Effects of planktonic copepods on transparent exopolymeric particles (TEP) abundance and size spectra

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[1]TO WHOM CORRESPONDENCE SHOULD BE ADDRESSED

Diatoms exude considerable quantities of polymers, mainly polysaccharides, that play an important role in the process of sestonic particle aggregation in the sea. We investigated the impact of copepods on transparent exopolymeric particles (TEP) generated by the diatom Thalassiosira weissflogii. Grazing experiments with ^{14}C-labelled algae exudates demonstrated that copepods typical of the Baltic Sea were not actively filtering TEP. Control experiments showed that 'uptake' of radioactivity could be ascribed to passive uptake, such as adsorption of radioactively-labelled particles to the body surface. Furthermore, we tested the effect of copepods on TEP size spectra. The abundance and size distribution of TEP (from 1.4 to 180 μm of Equivalent Spherical Diameter) were analysed in a 4 h incubation experiment. In the presence of copepods, the proportion of larger TEP was higher. An increase in total volume of TEP in jars containing copepods (~2 × 10^7 μm ml$^{–1}$) compared with control jars without copepods (~0.5 × 10^7 μm^3 ml$^{–1}$) was also observed. The process of aggregation of TEP demonstrated in this work, whereby copepods increase downward particle flux without consuming carbon, can have far-reaching consequences for carbon fluxes along the water column and for copepods feeding dynamics.

INTRODUCTION

Many marine organisms, e.g. fish, corals, macro and microalgae and bacteria (Decho, 1990), are known to secrete extracellular material. Diatoms are among the most important primary producers in the marine environment, and a considerable proportion of the organic material produced by diatoms consists of polysaccharides (Haug and Myklestad, 1976; Decho, 1990; Hoagland *et al.*, 1993). TEP are extremely hydrated particles (approximately 99% water) which are generated abiotically from dissolved extracellular polysaccharides (Mari and Burd, 1998; Mari, 1999; Passow, 2000) that coalesce via cation bridging (Passow and Alldredge, 1994). TEP are thought to have significant implications for flux processes in marine (Schuster and Herndl, 1995; Hong *et al.*, 1997) and freshwater environments (Grossart *et al.*, 1997; Worm and Sondergaard, 1998).

TEP might serve as an alternative food resource of nutritional value for zooplankton, because these particles may act as adsorption sites of solutes (Decho, 1990), change the chemical reactivity of many substances (Passow and Alldredge, 1995a), and are a substrate for bacteria (Passow and Alldredge, 1994; Schuster and Herndl, 1995; Mari and Kiørboe, 1996). In contrast, exopolymers have relevance in other processes related to zooplankton nutrition, as the extracellular coating may also enhance the survival of diatoms recently ingested (Decho, 1990), or clog the feeding apparatus of zooplankton (Malej and Harris, 1993; Young *et al.*, 1997; Dilling *et al.*, 1998; Liu and Buskey, 2000). There is evidence that many marine organisms ingest a variety of macromolecules (including carbohydrates and proteins). These observations include heterotrophic flagellates (Sherr, 1988; Tranvik *et al.*, 1993), planktonic tunicates (Flood *et al.*, 1992), euphausiids (Passow and Alldredge, 1999) and

copepods (Poulet, 1983; Decho and Moriarty, 1990). However, the role of algal exudates in zooplankton grazing has not been clarified. Experiments exposing copepods to cultures containing diatom exudates have provided contradictory conclusions. Cowles *et al.* (Cowles *et al.*, 1988) found higher ingestion rates when algal exudates from *Thalassiosira weissflogii* were added, whereas Malej and Harris (Malej and Harris, 1993) found inhibition of copepod grazing on *T. weissflogii* and *Skeletonema costatum* by diatom exudates. The results of Malej and Harris (Malej and Harris, 1993) could be attributed to copepods that selectively choose among different food. In a recent study of euphausiid grazing on TEP from the diatom *T. weissflogii*, Passow and Alldredge (Passow and Alldredge, 1999) found that these grazers fed on cells as well as on TEP clusters. This was probably the reason why euphausiids had lower ingestion rates on cells when cells and TEP were present at the same time. Nevertheless, Young *et al.* (Young *et al.*, 1997) observed by video analysis that *Daphnia* declines its filtering and ingestion rates when sticky aggregates of filamentous and gelatinous algae are presented.

Some studies on copepod assimilation have been carried out for other types of secretions than TEP. Richman *et al.* (Richman *et al.*, 1975) found that reef copepods ingested and assimilated 50% of the organic carbon of stressed coral mucus, and Gottfried and Roman (Gottfried and Roman, 1983) found 65% incorporation for *Acartia tonsa*, and 39% for *Mysidium intergrum*, using natural coral mucus. Decho and Moriarty bound ^{14}C-labelled bacterial exudates to bacterium-sized beads, thus imitating natural sediment bacteria (Decho and Moriarty, 1990). These beads mixed with sediment were then offered to the meiobenthic harpacticoid copepod, *Laophonte* sp., which readily ingested and retained the ^{14}C label with an efficiency of 80%. Hence, copepods can assimilate an important fraction of ingested secretions from corals and bacteria, but no information is yet available for pelagic copepods feeding on phytoplankton exudates.

