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Pyrene degradation in marine infauna and sediment

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Effects of sediment organic matter quality on bioaccumulation, degradation, and distribution of pyrene in two macrofaunal species and their surrounding sediment

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ABSTRACT

Sediment dwelling macrofauna (infauna) are important vectors for the transfer of sediment associated contaminants to higher trophic levels. Sedimenting organic matter constitutes an important food source for all benthic organisms and changes seasonally in terms of quantity and quality. Sediment organic matter (SOM) quality affects organism activity and feeding behaviour, and is therefore also likely to affect contaminant fate in benthic systems. We investigated the impact of SOM quality (enrichment with either labile *Tetraselmis* sp. or refractory lignin) on the accumulation and metabolism of sediment-associated pyrene in *Nereis diversicolor* (Annelida) and *Amphiura filiformis* (Echinodermata), as well as the combined effect of SOM quality and infauna on pyrene distribution and metabolism in the sediment. After 45 d of exposure, SOM quality almost doubled pyrene bioaccumulation in both species while pyrene metabolism remained unaffected. Metabolites comprised ~80 % of the total tissue pyrene in *N. diversicolor* and ~40 % in *A. filiformis*. *A. filiformis* arms contained one fifth of the disk pyrene concentration. Approximately 20 % of the pyrene found in *A. filiformis* arms was found to be covalently bound to, e.g. DNA, RNA or proteins, thus reducing pyrene bioavailability to arm-cropping predators. The sedimentary pyrene distribution and metabolism was species-dependent, but correlated poorly with prevailing knowledge on species-specific bioturbation patterns. This was attributed to the comparably high biodegradability of the contaminant thus altering its sorptive characteristics. Subduction of pyrene and metabolites occurred, and the fraction of pyrene covalently bound to SOM increased with depth, thereby removing pyrene from the bioavailable pool. Our results imply that bioaccumulation and trophic transfer of sediment-associated PAH should increase following fresh organic matter input, e.g. after phytoplankton blooms. We stress the importance of considering behavioural characteristics of infauna and the trophic situation of the system when assessing fate and effects of sediment-associated contaminants.

INTRODUCTION

Contaminated sediments constitute large environmental pollutant sources. Since sediment dwelling invertebrates (infauna) are an important food source for many other species, the extent to which they accumulate, metabolise, and affect distribution and microbial degradation of contaminants in the sediment is crucial not only for their own exposure but also for the propagation of contaminants along food chains. Commercial fish and crustacean species are important predators on infauna, and sediment processes are therefore important also for human health. Pyrene is a four-ringed high molecular weight polycyclic aromatic hydrocarbon (PAH) of pyrogenic origin, that together with fluoranthene, benzo(*b*)fluoranthene, and benzo(*e*)pyrene is one of the most abundant of the PAHs in estuarine and marine sediments (HELCOM, 1996, OSPAR-Commission, 2000). Since many PAHs, as well as their metabolites, are acutely toxic, mutagenic and carcinogenic, this contaminant group evokes high environmental concern (Jerina & Daly, 1974, OSPAR-Commission, 2000). Sediment associated organic contaminants are readily absorbed by sediment dwelling fauna both through ingestion of contaminated particles and through passive uptake via body surfaces.

In contrast to other important organic contaminant groups, e.g. polychlorinated biphenyls (PCBs), PAHs are more readily biotransformed by most organism groups, however, to a greatly varying extent (Cerniglia & Heitkamp, 1989, Livingstone, 1992). Like other eucaryotes, marine invertebrates use the cytochrome P-450 monooxygenase or mixed function oxygenase (MFO) enzyme system for the biotransformation of PAHs (James, 1989, den Besten, 1998, James & Boyle, 1998, Lee, 1998, Snyder, 2000). Aside from catalyzing a great variety of endogenous reactions, as for instance the metabolism of steroid hormones and fatty acids (Mansuy, 1998), the MFO system converts hydrophobic parent PAH to more easily eliminated water-soluble metabolites in a two-phase process. Oxidative phase I enzymes

introduce a functional group (e.g. O, OH, COOH) into the PAH molecule, providing it with a site for the subsequent attachment of larger polar molecules (such as carbohydrates, glutation, amino acids or sulfates) facilitated by conjugating phase II enzymes (Buhler & Williams, 1989, Foureman, 1989). The ability to metabolize PAH varies greatly both among and within marine invertebrate phyla, yet metabolic capacities appear to be more efficient in arthropods and annelids compared to molluscs, while information on echinoderms is scarce (Livingstone, 1992, Meador, Stein, Reichert & Varanasi, 1995, Kane Driscoll & McElroy, 1996, Livingstone, 1998). Although the regulation of MFO enzymes in marine invertebrates is poorly understood (Snyder, 2000, Hahn, 2002), a number of factors have been suggested to affect the biotransformation capacity of PAHs including; sex, age, contaminant pre-exposure, and dietary conditions (Buhler & Williams, 1989, Foureman, 1989, Forbes, Forbes & Holmer, 1996). The general importance of these factors remains yet to be resolved.

Since sedimenting organic matter constitutes an important food source and substrate for benthic macro - and microorganisms, seasonal variations in its quantity and quality affects organism growth, production, metabolism and activity (Smetacek, 1980, Christensen & Kanneworff, 1985, Graf, 1992, Gremare, Amouroux, Charles, Dinert, RiauxGobin, Baudart et al., 1997, Granberg, Hansen & Selck, 2005). Such physiological response due to changes in food value is likely also to affect the absorption, metabolism and general fate of sediment associated contaminants. Experimental enrichment with high quality SOM has previously been reported to stimulate the accumulation of sediment associated PCBs and PAHs in various marine invertebrates (Gunnarsson, Granberg, Nilsson, Rosenberg & Hellman, 1999a, Gunnarsson, Hollertz & Rosenberg, 1999b, Gunnarsson & Sköld, 1999, Granberg & Forbes, 2006), while effects of SOM quality on contaminant metabolism and toxicity are largely unexplored. Furthermore, the stimulating effect of increased SOM quality on general microbial mineralization is well documented (Graf, Schulz, Peinert & Meyer-Reil, 1983,

Meyer-Reil, 1983), while information is scarce on its effects on the microbial degradation of organic contaminants in sediments. Microbes, with emphasis on bacteria, are considered highly important for the biodegradation of PAHs in marine sediments (Cerniglia & Heitkamp, 1989, Neff, 2002). PAH degrading microbes (algae, fungi and bacteria) are largely oxygen dependent (Atlas, 1981, Cerniglia, 1993), and macrofaunal bioturbation (i.e. irrigation and particle mixing) is therefore an important process affecting sedimentary PAH degradation through the oxygenation of deeper sediment layers, and through the transport of contaminated particles between oxic and reduced layers (Gardner, Frez, Cichocki & Parrish, 1985, Bauer, Kerr, Bautista, Decker & Capone, 1988, Holmer, Forbes & Forbes, 1997, Kure & Forbes, 1997). Christensen et al. (2002) and Granberg et al. (2005) furthermore stress the importance of species specific bioturbation patterns for observed differences in microbial degradation and mineralization of pyrene in sediments. Although sparsely investigated, other factors suggested to affect sedimentary microbial PAH degradation are repeated PAH exposure and altered sediment organic matter (SOM) quantity and quality (ZoBell, 1946, Bauer & Capone, 1985, Cerniglia & Heitkamp, 1989, Granberg et al., 2005).

In the present study we investigated the importance of SOM quality for the uptake and metabolism of pyrene in the sediment dwelling brittle star *Amphiura filiformis* (Ophiuroidea, Echinodermata) and the common rag worm *Nereis diversicolor* (Polychaeta, Annelida). We also studied combined effects of SOM quality and pyrene contamination on somatic growth in *A. filiformis* as a measure of sediment toxicity. Furthermore we investigated combined effects of SOM quality and macrofaunal species on the distribution of pyrene and pyrene derived metabolites in surface, burrow and reduced bulk sediment compartments.

