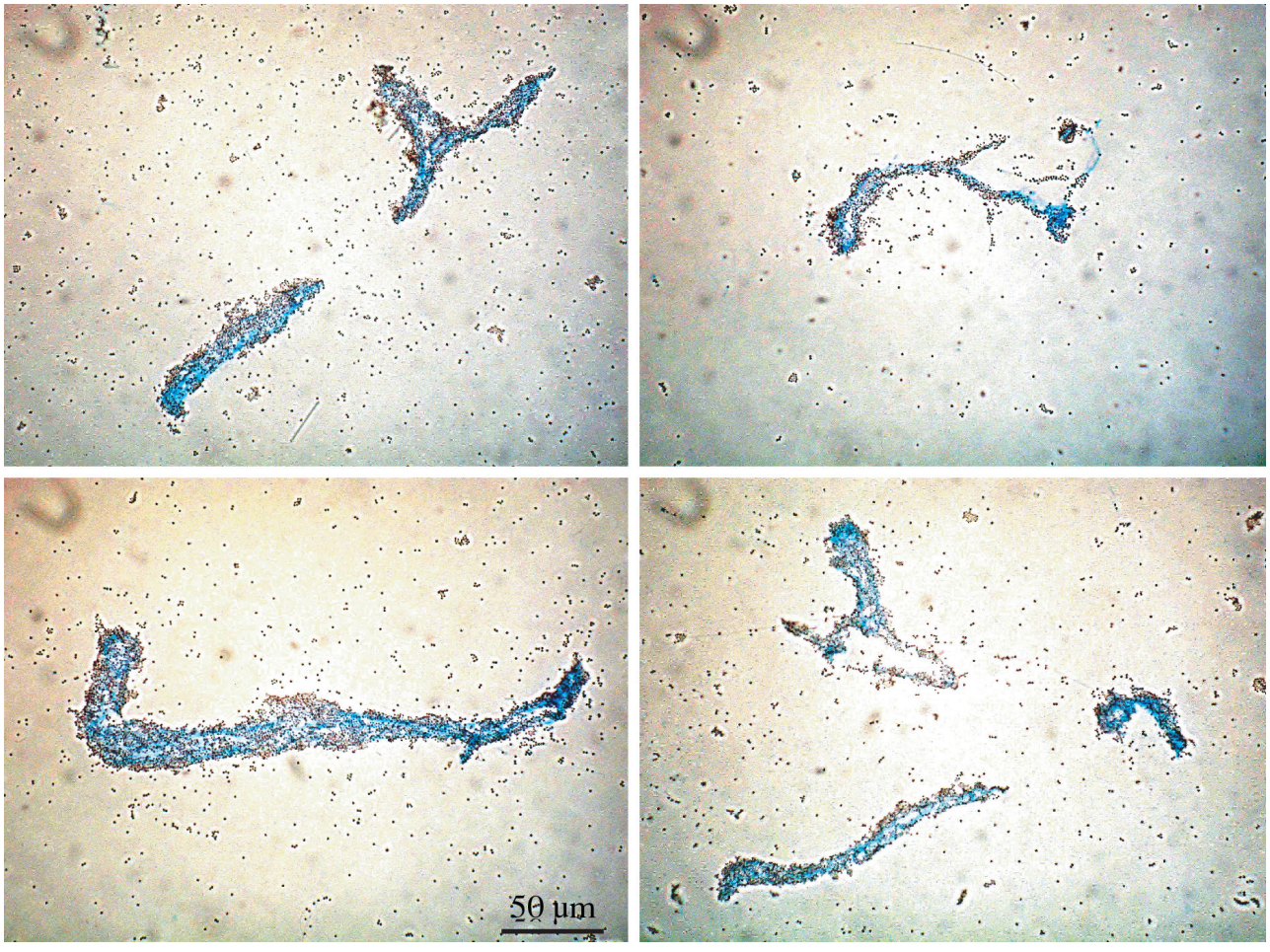

Fig. 2. Examples of large mixed aggregates of TEP-FMS

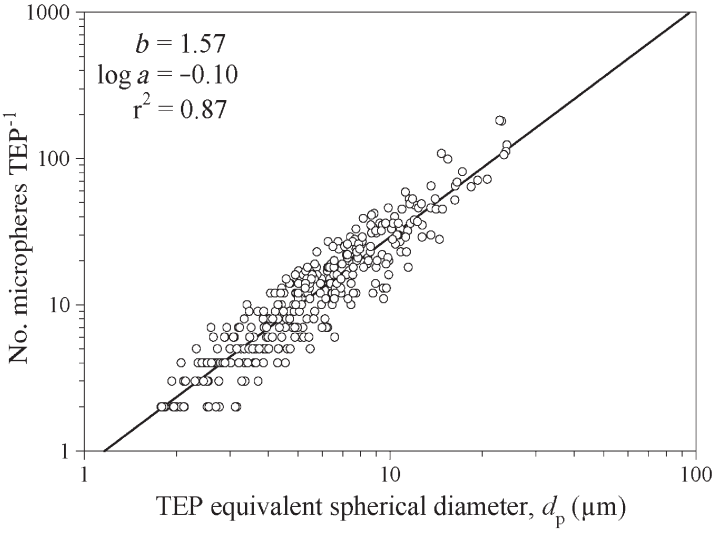

Fig. 3. Number of FMS attached to a TEP (n) as a function of its size (d_p, µm). Regressions were fitted to the observations ($n = a d_p^b$)

of 1 µm FMS per TEP (n) scaled with TEP diameter (d_p; µm) raised to an exponent of 1.57 (Fig. 3)—irrespective of the sampling location where TEP precursors were collected or of the treatment applied, i.e. LTCT or HTCT—is given as follows:

$$n = 0.79\ d_p^{1.57}$$

This relationship indicates that the number of attached 1 µm FMS increased with TEP size, while the volume-specific FMS density decreased with an increase in TEP size.

