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Influence of cockle *Cerastoderma edule* bioturbation and tidal-current cycles on resuspension of sediment and polycyclic aromatic hydrocarbons

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ABSTRACT: Experiments were performed to investigate the impact of cockle population density *Cerastoderma edule* on the resuspension of naturally contaminated sediments collected from the Tamar estuary (SW England). Annular flumes generated tidal-current cycles for 7 to 9 d. The suspended sediment concentration (SSC) at peak flow increased 5-fold with increasing cockle population density, although the 2 highest densities yielded similar resuspension. Polycyclic aromatic hydrocarbons (PAHs) in the sediment were analysed by gas chromatography/mass spectrometry (GC/MS) at the beginning of the experiment, and in the water column of each flume after 2 and 6 d at both the maximum and minimum current speeds. At the end of each experiment sediment erodability was measured as a function of a stepwise increase in current speed. Sediment erosion increased up to 10-fold with increasing cockle population density. However, at the highest density the SSC was lower than that observed for the medium density, probably due to binding resulting from higher mucus secretion and pseudofaeces production. Current-induced resuspension of contaminated sediment was dependent on the density of the cockles. The correlation between the suspended sediment concentrations and the concentrations of PAH was weak for low molecular weight PAHs (phenanthrene and anthracene) due to their higher water solubility. In contrast, higher molecular weight PAHs (fluoranthene, pyrene, benz(a)anthracene and chrysene) showed a strong correlation with suspended particulates as a result of their higher hydrophobicity.

KEY WORDS: Bioturbation · Tidal-current cycles · Sediment resuspension · *Cerastoderma edule* · Polycyclic aromatic hydrocarbons · PAHs · Contaminant remobilisation

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INTRODUCTION

Biota play an important role in determining the erosion potential and the dynamics of intertidal and subtidal sediments (Eckman 1985, Davis 1993, Paterson 1997, Widdows et al. 2000b, Widdows & Brinsley 2002, Orvain et al. 2004, Roast et al. 2004). The erodability of cohesive sediments is dependent on the interactions among physical, chemical and biological processes, and more specifically the balance of biostabilisation and bio-destabilisation mechanisms. Sediment stabilisation (i.e. an increase in the critical threshold for erosion) is influenced by biota, ranging

from benthic algal films (Paterson 1997), seagrass beds (Fonseca & Fisher 1986) and macroalgae mats (Romano et al. 2003) to epifaunal bivalves such as mussel beds/oyster reefs (Widdows et al. 1998b). For intertidal locations, sediment drainage/dehydration during prolonged air exposure is also important. Sediment destabilisation is induced by a decrease in the erosion threshold and an increase in the erosion rate. It can result from bioturbation (mixing activity produced by benthic species) and the burrowing and deposit-feeding activity of bivalves, such as *Macoma balthica* (Widdows et al. 1998b), *Cerastoderma edule* and *Ruditapes philippinarum* (Sgro et al. 2005) com-

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bined with physical disturbance by tidal currents, as well as intermittent storm events. Macrofauna have therefore been divided broadly into 2 functional groups: bio-stabilisers and bio-destabilisers (including bioturbators) (Rhoads & Young 1970, Rhoads 1974, Widdows & Brinsley 2002).

Estuaries and coastal waters, such as the Tamar estuary in SW England, receive a complex mixture of contaminants (metals, organic and organo-metal compounds) from sources including old mine workings, urban discharges, runoff, and inputs from major ports/harbours. Many of these contaminants are sufficiently stable to persist and accumulate in benthic sediments as well as in the tissues of local aquatic organisms (Langston et al. 2003). This storage can render the sediment itself an important source of remobilised contaminants, particularly after improved environmental controls and reductions in inputs to the aquatic environment. Two major processes can lead to contaminant remobilisation into the water column (Latimer et al. 1999): diffusive flux of dissolved chemicals and resuspension of sediments by both physical and biological disturbance. Among the contaminants of concern are petroleum hydrocarbons, particularly the polycyclic aromatic hydrocarbons (PAHs), because of their toxicity and the elevated concentrations found in many estuaries with industrial and urban developments. In the Tamar estuary, previous studies have shown significant levels of PAH contamination in the water column (Readman et al. 1982, Law et al. 1997), sediments (Readman et al. 1987, Law et al. 1997, Woodhead et al. 1999) and in mussel tissues with resultant adverse effects on scope for growth (Widdows et al. 1995). PAHs demonstrate a range of hydrophobicity with less watersoluble PAHs tending to adsorb onto particulate material and be deposited in the sediments. Environmental inputs of PAHs into the Tamar mainly originate from road run-off, shipping activity and oil storage facilities associated with the Devonport naval port.

The importance of interactions between bioturbation and physical processes (currents and bed shear stress) in sediment resuspension and sediment dynamics has been the subject of many investigations (Rhoads & Young 1970, Rhoads 1974, Davis 1993, Paterson 1997, Widdows et al. 2000b, Widdows & Brinsley 2002, Orvain et al. 2004). Various studies have analysed the role of benthic fauna on the release of contaminants from sediments (Reible et al. 1996, Madsen et al. 1997, Schaffner et al. 1997, Ciarelli et al. 1999, Gunnarsson et al. 1999, Rasmussen et al. 2000, Christensen et al. 2002). Other studies have investigated the importance of the physical disturbance of the sediment on the resuspension of contaminants (Boehm 1983, Domagalski & Kuivila 1993, Latimer et al. 1999, Eggleton & Thomas

2004). However, the process of contaminant release from natural sediments in the context of interactions between bioturbation and physical processes is less well understood. The present study is the first to investigate joint effects of tidal-current cycles and bioturbation on contaminant release from natural sediments.

The common cockle *Cerastoderma edule* (Mollusca: Pelecypoda) was used as the bioturbating/destabiliser organism, whereas the mussel *Mytilus edulis* (Mollusca: Pelecypoda) was used as a bioindicator of the potential toxicity of contaminants released into the water column. The aims of our study were to (1) quantify the impact of bioturbation by cockles on sediment destabilisation and resuspension when exposed to simulated tidal current cycles, (2) investigate the effects of bioturbation on the release of PAHs from environmentally contaminated sediments to the overlying water, (3) establish if degree of hydrophobicity affects PAH concentration in the water column and (4) analyse whether the PAH resuspension due to biotic and abiotic factors has any toxic effects on mussels suspended in the water column.

MATERIALS AND METHODS

Experimental design. Four environmentally realistic population densities of cockles *Cerastoderma edule* were studied in 2 successive experiments. In the first experiment, one annular flume contained no cockles (referred to as 'zero' density); 53 cockles were added to the second flume, representing a density of 312 ind. m⁻² ('high' density). We also added 53 cockles to a tank containing clean, washed sand collected from Exmouth (Exe estuary, Devon, SW England). This represented the control condition for the toxicological study because of the general difficulty in obtaining uncontaminated muddy sediment. In the second experiment, 8 cockles were added to one flume and 24 cockles to the other flume, representing densities of 47 and 141 ind. m⁻² ('low' and 'medium' densities, respectively). Bivalve feeding rate (or clearance rate) was measured as an indicator of the potential sublethal toxic effect of any toxicants in the water. Clearance rate (CR) of cockles could not be determined *in situ* in the flumes because of current-induced resuspension of sediments. Therefore 12 small mussels *Mytilus edulis* (10 mm shell length) were suspended in the water column of each flume, and in the control tank, to expose them to any contaminants released into the water column. The mussels were then temporarily removed from the flumes in order to measure their CR. The first experiment lasted 9 d (36 tidal-current sinusoidal cycles) and the second 7 d (28 tidal-current sinusoidal cycles). The experiments were performed at 15°C in a

temperature-controlled laboratory with a 10:14 h light:dark photoperiod, and were carried out during April and May 2004. A final erosion study was performed at the end of each experiment (see later sub-section).

