

Do large seston particles contribute to the diet of the bivalve *Cerastoderma edule*?

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ABSTRACT: The vertical distribution of suspended cellulose particles in the size range 60 to 500 μm was studied with artificial bivalve siphons in flume flow for a range of flow speeds and degrees of bed roughness. Significant vertical concentration gradients with an increase in particle concentration of 200 to 300% compared with their near-bed values were found for some combinations of flow speed and particle size in a 6 cm layer above the bed, which was an effect of flow velocity. In a second series of experiments, artificial siphons were replaced by the infaunal bivalve *Cerastoderma edule* (L.), and filtration was studied for large particles (60 to 500 μm). Corresponding experiments in a static system were also performed. *C. edule* readily ingested particles up to a maximum size of ca. 500 μm , and calculations of clearance for sizes between 60 and 300 μm were in the range of 0.5 to 3 $\text{l h}^{-1} \text{g}^{-1}$ dry weight, which is close to rates measured for microflagellates. Field studies on large particles in the near-bed layer (0 to 10 cm) show that this fraction contributes significantly to total seston. It is concluded that large particles like plant detritus, benthic diatoms and invertebrate larvae may accumulate in the near-bed layer and form a significant food source for benthic bivalves.

KEY WORDS: Suspension-feeding · Large particles · Flow · Bed roughness · *Cerastoderma edule*

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INTRODUCTION

Complex fluxes of organic matter, nutrients and contaminants occur across the interface between seawater and the sediment. Important, interrelated factors affecting these fluxes are the water flow (Herman et al. 1999), oxygen concentration (Diaz & Rosenberg 1995), sediment structure (Dade 1993) and biological activity (Graf & Rosenberg 1997). Benthic suspension-feeders, e.g. beds of bivalves, can substantially affect the exchange of material between the pelagic and the benthic system (e.g. Fréchette & Bourget 1985, Graf & Rosenberg 1997). In many coastal areas bivalves have the capacity of processing a daily water volume equivalent to the water column (Jørgensen 1990, Muschenheim & Newell 1992). These zoobenthic filter-feeders act as a sink for suspended particulate matter, increasing the particle flux from the water column down to the bottom with subsequent deposition of particulate matter (Graf & Rosenberg 1997). Ingested material changes its chemistry during gut passage, and indigestible parts are repackaged into faeces or pseudo-

faeces, changing the hydrodynamic properties of the particles (Brown 1986).

The supply of food to benthic organisms is not solely determined by the production in the photic zone and the rate of the vertical transport of potential food particles. The horizontal water transport in the near-bed region is also important in supplying particulate matter to suspension-feeders (Muschenheim 1987, Wildish & Kristmanson 1997). Any analysis of the available food resource for benthic suspension-feeders has to consider the supply rate and concentration of food particles where the organisms actually feed, i.e. in the near-bed layer. The vertical distribution of suspended particles in the near-bed region is mainly controlled by particle sinking velocity, turbulent vertical mixing, and the cohesiveness of the sediment (Middleton & Southard 1984). Thus, suspended particles are subjected to hydrodynamic sorting. In almost any natural situation, denser, often inorganic, particles exhibit concentration maxima closer to the bottom than less dense, often organic, fractions (Muschenheim 1987). Only in exceptional situations, when the sinking veloc-

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ity is insignificant or when the vertical mixing is about 2 orders of magnitude greater than the sinking velocity, is the near-bed suspension homogenous (Jumars & Nowell 1984). The feeding activity of suspension-feeders may further alter the vertical concentration gradient of food particles for down-stream individuals by depleting the near-bed layer of particles. Near-bed food depletion is counteracted by vertical mixing, which increases the time-averaged food concentration available for the organisms. The intensity of the vertical mixing depends on the velocity of the water flow, induced by physical forcing like wind and tide, and the roughness of the bottom (Fréchette et al. 1989, Møhlenberg 1995).

Nano- and microplankton (e.g. many autotrophic protists) are an important food resource for many suspension-feeders, and numerous studies report their clearance, both in the field and in the laboratory (e.g. Møhlenberg & Riisgård 1978, 1979, Fréchette et al. 1989, Riisgård et al. 1996, 1998), and on clearance of similar-sized mixtures of microalgae and non-living seston (e.g. Kiørboe et al. 1981, Navarro & Widdows 1997, Bacon et al. 1998, Hawkins et al. 1998). These studies have only treated clearance of particulate matter within a size range of a few micrometres, to about 60 μm . Few studies have investigated the role of large particles as a food resource for suspension-feeders. One potential food resource for suspension-feeders is resuspended microphytobenthos (Grant et al. 1990). This is emphasised by Newell et al. (1989), who found large (up to 110 μm), benthic algal species in the gut of *Mytilus edulis* and suggested that it may form a significant portion of the mussel diet. Similarly, Muschenheim & Newell (1992) found a significant decrease in the amount of large pelagic as well as benthic diatoms in the near-bed water mass over a blue mussel bed. *M. edulis* may also be a significant consumer of various mesozooplankton, occasionally as large as 3 to 6 mm in length (Davenport et al. 2000). Another potential food source is large detrital particles. Detrital particles and inorganic components coagulate and form large aggregates that provide a substantial surface for sorption of organic macromolecules as well as a site for bacterial attachment and growth, leading to increased organic content (Muschenheim et al. 1989, Kiørboe 2000). A better understanding of how large particles in the benthic boundary layer are captured and ingested will not only improve models of growth and survival of suspension-feeders, but it will also shed light on the biogeochemical fluxes of particles between the pelagic and the benthic system with consequences for transport of nutrients and hydrophobic organic contaminants and their turnover (Björk et al. 2000).

The aim of the present study was to test if large particles (60 to 500 μm) in the near-bed region could contribute to the diet of an infaunal bivalve, the cockle *Cerastoderma edule*. In a flume experiment we first explored the vertical distribution of these particles under different flow conditions and the supply of these potential food particles to the cockles. We then tested the capacity of *C. edule* to ingest large particles, both in a static system and in flume flow. The information from the laboratory-based experiments were then compared to field measurements of the size distribution and concentration of large particles (>100 μm) in the lower 10 cm of the benthic boundary layer.

MATERIALS AND METHODS

Particle distribution in flume flow. To study the particle distribution in different flow conditions we used a recirculating flume, 7 m long and 0.5 m wide (Jonsson & Johansson 1997). The flume was filled to a depth of 10 cm with filtered (125 μm) seawater (32‰). A turbulent boundary layer was tripped by a 10 mm bar 4.5 m upstream of the working section. Different flow conditions were created by changing flow speed and bottom roughness. Three different bottom substrates, smooth Plexiglas, sand (minor axis: 1.4 ± 0.4 mm, mean \pm SD) and gravel (minor axis: 7.3 ± 2.6 mm, mean \pm SD), were combined with 3 flow speeds (Table 1) leading to 9 different flow conditions. The sand and gravel were spread as a monolayer of grains on the floor of the flume.

