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Environmental Toxicology and Chemistry, Vol. 20, No. 5, pp. 1012–1021, 2001

Printed in the USA
0730-7268/01 $9.00 + .00

METABOLISM OF THE POLYCYCLIC AROMATIC HYDROCARBON FLUORANTHENE BY THE POLYCHAETE *CAPITELLA CAPITATA* SPECIES I

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(*Received* 29 *February* 2000; *Accepted* 18 *September* 2000)

Abstract—Previous studies have shown that infaunal deposit feeders may enhance the loss of organic contaminants from sediments. However, the extent to which this occurs as a result of sediment microbial stimulation, porewater flushing, or biotransformation by infauna remains unclear. The purpose of this study was to determine whether the infaunal polychaete *Capitella* sp. I is able to metabolize the polycyclic aromatic hydrocarbon (PAH) fluoranthene and to provide an initial characterization of the metabolites produced. Our results showed that *Capitella* sp. I is able to metabolize fluoranthene to more hydrophilic products and that, after 24 h in clean sediment, fluoranthene could no longer be detected in worm tissues whereas a number of fluoranthene-derived metabolites were present. None of the metabolites released or retained by worms resembled known bacterial metabolites, suggesting that *Capitella,* and not bacteria associated with its gut or body surface, were responsible for the biotransformation of fluoranthene in our system. On the basis of ultraviolet maxima, peak shape, relative height, and order of elution, tentative identities of two metabolites (i.e., 3- and 8-hydroxyfluoranthene) are proposed. The results demonstrate that, in addition to their effects on sediment geochemical properties, infaunal polychaetes such as *Capitella* can enhance the degradation of sediment-associated contaminants by directly metabolizing them.

Keywords—Detoxification Contaminant fate Cytochrome P450 Monooxygenase

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are produced from incomplete combustion of fossil fuels, oil spills, and production of oil-based products. They enter the marine environment by atmospheric deposition, industrial and domestic waste, surface runoff, and from shipping [1]. They are of concern because of their hydrophobic and persistent properties and because at least some of them have been identified as potent mutagens and carcinogens [1–4]. Mutagenic and carcinogenic effects are not associated directly with the parent compound but arise largely as a result of metabolic transformation processes that lead to the formation of reactive intermediates [5–10]. In species ranging from bacteria to vertebrates, metabolic transformation of PAHs involves the cytochrome P450 mixed-function oxygenase system. For most taxonomic groups, the details of this enzyme system, the structure and function of the enzymes involved, and the metabolites produced during transformation of xenobiotic chemicals such as PAHs remain poorly understood.

Metabolic transformation of PAHs can have an important influence on trophic transfer and/or removal of these chemicals from ecosystems. As a consequence of their hydrophobic properties, PAHs tend to partition to sediments, where they may accumulate to high concentrations [1]. Animals living in and feeding on sediments may be particularly susceptible to PAH exposure but likewise may play a key role in enhancing PAH degradation by mixing, irrigating, and consuming contaminated sediment [11]. Deposit-feeding infauna control the physical and chemical properties of sediments and are an important food source for higher trophic groups, including a variety of epifaunal species and commercial fish. Infaunal feeding and respiration can increase the burial rate of PAHs into the sediment column but may also increase the flux of contaminant from sediment porewater to the overlying water column [12,13]. In addition, metabolic transformation of PAHs by resident infauna can potentially reduce the contaminant concentration in the sediment and porewater but could alternatively lead to the production of reactive metabolites that are more hazardous than the parent compound.

The deposit-feeding polychaete *Capitella capitata* is well known as an opportunistic species complex often associated with organically enriched and/or polluted environments [14–16]. *Capitella capitata* represents a cryptic species complex in which adult morphology is so similar among species that no effort has been made to separate the complex with proper taxonomic species descriptions. Nevertheless, the so-called *Capitella* sibling species are reproductively isolated and differ substantially in a host of other parameters, e.g., in karyotypes and enzyme patterns [15,17,18]; in ultrastructure of eggs and ovarian follicle cells; in larval, genital spine, and sperm morphology [19]; in ecophysiological characters [20]; in sediment processing rates [21]; and in life-history features [22]. The species complex as a whole has a wide geographic distribution, and some of its members are among the first species to recolonize soft-bottom marine habitats following major oil spills [23].