Cockles and mussels. The cockle *Cerastoderma edule* is a suspension-feeding bivalve that lives and burrows in the top few centimetres of sediment. It disturbs the sediment by its vertical and horizontal movements (Richardson et al. 1993, Flach 1996) and creates roughness on the sediment surface. It is widely distributed and abundant in estuaries and sandy bays around the coasts of Britain and Ireland. Cockles were collected from the Exe estuary at low tide and allowed to acclimatise to laboratory conditions for 5 d prior to beginning the experiments. They were maintained in a system of recirculating seawater (temperature 15°C, salinity 30) and were fed with an algal culture of *Isochrysis galbana* (20 000 cells ml⁻¹). The 4 cockle population densities chosen for the experiments were within the range of natural densities (Widdows et al. 2000a), which can reach up to 1000 ind. m⁻² (Ferns et al. 2000). The main characteristics of the cockles introduced into the flumes were mean length = 2.81 ± 0.04 cm, minimum length = 2.11 cm, maximum length = 3.62 cm, mean fresh tissue weight = 1.63 ± 0.58 g, fresh wt/dry wt = 6.26 ± 0.12.

The mussel *Mytilus edulis* is a suspension-feeding bivalve commonly used in ecotoxicology to monitor pollutant effects (Widdows et al. 1981, 1995, Pruell et al. 1986, Eertman et al. 1995, Ciarelli et al. 1999). Small mussels (mean length 10.94 ± 0.11 mm, mean fresh wt = 54.37 ± 1.80 mg, fresh wt/dry wt = 5.99 ± 0.06) were collected at low tide in Whitsand Bay (Cornwall, SW England), a coastal site with relatively low levels of contamination. They were allowed to acclimatise to laboratory conditions for 5 d prior to beginning the experiments (i.e. the same conditions as for the cockles). Small mussels were chosen to minimise additional biomass and to avoid any spawning of adult mussels during the experiment in spring. Each mussel was glued with epoxy resin onto a stainless-steel wire 24 h before beginning the experiments; 12 mussels were then suspended in the water column of each flume and 12 in the control tank.

Annular flume description. A detailed description of the annular flume and the operating procedures has been provided by Widdows et al. (1998a, 2000b). The flume was a smaller, modified version of the design described by Fukada & Lick (1980). It was constructed of acrylic material with a 64 cm (outer) and 44 cm (inner) diameter, resulting in a 10 cm channel width with a total bed area of 0.17 m², a maximum water depth of 38 cm, and a maximum volume of 60 l. The flume was controlled and logged by a portable computer using LabView software. Water flow, ranging

from 0.01 to 0.50 m s⁻¹, was generated by 4 paddles (9 × 6.5 cm) attached to a rotating cylinder driven by a motor and gearbox with built-in rpm counter. Changes in suspended sediment concentration (SSC) were monitored every 15 s with an optical backscatter sensor (OBS-3M; D & A Instruments) flush-mounted in the outer wall. The OBS output (V) was calibrated against water samples taken for gravimetric analysis during each experimental run, and calibration curves were produced for each experiment. The relationship between current speed and bed shear stress (Pa) in both Plymouth Marine Laboratory's annular flume and in the field over relatively smooth intertidal mudflats (i.e. shallow water) is described by

$$\text{Bed shear stress} = 4.7608 U^3 - 1.2607 U^2 + 0.4264 U \quad (1)$$

where U is speed (m s⁻¹) at 5 cm above the bed. This relationship was established ($r^2 = 0.99$) in the flume and field using a Sontek micro-Acoustic Doppler Velocimeter (ADV) for measuring near-bed currents and bed shear stress (turbulent kinetic energy [TKE] method; Pope et al. 2006). Sediment erodability was quantified in terms of SSC (mg l⁻¹) and mass of sediment eroded (MSE, g m⁻²), where

$$\text{MSE} = \frac{(\text{SSC} \cdot \text{volume of water})}{(\text{surface area of sediment})} \quad (2)$$

and the critical erosion velocity (U_{crit} , m s⁻¹) defined as the current speed required to resuspend sediment above a MSE threshold of 1 g m⁻² (Roast et al. 2004).

Annular flume set-up. Muddy sediment was collected from the Tamar estuary at midshore height during low tide and 250 m south of the Tamar road bridge in Plymouth. This sediment was chosen for its location near Devonport dockyard and its relatively high content of contaminants, including polycyclic aromatic hydrocarbons (PAH concentrations are shown in Fig. 1). Sediment was cored from the sampling site to a depth of 7 cm by means of stainless-steel quadrant box cores (4 cores forming an annulus) designed to fit precisely into the flume. The quadrant box cores were pushed into the sediment and dug out, base plates were inserted and the cores lifted for transportation to the relevant flume. The quadrant box cores were carefully inserted into the annular flume and the stainless-steel boxes were then removed leaving the sediment and base plates in the flume. The 4 sediment blocks were then carefully pushed together to fill any small gaps and the final space filled with a slice of sediment from an additional core. Such coring methods enable the surface features including microphytobenthic film, and the natural sediment stratification to be retained. Earlier studies have demonstrated that there are no significant differences in sediment erodability between the *in situ* and laboratory-based flume measurements using

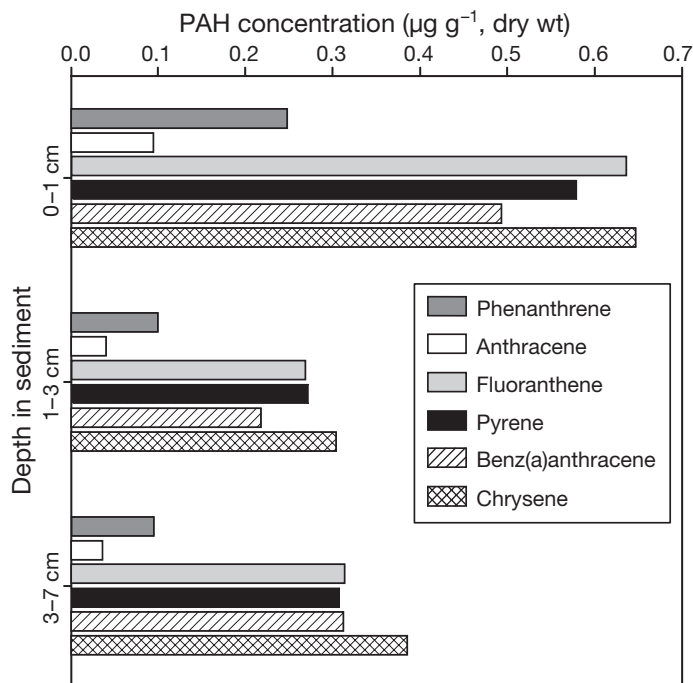


Fig. 1. Concentrations of polycyclic aromatic hydrocarbons (PAH) in Tamar estuary (Devon, SW England) sediments as a function of depth

quadrant box-cored sediment (Widdows et al. 2000a).