To characterise the flow in the flume, velocity profiles were measured by a hot-film anemometer (Dantec Stream Line). The friction velocity, u_* , was calculated from the slopes of regressions of the

Table 1. Flow conditions in the flume for the experiments on vertical particle distribution. The grain size (minor axis) of the bottom substrate is 1.4 ± 0.4 mm (mean \pm SD) for sand and 7.3 ± 2.6 mm (mean \pm SD) for gravel. Free stream velocity is measured 6 cm above the bottom

| Bottom substrate | Flow regime | Free-stream velocity U (cm s ⁻¹) | u_* (cm s ⁻¹) | z_0 (cm) |
|------------------|-------------|--|-----------------------------|------------|
| Plexiglas | Low | 3.8 | 0.19 | 0.0016 |
| | Medium | 6.0 | 0.29 | 0.0012 |
| | High | 8.5 | 0.40 | 0.0008 |
| Sand | Low | 3.2 | 0.23 | 0.015 |
| | Medium | 5.3 | 0.33 | 0.0068 |
| | High | 6.9 | 0.49 | 0.014 |
| Gravel | Low | 3.4 | 0.27 | 0.025 |
| | Medium | 5.8 | 0.40 | 0.014 |
| | High | 8.0 | 0.56 | 0.019 |

velocity profiles using the Karman-Prandtl equation (Schlichting 1979) for a logarithmic boundary layer:

$$u_z = \frac{u_*}{\kappa} \ln\left(\frac{z}{z_0}\right) \quad (1)$$

where u_z is the flow velocity at height z above the bottom, κ is von Karman's constant (0.4) and z_0 is the roughness height.

The vertical distribution of particles in the different flume flows was studied using a mixture of 2 size distributions of beaded cellulose (Sigma, 93% porosity, 100 to 250 and 250 to 500 μm). The particle distribution in the stock suspension, used in the experiments, was determined by measuring 599 particles in an inverted microscope (Nikon Diavert, 200 \times) and is shown in Fig. 1.

For each experiment 10 l of particle suspension containing about 5×10^5 particles with the size distribution shown in Fig. 1 was added uniformly across the flume just below the water surface through a perforated horizontally oriented pipe upstream of the flume collimators. Near-bed water with suspended particles was sampled using artificial bivalve siphons (Fig. 2A) at the working section 4.7 m downstream of the flume entrance. Samples were collected at 4 heights above the bed 0.4, 1.0, 3.0 and 6.0 cm (Fig. 2B), with 2 replicate siphons for each height. The different sampling heights were randomly distributed over the working section (length \times width: 0.4 \times 0.5 m), with the restriction that a higher level was never placed directly upstream of a lower level

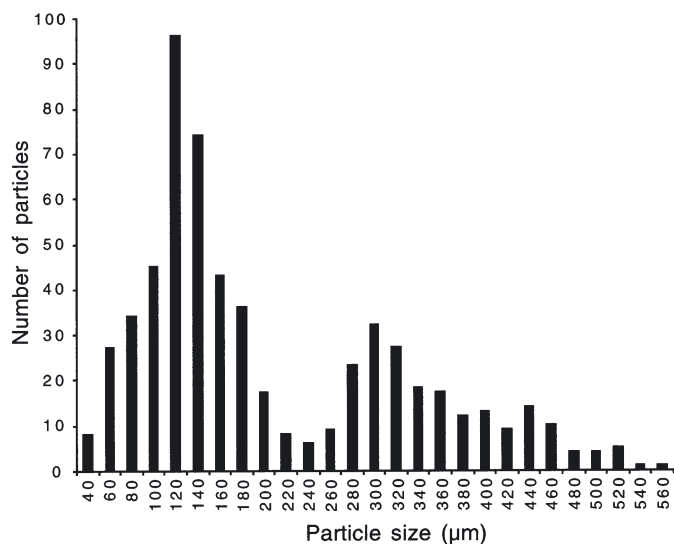


Fig. 1. Initial size distribution of cellulose particles in the stock suspension (10 ml). Mean size of the 2 size fractions 100 to 250 μm and 250 to 500 μm was $123 \pm 41 \mu\text{m}$ and $351 \pm 70 \mu\text{m}$ (mean \pm SD), respectively

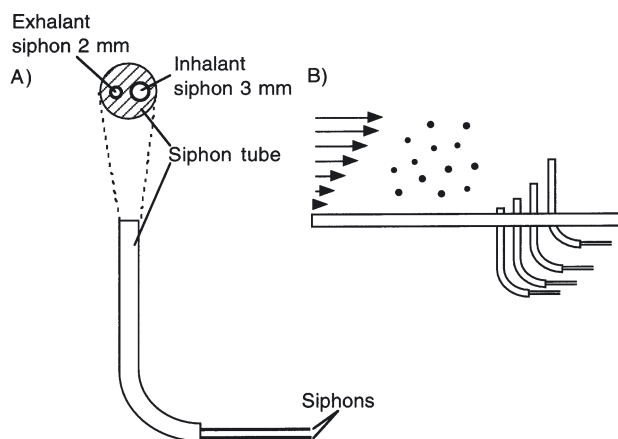


Fig. 2. Schematic drawing of artificial bivalve siphons. (A) Dimensions of inhalant and exhalant siphons (the exhalant siphon is not used in these experiments). (B) Side view of siphons placed in the working section of the flume at the 4 heights used (0.4, 1, 3 and 6 cm)

(Fig. 3). Dye-release tests showed no effect caused by a lower upstream siphon on the water flow at the sampling height of a downstream taller siphon. The siphons were not placed closer than 10 cm from the sidewall of the flume to avoid interaction between the circulation patterns around the siphons and the sidewall (Nowell & Jumars 1984). Water was sampled through the siphons by gravity with a flow rate of 30 ml min^{-1} , corresponding to a 35 to 40 mm *Cerastoderma edule* (André et al. 1993), and was collected in beakers. Sampling was continued as long as the 'cloud' of particles passed over the working section. The volume of the samples was measured, the particles were sieved, and their diameters were measured with a dissection microscope (Wild M5A, 50 \times). Every combination of flow velocity and bottom substrate was run twice.