Ingestion of FMS

The concentration of *Strombidium sulcatum* did not vary statistically during the incubation period or between treatments (t-test, $p > 5\%$) (overall average ± SD was 11.9 ± 2.0 *S. sulcatum* ml^{-1}). The number of FMS inside *S. sulcatum* digestive vacuoles differed according to TEP treatment (Fig. 4). In the LTCT, FMS

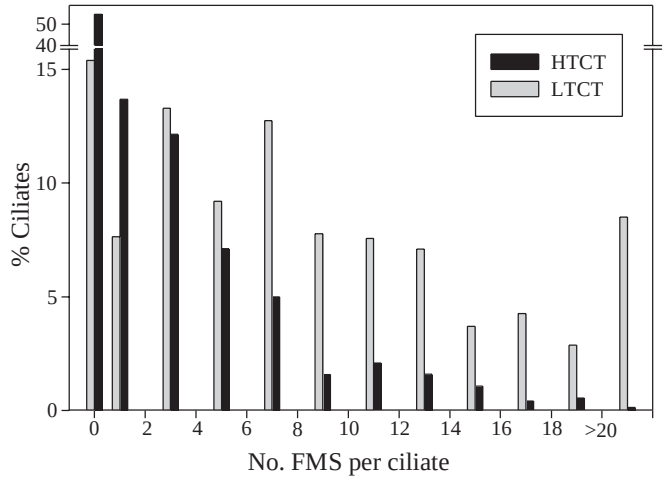

Fig. 4. Number of FMS ingested per individual ciliate for HTCT and LTCT. Data points are average values for the 3 TEP sources up to 45 min incubation, and data are grouped in intervals of number of FMS

