Journal of Plankton Research Vol.21 no.11 pp.2203–2217, 1999

Do transparent exopolymer particles (TEP) inhibit grazing by the euphausiid *Euphausia pacifica*?

Uta Passow and Alice L.Alldredge[1]

Marine Science Institute and [1]Department of Ecology, Evolution and Marine Biology, University of California at Santa Barbara, Santa Barbara, CA 93106, USA

Abstract. The hypothesis that ubiquitous, sticky transparent exopolymer particles (TEP) formed from phytoplankton exudates will adhere to and coat the feeding structures of marine zooplankton grazers, and thus depress feeding on phytoplankton, was tested using the euphausiid, *Euphausia pacifica*, as a model organism. During two feeding experiments, *E.pacifica* were offered cells of the diatom *Thalassiosira weissflogii*, TEP, or both TEP and *T.weissflogii* cells. Ingestion rates on cells were lower in the presence of TEP. However, contrary to the hypothesis, grazing on cells was not inhibited by TEP. Rather, TEP-clusters, aggregates which formed from TEP and nano-sized particles normally too small for the filtering apparatus of *E.pacifica* to retain, served as an alternative food source for *E.pacifica*, reducing their ingestion of cells. These clusters were very similar in form to the TEP actually available to marine grazers in nature. TEP-clusters were similar to cells in size and food quality, and were grazed at similar rates. When feeding on TEP-clusters, euphausiids short circuit the food web by feeding on nano- and picoplankton directly, bypassing the microbial loop. Thus, the presence of TEP appears to enhance, rather than depress, macrozooplankton grazing.

Introduction

Zooplankton play a pivotal role in controlling phytoplankton standing stocks in the ocean either directly through grazing (Bathmann *et al*., 1990) or indirectly through nutrient remineralization which impacts phytoplankton growth (Banse, 1994, 1995). Conversely, phytoplankton may deter zooplankton grazing through a variety of mechanisms, including bioluminescence (Esias and Curl, 1972) and the production of noxious chemical substances (Gill and Harris, 1987; Uye and Takamatsu, 1990). One type of chemical substance exuded copiously by marine phytoplankton is extracellular exopolymer material (Decho, 1990; Myklestadt, 1995). However, information on the impact of polysaccharide exudates on zooplankton grazing is scarce and contradictory. Exopolymers can be ingested and utilized as food by protozoans (Sherr, 1988; Shimeta, 1993; Tranvik *et al*., 1993), larvaceans (Flood *et al*., 1992) and, if they form a tight capsule around beads, by copepods (Decho and Moriarty, 1990). However, the presence of dissolved polysaccharide exudates is also known to depress feeding by copepods on diatoms (Malej and Harris, 1993). Malej and Harris (1993) suggest that diatoms may exude exopolymers as an adaptation to deter grazing and that the mechanism of inhibition may be partially mechanical since dissolved polysaccharides increase the viscosity of the water (Jenkinson, 1989) and, thus, the energy expenditure of feeding. Their suggestion is consistent with observations stemming back to Hardy and Gunther (1936) that zooplankton can be scarce in regions of high phytoplankton density.

Recent evidence regarding the physical nature of algal exudates may help clarify these incongruities. Some algal exopolymers spontaneously form

© Oxford University Press 1999

particles, called transparent exopolymer particles (TEP; Alldredge *et al*., 1993), through aggregation (Passow *et al*., 1994) or gelation (Chin *et al*., 1998). TEP are sticky (Logan *et al*., 1995), gel-like particles up to several hundred micrometers in size that exist as discrete particles or become attached to other particles in the water column (Alldredge *et al*., 1993; Passow and Alldredge, 1994). They exist at concentrations of tens to 10^6 per milliliter, especially when diatoms are abundant. TEP may be retained by filters with a pore size larger than the particles themselves (Logan *et al*., 1994), suggesting that TEP may stick to the fine feeding structures of planktonic grazers and alter their effectiveness. This could reduce grazing, consistent with the results of Malej and Harris (1993). We have previously observed stained TEP clinging to the appendages of euphausiids and copepods fed on rich diatom cultures. However, alternatively, it is also possible that the suppression of grazing on cells that Malej and Harris (1993) observed was an artifact of methodology. Polysaccharide particles may have suppressed feeding on cells by providing an alternate, unmonitored food source. Thus, the role of TEP as a food source or a grazing deterrent for zooplankton remains unresolved.

In this study, we investigated the hypothesis that TEP directly inhibits feeding of the euphausiid, *Euphausia pacifica*, by adhering to and interfering with its feeding appendages. *Euphausia pacifica* is one of the most abundant species of macrozooplankton off the California coast (Brooks and Mullin, 1983). It is omnivorous and feeds on a variety of particle types and sizes, including phytoplankton, zooplankton (Mauchline, 1980; Ohman, 1984; Willason and Cox, 1987) and all types of marine snow (Dilling *et al*., 1998). Adult *E.pacifica* feed on particles between 12 and 50 μm via compression filtration, and use raptorial feeding techniques on particles >50 μm or on zooplankton (Jørgensen, 1966; Hamner, 1988). Thus, this animal served as an excellent model organism with which to test the impact of TEP on zooplankton grazing.

Method

Experimental protocol

Two nearly identical experiments were conducted during a cruise in the Santa Barbara Channel, California, in June 1996 to investigate whether the presence of large quantities of TEP suppressed feeding of *E.pacifica* on phytoplankton. Feeding was measured by calculating clearance and ingestion rates from counts of food particles (Omori and Ikeda, 1984), and additionally by determining fecal pellet production (Honjo and Roman, 1978; Paffenhöfer and Knowles, 1979).