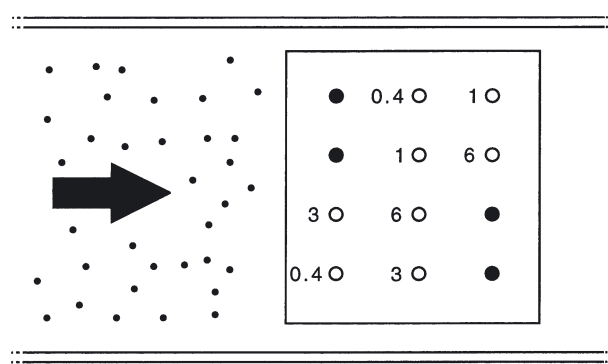


Fig. 3. Top view of typical locations of the artificial siphons in the working section of the flume. Numbers refer to siphon height (cm) above the flume floor

Particle clearance by the cockle *Cerastoderma edule* in a static system. Specimens of *C. edule* were collected close to the Tjärnö Marine Biological Laboratory, Sweden (58° 53' N, 11° 8' E). We estimated the particle clearance for a particle size range from 60 to 500 µm using beaded cellulose (Sigma, 93% porosity, 100 to 250 and 250 to 500 µm) or polystyrene particles (Nunc Biosilon dyed with Radglo fluorescent pigment, 140 to 250 and 250 to 340 µm). Clearance of the large-particle fractions was compared to the clearance of the autotrophic microflagellate *Isochrysis galbana* (4 µm), which is expected to be retained with ~100% efficiency by the cockles (Møhlenberg & Riisgård 1978). Each particle type and size fraction was run in separate experiments. A single cockle with a shell length of 35 ± 3 mm (mean ± SD, n = 38) was placed on top of a 4.7 cm diameter PVC cylinder filled with sand and was allowed to burrow into the sand. Glass beakers filled with 2 l of filtered (0.2 µm) seawater (32‰, 7.5 ± 1.5°C, mean ± SD) were placed in a flow-through water bath. Two cylinders were placed in each beaker on a grid 3 cm from the bottom. A magnetic stirrer below the grid kept the particles in suspension. The algae were added to a concentration of about 3000 to 5000 cells ml⁻¹ and allowed to mix for 30 s. A 10 ml sample was collected after 0, 5, 10, 20 min and finally a 20 ml sample after 30 min. The samples were filtered through a 25 µm sieve to remove any larger particles. Glutaraldehyde (6%) was added to preserve the algae, and the samples were filtered onto a 25 mm Nuclepore membrane filter (0.2 µm) stained with Irgalan-black and placed on top of immersion oil on microscope slides and then covered by immersion oil and a cover slip. Algae on the filter were counted in an epifluorescent microscope (Leitz Dialux, 250×).

By following the exponential decrease of the concentration of *Isochrysis galbana*, the clearance (F_t) was calculated using the equation:

$$F_t = \frac{V}{tN} \ln \frac{C_0}{C_t} \quad (2)$$

where V is the water volume in the experiment aquarium, t is the time the experiment lasted (=30 min), N is the number of cockles (=2), and C_0 and C_t are the algal concentrations at the experiment start and at time t , respectively (Coughlan 1969). The uptake of the larger size fractions was estimated by analysis of the stomach content of the cockles. Clearance (F_{ip}) was calculated using the equation:

$$F_{ip} = \frac{n}{\bar{C}tN} \quad (3)$$

where n is the number of particles filtered, during time t , N is the number of cockles, and \bar{C} is the average particle concentration in the experiment beaker

according to Frost (1972), where the concentration is corrected for settling particles during the experiment. Clearance values were then standardised to an equivalent of 1 g dry soft tissue weight, according to the formula (Navarro et al. 1992):

$$Y_s = Y_e \left(\frac{1}{W_e} \right)^b \quad (4)$$

where Y_s is the clearance value for the standardised animal, Y_e is the uncorrected clearance value, W_e is the weight of the individual cockle and b is the weight power (0.7, Møhlenberg & Riisgård 1979). To be able to find the cellulose particles when dissecting the cockles the particles were initially stained with calcofluor white (Sigma, Fluorescent brightener 28). Because the cockles showed some grinding of the cellulose particles in the stomach when kept for more than about 20 min after feeding, we also used polystyrene particles, which were not subjected to grinding, for comparison.

In the clearance experiments with large particles, it was important to avoid unrealistically high concentrations of these particles to avoid production of pseudo-faeces due to overloading of the digestive system. To prevent this we used a particle volume per litre equivalent to the volume of about 2×10^4 cells of *Isochrysis galbana*, which still may be a high concentration, but no production of pseudofaeces was observed during the experiment. This yields 500 particles l⁻¹ of the 100 to 250 µm fraction and 25 particles l⁻¹ of the 250 to 500 µm fraction. A 10 ml sample of a suspension of 10^5 particles l⁻¹ of the 100 to 250 µm fraction, or 50 particles of the 250 to 500 µm fraction, randomly picked out and measured with a dissection microscope (50×), were added. To assure that the cockles were continuously filtering, about 5000 cells ml⁻¹ of *I. galbana* were added at the same time as the large particles and the decrease of the algal concentration was followed as above. The experiments with cellulose beads were finished after 10 min by picking up the PVC cylinders and putting them in cellulose-free water for 5 min to be sure that all filtered particles had been ingested. The cockles were dissected, and the stomach content was analysed in UV-light using a dissection microscope (50×) for counting and measurements of ingested cellulose particles. The estimates of clearance of the polystyrene particles were accomplished by analysis of ingested particles in the faeces. After feeding on polystyrene particles for 30 min the cockles were picked up from the cylinders and individually put in plastic beakers filled with filtered seawater. *I. galbana* was added, and the cockles were left to produce faecal pellets. Particles were picked out from the faecal pellets and measured using a dissection microscope (50×). The cockles were

allowed to defecate until no particles were found in them. Control runs without cockles were made to be able to compensate for sedimentation in the clearance calculations, with the exception of the experiment with *I. galbana*, in which we assumed no settlement occurred under the present well-mixed conditions. Every experiment and control was replicated 4 times. After dissection the soft tissue of the cockles were dried at 70°C for 48 h and weighed.

Particle clearance by the bivalve *Cerastoderma edule* in flume flow. Clearance of large particles by *C. edule* was also studied in flume flow under different flow conditions. These experiments were similar to the study of particle distribution in flow using artificial bivalve siphons as described above. The artificial siphons were replaced by cockles (33 ± 3 mm; mean \pm SD, $n = 48$), which were allowed to burrow randomly in the sand-filled working section to a density of 500 cockles m^{-2} . In this series of experiments only sand was used as the bottom substratum. The water temperature in the flume was $7.6 \pm 0.7^\circ C$ (mean \pm SD), and the salinity was 32‰. Suspended cellulose particles were added, and particle uptake by the cockles was measured in the same way and under the same flow conditions as for the particle distribution experiments (Table 1). Every run was terminated when the same amount of particles had crossed the working section, equal to the time taken to add the 10 l bulk particle suspension into the flume (160 s). The time taken to add the bulk suspension was much shorter than the time taken for the water in the flume to recirculate, at any flow velocity. Each flow condition was replicated twice. Eight cockles that had been actively filtering during the experimental run were placed in filtered seawater for 5 min. The cockles were then dissected, and the stomach content was analysed in UV-light using a dissection microscope (50 \times) to pick out and measure all cellulose particles. Finally, dry weight of the cockle tissue was determined at 70°C for 48 h. Clearance was calculated by comparing the number of particles filtered with the concentration at the 0.4 cm level in the particle distribution experiment with sand bottom; 0.4 cm is the mean height at which cockles entrain the water by their inhalant current. Clearance data were standardised to 1 g soft tissue dry weight as in the static system experiment.