Furthermore, although the importance of abundance and size of TEP is known for the aggregation of marine particles (Alldredge *et al.*, 1993; Passow and Alldredge, 1994; Passow and Alldredge, 1995b; Schuster and Herndl, 1995; Mari and Kiørboe, 1996; Hong *et al.*, 1997), little is known about how the presence of zooplankton affects TEP size spectra. Most zooplankton repackage small particles into larger ones by production of fecal pellets, which sink faster than the primary particles, thus increasing the downward carbon flux. When the small particles are not consumed by the zooplankton, the effects of those organisms in the seston size spectrum and in sedimentation flux are not clear.

As feeding on TEP by copepods is unknown, the con-sequences of copepod activity on the TEP size spectrum and, consequently, on the dynamics of marine snow, need to be investigated. Therefore, the aims of the present study were as follows: to determine whether marine planktonic copepods ingest and assimilated free TEP produced by the diatom *T. weissflogii*, and then to evaluate the effect of copepod activity on TEP size spectra.

METHOD

Organisms and media

Copepods were collected with a 150 μm mesh size plankton net (95 cm mouth opening) from the upper 10 m of the water column in Kiel Bight, Germany. The contents of the net were transferred to a bucket containing surface seawater and promptly transported to the laboratory. Copepods were isolated and maintained at $14 \pm 0.5°C$ at dim light in 500 ml glass beakers. Algae (*T. weissflogii*) were supplied every 24 h during acclimation (3 days). Copepod species were *Paracalanus parvus*, *Pseudocalanus elongatus*, *Oithona similis*, *Acartia* sp., *Temora longicornis* and *Centropages hamatus*.

Stock cultures of *T. weissflogii* were maintained in f/2 medium (Guillard and Ryther, 1962) in batch cultures, using a 2 week transfer cycle. Studies have shown that this diatom provides high exopolymer production (Cowles *et al.*, 1988; Malej and Harris, 1993). For experiments, diatoms were grown in 0.6 l tissue culture flasks (Nunclon®) at $18 \pm 0.5°C$ and 80–100 μE m^{-2} s^{-1} light. One week before experiments, and coinciding with the exponential growth stage, some cultures were labelled with ^{14}C bicarbonate (Hartmann Analytic; 0.03 μCi ml^{-1} final concentration) to produce ^{14}C-labelled exopolymers. Cultures were not aerated, but were gently agitated manually once per day. The population of *T. weissflogii* had an average width of 17.3 μm (S.D. = 4.0, $n = 50$) and an average length of 10.9 μm (S.D. = 1.7, $n = 50$).

A cell-free culture medium containing algal exudates ('TEP medium') was created by low vacuum (150 mm Hg; 5 μm pore-size) just prior to experiments. A low filtration pressure minimizes cell rupture so that the medium contained mainly algal exudates (and not algal cell contents which would have entered the solution as a consequence of cell rupture). Therefore, this cell-free TEP was mainly re-formed from polymeric material having recently passed a 5 μm filter.

Samples for cell counts and bacterial abundance were collected from 'TEP medium' and from the cell culture of *T. weissflogii* ('Cells medium') in order to characterize them. *Thalassiosira weissflogii* cells were fixed (Lugol's solution) and enumerated using inverted microscopy [Leica Fluovert (Utermöhl, 1958)]. Cell concentrations were determined in three replicate subsamples. The number of

Table I: Thalassiosira weissflogii. Summary of biological and chemical composition before ('Cells medium') and after ('TEP medium') filtering a T. weissflogii culture through 5 μm

Medium	Thalass conc. (ml^{-1})	TEP conc. ($10^4 \times$ ml^{-1})	Bact conc. ($10^6 \times$ ml^{-1})	Organic C (μg ml^{-1})	C:N	TEP ESD (μm)
Cells medium	27319	7.1	12.2	13.1	10.3	6.47 ± 0.24
	23261	6.9	10.6	13.0	10.1	
TEP medium	60	1.9	2.0	1.4	9.3	3.76 ± 0.16
	10	3.0	1.6	1.4	9.4	

Values for two replicates are presented, except for TEP ESD, where values are average ± S.E., $n = 933$ and $n = 702$ for 'Cells medium' and 'TEP medium', respectively.

bacteria was determined by direct counts of previously preserved subsamples (glutaraldehyde 1.5%) using DAPI staining (Porter and Feig, 1980) and filtered through 0.2 μm Nuclepore® filters. Microscopic enumeration of bacteria was conducted with a Leica DMRB epifluorescence microscope. At least 400 bacteria were counted from each of two replicate slides.

Two replicates of both media were filtered onto precombusted glass-fibre filters (GF/F) and then combusted for carbon and nitrogren analysis in a Fisons NA 1500 N elemental analyser.

Two different experiments were conducted. One experiment investigated whether copepods fed on TEP when any other source of food was present. Feeding was measured by calculating clearance and ingestion rates from ^{14}C incorporated into copepods (Haney, 1971). A second experiment was performed to test the effect of copepods on TEP size spectra, measuring TEP on an image analysis system. TEP concentrations during this study were within the range of usual TEP concentrations in nature (Passow *et al.*, 1994) to ensure that TEP values were not limiting.

Grazing experiment

During this experiment, ^{14}C incorporated into copepods was measured in two different treatments: 1 (TEP Tr.) contained TEP, and 2 (Cells Tr.) contained *T. weissflogii* cells. Additionally, three replicates per treatment were incubated with dead copepods, and these functioned as passive-adsorption controls.