During each experiment, feeding was measured in four different treatments: 1 (Tr. 1) consisted of filtered sea water, 2 (Tr. 2) contained TEP, 3 (Tr. 3) contained cells (with as little TEP as possible) and 4 (Tr. 4) contained both cells and abundant TEP. Four active adult *E.pacifica* were added to each of three replicate 4 l bottles per treatment. Additionally, two 2 l bottles per treatment were incubated without animals and functioned as no-animal controls. All bottles were incubated for 6 h in the dark in environmental chambers set to *in situ* temperature (12–14°C).

Table I. Average initial concentrations of TEP, cells, TEP-clusters and POC in each treatment type. The majority of TEP (measured spectrophotometrically as concentration) is the essential component of TEP-clusters, which are measured as number abundance. The measurements of POC include TEP which are part of the TEP-clusters or which are attached to cells. Free TEP, however, are lost by GF/F filters and POC measurements

Treatment	TEP concentration (μg Xeq. l^{-1})	Cell abundance (no. ml^{-1})	TEP-cluster abundance (no. ml^{-1})	POC concentration (μg C l^{-1})
Experiment 1				
1. Sea water	37 ± 1	0	0	BD
2. TEP	397 ± 8	0	750 ± 25	773
3. Cells	135 ± 3	1555 ± 470	0	370
4. TEP + cells	477 ± 12	1385 ± 110	470 ± 25	813[a]
Experiment 2				
1. Sea water	121 ± 15	0	0	BD
2. TEP	442 ± 7	0	610 ± 25	686
3. Cells	141	1330 ± 85	0	486
4. TEP + cells	449 ± 27	1695 ± 165	780 ± 85	1447[b]

μg Xeq. l^{-1}, μg Gum Xanthan equivalent per liter; BD, below detection.
[a]Cells contributed 41% to POC.
[b]Cells contributed 39% to POC.

Preparations for experiments

Feeding mixtures for the different treatments were prepared as follows. Sea water for Tr. 1 without food was collected from below the euphotic zone, pre-filtered through 0.45 μm filters the day before the experiment and stored at *in situ* temperature. It is extremely difficult to generate TEP-free sea water, and small amounts of TEP were present in the water used for these no-food treatments (Table I).

Tr. 2, which contained large amounts of TEP, was prepared by mixing 2 l of 0.2-μm-pre-filtered sea water with 2 l of TEP-rich suspension. TEP-rich suspension was generated the day before from a dense multispecies diatom bloom grown in tinted 20 l bottles. The diatom bloom was grown at *in situ* temperature in natural light by incubating raw sea water from the chlorophyll (Chl) *a* maximum and adding nutrients to simulate upwelling conditions. The bloom used to generate TEP-rich suspension for experiment 1 was senescent, whereas an exponentially growing bloom was used for preparing TEP-rich suspension for experiment 2. TEP-rich suspension was prepared by first removing cells and larger particles with a 10 μm screen. Residual particles left in the filtrate were assumed to be too small to serve as food for *E.pacifica*, because *E.pacifica* can only graze particles >12 μm effectively (Parsons *et al.*, 1967). The filtrate was then incubated for 24 h in 285 ml centrifuge bottles on a rolling table (Shanks and Edmondson, 1989) to enhance the spontaneous formation of TEP from dissolved polysaccharide precursors (Alldredge *et al.*, 1993). During incubation on the rolling table, microaggregates, here called TEP-clusters, formed. TEP-clusters were on average 15 × 7 μm in size, and consisted of small detrital particles, nanoplankton

and bacteria embedded in a matrix of TEP. TEP promote aggregation and these TEP-clusters formed on the rolling table from the newly formed TEP and the residual particles, which passed the screen during preparation of the TEP-rich suspension. Since, in nature, TEP commonly exist with bacteria and particle inclusions (Passow and Alldredge, 1994), these clusters mimicked the form of TEP actually available to grazers *in situ*.

Tr. 3, which contained cells but as little TEP as possible, was generated by adding 0.2-μm-pre-filtered sea water to 40–70 ml of a batch culture of *Thalassiosira weissflogii* to yield an end concentration of ~1500 cells ml^{-1}. *Thalassiosira weissflogii* cells were 15–20 μm in size and are an adequate food for *E.pacifica* (Dilling *et al*., 1998). The culture used for experiment 1 was in late exponential phase and that for experiment 2 was in early exponential phase. Keeping cultures in exponential phase ensures that diatoms remain in suspension. The batch cultures of *T.weissflogii* were grown in F/2 media plus silica using standard culture techniques (Guillard, 1975). For the second experiment, the cultures were kept in early exponential phase for 3 days by daily dilution with fresh media.

Tr. 4 contained TEP + cells and was prepared by mixing 2 l of the TEP-rich suspension used for Tr. 2 with 0.2-μm-pre-filtered sea water and the same concentration of cells as in Tr. 3. This way initial concentrations of cells in Trs 3 and 4 and initial concentrations of TEP and TEP-clusters in Trs 2 and 4 were similar (Table I). Initial concentrations of TEP in the sea water (Tr. 1) and in the treatments with cells (Tr. 3) were kept as low as possible (Table I).

Large, adult *E.pacifica* were collected by vertical tows from 100 m depth using a 1-m-diameter, 333-μm-mesh, net after dusk and maintained at *in situ* temperature in the dark. Animals were collected 2–6 days before the experiments, fed with *T.weissflogii* if necessary, and starved for 36 h prior to experiments.