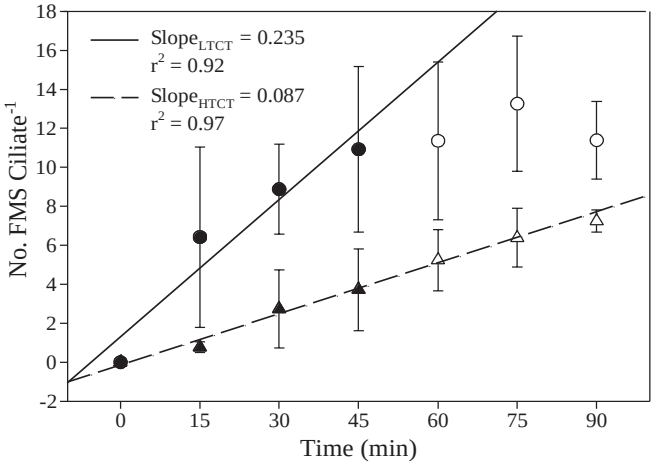

Fig. 5. Number of FMS ingested by the ciliate as a function of incubation time for HTCT (△) and LTCT (○). Regression lines were fitted to the data, but utilizing only the observations made within 1 h incubations (●, ▲)

were evenly distributed inside ciliate vacuoles, i.e. there were as many ciliates containing from 0 to 2 FMS as those containing more than 20. On the contrary, in HTCT the number of FMS inside digestive vacuoles showed a sharp decrease, i.e. 14% of the ciliates contained from 0 to 2 FMS, while none contained more than 20. In HTCT, the percentage of ciliates presenting less than 6 FMS in their digestive vacuoles averaged 88%, while it averaged 46% in LTCT. Additionally, the percentage of ciliates without FMS was higher in HTCT than in LTCT (55 and 15%, respectively), and the percentage with more than 20 FMS averaged ca. 10% in LTCT while it was 0 in HTCT.

Ingestion rates, calculated as the slope of the regression lines of number of FMS ingested versus time elapsed for the first 45 min, varied between 8.9 and 16.7 FMS ciliate^{-1} h^{-1} for the LTCT and between 1.7 and 8.3 FMS ciliate^{-1} h^{-1} for the HTCT (Table 1). Results obtained after more than 45 min incubation were excluded when estimating ingestion rates to avoid interference between the modification of the FMS size distribution due to TEP aggregation and due to ciliate ingestion.

The maximum ingestion rate, calculated from the maximum number of FMS ingested by *Strombidium sulcatum* after 15 min of incubation (Table 1), was found in the LTCT (Balsfjord data) and reached about 130 FMS ciliate^{-1} h^{-1}, corresponding to a maximum clearance rate of about 260 nl ciliate^{-1} h^{-1}.

On average, the ciliate *Strombidium sulcatum* ingested 14.1 FMS ciliate^{-1} h^{-1} and 5.2 FMS ciliate^{-1} h^{-1} (Fig. 5), corresponding to average clearance rates of 28.2 and 10.4 nl ciliate^{-1} h^{-1} in LTCT and in HTCT, respectively. The number of FMS ingested in LTCT was on average 2.7 times higher than in HTCT. This suggests that the formation of mixed aggregates of TEP-FMS could reduce the availability of FMS (and consequently of other micrometer-size particles likely to form mixed aggregates with TEP) for micro-grazers, thereby reducing their ingestion rate.

Table 1. Average ingestion and clearing rates as calculated from linear regressions of number of FMS vs. incubation time over 45 min, and maximum ingestion and clearing rates as calculated from the maximum number of FMS ingested by *Strombidium sulcatum* after 15 min of incubation

	Balsfjord		Malangen		Ullsfjord		Overall data	
	HTCT	LTCT	HTCT	LTCT	HTCT	LTCT	HTCT	LTCT
Ingestion rate, I_{45} (no. FMS ciliate^{-1} h^{-1})[a]	8.3	16.7	1.7	8.9	5.7	16.6	5.2[c]	14.1[c]
Clearing rate, F_{45} (nl ciliate^{-1} h^{-1})[a]	16.6	33.4	3.4	17.8	11.4	33.2	10.4[c]	28.2[c]
Maximum no. FMS[b]	12	32	12	27	4	13		
Ingestion rate, I_{15max} (no. FMS ciliate^{-1} h^{-1})[b]	48	128	48	108	16	52		
Clearing rate, F_{15max} (nl ciliate^{-1} h^{-1})[b]	96	256	96	216	32	104		