After inserting the sediment cores into each flume, a sheet of 'bubble wrap' the size and shape of the annulus was carefully placed on the sediment surface and seawater was gently pumped onto the sheet, which then gradually floated off without disturbing the sediment surface. The flumes were then filled with 45.9 l of water (27 cm depth) and gently aerated to maintain fully oxygenated water. Algal cells from a culture of *Isochrysis galbana* were introduced into the flumes and the control tank with a peristaltic pump to obtain a concentration of approximately 20 000 cells ml⁻¹. Cockles were then introduced into each flume and allowed to bury overnight at a low water current speed (3 cm s⁻¹); cockles were also introduced into the control tank. No cockles were added to the reference flume (0 cockles m⁻²). We also introduced 12 mussels suspended from stainless-steel wires into the flumes and the control tank. The following morning continuous sinusoidal cycles of currents were generated in the flumes to mimic tidal cycles: currents ranged from 3 to 18 cm s⁻¹ in 6 h, with 4 cycles per day. These current speeds are in the range of those recorded at the sampling site with a Sontek field ADV (1 to 19 cm s⁻¹).

Every 48 h the drive plate was stopped when the current speed was minimum (3 cm s⁻¹) and 10 l of water was removed from each flume (and from the control

tank) and replaced by new water. This partial water renewal was carried out to maintain water quality for the cockles and mussels. Precisely the same procedure was followed during the first and the second experiments. This water replacement occurred when SSC and PAH concentrations in the water column were minimum. It was anticipated that a new equilibrium between sediment and water would be re-established during the next tidal cycle (as confirmed by the water chemistry data). The PAH concentrations were not corrected for any removal of water.

Clearance rate measurements to assess sublethal toxic effects. The suspended mussels were temporarily removed from the control tank and the 2 flumes for measurement of clearance rate on Day 3 (Expts 1 and 2) and Day 8 (Expt 1). They were removed during periods of minimum current speed and each mussel (n = 12 individuals per condition) was placed in a separate beaker with 500 ml of seawater (15°C and salinity 30). Clearance rate is the volume of water (l) cleared of suspended particles h⁻¹ and was calculated from the exponential decline in cell concentration as

$$CR = V \cdot (\ln C_1 - \ln C_2) / t, \quad (3)$$

where V is the volume of water in the beaker, t is the time interval in hours, and C_1 and C_2 are the algal cell concentrations at the beginning and end of each time increment (40 min) with a total duration of 200 min. The initial algal (*Isochrysis galbana*) cell concentration was 20 000 cells ml⁻¹ and cell concentrations were determined every 40 min by means of an electronic particle counter (Coulter Counter Multisizer) using a 100 µm orifice tube (Widdows et al. 2002). After the clearance rate measurements, mussels were replaced in their respective flumes or tank when the current speed was at the lowest part of the cycle.

Final erosion study. At the end of each experiment, an erosion run was performed in each flume to quantify the sediment erodability over a wider range of current speeds/bed shear stresses. Current speed was increased stepwise from 0 to 40 cm s⁻¹ in 9 steps (0, 5, 10, 15, 19, 24, 29, 32, 37, 40 cm s⁻¹), each with a duration of 20 min. The sediment resuspension was continuously recorded by the OBS throughout the stepwise increase in current speeds. After the erosion run, cockles and mussels were removed from the flumes and tanks, maintained in clean seawater for 2 h to flush out pseudofaeces and sediment from the mantle cavity, and then the shells were rinsed in tap water and frozen at -20°C before measurement of fresh weight and dry weight.

Characterisation of sediment properties. When sediment was cored at the sampling site, surface sediment (1 cm depth) was collected and analysed for particle size (Beckman-Coulter Laser Diffraction Particle

Size Analyser LS 230), % of particulate organic matter, POM (loss on combustion at 450°C), bulk density (mass of wet sediment/volume of wet sediment) and % water content (mass of water/mass of wet sediment). Microphytobenthos density was measured by taking 1 mm-thick surface sediment and analysing it for chlorophyll *a* (extraction with acetone: excitation 436 nm, emission 680 nm; Perkin Elmer LS50B Spectrofluorometer; Welschmeyer 1994) and extra-cellular polymeric substance, EPS (phenol-sulphuric method: Jenway 6061 colorimeter; Underwood et al. 1995). The main sediment characteristics are described in Table 1. The mean grain-size was 58.8 µm, with 72% of particles below 63 µm in diameter.

Extraction of PAHs from water and sediments. PAHs in sediments were analysed according to the procedure described by King et al. (2004). All glassware, spatulas, filters and sodium sulphate were pre-cleaned thoroughly before use. Solvents were of glass-distilled grade (Rathburns Chemicals). Phenanthrene-d10 (Supelco, 10 µg) was used as an internal standard (IS) for both sediment and water analyses. In both experiments, water was sampled after 2 and 6 d, at minimum and maximum current speeds in each flume (3 and 18 cm s⁻¹, respectively), as well as from the control tank. Samples were also collected during the first maximum current speeds in the second experiment (making a total of 20 samples). Water samples of 1.8 l were collected in 2 l pre-cleaned glass bottles. The IS and 50 ml of Dichloromethane (DCM) were added to the samples before storing them in a dark, cool (5°C) room until analysis. Solvent extracts were dried with ashed sodium sulphate and then reduced in volume to 2 ml by rotary evaporation and then down to 250 µl under a gentle stream of high-purity nitrogen. Sedi-

ment samples were extracted into 10 ml DCM by ultrasonication for 1 h, and then filtered to remove particles (Whatman GF/C filters). The filtrate was dried with ashed sodium sulphate. Samples were reduced in volume by rotary evaporation followed by nitrogen blow-down to yield an extract of 250 µl volume.

Quantification of PAHs in water and sediments. Aliquots (1 µl) from the sample extracts were analysed using GC/MS (GC 6890N and MS 5973 mass selective detector; Agilent Technologies) fitted with an Rtx-5MS PTE-5 fused-silica capillary column ([5%]-diphenyl-[95%]-dimethylsiloxane, 0.25 mm × 30 m × 0.25 µm; Agilent Technologies). The injector port was operated in splitless mode with the oven temperature programmed from 40 to 300°C at a rate of 6°C min⁻¹. The MS was operated in electron impact mode, and target compounds were quantified using selected ion-monitoring of the molecular ions. Samples were also analysed in full-scan acquisition for compound identity confirmation. Blank extractions were performed with each batch of sediment and water samples to check for any analytical contamination. Blanks of DCM were run with each batch of samples. Concentrations were tested against the certified reference material IAEA-408 sediment, which had been assigned reference values for concentrations of selected PAH. It was extracted in the same way as samples. The mean recoveries were in the range of 70 to 110%. Calibration curves were established using a standard solution of 16 PAHs (TCL polynuclear aromatic hydrocarbon Mix, each at 2000 µg ml⁻¹; Supelco) including phenanthrene, anthracene, fluoranthene, pyrene, benz[a]-anthracene and chrysene.

Statistical analysis. We performed 1- and 2-way ANOVA on SSC data and on data from the clearance rate measurements of mussels. Fisher's least-significant difference (LSD) test was then used to determine significant differences amongst conditions. All data were tested for homoscedasticity (Cochran's *C*-test) and normality (χ^2 test). All tests were carried out using the statistical software Minitab® Statistical Software-8.