METHODS

Collection and preparation of sediment and organisms

Sediment and brittle stars (*Amphiura filiformis*) were collected from 40 m depth north-west of the Gullmarsfjord (58° 20'30'', 11°21'60'') on the Swedish west coast using a 0.062 m² box corer (Jonasson & Olausson, 1966). On deck, the upper oxidized sediment layer containing most of the macrofauna was removed from the lower reduced sediment, separating the sediment from each core into oxic and reduced pools. In the laboratory the reduced sediment was stored without sieving and covered with a layer of N₂ saturated water and a thick plastic film in darkness at 5 °C until use. Adult *A. filiformis* were carefully recovered from the oxic sediment, avoiding arm breakage, and placed on a layer of sieved (1 mm mesh size) surface sediment in aquaria with continuous water flow (32 psu, 5 °C). Remaining oxic sediment was sieved (1 mm mesh size) and stored with continuous flow as above until use. Polychaetes (*Nereis diversicolor*) were collected at a muddy sand flat on the shore of the Gullmarsfjord, and were kept in flow-through aquaria (32 psu, 5 °C) with a 5 cm layer of sieved surface sediment (1 mm mesh size) until use.

One month prior to the experiment, 24 one liter, wide mouth glass jars were filled with ~600 g wet weight (WW) of the reduced bulk sediment, which were homogenized by gently agitating filled jars on a shaking table. The jars were lidded, and placed together in a large water bath (5 °C) in a dark constant temperature room (5 °C). Two weeks prior to initiation of the experiment, one arm was cut off of each of 48 *A. filiformis* specimens. Predation on exposed arms, “arm-cropping”, by crustaceans and demersal fish is a common sub-lethal stress to this species in nature (Duineveld & Noort, 1986, Baden, Pihl & Rosenberg, 1990). Since amputated arms regenerate entirely, arm regeneration has been proven an excellent and fairly harmless tool for measuring somatic growth in brittle stars, which was the purpose of this exercise (Sköld & Gunnarsson, 1996, Thorndyke & Carnevali, 2001). Either the first, second, third or forth arm clockwise from the madreporite was cut between the seventh and eighth segments. Amputation of distinct arms separated individuals into four identifiable groups, and

only individuals differing in positioning of the cut arm were placed in the same experimental jar. Subsequent to amputation, brittle stars were returned to the sediment in flow through storage aquaria (32 psu, 5 °C) and left to heal and form scars, a procedure proven important for proper measurements of regeneration rates (Gunnarsson et al., 1999a, Selck, Granberg & Forbes, 2005).

The two carbon sources used for enrichment of oxic surface sediment were terrestrial lignin (Curan 100, Holmen paper AB, Vargön, Sweden) and a microalgae concentrate consisting of intact non-living cells of the chlorophyte *Tetraselmis* sp. (Reed Mariculture, Inc. San Jose, CA, USA). Lignin is a low quality, refractory carbon source of poor nutritional value to marine detritivorous macrofauna (e.g. Tenore, Cammen, Findlay & Phillips, 1982, Mayer, 1989, Gunnarsson et al., 1999a). *Tetraselmis* sp. has a high lipid and amino acid content (<http://www.instant-algae.com/microalgae/tetraselmis.htm>) and is thus a labile, high quality carbon source, of high nutritional value to marine macrofauna. Equal amounts of total organic carbon (TOC) of the two different carbon sources were added to the sediment. In order to achieve correct enrichments of the surface sediments, the contents of total carbon (TC) and total nitrogen (TN) were determined in freeze-dried samples of organic substrates and initial surface sediment using a Carlo Erba elemental analyzer (NA 1500 NC, Fisons Instruments). TOC was determined accordingly after treating samples with HCl (Hedges & Stern, 1984). Total amino acids (TAA) and enzymatically hydrolyzable amino acids (EHAA), representing the amount of amino acids available to sediment deposit-feeders, were also analyzed on freeze-dried enriched sediment samples according to the method of Mayer et al. (1995). Three days prior to the start of the experiment, oxic surface sediment was mixed with lignin powder and *Tetraselmis* sp. concentrate respectively increasing the sediment TOC content from ~2% dry weight (DW) (Table 1) to 3.0% DW. This resulted in a TN content 24 times higher in the sediment enriched with *Tetraselmis* sp. (156 mg kg^{WW}⁻¹ sediment) compared to the lignin

enriched sediment ($6.5 \text{ mg kg WW}^{-1}$ sediment). After enrichment the sediment was left for 24 h on a shaking table for thorough mixing at 5°C in darkness. For each carbon source, two methanol/acetone rinsed glass jars (3 liter) were prepared by adding a known volume of pyrene ($3.1 \text{ mg crystalline pyrene ml}^{-1}$ acetone, purity $>98\%$, Sigma-Aldrich) and radioactively labeled pyrene (^{14}C -4, 5, 9, 10- pyrene, $3.33 \mu\text{Ci ml}^{-1}$ methanol, purity $>98\%$, Sigma-Aldrich) to one and a corresponding volume of acetone/methanol only to the other. After solvent evaporation ($\sim 4 \text{ h}$), a known amount of the organically enriched wet sediment was added to the prepared glass jars, producing a pyrene/solvent and solvent only spiked set for each carbon source. The jars were vigorously agitated for 24 h in darkness allowing for the contaminant to mix and adsorb to the sediment. Sorption time was kept at a minimum to prevent the labile organic matter from being degraded (Granberg & Forbes, 2006). These conditions, which are not at thermodynamic equilibrium, closely resemble natural events of sedimenting phytoplankton blooms. The sediment reached a final nominal pyrene concentration of $30 \mu\text{g g DW}^{-1}$. The background concentration of surface sediment pyrene in the area where the sediment was collected varies between 48 and $120 \text{ ng pyrene g DW}^{-1}$ sediment (Å. Granmo pers. comm.).

Experimental setup

The experimental treatments were combinations of organic enrichment, lignin or *Tetraselmis* sp., and macrofaunal species, *Amphiura filiformis* or *Nereis diversicolor*, and consisted of a pyrene contaminated and an uncontaminated set (Table 2). The uncontaminated set was prepared as a control for measurements of somatic growth (i.e. arm regeneration) in *A. filiformis* therefore lacking combinations with *N. diversicolor*. One hundred g WW of the enriched and contaminated oxic surface sediment was added to each of 18 jars (1 liter, diameter: 10 cm, height: 15 cm) while enriched uncontaminated surface sediment was added to another 6 jars, all previously filled with 600 g WW homogenized reduced bulk sediment.

The jars were sealed with lids, perforated for in and out-flowing water, and were then carefully filled with seawater (32 psu, 5 °C) avoiding resuspension. The jars were placed in random order in the large water bath (5 °C) in a dark climate room (5 °C). Each jar was connected to an individual continuous supply of fresh deep fjord water (32 psu, 5 °C) passing via a sedimentation tank into the jars at a flow rate of 40 ml min⁻¹ giving a water residence time of 13 min. The jars were left to stabilize for 24 hours and subsequent addition of animals signified the start of the experiment. Seven medium sized (~7 cm long) *N. diversicolor* with a total biomass of ~3 g WW were added to each of 6 jars within the pyrene contaminated set. Before addition, brittle stars were video recorded under a dissection microscope to determine the initial length of the budding arm. Subsequently, four *A. filiformis* with disk diameters of 1 cm and a total biomass of ~1 g WW were added to each of another 12 jars including the pyrene contaminated and uncontaminated sets. The resulting treatment combinations including three replicate aquaria (jars) within each treatment are displayed in Table 2. The total exposure period lasted for 45 d under continuous water flow at 5 °C in darkness.

Analysis of sediment and organisms

Radioactively labeled pyrene was used to determine the amounts of unmetabolized pyrene (parent), water-soluble (aqueous-) pyrene metabolites, and water-insoluble (polar-) metabolites in sediment, and amounts accumulated and transformed by *A. filiformis* and *N. diversicolor*. The ratio between pyrene and radio-labeled pyrene was 0.42 ng pyren DPM⁻¹. Radioactivity associated with each phase was quantified by liquid scintillation counting (LSC) on a Beckman LS5000TD liquid scintillation system (Beckman instruments, Fullerton, CA, USA) and corrected for quench by the external standards ratio method after background subtraction. Subsequently DPM values were transformed to pyrene equivalents (i.e. µg pyrene gDW⁻¹) using the ratio of g pyrene DPM⁻¹.