[a]No. ciliates vs. incubation time over 45 min
[b]Maximum no. FMS ingested by *S. sulcatum* after 15 min incubation
[c]From Fig. 5

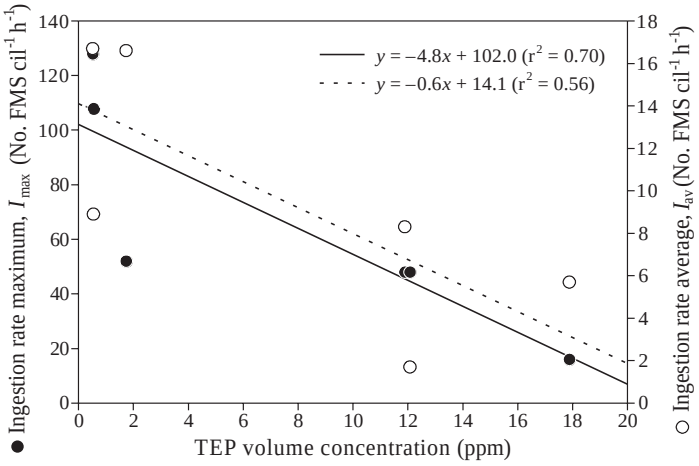

Fig. 6. Relationship between average (○) or maximum (●) ingestion rates and TEP volume concentration, for the 6 experiments conducted with contrasting TEP concentrations

For the 6 experiments conducted, estimates of ingestion rates (either average or maximum) decreased when TEP volume concentration increased (Fig. 6). In other words, the availability of FMS for grazing by *Strombidium sulcatum* is inversely proportional to the size of the TEP pool (i.e. to TEP volume concentration). This pattern suggests that grazing by *S. sulcatum* is suppressed above a critical value of TEP concentration.

FMS availability for *Strombidium sulcatum*

The percentage of FMS available for *Strombidium sulcatum* grazing was estimated as a function of the possible maximum particle size they could graze upon (Fig. 7). Maximum particle sizes were chosen in accordance with TEP size classes. The percentage of available FMS in LTCT was relatively independent from the maximum particle size *S. sulcatum* could graze upon, and varied between 89.4 ± 2.6 and 96.0 ± 3.6 % (i.e. less than about 10 % of FMS are unavailable for ciliates due to TEP-FMS formation). In contrast, the formation of TEP-FMS aggregates in HTCT rendered between 100 and 40 % of FMS unavailable for ciliate grazing, depending on the maximum size *S. sulcatum* can graze upon.

The ratio between the fractions of available FMS in LTCT and HTCT was calculated as a function of the maximum particle size available for *Strombidium sulcatum* (Fig. 8). As an example, assuming that *S. sulcatum* can only prey on particles <1.5 µm, then they should ingest 8 times more FMS in LTCT compared to HTCT. Since the number of FMS ingested in LTCT was on average 2.7 times higher than in HTCT (Fig. 5), the ratios suggest that *S. sulcatum* has optimal clearance for particles of ~3 µm ESD. Therefore, the formation of TEP-FMS aggregates seems to be responsible for the observed difference in ingestion rate. Over the range of observed values for ingestion rates in the different treatments (Table 1), the number of FMS ingested in LTCT was from 2.0 to 5.2 times higher than in HTCT, thus suggesting that *S. sulcatum* can graze particles of up to 6 µm ESD (Fig. 5).