Despite the fact that members of this species complex often dominate oil-contaminated sediments, surprisingly little is known about the metabolism and detoxification of oil components by these worms. Lee et al. [24] detected increased mixed function oxygenase activity in *C. capitata* sp. exposed continuously to benz[*a*]anthracene or crude oil for three generations. More recent studies of *Capitella* sp. I (the best known and most opportunistic of the sibling species so far described) indicated that this species takes up the PAH fluoranthene from contaminated sediment in a concentration-dependent manner

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but that, despite continued exposure to contaminated sediment, body burdens decrease and are undetectable after about 2 d of exposure [11]. Exposure of worms to ^{14}C-labeled fluoranthene determined that the production of dissolved radioactive metabolites was higher in worms that had been preexposed to unlabeled fluoranthene for a week compared with worms that had not been preexposed [11]. These results suggested the presence of an inducible detoxification system present in *Capitella* sp. I. However, the identities of the dissolved metabolites (other than to confirm that they were clearly not CO_2) were not determined. In addition, it could not be established with certainty that the metabolites produced were derived from worm metabolism and not produced by bacteria living in the gut or on the body surface of worms.

Following on our earlier work, the objectives of the present study were to determine whether *Capitella* sp. I is able to metabolize ingested fluoranthene to other products, to investigate whether the metabolites produced were released to the sediment or the water or were retained by the worms, and to provide an initial characterization of the metabolites produced by comparison with selected standards and with the published literature. An important component of this study was to distinguish worm metabolism of fluoranthene from metabolic transformation by bacteria associated with the gut and/or body surface of worms. Therefore, we employed the bacterial metabolites identified by Kelley et al. [25] as standards with which to compare any fluoranthene-derived metabolites produced in the presence of *Capitella.*

We have chosen to focus on fluoranthene because it is one of the most abundant PAHs identified in marine sediment samples [26,27], it tends to have a higher bioaccumulation potential relative to smaller or larger PAHs [28], it is highly toxic to benthic invertebrates (LC50 values for *Rhepoxynius abronius* and *Corophium spinicorne* between 15 and 50 μg/L; [29]), and it has been described as a potent mutagen [30].

METHODS

Experimental design

Capitella capitata were originally collected from Setauket Harbor, Long Island Sound, New York, USA, in 1984 and identified as species I by J.P. Grassle. Worms were cultured at 12°C in 10-L aquaria with glass fiber (type GF/C) filtered seawater (32‰ S) and prefrozen, sieved (to 500 μm) sediment collected from the Isefjord, Denmark. The fluoranthene concentration in the field-collected sediment was measured prior to use and was below the detection limit. Aquaria were aerated and maintained in darkness, and a supplementary food source consisting of an equal mixture of Tetramin© fish food (Tetrawerke, Melle, Germany), Milupa© baby cereal (Milupa A/S, Hørsholm, Denmark), and frozen, dried spinach was added weekly.

For the experiments, 500 worms were added to a glass beaker to which had been added 550 ml of 0.2-μm filtered seawater and 200 g of fluoranthene-contaminated sediment (<250 μm). Sediments were contaminated by adding 0.09 g fluoranthene dissolved in 1.0 ml acetone to 250 g sediment with filtered overlying water added to make a slurry. The slurry was shaken in the dark for 24 h, allowed to settle, and the overlying water removed. The nominal fluoranthene concentration was estimated as 554 μg/g dry weight sediment. The measured concentration was 426 ± 16 SD (n = 3) μg/g dry weight sediment. This concentration is around five times higher than concentrations found in polluted harbor sediments [26,27], but a high concentration was required so that adequate material could be obtained for metabolite detection.