At termination of the 45 d exposure, detailed sampling of sediment (surface-, burrow - and reduced bulk sediments) was performed along with the recovery of polychaetes and brittle stars. Burrows were sampled according to Granberg et al (2005). *Nereis diversicolor* were rinsed in clean seawater, left to purge their guts for 2-4 h in filtered seawater, blotted dry and weighed (blotted WW), then frozen (-20 °C) in individual glass test tubes until further pyrene- and lipid analysis. *Amphiura filiformis* were recovered from the sediment, rinsed in clean seawater, individually video recorded under a dissection microscope, and left to purge their guts for 2-4 h in filtered seawater. The brittle stars were then dissected into arms and disk, which were weighed (WW) and frozen (-20°C) separately in glass test tubes until further pyrene- and lipid analysis.

Pyrene analysis included separation and determination of total radioactivity into concentrations of parent pyrene, polar metabolites, aqueous metabolites, and residual pyrene (i.e. un-extractable fraction). Sediment and macrofauna samples were extracted using a modification of the Bligh and Dyer (1959) lipid extraction method, adapted for the extraction and analysis of PAHs and metabolites. Briefly, this two step extraction initially produces a chloroform phase (containing parent pyrene and organic-soluble metabolites: i.e. polar pyrene-metabolites) and a methanol/water phase (containing water-soluble metabolites: i.e. aqueous pyrene-metabolites). The chloroform phase was evaporated during the second step and re-extracted in hexane, potassium hydroxide (KOH) and dimethyl sulfoxide (DMSO) to separate parent pyrene from polar metabolites. Parent pyrene was retained in the hexane phase, polar pyrene metabolites in the DMSO/KOH phase and aqueous pyrene metabolites in the methanol/water phase. Extraction was facilitated by use of an ultrasonic rod (Misonix, Farmingdale, New York) immersed in the sample-solvent mixture. For a detailed description of the extraction procedure see Selck et al. (2005). Pyrene-derived radioactivity was determined in surface, burrow, and reduced bulk sediments of all contaminated treatments at

the end of the experiment. Amounts of radioactive parent pyrene, polar- and aqueous pyrene metabolites and pyrene tissue residues were determined in whole *Nereis diversicolor*, and *Amphiura filiformis* disks and arms separately at the end of the experiment. Individuals (disks and arms separately) were pooled within jars resulting in three replicate samples within each treatment. Radioactivity was determined by LSC 24 h after addition of scintillation cocktail (Ultima Gold XR, Packard). Pyrene body burdens, i.e. in *A. filiformis* disks and arms and in whole *N. diversicolor*, were assessed as weight-specific body burdens expressed in pyrene equivalents ($\mu\text{g pyrene gDW}_{\text{tissue}}^{-1}$), and as fractions (in percent) of parent pyrene, polar pyrene-metabolites, aqueous pyrene-metabolites and residual tissue pyrene in relation to the total amount i.e. Σ pyrene (parent pyrene, polar metabolites, aqueous metabolites, and residual pyrene). Polar pyrene metabolites are considered phase I products, aqueous pyrene metabolites phase II products (i.e. polar metabolites conjugated with endogenous cell compounds) and residual tissue pyrene as unextractable or phase I products that are further oxidized and bound intracellularly e.g. to DNA, RNA or proteins (Kulkarni & Hodgson, 1980). Total lipid contents of *A. filiformis* and *N. diversicolor* samples were determined at the end of the experiment according to the micro-gravimetric method of Gardner et al. (1985) modified from Bligh and Dyer (1959). Lipid content was not determined in brittle star arms since earlier studies have shown that the content was below detection (Gunnarsson & Sköld, 1999). Biota-sediment accumulation factors (BSAFs) were calculated according to Lee II et al. (1989), i.e. $\text{BSAF} = (C_{\text{tissue}}/\text{lipid}) / (C_{\text{sediment}}/\text{TOC})$; where C = total concentration of pyrene equivalents (Σ pyrene) in tissue ($C_{\text{tissue}}: \mu\text{g pyrene gDW}_{\text{tissue}}^{-1}$) or sediment ($C_{\text{sediment}}: \mu\text{g pyrene gDW}_{\text{sediment}}^{-1}$), lipid = total tissue lipid concentration ($\text{g lipid gDW}_{\text{tissue}}^{-1}$), and TOC = sedimentary total organic carbon content ($\text{g organic carbon gDW}_{\text{sediment}}^{-1}$). A video camera mounted on a dissection microscope and the SigmaScanPro software (ver. 5.0.0) were used to record live animals. Brittle stars from each jar were identified by the position of the regenerated arm, and individual lengths (L, mm) and areas (A, mm^2) of cut and regenerated

arms were determined from measurements of projected animals. Each length and area estimate used in the analysis was a mean of three replicate measurements of the same arm. Regenerated arm length and area were calculated from the difference between start and end measurements. The uncontaminated set (Li-Amf, Te-Amf) served as a control, and when comparing contaminated exposures, arm regeneration was tested as a possible tool for measuring sediment toxicity.

Statistical Analyses

The effect of macrofaunal species and SOM quality was tested in two-way orthogonal ANOVAs on log-transformed (\log_{10}) values of Σ pyrene, fractional amounts of parent pyrene, polar metabolites, aqueous metabolites and residual pyrene, as well as on measured total tissue lipid concentrations and calculated BSAF values in organisms. Comparisons between species and between tissues within *A. filiformis* were performed separately. The effect of the two factors on Σ pyrene, fractional amounts of parent pyrene, polar and aqueous metabolites and residual pyrene in sediment was tested accordingly in surface, burrow and reduced bulk sediments separately. In cases where only two samples remained due to accidental loss of replicates the ANOVAs were balanced using the average value of the two remaining replicates according to Underwood (1997). The degrees of freedom were adjusted accordingly prior to statistical testing. Ranking among levels of significant factors was identified by pairwise comparisons using Tukey post hoc tests. Levene Median and Kolmogorov-Smirnov tests were used for verification of homogeneity of variances and normality of data sets respectively. Effects of pyrene and SOM quality on regenerated arm length and area in *A. filiformis* were tested on log-transformed (\log_{10}) data using an orthogonal two-way ANOVA along with above mentioned complementary tests.

RESULTS

Macrofaunal bioaccumulation and biodegradation

All brittle stars originally added to experimental jars were recovered at the end of the experiment, while single polychaetes (~16 %) were lost, i.e. escaped or died, from some of the replicate jars. There was no difference in total accumulated pyrene body burdens (?pyrene) between the two species. However, ?pyrene was dependent on SOM quality (2-way ANOVA $F_{1,8}=16.841$, $p=0.003$) with highest body burdens, 22.7 ± 2.4 (mean \pm SD) and $21.8 \pm 6.8 \mu\text{g}$ pyrene $\text{gDW}_{\text{tissue}}^{-1}$ found in *N. diversicolor* and *A. filiformis* disks respectively when exposed to sediment enriched with *Tetraselmis* sp. (Fig. 1A, Table 3). The fraction of parent pyrene depended both on fauna and SOM quality, being greatest in *A. filiformis* (49-51 %), and elevated in both species when exposed to sediment enriched with *Tetraselmis* sp. (Table 3). Fractions of polar and aqueous metabolites both depended on species, where the fraction of polar metabolites was greater in *A. filiformis* (2-way ANOVA $F_{1,8}=15.0$, $p=0.0050$) and the fraction of aqueous metabolites was greater in *N. diversicolor* (2-way ANOVA $F_{1,8}=516.3$, $p<0.001$). The fraction of residual pyrene was also dependent on fauna (2-way ANOVA $F_{1,8}=7.041$, $p=0.029$) and was greatest in *N. diversicolor*. Thus, SOM quality was mainly important for the regulation of ?pyrene accumulation in the organisms while metabolic differences were species dependent

Organism-lipid and sediment-TOC normalized biota sediment accumulation factors (BSAFs) did not diverge from the observed effect of SOM quality on DW ?pyrene in the two species (2-way ANOVA $F_{1,8}=6.406$, $p=0.035$), giving increased bioaccumulation in *Tetraselmis* sp. enriched sediment (Fig. 1B). BSAFs did however, also differ between species (2-way ANOVA $F_{1,8}=7.911$, $p=0.023$) with higher values observed for *A. filiformis* than for *N. diversicolor*. This difference was mainly attributed to the significantly greater amount of

lipids found in *N. diversicolor* tissue (7.4 ± 2.2 %DW, n=6) compared to *A. filiformis* disks (4.7 ± 1.2 %DW, n=6) independent of SOM quality (2-way ANOVA $F_{1,8}=7.23$, $p=0.028$).