Field measurements of near-bed particle concentration. Measurement of particle flux in the near-bed layer requires a method that allows high spatial resolution of particle analysis, avoids any breakage of large, fragile particles and prevents interference with the local flow pattern. To meet these demands we developed an *in situ* optical technique based on a rod-lens borescope (Karl Storz, 88392 DF 90°) connected to a video camera (Mintron, CCD-camera with an

interference filter, 830 nm). Particle images were produced within a thin layer of infrared light from a scanning laser (Latronix, LD20-830). All electronic equipment was built into a waterproof casing and was connected to a power supply and a video recorder (Panasonic AG-7450) at the surface by cables. With this technique it was possible to detect particle sizes from about 100 μm up to a few millimetres, and measurements could be made as close as 1 mm above the sediment surface without interference with the water volume of interest (Fig. 4).

Images were later processed with computer-aided image analysis. First, they were sequentially and automatically grabbed using the Media Grabber (Raster Ops) software and stored. The sequence of image files was then automatically processed with image analysis software (IPLab 2.5, Signal Analytics Corp. for the Macintosh), which makes it possible to estimate particle flux, concentration gradients and size distribution of particles near the bottom (P. R. Jonsson, Ö. Karlsson & L. O. Loo unpubl. data).

The particle image technique was used to estimate the distribution of suspended particulate matter in the near-bed region in the field at 2 different locations, in Oosterschelde, The Netherlands (51° 33' N, 3° 50' E), where hydrodynamics is mainly forced by tides, and in a small bay, Knebel Vig, in the Århus Bight, Denmark (56° 12' N, 10° 30' E), where wind forcing dominates. Particle size and concentration in a ca. 10 cm layer above bare sediment without bivalves were estimated by video recording at 5 heights with 0.58 cm between each vertical field of view. Every height was recorded for 3 min.

Statistical analysis. Data were analysed with multi-factorial analysis of variance (ANOVA) to test the effects of substrate, flow velocity and height above the bottom on particle concentration. *F*-ratios were constructed according to Confield & Tukey (Winer et al. 1991). The relationship between relative particle concentration and height above the bottom was tested by linear regression. Clearance was also analysed with multi-factorial ANOVA to test the method of clearance estimate, particle type and flow velocity. Before analysis, data were inspected for departure from the assumption of homoscedasticity using Cochran's *C*-test (Underwood 1996), and when this was detected a \log_{10} transformation was used, which stabilised the variances. In most statistical tests an α of 0.05 was used. When non-orthogonal comparisons were performed the probabilities of a Type I error were adjusted using the Dunn-Sidak procedure (Underwood 1996). The particle distribution experiment was analysed both with the total amount of particles and after dividing the dataset into 3 size groups: 100 to 200, 200 to 300 and 300 to 400 μm .

RESULTS

Particle distribution in flume flow

Fig. 5 summarises the particle concentration distribution in the flume as a function of particle size and flow velocity. In the 3-factor ANOVA, no effects of substrate or of its interactions with other factors were detected ($p > 0.05$). Therefore all substrates were combined in every flow regime (low, medium, high). As expected, an increase in flow speed and friction velocity (Table 1)

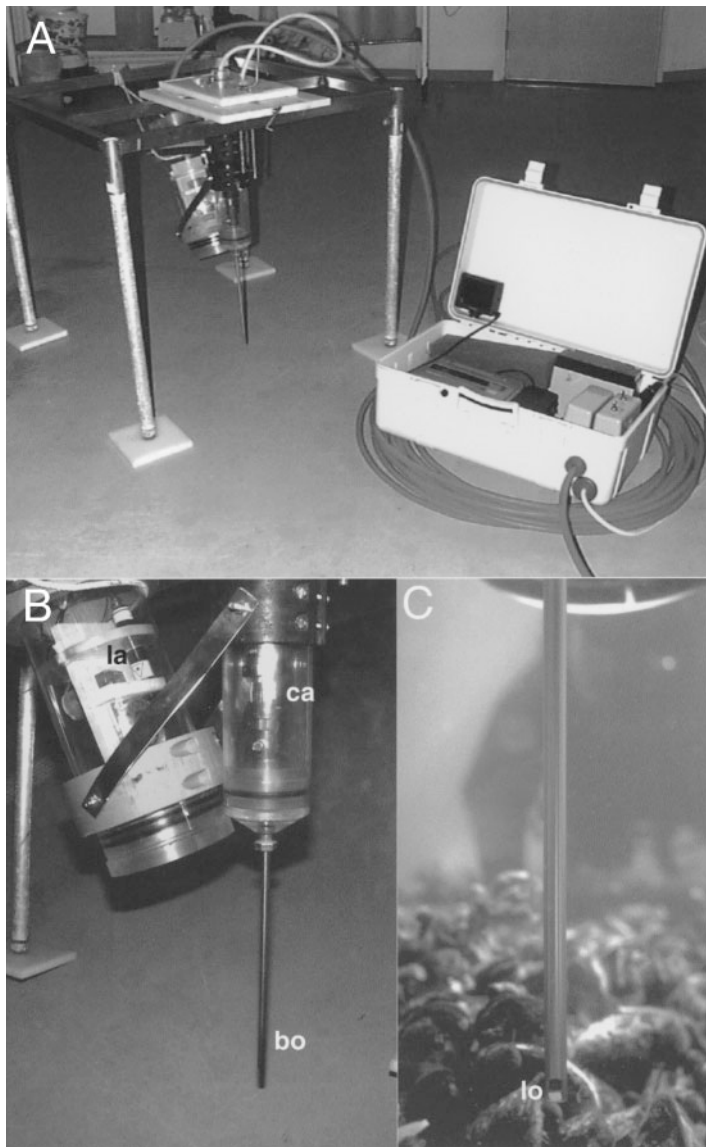


Fig. 4. Particle image system for *in situ* recording of near-bed particle flux. (A) overview showing the recording unit mounted on a table and the surface unit containing video-recorder, monitor and power unit. (B) Close-up showing video-camera (ca), the borescope (bo) and the laser unit (la). (C) *In situ* view of the borescope above a bed of mussels, *Mytilus edulis*; the lens opening (lo) is directed towards the viewer

caused increased concentration of the larger particles (200 to 400 μm). Simultaneously, the concentration of the smallest size fraction (100 to 200 μm) decreased, leading to a decrease in the total number of particles with flow speed (Table 2). In particular, for the larger particles a vertical concentration gradient was evident, with concentration increasing towards the bed. This is more clearly seen if particle concentrations are normalised as shown in Fig. 6, where the largest particle fraction occurring in the 2 slower flow velocities shows a significant concentration gradient (linear regression: low velocity 200 to 300 μm particles, $F_{1,22} = 6.2$, $p < 0.05$; medium velocity 300 to 400 μm particles: $F_{1,22} = 7.3$, $p < 0.05$). Note that the largest size fraction (300 to 400 μm) is missing in the slowest flow velocity.

Particle clearance in a static system

Clearance rates for *Cerastoderma edule*, 760 ± 20 mg soft tissue dry weight (mean \pm SD), feeding on the microalga *Isochrysis galbana* are shown in Fig. 7. Although clearance decreased rapidly for the larger particles, the cockles readily ingested cellulose and polystyrene particles, and the maximum size found was 240 μm (Fig. 8). Clearance of polystyrene particles was slightly higher compared to cellulose particles, but not significantly so (Table 2).