Worms were exposed to contaminated sediment for one week in the dark, after which time 15 worms were transferred to each of twenty-one 100-ml glass Erlenmeyer flasks to which 3 g dry weight clean sediment and 8 ml filtered seawater had been added. The worms were maintained in the covered flasks for a total of 96 h in the dark at 12°C. During this period, three flasks were harvested each at 1, 3, 6, 24, 48, 72, and 96 h and two samples of water and sediment were taken from each replicate for metabolite analysis (giving six samples each of water and sediment per collection time). Worms were separated from the sediment and the 15 worms from each flask pooled for metabolite analysis (× 3 replicates per collection time). In addition to the above treatments, a control group of worms was exposed to uncontaminated sediment for a week. This group was used to identify metabolites and other compounds unrelated to fluoranthene metabolism that would be omitted from further consideration. In addition, samples from a flask with fluoranthene-contaminated sediment without added worms were used to identify fluoranthene breakdown products that were not associated with worm metabolism (i.e., from sedimentary bacterial metabolism and/or abiotic breakdown). No reproduction took place during the course of the exposure period or the experiment, and worm densities were the same throughout.

Extraction and analysis of fluoranthene and metabolites

Sediments were extracted by taking a 0.5-g sediment sample, to which 1 ml methanol, 2 ml 95% acetic acid, and 2 ml ethyl acetate were added. After stirring for 5 s, the sample was exposed to ultrasonic treatment for 10 min, stirred again for 5 s, and finally centrifuged for 10 min at 3,000 *g* at 4°C. The supernatant was transferred to a new glass tube and the extraction in ethyl acetate repeated twice. Three replicate samples were measured at each collection time.

Water samples were extracted by adding seven drops of acetic acid and 2 ml of ethyl acetate to 3 ml of water in an 8-ml pyrex tube. After stirring for 5 s, the sample was centrifuged for 10 min at 3,000 *g* at 4°C. The supernatant was transferred to a new tube and the extraction in ethyl acetate repeated twice. Three replicate samples were measured at each collection time.

Worm tissues were extracted by placing the 15 worms from each replicate in an 8-ml pyrex tube, giving ~51 mg wet tissue per sample and three replicate samples per collection time. One milliliter of 4 M NaOH and 1 ml of methanol were added, and the tube was covered and placed at 40°C overnight. After cooling to room temperature, 1 ml of 4 M HCl was added and the tube briefly shaken. Two milliliters of ethyl acetate was added, the sample was placed in an ultrasonic bath for 10 min, and then centrifuged at 3,000 *g* for 10 min at 4°C. The supernatant was transferred to a new tube and the extraction in ethyl acetate repeated twice.

For all samples, the supernatant in the extracted samples was stirred again for 30 s, exposed to ultrasonic treatment for 10 min, and stirred for another 30 s. A known volume (~7 ml) was transferred into another glass tube, and all samples were evaporated to near dryness under a nitrogen atmosphere in a 35°C water bath. The samples were resuspended in 0.5 ml of a 30:70 (vol:vol) mixture of methanol and water. The samples were then centrifuged at 3,000 *g* for 10 min and frozen at −80°C until analysis.