A. filiformis arms and disks were analyzed separately. This enabled making a crude estimate of trophic transfer through arm cropping by predators. β pyrene was significantly lower in arms compared to disks (2-way ANOVA $F_{1,8}=151.5$, $p<0.001$), and was also higher in both tissues when *A. filiformis* was exposed to sediment enriched with *Tetraselmis* sp. (2-way ANOVA $F_{1,8}=8.744$, $p=0.018$) (Fig. 1A, Table 3). Fractions of parent pyrene, polar and aqueous metabolites did not differ between arm and disk tissues and were independent of SOM quality. However, the residual fraction of pyrene was consistently higher in arms (20-21 %) compared to disks (8-10 %) (2-way ANOVA $F_{1,8}=5.788$, $p=0.043$).

Somatic growth

Regenerated arm length and area were compared among brittle stars exposed to pyrene contaminated and uncontaminated sediment enriched with either quality carbon source after 45 d of exposure. Neither regenerated arm length nor area differed between treatments and the regenerated arm length averaged 5.85 ± 1.38 (mean \pm SD) mm, and arm area 1.38 ± 0.37 mm² (n=48) yielding arm regeneration rates of 0.13 ± 0.03 mm d⁻¹ and 0.03 ± 0.008 mm² d⁻¹.

Surface sediment

β pyrene and the fraction of parent pyrene did not differ between treatments (Fig. 3, Table 4).

After 45 d β pyrene ranged between 19.2 ± 7.2 (mean \pm SD) and 34.8 ± 4.1 μ g pyrene gDW_{sediment}⁻¹ in *Tetraselmis* sp. enriched sediment with *A. filiformis* and lignin enriched sediment with *N. diversicolor* respectively. Due to great variation among samples within treatments, β pyrene did not differ from the initial 30 μ g pyrene added per gDW sediment (Table 4). The fraction of aqueous metabolites was dependent on SOM quality (2-way

ANOVA $F_{1,12}=12.084$, $p=0.005$) and was greater with lignin (7-12 %) than with *Tetraselmis* sp. (3-7 %) (Fig. 3a, Table 4). Fractions of polar metabolites or residual pyrene did not differ between treatments.

Burrow sediment

Pyrene depended on fauna (2-way ANOVA $F_{1,8}=9.186$, $p=0.016$) and was highest in *N. diversicolor* burrows (Fig. 3, Table 4). Fractions of parent pyrene and aqueous metabolites did not differ between treatments. The fraction of polar metabolites depended on fauna (2-way ANOVA $F_{1,8}=17.486$, $p=0.003$) and was greatest in *N. diversicolor* burrows. The fraction of residual pyrene was dependent on fauna (2-way ANOVA $F_{1,8}=5.7997$, $p=0.043$) and was highest in *A. filiformis* burrows (Table 4).

Bulk sediment

Pyrene was lowest in reduced bulk sediment of all compartments, and depended significantly on fauna (2-way ANOVA $F_{2,18}=11.431$, $p=0.002$) with the highest concentrations found in controls and in sediment inhabited by *N. diversicolor* (Tukey, $p<0.05$) (Table 4, Fig. 3). The fraction of parent pyrene was dependent on SOM quality and fauna simultaneously (2-way ANOVA $F_{2,18}=5.322$, $p=0.022$). It was greatest in *Tetraselmis* sp. enriched sediment with *N. diversicolor* (36 %) and smallest in *Tetraselmis* sp. enriched sediment with *A. filiformis* (0.1 %) (Fig. 3, Table 4). The fraction of aqueous metabolites did not differ between treatments while the fraction of polar metabolites was dependent on fauna (2-way ANOVA $F_{2,18}=10.504$, $p=0.002$), being greatest in uninhabited control sediment (12-16 %) and in sediment inhabited by *N. diversicolor* (10-11 %) (Tukey, $p<0.05$). The fraction of residual pyrene was dependent on fauna (2-way ANOVA $F_{2,12}=9.349$, $p=0.004$) and was greatest in sediment inhabited by *A. filiformis* (74-75 %) (Tukey, $p<0.05$) (Fig. 3, Table 4).

DISCUSSION

Bioaccumulation of pyrene

Nereis diversicolor and *Amphiura filiformis* accumulated pyrene from the sediment to a similar extent. The lack of difference in accumulation is interesting when considering the apparent difference in biotransformation capacity observed between the two species (Table 3). In this respect *N. diversicolor* would be expected to contain lower total pyrene concentrations than *A. filiformis* due to a higher biotransformation capacity. Contaminant metabolites have, however, been observed to be excreted quite inefficiently by marine invertebrates (James, 1989), and Kane Driscoll & McElroy (1997) even report a more rapid excretion of parent benzo(a)pyrene compared to metabolites in both *N. diversicolor* and another polychaete species, *Marenzelleria viridis*. Furthermore, *N. diversicolor* and *A. filiformis* both accumulated significantly more pyrene when exposed to sediment enriched with high quality *Tetraselmis* sp. than with refractory lignin. Enrichment with high quality organic matter (OM) has been reported to stimulate accumulation of sediment associated PCB and PAH in various sediment dwelling marine invertebrates including *A. filiformis* and *N. diversicolor* (Gunnarsson, Schaanning, Hylland, Sköld, Eriksen, Berge et al., 1996, Gunnarsson et al., 1999b, Gunnarsson & Sköld, 1999). In all of these studies, however, enriched treatments contained more total organic carbon (TOC) than controls, thus confounding effects of SOM quality and quantity and complicating mechanistic interpretations. In the present study, we specifically tested the effect of SOM quality by keeping TOC constant and altering nitrogen and amino acid levels through the choice of organic matter sources. Other studies with this design report positive correlations between SOM quality and accumulation of both PCB and cadmium in *A. filiformis* and of pyrene in the mud snail *Hydrobia ulvae* (Maloney, 1996, Gunnarsson et al., 1999a, Granberg & Forbes, 2006). The important message supported by all these studies is, however, that during a labile SOM enrichment situation, such as associated with the occurrence and sedimentation of a phytoplankton bloom, accumulation of sediment-

associated contaminants increases. This phenomenon will be overlooked if methods based on equilibrium partitioning (EPT) (DiToro, Zarba, Hansen, Berry, Swartz, Cowan et al., 1991) are used to predict bioaccumulation from sediments. Enhanced contaminant bioaccumulation stimulated by enrichment with high quality SOM has in most studies been explained by various macrofaunal feeding responses, such as selective ingestion of contaminated food particles (e.g. Kukkonen & Landrum, 1994, Gunnarsson et al., 1996, Gunnarsson & Sköld, 1999, Magnusson, Agrenius & Ekelund, 2003) or increased ingestion rate as a function of food value (Granberg & Forbes, 2006). Since pore water concentrations of truly dissolved contaminant normally decrease as a result of association with added organic matter (Landrum, Reinhold, Nihart & Eadie, 1985, Swartz, Schults, Dewitt, Ditsworth & Lamberson, 1990, DeWitt, Ozretich, Swartz, Lamberson, Schults, Ditsworth et al., 1992, Gunnarsson & Rosenberg, 1996, Maloney, 1996, Timmermann & Andersen, 2003), feeding behaviour is likely to be the key process determining bioaccumulation. It is important to note that this decrease in truly dissolved contaminant concentrations in pore water is predicted by EPT while enhanced bioaccumulation due to stimulated feeding, driven by SOM quality, lacks consideration in this model. The effect of SOM quality furthermore remained when bioaccumulation was expressed as biota-sediment accumulation factors (BSAFs) (Fig. 1), indicating that the system was not in equilibrium or that other factors than passive equilibration between sedimentary TOC and organism lipids governed bioaccumulation. The BSAF was higher in *A. filiformis* than in *N. diversicolor*, a species-specificity not found for pyrene. The difference was attributed to the lipid content being higher in *N. diversicolor* compared to *A. filiformis*. The species-specific increase in lipid content during the experiment likely resulted in a growth dilution scenario in *N. diversicolor*, where contaminant accumulation did not keep up with the storage of lipids. Average pyrene BSAF values were consistent with those reported for other PAHs in sediment dwelling organisms (Kane Driscoll & McElroy, 1996, Neff, 2002, Selck et al., 2005). In some cases SOM quality and

accumulation lack correlation as was reported for fluoranthene accumulation in *A. filiformis* measured by Selck et al. (2005) in an experiment designed much like the present. The authors attributed the lack of response to hypoxic stress occurring in the high quality SOM enrichment. Hypoxia may have reduced ingestion rate (Weston, 1990), consequently hampering bioaccumulation in *A. filiformis* specifically in high quality SOM treatments.