Experimental procedure

At the beginning of each experiment, all bottles were prepared by mixing the different components for the respective treatments as described above. Then subsamples to measure initial concentrations of TEP and particles were collected from each bottle and finally large, healthy looking adult *E.pacifica* were carefully added (except to no-animal controls). Two extra bottles were prepared, one identical to Tr. 2 which contained TEP and TEP-clusters, and one which contained only cells (Tr. 3). These were used to determine the initial concentrations of particulate organic carbon and nitrogen (POC, PON) of cells and TEP-clusters, respectively.

After the 6 h incubation period, the animals were checked for activity, the presence of eggs and molts noted, and animals removed from bottles. The length of each animal was measured and the animals from each bottle were transferred to a pre-weighed 0.4 μm Nucleopore filter to determine their average weight. Then, 200–250 ml subsamples were taken from the suspension to measure the final concentration of TEP and particles. Finally, fecal pellets were collected to quantify fecal pellet production by carefully screening the rest of the solution through a 35 μm mesh.

Analysis of samples

Subsamples (10–20 ml) for particle counts were preserved in formalin and changes in particle concentration determined later in the laboratory by counting initial and final concentrations in a Sedgewick-Rafter chamber at ×100 magnification with a compound microscope. Two types of particles were counted: *T.weissflogii* cells and TEP-clusters. TEP-clusters consisted of small particles (<5 μm) enclosed in a TEP matrix. The abundance of small particles made TEP-clusters visible without staining. *Thalassiosira weissflogii* cells were rarely part of TEP-clusters (possible in Tr. 4), presumably because TEP-clusters formed in the TEP-rich suspension before *T.weissflogii* was added. At least 250 cells and 120 TEP-clusters were counted per sample.

The initial POC and PON content of cells and TEP-clusters was determined from the suspensions identical with treatments containing TEP or cells, respectively (Tr. 2 and Tr. 3). Between 300 and 500 ml of three replicate subsamples of these respective suspensions were filtered onto muffled 25 mm GF/F filters. The filters were dried and stored in a desiccator before analysis for POC and PON with a Leeman Labs Inc. CE CHN Analyzer (Model 440) according to Sharp (1992).

The concentration of TEP was determined immediately on board using the colorimetric method of Passow and Alldredge (1995). Briefly, three replicate 60–70 ml samples were filtered onto 0.4 μm polycarbonate filters, then TEP was stained with an Alcian Blue solution and the dye redissolved in sulfuric acid. Gum Xanthan was used to calibrate the dye (calibration factor $f = 166$) and results are expressed as micrograms of Gum Xanthan staining equivalent per liter (μg Xeq. l^{-1}). Fecal pellets were sized and counted under a dissecting microscope (Leitz) on board ship. Filters containing animals were dried in a 65°C oven, stored in a desiccator and reweighed on a Cahn electrobalance after returning to the laboratory.

Calculations of ingestion rate and clearance rate

Ingestion and clearance rates of TEP were calculated from results of spectrophotometric measurements and concentrations of *T.weissflogii* cells and TEP-clusters from microscopy counts. All ingestion rates were calculated separately for each sample according to Omori and Ikeda (1984) and then averages of replicate bottles were calculated.

First, the growth coefficient k (h^{-1}) was calculated for each treatment from the respective no-animal controls assuming exponential growth:

$$k = (\ln C_t - \ln C_0)\, t^{-1} \tag{1}$$

where C_0 and C_t are concentrations in no-animal controls at the beginning and end of the experiment, respectively, and t is the incubation time. The feeding coefficient, f (h^{-1}), was then calculated assuming exponential decrease of particles:

$$f = k + ((\ln C_{a0} - \ln C_{at})\, t^{-1}) \tag{2}$$

with C_{a0} and C_{at} representing concentrations of particles in treatments with animals at the beginning and end of the experiment, respectively. The clearance rate, F (ml animal^{-1} h^{-1}), represents the amount of water swept clear of a particular food item and is calculated as:

$$F = V\,N^{-1}\,f \tag{3}$$

where V is the volume of experimental vessels and N the number of animals per vessel. The ingestion rate, I (no. of cells animal^{-1} h^{-1}), was calculated from the clearance rate by:

$$I = F\,(wt)^{-1}\,(C_{at} - C_{a0})\,((k - f)\,t)^{-1} \tag{4}$$

where wt (mg) equals the average dry weight of euphausiids. Using measurements of POC content of cells and TEP-clusters, the ingestion rate can also be calculated as the amount of carbon ingested.

Selective feeding

Selective feeding (differential ingestion or avoidance of certain types of foods) on cells in Tr. 4, where both cells and TEP-clusters were offered simultaneously, was calculated by three different indices. Clearance rates were used to compute electivity (W) according to Vanderploeg and Scavia (1979). The electivity index E was calculated from proportions of cells in the diet compared to their availability (Omori and Ikeda, 1984). Third, the measure of preference, α, was derived from a stochastic model involving probabilities of encounter of cells and ingestion upon encounter (Chesson, 1978).

Results

Euphausia pacifica

The average weight of animals was 4.2 ± 1.2 mg and their average size was 1.6 ± 0.1 cm. All animals, except two, appeared healthy and active at the end of experiments. However, with only four animals per container, variations in stages of molting or reproduction cycle have large effects on measured feeding rates, as feeding is often depressed when animals are molting or generating eggs (Lasker, 1966). During our experiments, several animals produced eggs and one animal molted, which caused variability in ingestion rates between replicate bottles. During experiment 1, at least one and sometimes two animals per treatment produced eggs, but as measurable feeding was observed in all bottles of experiment 1, all replicates were incorporated for calculations of average ingestion rates. Two dead or egg-carrying animals were found in three bottles of experiment 2, whereas all four animals were alive and without eggs in all other vessels. As no grazing was measurable in the three bottles containing handicapped animals, the data of these bottles (one replicate of Tr. 1 and two replicates of Tr. 3) were omitted from averages.

