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Impact of sediment organic matter quality on the fate and effects of fluoranthene in the infaunal brittle star *Amphiura filiformis*

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Abstract

Hydrophobic contaminants, such as polycyclic aromatic hydrocarbons (PAHs) readily adsorb to organic matter. The aim of this study was to determine the importance of the quality of sedimentary organic matter for the uptake, biotransformation and toxicity of the PAH, fluoranthene (Flu), in the infaunal brittle star *Amphiura filiformis*. Brittle stars were exposed to a base sediment covered by a 2 cm Flu-spiked top layer (30 μg Flu/g dry wt. sed.), enriched to the same total organic carbon content with either refractory or labile organic matter. The labile carbon source was concentrated green flagellate: *Tetraselmis* spp. The refractory carbon source was lignin from a paper mill. Tissue concentrations of Flu both in disk and arm-fractions were determined as total Flu, parent Flu (i.e. untransformed), aqueous Flu-metabolites, polar Flu-metabolites and tissue residue Flu (i.e. unextractable). Our results showed that sediment particle ingestion is a pathway by which Flu can enter benthic food webs. Flu toxicity (measured as arm-regeneration), but not net accumulation, was dependent on the nutritional quality of the ingested sediment particles. Flu bioaccumulation could not be attributed solely to equilibrium partitioning between organism lipid content and organic content of the sediment. Biotransformation of Flu by brittle stars was very limited and unaffected by organic matter quality. *A. filiformis* contributed to the downward transport of Flu from the surface sediment to the burrow lining. The limited breakdown of parent Flu by brittle stars and/or microorganisms was relatively higher in burrows compared to surface sediment, and highest in the presence of labile organic matter. Tissue concentrations were higher in disk than

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in arms, but the proportion of metabolic products relative to parent Flu was higher in arms than in the disk fraction. We estimate that the yearly mobilization of sediment-associated Flu by arm-regeneration in *A. filiformis* is in the range of 3.8–29.4 μg total Flu eq. m^{-2} $year^{-1}$ at a sediment concentration of 30 μg Flu/g dry wt. sed.

Keywords: PAH; Biotransformation; Sediment contamination; Deposit feeders; Organic carbon quality

1. Introduction

Due to their hydrophobic character, polycyclic aromatic hydrocarbons (PAHs) readily adsorb to organic matter in the water column and tend to partition preferentially to sediments in the marine environment where they may persist and cause ecological damage (Karickhoff, Brown, & Scott, 1979; Neff, 1985). Especially deposit-feeding organisms may be exposed to high concentrations of such toxicants, because their feeding strategy includes processing large quantities of fine-grained and organic-rich sediment (Lopez & Levinton, 1987). Recent studies have shown that uptake during ingestion from the sediment-bound fraction is an important route of cadmium and fluoranthene (Flu) absorption for the deposit-feeding polychaete *Capitella* species I (Selck, Forbes, & Forbes, 1998; Selck, Palmqvist, & Forbes, 2003a), and of tetrachlorobiphenyl (TCB), benzo[*a*]pyrene (BaP) and polychlorinated biphenyl (PCB) absorption in the echinoderm *Amphiura filiformis* (Gunnarsson, Granberg, Nilsson, Rosenberg, & Hellman, 1999; Gunnarsson et al., 1996; Gunnarsson & Sköld, 1999). Although uptake by benthic organisms may be one of the most important factors influencing transfer of sediment-associated contaminants to demersal fish and higher trophic levels, there is still a lack of understanding of the factors that control bioavailability of sediment-associated contaminants. It has been documented that benthic organisms accumulate contaminants associated with easily digestible food with higher efficiency than those associated with more refractory food (Gunnarsson et al., 1999; Selck, Decho, & Forbes, 1999; Wang & Fisher, 1996).

Once taken up by an organism the fate and effects of PAHs are especially dependent on their susceptibility to biotransformation. Generally, biotransformation of PAHs involves a two-phase process in which more water-soluble metabolites are produced (e.g. Livingstone, 1998; Van den Berg, van de Meent, Peijnenburg, Sijm, & Tas, 1995). A lipophilic compound is transformed into more polar metabolites in phase I (resulting in only a slight increase in water-solubility) and then to more water soluble conjugates during phase II (resulting in an extensive increase in water-solubility), generally forming products that are ionizable and readily excreted (Di Giulio, Benson, Sanders, & Van Veld, 1995; Van den Berg et al., 1995; Walker, Hopkin, Sibly, & Peakall, 1996, Chap. 5). PAHs, such as Flu, are biotransformed by a variety of bacteria and invertebrates. However, biotransformation capacity varies greatly among species. For example, the polychaete *Capitella* sp. I is able to extensively biotransform PAH (Selck, Palmqvist, & Forbes, 2003b). The same

extensive biotransformation was observed in the polychaetes *Nereis diversicolor* and *Marenzellaria viridis*, whereas another polychaete *Leitoscoloplos fragilis* showed a very low PAH biotransformation capacity (Kane Driscoll & McElroy, 1996). Studies of PAH biotransformation by echinoderms have to our knowledge been limited.

PAHs are known to exert acutely toxic effects as well as to have mutagenic or carcinogenic properties (Kanaly & Harayama, 2000; Rand, Wells, & McCarty, 1995, Chap. 1; Swartz, Schults, DeWitt, Ditsworth, & Lamberson, 1990). Since metabolic products of PAHs are thought to be more toxic than the parent compound (Rand et al., 1995; Di Giulio et al., 1995; Ma, Van Kleunen, Immerzeel, & de Maagd, 1998), it is important to examine the mechanisms of contaminant mobilization from sediment, including the relationship between bioavailability and biotransformation in benthic organisms in order to assess contamination risks through trophic transfer. In this study we specifically examined how the quality of the sedimentary organic matter affected Flu uptake, biotransformation and toxicity in the brittle star *A. filiformis*. Special emphasis was placed on the ratio of parent to metabolic products of Flu in disk- and arm-tissue in relation to organic matter quality. Tissue homogenates were extracted into four fractions: (1) parent Flu (i.e. untransformed), (2) aqueous Flu-metabolites, (3) polar Flu-metabolites and (4) tissue residue Flu. Polar Flu-metabolites are considered phase I products, aqueous Flu-metabolites phase II products (i.e. polar metabolites covalently bound to endogenous cell compounds) and tissue residue Flu as unextractable or phase I products that are further oxidized and bound intracellularly (e.g. to DNA, RNA, proteins; Kulkarni & Hodgson, 1980). Two experiments were conducted in which brittle-stars were exposed to sediment-associated Flu either adsorbed to refractory- or easily digestible organic matter.

Amphiura filiformis was chosen because it lives with its body buried in the sediment and because it selects and ingests fine-grained sediment particles when deposit-feeding. *A. filiformis* is one of the most abundant infaunal species in marine soft bottom habitats in the Kattegat, Skagerrak and North Sea region and is also one of few species that has increased in number and biomass, presumably in response to eutrophication in the Kattegat Sea (Duineveld, Künitzer, & Heyman, 1987; Josefson, Jensen, & Aertebjerg, 1993; Pearson, Josefson, & Rosenberg, 1985; Rosenberg, 1995). Furthermore, *A. filiformis* was chosen because it provides an important food source for many fish (Duineveld & Noort, 1986) and invertebrate predators (Baden, Loo, Pihl, & Rosenberg, 1990) that feed largely on the arms of the brittle stars, which subsequently regenerate (e.g. Nilsson, 1999; Sköld, Loo, & Rosenberg, 1994). Flu, one of the most abundant PAHs in marine sediment samples, was chosen as a model compound because it tends to have high bioaccumulation potential compared to smaller or larger PAHs (Gao, Maguhn, Spitzauer, & Kettrup, 1998; Landrum, 1989), is known to exert toxic effects in benthic invertebrates (Swartz et al., 1990) and because a ^{14}C-labelled form is commercially available. Two organic carbon sources that occur in estuarine environments and that differ in digestibility were chosen. Lignin is refractory and derived from terrestrial plants. *Tetraselmis* spp. is a green flagellate with a high nutritive value (www.instant-algae.com).

2. Material and methods

This study was conducted at the Kristineberg Marine Research Station, Sweden, during spring (13/3–13/6) 2000 and is part of a larger research project investigating the fate and effects of organic contaminants in marine sediments.

2.1. Collection and preparation of sediment and brittle stars

Sediments and *A. filiformis* were collected (15/3 2000) in the Gullmar Fjord at 40 m depth. Approximately 40 samples were collected with a 0.1 m^2 box corer (Jonasson & Olausson, 1966). Sediment cores were separated into a light oxic- and a dark anoxic layer. The anoxic sediment was stored (1 m^3 plexi-glass boxes, 5 °C) in the dark under anoxic conditions (i.e. placed under a layer of N_2 saturated water covered with thick plastic bags) until further use in the experiments. Additional oxic sediment was collected (12/4) from the same location, sieved (<1 mm) and kept aerated until spiking with organic matter and Flu. Percent particulate organic matter was 2.3% as determined by loss on ignition (500 °C, 6 h). Oxic sediment was mixed before use to homogenize and disrupt particle aggregates.