Particle clearance in flume flow

Fig. 9 shows clearance as a function of flow velocity and particle size. Higher clearance rates were found for *Cerastoderma edule*, 649 ± 26 mg soft tissue dry weight (mean \pm SD), feeding on cellulose particles in flume flow compared to the static situation (Table 2, SNK, $p < 0.01$), but with no differences between flows in the flume (Table 2). The size range of the particles ingested was between 80 and 500 μm , and the maximum size found increased with flow velocity. At the lowest flow velocity the largest particles found were 200 μm (Fig. 9A). Higher velocities made larger particles available through resuspension, and particles up to 500 μm were found in the stomach of the cockles in the highest flow velocity (Fig. 9B,C). Clearance was highest for the smallest particles and then gradually decreased with increasing particle size, from about 3 to 0.5 $\text{l g}^{-1} \text{h}^{-1}$.

Table 2. Summary of statistical tests of particle distribution and clearance based on analysis of variance (ANOVA) showing degrees of freedom (df), the *F*-ratio statistics (*F*) and the probability of the null hypothesis (*p*)

| Test | df | <i>F</i> | <i>p</i> |
|---|------|----------|----------|
| Velocity on concentration of 100 to 200 μm particles | 2,9 | 16.9 | <0.05 |
| Velocity on concentration of 200 to 300 μm particles | 2,9 | 77.3 | <0.05 |
| Velocity on concentration of 300 to 400 μm particles | 2,9 | 116 | <0.05 |
| Particle type (cellulose, polystyrene) on clearance | 1,30 | 2.28 | >0.05 |
| Method (static, flume) on clearance | 3,24 | 5.94 | <0.05 |
| Velocity on clearance in flume flow | 2,18 | 0.722 | >0.05 |

Field measurements of near-bed particle concentration

The flow velocity, 8 cm above the bottom, in Oosterschelde and Knebel Vig was 13.5 cm s^{-1} (range: 7.0 to 23.8 cm s^{-1}) and 5.3 cm s^{-1} (range: 2.7 to 7.5 cm s^{-1}), respectively. The particle distributions in the lower

10 cm of the benthic boundary layer in Oosterschelde and Knebel Vig are shown in Fig. 10. The data were pooled because each profile was recorded during 15 min, which makes it impossible to give an instantaneous picture of the particle profile. Particle size and concentration showed a slight decrease with distance to the bottom in both areas, with a higher particle concentration in Knebel Vig compared to Oosterschelde (around 330 and 90 particles l^{-1} , respectively, in the sampled water volume, Fig. 10A,C). The mean particle sizes (major axis) were around 300 and 240 μm in Oosterschelde and Knebel Vig, respectively (Fig. 10B,D).

DISCUSSION

Vertical particle concentration gradients in the boundary layer are expected from sediment theory and have been empirically quantified in both field (Gloor et al. 1994) and flume studies (Muschenheim 1987). Large differences in particle loading in the near-bed layer compared to the bulk water may lead to serious underestimations of feeding rates of benthic suspension-feeders. Few studies exist in the near-bed layer on scales relevant to bivalves using siphonal feeding currents.

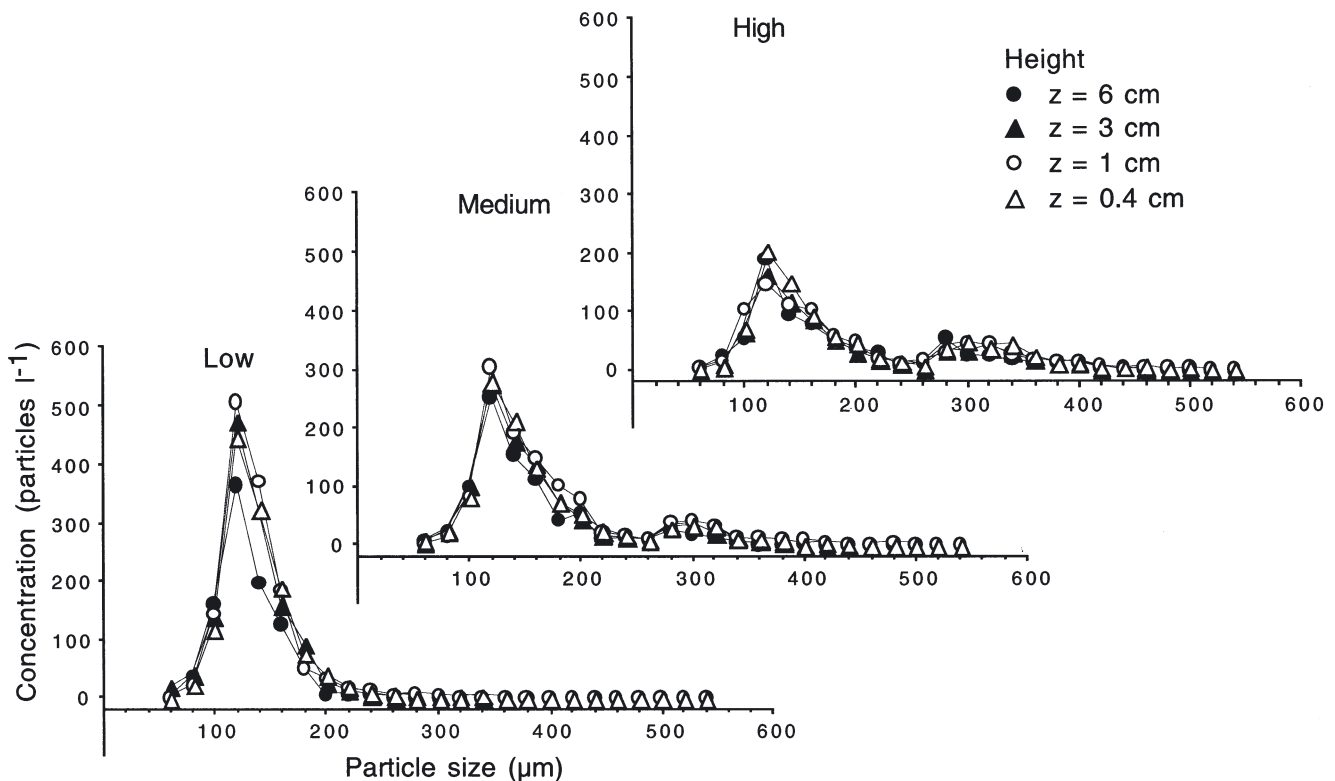


Fig. 5. Summary of sampled size distributions of cellulose particles, mean concentration as a function of particle size, at 3 levels of flow speeds (low, medium, high) averaged over all substrates and at 4 heights above the flume floor using artificial siphons