DISCUSSION

Optimum food size range for *Strombidium sulcatum*

The difference between the number of FMS ingested by *Strombidium sulcatum* in LTCT and HTCT is best explained by an optimum clearance rate for TEP-FMS aggregates of ca. 3 µm. Fenchel & Jonsson (1988) studied particle selection and ingestion rates as a function of prey size during grazing experiments with latex beads of various sizes. They demonstrated that *S. sulcatum* have maximum clearance rate when feeding on particles of 2.83 µm diameter. A later study (Bernard & Rassoulzadegan 1990) demonstrated, using natural food items of various sizes, that *S. sulcatum* feeding was most efficient for prey in the 2.5 µm size range. Although we did not follow prey ingestion as a function of prey size, our data confirm the preference of *S. sulcatum* for particles ranging from 2.5 to 3 µm. Furthermore, we indirectly showed that *S. sulcatum* could graze particles of up to 6 µm. This finding is consistent with that of Bernard & Rassoulzadegan (1990), which showed that the feeding efficiency of *S. sulcatum* decreased down to zero for particles within the size range of 6 to 12 µm.

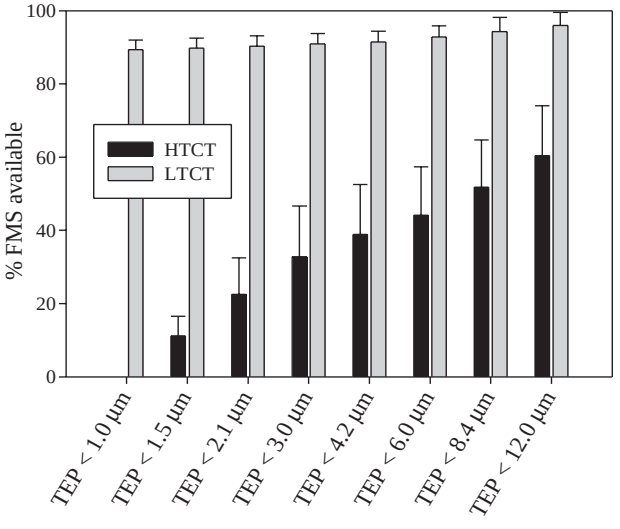

Fig. 7. Fraction of available FMS as a function of TEP concentration, according to the possible maximum aggregate size the ciliate *Strombidium sulcatum* can graze upon

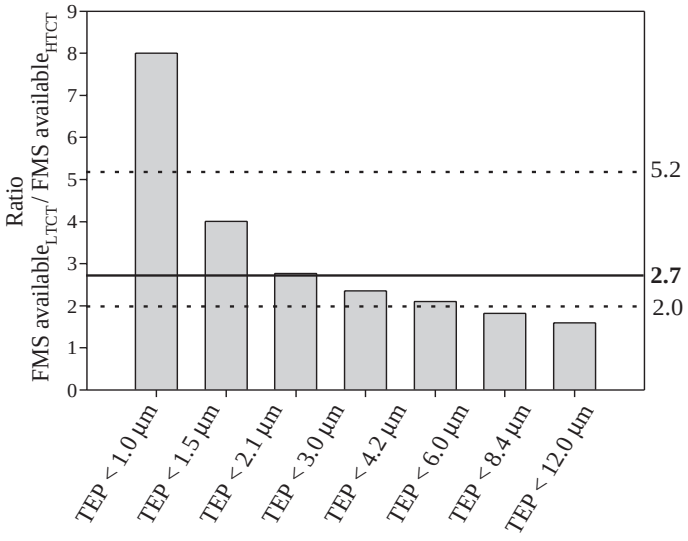

Fig. 8. Ratio between the fraction of available FMS in LTCT versus the fraction of available FMS in HTCT, according to the maximum particle size ciliates can graze upon. Lines correspond to the average ratio (——) or to the maximum and minimum ratios (- - -) of $Slope_{LTCT}$ versus $Slope_{HTCT}$ (cf. Fig. 5)

Strombidium sulcatum ingestion and clearance rates

Ingestion rates of 1 µm fluorescent microspheres averaged ~5 FMS ciliate^{-1} h^{-1} in HTCT and ~14 FMS ciliate^{-1} h^{-1} in LTCT. This observed range fits well with previous estimates obtained in similar conditions using the same *Strombidium sulcatum* strain (~10 FMS ciliate^{-1} h^{-1}; Dolan & Šimek 1997). Average clearance rates for *S. sulcatum* ranged from 10 to 30 nl ciliate^{-1} h^{-1} (maximum ~260 nl ciliate^{-1} h^{-1}). This agrees with estimates obtained by following ingestion of prey analogs of similar size and using the same *S. sulcatum* strain (ca. 100 nl ciliate^{-1} h^{-1}; Christaki et al. 1998).

Our study clearly showed a marked decrease in ingestion rates with increasing TEP concentrations. By comparing ingestion rates in the different treatments with the corresponding FMS availability, we showed that micro-particle availability for grazers is reduced because high TEP concentrations modify the food size spectra due to TEP-FMS formation. However, other mechanisms may explain the inverse relationship between FMS ingestion rates and TEP concentrations. One could argue that, due to their high sticking properties, TEP may trap micro-grazers into a mucous matrix. During our experiments, we commonly observed several *Strombidium sulcatum* embedded into large gel-like aggregates, especially in HTCT. Finally, the presence of fibrillar polymers in seawater influences its viscosity and rheological properties (Jenkinson 1986, 1993). Assuming that increasing TEP concentration is accompanied by an increase in fibrillar polymers, variations in TEP concentration may modify the viscoelasticity of the medium. Therefore, *S. sulcatum* swimming pattern, speed and, ultimately, their feeding behavior may change according to medium viscosity and, thus, to TEP concentration.

Estimation of ingestion and clearance rates as a function of TEP concentration

Considering that *Strombidium sulcatum* have maximum clearance rate for particles of about 3 µm diameter, ingestion and clearance rates calculated directly from the number of FMS inside digestive vacuoles may not be correct, especially for high TEP concentrations. Estimates of ingestion and clearance rates based on the number of FMS inside *S. sulcatum* and on the concentration of FMS in the medium are valid only if FMS are ingested individually. Since ≥90% of FMS are available in LTCT, irrespective of assumptions concerning the maximum food size available for *S. sulcatum*, it is to be expected that *S. sulcatum* will mostly feed on free FMS. In contrast, 100% of the total FMS are attached to TEP in HTCT and ca. 70% are attached to TEP >3 µm (cf. Fig. 7). Since optimum clearance for *S. sulcatum* is obtained with particles of ~3 µm, we argue that at high TEP concentrations the main source of FMS for this ciliate derives from the ingestion of 3 µm TEP-FMS aggregates (a 3 µm TEP-FMS aggregate hosts between 4 and 5 FMS). Under the above assumptions, ingestion and clearance rates in HTCT should be estimated from the concentration of 3 µm TEP-FMS aggregates. Concentrations of 3 µm TEP-FMS in the medium were obtained from the TEP-FMS size spectra, and the theoretical number of 3 µm TEP-FMS aggregates inside vacuoles was estimated from the total number of FMS inside vacuoles. As a result, in HTCT, ingestion rates dropped from 8.3 FMS ciliate^{-1} h^{-1} to 1.9 TEP-FMS of 3 µm ciliate^{-1} h^{-1}, from 1.7 FMS ciliate^{-1} h^{-1} to 0.4 TEP-FMS of 3 µm ciliate^{-1} h^{-1}, and from 5.7 FMS ciliate^{-1} h^{-1} to 1.3 TEP-FMS of 3 µm ciliate^{-1} h^{-1}, for Balsfjord, Malangen and Ullsfjord data, respectively. Over the observed concentration range of TEP-FMS aggregates of 3 µm ESD in HTCT, we estimated clearance rates 1 order of magnitude higher than those obtained directly from FMS counts. This means that, in HTCT, *S. sulcatum* have to sweep clear 10 times the volume they have to clear in LTCT, in order to catch the same quantity of FMS. This finding has strong implications when estimating clearance of an organism from microsphere ingestion, as clearance rate calculation strongly depends upon TEP concentration.