RESULTS

Sediment resuspension during simulated tidal current cycles

The flumes were effective in simulating continuous tidal cycles of currents. As a result they were able to induce resuspension of sediments and contaminants at environmentally realistic current speeds and the de-

Table 1. Main characteristics of surficial sediment sampled from Tamar estuary (SW England) mudflats at mid-tide level, about 250 m south of Tamar Road Bridge

Sediment characteristics	Mean ± SD
Chlorophyll <i>a</i> (µg g ⁻¹ , wet wt)	19.62 ± 0.92
Extra-cellular polymeric substances (EPS) as glucose equivalent (µg g ⁻¹ , wet wt)	376.4 ± 90.6
Bulk density (g ml ⁻¹ , wet wt)	1.33 ± 0.01
Water content (% wet wt)	59.44 ± 0.20
Particulate organic matter (POM; % dry wt)	6.69 ± 0.04
Grain-size (% of total)	
fine silt and clay (<15 µm)	33.3 ± 1.3
medium silt (15–30 µm)	16.2 ± 1.5
coarse silt (30–63 µm)	22.8 ± 1.1
very fine sand (63–125 µm)	16.3 ± 2.0
fine sand (125–250 µm)	7.4 ± 1.7
medium sand (250–500 µm)	3.2 ± 0.8
coarse sand (500–1000 µm)	0.8 ± 0.0
very coarse sand (>1000 µm)	0.0 ± 0.0

gree of resuspension was a function of cockle density. Fig. 2 illustrates the suspended sediment concentrations (SSC) measured in the flumes during the 4 tidal cycles of the 3rd day of the 2 experiments; the graphs are representative of events during all the simulated tidal cycles. For each flume condition, and even without cockles, the SSC increased with increases in current speed above 12.3 cm s^{-1} , and decreased when the current speeds declined. The presence of cockles enhanced this effect. Suspended sediment concentrations at the peak current speeds increased as a function of cockle density from zero to medium density, but the average maximum SSC for the high density of cockles was similar to that for the medium density.

Mean SSC and mass of sediment eroded (MSE) measured at minimal and maximal current speeds throughout Expts 1 and 2 for the 4 cockle densities are shown in Fig. 3. SSC and MSE at minimal current speeds were significantly lower than those at maximal current speed for all experimental conditions ($p < 0.001$). SSC and MSE at the minimum current speeds for the condition without cockles (SSC: $6.4 \pm 0.3 \text{ mg l}^{-1}$; MSE: $1.8 \pm 0.5 \text{ g m}^{-2}$) were significantly lower ($p < 0.05$) than those recorded for the 3 conditions with cockles (SSC: low = 10.1 ± 0.3 , medium = 9.4 ± 0.3 , high = $8.3 \pm 0.3 \text{ mg l}^{-1}$; MSE: low = 2.8 ± 0.4 , medium = 2.6 ± 0.5 , high = $2.3 \pm 0.6 \text{ g m}^{-2}$). There was also a statistical difference ($p < 0.05$) between the low and high densities. The SSC and MSE at maximum currents increased significantly with increasing cockle density from zero to medium density (SSC: zero = 12.7 ± 0.6 , low = 36.0 ± 2.1 , medium = $67.7 \pm 4.2 \text{ mg l}^{-1}$; MSE: zero = 3.6 ± 1.1 , low = 10.1

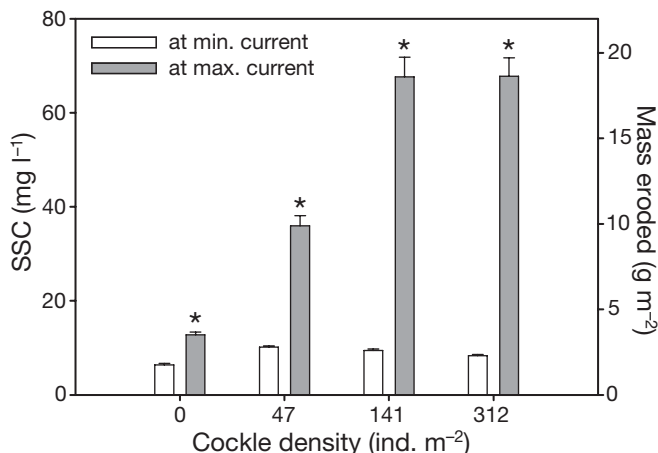


Fig. 3. Mean (\pm SE; $n = 28$) maximum and minimum suspended sediment concentration (SSC) and mass of sediment eroded throughout Expts 1 and 2 as a function of cockle *Cerastoderma edule* density. *Significant difference ($p < 0.001$) between SSC at max. current speed (18 cm s^{-1}) compared to min. current speed (3 cm s^{-1}); ANOVA followed by LSD test

± 3.1 , medium = $18.9 \pm 6.2 \text{ g m}^{-2}$), but there were no significant differences between the medium and high densities (for high density, SSC = $67.8 \pm 4.0 \text{ mg l}^{-1}$ and MSE = $19.0 \pm 6.7 \text{ g m}^{-2}$; Fig. 3).

The 8 tidal cycles corresponding to the 3rd and 4th days of the experiments were studied in detail to gain more insight into the erosion and deposition processes (Fig. 4). The most important fact is that the ascending resuspension curves and the descending deposition

curves were asymmetric. At a given current speed the SSC was lower during the phase of increasing current (e.g. at 14 cm s^{-1} , SSC zero = 7.7 ± 0.3 , low = 13.3 ± 0.4 , medium = 15.0 ± 1.1 , high = $19.5 \pm 1.1 \text{ mg l}^{-1}$) than during the phase of decreasing current (at 14 cm s^{-1} , SSC zero = 12.0 ± 0.9 , low = 29.3 ± 2.6 , medium = 41.5 ± 5.8 , high = $49.1 \pm 6.2 \text{ mg l}^{-1}$) (Fig. 4A). A current speed of 16 cm s^{-1} was required to resuspend 50% of the maximum SSC at 18 cm s^{-1} for all cockle densities during the erosion phase, whereas during the deposition phase 50% of the maximum SSC occurred at 13 cm s^{-1} (Fig. 4A). Therefore, the onset and peak of sediment fluxes (g m^{-2}) occurred at a higher current speed for sediment erosion than for deposition (Fig. 4B). This is consistent with the fact that critical erosion thresholds are higher than critical deposition thresholds. The critical

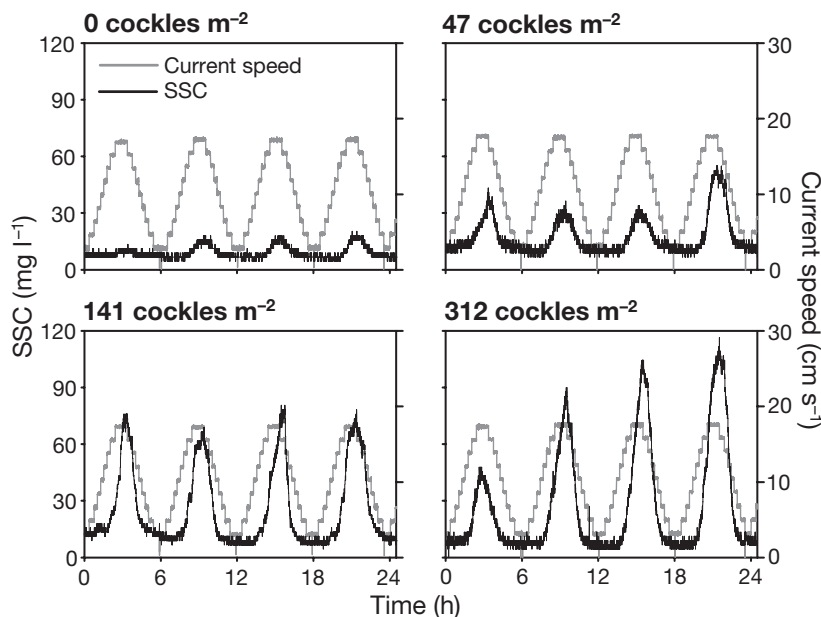


Fig. 2. Examples of the 4 tidal-current cycles during Day 3 of Expts 1 and 2. SSC: suspended sediment concentration

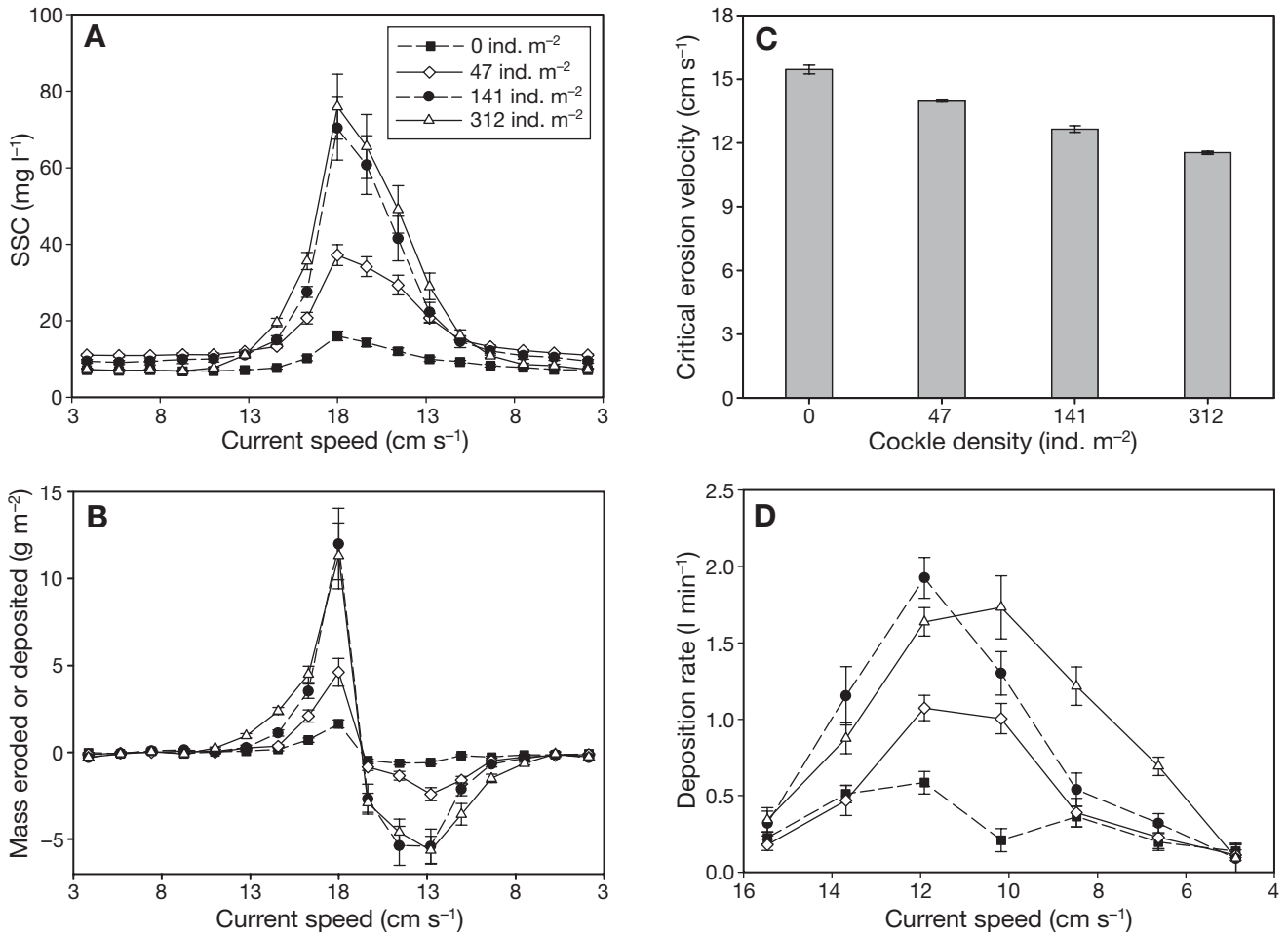


Fig. 4. (A) Mean (\pm SE) suspended sediment concentration (SSC) during 8 tidal cycles (corresponding to 3rd and 4th days of each experiment) as a function of current speed and cockle *Cerastoderma edule* density. (B) Mean (\pm SE) mass of sediment eroded (positive values) or deposited (negative values) as a function of current speed (according to the stage in the cycle) and cockle density, based on mean of the 8 tidal cycles. (C) Mean (\pm SE) critical erosion velocity, U_{crit} (current speed required to erode 1 g of sediment bed m^{-2}), at the 4 cockle densities during the 8 tidal cycles. (D) Mean (\pm SE) sediment deposition rate as a function of current speed during descending component of the 8 tidal cycles; total deposition rate includes both physical deposition rate and biodeposition rate due to cockles (when present), and was calculated for the flume using Eq. (3) with C as SSC and t in min

erosion velocity U_{crit} was also calculated during these 8 tidal cycles. U_{crit} decreased from zero to high density of cockles: zero = 15.5 ± 0.2 , low = 14.0 ± 0.1 , medium = 12.7 ± 0.2 , high = $11.5 \pm 0.1 \text{ cm s}^{-1}$ (Fig. 4C).

The deposition rates during the descending part of the current cycles (from 18 to 3 cm s^{-1}) were calculated and represent the flux of sediment from the water column to the bed due to both the physical sedimentation rate and the biodeposition rate when cockles were present. The results show that the maximum deposition rates were 0.59, 1.07, 1.93 and $1.73 \text{ l min}^{-1} \text{ flume}^{-1}$ for zero, low, medium and high densities respectively (Fig. 4D). The deposition rate is a function of both the SSC and the suspension-feeding activity of the cockles, both of which are dependent on cockle density.

Sediment resuspension during final erosion experiment

The erosion potential was measured in terms of suspended sediment concentration (SSC, mg l^{-1}), critical erosion velocity (U_{crit} , cm s^{-1}) and mass of sediment eroded (MSE, g m^{-2}) in relation to increasing current speed. The influence of cockle density on the time course of SSC and mass MSE following a stepwise increase in current speeds are presented in Fig. 5A and B, respectively. Sediment resuspension increased as a function of increasing current speed and cockle density from zero to medium density. At the maximum current speed of 40 cm s^{-1} , SSC reached 213, 1055 and 2085 mg l^{-1} and MSE reached 60, 295 and 584 g m^{-2} for zero, low and medium densities, respectively. The sediment

resuspension for the high cockle density was lower than that for medium density at all current speeds. The values at 40 cm s^{-1} were 1792 mg l^{-1} for SSC and 502 g m^{-2} for MSE. U_{crit} values calculated for each cockle density decreased from 15 to 14 and 8 cm s^{-1} at zero, low and medium densities, respectively, and then increased slightly at the high density (11 cm s^{-1}).

PAH concentrations in Tamar Estuary sediment

The sediment collected from the Tamar mudflat was analysed for PAHs and numerous other contaminants (including saturated hydrocarbons, phthalates and phenolics). Phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene and chrysene were selected as representative PAHs for quantification (Fig. 1), with highest concentrations in the 0 to 1 cm layer (0.25 , 0.10 , 0.64 , 0.58 , 0.49 and $0.65 \text{ } \mu\text{g g}^{-1}$ dry wt, respectively). PAH concentrations were approximately 50% lower at both 1 to 3 cm and 3 to 7 cm depth intervals, although the relative proportions of the individual PAHs was similar in the 3 layers.

PAH concentrations in water column following re-suspension during simulated cycles in tidal currents

Fig. 6 shows the concentrations of phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene and chrysene in the water column as a function of the SSC in the flumes when the water samples were collected. The plots correspond to all water samples collected during the experiments. The SSC ranged from 6.8 to 73.5 mg l^{-1} . Linear regressions have been plotted to study the link between the SSC and individual PAH concentrations. The relationships between the concentrations of 3-ring PAHs in the water column and the SSC were either not significant (phenanthrene) or had a p -value > 0.01 (anthracene). Concentrations ranged from 2.9 to 26.5 ng l^{-1} for phenanthrene and 0.4 to 3.1 ng l^{-1} for anthracene. In contrast, there were highly significant relationships between concentrations of the 4-ring PAHs (fluoranthene, pyrene, benz(a)anthracene and chrysene) in the water column and the SSC ($r^2 > 0.80$; $p < 0.0001$). The concentrations of these PAHs increased with increasing SSC. Values ranged from 1.5 to 21.6 ng l^{-1} for fluoranthene, from 1.4 to 19.1 ng l^{-1} for pyrene, from < 0.1 to 28.5 ng l^{-1} for benz(a)anthracene and from < 0.1 to 32.8 ng l^{-1} for chrysene.

Sublethal effects of resuspended contaminants

The clearance rates of the suspended mussels were surprisingly unaffected by the different experimental

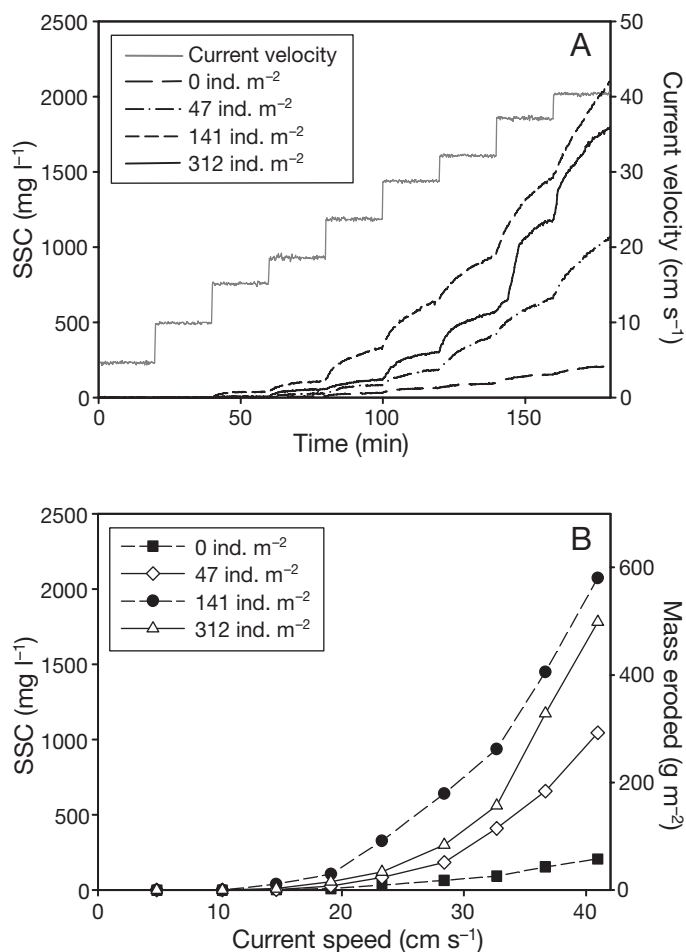


Fig. 5. (A) Suspended sediment concentration (SSC) during final erosion run as a function of time; (B) SSC and mass of sediment eroded at end of each incremental step in current speed in final erosion run

treatments. There were no significant differences between the flumes or tanks containing different sediment types and different cockle densities after 3 or 8 d exposure to different contaminant concentrations (Table 2). The mean values for the clearance rates ranged from 0.21 ± 0.02 to $0.29 \pm 0.02 \text{ l h}^{-1} \text{ mussel}^{-1}$.

DISCUSSION

The flumes successfully recreated the ~12 h diurnal tidal cycles, with peak flows associated with the flood and ebb tides, and intermediate periods of slack water. These flume experiments not only quantified the increase in sediment resuspension with increasing current speed, but also showed a marked increase in sediment erodability with increasing population density of the cockle *Cerastoderma edule*. In addition, the

results demonstrated the combined effects of bioturbation and tidal-current cycles on the resuspension of contaminants. The current cycling regime was well within the range that cockles and mussels experience in the environment, and previous studies have shown that their feeding/clearance rates are maintained independent of current speeds up to 0.45 m s^{-1} (*C. edule*; Navarro & Widdows, in press) and 0.8 m s^{-1} (*Mytilus edulis*; Widdows et al. 2002).

Sediment destabilisation by cockles

After burrowing into the sediment, cockles *Cerastoderma edule* remain active and continue to move periodically, as demonstrated by Flach (1996). Flach (1996) also noted that cockles 'shake' themselves regularly (i.e. sudden valve adduction), thus disturbing the surrounding sediment. Richardson et al. (1993) demonstrated that when cockles *C. edule* were exposed to tidal cycles of emersion and immersion and a light regime of 12:12 h light:dark, up to 40% of the group emerged onto the surface of the substratum when the period of air exposure coincided with the onset of darkness. Therefore cockles have a range of behavioural activities within the sediment that loosen the sediment and increase bed roughness, resulting in higher sediment erodability and thus resuspension during the tidal current cycles.

At the medium and high cockle population densities of the present study, the mean amount of sediment resuspended was similar throughout the experiment (9 d). However, during the course of the experiment there was evidence of an overall decline in sediment resuspension at the highest density, reflecting a gradual reduction in the destabilisation of the sediment bed by cockles at high density. At the beginning of the experiment, sediment resuspension was approximately 2-fold higher than at the end (i.e. $112 \pm 14 \text{ mg l}^{-1}$ compared to $55 \pm 3 \text{ mg l}^{-1}$ at 18 cm s^{-1}). This gradual reduction in the degree of sediment destabilisation was probably associated with enhanced mucus production by the highest density of cockles.

The gills of suspension-feeding bivalves produce copious amounts of mucus in response to elevated SSC and as a result produce large quantities of pseudofaeces. This is the primary mechanism for dealing with the excess sediment (filtered by the gills), which the cockles are unable to ingest. These pseudofaeces are

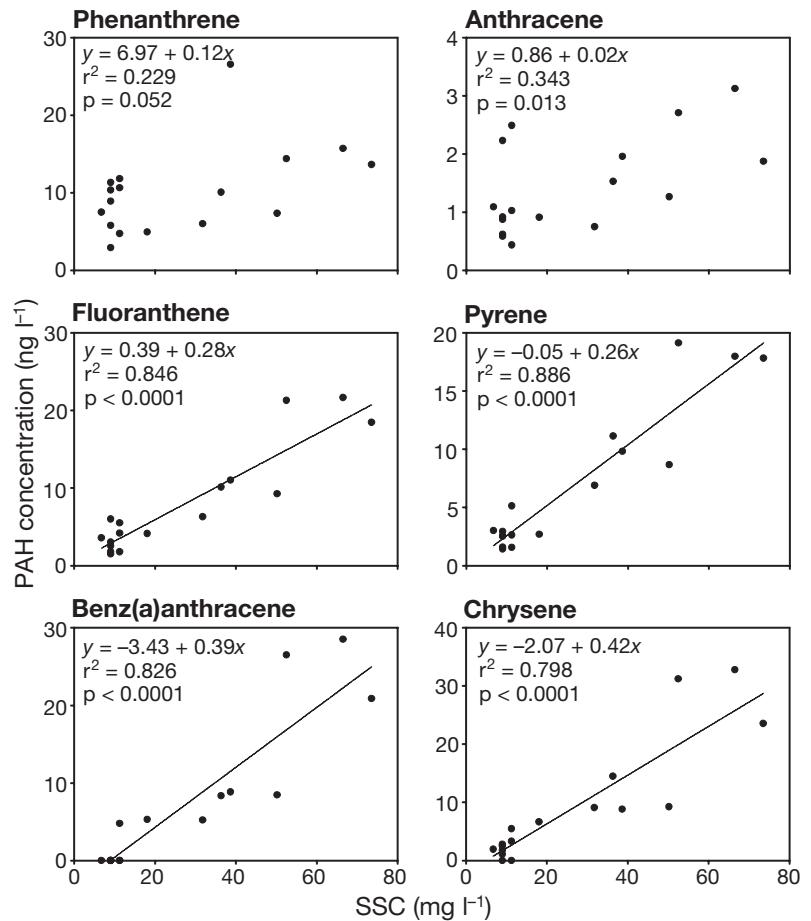


Fig. 6. Polycyclic aromatic hydrocarbons (PAH) concentrations in water column as a function of suspended sediment concentration (SSC)