One month prior to the experiment, portions of the anoxic sediment (ca. 600 g wet wt.) were transferred to each of 120 one liter glass jars covered with a lid and parafilm, homogenized on a shaking table and finally placed together in a water bath at 5 °C. This was done to preserve a natural microbial community adapted to anoxic conditions. Oxic sediments (<1 mm) were enriched to the same total organic carbon (OC) content (1 g OC/g dry wt. sed.) by adding either refractory- or easily digestible organic matter to the sediment. A commercially available green flagellate (Instant algae 2000 marine microalgae concentrate; species '*Tetraselmis*'; 1.1 billion cells/ml; Reed Mariculture, Inc. San Jose, CA, USA) was used as the labile food source and terrestrial lignin as the refractory food source. *Tetraselmis* spp. belongs to the Micromonadophyceae (previously Prasinophyceae) which is a primitive group of green flagellates (www.ucmp.berkeley.edu/greenalgae/micromonads.html). These flagellates have a high nutritional value (high content of lipids and amino acids) and are easily digested by benthic animals (www.aquatext.com/tables/tetrasel.htm, www.instant-algae.com/microalgae/tetraselmis.htm). Terrestrial lignin was provided from a paper mill (Curan 100; Holmen Paper AB, Wargön, Sweden). Curan 100 is a brown powder composed of insoluble, refractory, structural lignins remaining after the separation of cellulose during the manufacture of unbleached paper (Gunnarsson et al., 1999). The total nitrogen (N) content was higher (ca. 12 times) and the organic carbon content lower (1.4 times) in *Tetraselmis* spp. compared to lignin (Table 1). Enriched sediment was left on a shaking table for 24 h. A known volume of Flu – (3.1 μg crystalline Flu/ml methanol; 98% GC grade, Sigma–Aldrich, Denmark) and radioactively marked Flu- (3.33 μCi/ml acetone; Fluoranthene-3-^{14}C (F6147), Sigma–Aldrich, Sweden) stock solutions were added to methanol/acetone rinsed glass jars. The glass jars were left on a shaking table until the methanol and acetone had evaporated (ca. 4 h). Finally, a known volume of organically enriched wet sediment was added to each jar and left on a shaking table for another 24 h. The relation

Table 1
Quality characteristics of the carbon sources used for enrichment of the original sediment

Carbon source	OC (% dry wt.)	N (% dry wt.)	C/N
Lignin	52.5	0.18[a]	349[a]
Tetraselmis	38.09 (1.21)	2.18 (0.54)	17

N: total nitrogen ($n = 3$), OC: total organic carbon ($n = 3$), C/N: carbon/nitrogen ratio. Data are presented as means (±SD).

[a] Percent dry weight lignin was obtained from Gunnarsson et al. (1999).

between volume acetone/methanol and sediment was equal (3 ml acetone + 3 ml methanol per kg wet wt. sed.) in all treatments including controls.

Adult (disk diameter: up to 7 mm) *A. filiformis* were sieved from the oxic sediment and kept (8 days) in small plastic aquaria on sieved sediment in a flow-through (5 °C) system until cutting of arms. Predation on arms, protruding from the sediment, is a common sub-lethal stress to *A. filiformis*. Amputated arms regenerate, and arm regeneration can be used as an efficient measure of somatic growth (Sköld & Gunnarsson, 1996). One arm was amputated per brittle star 2 weeks prior to the start of the experiment. By cutting different arms, we could distinguish among individual animals added to the same jar. Arms were cut between the seventh and eighth arm segment by cutting the first, second, third, fourth or fifth arm clockwise from the madreporite. After arm amputation brittle stars ($n = 381$) were kept in flow-through aquaria (5 °C) with sieved sediment until the beginning of an experiment. Earlier studies suggested that transfer of *A. filiformis* to experimental containers directly after arm amputation reduces the subsequent arm-regeneration rate compared to experiments in which animals were left to form a scar and heal for 3 weeks before the start of the experiment (Gunnarsson et al., 1999; Sköld & Gunnarsson, 1996). Therefore, brittle stars were allowed to develop a scar subsequent to arm-amputation before being introduced to the experimental jars.

2.2. Experimental setup

The experiments were run in a flow-through system, with 120 glass jars (1 l) placed randomly in a water bath at 5 °C in a climate room and kept in darkness to minimize Flu degradation. Each jar was supplied with homogenized anoxic sediment, giving a base sediment of ca. 6 cm depth and 0.008 m^2 surface area. A Flu-contaminated and/or organically enriched surface layer of 100 g wet wt. oxic sediment (ca. 2 cm) was placed on top of the base sediment and covered by a water column of 7 cm. The jars (∅: 10 cm, h: 15 cm) were sealed with lids perforated for in- and out-flowing water. A head tank supplied seawater from the Gullmar fjord pumped from 35 m depth to a horizontal duct connected via silicone tubes to each jar. A water flow of 40 ml/min gave an approximate water residence time of 13 min. After addition of the top sediment layer, the jars were allowed to settle for 24 h with running seawater before addition of animals.

Two experiments were conducted (i.e. expt. A and B). Each experiment was subdivided into a control- (only organic enrichment) and a Flu (organic enrichment

plus Flu) treatment. The latter was further separated into a treatment with and without addition of brittle stars, giving a total of three treatments per experiment (Table 2). Brittle stars were either exposed to oxic sediment with added lignin ('Li') or *Tetraselmis* ('Te') (i.e. the control groups) or to oxic sediment to which both Flu and lignin ('Li-Flu') or Flu and *Tetraselmis* ('Te-Flu') were added. The Flu concentration was equal between the Flu-treatments (i.e. 30 μg Flu/g dry wt. sed.). In addition, three jars without brittle stars were included per organic treatment (i.e. Li-Flu Control, Te-Flu Control). Details of exposure conditions and the sources of organic matter are given in Tables 1 and 2.

One day prior to the start of an experiment, individual *A. filiformis* were sieved from the flow-through aquaria, placed under a dissection microscope, video-filmed, and placed in small 250 ml glass jars with added water (5 °C) overnight. Animals were separated into groups, differing with regard to location of the amputated arm from the madreporite (arm number 1, 2, 3, 4 or 5). This allowed us to distinguish among individuals within glass jars and thus to determine individual length- and area changes. The experiment was started by introducing four brittle stars to each jar.

2.3. Sampling of sediment and brittle stars

Radiolabelled Flu was used to determine the amounts of unmetabolized Flu (parent), water-soluble (aqueous-) Flu metabolites, water-insoluble (polar-) Flu metabolites in sediment and amounts taken up and biotransformed by *A. filiformis*. It was assumed that radioactive Flu behaved identically to non-radioactive Flu. The ratio between Flu and radioactively marked Flu was 4.2×10^{-4} μg Flu/dpm. Radioactivity associated with each of the phases was quantified by liquid scintillation counting (LSC) on a Wallac 1409 liquid scintillation system (Wallac Inc., Gaithersburg, MD, USA), corrected for quench by the external standards ratio method, and transformed to Flu equivalents (i.e. μg Flu eq.) using the ratio of μg Flu/dpm.

Detailed sediment (surface-, burrow- and anoxic sediment layers) and brittle star sampling were conducted at selected time intervals during the Flu-treatments (i.e. Li-Flu, Te-Flu) and at the end of the control treatments (Li, Li-Flu Control, Te, Te-Flu

Table 2
Amount of organic matter, fluoranthene (Flu) and number of *Amphiura filiformis* added in the different experimental treatments

Experiment	Treatment	Li added (g/g dry wt. sed.)	Te added (g/g dry wt. sed.)	Nominal [Flu] (g/g dry wt. sed.)	*A. filiformis* (number per jar)
A	Li	0.019	–	0	4
	Li-Flu	0.019	–	30	4
	Li-Flu Control	0.019		30	0
B	Te	–	0.027	0	4
	Te-Flu		0.027	30	4
	Te-Flu Control	–	0.027	30	0

Organic matter: Lignin (Li) and *Tetraselmis* spp. (Te).

Control). There were three replicate jars for each sampling occasion. The overlying water was slowly removed by use of a peristaltic pump to avoid disturbance of the sediment. Subsequently, burrow sediment was sampled by gently introducing the tip of a syringe (diam. 1.5 mm) in the visible burrows of *A. filiformis* (seen through the glass jars as a light U-shaped layer (Fig. 1)) and slowly drawing the burrow sediment into the syringe. Surface sediment was collected, and the remaining sediment was poured onto a sieve (<1 mm) from which anoxic sediment samples were taken. All sediment samples were frozen (−20 °C) in zip-lock bags until further analysis. Finally, animals were sieved from the sediment, rinsed in clean seawater to remove sediment, individually video-filmed under a dissection microscope, and left to purge their guts for 2–4 h in filtered seawater. Pilot experiments as well as personal observation of individuals ensured that each animal had purged its gut before further handling. Since arms, rather than disks of *A. filiformis* constitute an important food for demersal fish we distinguished between these two compartments in our analysis. Therefore, animals were dissected into arms and disk, weighed (wet wt.) and frozen

Fig. 1. Experimental jar. Anoxic base sediment covered by a top layer of oxic sediment (light layer) enriched to an OC content of 3% with either lignin or *Tetraselmis* spp. (a green flagellate). The light colored sediment in the dark anoxic sediment layer shows the burrow linings of *A. filiformis*.

(−20 °C) in ultra centrifuge tubes (three replicates of four brittle star disks or arms) until further Flu- and lipid analysis.

2.4. Analysis of sediment and brittle stars

Samples of burrow-, anoxic- and surface sediment layers were analyzed for organic carbon and nitrogen content at the start (day 1), and end (day 45) of the experiments. Briefly, total carbon (C), and total nitrogen (N) concentrations were measured on freeze-dried samples with a Carlo Erba element analyzer (EA 1110 CHNS). Total organic carbon (OC) was determined after treating samples with HCl following Hedges and Stern (1983).

Flu analysis of sediment and brittle stars included separation of total radioactivity into concentrations of parent Flu (P), polar- (P–M) and aqueous (A–M) Flu-metabolites as well as residual Flu (i.e. unextractable fraction; R). Samples from each replicate beaker were extracted using a modification of the Bligh and Dyer (1959) lipid extraction method, adapted for analysis of PAHs and metabolites (Kane Driscoll & McElroy, 1996, 1997; McElroy, 1990; Selck et al., 2003b). Briefly, this two step extraction scheme initially produces a chloroform phase (containing parent Flu and organic-soluble metabolites: i.e. polar Flu-metabolites) and a methanol/water phase (containing water-soluble metabolites: i.e. aqueous Flu-metabolites). The chloroform was evaporated during the second step and re-extracted in hexane, potassium hydroxide (KOH) and dimethyl sulfoxide (DMSO) to separate parent Flu from polar metabolites (Van Cantfort, DeGraeve, & Gielen, 1977). Parent Flu was contained in the hexane phase, polar Flu-metabolites in the DMSO/KOH phase and aqueous Flu-metabolites in the methanol/water phase.

Flu concentrations were determined in surface- and burrow sediments at the start and end of a treatment (Li-Flu, Te-Flu, Li-Flu Control, Te-Flu Control) as well as at two times during the experimental period in Li-Flu and Te-Flu (days 2 and 16). Methanol (2 ml), chloroform (1 ml) and demineralized water (0.8 ml) were added (one phase) to each sediment sample (1.2–2.8 g dry wt. surface sediment; 0.3–1.1 g dry wt. burrow sediment). After stirring (30 s), the sample was exposed to ultrasonic treatment (20 min), and additional chloroform (1 ml) and demineralized water (1 ml) were added. The solution was stirred again (30 s), and finally centrifuged (5 min, 250*g*, 18 °C). The supernatant (i.e. water/methanol and chloroform phases) was transferred to new glass tubes, and the extraction from the sediment was repeated twice with half the volumes of solvent as described above. The methanol/water phase was mixed and 3 ml transferred to a scintillation vial containing 15 ml Ultima Gold XR scintillation cocktail (Packard, Denmark). The chloroform phase was evaporated (w. O_2) to almost dryness (ca. 2 h) and resuspended in 600 μl DMSO (HPLC purity 99%), 250 μl demineralized water and 150 μl KOH (1 M). The solution was stirred (whirlmixer, 30 s), 2 ml hexane was added, stirred again (30 s) and centrifuged (5 min at 250*g*). The hexane phase was transferred to a new pyrex tube, and the procedure was repeated twice from the addition of hexane. Three milliliters of the hexane phase, 0.6 ml of the DMSO phase and the remaining sediment sample were transferred separately to three scintillation vials with 15 ml Ultima Gold XR.

The amounts of parent Flu, aqueous- and polar Flu metabolites and tissue residue Flu were determined in three groups of four brittle stars (i.e. four disks or 16 arms) on each sampling occasion (day 1, 2, 4, 6, 8, 11, 16, 26, 37, 45) during the experiment (Li-Flu, Te-Flu) and at experimental termination (Li, Te). Brittle star samples (0.15–0.24 g dry wt. tissue) were thawed, transferred to pre-weighed pyrex centrifuge tubes (10 ml) and homogenized (2 min) in 0.8 ml demineralized water using a microhomogenizer (Jencons, Bedfordshire, UK). The amount of demineralized water added was corrected for water content in brittle star tissue. The dry weight content of arms and disks was determined to be 41.3 (±1.5) % and 33.8 (±2.6) % of the wet weight ($n = 3$), respectively. Subsequently, 1 ml chloroform and 2 ml methanol were added, and the same procedures as described for sediment extraction were used to extract parent Flu, aqueous- and polar Flu metabolites from the brittle stars. Radioactivity remaining in the tissue after extraction (unextractable or residual activity) was determined after the tissue homogenate had been left in 1 ml tissue solubilizer (Soluene) overnight. Flu body burdens (i.e. disk and arms) in *A. filiformis* were assessed as weight-specific body-burdens (BB: μg Flu eq./g dry wt. tissue) and as proportional fraction (in %) of parent Flu, polar Flu-metabolites, aqueous Flu-metabolites and residue tissue Flu. The percentage of each fraction was calculated from the total radioactivity in disk or arms (i.e. $\sum$(P, A–M, P–M, R)). Uptake/biotransformation kinetics was calculated for each compartment separately. Net 'appearance' (i.e. uptake rate for parent Flu and formation rate for metabolites) rate constants [net $k_{app.}$, μg Flu equivalents (g dry wt. tissue)$^{-1}$ day^{-1}] of total Flu (i.e. $\sum$(P, A–M, P–M, R)) were calculated by measuring the tangent to the uptake curves between each sampling occasion following Spacie and Hamelink (1995). Thus, net appearance rate is a measure of uptake/biotransformation rate minus disappearance rate (i.e. depuration or further biotransformation) for each of the four fractions.

Total lipid content in brittle-star samples was determined on days 37 and 45 according to the microgravimetric method of Gardner, Frez, Cichocki, and Parrish (1985) modified from Bligh and Dyer (1959). This method permitted measurement of total lipid content in disk subsamples as small as 2–3 mg of dry weight tissue. Lipid content in brittle star arms was not determined since earlier studies have shown that the content was below detection (Gunnarsson & Sköld, 1999). The biota-sediment-accumulation-factor was calculated according to: $BSAF = (C_{tissue}/lipid)/(C_{sediment}/OC)$; where C = total Flu concentration in tissue (C_{tissue}: μg Flu eq./g dry wt. tissue) or sediment ($C_{sediment}$: μg Flu eq./g dry wt. sed.) on days 37 and 45, and OC = total organic carbon content on day 45.

The effects of Flu and/or organic matter quality (i.e. Li vs. Te) on the growth of brittle stars were assessed by measuring regenerated arm length and area. Individual length (L, mm) and area (A, mm^2) of the regenerating arms were determined from measurements of projected animals using SigmaScanPro software (ver. 5.0.0). Each length and area estimate used in the analysis was the mean of three replicate measurements of the same arm. Regenerated arm length and area were calculated from the difference between start and end measurements on each sampling occasion. Brittle stars collected on days 1 and 2 were excluded from length- and area analysis since individual length and area changes could not be determined after such a short period.

2.5. Statistics

One-way ANOVA was used to test for differences when more than two groups were involved. Tukey's HSD test was used to test for significant pairwise differences among groups. Student's *t*-tests were performed when only two groups were compared. When necessary, data were $\log_{(10)}$ transformed to either homogenize the variances or to normalize the data prior to analysis. Significance level: significant: $P \leqslant 0.05$; marginally significant: $0.05 < P \leqslant 0.1$.

3. Results

3.1. Analysis of sediment organic matter

The boundary between the oxic- and the anoxic layers was well defined in all glass jars regardless of organic treatment (Fig. 1). Initially (day 1) the surface sediment in Li-Flu appeared to contain lower mean levels of OC (46.3%) and N (18.1%) relative to in Te-Flu (Table 3), but the difference was only statistically significant for N (OC: $P = 0.144$; N: $P = 0.038$). Time had no significant impact on OC content of surface sediment in Li-Flu ($P = 0.230$). In contrast, a significant decrease of 80.1% was observed in the OC content in Te-Flu (Table 3) ($P = 0.031$). No time-dependent change was observed in N content in either Li-Flu or Te-Flu (Table 3) (Li-Flu: $P = 0.834$; Te-Flu: $P = 0.328$). The OC levels were similar between control treatments at the end of the experiment (day 45) (i.e. Li vs Te: $P = 0.259$). Organic matter quality did not influence the N content in systems with added Flu (i.e. Li-Flu vs. Te-Flu: N: $P = 0.174$). In contrast, the content of OC in surface sediments was significantly lower (76.0%) in treatments with *Tetraselmis* and Flu compared to with lignin and Flu (Table 3) ($P = 0.002$). The presence of Flu marginally reduced the OC content in systems with labile organic matter (Te vs. Te-Flu: $P = 0.064$), whereas no effect was observed in systems with added lignin (Table 3) (Li vs. Li-Flu: $P = 0.197$).

Table 3
Total organic carbon (OC) and total nitrogen (N) in surface sediment samples (% of g dry wt. sed.) on day 1 and 45 in systems with added fluoranthene (Flu) and/or lignin (Li) or *Tetraselmis* (Te)

Treatment	Day	OC	N
Li	45	1.7 (0.9)	–
Te	45	1.0 (0.2)	–
Li-Flu	1	1.6 (1.0)	0.3 (0.0)
	45	2.5 (0.3)	0.3 (0.0)
Te-Flu	1	3.0 (0.9)	0.4 (0.0)
	45	0.6 (0.1)	0.4 (0.0)

Values are presented as means (±SD).
$n = 3$.

3.2. Analysis of fluoranthene in surface- and burrow sediments

In sediment with brittle stars, the total measured Flu concentration ($\sum$P, A–M, P–M; μg Flu eq./g dry wt. sed.) in surface sediment did not change during the course of the experiment regardless of organic carbon treatment (Li-Flu: $P = 0.275$; Te-Flu: $P = 0.779$). Day 2 was excluded from the analysis of surface sediment in Li-Flu due to contamination of samples. The final total Flu concentrations were 21.25 ($\pm$ 5.43) μg Flu eq. (g. dry wt. sed.)$^{-1}$ in Li-Flu and 22.20 ($\pm$9.92) μg Flu eq. (g dry wt. sed.)$^{-1}$ in Te-Flu. After 45 days of exposure parent Flu constituted the highest percentage (Li-Flu: 92.3%, Te-Flu: 96.7%) whereas aqueous- and polar Flu-metabolites contributed very little (Li-Flu: 2.1–2.7%, Te-Flu: ca. 1.5%) to the total Flu concentration in surface sediment (Table 4). Aqueous- and polar Flu-metabolites increased significantly in the surface sediment from day 16 to day 45 in treatments with lignin (A–M: 46.7%; $P = 0.034$, P–M:

Table 4
Measured concentrations of parent Flu, aqueous- and polar Flu-metabolites in surface- (S) and burrow (B) sediment inhabited by *Amphiura filiformis* (Li-Flu, Te-Flu) or without animals (Li-Flu Control, Te-Flu Control)

Treatment	Day	Parent Flu		Aqueous Flu-metabolites		Polar Flu-metabolites		*n*
		(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	
Li-Flu								
S	2	–	–	–	–	–	–	–
S	16	15.95 (0.74)	96.0	0.30 (0.05)	1.80	0.34 (0.07)	2.20	3
S	45	20.26 (5.36)	95.4	0.44 (0.06)	2.14	0.55 (0.02)	2.72	3
B	2	0.55 (0.07)	50.7	0.26 (0.03)	23.6	0.28 (0.08)	25.8	2
B	16	3.40 (0.80)	75.3	0.57 (0.14)	12.7	0.54 (0.13)	12.0	2
B	45	5.85 (0.07)	92.3	0.25 (0.07)	3.90	0.24 (0.08)	3.80	3
Te-Flu								
S	2	17.43 (6.91)	96.7	0.26 (0.08)	1.47	0.21 (0.03)	1.81	2
S	16	23.00 (7.87)	97.1	0.28 (0.04)	1.35	0.31 (0.08)	1.53	3
S	45	21.57 (9.84)	96.9	0.31 (0.04)	1.54	0.32 (0.06)	1.57	3
B	2	0.77 (0.13)	58.1	0.29 (0.07)	21.8	0.27 (0.07)	20.1	3
B	16	4.09 (1.33)	84.2	0.39 (0.11)	7.98	0.37 (0.07)	7.84	2
B	45	4.81 (2.56)	83.4	0.48 (0.26)	8.79	0.41 (0.17)	7.81	3
Li-Flu Control								
S	45	15.35 (7.95)	90.8	0.67 (0.17)	4.70	0.64 (0.13)	4.50	3
Te-Flu Control								
S	45	4.20 (0.55)	75.3	0.72 (0.15)	13.0	0.64 (0.08)	11.7	3

Surface sediment was enriched with lignin (Li) or *Tetraselmis* (Te). Concentrations are presented as mean Flu equivalents (μg Flu eq./g dry wt. sed. ($\pm$SD)). The Flu concentration was below detection in anoxic sediments. Data for day 2 in Li-Flu surface sediment were excluded from analysis due to contamination of samples.

61.7%; $P = 0.013$). The apparent increase in average concentration of A–M and P–M during the experiment in Te-Flu (A–M: 19.2%, P–M: 52.4%) was not statistically significant (A–M: $P = 0.515$; P–M: $P = 0.974$). The concentration of parent Flu did not change in either Li-Flu ($P = 0.263$) or Te-Flu ($P = 0.779$). In systems with brittle stars organic matter quality had no influence on the total radioactivity or the concentration of parent Flu ($P > 0.500$) in surface sediment at experimental termination (i.e., day 45). However, the amounts of aqueous- ($P = 0.035$) and polar ($P = 0.002$) Flu-metabolites were higher (A–M: 1.4 times; P–M: 1.7 times) in Li-Flu compared to in Te-Flu (Table 4). Brittle stars (Li-Flu vs. Li-Flu Control) had no effect on the amount of total Flu ($P = 0.459$), parent Flu ($P = 0.426$) or polar Flu-metabolites ($P = 0.311$) whereas the aqueous Flu-metabolites were marginally affected ($P = 0.090$) in systems with added lignin (Table 4). In contrast, brittle stars significantly affected sediment Flu concentrations in systems with added *Tetraselmis* (Te-Flu vs. Te-Flu Control). The total Flu concentration was higher (4 times) in sediment with- than without brittle stars ($P = 0.007$). The proportion of parent Flu ($P = 0.004$) was ca. 5 times higher and aqueous- ($P = 0.005$) and polar Flu-metabolites ($P = 0.004$) were lower (2–2.3 times) in Te-Flu compared to sediment without animals (i.e. Te-Flu Control) (Table 4).

Total radioactivity in the burrows of *A. filiformis* increased significantly with time in sediment with added lignin (5.9 times; $P = 0.008$) and marginally in sediment with added *Tetraselmis* (4.3 times; $P = 0.091$) (Table 4). In Li-Flu the concentration of parent Flu increased (10.6 times) significantly in the burrow sediment ($P = 0.003$) whereas the changes in aqueous Flu-metabolites and polar Flu-metabolites with time were marginally significant (A–M: $P = 0.062$, P–M: $P = 0.100$) (Table 4). Parent Flu constituted the highest percentage (92.3%) and metabolites less than 4% each on day 45 (Table 4). In Te-Flu a marginally significant increase in parent Flu (6.2 times) was observed in the burrow sediment during the experiment ($P = 0.075$). Despite a mean increase of 65.5% for A–M and 51.9% for P–M in the burrow sediment in Te-Flu, these time-dependent changes were not statistically significant (A–M: $P = 0.484$; P–M: $P = 0.395$). Burrow sediment was dominated by parent Flu (83.4%), and the remainder was equally distributed between A–M and P–M (Table 4). Organic treatment had no significant influence on the total Flu concentration (Li-Flu: 6.33 ($\pm$0.22) μg Flu eq./g dry wt. sed.; Te-Flu: 5.70 ($\pm$2.94) μg Flu eq./g dry wt. sed.) ($P = 0.687$), the amount of parent Flu or metabolites in burrow sediment at experimental termination (day 45) (P: $P = 0.778$, A–M: $P = 0.235$, P–M: $P = 0.229$). However, the results suggest that parent Flu constituted a greater proportion and aqueous- and polar Flu-metabolites a smaller fraction in treatments with lignin compared to *Tetraselmis* (Table 4).

Amphiura filiformis transported the surface sediment into their burrows, thus into the non-contaminated anoxic sediment in systems with both added labile- and refractory organic matter. The total radioactivity of burrow sediment was lower than in surface sediment (Li-Flu: $P = 0.010$, 3.4 times; Te-Flu: $P = 0.029$, 3.9 times). The average concentration of parent Flu was higher whereas the metabolites were approximately at the same level in surface – relative to burrow

sediment both in systems with added lignin and *Tetraselmis*. However, the proportion of metabolites relative to parent was higher in burrow – compared to surface sediment (Li-Flu: A–M: 1.4 times, P–M: 1.8 times; Te-Flu: A–M: 5.7 times, P–M: 5.0 times) (Table 4).

3.3. Effect of fluoranthene and organic matter on arm-regeneration rate

There were no significant differences in mean regenerated arm length (0.399 ± 0.222 mm, $n = 287$) or area (0.091 ± 0.054 mm^2, $n = 287$) ($n = 287$; length: $P = 0.949$; area: $P = 0.680$) among replicate glass jars ($n_{\text{jars}} = 72$; $n_{\text{animals/jar}} = 4$) in the four treatments (Li: $n_{\text{jars}} = 3$, Te: $n_{\text{jars}} = 3$, Li-Flu: $n_{\text{jars}} = 33$, Te-Flu: $n_{\text{jars}} = 33$) following scar formation and before the animals were introduced to the experimental treatments ($t = 0$). Arm-regeneration was affected by Flu in sediment with labile, but not refractory, organic matter measured after 45 days of exposure. In the presence of Flu the mean regenerated area was reduced ($P = 0.049$) by 22.9% whereas no effect was observed on the regenerated length ($P = 0.120$) in sediment with added *Tetraselmis* (i.e. Te vs. Te-Flu) (Fig. 2). The presence of Flu did not affect the final regenerated length ($P = 0.602$) or area ($P = 0.917$) in systems with added lignin (Li vs. Li-Flu). In the absence of Flu, average length and area were 17.2% and 15.1% higher, respectively, in systems with labile organic matter (Te) than in systems with refractory organic matter (Li), but these differences were not statistically significant (length: $P = 0.198$; area: $P = 0.332$) (Fig. 2). No difference in regenerated arm length ($P = 0.345$) or arm-area (students t test: $P = 0.243$) was detected between Li-Flu and Te-Flu.

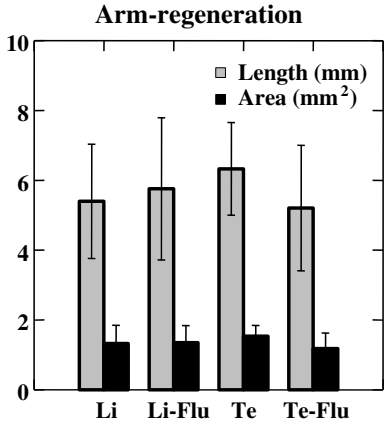

Fig. 2. Individual regenerated arm-length (mm) and -area (mm^2) of *Amphiura filiformis* at experimental termination (day 45) (mean ± SD). Treatments: lignin (Li), lignin and fluoranthene (Flu) (Li-Flu), *Tetraselmis* spp. (Te) or *Tetraselmis* and Flu (Te-Flu).

3.4. Fluoranthene uptake and biotransformation

3.4.1. Weight-specific Flu body-burden (BB)

Amphiura filiformis accumulated Flu regardless of the quality of organic matter added to the sediment. The total body-burden (i.e. total radioactivity in brittle-star tissue) in the disk increased with exposure time both in Li-Flu and Te-Flu treatments (Fig. 3(a)). The time-dependent accumulation in the disk was delayed (approximately 6 days) for brittle stars in Li-Flu compared to Te-Flu. Net uptake was approximately zero during these first 6 days after which the rate increased significantly in Li-Flu (Fig. 3(a), Table 5). In contrast, brittle stars in sediment enriched with easily digestible organic matter showed the highest uptake rate during the first days of exposure (Fig. 3(a), Table 5). There was no significant difference in the total disk tissue concentration of Flu between Li-Flu (23.45 ± 3.67 μg Flu eq./g dry wt. disk tissue) and Te-Flu (19.35 ± 1.39 μg Flu (g dry wt. disk tissue)) after 45 days of exposure ($P = 0.244$). Brittle-star weight-specific Flu body-burden in the arms was independent of exposure time in Te-Flu, whereas the arm body-burden increased with time until day 16 before reaching a constant level in Li-Flu (Fig. 3(b), Table 5). The total Flu concentration in arm tissue on day 45 was 3.70 ± 0.59 μg Flu (g dry wt. arm tissue) ($n = 2$) in Li-Flu and 5.29 μg Flu (g dry wt. arm tissue) ($n = 1$) in Te-Flu. A comparison of body-burden between arms and disk clearly demonstrates that accumulation of Flu was considerably higher (ca. 3.6–6.3 times) in brittle star disk fractions compared to arm fractions in both organic treatments (Fig. 3). This was reflected in uptake rates (Table 5).

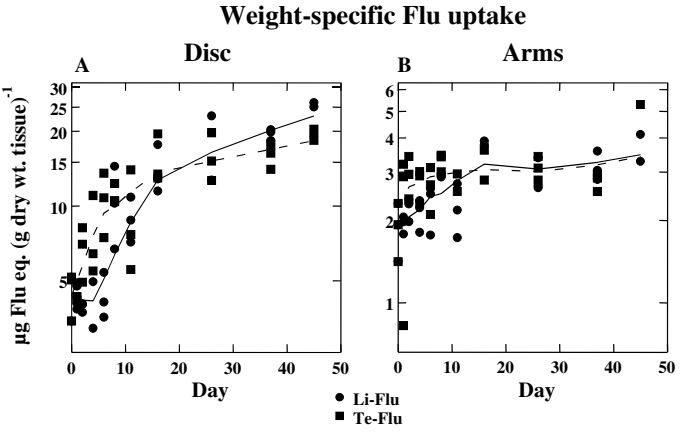

Fig. 3. Relation between weight-specific body-burden (BB: μg Flu equivalents/g dry wt. tissue) and exposure time in *Amphiura filiformis* disk- (A) and arm (B) fractions. Circles, exposure to sediment enriched with lignin and Flu (Li-Flu); squares, exposure to sediment enriched with *Tetraselmis* and Flu (Te-Flu). Lines are locally weighted scatterplot smooths.

Table 5
Net appearance rate constants ($k_{app.}$: net radioactivity) of weight-specific body-burden (BB: $\sum$ μg Flu eq. g dw. tissue^{-1} day^{-1}) in disk and arms of *Amphiura filiformis* calculated as the tangent (dC/dt) between means at each time interval for the two organic treatments (i.e. lignin, *Tetraselmis*)

Day	Li-Flu		Te-Flu	
	Arm	Disk	Arm	Disk
1	0.06	−0.33	0.42	−0.08
2	0.28	−0.30	***0.62***	***2.23***
4	−0.04	0.08	−0.09	0.47
6	0.08	0.14	−0.05	1.48
8	*0.31*	***3.06***	0.33	0.24
11	−0.24	−0.52	−0.18	−0.51
16	***0.33***	1.01	0.09	1.25
26	−0.09	0.45	−0.01	0.06
37	0.02	0.11	−0.04	0.02
45	0.07	0.50	0.31	0.42

The highest rate in each column is highlighted.

3.4.2. The biota-sediment-accumulation-factor

Lipid data for days 37 and 45 were pooled within organic treatments, since no change in disk lipid content was observed between days 37 and 45. Organic matter quality did not affect the lipid content in the disk fraction of *A. filiformis* ($P = 0.849$, $n = 8$; Li-Flu: $3.74 \pm 0.40\%$ lipid; Te-Flu: $3.65 \pm 0.97\%$ lipid). Accumulated total Flu normalized to tissue lipid concentrations was marginally higher ($P = 0.067$) in brittle stars from sediment with added lignin (591.8 ± 66.2 μg Flu eq./lipid, $n = 4$) compared to brittle stars from sediment with added *Tetraselmis* (443.3 ± 108.5 μg Flu eq./lipid, $n = 4$). The mean biota-sediment-accumulation-factor (BSAF) was 33.5% higher in brittle stars in sediment with added lignin (BSAF: 0.67 ± 0.08) compared to sediment with added *Tetraselmis* (BSAF: 0.51 ± 0.12).

3.4.3. Flu biotransformation

Amphiura filiformis appear to metabolize Flu but at a slow rate (Table 6, Fig. 4). Overall, parent Flu constituted the highest concentration whereas metabolites contributed minimally to the total Flu body burden in both disks and arms of *A. filiformis* regardless of organic matter quality throughout the course of the experiment (Table 6, Fig. 4(a)–(d)). Only the increases in weight-specific body-burden (μg Flu eq./g dry wt. tissue) of parent Flu and aqueous Flu-metabolites were significant in both arms and disk in the two treatments (Table 7, Fig. 4). A marginally significant increase was detected in tissue residue in Li-Flu arms and disk and polar Flu-metabolites in disk (Table 7). However, the pairwise comparisons did not detect any significant differences (all $P > 0.100$). Accordingly, we conclude that no significant increase in weight-specific body-burdens of polar Flu-metabolites or tissue residue Flu could be detected (Table 7). Organic matter quality (Li-Flu vs. Te-Flu) did not have a marked influence on the tissue concentrations of parent Flu, aqueous Flu-metabolites, polar Flu-metabolites or tissue residue Flu in disk or arms of *A. filiformis* (Table 6). A comparison between disk and arms reveals that the average body-burdens of parent Flu, aqueous

Table 6
Fractional tissue concentration of parent Flu (P), aqueous Flu-metabolites (A–M), polar Flu-metabolites (P–M) and tissue residue Flu (R) in disk (D) and arms (A) of *Amphiura filiformis* on different days

Treatment	Day	Parent Flu		Aqueous Flu-metabolites		Polar Flu-metabolites		Tissue residue		*n*	Total Flu
		(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)	(μg/g)	(%)		
Li-Flu											
D	2	1.54 (0.11)	39.2	1.08 (0.01)	27.6	0.83 (0.04)	21.0	0.48 (0.02)	12.2	3	3.93 (0.17)
D	16	10.3 (2.44)	73.9	1.70 (0.37)	12.2	1.25 (0.36)	8.84	0.70 (0.09)	5.06	3	14.0 (3.24)
D	45	18.6 (3.14)	79.2	2.65 (0.17)	11.4	1.36 (0.22)	5.78	0.85 (0.16)	3.60	3	23.4 (3.67)
A	2	1.01 (0.09)	45.5	0.54 (0.07)	24.2	0.42 (0.05)	18.9	0.25 (0.04)	11.4	3	2.22 (0.21)
A	16	2.31 (0.14)	59.7	0.66 (0.09)	16.6	0.46 (0.01)	11.6	0.31[a]	12.1	2	3.83 (0.09)
A	45	2.30 (0.52)	62.6	0.70 (0.02)	18.6	0.41 (0.05)	10.9	0.29 (0.00)	7.87	2	3.70 (0.59)
Te-Flu											
D	2	3.91 (1.59)	56.6	1.30 (0.06)	20.2	0.95 (0.05)	14.8	0.55 (0.03)	8.44	3	6.70 (1.64)
D	16	12.1 (3.57)	78.3	1.49 (0.16)	9.97	1.13 (0.06)	7.65	0.59 (0.06)	4.04	3	15.3 (3.63)
D	45	15.2 (0.87)	78.4	2.20 (0.25)	11.4	1.25 (0.10)	6.44	0.73 (0.17)	3.75	2	19.4 (1.39)
A	2	1.62 (0.34)	55.1	0.57 (0.07)	19.7	0.45 (0.07)	15.4	0.28 (0.04)	9.73	3	2.92 (0.51)
A	16	2.10 (0.46)	66.9	0.49 (0.05)	14.8	0.38 (0.04)	11.4	0.23 (0.01)	6.86	2	3.20 (0.56)
A	45	3.41 (0.59)	72.3[a]	0.79 (0.18)	12.6[a]	0.54 (0.06)	9.39[a]	0.30[a]	5.69	2	5.29[a]

Fluoranthene concentrations are presented as Flu equivalents (μg Flu eq./g dry wt. tissue) and as a percent of total Flu eq. Data are presented as means (±SD).

[a] $n = 1$.

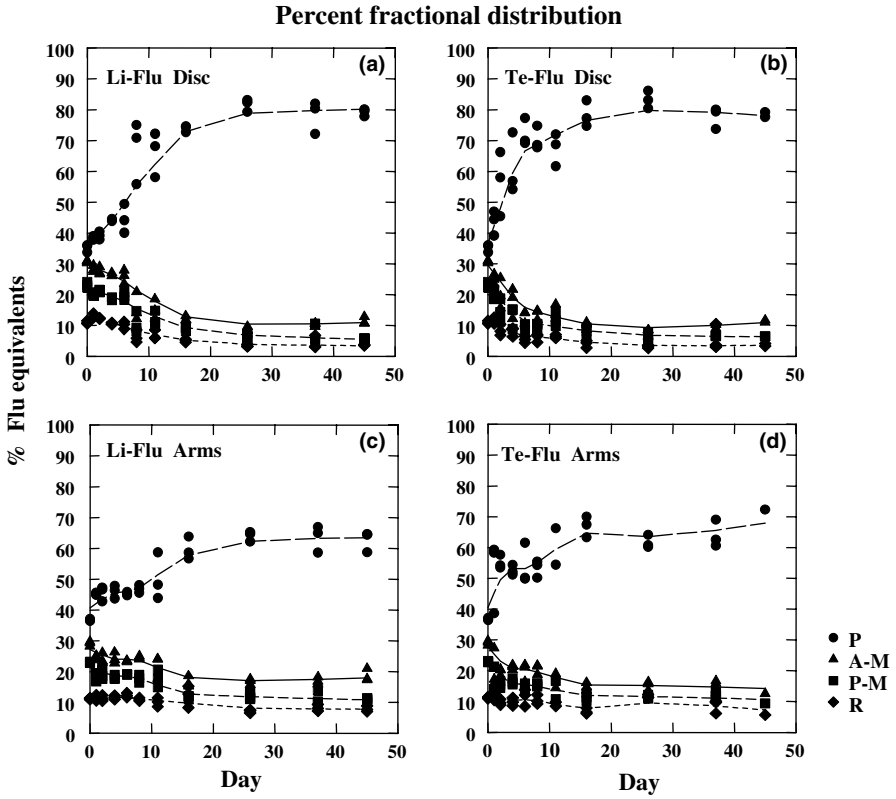

Fig. 4. Proportional distribution of Flu equivalents was determined as percent parent Flu (P: circles), aqueous Flu-metabolites (A–M: triangles), polar Flu-metabolites (P–M: squares) and residual Flu (i.e. unextractable, R: diamonds) in *Amphiura filiformis* disk- (top row) and arm (bottom row) fractions during the exposure period. Treatments: lignin (Li) and fluoranthene (Flu) (Li-Flu: left column) and *Tetraselmis* (Te) and Flu (Te-Flu: right column).

Flu-metabolites, polar Flu-metabolites and tissue residue Flu were substantially higher (P: 5.9 times; A–M: 3.2 times; P–M: 2.8 times; R: 2.7 times) in disk compared to arms (Table 6). However, the mean proportions of aqueous Flu-metabolites, polar Flu-metabolites and tissue residue Flu were higher in the arms relative to the disk (A–M: 1.4 times; P–M: 1.7 times; R: 1.8 times).

4. Discussion

4.1. Sediment organic matter

We chose two organic carbon sources that differed in nutritional quality, as defined by Gunnarsson et al. (1999). These authors suggested that the nutritional quality of organic carbon sources was primarily dependent on the content of N and

Table 7
Percent increase in weight-specific body-burden (disk & arm-fraction) of parent Flu (P), aqueous Flu-metabolites (A–M), polar Flu-metabolites (P–M) and tissue residue Flu (R) in disk and arms of *Amphiura filiformis* after 45 days of exposure to fluoranthene associated with lignin (Li-Flu) or *Tetraselmis* (Te-Flu)

Fraction	Disk					Arms				
	% increase	*P*	*F* ratio	d.f.	*n*	% increase	*P*	*F* ratio	d.f.	*n*
Li-Flu										
P	1055.7	<0.001	24.9	10,22	33	231.8	<0.001	16.4	10,20	31
A–M	89.5	<0.001	12.7	10,22	33	28.5	0.009	3.44	10,20	31
P–M	5.00	0.080	2.03	10,22	33	–	0.233	1.44	10,20	31
R	24.4	0.057	2.25	10,21	32	37.1	0.044	2.45	10,19	30
Te-Flu										
P	842.8	<0.001	11.4	10,21	32	392.8	<0.001	6.71	10,20	31
A–M	57.7	0.013	3.16	10,21	32	45.0	0.039	2.50	10,20	31
P–M	18.9	0.608	0.83	10,21	32	24.2	0.108	1.89	10,20	31
R	47.5	0.531	0.92	10,21	32	42.6	0.256	1.40	10,18	29

Percent increase was calculated as: $\{([t_{45}] - [t_0])/[t_0]\} \times 100\%$; t_0: background concentration, t_{45}: final tissue concentration. ANOVA was used to test for changes in weight-specific body-burden (μg Flu eq./g dry wt. tissue). d.f.: degrees of freedom; *n*: number of replicates.

amino-acids. The sea lettuce, *Ulva lactuca*, had the highest (2.16%) and lignin the lowest (0.18%) N content of five tested organic materials, and these were thus considered the most labile and refractory organic matter, respectively (Gunnarsson et al., 1999). Following this definition, *Tetraselmis* spp. can be considered a highly labile organic material ($N = 2.18\%$) and lignin as refractory. In the present study, mineralization was only detected in systems with labile organic matter, evidenced as a decline in the OC content during the course of the experiment. This is in good agreement with results presented by Gunnarsson et al. (1999), who found that OC mineralization was highest in sediments with the most labile organic matter (*Ulva*) and lowest in sediments with the most refractory organic matter (i.e. lignin).

4.2. Fluoranthene distribution in sediment

Organic matter quality did not affect the total radioactivity in surface sediment in the presence of brittle stars. In contrast, the total Flu concentration was higher in systems with- than without brittle stars in sediment with added labile organic matter (Te). Nearly all extracted radioactivity from surface sediment consisted of parent fluoranthene (>95% at all time intervals), and only a small amount was determined to be aqueous- (<2.2%) or polar Flu-metabolites (<2.8%). However, there was less degradation of parent Flu (to aqueous and polar metabolites) in surface sediment with labile organic matter compared to sediment with refractory organic matter. An increase in both parent Flu and metabolites was observed in the linings of the burrows both in sediment with lignin and *Tetraselmis*. Surface concentrations were higher than in the burrows, but aqueous- (>3.9%) and polar (>3.8%) Flu-metabolites constituted a greater part and parent Flu (>83%) a smaller part of the total Flu content in burrow compared to surface sediment.

It was obvious from the photographs of the experimental jars that *A. filiformis* buries into the sediment to a depth below the surface layer (i.e. the top 2 cm) (Fig. 1). A light oxic sediment layer surrounded the burrows of *A. filiformis*, indicating that they transport surface sediment deeper into the anoxic sediment or that they irrigate their burrows and thereby oxygenate the burrow lining without transporting surface sediment to depth. The time-dependent increase in the concentration of parent Flu in the burrows suggests particle transport. The accumulation of organic contaminants has been found to be up to one order of magnitude higher in the burrow walls of infaunal species, compared to in the adjacent bulk sediment (Gunnarsson et al., 1999). For example, TCB concentrations were higher in the linings of arm burrows and disk chambers than in the bulk sediment sampled just outside the burrows of the brittle star *A. filiformis* at the same depth (Gunnarsson et al., 1999). We only detected Flu in surface- and burrow sediment and not in the anoxic sediment, suggesting that *A. filiformis* does not redistribute contaminants deeper into the sediment other than by the burrows. However, *A. filiformis* does not have a permanent burrow construction. Thus, its movement may cause a more or less continuous displacement of the top sediment, and this may create a net downward transport of sediment-associated contaminants. It was clearly demonstrated that parent Flu contributed by far the highest concentration (>75%) in sediments (burrow and/or surface) regardless of organic matter quality and the presence or absence of *A. filiformis*. In the presence of brittle stars, surface sediment contained higher concentrations of parent Flu compared to burrow sediment whereas the metabolites were at about the same levels. Interestingly, the proportions of metabolites were higher in burrow- (3.8–8.8%) compared to surface (1.5–2.7%) sediment, indicating that the breakdown of parent Flu by brittle stars and/or microorganisms is relatively higher in burrows compared to surface sediment and especially so in systems with added labile organic matter. The stimulating effect of macrofaunal bioturbation on the oxygenation of deeper anoxic sediments, bacterial activity and organic matter mineralization is well documented (Aller, 1982; Banta, Holmer, Jensen, & Kristensen, 1999; Kristensen & Blackburn, 1987). The importance of these burrow linings for the breakdown of organic contaminants is poorly investigated. However, burrow-, anoxic- and surface sediments were collected at the termination of a similar experimental system (with- and without brittle stars) using pyrene instead of Flu (Granberg, Hansen, & Selck, in press). Sediment samples were transferred to CO_2-traps, were spiked with ^{14}C-pyrene, and $^{14}CO_2$ production (i.e. microbial degradation) was followed for 5 months. Briefly, Granberg et al. (in press) found an increased microbial degradation of pyrene in the burrow- and surface sediment compared to anoxic sediment, and that pyrene degradation in burrow sediment was higher in systems with added lignin than with *Tetraselmis*. At the end of the present study the lowest concentrations of parent Flu and the highest metabolite concentrations were detected without brittle stars in sediments enriched with labile organic matter. Our results suggest that the presence of *A. filiformis* suppresses Flu degradation by sediment microorganisms in the presence of *Tetraselmis*. Evidence for both stimulation and depression of microbial activity in response to the presence of benthic organisms has been documented. For example, Christensen, Banta, and Anderson (2002) found that the presence of

Arenicola marina stimulated the microbially mediated degradation of sediment-associated pyrene, whereas the presence of *Nereis diversicolor* depressed degradation after 42 days of exposure. Moreover, Chung and King (1999) found that degradation of PAHs was sediment-specific (burrow- vs. surface sediment) and in some cases dependent on the presence of a specific benthic organism. Amphiurids have been found to feed on bacteria in addition to other nutritious particles and dissolved organic carbon (Clements, Fielman, & Stancyk, 1988; Lopez & Levinton, 1987). Our results suggest that the presence of *A. filiformis* (either directly or indirectly) alters the production of Flu metabolites in sedimentary systems, but that this effect is complicated by the quality of the sedimentary organic matter.

4.3. Effect of fluoranthene and organic matter quality on arm-regeneration rate

In the absence of Flu, organic matter quality did not have a significant influence on the regeneration of brittle star arms. Earlier published results showed that arm-regeneration rate, measured as increase in regenerated arm-length divided by experimental time, was positively correlated to organic matter quality in *A. filiformis* (Gunnarsson et al., 1999; Gunnarsson & Sköld, 1999; Sköld & Gunnarsson, 1996). Regeneration rates were found to range from 0.31 mm day^{-1} (lignin) to 0.42 mm day^{-1} (*Ceratium* spp.) (Gunnarsson et al., 1999). In the present study regeneration rates (length) for brittle stars in sediment with added lignin (0.12 ± 0.04 mm day^{-1}) and *Tetraselmis* (0.14 ± 0.03 mm day^{-1}) were 2.6–3 times lower than those reported by Gunnarsson et al. (1999). We expect that this difference is due to the lower temperature (i.e. 5 °C) in the present study relative to a temperature of 10 °C used by Gunnarsson et al. (1999). In another study conducted at a temperature of 6.2–7.6 °C the regeneration rates of *A. filiformis* were between 0.12 and 0.15 mm day^{-1} in control sediment and between 0.12 and 0.18 mm day^{-1} in phytoplankton enriched sediment (Sköld & Gunnarsson, 1996). During the time of the experiment (March–April), the somatic growth of *A. filiformis* is known to be slower than during summer and autumn (Michael Thorndyke pers comm.). Somatic growth has been shown to correlate well with water temperature, and in March the bottom water temperature is approximately 6 °C. Because of the slow growth and the limited duration of the experiment, small differences in growth may be difficult to detect statistically.

Organic matter quality and Flu treatment interacted such that Flu suppressed arm-regeneration in systems with added labile organic matter, whereas Flu had no effect on regeneration in systems with added refractory organic matter. These results suggest that Flu was more toxic when the organic matter in the sediment was labile (i.e. *Tetraselmis*) than when it was refractory (i.e. lignin). We observed a greater sensitivity of regenerated arm area compared to arm length which is consistent with previous studies (e.g. Nilsson & Sköld, 1996). *A. filiformis* initially regenerates arm length which is then followed by an increase in arm width (Salzwedel, 1974). Thus, regenerated arm area may be more sensitive than length as an effect endpoint.

Nilsson (1999) investigated the combined effects of hypoxia and organic enrichment on regeneration in *A. filiformis*. He found that increasing organic content stimulated regeneration to a certain point but that a further increase in organic

content affected regeneration negatively (Nilsson, 1999). Highly labile organic matter, such as *Tetraselmis*, may stimulate bacterial mineralization to a higher degree than more refractory organic matter such as lignin. An increased bacterial respiration, as observed in the presence of *Tetraselmis*, is likely to increase sulphide levels in the upper sediment layer. We found that the presence of Flu increased mineralization in sediment with labile organic matter, and preliminary studies indicate that microbial degradation of pyrene in the surface sediment is elevated in sediment with *Tetraselmis* compared to sediment with lignin (Granberg et al., in press). Thus, hypoxic conditions may have developed in sediment with *Tetraselmis* and Flu. *A. filiformis* are capable of preventing acute toxic concentrations of sulphide occurring in their burrow system, at least when the overlying water is non-sulphidic as it was in the present study due to the flow-through design of the exposure system. Tolerance to sulphide can be achieved by internal detoxification mechanisms, such as exclusion, water-flow, creation of an oxidized layer around burrows and mucus production. However, most of these mechanisms cost energy in the form of increased irrigation of burrows or mucus production, which could reduce the amount of energy available for growth (Forbes & Calow, 1996). Therefore, one possible explanation for our results is that *Tetraselmis* caused elevated sediment sulphide levels, and that further stress by addition of Flu caused a reduction in regeneration rates compared to in systems without Flu.

4.4. Fluoranthene uptake and biotransformation

4.4.1. Weight-specific Flu body-burden (BB)

Flu body burdens in the disk fraction were much higher than in the arm fraction. Neither uptake rates nor weight-specific body-burdens in disk- and arm fractions differed between brittle stars in sediment with *Tetraselmis* or lignin at experimental termination, indicating that organic matter quality did not affect net Flu accumulation in brittle-stars in this study. However, the time-dependent accumulation was delayed approximately 6 days for brittle-stars in sediment with lignin, implying that Flu associated with *Tetraselmis* was bound in a readily bioavailable form whereas lignin may have formed more stable Flu-lignin complexes. A similar time-lag for uptake of TCB was observed by Gunnarsson et al. (1999), who found that the time-lag was highest in sediment enriched with lignin and decreased with increasing organic matter quality. These authors suggested that the short half-life and rapid uptake rate from the most labile organic matter could have been triggered by an initial leaching of bioavailable TCB-contaminated matter. Furthermore, brittle stars were found to be less active in sediment with refractory organic matter compared to with labile organic matter (Gunnarsson et al., 1999).

4.4.2. The biota-sediment-accumulation-factor

Amphiura filiformis have detectable amounts of lipid only in the disk fraction (Gunnarsson & Sköld, 1999). Organic matter quality did not affect lipid concentration in disk tissue, which is in contrast to earlier results showing an increase in lipid content of the disk with increasing organic matter quality (Gunnarsson et al., 1999).

However, the lipid content in the disk depends on factors such as gonad production, season and temperature. The low temperature used in the present study, resulting in slow growth rates, may have prevented detection of differences in lipid content in response to organic matter quality. There was a strong indication that brittle stars in sediment with refractory organic matter had a higher concentration of Flu to lipid ratio compared to brittle stars from sediment with *Tetraselmis*. Although Gunnarsson and Sköld (1999) found no evidence for differences in OC (abbreviated by these authors as TOC) or TCB content of the sediment surface between control treatments (i.e. no phytoplankton) and treatments with added phytoplankton, they detected a significantly higher TCB accumulation in brittle stars (i.e. *A. filiformis*, *Amphiura chiajei*) in the phytoplankton treatment. The authors suggested that selective feeding, rather than passive equilibration between tissue lipids and sediment OC, was the cause of the increased TCB burden because the difference was significant even after normalization to lipid content. We did not detect any changes in N or Flu content in surface sediment during the experiment. However, the OC content decreased only in sediment with *Tetraselmis* and was lower compared to Li-Flu at experimental termination. To take account of the different OC contents between organic treatments at the end of the experiment we calculated the BSAF. BSAF was higher in brittle stars in sediment with lignin compared to in sediment with *Tetraselmis*. Therefore, our results suggest that Flu bioaccumulation in *A. filiformis* is controlled by other factors than equilibrium partitioning between lipid phases of the organism and the organic carbon pool in the sediment. The same positive relation between OC and PAH BSAFs has been reported for other invertebrates such as the lugworm *Abarenicola pacifica* (Weston, 1990), whereas a negative correlation was observed for the polychaete *Armandia brevis* (Meador, Casillas, Sloan, & Varanasi, 1995).

4.4.3. Flu biotransformation

Overall, the concentration of parent Flu and aqueous Flu-metabolites increased during the exposure period in disk and arms of *A. filiformis* regardless of the quality of the organic matter to which Flu was associated. The present experiments strongly indicate that the biotransformation capability of *A. filiformis* is very limited since the concentration of parent Flu by far contributed the largest fraction even after 45 days of exposure. Specific activities both of phase I and II biotransformation enzymes are very low in echinoderms compared to other invertebrate groups including fish, crustaceans and molluscs (Den Besten, 1998; Livingstone, 1998). This relatively low enzyme activity may be responsible for the low biotransformation of Flu in *A. filiformis*. In comparison, the marine polychaete *Capitella* sp. I biotransformed Flu taken up from the sediment-associated pool (30 μg Flu/g dry wt. sed.) at a very high rate, such that about half of the body-burden after 5 days of exposure was present as aqueous Flu-metabolites (i.e. 46.2%) (Selck et al., 2003b). Furthermore, total Flu body-burden in *A. filiformis* after 45 days of exposure was very low (ca. 20 μg Flu eq./g dry wt. tissue) compared to the body-burden in *Capitella* sp. I after 5 days of exposure (ca. 140 μg Flu eq./g dry wt. tissue) despite exposure to a similar sediment concentration of Flu. For a contaminant to become bioavailable for digestive uptake it has to be solubilized (Mayer, Weston, & Bock, 2001). Gut fluids vary in their

content of solubilizing agents leading to differences in contaminant solubilization among taxa (Mayer et al., 2001). In this respect, cnidaria and echinoderms generally show the weakest digestive intensity whereas annelids and echiurans exhibit the strongest digestive intensity (Mayer et al., 2001). In accordance with the differences in Flu accumulation between *A. filiformis* and *Capitella* sp. I, Mayer et al. (2001) detected the lowest BaP solubilization (range: 6–45%) in echinoderms and the highest in annelids. Nevertheless, the presence of aqueous Flu-metabolites indicates that *A. filiformis* is capable of biotransforming Flu through both phase I and II pathways. The lack of polar Flu-metabolites along with an increase in aqueous Flu-metabolites suggests a slow transformation by phase I enzymes (e.g. P450) followed by a relatively rapid transformation by phase II enzymes (e.g. glutatione *S*-transferase; GST).

4.4.4. Trophic transfer

Brittle-stars provide an important food source for many fish and invertebrate predators, including haddock *Melanogrammus aeglefinus* (Mattson, 1992) and Norway lobster *Nephrops norvegicus* (Baden et al., 1990). These predators do not generally consume the entire brittle star but crop the arms, which are later regenerated (Sköld et al., 1994). For example, *Amphiura* arms constituted approximately 60% of the stomach content in the dab *Limanda limanda* (Duineveld & Noort, 1986). Accumulation of PCBs by infaunal organisms has been shown to be the first step in their transfer to higher trophic levels, including human consumers (Thomann, Connoly, & Thomas, 1986). Loizeau and Menesguen (1993) showed that 8–15% of the PCB burden in dab from the Bay of Seine could be explained by ophiuroid consumption. In *A. filiformis* the tissue concentration of Flu was lower in the arm – compared to the disk-fraction. However, the proportion of metabolites was higher in the arms – compared to the disk. Even though biotransformation is often considered a detoxification mechanism, many examples of increased toxicity as a result of biotransformation have been reported. For example, PAHs become carcinogenic, mutagenic or both when activated through biotransformation (Di Giulio et al., 1995; Ma et al., 1998; Rand et al., 1995). Therefore, *Amphiura* communities may play an important role in the remobilization of sediment-associated PAHs and their metabolites to higher trophic levels. The importance of *A. filiformis* arms for food chain transfer of Flu can be roughly estimated as suggested by Gunnarsson and Sköld (1999) by including information given in Duineveld and Noort (1986). The annual food consumption of *Amphiura* arms by an average standing stock of dab in the North Sea is 0.84 g arms m^{-2} $year^{-1}$ (Duineveld & Noort (1986)). Thus, a yearly remobilization of total Flu by trophic transfer to dab can be estimated by using the average arm concentration of total Flu given in Table 6 (i.e. 4.5 μg total Flu eq./g dry wt. arm tissue × 0.84 g arms m^{-2} $year^{-1}$). Accordingly, 3.8 μg total Flu eq. m^{-2} $year^{-1}$ would be removed from the sediment by consumption of *A. filiformis* arms by *Limanda limanda*. However, other organisms consume brittle star arms. Thus, removal by trophic transfer can also be calculated from the estimated yearly production (P) to biomass (B) ratio of *A. filiformis* (P/B: 0.46 $year^{-1}$) of which 13.3% is due to arm regeneration (i.e. 0.06 $year^{-1}$) (Sköld et al., 1994). By including a coastal biomass of *A. filiformis* of 24.2 g dry wt. m^{-2} (Gunnarsson & Sköld, 1999) and the concentration of total Flu eq. in the

arms of *A. filiformis* (0.06 $year^{-1} \times 24.2$ g dry wt./$m^2 \times 4.5$ μg total Flu eq./g dry wt. arm tissue) we estimate that 6.7 μg total Flu eq. m^{-2} $year^{-1}$ of the sediment-associated Flu may be remobilized by arm-regeneration in *A. filiformis*. Notably, the offshore abundance of *A. filiformis* (1917 ind./m^2) reported by Gunnarsson and Sköld (1999) corresponds to a biomass of 108.9 g dry wt./m^2. Hence, in this case remobilization of sediment-associated Flu (30 μg Flu/g. dry wt. sed.) by *A. filiformis* arm-regeneration would be on the order of 29.4 μg total Flu eq. m^{-2} $year^{-1}$. By assuming the same distribution of Flu among parent Flu, polar-, aqueous Flu-metabolites and tissue residue in brittle star arms as found in the present study we estimate a trophic transfer of parent Flu and metabolites in concentrations in the range of about: P: 2.5–19.7; A–M: 0.7–5.3; P–M: 0.4–2.6; R: 0.2–1.8 μg Flu eq. m^{-2} $year^{-1}$.

5. Conclusions

Our results show that bioturbation by *A. filiformis* enhances the transport of Flu from surface to deeper sediment layers by burrow building and alters the distribution of total Flu between parent and metabolites. Organic enrichment with highly labile, but not with refractory, organic material interacted with Flu to reduce growth. However, it was not clear if the reduced growth was due solely to Flu or due to the higher energy costs associated with increased ventilation in a sulphide rich environment. This study emphasizes sediment particle ingestion as a pathway whereby Flu can enter benthic food webs, and that net accumulation of Flu by *A. filiformis* was not markedly influenced by the quality of the sedimentary organic material. We propose that Flu bioaccumulation in *A. filiformis* is controlled by other factors than equilibrium partitioning between lipid phases of the organism and the organic carbon pool in the sediment, and that bioavailability is not as straightforward as is usually considered in sediment quality models.

Biotransformation was very limited and not related to organic matter quality. We suggest that the relatively low uptake and limited biotransformation capability observed in *A. filiformis* exposed to dietary Flu is related to both the low digestive intensity (i.e. solubilizing agents) (Mayer et al., 2001), and the low activity of phase I and II enzymes (Livingstone, 1998) reported for echinoderms.

Trophic transfer of both parent Flu and metabolites is likely to occur since *A. filiformis* arms constitute a major part of food for demersal fish. We estimate that the yearly mobilization of sediment-associated Flu by arm-regeneration in *A. filiformis* is in the range of 3.8–29.4 μg total Flu eq. m^{-2} $year^{-1}$ at a sediment concentration of 30 μg Flu/g dry wt. sed. Thus, it is clear that a better understanding of the dietary transfer of PAH and their metabolites to higher trophic levels is needed.

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