Feeding in filtered sea water

No cells or clusters were present in filtered seawater treatments and initial concentrations of TEP were very low (Table I). The production of fecal material was negligible (Figure 1D and H), indicating that the small amount of TEP present was of no significance as a food source and that the starvation time of animals before each experiment was adequate. However, TEP was removed from sea water in the presence of animals and may be used as a tracer to estimate clearance rates in the absence of food. Clearance rates in seawater treatments were appreciably higher compared to clearance rates in other treatments (Table II).

Feeding on cells and TEP-clusters

Initial food concentrations (Table I) were designed to lie above the critical food concentration of 300 μg C l^{-1} observed for large adult *E.pacifica* grazing on diatoms (Ross, 1982; Ohman, 1984) in order to reduce any ambiguity that might arise from effects of food concentration in determining whether *E.pacifica* consumed TEP. Even so, despite the high food concentrations relative to those found in nature (POC ranging from 370 to 1446 μg C l^{-1}), maximal rates of ingestion were still not achieved. Ingestion rates were a function of initial food concentrations even at these high concentrations (Figure 2), suggesting that the critical food concentration of *E.pacifica* can be higher than originally estimated.

Ingestion rates of cells calculated from Trs 3 and 4 ranged from 3 to 35 $\times 10^3$ cells · mg euphausiid^{-1} h^{-1} (Figure 1A and E) and varied both as a function of initial cell concentration and TEP concentration (Figure 2). Ingestion rates of cells were lower in treatments where TEP had been added (Tr. 4) compared to treatments without TEP (Tr. 3), but the reduction in average clearance and ingestion rates of cells in the presence of TEP (Figure 1, Tables II and III) was not statistically significant (Mann–Whitney *U*-test, $P = 0.1$) because of the large variability between replicates (small data set). However, if the individual ingestion

Table II. Average clearance rates, F (ml euphausiid^{-1} h^{-1}), calculated from colorimetric determination of TEP, and the enumeration of cells and TEP-clusters

Treatment	Clearance rate of TEP	Clearance rate of cells	Clearance rate of TEP-clusters
Experiment 1			
1. Sea water	106 ± 17	0	0
2. TEP	12 ± 2	0	54 ± 21
3. Cells	13 ± 7	75 ± 62	0
4. TEP + cells	–11 ± 12[a]	23 ± 14	52 ± 33
Experiment 2			
1. Sea water	150 ± 14	0	0
2. TEP	40 ± 12	0	30 ± 14
3. Cells	26	54	0
4. TEP + cells	–131 ± 21[a]	35 ± 21	74 ± 62

[a]Negative clearance rates indicate the production of TEP.

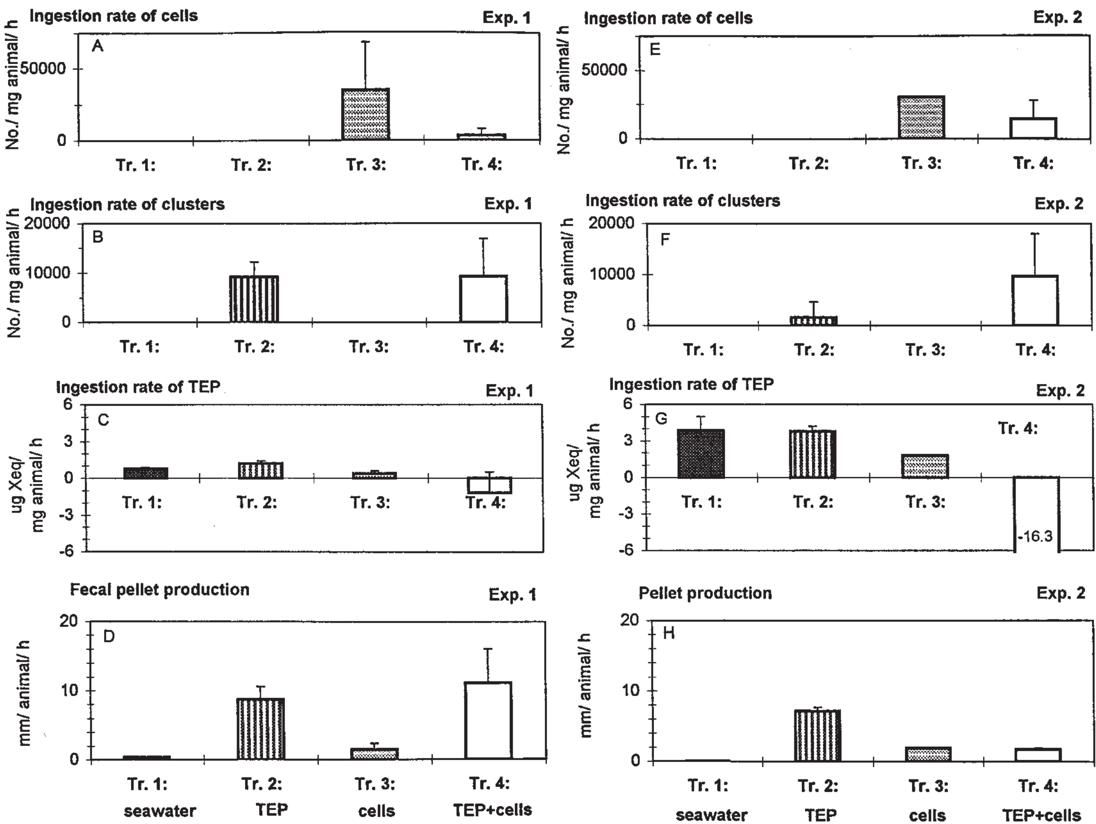

Fig. 1. Average ingestion rates of cells, TEP-clusters and TEP and fecal pellet production during experiments 1 and 2. Ingestion rates of cells (**A**, **E**) and TEP-clusters (**B**, **F**) for experiments 1 and 2, respectively, were calculated from changes in number abundance. Ingestion rates of TEP (**C**, **G**) were calculated from colorimetric determinations. Fecal pellet production (**D**, **H**) for both experiments was measured as the total length of generated fecal material.