Table 2. *Mytilus edulis*. Maximum clearance rates (CR; mean \pm SE) during 40 min period, as a measure of toxicity induced by bioaccumulated contaminants in different experimental conditions (4 cockle *Cerastoderma edule* population densities in contaminated Tamar sediment and 1 control with 312 cockles m⁻² in clean sand). nd: not determined. Eq. (3) used for calculation of CR

Cockle density (ind. m ⁻²)	Max. clearance rate (l h ⁻¹ mussel ⁻¹)	
	after 3 d	after 8 d
Control (312)	0.276 ± 0.051	0.214 ± 0.020
0	0.253 ± 0.040	0.262 ± 0.014
47	0.259 ± 0.018	nd
141	0.291 ± 0.024	nd
312	0.278 ± 0.017	0.266 ± 0.014

largely mucus-bound sediment particles, which are ejected from the mantle cavity and deposited on the sediment surface. The addition of large quantities of mucus is likely to enhance sediment cohesion. The rate of pseudofaeces production by *Cerastoderma edule* increases with increasing SSC and/or the inorganic

content of the seston (Iglesias et al. 1996, Navarro & Widdows 1997, Ibarrola et al. 2000, Urrutia et al. 2001). Therefore, increasing cockle population density enhances SSC, which in turn increases mucus and pseudofaeces production. At the highest cockle density there was some indication that high mucus production partially dampens or reduces sediment resuspension. Hence, there was a gradual lowering of SSC at the higher current speeds during the course of the 9 d experiment with the high cockle density. Furthermore, this is consistent with the lower sediment resuspension in the final erosion experiment at the high cockle density compared to the medium cockle density (see below). The hypothesis that mucus production by molluscs acts to bind sediment particles is supported by the study of Hannides et al. (2005), who showed that the marine snails *Neverita duplicata* and *Euspira heros* crawling over glass beads increased the cohesiveness of the beads compared to clean beads.

Detailed examination of the 8 tidal cycles corresponding to the 3rd and 4th days of the experiment gives more insight into the erosion and deposition processes occurring during each tidal-current cycle. The present study demonstrates the asymmetrical nature of the erosion and deposition processes in relation to current speed. Regardless of cockle population density, the current speed that resuspends 50% of the maximum SSC at 18 cm s^{-1} is higher than that at which 50% of the maximum SSC is deposited. This is consistent with the notion that the critical erosion threshold is higher than the deposition threshold (Partheniades 1971). Furthermore, the critical erosion threshold decreased with increasing cockle density, and the current speed corresponding to the end of sediment deposition decreased with increasing cockle density. Consequently, sediment was resuspended for a longer period of time as a function of cockle density (i.e. the sediment was resuspended earlier and deposited later with increasing cockle density). The increased mass of sediment eroded as well as the time in suspension may have important environmental implications in terms of enhancing the mobilisation and transport of contaminated sediments.

The process of sediment deposition during the decelerating part of the current cycles will involve 2 components, the physical sedimentation rate and the biodeposition rate when cockles are present. To estimate the role of the cockles' filtration rate in this sediment deposition process, the mean flume clearance rates (i.e. the volume of water cleared of particles h^{-1} due to both physical and biological processes) during the same 8 tidal cycles (corresponding to the 3rd and 4th days) were calculated. Navarro & Widdows (1997) measured the clearance rates of individual cockles in response to a wide range of SSC and demonstrated a

logarithmic decline with increasing SSC. Our calculation of the total biodeposition rates was based on the number of cockles in the flume and their clearance rates at the corresponding SSC (from Navarro & Widdows 1997), as the mean SSC declined during the deceleration part of the current cycles. Depending on the stage in the deceleration part of the current cycles and the SSC (i.e. cockle clearance rates increased with declining SSC), the cockles' biodeposition rate varied as a proportion of the total deposition rate. At the low cockle density the biodeposition rate accounted for 12 to 38% of the total deposition rate, at the medium density it was 20 to 84%, and at the high density it was 49 to 84%. The maximum contribution occurred at an SSC of approximately 11 mg l^{-1} and current speeds of 8.5 cm s^{-1} . Consequently, under these conditions cockles can provide a major contribution to the total sedimentation process (i.e. up to 84% through filtration and biodeposition at higher cockle densities).

The final erosion studies allowed further quantification of the sediment destabilisation induced by the cockles. The critical erosion velocity (U_{crit}) decreased with increasing cockle population density from zero to medium density, but at high density increased slightly and was greater than the U_{crit} for medium density. Expressed as a percentage of the U_{crit} under control conditions, the U_{crit} is reduced by 21% for the low density, by 40% for the medium density and by 30% for the high density. Cockles appeared to have a strong impact on the potential for resuspension of intertidal sediments, even at lower densities, but this effect was not linear. The final erosion experiments also clearly demonstrated that the sediment destabilisation induced by the high population density was lower than at the medium density. At low and medium densities, bioturbation by cockles clearly resulted in sediment destabilisation with little evidence of mucus associated with moderate quantities of biodeposits reducing sediment resuspension. At the high density, mucus production appeared to contribute to the partial stabilisation of the sediment, thus decreasing the full impact of bioturbation. It is interesting that for zero, low and high densities the U_{crit} values for the 8 tidal cycles on the 3rd and 4th days of the experiment were very similar to those during the final erosion run. These results provide evidence of good agreement between the erodability data obtained from tidal cycles and stepwise increases in current speed.

The values for U_{crit} and the MSE in the present study are within the range reported in earlier studies and are dependent on the nature of the sediment and the abundance of the bio-stabilisers and destabilisers present (Widdows et al. 2000b, 2004). When sediments are dominated by bio-stabilisers, U_{crit} values are high ($>18 \text{ cm s}^{-1}$) and MSE values are low. Previous experi-

mental studies (Widdows et al. 1998b) with the bioturbating clam *Macoma balthica* showed U_{crit} values of 10 to 15 cm s⁻¹ with current-induced sediment resuspension increasing with increasing clam density; however, the effect was non-linear with evidence of an asymptote at the highest densities. Other studies have recorded non-linear effects of organism density on sedimentary processes (for example the effect of increasing *M. balthica* density on sediment geochemistry; Marinelli & Williams 2003), and rates of N remineralisation and denitrification are highly dependent on sedimentary biogenic structures and the geometries and distribution of irrigated burrows (Gilbert et al. 2003).

PAH concentrations in Tamar sediments

The 6 PAHs analysed in the water and sediment samples of this study only represent 'marker' compounds within a complex mixture of contaminants. The concentrations of PAHs we found in the Tamar Estuary sediment were comparable to those reported in previous studies in the same area. Our values are, however, 18 to 33% lower than the concentrations reported by Readman et al. (1982) and 21 to 49% lower than those reported by Woodhead et al. (1999) for Tamar estuary sediments taken in 1993 at 2 sites near the present sampling location. The reductions in PAHs in 2004 compared with those in the previous studies (in 1980 and 1993) are consistent with a decline in sedimentary PAH contamination reported by Readman et al. (1987), and probably reflect improved environmental regulations and management. The distribution pattern of the PAHs measured in this study is characteristic of a pyrogenic source (i.e. fuel combustion) rather than a petrogenic source (i.e. spillage of crude or refined oil).