Macrofaunal pyrene degradation

Biodegradation of pyrene was in general unaffected by SOM quality in both species but differed greatly between them. After the 45 d exposure period, ~80 % of the total tissue pyrene consisted of pyrene metabolites (both polar and aqueous) in *N. diversicolor*, while metabolites comprised ~40 % of the total tissue pyrene in *A. filiformis*. The polychaete *N. diversicolor* is known to efficiently metabolize both benzo(a)pyrene and pyrene, with 1-hydroxypyrene as the only identified phase I product (Kane Driscoll & McElroy, 1996, Christensen, Andersen & Banta, 2002, Giessing, Mayer & Forbes, 2003). Pyrene metabolism has to our knowledge never previously been measured in *A. filiformis*, and information on PAH metabolism in ophiurids is scarce in general despite their dominance in many habitats (Pearson, Josefson & Rosenberg, 1985, Duineveld & Noort, 1986, Rosenberg, Gray, Josefson & Pearson, 1987, den Besten, 1998). Selck et al. (2005) measured biodegradation of a closely resembling PAH, fluoranthene, in *A. filiformis* but found polar and aqueous metabolites to comprise only ~15 % of the total fluoranthene accumulated after 45 d, consequently classifying degradation as slow. In comparison, degradation of pyrene appears to be fairly efficient in *A. filiformis* and also comparable to degradation measured in some polychaetes (Kane Driscoll & McElroy, 1996). From a long term study (29 weeks), Magnusson et al. (2003) reported water soluble metabolites to comprise 5-25 % of the total accumulated tetrabrominated diphenyl ether (TBDE) in *A. filiformis*, reflecting intermediate levels compared to concentrations found in other invertebrate species analyzed. The fraction of

TBDE metabolites further increased with time indicating slow elimination. Consequently, *A. filiformis* apparently has the ability to metabolize various kinds of organic contaminants. Polar and aqueous metabolites were both present in *N. diversicolor* and *A. filiformis* but aqueous metabolites constituted the largest metabolite fraction. In *A. filiformis* however, levels were more evenly distributed between polar and aqueous metabolites than in *N. diversicolor*. This information indicates that both phase I and II enzymes are functional in both species. The fraction of parent pyrene was higher in both species when exposed to sediment enriched with *Tetraselmis* sp. suggesting that uptake rates override metabolic rates when SOM quality is good. Metabolism remained unaffected by SOM quality, indicating that MFO activity is decoupled from general metabolic processes. When interpreting results on biodegradation capacities it is important to recognize that this investigation only gives a snapshot of bioaccumulation and biodegradation processes. This experiment was, however, performed simultaneously to, and under identical conditions as a kinetically designed experiment investigating bioaccumulation and biodegradation of the PAH fluoranthene in *A. filiformis* (Selck et al., 2005). Comparing the kinetic data set from the fluoranthene study with the static dataset for pyrene from this investigation confirmed the solidity of the interpretation of biodegradation results in this investigation.

Pyrene distribution and arm regeneration in A. filiformis

The total pyrene concentration in *A. filiformis* arms increased in response to SOM quality and was ~20 % of the concentration found in disks. In general, the fractional distribution of pyrene and metabolites in arm tissue mirrored disk conditions except for the fraction of residual pyrene, which was twice as high in arms compared to disks, constituting ~20 % of the total arm tissue pyrene. The activity of MFO related contaminant degradation in echinoderms is mainly located to digestive tissues such as the pyloric caeca and stomach (den Besten, Herwig, Donselaar & Livingstone, 1990). Carnevali et al. (2001b) do however, also

report increased amounts of Cytochrome P-450 enzymes in regenerating arm tissue of feather stars (*Antedon mediterranea*, Crinoidea) when exposed to the PCB mixture Arochlor 1260, as identified with immuno-fluorescence techniques using vertebrate Cytochrome P-450 antibodies. Although MFO enzymes have been detected in various invertebrates, confusion exists about their relevance in the metabolism of contaminants, and their functions may differ between phyla (Snyder, 2000). Arm regeneration involves growth and differentiation of several types of tissues intricately regulated on a molecular level (Thorndyke & Carnevali, 2001), and MFO enzymes catalyze a plethora of endogenous reactions (Mansuy, 1998). The coupling between observed amounts of Cytochrome P-450 enzymes in arm tissue and xenobiotic biodegradation activity is therefore not straightforward. Our results suggest, however, that contaminants most likely were ingested, absorbed, and degraded in digestive tissues with subsequent internal distribution to arm tissue, giving the apparent mirror image of fractional patterns in disk tissue.

Arm regeneration has been suggested as a possible screening tool for contaminant exposure (Carnevali, Bonasoro, Patruno, Thorndyke & Galassi, 2001a). Arm regeneration was measured in terms of length and area to estimate somatic growth in *A. filiformis* as affected by SOM quality and pyrene. None of the factors affected arm regeneration. Similar results were obtained for *A. filiformis* exposed to fluoranthene and varying SOM quality reported by Selck et al. (2005), however, reduced regenerated arm area was observed in fluoranthene contaminated sediment enriched with labile *Tetraselmis* sp. Arm area is a more sensitive measure than arm length. Previous studies consistently report enhanced arm regeneration in *A. filiformis* as a function of increased SOM quality while no effects have been observed of either PCB or benzo(a)pyrene (Hylland, Sköld, Gunnarsson & Skei, 1996, Sköld & Gunnarsson, 1996, Gunnarsson et al., 1999a, Nilsson, 1999). Carnevali et al. (2001b) do however, report hormesis like increased arm regeneration in *A. mediterranea* after chronic

low dose PCB exposure, and pyrene exerted a vague growth stimulation in *A. filiformis* at low concentrations, while reduced arm regeneration was reported at exposure concentrations exceeding that used in this study (Bannister, 2004). The coupling between arm regeneration and environmental conditions thus appear highly complex. Bannister (2004) furthermore report arm regeneration to be highly temperature dependent in *A. filiformis*, a feature correlating with field measurements of seasonal variations in regeneration rates observed among ophiurids (Sköld, Loo & Rosenberg, 1994, Stancyk, Golde, Papelindstrom & Dobson, 1994). Arm regeneration rates were comparably low in our study (0.13 mm d^{-1}) (Nilsson & Sköld, 1996, Sköld & Gunnarsson, 1996, Gunnarsson et al., 1999a). Consequently, as experiments were performed at low temperatures ($+5 \text{ }^{\circ}\text{C}$) in both the present study and in the study by Selck et al. (2005), possible treatment effects might not have had sufficient time to develop during the 45 d exposure due to a general slow growth. Using a more sensitive tool such as histological examinations might have enabled the identification of possible pollutant or enrichment effects (see Carnevali et al., 2001b). The lack of response to the studied factors should therefore be interpreted with caution.