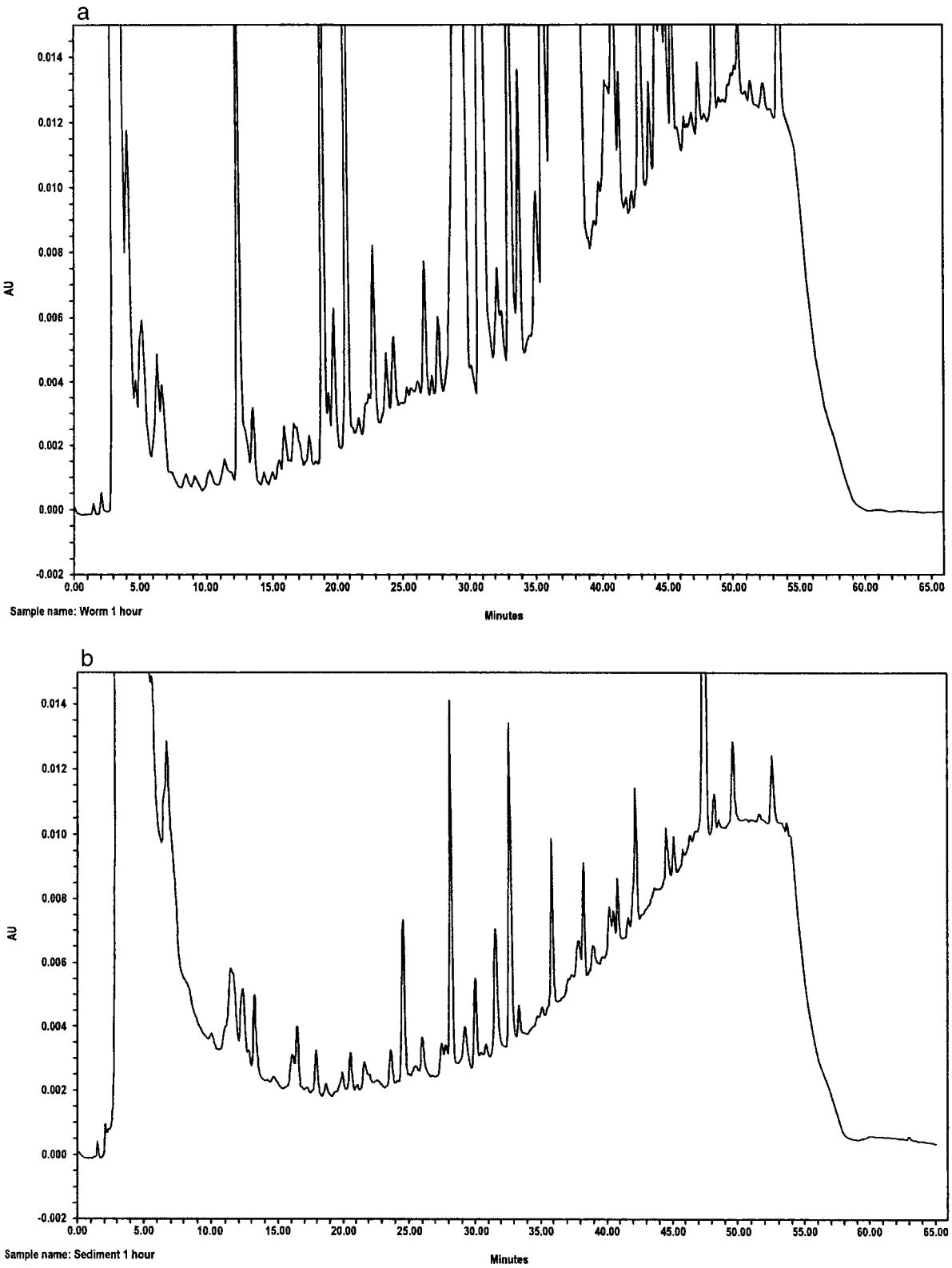


Fig. 1. Representative high-performance liquid chromatography traces for (**a**) worm tissue, (**b**) sediment, and (**c**) water.

Fluoranthene concentrations were measured by reversed-phase high-performance liquid chromatography (HPLC) modified after Kelley et al. [25]. The HPLC system was equipped with a Waters 600 E pump, a Waters Wisp 700 autosampler, a Waters 994 photodiode array detector (all from Millipore A/S, Glostrup, Denmark), a Nucleosil precolumn (10 C18), and a Primesphere 4.6-mm by 25-cm by 5-μm C18-HC 110 A column (both from Mikrolab, Aarhus, Denmark). The mobile phase was a linear gradient of methanol-water (three solvents, 30–90% methanol [vol/vol], less than 0.5% acetic acid) run for 65 min at 0.85 ml/min. The ultraviolet (UV) spectra was scanned over the range 200 to 370 nm and UV absorbance