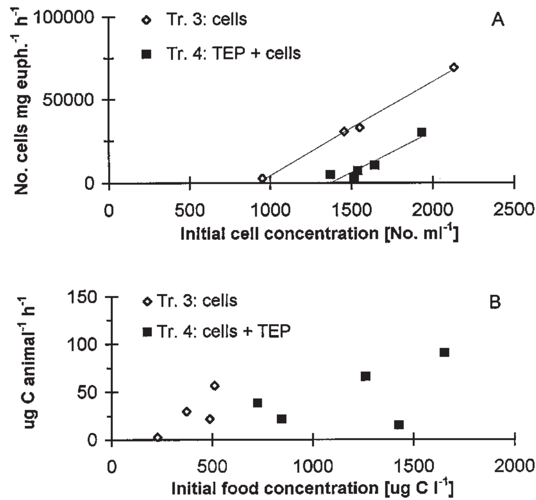

Fig. 2. Ingestion rates as a function of food concentration. (**A**) Ingestion rates of cells in Treatments 3 and 4 of both experiments as a function of initial concentrations of cells. A significant relationship was found for each treatment (Tr. 3: $y = 56x - 52011$, $r^2 = 0.99$, $P < 0.01$; Tr. 4: $y = 48x - 65918$, $r^2 = 0.89$, $P < 0.05$). Ingestion rates of cells were consistently lower in the presence of TEP (Tr. 4). If total ingestion rates, which include feeding on TEP, had not been monitored, these results would have suggested a feeding inhibition due to TEP. (**B**) Total ingestion rates of cells + TEP-clusters in Treatments 3 and 4 of both experiments as a function of initial concentrations of total carbon. Ingestion rates of cells + TEP-clusters varied appreciably, but the presence of TEP did not affect total ingestion rates negatively.

rates of replicates are depicted as a function of initial cell concentration (Figure 2), it becomes clear that ingestion of cells was indeed reduced in the presence of TEP. The elevation of the regression line of ingestion rate versus cell concentration was significantly lower in the presence of TEP (analysis of covariance, $P < 0.001$; Snedecor and Cochran, 1989) (Figure 2A), indicating that for any given initial cell concentration, fewer cells were consumed when TEP were present.

Euphausiids also grazed on TEP-clusters which had formed in treatments where TEP had been added (Tr. 2 and 4), clearly demonstrating that these animals did ingest TEP. The average ingestion rates of TEP-clusters varied between 3000 and 17 000 clusters · mg euphausiid^{-1} h^{-1} (Figure 1B and F). Ingestion rates of TEP-clusters expressed as carbon were not significantly different from ingestion rates on cells (Mann–Whitney *U*-test) (Table III).

Total ingestion rates of POC (cells + TEP-clusters) increased with initial food concentration below 600 μg carbon l^{-1} (Figure 2B). At higher food concentrations, total ingestion rates varied around a maximum ingestion of ~60 μg carbon euphausiid^{-1} h^{-1}. Ingestion rates of 60 μg carbon euphausiid^{-1} h^{-1} lie well above the maximal ingestion rates calculated from weight (20 μg carbon euphausiid^{-1} h^{-1}) according to Ross (1982). However, ingestion rates measured by Ross (1982) were averaged over a 24 h period, whereas animals in our experiments fed for only 6 h.

Table III. Average ingestion rates, *I*, as μg carbon · mg euphausiid^{-1} h^{-1}. Ingestion of TEP was predominantly included in ingestion rate measurements of TEP-clusters, as most TEP were contained in TEP-clusters

Treatment	Ingestion rate of cells	Ingestion rate of TEP-clusters	Total ingestion rate
Experiment 1			
1. Sea water	0	0	0
2. TEP	0	9.7 ± 2.6	9.7 ± 2.6
3. Cells	8.4 ± 6.5	0	8.4 ± 6.5
4. TEP + cells	0.9 ± 0.7	9.8 ± 6.5	10.7 ± 6.5
Experiment 2			
1. Sea water	0	0	0
2. TEP	0	2.5 ± 1.8	2.5 ± 1.8
3. Cells	10.2	0	10.2
4. TEP + cells	4.8 ± 3.7	10.9 ± 7.7	15.7 ± 8.5

A higher maximal ingestion rate for hungry animals right after they are fed would be expected. Total ingestion rates of POC in treatments with cells + TEP were slightly higher than those in treatments with cells only (Table III) because initial food concentrations were higher in treatments with cells + TEP (Figure 2B). The two very low total ingestion rates at high initial food concentrations reflected low ingestion rates of cells; the ingestion rates of TEP-clusters were high in those treatments (Table III). Total ingestion rates were a function of initial food concentration and the data clearly indicate that the presence of TEP did not depress total ingestion rates.

To evaluate whether animals grazed preferentially on cells or on TEP-clusters when given the choice (Tr. 4), three selectivity indices were calculated. Results of all three indices matched, and indicated that TEP-clusters, which consisted predominately of TEP, were definitely not avoided. Euphausiids fed preferentially on TEP-clusters (selecting against cells) in 50% of bottles, on cells in 17% of the bottles and showed no preference in 33% of the bottles. The C:N ratios of cells and TEP-clusters were similar. Cells and TEP-clusters had a C:N ratio of 6.3 and 6.5, respectively, during experiment 1, and 5.7 and 5.5, respectively, in experiment 2.