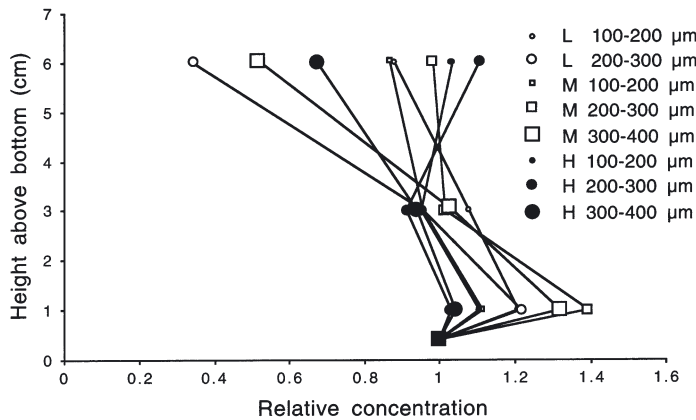


Fig. 6. Normalised vertical distribution for cellulose particles in 3 size classes (100 to 200, 200 to 300, and 300 to 400 µm) and at 3 levels of flow speeds (low, medium, high: L, M, H, respectively) including all substrates. Note that the largest size fraction, 300 to 400 µm, is missing in the slowest flow velocity

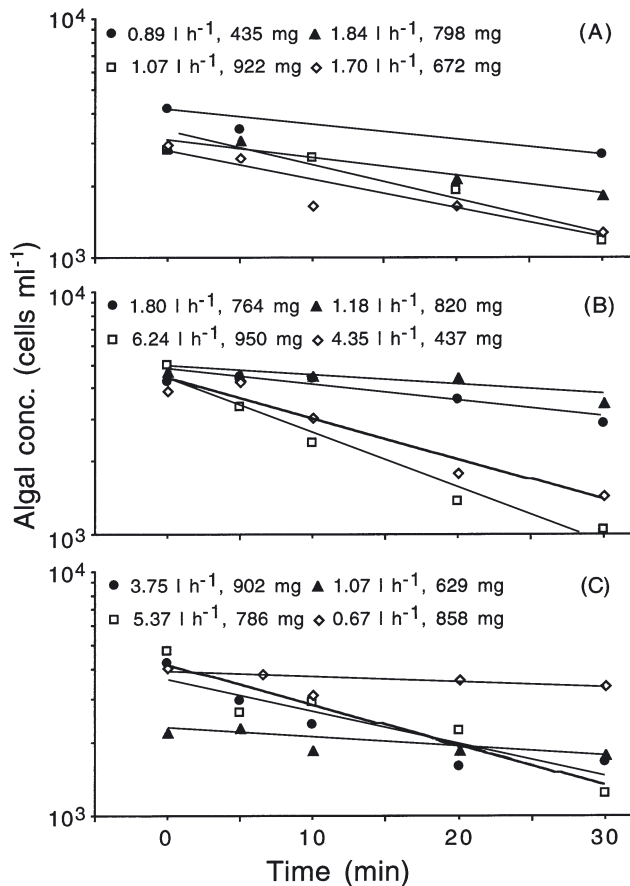


Fig. 7. Clearance of *Isochrysis galbana* by *Cerastoderma edule* in a static system. Experiments with (A) pure *I. galbana* (B) *I. galbana* + polystyrene (100 to 250 µm) and (C) *I. galbana* + polystyrene (250 to 500 µm). Symbols represent each run. Regression lines, estimated clearance per mussel and mean dry weight (n = 2) are shown for every experimental run. Some data are missing because of loss of samples

Vertical concentration gradients are mainly expected for large detrital particles at flow speeds characteristic of many shallow sediments. A major question is if particles larger than 60 µm are available to bivalves, since very few studies have measured clearance of this type of particles. In this study we first explored the vertical distribution of large particles using artificial bivalve siphons in flume flow, and finally measured the clearance rates for the infaunal bivalve *Cerastoderma edule*.

Vertical distribution of suspended particles

The cellulose particles used in this study were considered a good model for many detrital particles, e.g. of algal origin. The particle density (1049 kg m⁻³) is also similar to estimates of organic-mineral aggregates (1055 kg m⁻³, Chase 1979) and large phytoplankton (100 µm; 1030 kg m⁻³, Jackson 1989). For the largest particle size fraction at low and medium flow velocities, significant concentration gradients over the 6 cm near-bed layer were detected with a decrease of the particle concentration from 200 to 300% compared with their near-bed values (Fig. 7). As expected from sediment theory (Muschenheim 1987), higher flow velocities and smaller particles with low sinking velocities resulted in more homogeneous particle distributions. An attempt was made in this study to alter the bed roughness by using mineral grains of different sizes in addition to a smooth Plexiglas surface. Increasing bed roughness only had small effects on the friction velocity (Table 1) and did not change the apparent particle distribution. The effect of bed roughness should be studied in more detail. As flow speed and friction velocity increased, larger particles were sampled in the artificial siphons, which we interpret as resuspension of particles when a critical shear stress is reached. Another effect at higher flow speeds was a slight reduction in overall particle concentration. This is certainly because the bulk particle suspension was added at the same rate irrespective of the flow speed in the flume. This causes lower particle concentrations at higher flow speeds, since the water volume into which the particles are added increases with flow speed. However, this will not hazard the interpretation of the particle concentration gradients and bivalve ingestion in the flume.

Compared with field data, the particle concentration in the flume (Fig. 5) was between 3 and 10 times the particle concentration at Knebel Vig and Oosterschelde (Fig. 10), respectively. Muschenheim (1987) found near-bed concentrations of particles >300 µm from 1700 to 8000 particles l⁻¹ in Eastern Passage, Nova Scotia. This emphasises that the particle loading in the flume study may be representative for field conditions.

Particle filtration

The bivalve *Cerastoderma edule* ingested both cellulose and polystyrene particles up to a size of 500 μm . (Note: sand grains up to a size of 600 μm were found in the intestine of the cockles.) The maximum size ingested in the static system was only 240 μm , which could be explained by the small probability of capturing the larger and less common particles. Clearance for particles in the size range of 60 to 300 μm was surprisingly efficient, with clearance rates of up to 3 l h^{-1} , close to that found for a small (4 μm) microflagellate (Figs. 8 & 9). However, the clearance of *Isochrysis galbana* was obviously lower than the rates in other clearance experiments performed with monocultures of microalgae, and is 3 to 4 times lower than clearance rates found by Møhlenberg & Riisgård (1979). The pumping activity of the cockles was not checked closely during the experiment; therefore, one possible reason for low clearance may be that the cockles were not fully open during the experiment, which would lead to reduced pumping rate (Jørgensen et al. 1988). The temperature was lower (6 to 8°C) in the present study compared to 10 to 13°C in a study by Møhlenberg & Riisgård (1979), which, if comparable to *Mytilus edulis*, could cause a reduction in the pumping rate of about 20% (Jørgensen et al. 1990). However, our clearance estimations are in accordance with the results from the semi-field conditions in Smaal et al. (1997), but this comparison should be made with caution due to presumed refiltration in their experimental set-up. The duration of the cellulose particle clearance experiments was assumed to be shorter than the particle transit time through the stomach. Violation of this assumption should lead to underestimation of the clearance rate. However, this does not seem to be the case, as the clearance rate of cellulose particles corresponds with the clearance rate of polystyrene particles. Figs. 8 & 9 show a decrease in clearance rate with particle size. This is most likely an artefact of the way we measured the clearance rate for the large particles. It is unlikely that the gills retained the large particles with less efficiency than microalgae. Our way of estimating clearance rates by counting ingested particles has its restrictions. Ingestion rate will only be equivalent to clearance rate if no preingestive sorting is occurring (Wildish & Kristmanson 1997). Even if we did not observe any rejection of pseudofaeces, it could still have been the case. Because of the low concentration of the large particles, they may have been rejected as single particles and not as strings of mucous-bound particles, and would then have been more difficult to detect. A significantly higher clearance rate of cellulose particles was found in the flume compared to the static situation. This may be due to flow-enhanced