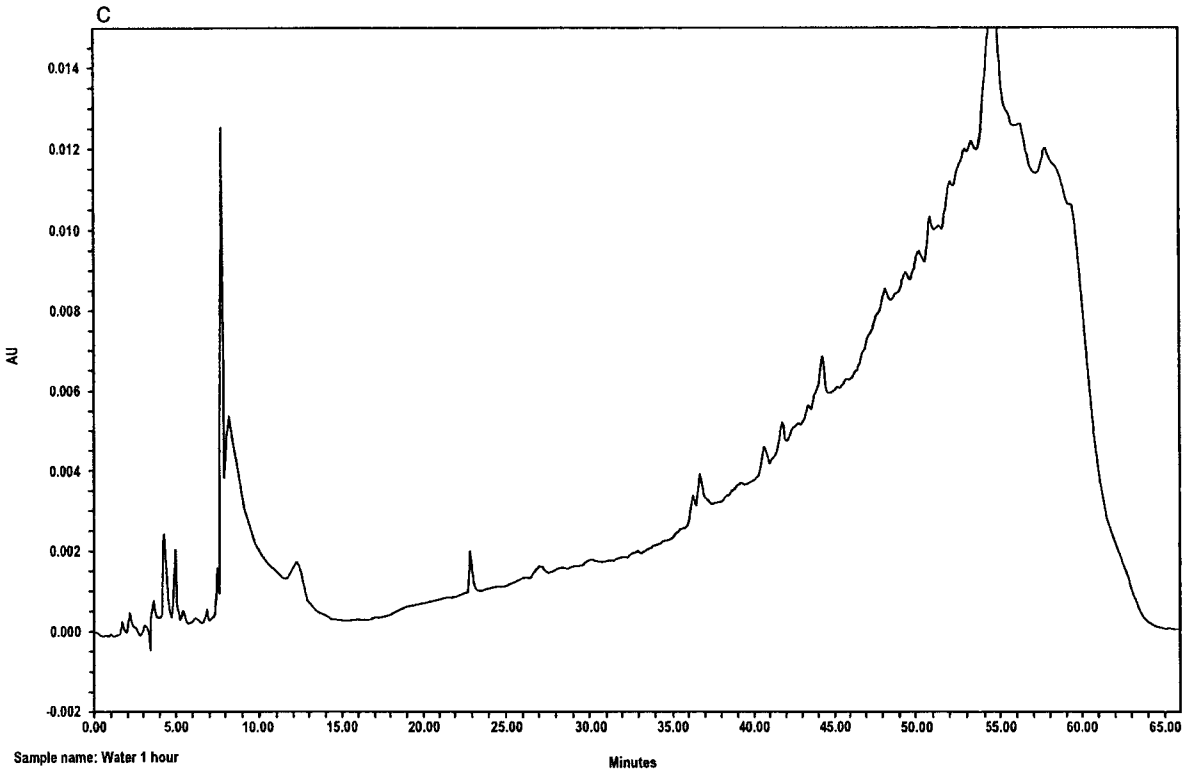


Fig. 1. Continued.

was measured at 254 nm. Peak areas were integrated with a Millenium computer program (Version 2.15, Waters A/S, Hedehusene, Denmark). Initially, all samples were run twice, but due to very low variability between runs, later samples were run only once.