Changes in TEP concentration

TEP concentrations were decreased by grazing in Trs 1–3 and did not change in no-animal controls. Surprisingly, however, concentrations of TEP increased in Tr. 4 in both experiments. Negative ingestion rates of TEP in treatments with TEP + cells (Figure 1C and G) indicate the production of TEP due to interactions of the animals with the cells and TEP-clusters contained in this treatment. The animals alone were not generating TEP since TEP were not generated in seawater controls.

Fecal pellet production

Fecal pellet production, expressed as total length of feces produced (feces had a radius of ~50 μm), varied between 0.1 and 11 mm h^{-1} euphausiid^{-1} (Figure 1D

Table IV. Three different selectivity indices for selection of cells over TEP-clusters by *E.pacifica* in the six replicates of Treatment 4. All three indices gave consistent results. No selectivity for cells indicated when $W = 0.4–0.6$, $E = 0$, $\alpha = 0.4–0.6$; selection for cells indicated when $W > 0.6$, $E > 0$, $\alpha > 0.6$ (*); selection against cells (e.g. selection for TEP-clusters) indicated when $W < 0.4$, $E < 0$, $\alpha < 0.4$ (**)

Experiment	Replicate	*W*	*E*	α
1	1	0.6	0.0	0.5
1**	2	0.2	–0.7	0.0
1**	3	<0	–1.4	0.0
2**	1	0.1	–0.7	0.1
2	2	0.5	0.0	0.5
2*	3	1.0	0.4	1.0

and H). This feces production is within the range of fecal pellet production measured during feeding experiments on marine snow, although ingestion rates of marine snow were slightly lower (Dilling *et al*., 1998). Production of fecal matter was relatively high in treatments with TEP only (Tr. 2) and comparatively lower when animals were feeding on cells only, although ingestion rates were similar in both treatments. Fecal pellets generated in treatments with TEP only (Tr. 2) appeared looser and less dense than those generated in treatments with cells only (Tr. 3). The observed differences in appearance and production of fecal pellets suggest that the volume of feces may vary with food source. The gel-like properties of TEP may generate very fluffy fecal pellets with a large volume compared to dense pellets containing broken diatom frustules. Thus, fecal pellet production, especially when measured as volume rather than as carbon or weight, is clearly not a good indicator of ingestion rates when different types of food are compared.

Comparison between experiments

Differences in the C:N ratios of food in experiments 1 and 2 reflected differences in the age of the cells and the blooms used to prepare TEP-rich suspensions. This difference had no impact on ingestion rates of cells and TEP-clusters or on fecal pellet production, but ingestion and clearance rates of TEP in Trs 1–3 and production of TEP in Tr. 4 were higher during the second experiment (Figure 1).

Discussion

Results from both experiments clearly allow us to reject our initial hypothesis. The presence of TEP does not inhibit the grazing of *E.pacifica*. Although ingestion rates on cells were reduced in the presence of TEP, total ingestion rates were not. Rather than inhibit grazing, TEP provided an alternate food source by aggregating nanoplankton-sized particles into TEP-clusters, which were eaten by euphausiids at similar rates as cells. The TEP-clusters that formed in this study were very similar to TEP-particle aggregations available to grazers in nature. Natural TEP commonly enclose bacteria and nano-sized particles. TEP-clusters were similar in size and nutritional quality (assessed as C:N ratios) to cells, and

were grazed at comparable rates, implying that they provided an adequate food source for *E.pacifica.* Exopolymers have been shown to be a highly labile carbon source for harpacticoid copepods and, since extracellular polysaccharides easily bind compounds such as amino acids or fatty acids, they may also be a source of nitrogen and other nutrients (Decho and Moriarty, 1990; Schuster *et al.*, 1998). However, the carbon content of pure TEP is not known, and is likely to be low since gels are predominantly water. The absence of fecal matter in our seawater treatments, despite high clearance rates resulting in some TEP ingestion, suggests that TEP themselves did not contribute significantly to the food of the euphausiids. Nano-sized particles, embedded in the exopolymer matrix of TEP-clusters, are likely to be the major source of carbon and nitrogen in these particles. Future investigations will have to demonstrate the relative contribution of this food for euphausiids *in situ*.

Food concentrations offered in these experiments were deliberately higher than *E.pacifica* would commonly encounter in nature. Pelagic crustaceans demonstrate considerable flexibility in the types of food chosen when food concentrations are limited (Landry, 1981). We wished to be certain that neither cells nor TEP were scarce in order to determine unambiguously whether *E.pacifica* consumed TEP. The consumption of TEP by *E.pacifica* even in the presence of abundant cells suggests that in nature *E.pacifica* may commonly consume TEP-clusters. TEP are usually most abundant when phytoplankton are also abundant (Passow and Alldredge, 1994), but TEP can also be abundant in oligotrophic waters and at midwater depths (Kumar *et al.*, 1998). In environments where phytoplankton are scarce, TEP and the detrital particles embedded in them may serve as an alternate food source sustaining zooplankton until the next phytoplankton bloom.

Individual TEP are generally smaller than traditional food particles of *E.pacifica* and, based on their small size, grazing of free TEP (not associated with other particles) by *E.pacifica* should be negligible. However, high clearance rates of TEP in seawater treatments suggest that *E.pacifica* can collect free TEP. The removal of free TEP by filtering euphausiids may be possible, as sticky particles can be retained by filters and screens with a pore size much larger than the particles themselves (Logan *et al.*, 1994). As a simple test, we carefully filtered *T.weissflogii* through a 10 µm screen and traced TEP in the different fractions. Only 20% of the total amount of TEP was recovered in the filtrate and 37% was associated with cells (fraction >10 µm), suggesting that the remaining 43% of the TEP stuck to the screen. We suggest that, in sea water, TEP may similarly be collected by euphausiid feeding appendages. The high clearance rates of trace amounts of TEP from the seawater treatments indicates that animals did not stop filtering in the absence of food, as is commonly assumed. The alternative explanation, that TEP may have disappeared from seawater treatments because they were utilized by bacteria attached to the animals (Carman, 1990), is unlikely given the short incubation times and the relative resistance of TEP to microbial degradation (Passow *et al.*, 1994).

The grazing on TEP-clusters enabled euphausiids to ingest bacteria and nanoplankton, particle sizes not normally available to them. Grazing of these tiny

particles via TEP-clusters by euphausiids represents a short cut within the food web. These results imply that macrozooplankton may feed directly on nanoplankton when they consume TEP-clusters containing these small cells. Such a shortcut in the food web would result in a higher efficiency of carbon transfer. The main importance of TEP would thus not necessarily be as a food source *per se*, but in making other, previously inaccessible, particles available as food.

The observed negative ingestion rates of TEP in treatments with TEP + cells (Tr. 4) suggest that new TEP were generated by animal/food interactions during the experiments. TEP were formed especially in treatments containing cells. Polysaccharides within cells, including TEP or their precursors, may be released into the water by planktonic grazers due to sloppy feeding and cell breakage, increasing the amount of free polysaccharides (Strom *et al*., 1997), which in turn form TEP abiotically (U.Passow, unpublished). The production of TEP by suspension feeders through sloppy feeding facilitates the aggregation of tiny particles into TEP-clusters, mitigating the loss of carbon potentially resulting from the sloppy feeding. Since food concentration *in situ* is thought often to lie below the critical concentration for maximal feeding of euphausiids, we suggest that the aggregation of small particles, which are individually inaccessible to euphausiids, by TEP supplements *in situ* food supply significantly.

Acknowledgements

We thank Jesse Silguero, Stephanie Oakes and Darlene Randalls for help with the data collection, and Karen Habermann and Avan Antia for helpful comments on the manuscript. U.P. was funded in part by NSF grant OCE94-00698 and partly by the Deutsche Forschungsgemeinschaft.

References

Alldredge,A.L., Passow,U. and Logan,B.E. (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res. I*, **40**, 1131–1140.

Banse,K. (1994) Grazing and zooplankton production as key controls of phytoplankton production in the open ocean. *Oceanography*, **7**, 13–20.

Banse,K. (1995) Zooplankton: Pivotal role in the control of ocean production. *ICES J. Mar. Sci.*, ***52***, 265–277.

Bathmann,U.V., Noji,T.T. and von Bodungen,B. (1990) Copepod grazing potential in late winter in the Norwegian Sea—a factor in the control of spring phytoplankton growth. *Mar. Ecol. Prog. Ser*., **60**, 225–233.

Brooks,E.R. and Mullin,M.M. (1983) Diel changes in the vertical distribution of biomass and species in the Southern California Bight. *Calif. Coop. Fish. Invest. Rep.*, **24**, 210–215.

Carman,K.R. (1990) Mechanisms of uptake of radioactive labels by meiobenthic copepods during grazing experiments. *Mar. Ecol. Prog. Ser*., **68**, 71–83.

Chesson,J. (1978) Measuring preference in selective predation. *Ecology*, **59**, 211–215.

Chin,W.-C., Orellana,M.W. and Verdugo,P. (1998) Spontaneous assembly of marine dissolved organic matter into polymer gels. *Nature*, **391**, 568–571.

Decho,A.W. (1990) Microbial exopolymer secretion in ocean environments: Their role in food webs and marine processes. *Oceanogr. Mar. Biol. Ann. Rev*., **28**, 73–153.

Decho,A.W. and Moriarty,D.J.W. (1990) Bacterial exopolymer utilization by a harpacticoid copepod: A methodology and results. *Limnol. Oceanogr*., **35**, 1039–1049.

Dilling,L., Wilson,J., Steinberg,D. and Alldredge,A. (1998) Feeding by the Euphausiid, *Euphausia pacifica* and the Copepod *Calanus pacificus* on marine snow. *Mar. Ecol. Prog. Ser*., **170**, 189–201.

Esias,W.E. and Curl,H.C. (1972) Effect of dinoflagellate bioluminescence on copepod ingestion rates. *Limnol. Oceanogr.*, **17**, 901–906.
Flood,R., Deibel,D. and Morris,C.C. (1992) Filtration of colloid melanin from seawater by planktonic tunicates. *Nature*, **355**, 630–632.
Gill,C.W. and Harris,R.P. (1987) Behavioral responses of the copepods *Calanus helgolandicus* and *Temora longicornis* to dinoflagellate diets. *J. Mar. Biol. Assoc. UK*, **67**, 785–801.
Guillard,R.R.L. (1975) Culture of phytoplankton for feeding marine invertebrates. In Smith,W.L. and Chanley,M.H. (eds), *Culture of Marine Invertebrate Animals*. Plenum Press, New York, pp. 29–60.
Hamner,W.M. (1988) Biomechanics of filter feeding in the Antarctic krill *Euphausia superba*: Review of past work and new observations. *J. Crust. Biol.*, **8**, 149–163.
Hardy,A. and Gunther,E.R. (1936) The plankton of the South Georgia whaling grounds and adjacent waters, 1926–1927. *Discovery Rep.*, **11**, 1–456.
Honjo,S. and Roman,M.R. (1978) Marine copepod fecal pellets: production, preservation and sedimentation. *J. Mar. Res.*, **36**, 45–57.
Jenkinson,I.R. (1989) Increase in viscosity may kill fish in some blooms. In Okaichi,T., Anderson,D.M. and Nemoto,T. (eds), *Red Tides: Biology, Environmental Sciences and Toxicology.* Elsevier, New York, pp. 435–438.
Jørgensen,C.B. (1966) *Biology of Suspension Feeding*. Pergamon, Oxford.
Kumar,M.D., Sarma,V.V.S.S., Ramaiah,N., Gauns,M. and de Sourda,S.N. (1998) Biogeochemcial significance of transparent exopolymer particles in the Indian Ocean. *Geophys. Res. Lett.*, **25**, 81–84.
Landry,M.R. (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. *Mar. Biol.*, **65**, 77–82.
Lasker,R. (1966) Feeding, growth, respiration and carbon utilization of a euphausiid crustacean. *J. Fish. Res. Board Can.*, **23**, 1291–1371.
Logan,B.E., Passow,U. and Alldredge,A.L. (1994) Variable retention of diatoms on screens during size separations. *Limnol. Oceanogr.*, **39**, 390–395.
Logan,B.E., Passow,U., Alldredge,A.L., Grossart,H-P. and Simon,M. (1995) Rapid formation and sedimentation of large aggregates is predictable from coagulation rates (half lives) of transparent exopolymer particles (TEP). *Deep-Sea Res.*, **42**, 203–214.
Malej,A. and Harris,R.P. (1993) Inhibition of copepod grazing by diatom exudates: a factor in the development of mucus aggregates*?* *Mar. Ecol. Prog. Ser.*, **96**, 33–42.
Mauchline,J. (1980) The biology of mysids and euphausiids. *Adv. Mar. Biol.*, **18**, 373–677.
Myklestadt,S. (1995) Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Sci. Total Environ.*, **165**, 155–164.
Ohman,M.D. (1984) Omnivory by *Euphausia pacifica*: the role of copepod prey. *Mar. Ecol. Prog. Ser.*, **19**, 125–131.
Omori,M. and Ikeda,T. (1984) *Methods in Marine Zooplankton Ecology*. Wiley and Sons, New York.
Paffenhöfer,G.A. and Knowles,S.C. (1979) Ecological implications of fecal pellet size, production and consumption by copepods. *J. Mar. Res.*, **37**, 35–49.
Parsons,T.R., LeBrasseur,R.J. and Fulton,J.D. (1967) Some observations on the dependence of zooplankton grazing on cell size and concentration of phytoplankton blooms. *J. Oceanogr. Soc. Jpn*, **23**, 10–17.
Passow,U. and Alldredge,A.L. (1994) Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. *Mar. Ecol. Prog. Ser.*, **113**, 185–198.
Passow,U. and Alldredge,A.L. (1995) A dye binding assay for the colorimetric measurement of transparent exopolymer particles (TEP). *Limnol. Oceanogr.*, **40**, 1326–1335.
Passow,U., Alldredge,A.L. and Logan,B.E. (1994) The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep-Sea Res.*, **41**, 335–357.
Ross,R.M. (1982) Energetics of *Euphausia pacifica* I. Effects of body carbon and nitrogen and temperature on measured and predicted production. *Mar. Biol.*, **68**, 1–13.
Schuster,S., Arrieta,J.M. and Herndl,G.J. (1998) Adsorption of dissolved free amino acids on colloidal DOM enhances colloidal DOM utilization but reduces amino acid uptake by orders of magnitude in marine bacterioplankton. *Mar. Ecol. Prog. Ser.*, **166**, 99–108.
Shanks,A.L. and Edmondson,E.W. (1989) Laboratory-made artificial marine snow: a biological model of the real thing. *Mar. Biol.*, **101**, 463–470.
Sharp,J.H. (1992) Total mass and particulate carbon, nitrogen and phosphorus. In Hurd,D.C. and Spencer,D.W. (eds), *Marine Particles: Analysis and Characterization. Geophysical Monograph 63.* American Geophysical Union, Washington, DC, pp. 87–91.
Sherr,E.B. (1988) Direct use of high molecular weight polysaccharide by heterotrophic flagellates. *Nature*, **335**, 348–351.

Shimeta,J. (1993) Diffusional encounter of submicrometer particles and small cells by suspension feeders. *Limnol. Oceanogr.*, **38**, 456–465.
Snedecor,G.W. and Cochran,W.G. (1989) *Statistical Methods.* Iowa State University Press, Ames, IA.
Strom,L.S., Benner,R., Ziegler,S. and Dagg,M.J. (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnol. Oceanogr.*, **42**, 1364–1374.
Tranvik,L.J., Sherr,E.B. and Sherr,B.F. (1993) Uptake and utilization of 'colloidal DOM' by heterotrophic flagellates in seawater. *Mar. Ecol. Prog. Ser.*, **92**, 301–309.
Uye,S. and Takamatsu,K. (1990) Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters. *Mar. Ecol. Prog. Ser.*, **59**, 97–107.
Vanderploeg,H.A. and Scavia,D. (1979) Two electivity indices for feeding with special references to zooplankton grazing. *J. Fish. Res. Board Can.*, **36**, 362–365.
Willason,S.W. and Cox,J.L. (1987) Diel feeding, laminarinase activity and phytoplankton consumption by euphausiids. *Biol. Oceanogr.*, **4**, 1–27.

Received on March 29, 1999; accepted on July 1, 1999