Sedimentary distribution of ?pyrene

Since the contaminant was added to surface sediment only, the total amount of pyrene (?pyrene) was not surprisingly almost an order of magnitude higher in surface compared to burrow and reduced bulk sediments. Burrows of *N. diversicolor* contained a larger amount of ?pyrene than did *A. filiformis* burrows, while ?pyrene in reduced bulk sediments was highest in controls and in sediment inhabited by *N. diversicolor*. There was thus an obvious downward transport of pyrene, which was more pronounced in sediment with *N. diversicolor* than with *A. filiformis*. Pyrene is a particle reactive compound, $\log K_{ow}$ between 4.50 and 5.32 (Meador et al., 1995), and its distribution is thus highly dependent on forces affecting particle mixing, such as macrofaunal feeding and burrowing. Species-specific differences in

bioturbation behaviour often appear to leave finger prints in the distribution of contaminants in the sediment (Holmer et al., 1997, Kure & Forbes, 1997, Gunnarsson et al., 1999a, Gunnarsson et al., 1999b, Sandnes, Forbes, Hansen & Sandnes, 2000). *N. diversicolor* build, and continuously re-route, extensive burrow galleries (Davey, 1994), while *A. filiformis*, although sometimes observed to be highly mobile, construct only as much burrow needed for keeping disks buried at ~5 cm depth with 2-4 arms protruding from the sediment for feeding (Woodley, 1975, Jonsson, Broman, Gunnarsson, Lagergren, Larsson, Ohlsson et al., 1997) (Fig. 2). *Amphiura filiformis* switches between filter and surface deposit feeding, generating a continuous flow of particles to the buried mouth (Woodley, 1975). *Nereis diversicolor* is omnivorous, sometimes filter and deposit feeding within the burrow, and occasionally ascending for epibenthic feeding on everything from detritus to living prey (Fauchald & Jumars, 1979). In our study, Σ pyrene was greater in burrows made by *N. diversicolor* than in burrows made by *A. filiformis*. This contradicts both theory and previous observations of bioturbation and sedimentary PCB distribution (Gunnarsson et al., 1999a, Gunnarsson et al., 1999b). Pyrene is however, more readily biodegraded than PCB. Its fate is therefore also determined by metabolism and mineralization processes, and degradation product may subsequently be eliminated from burrow sediment with exhaust water facilitated by macrofaunal irrigation. Granberg et al. (2005) found microbial pyrene mineralization rates to be 1.5-2.5 times higher in *A. filiformis* burrows compared to *N. diversicolor* burrows. Observed Σ pyrene patterns are thus likely a result of combined effects of particle mixing, biodegradation and irrigation as well as macrofaunal excretion. Elevated bulk sediment Σ pyrene levels in sediment inhabited by *N. diversicolor* correlate well with this species' extensive burrowing behaviour, while the higher levels detected in control sediment must be caused by accidental contamination.

Sedimentary distribution of pyrene metabolites

Interpreting differing patterns in fractional pyrene metabolite distributions in sediments is ambiguous since metabolites are not necessarily produced where they are found and may be produced by various sedimentary organisms. More than 72 % of surface sediment pyrene still remained in parental form after 45 d, while aqueous and polar metabolites together constituted between 8 and 20% of the surface sediment pyrene. Kane Driscoll & McElroy (1997) found little evidence of surface sediment benzo(a)pyrene metabolism but substantial amounts of metabolites were detected in polychaete fecal casts produced epibenthically. Although not statistically significant, slightly higher Σ pyrene was observed in surface sediment with *N. diversicolor* in our study (Fig. 3), which may be attributed to epibenthic defecation. The fraction of aqueous metabolites was consistently higher in surface sediment enriched with lignin compared to *Tetraselmis* sp., suggesting either that contaminant degradation was greater with *Tetraselmis* sp. or that diffusion into water was slower with lignin, or both. Contaminants generally associate stronger with refractory organic matter (Xing, 1997, Lueking, Huang, Soderstrom-Schwarz, Kim & Weber, 2000, Lyytikäinen, Hirva, Minkinen, Hämäläinen, Rantalainen, Mikkelsen et al., 2003). Enhanced retention of pyrene metabolites in lignin enriched sediment may thus give the impression of higher apparent pyrene biodegradation. Unaltered parent pyrene constituted the largest fraction followed by residual pyrene in *N. diversicolor* burrows, while *A. filiformis* burrows contained mostly residual pyrene. The residual pyrene fraction likely consists of metabolites which have become covalently bound to decaying refractory sedimentary organic matter (Richnow, Seifert, Hefter, Kastner, Mahro & Michaelis, 1994). This fraction, being larger with *A. filiformis*, may in part be attributed to the specific feeding behaviour of this species, generating elevated concentrations of decaying SOM in burrows, scavenging metabolites. *Amphiura filiformis* maintain substantial amounts of partly digested organic matter in a "feeding pocket" around the disk, where sedimentary food items repeatedly are egested and re-ingested (Fig. 2).

Amphiura filiformis do not appear to apply a defecation strategy as specific as *N. diversicolor*'s, and consequently much of excreted metabolites may be left for sedimentary association within the burrows. Although not statistically significant, the fraction of parent pyrene was much larger in *A. filiformis* burrows with *Tetraselmis* sp. than with lignin (Fig. 3). Enhanced feeding, stimulated by high SOM quality (Kofoed, Forbes & Lopez, 1989, Granberg & Forbes, 2006), likely resulted in facilitated downward transport of contaminated surface sediment particles, overriding metabolic rates, particularly pronounced in sediment with *A. filiformis*. The fraction of residual pyrene further increased with depth and was consistently higher with *A. filiformis*. Since PAH biodegradation is much slower in anoxic or reduced environments, such as deep sediments (Gardner, Lee, Tenore & Smith, 1979, Cerniglia, 1993, Rockne & Strand, 1998, Granberg et al., 2005), metabolites are likely transported there by macrofaunal bioturbation, while fractional distributions probably are determined elsewhere. The fraction of residual pyrene is, however, stimulated by bulk sediment humification processes and is likely to increase with time, constantly removing pyrene from the bioavailable sedimentary pool.

Ecological implications

Amphiura filiformis is one of the dominating species below the halocline in the Kattegat, Skagerrak and North Sea regions (Pearson et al., 1985, Duineveld, Künitzer & Heyman, 1987, Rosenberg et al., 1987), commonly occurring at densities of 100-400 ind. m⁻² with localized maxima of ~3500 ind. m⁻² (Pearson et al., 1985, Rosenberg, 1995, Sköld & Gunnarsson, 1996). Likewise *Nereis diversicolor* is among the most common and abundant species inhabiting shallow estuarine soft bottoms of north western Europe and north eastern America, with densities varying from 35 to 3700 ind. m⁻² (Smith, 1977, Kristensen, 1983, Riisgård, 1991, Scaps, 2002). Since the species are so abundant and also constitute important food sources for many other species (Evans, Herdson, Knights & Pienkowski, 1979, Davey &

George, 1986, Duineveld & Noort, 1986, Baden et al., 1990), *A. filiformis* and *N. diversicolor* are significant vehicles for contaminant transfer between sediment and higher trophic levels in their respective habitats. Organic matter quality had a strong impact on bioaccumulation in the two species. Total pyrene concentrations in *A. filiformis* arm tissue doubled and concentrations more than doubled in whole *N. diversicolor* tissue with the addition of labile *Tetraselmis* sp., which likely is attributed to enhanced ingestion as food value increases. The fraction of pyrene metabolites decreased with SOM quality indicating that metabolism did not keep up with ingestion. Consequently more tissue pyrene will be in parental form when SOM quality is high. Approximately 20 % of the total pyrene in *A. filiformis* arms resided in a form covalently bound to, e.g. DNA, RNA or proteins irrespective of SOM quality. Such molecular association likely hampers uptake of the contaminant in guts of predators, indicating that less than 80 % of the measured arm tissue pyrene actually is bioavailable to predators.

Sedimentary distribution of total pyrene and pyrene metabolites was largely unaffected by SOM quality at present levels but differed, fairly ambiguously, depending on inhabiting macrofaunal species. Observed differences are not surprising since *A. filiformis* and *N. diversicolor* display very diverging bioturbation behaviours. Difficulties in coupling contaminant distribution with bioturbation behaviour are likely due to the biodegradability of pyrene giving it variable hydrophobicity in turn affecting contaminant sorption. Furthermore, the association of metabolites with sedimentary organic matter was stimulated by the presence of *A. filiformis*. This residual fraction became greater with sediment depth, likely stimulated by general humification. This process should act to remove toxic pyrene and pyrene metabolites from the bioavailable pool. Ultimately, bioaccumulation and trophic transfer of sediment-associated PAH are likely to be enhanced during periods of sedimentation of fresh organic matter, such as after phytoplankton blooms. Our results strongly suggest that the fate of sediment-associated PAH should not be assessed without considering behavioural characteristics of the macrofauna present, along with the trophic situation of the system.

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Table 1. Specifications of total nitrogen (TN), total carbon (TC), and total organic carbon (TOC) in initial sediment and organic carbon sources, lignin and *Tetraselmis* sp. Contents of total amino acids (TAA), and enzymatically hydrolyzable amino acids (EHAA) was measured in sediment after enrichment with respective organic carbon source. DW indicates dry weight, – indicates not analyzed. All values are means of 3 replicates (\pm SD), *n=2.

Source	TN (% DW)	TC (% DW)	TOC (% DW)	TAA ($\mu\text{mol g}^{-1}$ DW)	EHAA ($\mu\text{mol g}^{-1}$ DW)
Sediment	0.33 ± 0.03	4.25 ± 0.15	2.15 ± 0.41	-	-
Lignin	0.11 ± 0.004	61.1 ± 0.9	$52.5 \pm 2.9^*$	699 ± 51	101 ± 6
<i>Tetraselmis</i> sp.	$1.88 \pm 0.13^*$	$37.7 \pm 0.7^*$	$37.5 \pm 1.0^*$	799 ± 40	160 ± 2

Table 2. Schematic diagram of experimental exposure combinations. X indicates an existing combination while – indicates absence of the combination, n=3.

	Pyrene contaminated		Uncontaminated	
	<i>Tetraselmis</i> sp.	Lignin	<i>Tetraselmis</i> sp.	Lignin
No fauna	X	X	-	-
<i>Amphiura filiformis</i>	X	X	X	X
<i>Nereis diversicolor</i>	X	X	-	-

Table 3. Fractional tissue concentrations of pyrene in disks and arms of *Amphiura filiformis* and whole *Nereis diversicolor* exposed to sediment enriched with either lignin or *Tetraselmis* sp.. Pyrene concentrations are expressed as pyrene equivalents ($\mu\text{g pyrene g}^{-1}$ dry weight tissue) and as percentages of total pyrene equivalents. Data are presented as means of three replicates \pm SD. *n=2.

Organism/tissue	Parent		Polar metabolites		Aqueous metabolites		Residual		?pyrene
	$\mu\text{g gDW}^{-1}$	%	$\mu\text{g gDW}^{-1}$	%	$\mu\text{g gDW}^{-1}$	%	$\mu\text{g gDW}^{-1}$	%	$\mu\text{g gDW}^{-1}$
Lignin									
<i>A. filiformis</i> arm	1.2 \pm 0.04	46	0.26 \pm 0.13	10	0.7 \pm 0.2	23	0.57 \pm 0.33	20	2.7 \pm 0.4
<i>A. filiformis</i> disk*	7.4 \pm 0.4	49	2.7 \pm 0.1	18	3.7 \pm 0.4	25	1.2 \pm 0.1	8	15.0 \pm 0.7
<i>N. diversicolor</i>	0.4 \pm 0.2	5	0.72 \pm 0.45	8	6.7 \pm 3.6	71	1.3 \pm 0.3	16	9.2 \pm 4.1
<i>Tetraselmis</i> sp.									
<i>A. filiformis</i> arm	1.9 \pm 0.7	45	0.46 \pm 0.19	11	1.0 \pm 0.3	23	1.0 \pm 0.7	21	4.7 \pm 1.5
<i>A. filiformis</i> disk	11.0 \pm 3.3	51	3.0 \pm 0.8	14	5.3 \pm 1.6	25	2.4 \pm 1.5	10	21.8 \pm 6.8
<i>N. diversicolor</i>	1.5 \pm 0.4	6	2.4 \pm 0.6	11	16.4 \pm 1.4	72	2.4 \pm 0.3	11	22.7 \pm 2.4

Table 4. Fractional concentrations of pyrene in surface, burrow, and reduced bulk sediments as a function of carbon source (C), either lignin (L) or *Tetraselmis* sp. (T), and fauna, either *Amphiura filiformis*, *Nereis diversicolor* or controls without fauna. Pyrene concentrations are expressed as pyrene equivalents (μg pyrene (g dry weight sediment) $^{-1}$) and as percentages of total pyrene equivalents. Data are presented as means of three replicates \pm SD. *n=2.

Treatment	C	Parent		Polar metabolites		Aqueous metabolites		Residual		?pyrene
		µg gDW ⁻¹	%	µg gDW ⁻¹	%	µg gDW ⁻¹	%	µg gDW ⁻¹	%	µg gDW ⁻¹
Surface										
Control	L	16.3 ± 3.5	78	0.94 ± 0.19	5	2.3 ± 1.3	12	1.2 ± 0.31	6	20.8 ± 2.8
Control	T	26.3 ± 11.1	83	1.1 ± 0.7	4	2.0 ± 0.9	7	1.6 ± 0.38	6	30.9 ± 10.3
A. filiformis	L	18.8 ± 6.8	85	0.55 ± 0.14	3	1.4 ± 0.4	7	1.1 ± 0.29	5	21.8 ± 6.7
A. filiformis	T	16.6 ± 7.36	85	0.74 ± 0.31	4	0.58 ± 0.24	3	1.2 ± 0.13	7	19.2 ± 7.2
N. diversicolor	L*	25.1 ± 1.0	72	2.8 ± 1.1	8	4.2 ± 1.0	12	2.7 ± 0.95	8	34.8 ± 4.1
N. diversicolor	T*	22.0 ± 13.5	84	1.2 ± 0.2	5	1.1 ± 0.7	4	1.3 ± 0.17	6	25.6 ± 14.1
Burrow										
A. filiformis	L	0.30 ± 0.28	18	n.d.	-	0.15 ± 0.13	8	1.3 ± 0.31	74	1.8 ± 0.26
A. filiformis	T	2.0 ± 1.8	43	0.14 ± 0.24	4	0.34 ± 0.07	10	1.3 ± 0.37	43	3.6 ± 1.6
N. diversicolor	L	3.8 ± 3.9	46	0.38 ± 0.29	6	0.81 ± 0.42	15	1.5 ± 0.11	33	6.5 ± 4.6
N. diversicolor	T	2.0 ± 0.6	52	0.91 ± 0.14	15	0.63 ± 0.10	11	1.3 ± 0.08	22	4.9 ± 0.3
Reduced bulk										
Control	L	0.54 ± 0.81	23	0.24 ± 0.14	12	0.61 ± 0.23	30	0.71 ± 0.21	35	2.1 ± 0.3
Control	T	0.18 ± 0.18	7	0.37 ± 0.13	16	0.85 ± 0.34	36	0.92 ± 0.18	41	2.3 ± 0.7
A. filiformis	L	0.06 ± 0.10	5	0.02 ± 0.04	1.5	0.24 ± 0.11	19	0.93 ± 0.17	75	1.2 ± 0.13
A. filiformis	T	0.002 ± 0.003	0.1	0.06 ± 0.11	4	0.29 ± 0.20	21	0.92 ± 0.09	74	1.3 ± 0.2
N. diversicolor	L	0.02 ± 0.02	1	0.16 ± 0.03	10	0.50 ± 0.06	32	0.91 ± 0.08	57	1.6 ± 0.1
N. diversicolor	T*	1.2 ± 1.2	36	0.31 ± 0.14	11	0.41 ± 0.05	16	0.85 ± 0.07	36	2.7 ± 1.4

FIGURE CAPTIONS

Fig. 1

Average accumulation of pyrene in *Amphiura filiformis* and *Nereis diversicolor* from contaminated sediment enriched with either lignin or microalgae (*Tetraselmis* sp.) after 45 d exposure. A. total pyrene concentrations in arms and disks of *A. filiformis* and whole body tissue of *N. diversicolor*. B. Biota sediment accumulation factors (BSAFs) in *A. filiformis* disks and whole body *N. diversicolor*. * and ** indicate $p < 0.05$ and $p < 0.001$ levels of significance from ANOVAs. Error bars indicate SD, $n=3$.

Fig. 2

Sediment images taken at the end of the experimental exposure. A. Burrow gallery of *Nereis diversicolor* in sediment enriched with labile *Tetraselmis* sp. B. Burrow of *Amphiura filiformis* in sediment enriched with labile *Tetraselmis* sp. Arrow indicates feeding pocket with darker reduced sediment and visible disk. Bar indicates one cm.

Fig. 3

Fractional distribution of pyrene and metabolites in surface, burrow and reduced bulk sediments after 45 days of exposure. Sediments were enriched with either a) refractory lignin or b) labile microalgae (*Tetraselmis* sp.) and inhabited by either *Amphiura filiformis* (A), *Nereis diversicolor* (N), or no fauna (C). The size of each pie-circle is proportional to the total amount of pyrene, i.e. ?pyrene (parent pyrene, polar metabolites, aqueous metabolites and residual pyrene), found in respective compartment within each treatment.

Fig 1

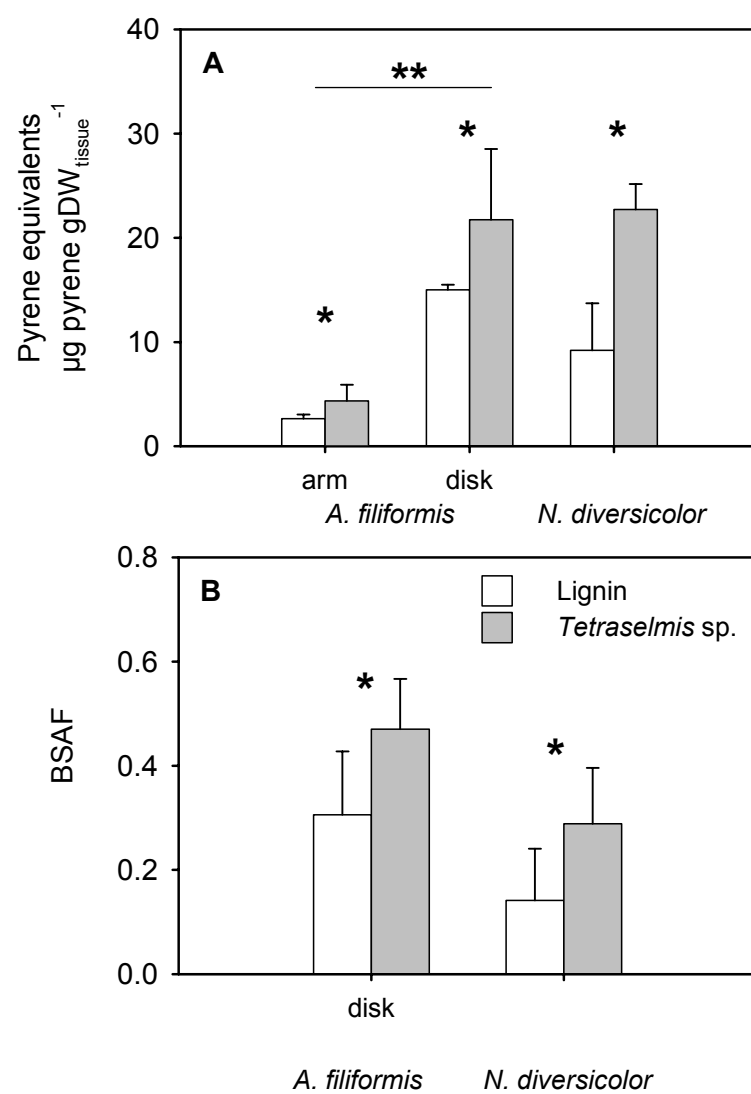


Fig. 2

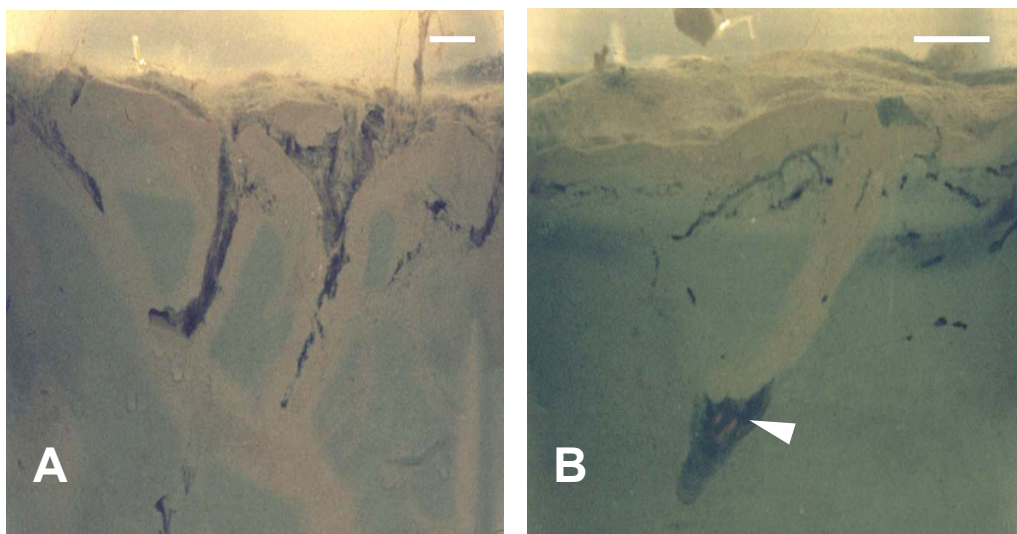
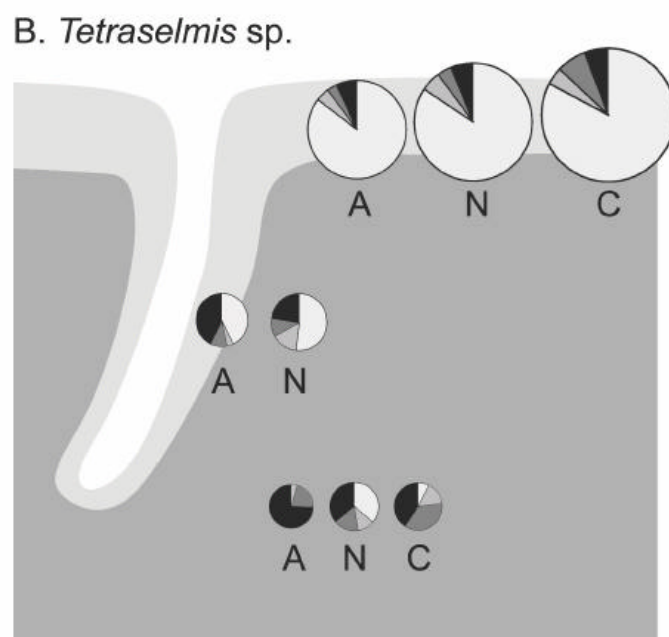
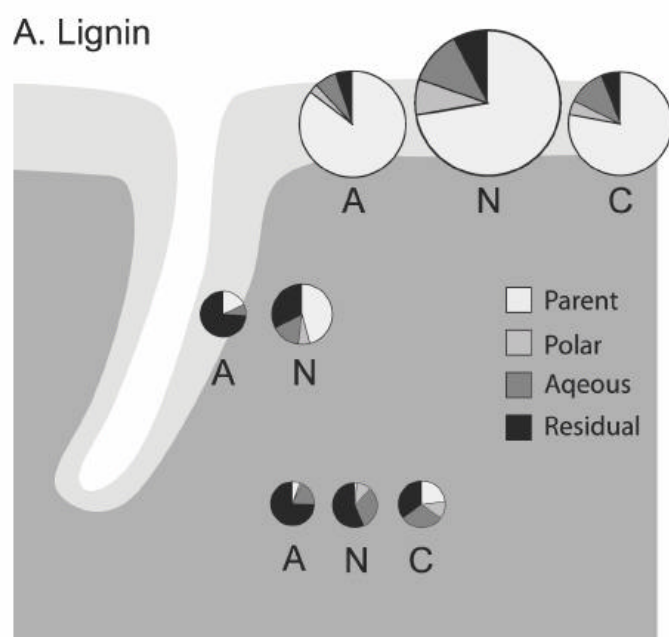


Fig 3:



A