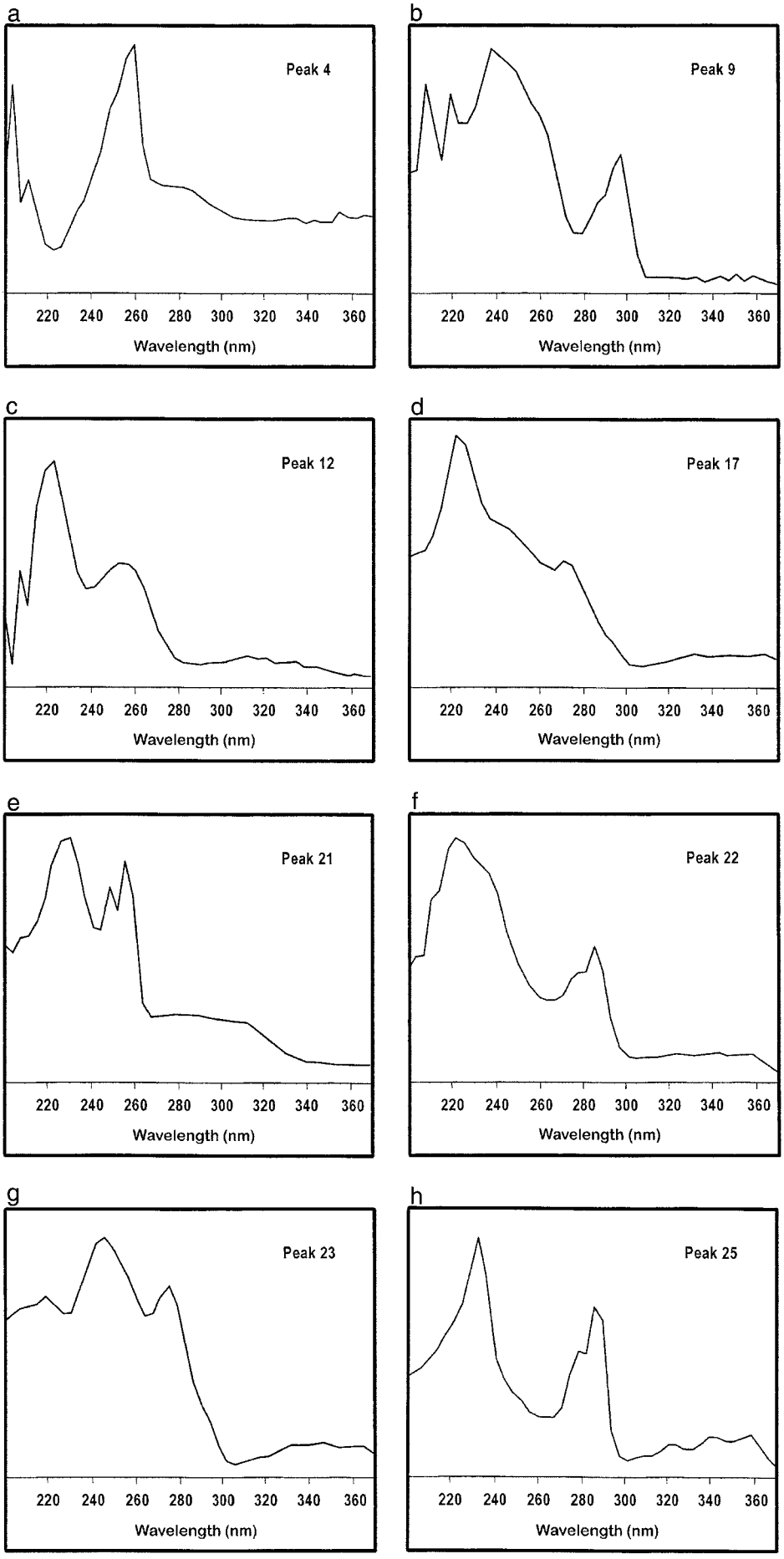
We used external standards for fluoranthene (crystalline fluoranthene, 98% purity) and nine metabolites that were previously identified by Kelley et al. [25] to be produced from bacterial metabolism of fluoranthene (Table 1). The nine metabolites were phenylacetic acid, 2-carboxybenzaldehyde, 9-hydroxyfluorene, 9-fluorenone-1-carboxylic acid, 9-hydroxy-1-fluorenecarboxylic acid, adipic acid, benzoic acid, 9-fluorenone, and phthalic acid. Fluoranthene and the first five metabolites were obtained from Sigma-Aldrich (Vallensbæk Strand, Denmark). Adipic acid and acetic acid were obtained from Struers (Albertslund, Denmark), benzoic acid and 9-fluorenone from Merck (Virum, Denmark), and phthalic acid from BDH Chemicals Ltd. (Pool, England). Methanol, ethyl acetate, and acetone were obtained from Niels Peter Mark (Valby, Denmark). All chemicals were of reagent grade or better.