Resuspension of PAH-contaminated sediment

In this study, the release of PAHs from the sediment to the overlying water appears to be a function of the molecular weight (MW) and associated solubility/hydrophobicity of the relevant molecule. The concentrations of 4-ring PAHs in the overlying water column were strongly correlated with sediment resuspension because they are hydrophobic and tightly bound to the sediment particles. In contrast, the concentrations of the more water-soluble 3-ring PAHs, which are less tightly bound to sediment particles, were not significantly correlated with the SSC. Consequently, the lower MW PAHs (phenanthrene and anthracene) may have been released into the overlying water by direct

diffusion from the sediment pore water, and/or by resuspension with the particles and desorbed once in the water column. This behaviour of the PAHs with respect to molecular weight is consistent with observations on particulate-PAH interactions reported for the Tamar Estuary water column by Readman et al. (1982). Water column concentrations recorded by Readman et al. (1982) and by Law et al. (1997) were generally within the same range as those measured in our flume experiments.

Several studies have demonstrated up to a 10-fold increase in the concentration of organic contaminants (polychlorinated biphenyls, PAHs, coprostanol) in the water column due to physical disturbance and tidal resuspension processes (Boehm 1983, Domagalski & Kuivila 1993, Latimer et al. 1999, Eggleton & Thomas 2004). Other studies have demonstrated the important role of both estuarine and marine benthic fauna in the resuspension of contaminants from sediments, e.g. metals (Rasmussen et al. 2000) and organic pollutants (Reible et al. 1996, Madsen et al. 1997, Schaffner et al. 1997, Ciarelli et al. 1999, Gunnarsson et al. 1999, Christensen et al. 2002). However, to our knowledge, the present study is the first to reveal the combined effects of bioturbation and tidal-current cycles on contaminant resuspension.

Predicted PAH concentrations in the water column have been calculated from the product of PAH concentrations measured in the bed sediment and the SSC recorded during the water sampling for PAH measurements. The measured concentrations were 1.3- to 2-fold lower than the predicted concentrations. The lower than predicted PAH concentrations are unlikely to be due to the partial water changes, because these occurred when the currents, SSC and PAH concentrations were at their lowest in the water column, thus minimising the removal of PAHs from the system. The fact that the measured concentrations were lower than the predicted concentrations may indicate differential binding of PAHs to particles of varying size/density with different resuspension characteristics. The combined effects of bioturbation and water flow led to the selective resuspension of smaller sediment particles, which stayed longer in suspension than the larger particles. The mean grain-size of particles in suspension was 33 µm, with 88% of the particles below 63 µm in diameter, while the mean grain-size of the sediment was 59 µm, with 72% of the particles below 63 µm in diameter. Therefore the results suggest that the 4 higher MW PAHs (fluoranthene, pyrene, benz[a]anthracene and chrysene) were preferentially bound to, or associated with, the larger sediment particles. This is consistent with results of Readman et al. (1984) and is also in agreement with the study of Ahrens & Depree (2004), who showed that the highest PAH concentra-

tions occurred in the larger grain-size fractions (125 to 1000 μm). At the Tamar site, these grain-size fractions contributed only 11% of the total sediment volume and would be less easily resuspended, thus accounting for the lower measured versus predicted PAH concentrations in the water column.

PAH toxic effects on mussels

The mussel clearance or feeding rate provides a sensitive response to sublethal stress and toxicants (Widdows & Donkin 1991). The clearance rate measurements for mussels of 1 cm shell length in this study were between 0.21 ± 0.02 and 0.29 ± 0.02 l h^{-1} mussel $^{-1}$. When converted to a standard 1 g mussel using a weight exponent of 0.67, the clearance rates range between 5.0 and 6.8 $\text{l h}^{-1} \text{g}^{-1}$, which is representative of healthy unstressed mussels (Widdows et al. 1995). In the present study, clearance rate was surprisingly unaffected by exposure to any contaminants released from the Tamar sediments into the overlying water column during the 3 and 8 d exposure to tidal current cycles. This is in contrast to previous studies of mussels from the Tamar estuary which recorded marked reductions in clearance rate and scope for growth (Widdows et al. 1995) in response to elevated concentrations of 2- and 3-ring PAHs (reflecting a more petrogenic source). However, these lower MW PAHs and their alkylated homologues were not detected at measurable concentrations in the Tamar sediment in the present study, indicating that there had been no significant and recent oil contamination prior to sampling the sediment. The lack of a measurable toxic effect on mussel clearance rate is consistent with the bioavailability and toxicity of the PAHs found in the water and sediment (Donkin & Widdows 1990, Widdows & Donkin 1991). The bioavailable and toxic lower MW PAHs originating from petrogenic sources were not significant contaminants in the sediment, whereas the predominantly higher MW and sediment-bound PAHs were only released into the water column when there was resuspension during periods of higher current speeds. Speciation (for example occlusion) of the PAHs would also explain these observations (Readman et al. 1984).

A few studies have also examined sediment resuspension and PAH effects on marine invertebrates. Ciarelli et al. (1999), using sediment artificially spiked with very high concentrations of fluoranthene, demonstrated that bioturbation by *Corophium volutator* led to a significant increase in sediment resuspension within 2 d. Uptake of fluoranthene into the tissues of *C. volutator* and mussels *Mytilus edulis* held in the same tanks was shown to be dependent on *C. volu-*

tator density (op. cit.). Eertman et al. (1995) found an increase in fluoranthene and benzo(a)pyrene accumulation by *Mytilus edulis* with time and water concentration (1 and 6 $\mu\text{g l}^{-1}$). They also demonstrated that fluoranthene and benzo(a)pyrene significantly reduced the clearance rate of mussels at these relatively high water and tissue concentrations. Pruell et al. (1986) showed that several 4-, 5- and 6-ring PAHs were accumulated at high levels in mussels exposed to contaminated sediment (0.2 to 1.5 $\mu\text{g g}^{-1}$ dry wt depending on the compound). These different results indicate that various factors can influence contaminant resuspension as well as their bioavailability, uptake and toxic effect. These include the concentration of PAHs in the sediment, the way in which they are bound (spiked or naturally bound), the sediment:water ratio, the number and biomass of organisms and exposure time.

Environmental implications

This flume study has simulated the tidal cycling of currents in estuaries and demonstrated that the interaction between bioturbation by cockles and currents can result in a more than 5-fold increase in sediment erosion and contaminant flux to the water column. Sediment resuspension is likely to be increased further (10-fold) at higher current speeds and bed shear stresses associated with more extreme conditions (e.g. high fluvial flows and storm events). Depending on the intensity of physical forces and the extent to which biota modify sediment stability, sediments can act as both a 'sink' or storage reservoir for contaminants and a 'source' of remobilised contaminants. When contaminant levels are sufficiently high to induce toxic effects, any sediment resuspension will disperse pollutants more widely within an estuary with the potential for adverse effects over a larger area. However, physical and biological processes enhancing the resuspension of more moderately contaminated sediments, such as those in the Tamar, are likely to form an important part of the natural bioremediation process. Bioturbation will lead to oxygenation of the surface sediments, allowing microbial degradation and photo-oxidation of the contaminants, and their subsequent transport and dispersion within the estuarine and coastal zone.

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