Three hours before the experiment, copepods were placed at a density of 0.2–0.3 ind. ml^{-1} in glass jars containing filtered sea water and acclimated to experimental conditions at 18 ± 0.5°C and 80–100 μE m^{-2} s^{-1} light. Six replicate grazing trials were run for each treatment.

TEP treatment. An 80 ml sample of the ^{14}C-labelled cell-free medium ('TEP medium') was added to experimental jars, thus creating a final volume of 230 ml in the grazer jars (150 ml 0.2 μm-filtered sea water plus 80 ml cell-free labelled medium). After the dilution, the final concentration of TEP in the experimental jars was 8.5×10^3 TEP ml^{-1}. After exposing copepods to a ^{14}C-labelled algal exudate for 30 min, copepods of three experimental jars were rapidly anaesthetized with carbonized water, collected on a 100 μm screen and thoroughly rinsed with 0.2 μm-filtered sea water (measurement of TEP ingestion rates). Copepods in the remaining three jars were transfered to a non-labelled suspension of equal TEP concentration, in which copepods were allowed to empty their digestive systems of ingested label (measurement of TEP carbon retention). After 30 min of exposure to this non-labelled suspension, the copepods were anaesthetized with carbonized water, collected and rinsed as described above. All copepods were immediately counted under a Leica WILD M3Z dissecting microscope and subsequently pipetted into scintillation vials (20 ml). Immediate separation reduces loss of radioactive label by fixation and storage (Holtby and Knoechel, 1981). Then, 0.5 ml tissue solubilizer (Soluene-350) was added to the vials and digestion accelerated by exposing the vials to 45°C for 24 h. The vials were allowed to cool, and then 10 ml scintillation cocktail (Ultima Gold, Packard) were added to each of the scintillation vials. The ^{14}C radioactivity (dpm) was measured by means of a liquid scintillation analyser (TRI-CARB 2100 TR). Correction for quenching was done using an external standard.

Besides labelled algal exopolymers, the 'TEP medium' also contained labelled bacteria, and labelled particulate and dissolved organic matter (Table I). In order to correct for passive uptake of label by copepods, either by direct adsorption of ^{14}C compounds or by attachment of labelled bacteria to the external surface of the copepods (all of these representing sources of non-ingested radioactivity), we conducted simultaneous control trials. These controls (triplicates) were done by exposing formalin-killed copepods at a density of 0.2–0.3 ind. ml^{-1} to

radioactive labelled algal exudates at the same concentration as with live copepods. Radioactive values assessed for passive adsorption of radioactive tracer by formalin-killed copepods were used to correct for values obtained in grazing trials.

In order to determine the amount of radioactivity added to glass jars, triplicate samples of 10 ml of 'TEP medium' were filtered onto GF/C filters. Filters were exposed to vapours of concentrated HCl in order to remove non-incorporated residual radioactive bicarbonate as $^{14}CO_2$. After 2 h, filters were placed into scintillation vials with 5 ml scintillation cocktail (Lumagel SB, Baker), and ^{14}C radioactivity (dpm) was measured as mentioned above.

Cells treatment. A 10 ml volume of the ^{14}C-labelled cell medium ('Cells medium') was added to experimental jars, giving a final concentration of cells of 1205 cells ml^{-1}. After dilution, TEP were present at 3.3×10^3 TEP ml^{-1}. The total volume in the grazer jars was 210 ml (200 ml 0.2 μm-filtered sea water plus 10 ml labelled cell medium). Incubation methods and control treatments for adsorption of ^{14}C were identical to those used in the TEP medium treatment. To determine the amount of radioactivity added to grazer jars, 1 ml 'Cells medium' was filtered and the same methodology used as in the previous treatment.

Calculations of clearance rates, ingestion rates and retention efficiency. Individual clearance rate, F (ml ind^{-1} h^{-1}), represents the volume of water swept clear of particulate food in unit time and was computed using the equation provided by Haney (Haney, 1971):

$$F = (\text{dpm ind.}^{-1}/\text{dpm ml}^{-1} \text{ food suspension}) \times (60/t) \quad (1)$$

where t is the grazing time (in minutes). The ingestion rate, I (TEP ind.$^{-1}$ h^{-1} or cell ind.$^{-1}$ h^{-1}), was obtained from the clearance rate by:

$$I = F \times C \quad (2)$$

where C is the concentration of TEP or cells (depending on the treatment) in the food suspension. Retention efficiencies of TEP ^{14}C, RE (%) were calculated as:

$$RE = (\text{dpm retained ind.}^{-1}/\text{dpm ingested ind.}^{-1}) \times 100 \quad (3)$$

where dpm ingested ind^{-1} corresponded to the radioactivity accumulated after the 30 min exposure to radioactive food, and dpm retained ind^{-1} was the radioactivity of copepods after the depuration process in non-labelled suspension.

Care should be taken to avoid experimental artefacts that may result in an overestimation of radioactive uptake. The methods used in this research followed the suggestions of Douillet (Douillet, 1993). Controls are necessary to evaluate passive uptake of label by the animals. A short feeding period on labelled food and a feeding period on non-labelled suspension depurates the organism digestive system and reduces the possibility of label recycling.

TEP size spectra experiment

We tested the effect of copepods on TEP size spectra. The experiment of 4 h duration was done at 14 ± 0.5°C under dim light (<15 μE m^{-2} s^{-1}). Polycarbonate bottles (100 ml) filled with TEP medium were placed on a plankton wheel (1.24 rev min^{-1}) and different treatments were applied. Copepod treatments (Copepod Tr.) used animals at densities of 25, 41 and 54 (one replicate each) and the control treatment (Control Tr.) (three replicates) used no animals. Samples were taken initially (t_0) and after this experiment.

From each treatment, slides of TEP were prepared singly (Control Tr.) or in duplicate (t_0 and Copepod Tr.) by filtering 1–7 ml sample onto Nuclepore® filters (0.4 μm) following Passow *et al.* (Passow *et al.*, 1994). All samples were freshly prepared to avoid modification of the size of the polymers (Chin *et al.*, 1998). Blanks were prepared to account for the background of stained particles derived from the reagent and other sources, and were always insignificant.

The abundance and size of TEP were determined with a compound light microscope at 100×, 200× and 400× magnifications. At each magnification, 100–700 particles (10–150 frames) were counted and sized. The microscope was connected to a video recorder S-VHS through a colour video camera. Frames were recorded on a Super VHS tape and later digitized on a Macintosh Power PC. TEP contour lines were traced manually so that individual TEP were distinguishable from other particles (i.e. cells). The image analysis software used was NIH Image 1.60, with an optical resolution of 0.20, 0.41 or 0.83 μm pixel^{-1}, depending on whether the magnification was 100×, 200× or 400×, respectively. This system measures the area of each particle from which the Equivalent Spherical Diameter (ESD) and Equivalent Spherical Volume (ESV) were calculated. Using this technique we were able to study TEP in a size range from 1.4 to 180 μm of EDS.

The size measurements obtained were classified into increasing size classes arranged in octaves (2^n) of the individual volume. Nominal volumes (v) of size classes have been defined as $v = 1.44w$, w being the lower limit of the size class (Blanco *et al.*, 1994). Following the techniques of Platt and Denman (Platt and Denman, 1977, 1978), a linear function was fitted to the log-transformed normalized volume (ß).

Table II: Summary of results of ^{14}C grazing experiments conducted with copepods feeding on Thalassiosira weissflogii cells and TEP

Feeding on	^{14}C 'control' copepods (dpm ind.$^{-1}$)	^{14}C 'ingestion' copepods (dpm ind.$^{-1}$)	^{14}C 'retention' copepods (dpm ind.$^{-1}$)	Ingestion rate (Cell ind.$^{-1}$ h^{-1})	Retention efficiency (%)
T. weissflogii	9.96 ± 5.38	59.0 ± 33.9	42.2 ± 13.5	640.2 ± 441.6	65.62
TEP	5.78 ± 0.82	5.54 ± 0.56	6.80 ± 0.70	0	0

Ingestion rates and retention efficiencies are corrected for passive uptake of radioactive label.

$$\log (\text{ß}) = a - b \log (w) \qquad (4)$$

The slope of the normalized size–volume spectrum (b) can be used to assess the size characteristics of TEP. Thus, with increasing proportions of small-sized particles, the value of b becomes greater. Much of the discussion in the TEP size spectra experiment was based on the value of parameter b, which summarizes information on the size distribution of TEP in a single number.

RESULTS

Characteristics of the organisms and media

Microscopic examination of the 'TEP medium' revealed that 99.9% of the phytoplankton cells were removed by filtration (Table I). Bacterial abundance and Particulate Organic Carbon (POC) were reduced by 84% from the initial values, and the carbon to nitrogen ratio was maintained. This important reduction of bacterial abundance was probably due to the sticky and jelly nature of TEP, which clogged the filter and made the real pore size much smaller. Also, the close relation between bacteria and TEP explains some of the bacterial reduction. Due to their labile nature and wide size range, the abundance of TEP was only decreased by 65%, but TEP size (expressed as average EDS) was reduced to nearly half of the original size (Table I). The average dry weight of animals was 204 µg (S.D. = 63, n = 120) and all animals appeared active before the experiments.

Grazing experiment

Feeding on TEP. Ingestion of ^{14}C TEP showed 5.54 dpm per individual (S.D. = 0.56, n = 3) at the end of the 30 min feeding period (Table II). After a subsequent 30 min exposure time of copepods to a non-labelled suspension, the copepods retained 6.80 dpm per individual (S.D. = 0.70, n = 3).

The ^{14}C present in the formaldehyde-killed copepods at the end of the 30 min incubation period was 5.78 dpm ind.$^{-1}$ (S.D. = 0.82, n = 3), which was similar to the ^{14}C present in the non-control copepods. The ^{14}C ingestion and ^{14}C retention values were not statistically different to control values (0.25 > P > 0.1, one-way ANOVA), therefore after doing the correction for controls, ingestion of the ^{14}C TEP and ^{14}C TEP retained were negligible.

This experiment showed that the copepod community was not significantly grazing TEP, since there was no significant difference in the uptake of radioactive label in jars containing live copepods and in control jars. Measurable radioactivity of copepods feeding on TEP can, therefore, be ascribed to passive uptake of radioactive label, such as adsorption of radioactive-labelled particles to the body.

Feeding on cells. The clearance rate (F) of copepods feeding on *T. weissflogii* was 0.53 ml ind.$^{-1}$ h^{-1} (S.D. = 0.37, n = 3; Table II). An ingestion rate (I) of 640.2 cells ind.$^{-1}$ h^{-1} (S.D. = 441.64, n = 3) was calculated from the concentration of cells in the experimental jars. The I expressed in terms of carbon was 0.33 µg C ind.$^{-1}$ h^{-1} (S.D. = 0.23, n = 3). Retention efficiency (RE) was 65.62% of the initially ingested ^{14}C. These results for the copepod feeding experiment on *T. weissflogii* cells are already corrected for controls of formaldehyde-killed copepod incubations.

TEP size spectra experiment

No significant differences were found among initial and control treatments (Figures 1a, b), either in abundance or total volume of TEP (0.5 > P > 0.2, t-test between means in both tests). Although the abundance of TEP (Figure 1a) was not significantly different between Control Tr. and Copepod Tr. (P > 0.25, nested one-way ANOVA), a significant increase in the total volume of TEP (P < 0.05, nested one-way ANOVA) in the presence of grazers was found (Figure 1b).

Although no significant differences were found within Copepod Tr. in either abundance or total volume of TEP
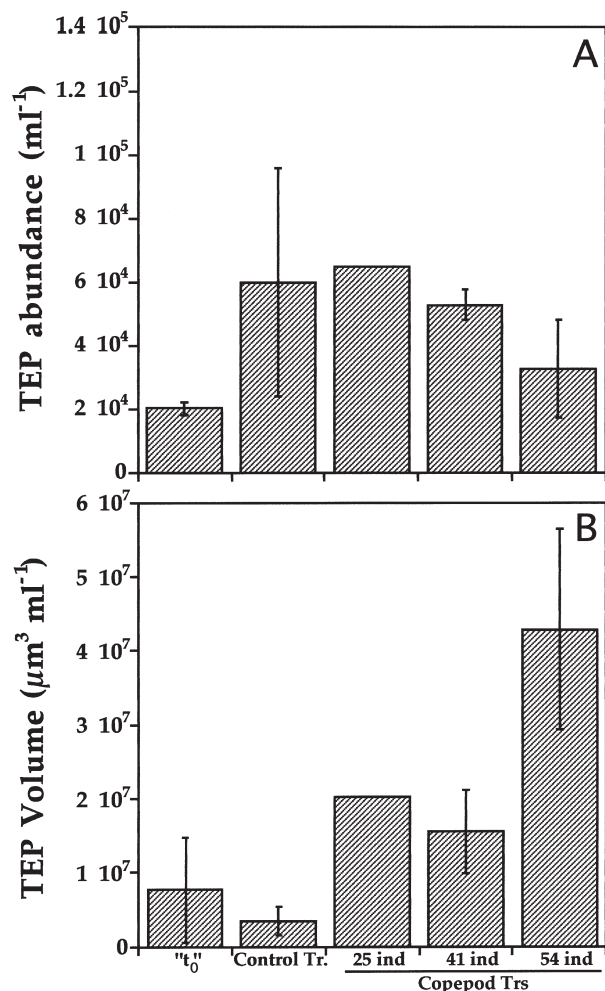
Fig. 1. Average total abundance (A) and total volume (B) of TEP. Bars represent the range of values of two replicates, except in Control Tr., where $n = 3$, and in the bottle with 25 individuals, where $n = 1$ (as one replicate of the Copepod Tr. with 25 individuals was lost).

Fig. 2. Average of TEP size expressed as ESD (μm) from each sub-sample. Error bars represent ± S.E. The number of individual TEP (n) measured varied in each subsample but in all cases $n > 400$.

(nested one-way ANOVA, $0.25 > P > 0.1$ and $P > 0.1$, respectively), the abundance of TEP seems to decrease with increasing density of copepods.

The average ESD of TEP showed significant differences for the average size between Control Tr. ($0.005 > P > 0.001$, Tukey's HSD Test) and the Copepod Tr. with the highest number of copepods (54 individuals). In this case the average size of TEP increased from 8.7 μm (in control jar) to 17.8 μm of ESD in the jar with 54 copepods (Figure 2).

A more detailed study of the effect of planktonic copepods on the size of TEP was based on the size spectra of TEP (the results of each linear function are in Table III). All size spectra of TEP were first pooled according to whether they were from the onset of the experiment,
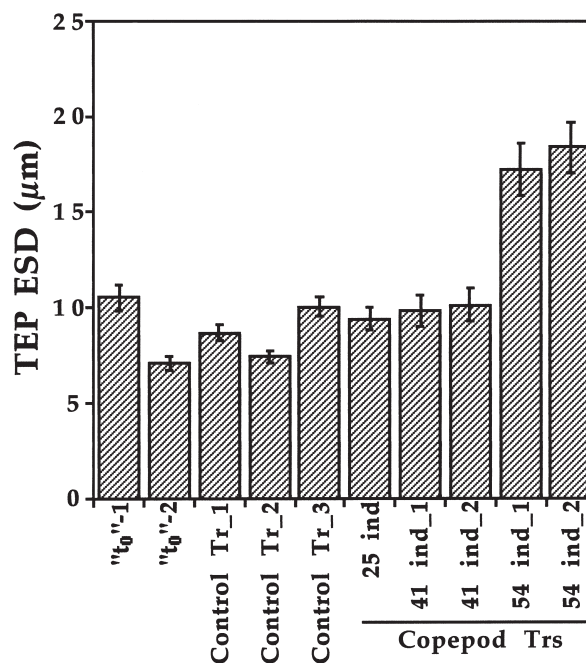
Table III: Equation parameters and coefficients of determination of the linear models of the TEP size spectra for the onset of the experiment ('t_0'), Control Tr., and Copepod Tr

Subsample	a	b	r^2
't_0' –1	3.77	0.16	0.91
't_0' –2	3.99	0.70	0.97
Control Tr. 1	4.23	0.76	0.97
Control Tr. 2	4.57	0.73	0.98
Control Tr. 3	4.58	0.79	0.98
Copepod Tr. 25 ind.	3.78	0.50	0.81
Copepod Tr. 41 ind. 1	4.26	0.67	0.96
Copepod Tr. 41 ind. 2	4.12	0.61	0.95
Copepod Tr. 54 ind. 1	3.90	0.52	0.87
Copepod Tr. 54 ind. 2	3.82	0.49	0.97

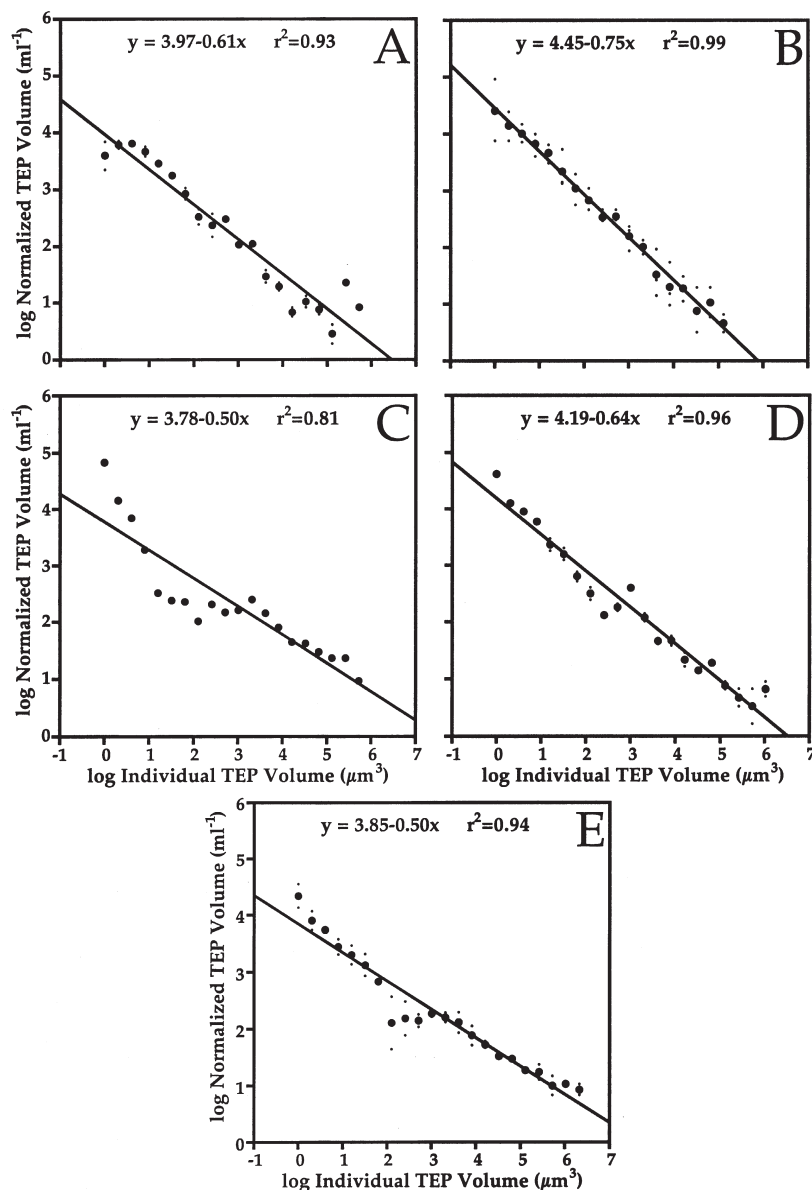A linear model was fitted for each subsample separately.

Fig. 3. Grouped size spectrum of TEP for 't_0' of the experiment (A), Control Tr. (B), and Copepod Tr. (C–E). See text for details of grouping. Smaller dots represent the observations for each subsample, whereas large dots represent the mean value. A linear model was fitted for each grouped size spectrum, whose equation is shown.

Control Tr. or Copepod Tr. with the same number of grazers. Within all of these groups, linear functions seemed to fit to the same size spectra, so a test of multiple comparison among slopes (Zar, 1984) was made for those spectra belonging to the same group. Thus, we were able to check whether the same linear model fitted to the spectra within the same group. This being the case, only one size spectrum was obtained for each group (Figure 3).

The size spectra of TEP changed significantly [0.001 < P < 0.005 (Zar, 1984)] from 't_0' to Control Tr. (Figure 3a,

b) towards an increase in abundance of small particles, denoted by a greater b value (from 0.615 to 0.755). Hence, after 4 h in a plankton wheel, TEP disaggregated in bottles without copepods. The effect was different when copepods were present (Figure 3c, d, e). The comparison among b values obtained from the Control Tr. and Copepod Tr. is shown in Table IV. The probability of the null hypothesis (slopes are equal) of the test of multiple comparison among slopes confirmed that slopes of size spectra of control and copepods were significantly different [P < 0.001 (Zar,

Table IV: Comparison of the value of slopes from TEP size spectra of Control Tr. and Copepods Tr.