Percent recovery (determined by spiking a known amount of each standard into sediment and extracting as above) and detection limits (determined by creating a standard curve with pure standard) for fluoranthene and the metabolite standards measured in sediment are shown in Table 1. All HPLC samples involved large numbers of peaks, and it was necessary to develop a systematic approach for deleting irrelevant peaks from further analyses. To identify metabolites produced by sediment-associated bacteria, we compared the peaks produced in the vials containing fluoranthene-contaminated sediment but no worms with the bacterial metabolite standards. To identify worm-produced metabolites, we subtracted peaks deriving from fluoranthene, from the known bacterial metabolites shown in Table 1, from the control (sediment with worms but no fluoranthene), from the sediment plus fluoranthene (without worms) samples, and from the solvents (methanol, water, and ethyl acetate). After initial selection of relevant peaks, we determined how often each of the peaks appeared in the sample runs for each medium separately; peaks that appeared in fewer than half of the sample runs in at least one of the collection times were omitted from further analyses. Finally, peaks were evaluated for their purity (visually, by the absence of a shoulder and with the aid of the Millenium software program).

RESULTS

In the present study, preexposed worms were transferred to clean sediment and water at the start of the experiment. Thus, in vials containing worms, the only source of fluoranthene to the system during the 4-d experiment would have been from the worms. We estimated the fluoranthene concentration in worms after the first hour of the experiment (which should have allowed worms to empty their guts of fluoranthene-contaminated sediment) to be 598 μg fluoranthene/g worm wet weight. Since we had an average of 51.27 mg worm wet weight per sample, this gives a total of 30.7 μg fluoranthene potentially introduced to the experimental flasks from worms. Divided by the weight of sediment in each flask, this gives a concentration of 10.2 μg fluoranthene/g dry weight sediment (which is equal to 10.2 μg/g × 0.72 g/ml sample = 7.3 μg fluoranthene/ml sample), which is well above our detection limit of 0.31 μg fluoranthene/ml sample.

The HPLC analysis of extracts of seawater, sediment, and worm tissues detected a large number of peaks (Fig. 1) due to the presence of a variety of naturally occurring organic compounds not related to fluoranthene or its metabolites (determined by comparison with HPLC profiles of controls without added fluoranthene). In flasks containing fluoranthene-con-


a
Peak 4
220 240 260 280 300 320 340 360
Wavelength (nm)
b
Peak 9
220 240 260 280 300 320 340 360
Wavelength (nm)
c
Peak 12
220 240 260 280 300 320 340 360
Wavelength (nm)
d
Peak 17
220 240 260 280 300 320 340 360
Wavelength (nm)
e
Peak 21
220 240 260 280 300 320 340 360
Wavelength (nm)
f
Peak 22
220 240 260 280 300 320 340 360
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g
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h
Peak 25
220 240 260 280 300 320 340 360
Wavelength (nm)