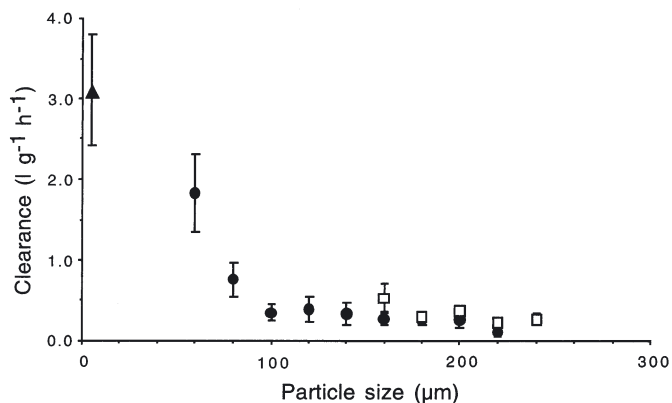


Fig. 8. Clearance (mean \pm SE) of *Cerastoderma edule* as a function of particle size and for different particle types in a static system. \bullet : cellulose particles; \square : polystyrene particles; \blacktriangle : microflagellate *Isochrysis galbana*

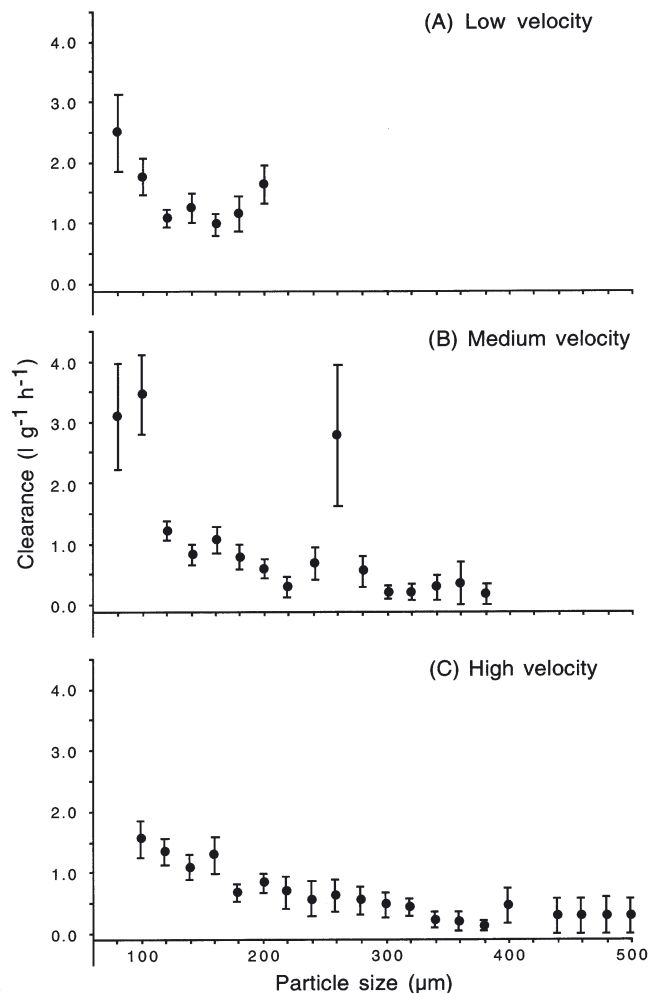


Fig. 9. Clearance (mean \pm SE) of *Cerastoderma edule* as a function of particle size and flow speed: (A) low, (B) medium and (C) high, in flume flow

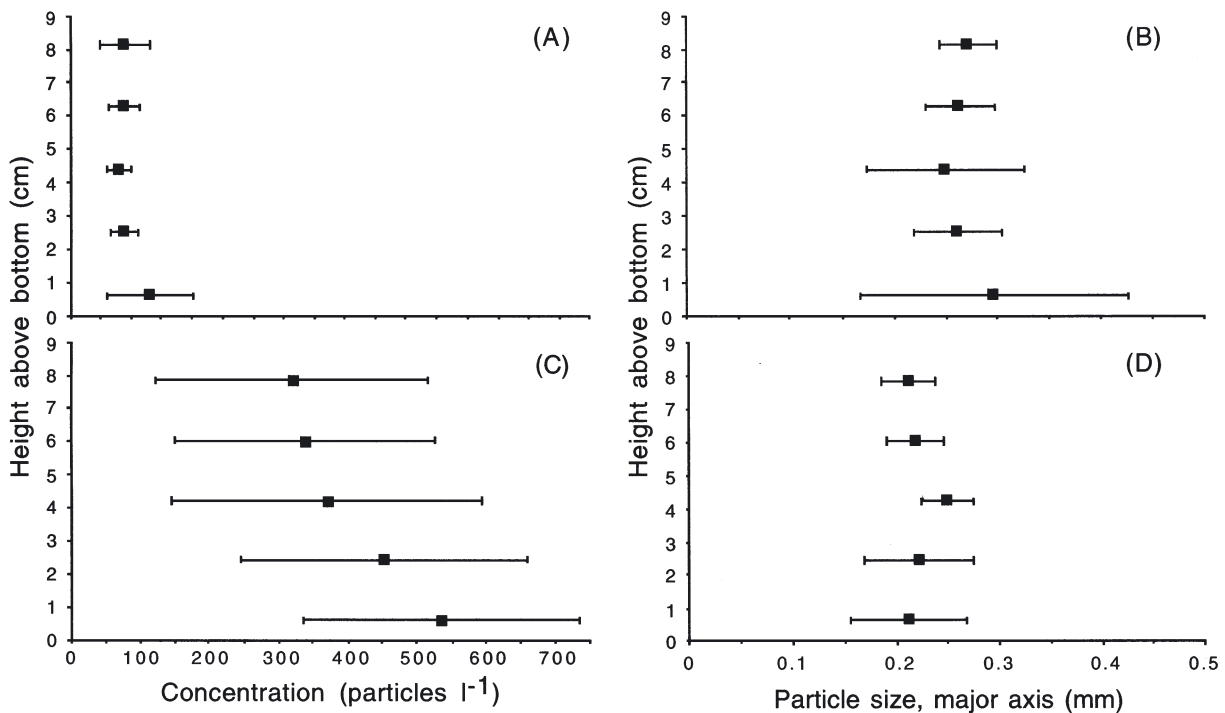


Fig. 10. Field measurements of concentration and particle size (mean \pm SD) above a bare sediment surface without bivalves from (A,B) Oosterschelde (The Netherlands) and (C,D) Knebel Vig (Denmark)

activity and a less disturbed environment in the flume compared to the glass beakers. Another conceivable explanation is that *C. edule* appears to have high selection efficiency for chlorophyll, leading to higher

rejection of cellulose particles in the static system where microalgae were present (Urrutia et al. 1996).

Whether the use of inert particles gives reliable estimates of bivalve clearance is questionable. Hawkins et al. (1998) argue that natural feeding behaviour in filter-feeders can only be studied using naturally occurring suspended particles. Tamburri & Zimmer-Faust (1996) found that large particles of polystyrene or glass were rejected to a higher extent than invertebrate larvae of the same size by the oyster *Crassostrea virginica*. Their conclusion was that chemical stimuli are needed to make the oysters ingest the particles. However, in this study the particles consisted of cellulose, which is a naturally occurring component of natural seston, and clearance of the smaller size fraction of these particles and *Isochrysis galbana* were similar.

As mentioned earlier very few previous data exist on bivalves feeding on large particles (Newell et al. 1989, Davenport et al. 2000). This study shows that capture by the cockle may be a significant route for this size class of particles. If the effect of near-bed accumulation of large particles is combined with the measured size-specific clearance rates, it is possible to estimate ingestion rates. In Fig. 11 ingestion rates in terms of particle volume h⁻¹ are shown for different flow speeds. This summarises the interaction found between particle distribution and flow speed. Even if the total particle concentration decreased with flow velocity, the volume

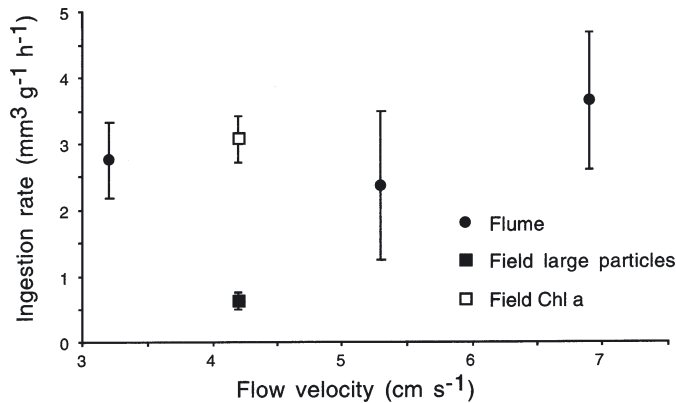


Fig. 11. Calculated ingestion rates (mean \pm SE) for *Cerastoderma edule* assuming observed size-specific clearance rates and flume concentrations of cellulose particles from experiment with sandy substrate (●). ■ shows the estimated ingestion rate based on field measurements in Knebel Vig of large particles (100 to 3000 μ m). □ ingestion of phytoplankton material by *Mytilus edulis* (mean 593 mg dry weight) based on faecal production of chlorophyll *a* in Knebel Vig at the same time period and depth as the measurements of large particles (F. Møhlenberg pers. comm.)

concentration increased due to the increased amount of larger cellulose particles. Thus, there were small changes in the ingestion rate with flow velocity. To put the flume data in perspective, Fig. 11 also shows the ingestion calculated by combining flume clearance data with field data from Knebel Vig on large particles in the near-bed layer, and the volume of ingested phytoplankton material based on faecal production of chlorophyll *a* for *Mytilus edulis* from the same area (F. Møhlenberg pers. comm.). We conclude that ingestion in our flume experiment corresponds with field assumptions, for both phytoplankton and large seston particles. Furthermore, we conclude from our data that large particles could contribute to the diet of bivalves. However, the importance of these particles as a food resource is to a great extent dependent on the organic content of the particles and how degradable this organic matter is for the suspension-feeders. Duggins & Eckman (1994) found that kelp debris could be an important source of organic carbon for suspension-feeders, and in some species >80% of the carbon ingested by suspension-feeders showed a kelp origin rather than a phytoplankton origin. The digestive enzymes of *Cerastoderma edule* include cellulase and laminarinase, indicating that they are able to ingest the major components of macroalgal debris, and Ibarrola et al. (1998) found adjustment of the activity of the different enzymes, in the digestive organs in *C. edule*, in relation to the sources of food available. A peak of cellulase activity in May corresponded to a diet based mainly on phytoplankton, and later in the autumn a laminarin-rich kelp detritus diet induced higher laminarinase activity. Cranford & Grant (1990) showed that the scallop *Placopecten magellanicus* has the ability to absorb aged kelp detritus with an efficiency of up to 87%, and concluded that even if phytoplankton is the primary food source, particles of detrital origin could be an additional source to meet energy demands when phytoplankton is scarce.

Possible sources of food with high digestibility are larvae and other zooplankton. The oyster *Crassostrea virginica* has the capability to ingest invertebrate larvae as large as 1500 μm (Tamburri & Zimmer-Faust 1996). However, Tamburri & Zimmer-Faust introduced the larvae by pipette directly into the mantle cavity of the oysters, preventing the escape of larvae. This could overestimate the role of invertebrate larvae as a food source, because larvae are motile and may escape the inhalant current of bivalves under natural conditions. Under more realistic conditions in flume flow, André et al. (1993) found that larvae of *Cerastoderma edule* (280 μm) were readily ingested by conspecific adults. *Mytilus edulis* can ingest substantial amounts of mesozooplankton, which could result in a net energy gain for the mussels (Davenport et al. 2000).

Some caution is called for when predicting ingestion from clearance rates of large particles and measured size distributions in the near-bed layer. Many fragile particulate aggregates possibly break upon being inhaled by bivalves and processed on the gills and by the labial palps, thus reducing the actual particle size ingested. Even when the contribution to the bivalve diet by large particles is less important, the filtration activity changes the chemical and hydrodynamic properties of the particulate matter by repackaging it into faeces or pseudofaeces. This could improve the digestibility and affect the subsequent fate of particles entering the benthic system. Further research is needed to estimate the particle distribution and flow conditions in the near-bed layer in the vicinity of benthic suspension-feeders. Studies of particle size and concentration in non-intrusive ways, combined with estimates of food quality of the particulate matter, should improve our knowledge about the role of large seston particles as a food source.

CONCLUSIONS

We conclude that large particles with low settling velocities, typical of many detrital particles, benthic diatoms and larvae may show substantial accumulation in the near-bed layer, where bivalves feed. It is further shown that the bivalve *Cerastoderma edule* has a relatively high capacity to ingest large particles (60 to 500 μm). It is suggested that large particles may be an important food source for many bivalves, depending on the nutritive value of the particle content. Ingestion by bivalves may further be an important sink for particles with implications for chemical transformation and repackaging, with hydrodynamic consequences for the transport of particulate matter. This may have important consequences on the regeneration of nutrients and the spread of particle-bound contaminants in coastal areas.

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