	Control	25 individuals	41 individuals	54 individuals
Control	–	$P < 0.001$	$P < 0.001$	$P < 0.001$
25 ind.	–	–	$P < 0.001$	$P < 0.5$
41 ind.	–	–	–	$P < 0.001$
54 ind.	–	–	–	–

The probability of the null hypothesis (slopes are equal) of the test of multiple comparison among slopes is shown (Zar, 1984).

1984)]. Slopes of size spectra from Copepod Tr. were lower than the slope of the Control Tr. Consequently, copepods apparently had an aggregation effect on TEP.

DISCUSSION

Our results clearly indicate that the copepod community formed by *P. parvus*, *P. elongatus*, *O. similis*, *Acartia* sp., *T. longicornis* and *C. hamatus* does not ingest algal exudates from the diatom *T. weissflogii*, since there was no significant difference between the values of ingested radioactive label of live copepods that had been feeding on labelled algal exudates and controls (passive uptake). The reason why copepods did not use TEP as a food resource when any other food was presented is controversially discussed in the literature. Copepods might suffer a hydrodynamic disadvantage, as an increased viscosity due to polysaccharides may reduce turbulence (Jenkinson, 1990) and consequently, reduce encounter rate (leading to lower ingestion rates). Copepods use comparatively more energy for swimming than other zooplankton (Davenport and Trueman, 1985), and in a more viscous and less turbulent medium, they would require a higher energy output when feeding (Granata and Dickey, 1991). In this less favourable medium, copepods need a rich food source to compensate for the additional expenditure of energy. In this sense, the carbon content of *T. weissflogii* cells [according to (Falkowski *et al.*, 1985), and the individual cell volume of our population] was ~2.6×10^{-7} µg C µm^{-3}, while TEP carbon content was in the order of 10^{-8} µg C µm^{-3} (Mari, 1999). Thus, TEP represent a much lower carbon source than cells, and it is not favourable for copepods to spend energy on catching TEP. As feeding selectivity by calanoid copepods occurs both via post-capture and pre-capture selection (Cowles *et al.*, 1988), it is possible that copepods did not ingest TEP because these particles were not chemically stimulating for them, may have released fewer olfactory cues, or may have been poorer in required vitamins. It is important to note that TEP used in this study were recently formed and cell-free TEP, whereas in previous experiments, which indicate that grazers do eat sticky aggregates from phytoplankton (Young *et al.*, 1997; Passow and Alldredge, 1999), the aggregates were aged. As mucilaginous aggregates behave like an absorption lattice (Olianas *et al.*, 1996), they may accumulate phytoplankton, protozoans, flagellates, bacteria and marine metabolites during ageing. Therefore, in the present study, TEP were probably less attractive to copepods than the aggregates offered in these previous studies, as the lack of cells may make TEP 'invisible' for copepods. Another reason could be attributed to mechanical effects of sticky particles on the filter limbs (Young *et al.*, 1997) rather than a neurally-controlled selection mechanism.

Although copepods did not feed on TEP, their ingestion (*I*) rate and retention efficiency (*RE*) when feeding on *T. weissflogii* were within the range reported in the literature. In our experiments, the *I* for copepods was 640 cells ind.$^{-1}$ h^{-1} when feeding at 1200 cells ml^{-1} concentration of *T. weissflogii*. Cowles *et al.* reported ingestion rates varying from 650 to 2000 cells ind.$^{-1}$ h^{-1} for *Acartia tonsa* feeding on *T. weissflogii* at 1500 cells ml^{-1}, depending on which type of algae animals had been acclimated to (Cowles *et al.*, 1988). Gifford *et al.* found similar ingestion rates for *Calanus finmarchicus* feeding on *T. weissflogii* cells, with or without chitan filaments (Gifford *et al.*, 1981). An ingestion rate of 560–670 cells ind.$^{-1}$ h^{-1} was estimated by Malej and Harris (Malej and Harris, 1993) for *Temora longiformis* feeding on *T. weissflogii* at 2000 cells ml^{-1} and at different concentrations of algae exudates. In terms of carbon, in the present study, we measured 0.33 µg C ind.$^{-1}$ h^{-1}, and Frost (Frost, 1975) found and *I* of 0.34 µg C ind.$^{-1}$ h^{-1} of the copepod *G. pacificus* fed on *T. weissflogii* at 1010 cells ml^{-1}. Similarly, we found a retention efficiency of 65.62%, close to the *RE* reported by Runge (Runge, 1980) for *C. pacificus* feeding on *Thalassiosira* sp. Therefore, our results for grazing of copepods fed on *T. weissflogii* confirmed the good health and activity level of the copepods used in our experiments.

Even though copepods do not feed on TEP, we provide evidence that copepods affect TEP size distribution, generating larger particles. As the increase in TEP size cannot be attributed to the effects of grazing, we suggest that copepods are either exuding new TEP of greater size or/and generating aggregation of already existing TEP. Exudation of polymers by zooplankton is known (Alldredge and Silver, 1988; Schuster and Herndl, 1995). These polymers would be stained equally with Alcian Blue and therefore, be indistinguishable from algal

exudates. With regard to the process of aggregation, Bundy *et al.* (Bundy *et al.*, 1998) presented evidence that free-swimming calanoid copepods attack non-motile inert particles (beads) located outside the influence of the feeding current, and all beads were rejected after capture. Young *et al.* observed by video analysis how copepods capture and reject particles (Young *et al.*, 1997). Copepods beat filtering limbs to force a current of water through the carapace gape and onto the feathery combs which trap particles. Those particles were then scraped into the food groove. This can be cleared of particles by a ranking movement of the post-abdominal claw, which rejects accumulated and inedible material, pushing it out again through the carapace gape. If the copepods show a similar feeding behaviour on TEP, then the size of TEP is probably likely to be affected because of their sticky nature. Moreover, swimming copepods introduce kinetic energy inside jars compared with controls, and exudates have been shown to increase swimming activity of copepods (Buskey, 1984). Schuster and Herndl studied the formation and dynamics of TEP in relation to turbulence and found that turbulence $(0.06 \, \text{cm}^2 \, \text{s}^{-3})$ was important for TEP formation (Schuster and Herndl, 1995). Yen *et al.* found that viscous energy dissipation within the feeding current of the copepod *Euchaeta rimana* was higher than that due to natural turbulence (Yen *et al.*, 1991). However, these authors found that the spatially averaged vertical eddy diffusivity caused by feeding currents of copepods was several orders of magnitude lower than that caused by turbulence, the possible exception being within a very dense swarm of copepods. Even though the copepod species of this study have shorter lengths (6.7–9.7 mm) than *E. rimana* (25 mm), they generate feeding currents $[0.4–1 \, \text{cm s}^{-1}$; see (Kiørboe *et al.*, 1999)] that are slightly higher than *E. rimana* $(0.3 \, \text{cm s}^{-1})$. Also, the fact that copepod feeding currents have an important component of elongational deformation (Visser and Jonsson, 2000) and due to the physical properties of polymers in aqueous solutions the expolymers may align in the direction of the flow (Christensen and Characklis, 1990) enhancing this type of deformation. Considering similar viscous energy dissipation per individual than those calculated by Yen *et al.* (Yen *et al.*, 1991) and the total volume inside the jars, the energy dissipation rates due to copepods inside the experimental jars were 2.3×10^{-5}, 3.8×10^{-5} and $5 \times 10^{-5} \, \text{cm}^2 \, \text{s}^{-3}$ for 25, 41 and 54 individuals, respectively. The levels of turbulence introduced by copepod movements were obviously much lower than those utilized by Schuster and Herndl (Schuster and Herndl, 1995) but of the same order as oceanic turbulence and therefore, turbulence could favour aggregation of TEP. The density of copepods in the present experiment was high compared with usual open sea concentrations, where, in that sense,

the effects of copepods in TEP size spectra would be lower. However, dense patches of copepods of 500–1500 ind.1^{-1} have been recorded on reefs in Australia and Palau (Hammer and Carleton, 1979), which are in the upper range of the densities used here. Evans and Granger (Evans and Granger, 1980) in Eskimo Bay, Canada, and Northcole and Hall (Northcole and Hall, 1990) in a saline lake, found copepod abundances in the same order of magnitude as our study. Therefore, in some cases, TEP size spectra would be affected by copepod activity at the same degree as in the present study.

Besides the increase in the proportion of larger particles, the presence of copepods produces further consequences on TEP dynamics. Our results showed an increase in total volume of TEP in jars containing copepods when comparing them with controls (Figure 1b). This would result from both processes, exudation and aggregation. The reason why exudation causes this effect is clear (new TEP, more total volume) but with aggregation, it is not so explicit. The process of aggregation might imply an increase in total volume of TEP inside the jars due to three facts. First, TEP aggregates are highly porous and have fractal geometry (Passow *et al.*, 1994; Li and Logan, 1995). For particles with a fractal dimension (D_3) less than 3, porosity is not constant due to the heterogeneous porous structure (Jackson *et al.*, 1995). These characteristics of TEP mean that the new particle formed by aggregation has a total volume greater than the sum of the volumes of the two particles from which it was formed (a greater proportion of water is incorporated into the new and larger particle).

Second, new exopolymeric particles can be formed from dissoved material and the assembly of precursors (dissolved polymers) to form particles increases with polymer length (Chin *et al.*, 1998) and shear (Passow, 2000); both particle size and shear have been shown above to be increased by copepod activity.

The third reason for an increase in total volume is of a methodological nature. It is related to the fact that we see particles in two dimensions, whereas ESV (Equivalent Spherical Volume) is an estimate for three dimensions. An example can help to clarify this methodological artefact. Thus, if we image a particle of 25 μm^2 (particle '*a*'), following the relation $\text{ESV} = (\pi/6)*(4A/\pi)^{3/2}$ (*A* being the area), that particle has an ESV of 94 μm^3. The sum of volumes of two identical particles such as the one just described is 188 μm^3. Now, one particle with an area of 50 μm^2 (particle '*b*') has an ESV of 266 μm^3. The relation between both total ESV of particles ('2*a*' and '*b*') increases by a factor of $\sqrt{n}$, *n* being the number of single particles that have aggregated to form the new particle.

Regardless of the origin of this effect on TEP volume, our preliminary study provides evidence that marine

planktonic copepods do not graze significantly on TEP in the absence of all other food sources. Although copepods do not desire energetic benefits from these exopolymers, they alter TEP size distribution and increase the total volume of TEP, an effect that might have consequences for particle fluxes in the ocean as it implies an increase in the downward flux of carbon, due both to the higher settling velocities of larger particles and the implications of TEP in the aggregation dynamics of marine particles.

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