Implications for the microbial loop structure and function

TEP-FMS aggregates showed a structure similar to that observed for TEP-bacteria aggregates (Mari & Kiørboe 1996), i.e. FMS were both attached to the surface and embedded inside TEP. Re-examination of data from Mari & Kiørboe showed that during the spring bloom observed in the Kattegat, the number of attached bacteria (n) scaled with TEP size (d; µm) raised to an exponent of 1.48 ($n = 0.45\ d^{1.48}$; $r^2 = 0.82$; n = 340) during the growth phase of the bloom, while it scaled with TEP size raised to an exponent of 1.05 ($n = 1.38\ d^{1.05}$; $r^2 = 0.67$; n = 140) during the senescent phase of the bloom. Interestingly, the slopes of the regression lines of number of attached FMS versus TEP size, and number of attached bacteria during the growth phase of the bloom versus TEP size do not differ statistically. As previously discussed by Mari & Kiørboe (1996), exponents of the relationships between micrometer-size particles attached to TEP and TEP size can be considered as estimates of TEP fractal dimension, under the assumption that the number of attached micrometer-size particles is directly proportional to TEP porosity. An exponent of ~1.5 for the number of bacteria and of FMS per TEP is consistent with fractal dimensions estimated for marine snow particles, and is consistent with the dimensions of aggregates formed through shear coagulation (Logan & Wilkinson 1990, Logan & Kilps 1995). Although bacteria cannot be considered as conservative tracers for TEP porosity (Mari & Kiørboe 1996), during the phase of active TEP production (i.e. growth phase of the bloom) newly produced TEP precursors may combine with bacteria according to shear coagulation models to form TEP-bacteria aggregates of a similar structure to that observed for TEP-FMS aggregates. This suggests that, as for FMS, bacteria may collide with TEP precursors, thus forming small mixed clusters which in turn may join to form larger and larger TEP-bacteria aggregates. Therefore, a TEP-bacteria aggregate for which the number of attached bacteria scales with TEP size raised to an exponent of ~1.5 may represent the infancy of marine snow aggregates. Deviation from this exponent may translate into 'aging' of the aggregate and subsequent evolution of its size due to degradation and modification of the number of attached bacteria as a result of increased heterotrophic activity (Herndl 1988, Smith et al. 1992, Grossart & Simon 1993,1998, Grossart et al. 1998, Grossart & Ploug 2000) and active bacterial colonization (Kiørboe & Jackson 2001).

Finally, we demonstrated that increasing TEP concentration had a negative effect on pelagic bacterivorous ciliates, by modifying prey size spectra (Fig. 9). This process leads to a diminution of ingestion rates, which ultimately may cause the disappearance of *Strombidium sulcatum* at a critical TEP volume concentration. While the disappearance of a bacterivorous ciliate species may be due to transfer of its food source to unattainable size classes, the newly formed food size spectra may in turn become accessible for and benefit other organisms. As an example, the predacious ciliate *Euplotes* spp., known to graze on large particles (Artolozaga et al. 1997, 2000, Jonsson & Johansson 1997), may theoretically succeed *Strombidium* spp. during accumulation of the TEP pool and subsequent formation of large aggregates.

Partitioning of the carbon source

Ingestion rates (in pg C ciliate^{-1} h^{-1}) can be estimated considering a bacterial carbon content of 20 fg C bacterium^{-1} (Lee & Fuhrman 1987) and a TEP carbon content given by TEP-C = $0.25\ r^{2.55}$ pg C TEP^{-1} (Mari 1999),

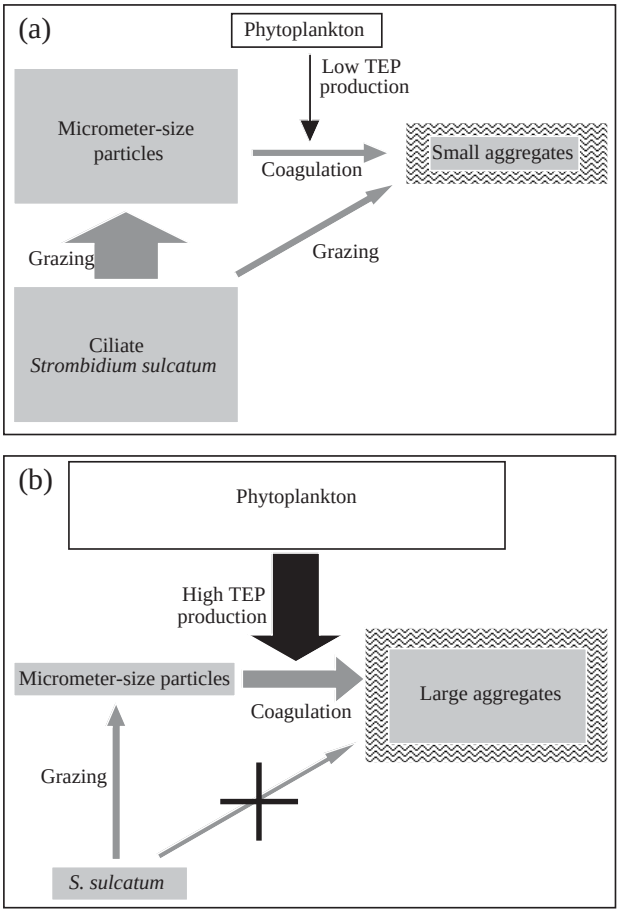

Fig. 9. *Strombidium sulcatum*. Conceptual representation of the effect of (a) low and (b) high TEP concentrations on grazing behavior. Difference in box size between (a) and (b) represents an evolution of the compartment size

where r is the equivalent spherical TEP radius. The number of TEP-attached bacteria can be estimated either from the relationships obtained from re-examination of Mari & Kiørboe (1996) or from the TEP size versus number of FMS, assuming that bacteria follow the same relationship.

For a TEP of 3 µm ESD, 2 to 5 bacteria should theoretically be associated with the particle. Five bacteria per TEP of 3 µm ESD can be considered as a maximum since other estimates of bacterial density (Passow & Alldredge 1994, Schuster & Herndl 1995) predict even lower numbers of attached bacteria. As a result, a *Strombidium sulcatum* ingesting one 3 µm TEP-bacteria aggregate gains the major fraction of its organic carbon from TEP (≥86 %), while bacterial carbon would only represent a minor fraction (≤14 %) of the total amount ingested. This is a crude estimate assuming that TEP are highly digestible, but it shows that TEP may represent a significant source of carbon for microzooplankton as compared to bacterial carbon. The relative significance of TEP-C to the ciliate energy budget may depend upon TEP concentration rather than on bacterial concentration, as at relatively high TEP concentrations food size spectra are shifted towards larger size classes. Such a mechanism may shunt the microbial loop for the transfer of energy from the dissolved pool back to higher trophic levels. In other words, ciliate, and maybe up to micro- and mesozooplankton, bacterivory via TEP aggregation may short circuit the microbial loop in aquatic ecosystems. This hypothesis is supported by recent work conducted on TEP ingestion by copepods (Ling & Alldredge 2003) and by euphausiids (Passow & Alldredge 1999), and on lake snow (formed by TEP aggregation) grazing by juvenile fish (Grossart et al. 1998).

Furthermore, it has been suggested that the nutritional status of the medium controls TEP accumulation by determining the efficiency of the microbial loop in metabolizing the TEP formed (Mari & Burd 1998). On the other hand, TEP accumulation appears to control the functioning of the microbial loop by unpriming it at the bacterial level. The result would be a switch from the microbial loop to a direct lift from the dissolved pool to higher trophic levels, controlled by nutrient conditions. This pathway for carbon via TEP accumulation represents a positive feedback mechanism whereby carbon flux towards the classical food chain is optimized as intermediary levels are bypassed, thus minimizing energy losses (Sherr & Sherr 1988).

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