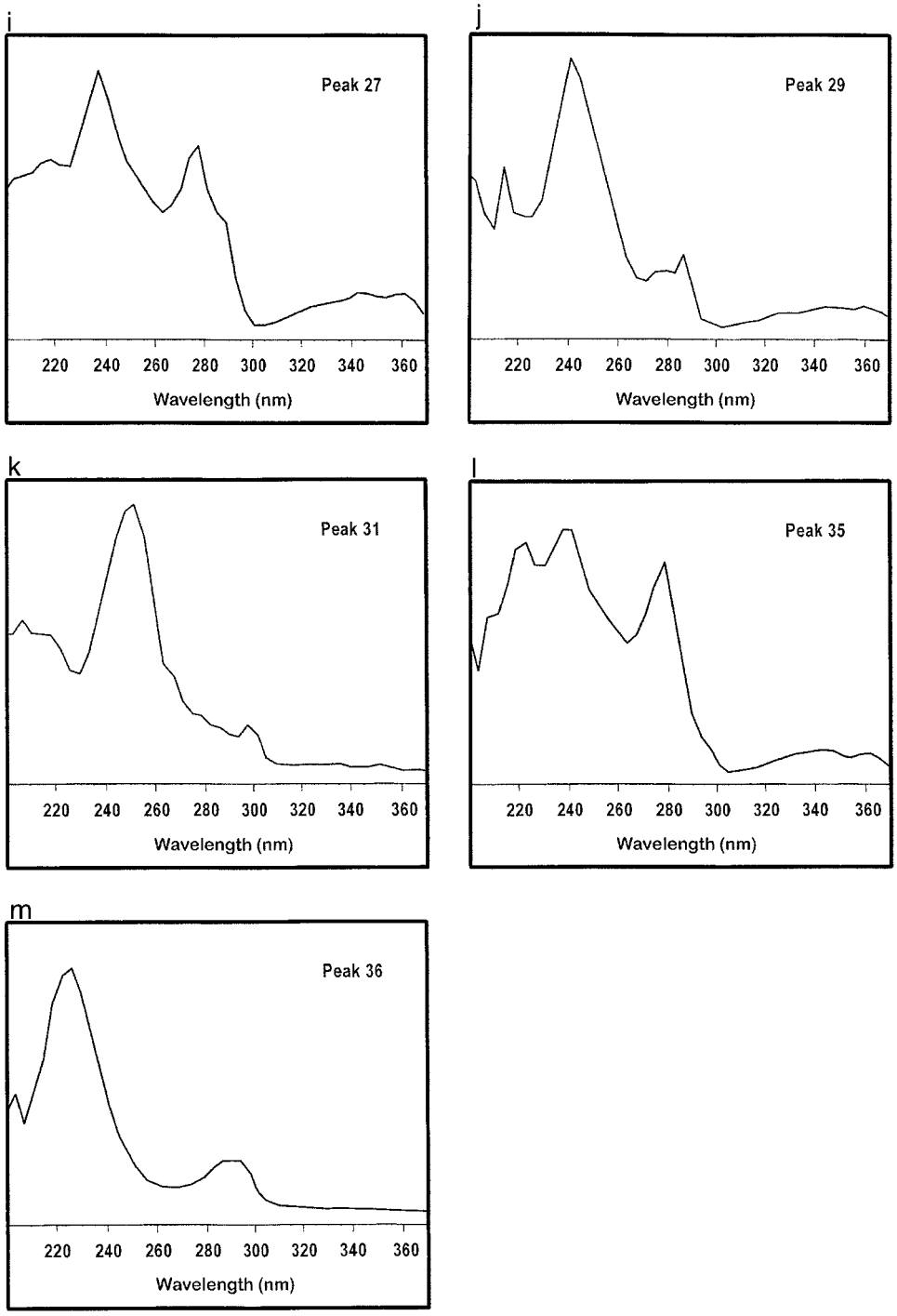

Fig. 2. Continued.

taminated sediment but no worms, we could not detect any of the metabolites identified by Kelley et al. [25] during the 96-h experimental period, suggesting that sediment-associated bacteria did not contribute measurably to the biotransformation of fluoranthene in our system. Likewise, none of the peaks obtained in the presence of worms matched the spectra for the bacterial metabolites described by Kelley et al. [25]. After subtracting peaks from other sources (see Methods), there remained a total of 36 peaks that we attributed to worm metabolism of fluoranthene (Table 2). Of these, 14 were found in sediment, water, or both but not in worm tissues at any point during the experiment. The remaining 22 metabolites were

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Fig. 2. Ultraviolet spectra of fluoranthene metabolites measured in worm tissue. Peak 4 (**a**), peak 9 (**b**), peak 12 (**c**), peak 17 (**d**), peak 21 (**e**), peak 22 (**f**), peak 23 (**g**), peak 25 (**h**), peak 27 (**i**), peak 29 (**j**), peak 31 (**k**), peak 35 (**l**), peak 36 (**m**). The *y*-axis shows absorbance and is the same scale for all figures. See text for details.

Table 1. Properties of standards used in the present study. Percent recoveries were determined in sediment extracts for fluoranthene and nine bacterial metabolites identified by Kelley et al. [25]. