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Transparent exopolymer particles (TEP) from marine suspension feeders enhance particle aggregation

Binglin Li[1,2,*], J. Evan Ward[1], Bridget A. Holohan[1]

[1]Department of Marine Sciences, University of Connecticut, 1080 Shennecosset Road, Groton, Connecticut 06340, USA

[2]*Present address:* Department of Oceanography, University of Hawaii, 1000 Pope Rd, Honolulu, Hawaii 96822, USA

ABSTRACT: Transparent exopolymer particles (TEP) are known to form from dissolved precursors released by phytoplankton and bacteria. Many benthic suspension feeders also have been shown to produce significant amounts of TEP under field and laboratory conditions. Although TEP from phytoplankton have been implicated in enhancing aggregation and sedimentation of suspended material, the role of TEP from suspension feeders in aggregate formation is still unknown. In order to determine if TEP produced by suspension feeders enhance the formation of marine aggregates, organic matter from actively feeding blue mussels *Mytilus edulis* and sea vases *Ciona intestinalis* held in recirculating chambers was collected. Control water was collected from chambers without animals. Animal-conditioned and control water were then mixed with 1 of 2 non-sticky particles (silica-amino beads or diatomaceous earth). The suspensions were transferred to 250 ml bottles, which were placed on a roller table to generate aggregates. Particle aggregation was quantified by examining the size distribution of particles over time by means of an electronic particle counter (particles between 6 and 90 µm), and by counting large (greater than ca. 100 µm) aggregates under a microscope at the end of the experiment. We found that significantly more aggregates formed in the animal-conditioned water than in control water after 48 h, for both species and both kinds of added particles. The number of large aggregates that formed was related to TEP concentration, and not related to total or dissolved organic carbon. Our results suggest that suspension feeders produce significant quantities of TEP that can enhance particle aggregation.

KEY WORDS: Transparent exopolymer particles · TEP · Suspension feeders · Aggregation · Aggregates · Organic matter · Bivalves · Tunicates

INTRODUCTION

Many marine organisms, including phytoplankton and bacteria, generate large amounts of extracelluar polymeric substances (EPS). These substances are mainly composed of polysaccharides (Hoagland et al. 1993), generally heteropolymers formed from a wide variety of simple sugars (Decho 1990). EPS can be found in 3 pools in the aquatic environment: cell coatings, colloidal EPS, and transparent exopolymer particles (TEP) (Thornton 2002). TEP can be described as discrete, gel-like, transparent particles containing acidic polysaccharides that are stainable with Alcian blue (pH 2.5; Alldredge et al. 1993, Passow & Alldredge 1995a).

Over the past 20 yr, research on exopolymer particles has changed our understanding of the mechanisms promoting aggregation, the process by which large particles are produced from small particles. Aggregation is an important process of particle dynamics (McCave 1984) that can significantly affect the vertical transport of carbon in the ocean. Aggregation rate is dependent upon the size distribution and abundance of colliding particles, the intensity of mechanisms that cause collisions between particles (e.g. shear), and the probability that particles will stick

*Email: lbinglin@gmail.com

together once they have collided. This probability is quantified as the stickiness coefficient, α (O'Melia & Tiller 1993). The stickiness coefficient of TEP is usually much higher than that of most other particles; thus, the presence of TEP probably increases the aggregation rate by increasing overall stickiness of suspended particles (Dam & Drapeau 1995, Jackson 1995). Results of laboratory and field studies strongly suggest that blooms of many phytoplankton species are mediated by TEP, and TEP forms the matrix of most aquatic aggregates (Alldredge et al. 1993, Passow et al. 1994, 2001, Logan et al. 1995, Passow & Alldredge 1995b, Grossart et al. 1997, Engel 2004).

Although phytoplankton appear to be the main producers of TEP and TEP precursors in the ocean (e.g. Alldredge et al. 1998, Passow et al. 2001, Passow 2002a,b), other organisms that produce mucoid material also can contribute to the concentration of TEP in the water column. For example, some macrozooplankton and nekton contribute mucus material to the pelagos in the form of protective sheaths, mucus 'houses', fecal pellets, and feeding webs (Alldredge & Silver 1988). Many benthic suspension feeders have been shown to produce significant amounts of TEP under field and laboratory conditions, including the eastern oyster *Crassostrea virginica* (McKee et al. 2005), the bay scallop *Argopecten irradians*, blue mussel *Mytilus edulis*, slipper snail *Crepidula fornicata*, and 2 species of solitary ascidians (*Ciona intestinalis* and *Styela clava*) (Heinonen et al. 2007). Mucus released by macroalgae and corals can also form TEP (Wild 2000, Ramaiah et al. 2001). These studies demonstrate that many marine organisms contribute to the TEP pool; however, the chemical composition of exopolymers can be diverse and dependent on the species releasing them and, for phytoplankton, the prevailing growth conditions (Myklestad 1977, Hoagland et al. 1993, Myklestad 1995, Biddanda & Benner 1997, Engel & Passow 2001). Although TEP from phytoplankton have been implicated in enhancing aggregation and sedimentation of suspended material (e.g. Alldredge & Jackson 1995), the role of TEP from suspension feeders in aggregate formation is still unknown.

In this laboratory study, we examined the role of TEP from benthic suspension feeders in the formation of marine aggregates. Exopolymers from 2 suspension-feeding animals were examined: the blue mussel *Mytilus edulis* (Bivalvia) and the sea vase *Ciona intestinalis* (Ascidiacea). Both species are abundant along the Atlantic coast of North America and are important in near-shore ecosystem processes (Dame 1996, Lambert 2005). We hypothesized that TEP from these animals enhance particle aggregation, and therefore indirectly affect carbon deposition and benthic–pelagic coupling.

MATERIALS AND METHODS

TEP production experiments. Blue mussels *Mytilus edulis* and sea vases *Ciona intestinalis* were collected from subtidal populations in waters adjacent to the University of Connecticut's marine campus at Avery Point (72° 03′ 54″ N, 41° 19′ 01″ W). Shells of the mussels were cleaned and treated with a 1.2% solution of sodium hypochlorite to remove fouling organisms and reduce the amount of adhering bacteria. Because of the soft-bodied nature of the sea vase, fouling organisms were removed from the tunic only with mild scrubbing. After cleaning, animals were rinsed and placed in filtered seawater for 24 h prior to experimentation. This period allowed the animals to clear their digestive tracts and reduced the production of feces during the experiment.

We used a recirculating system to collect TEP. Each system consisted of a 17 l rectangular plastic reservoir and a 1.4 l PVC cylindrical chamber that held the experimental animals. Tygon tubing was used to attach the reservoir to the chamber, the chamber to the input of a peristaltic pump, and the output of the pump to the reservoir. This design allowed 14.5 l of seawater to be pumped from the reservoir, through the chamber, and back into the reservoir at a rate of 200 ml min^{-1} (Heinonen et al. 2007). Seawater used for the experiments was pumped from Long Island Sound and passed through a sand filter and a series of cartridge filters, the finest having a nominal pore size of 1 µm. Based on the analysis of filtered water with an electronic particle counter (Coulter Multisizer II, fitted with 100 and 140 µm apertures), the filtration process removed ca. 95% of particles >10 µm, and ca. 60% of particles between 2 and 10 µm (e.g. sediment, detritus, large bacteria, plankton). The process also decreased the background concentration of TEP. The remaining particles were sufficient to stimulate experimental animals to open and pump water actively. Experiments were conducted from September to October 2004, with seawater temperature ranging from 15 to 20°C.

Each experiment consisted of 2 treatments, one in which animals were present in the chambers (animal treatment) and one in which no animals were in the chambers (control treatment). Duplicate trials were conducted sequentially for each experiment. For each trial there were 4 to 5 animal chambers and 4 to 5 control chambers, for a total of 9 to 10 replicate chambers $treatment^{-1}$ $experiment^{-1}$. In experiments with *Mytilus edulis*, 15 mussels with an average shell length of 65 ± 12 mm were placed in each chamber. In experiments with *Ciona intestinalis*, 10 sea vases with an average body length of 70 ± 20 mm were placed in each chamber (Heinonen et al. 2007). Control treatments contained no animals so that background concentrations

of TEP, dissolved organic carbon (DOC) and total organic carbon (TOC) could be measured. In one trial we used empty mussel shells instead of live animals to determine if shell material affected the organic concentration of seawater.

At the start of each experiment, the reservoirs were filled with filtered seawater, animals were placed in their chambers, and the pumps were started. In order to measure changes in TEP, DOC, TOC, and bacterial concentrations, 300 ml of water were taken from each animal and control chamber at the start (t = 0 h) and termination (t = 5 h) of the experiment. Then, 20 ml subsamples were separated into acid-washed, muffled scintillation vials and preserved with 0.5 ml formaldehyde (37%) for bacterial counts. Two additional 20 ml subsamples were collected in acid-washed, muffled scintillation vials with Teflon caps for DOC and TOC analyses. The remaining water was used for TEP measurements. In addition, 20 ml samples were collected in Coulter vials at the beginning (t = 0) and middle of the experiments (t = 2.5 h) for particle counts, to evaluate animal feeding activity. Finally, 2.5 l of water were collected from each chamber at the end of every trial, frozen and stored at –20°C until used for aggregation experiments. Water that was recirculated over the animals was designated as either mussel water or sea vase water, whereas the water that recirculated in chambers with no animals was designated as control water.

At the end of every trial, animals were separated and frozen for later measurements of dry tissue mass. TEP concentrations were analyzed and bacterial slides prepared within 24 h of the end of the experiment. DOC and TOC samples were frozen for later analyses.

Aggregation experiments. The roller-table method of Shanks & Edmondson (1989) was used to generate aggregates. After preliminary experiments (see Li 2006), 2 types of non-sticky particles were selected. One was an artificial particle (silica-amino bead; nominal diameter 6 to 16 µm) and the other was a natural particle (diatomaceous earth; nominal diameter 6 to 20 µm). These particles were chosen because in filtered seawater they exhibited little, if any, aggregation. Hence, they were good models to examine the effect of organic matter from suspension feeders on particle aggregation.

Experiments were conducted at 15°C using water collected from chambers in the aforementioned treatments. Both particle types were tested in water from each of the 2 animal sources and their corresponding controls, resulting in 4 experimental conditions. For each experiment, particles of 1 type (silica-amino bead or diatomaceous earth) were added to 2.5 l of mussel or sea vase water and to the corresponding control water to obtain a concentration of ca. 5000 particles ml^{-1}. Each suspension was then poured into seven or eight 250 ml polycarbonate bottles. The bottles were placed on the roller table and rotated at a constant velocity (12 rpm) for 48 h. At t = 0, 24, and 48 h, bottles were removed from the roller table and the concentration and size distribution of particles in the water were determined by means of a Multisizer fitted with a 140 µm orifice. DOC, TOC, and bacteria samples (20 ml each) were collected as described above from haphazardly selected bottles at the beginning (t = 0 h) and end (t = 48 h) of the rolling period. In addition, three 1 ml samples were collected from each bottle at the end of the rolling period to enumerate aggregates larger than ca. 100 µm. The experiment in which silica-amino beads were added to sea vase water was repeated to verify certain data, resulting in up to 16 replicates for some analyses.

Sample analyses. Feeding activity of mussels and sea vases in the TEP-production experiments was evaluated by counting the number of particles in samples from animal and control chambers using the Multisizer. Bacterial samples were stained with DAPI (4′,6-diamidino-2-phenylindoledihydrochloride hydrate) and passed through black, polycarbonate filters with 0.22 µm pore size. The stained cells (free and attached) were viewed and enumerated under an epifluorescent microscope.

TEP concentration in water samples from animal and control chambers was determined using the spectrophotometric method of Passow & Alldredge (1995a). Each water sample was divided in 2 and passed through 0.4 µm pore size polycarbonate filters at a constant, low pressure of 150 mm Hg (100 ml $filter^{-1}$). The TEP on each filter were stained with a 0.5 ml aliquot of 0.02% Alcian blue solution (pH 2.5) for <2 s. After staining, the filter was rinsed with Milli-Q water to remove excess dye. Because the bound pigment is not soluble in water, the stained material on the filter is not affected by rinsing. The 2 filters were placed into a scintillation vial containing 6 ml of 80% H_2SO_4 and agitated gently 3 to 5 times over a 2 h period. A 3.5 ml aliquot of this extract was then placed in a glass cuvette, and the absorption at 787 nm was determined on a spectrophotometer. Milli-Q water was used as a blank. The absorption reading was then converted to a concentration of TEP in gum xanthan (GX) equivalents using a 4-point standard curve (Passow & Alldredge 1995a, Heinonen et al. 2007). Concentration of TEP in collected samples was expressed as micrograms of GX per liter.

A high-temperature catalytic oxidation (HTCO) method (Skoog et al. 1997), performed on a Shimadzu TOC analyzer, was used to determine the concentration of non-volatile organic carbon in each water sample. DOC samples were passed through a GF/F filter before measurement, whereas TOC samples were

used unfiltered. Each water sample was acidified with hydrochloric acid (pH < 4) and sparged with air for 10 min prior to the high-temperature, catalytic (Pt) oxidation. Milli-Q water was used as a blank. The concentration of TOC and DOC in samples was quantified using a 4 point potassium-hydrogen-phthalate standard curve, with values ranging from 0 to 5 mg C l^{-1}.

Aggregation of particulate matter was determined by analyzing the concentration and size distribution of particles in the range of 6 to 90 µm using an electronic particle analyzer (Kiørboe et al. 1990, Dam & Drapeau 1995). Because the concentrations of added particles in our experiment were constant, and assuming that mass was conserved, the result of aggregation would be a decrease in the number of smaller particles and an increase in the number of larger particles (e.g. Li & Logan 1995). During the generation of TEP by mussels and sea vases, the number of natural particles in the animal chambers decreased compared to the controls, due to suspension-feeding activity of the animals. Therefore, aggregation was determined by comparing the percentage of particles larger than a set size at time 0 h with that at time 24 h and 48 h. The set sizes corresponded to the upper size limits of the added monodispersed particles, and were as follows: silica-amino beads = 16 µm and diatomaceous earth = 20 µm. The percentage of large particles was determined by the following equation:

$$\frac{\text{Number of particles} > \text{set size limit}}{\text{Total number of particles } (6-90\ \mu\text{m})} \times 100$$

Particles <6 µm (lower size limit of both particle types) were disregarded because we were predominantly interested in the behavior of the added particles. Moreover, this procedure minimized the effect of colloidal aggregation, which produced particles ca. 3 µm in size. Finally, aggregates larger than ca. 100 µm (longest axis) were counted on a rafter cell under a compound microscope (Olympus CH-30, 100×). The 3 subsamples from each bottle were examined individually, and the number of aggregates in each was determined.

Data analyses. Data were first analyzed for normality and homogeneity of variance using a 1-sample, Kolmogorov-Smirnov test (Lilliefors option; SYSTAT 1999) and an F_{max} test (Zar 1984), respectively. In most cases our raw data violated these assumptions and were transformed prior to statistical analyses. Percentage data were normalized using an arcsine transformation; other data were normalized using a square-root transformation. For the TEP-production data, we first compared the TEP concentrations from the 2 trials within each experiment using a *t*-test. If there was a significant difference between the 2 trials, then a nested design (trials nested within treatment) was used in all subsequent analyses of data generated in experiments that used this water (TEP production and aggregation).

A 2-way, repeated-measures analysis of variance (ANOVAR) was used to analyze TEP, DOC, TOC, bacterial abundance, and aggregation data, with time as the repeated factor (within subject) and treatment as the independent factor (between subject). The Greenhouse-Geiser's epsilon index was used to evaluate homogeneity of the data (LaTour & Miniard 1983). In all cases, the Greenhouse-Geiser and Huynh-Feldt corrected significance levels agreed, so the univariate analysis was used to examine the within-subject effects (Potvin et al. 1990). If the 2-way model indicated a significant between-subject effect, *a posteriori* contrasts were performed to examine differences between treatments. In most cases, within-subject effects (time) were further analyzed using a 1-way ANOVAR. For the direct counts of large aggregates, a nested ANOVA was applied, with the 3 replicate counts for each bottle nested within trials (if appropriate) and treatment.

RESULTS

TEP production experiments

Most animals in the recirculation chambers appeared to be feeding immediately after the start of the experiment, and all animals appeared to be feeding after 20 min (valves open, siphons extended). Decreasing particle counts in the animal chambers (>50% over 2.5 h), corrected for changes in control chambers, confirmed that the animals were indeed feeding.

In the experiment with mussels *Mytilus edulis*, water from 9 animal chambers and 10 control chambers was collected (total for the 2 trials). No significant difference in TEP concentrations was found between the 2 trials at $t = 0$ h or $t = 5$ h ($p > 0.05$, *t*-test), and trial was removed as a factor. In the experiment with sea vases *Ciona intestinalis*, water from 9 animal chambers and 9 control chambers was collected (total for the 2 trials). Statistical comparison indicated small, but significant differences in TEP concentration between the 2 control trials at $t = 0$ h, and between the 2 sea vase trials at $t = 0$ and $t = 5$ h ($p < 0.05$, *t*-test). Although differences between trials were not of interest, this finding necessitated the use of a nested design for further analyses (trial nested within treatment).

Similar results were obtained from both mussel and sea vase experiments. Comparison of TEP concentrations in samples from animal and control chambers revealed significant treatment, time, and interaction effects ($p < 0.01$, 2-way ANOVAR; Fig. 1). *A posteriori* analyses indicated that TEP concentrations in the con-

trol and animal (mussel or sea vase) water were not different at $t = 0$ h ($p > 0.1$), but were significantly higher in the animal water at $t = 5$ h (mussel: $p < 0.01$, sea vase: $p < 0.05$, post hoc contrasts; Fig. 1). Furthermore, TEP concentrations increased significantly over time in water from the animal chambers ($p < 0.01$), but did not change in water from the control chambers ($p > 0.1$, 1-way ANOVAR; Fig. 1).

The biomass-specific production of TEP was determined using the difference in TEP concentration between $t = 0$ h and $t = 5$ h in animal chambers divided by total dry mass of animals in each chamber. Sea vases demonstrated a higher biomass-specific TEP production rate (6.7 ± 0.9 µg GX g^{-1} h^{-1}) than blue mussels (1.1 ± 0.3 µg GX g^{-1} h^{-1}).

There were no significant effects of treatment, time, or interaction on DOC concentrations in either mussel or sea vase experiments ($p > 0.05$; 2-way ANOVAR; Table 1). Similar results were found for TOC concentrations ($p > 0.1$, 2-way ANOVAR), except that in the mussel experiment there was a significant effect of time. One-way ANOVAR tests confirmed a slight but significant increase in TOC concentration over time for both mussel and control water ($p < 0.05$; Table 1).

Bacterial concentrations in the water from control and animal chambers (2.55 to 3.10 × 10^5 cells ml^{-1}) were lower than those observed in Long Island Sound (Capriulo et al. 2002), due to the filtration process. There were no significant treatment, time, or interaction effects on bacterial abundance in the mussel experiment ($p > 0.1$, 2-way ANOVAR; Table 1). In the sea vase experiment, only time affected bacterial abundance ($p < 0.05$, 2-way ANOVAR). One-way ANOVAR tests confirmed a slight but significant increase in bacterial numbers in both sea vase and control water during the experiment ($p < 0.05$; Table 1).

Finally, in the mussel-shell experiment, there were no significant time, treatment, or interaction effects on

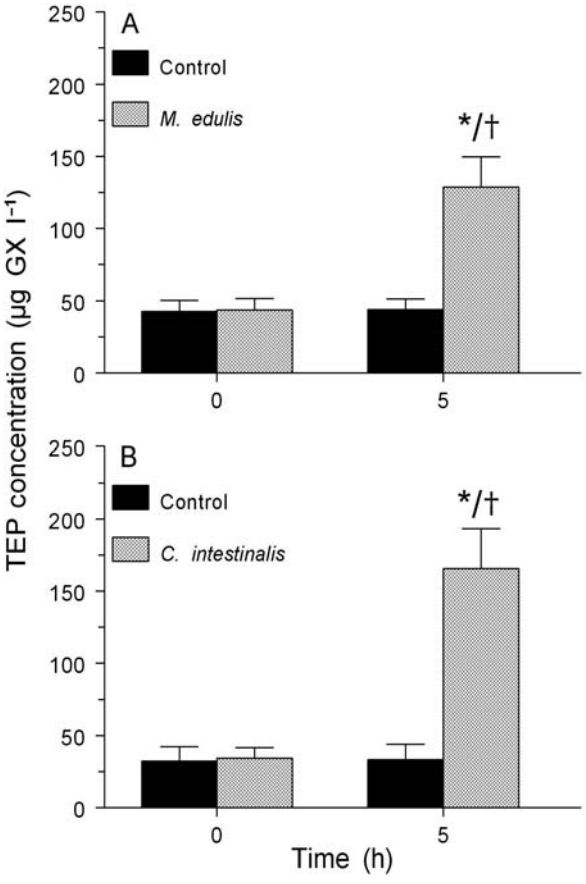

Fig. 1. Production of transparent exopolymer particles (TEP) by (A) mussels *Mytilus edulis* and (B) sea vases *Ciona intestinalis*. TEP concentration is given in micrograms of gum xanthan (GX) equivalents per liter. For both sets of experiments, the concentration of TEP in the animal treatments was significantly higher than that of the corresponding controls at 5 h (*, mussel: $p < 0.01$, sea vase: $p < 0.05$), but not at 0 h. TEP concentration in the animal water also increased significantly over time (†, $p < 0.01$). No significant change over time was found for the controls. Data are means ± SD for 9 to 10 replicate chambers

Table 1. Concentrations of dissolved (DOC) and total (TOC) organic carbon and bacteria at the beginning and end of experiments in which animals (mussels and sea vases) produced transparent exopolymer particles (TEP). In the experiment with mussels, both animal and control treatments displayed a slight but significant increase in TOC after 5 h (†, $p < 0.05$). In the experiment with sea vases, both animal and control treatments displayed a slight but significant increase in bacterial abundance after 5 h (†, $p < 0.05$). No other significant treatment, time, or interaction effects were found for these parameters ($p > 0.05$). Data are means ± SD for 6 (DOC, bacteria) or 10 (TOC) replicate samples

Experiment, treatment	DOC (mg l^{-1}) Time (h)		TOC (mg l^{-1}) Time (h)		Bacteria (× 10^5 cells ml^{-1}) Time (h)	
	0	5	0	5	0	5
Mussel						
Control	1.38 ± 0.1	1.41 ± 0.1	1.46 ± 0.1	1.85 ± 0.4†	2.56 ± 0.3	2.62 ± 0.04
Animal	1.39 ± 0.2	1.58 ± 0.1	1.43 ± 0.1	1.83 ± 0.1†	2.55 ± 0.03	2.89 ± 0.1
Sea vase						
Control	1.60 ± 0.1	1.41 ± 0.1	1.52 ± 0.2	1.57 ± 0.2	2.64 ± 0.2	3.10 ± 0.1†
Animal	1.48 ± 0.3	1.42 ± 0.1	1.64 ± 0.5	1.66 ± 0.4	2.59 ± 0.2	2.95 ± 0.1†

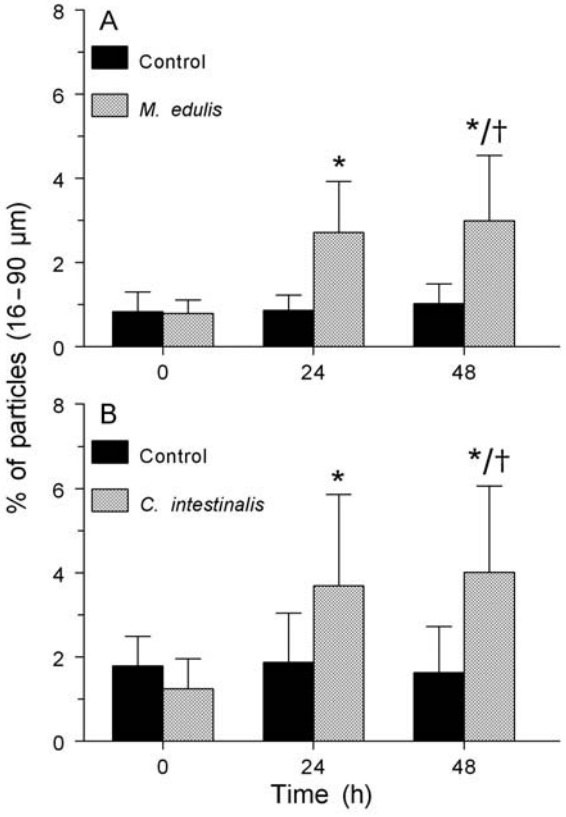

Fig. 2. Aggregation of silica-amino beads as determined by means of an electronic particle counter (Multisizer). The percentage of particles between 16 and 90 µm (diameter) is shown for experiments in which organic matter from (A) mussels *Mytilus edulis* and (B) sea vases *Ciona intestinalis* was tested. For both sets of experiments, there was no significant difference between the animal and corresponding control treatments at $t = 0$ h, but a significant difference at $t = 24$ h and $t = 48$ h (*, $p < 0.01$). The number of aggregates that formed over time significantly increased in the mussel and sea vase treatments (†, $p < 0.01$), but did not increase in the control treatments. Data are means ± SD for 7 (A) or 16 (B) replicate bottles

TEP concentrations ($p > 0.1$, 2-way ANOVAR), a result similar to that previously reported by Heinonen et al. (2007). There was also no significant difference in bacterial abundance between the mussel-shell treatment and control treatment at either $t = 0$ h or $t = 5$ h ($p > 0.1$, 2-way ANOVAR).

Aggregation experiments

Multisizer data

The concentration and size distribution of particles in the range of 6 to 90 µm were analyzed by means of the Multisizer. Similar results were obtained for both mussel and sea vase experiments in which silica-amino beads were used. Statistical analysis indicated significant treatment (mussel vs. control, sea vase vs. control), time, and interaction effects on particle aggregation ($p < 0.01$, 2-way ANOVAR; Fig. 2). No significant trial effect within treatments was found ($p > 0.1$, nested-2-way ANOVAR, applied to sea vase data only). *A posteriori* analyses indicated that the percentage of aggregated particles (16 to 90 µm diameter) in the animal (mussel or sea vase) and corresponding control water was the same at $t = 0$ h, but significantly higher in the animal water at both $t = 24$ h and $t = 48$ h ($p < 0.01$, post hoc contrasts). Furthermore, in both mussel and sea vase water, there was a significant increase in the percentage of aggregates that formed over time ($p < 0.01$), but no significant change in the controls ($p > 0.05$, 1-way ANOVAR; Fig. 2).

Similar results were also obtained for mussel and sea vase experiments in which diatomaceous earth was used. Statistical analysis indicated a significant effect of

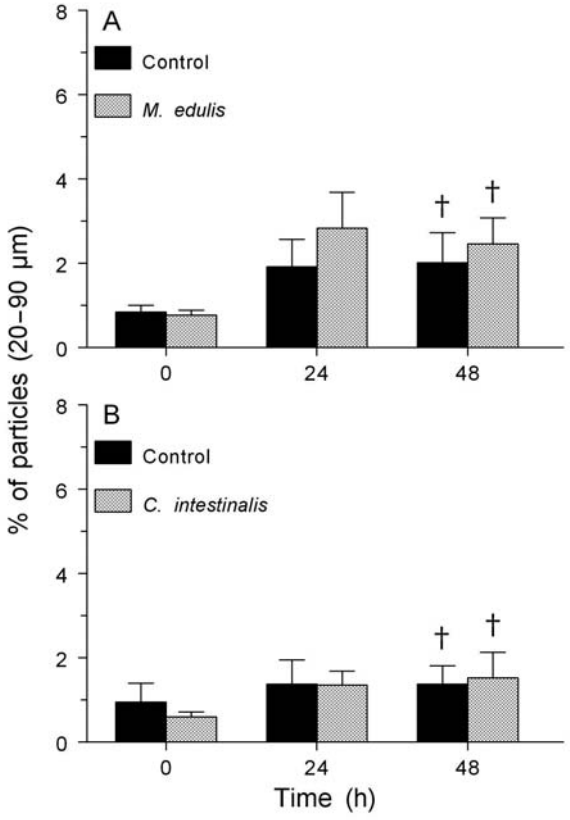

Fig. 3. Aggregation of diatomaceous earth as determined by means of an electronic particle counter (Multisizer). The percentage of particles between 20 and 90 µm (diameter) is shown for experiments in which organic matter from (A) mussels *Mytilus edulis* and (B) sea vases *Ciona intestinalis* was tested. For both sets of experiments, no treatment effects were found. There was, however, a slight but significant increase in the number of aggregates that formed over time in the mussel, sea vase, and control treatments (†, $p < 0.01$). Data are means ± SD for 7 to 8 replicate bottles

time on particle aggregation ($p < 0.01$), but no significant treatment (mussel vs. control, sea vase vs. control) or interaction effects ($p > 0.05$; 2-way ANOVAR; Fig. 3). Again, no significant trial effect within treatments was found ($p > 0.1$, nested 2-way ANOVAR, applied to sea vase data only). Further analysis indicated a slight but significant increase in the percentage of aggregated particles (20 to 90 µm in diameter) that formed over time in mussel, sea vase, and control water ($p < 0.01$, 1-way ANOVAR; Fig. 3).

Microscope data

Aggregates larger than ca. 100 µm (longest axis) were counted on a rafter cell under a compound microscope. After 48 h of rolling suspensions of silica-amino beads or diatomaceous earth, significantly more large aggregates formed in mussel and sea vase water than in the corresponding control water ($p < 0.01$, nested ANOVA; Fig. 4). In the sea vase experiment with diatomaceous earth, no significant trial effect within treatments was found ($p > 0.1$). In the experiment with silica-amino beads, however, there was a significant trial effect ($p < 0.01$). *A posteriori* analyses indicated that a greater number of large aggregates formed in the sea vase water with the higher mean TEP concentration (Trial 1, $p < 0.01$, Tukey HSD); no difference in aggregate number was found between the 2 control trials ($p > 0.05$, Tukey HSD).

DOC, TOC, and bacteria

The concentration of DOC, TOC, and bacteria varied little over the 48 h rolling period. In most experiments there were no significant treatment, time, or interaction effects on the concentration of these parameters ($p > 0.05$, 2-way ANOVAR; Table 2). In the mussel experiment with silica-amino beads, there was a slight but significant difference in TOC concentration between mussel and control water at $t = 0$ h ($p < 0.05$), but no significant difference at $t = 48$ h ($p > 0.1$, post hoc contrasts; Table 2). In the mussel experiment with diatomaceous earth, there was a slight but significant increase in bacterial abundance over time in control water ($p < 0.05$), but no significant change in mussel water ($p > 0.05$, 1-way ANOVAR; Table 2).

DISCUSSION

Our research demonstrates 2 important points regarding benthic suspension feeders. First, blue mussels *Mytilus edulis* and sea vases *Ciona intestinalis* release significant quantities of TEP into seawater; second, the organic matter released from these suspension feeders can enhance particle aggregation.

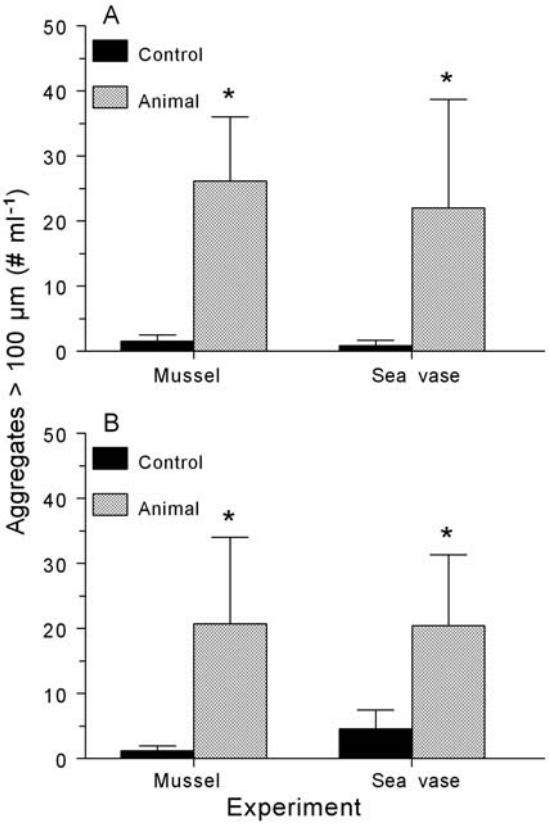

Fig. 4. Aggregation of (A) silica-amino beads and (B) diatomaceous earth as determined by means of direct counts under a compound microscope. The number of aggregates larger than ca. 100 µm in diameter (no. ml^{-1} of water) that formed after 48 h of rolling is shown for experiments in which organic matter from mussels *Mytilus edulis* and sea vases *Ciona intestinalis* was tested. In all experiments there were significantly more aggregates in the mussel and sea vase treatments compared to the corresponding control treatments (*, $p < 0.01$). Data are means ± SD for 7 (mussel), 12 (sea vase, A), or 8 (sea vase, B) replicate bottles

Our first result is consistent with the findings of Heinonen et al. (2007), who reported that several species of bivalves and ascidians produced significant quantities of TEP over a 5 h period, and that DOC concentrations did not change over time in chambers that held suspension-feeding animals. Similarly, we found no significant difference in DOC and TOC concentrations between the animal and control water, and little or no change in concentration over time. These results suggest that mussels and sea vases release little organic matter other than TEP, and that TEP is a small fraction of the TOC pool. In addition, similar to the contention of Heinonen et al. (2007), we suggest that the effects of bacteria in our experiments were minor.

Table 2. Concentrations of dissolved (DOC) and total (TOC) organic carbon, and bacteria at the beginning and end of experiments in which organic matter from mussels and sea vases was tested for its effect on aggregation of silica-amino beads and diatomaceous earth (DE). In experiments with silica-amino beads, a significant difference in TOC concentration was found between mussel and control treatments at $t = 0$ h (*, $p < 0.05$). In experiments with DE, a slight but significant increase in bacterial abundance was found in one control treatment after 48 h (†, $p < 0.05$). No other significant treatment, time, or interaction effects were found for these parameters ($p > 0.05$). Data are means ± SD

Particle type, treatment	DOC (mg l^{-1}) Time (h)		TOC (mg l^{-1}) Time (h)		Bacteria ($\times 10^5$ cells ml^{-1}) Time (h)	
	0	48	0	48	0	48
Silico-amino beads						
Control (n = 4)	1.68 ± 0.7	1.87 ± 0.2	1.60 ± 0.3*	1.58 ± 0.4	1.67 ± 0.2	1.95 ± 0.2
Mussel (n = 4)	1.50 ± 0.4	1.83 ± 0.4	2.05 ± 0.1	2.08 ± 0.3	1.52 ± 0.1	1.95 ± 0.3
Control (n = 8)	1.54 ± 0.4	1.48 ± 0.5	2.06 ± 0.5	1.25 ± 0.6	2.53 ± 0.3	2.72 ± 0.1
Sea vase (n = 8)	2.20 ± 0.7	2.29 ± 0.8	1.99 ± 0.6	1.51 ± 0.5	2.47 ± 0.3	2.48 ± 0.2
Diatomaceous earth						
Control (n = 4)	1.63 ± 0.6	1.64 ± 0.7	1.38 ± 0.7	2.04 ± 0.6	1.68 ± 0.1	2.03 ± 0.2†
Mussel (n = 4)	2.07 ± 0.6	1.77 ± 0.9	1.84 ± 0.6	1.76 ± 0.5	1.90 ± 0.0	1.88 ± 0.1
Control (n = 4)	1.58 ± 0.6	2.09 ± 0.7	1.54 ± 0.4	1.38 ± 0.4	1.74 ± 0.3	1.79 ± 0.1
Sea vase (n = 4)	1.74 ± 0.5	2.16 ± 0.2	1.60 ± 0.4	1.54 ± 0.5	1.46 ± 0.2	1.62 ± 0.3

Although bacteria are known to produce exopolymer particles (Stoderegger & Herndl 1999), their contribution to the total TEP pool is unclear (Passow et al. 2001, Passow 2002b). Bacteria can also degrade and modify TEP, which may influence the aggregation process (Smith et al. 1995). Thus, the interaction between bacteria and TEP is complex, and the impacts of bacteria on aggregation are difficult to evaluate. In our study, however, bacterial biomass remained low in the experimental chambers, and was similar between the control and animal treatments (Table 1).

The abundance and distribution of TEP in the marine environment has been measured in numerous studies. In general, TEP concentrations are lower in open-ocean waters (e.g. 29 µg GX l^{-1}; Engel 2004) and higher in coastal waters (e.g. 100 to 2800 µg GX l^{-1}; see Passow 2002b for review). Peak concentrations are associated with phytoplankton blooms, and high concentrations have been measured in near-shore waters inhabited by dense assemblages of corals, macroalgae, sea grasses, and suspension feeders (e.g. 700 to 900 µg GX l^{-1}; Wild 2000, Ramaiah et al. 2001, McKee et al. 2005). The concentration of TEP at any specific location, however, can vary widely depending on local physical and biological conditions (e.g. up-welling, phytoplankton assemblages, benthic assemblages). By comparing the published values of TEP standing stock with production rates measured for a variety of suspension feeders, we can probe the potential importance of these animals in contributing to the TEP pool. In laboratory studies, biomass-specific production rates fall within the range of 0.8 to 6.7 µg GX g^{-1} h^{-1} (McKee et al. 2005, Heinonen et al. 2007, present study), but can vary due to, perhaps, differences in water-pumping activity and physiological status of the animals (cf. rates obtained in this study for mussels and sea vases with those obtained by Heinonen et al. 2007). Species-specific TEP production rates, however, may be higher under natural conditions. For example, in a laboratory study, McKee et al. (2005) reported a mean biomass-specific production rate for the oyster *Crassostrea virginica* of 5.2 µg GX g^{-1} h^{-1}. In a field study, McKee et al. (2005) measured a production rate for the same species of 34 µg GX g^{-1} h^{-1}. Using this field production rate, an oyster with a dry tissue mass of 1 g (ca. 8 cm shell height; Jordan et al. 2002) could produce about 816 µg of TEP in 24 h. Similarly, Heinonen et al. (2007) estimated that a dense bed of mussels (ca. 654 individuals m^{-2}) could produce 13.7 mg TEP in 24 h, or the total standing stock of TEP measured in a 1 m^3 volume overlying the bed in about 8 d. Given that many other suspension-feeding organisms are often found within a mussel bed (e.g. tunicates), the actual amount of time necessary to produce the concentration of TEP measured in the field is probably much shorter. These comparisons, however, should be viewed with caution, as we do not fully understand the complex interactions that govern the production, fate, and turn-over rate of TEP (see Passow 2002b for review). Nonetheless, our results suggest that suspension feeders may be important contributors to the TEP pool in near-shore waters.

Our second result demonstrates that organic material released by mussels and sea vases enhances particle aggregation, which was clearly shown in the data from direct counts of aggregates >100 µm. Microscope counts revealed that significantly more aggregates formed in the animal water, which had significantly higher TEP concentration, than in the control water,

with lower TEP concentration, for both species and both particle types (Fig. 4). Analyses of particle aggregation using the Multisizer, however, indicated a difference between the 2 types of particles used in the experiments. With silica-amino beads, the Multisizer data indicated significantly higher aggregate formation in animal water than in control water (Fig. 2). With diatomaceous earth, the Multisizer data did not show a significant difference in aggregation between the animal and control water (Fig. 3). These disparate results could be caused by how measurements are made by the Multisizer and the different surface properties of the 2 particle types. TEP are anionic particles, silica-amino beads are cationic, and diatomaceous earth is anionic in seawater (pH ≈ 8; Johnson & Stevenson 1978, Stumm 1992). Therefore, the interaction between TEP and silica-amino beads would be much stronger than that of TEP and diatomaceous earth. Microscopic observations confirm that aggregates of diatomaceous earth were less cohesive than aggregates of silica-amino beads. Furthermore, the Multisizer is an electronic device that determines the number and size of particles by monitoring the electrical current between 2 electrodes. Particles are drawn through an aperture and disturb an electric field as they pass from the outside to the inside of the aperture. The pressure differential across the aperture creates shear that could disrupt aggregates (Kiørboe et al. 1990). Thus, the diatomaceous earth aggregates, which are less cohesive, would more likely be broken and disaggregated compared to the silica-amino-bead aggregates. Disaggregation would lead to spurious counts, with few large aggregates actually being enumerated by the Multisizer.

Our data demonstrate that organic matter released from suspension feeders enhances aggregation of natural and synthetic particles, and several lines of evidence strongly suggest that TEP was responsible for these findings. First, in generating the sea vase organic matter, a significantly higher concentration of TEP was produced in 1 of the trials (Trial 1 = 183.8 ± 21.7 µg GX l^{-1}; Trial 2 = 143.2 ± 13.1 µg GX l^{-1}). In the aggregation experiments with silica-amino beads, significantly more large aggregates (largen than ca. 100 µm) formed in the water from Trial 1, which had the higher TEP concentration (data not shown, see Li 2006). Second, the number of large aggregates that formed was related to the TEP concentration in all experiments (control water vs. animal water), and not related to DOC or to TOC concentrations. Although TEP was a small fraction of the total carbon pool, particle aggregation was related to the TEP concentration. Such indirect evidence forms the basis of current assumptions concerning the importance of phytoplankton-generated TEP in particle aggregation. To conclusively confirm that TEP from suspension feeders enhances aggregation, the method developed by Mari & Dam (2004) to extract and concentrate TEP could be applied. The isolated TEP could then be used in aggregation experiments.

Silica-amino beads and diatomaceous earth are non-sticky particles that demonstrate low aggregation in filtered seawater (Li 2006). Our experiments show that aggregation of these particles increases dramatically in the presence of TEP. Considering that TEP concentration in our experiments was a small fraction of the TOC pool, the enhanced aggregation was probably caused by a change in the stickiness of added particles and not simply by an increase in particle concentration. Thus, results of our research agree with the contentions that: (1) particle stickiness is related to TEP concentration (Dam & Drapeau 1995) and (2) the dominant effect of TEP on aggregate formation is to increase particle stickiness rather than to aggregate themselves (Jackson 1995).

Finally, our experiments suggest another way in which suspension feeders may couple the benthic and pelagic realms. In the traditional view of benthic–pelagic coupling, suspension feeders remove seston from the water column and deposit feces and pseudofeces to the benthos. Some benthic organisms, however, may also interact with the pelagos through the production of EPS. Several studies have shown that suspension feeders can produce TEP (McKee et al. 2005, Heinonen et al. 2007, present study), and our current results demonstrate that these TEP can enhance the aggregation of particles. Such aggregation would increase deposition of particulate matter and potentially increase food supply to the benthos, thereby enhancing benthic–pelagic coupling. Using stable isotope techniques, Alber & Valiela (1994, 1995) found that bay scallops and mussels can utilize organic aggregates as a food source. Recently, Ward et al. (2006) reported that aggregates can significantly enhance the uptake of picoparticles and colloidal material by several species of bivalves. Therefore, we propose that in near-shore waters dense populations of suspension feeders produce TEP that leads to enhanced aggregation, deposition, and perhaps utilization of particulate matter by the benthos. Such a cycle could be an important pathway for organic carbon in coastal ecosystems.

In conclusion, this research is the first to demonstrate that organic matter from suspension feeders, such as TEP, enhances particle aggregation. Our future studies will extend laboratory results to the natural environment, and determine if the abundance of marine aggregates is correlated with TEP concentrations near dense beds of suspension feeders in coastal waters.

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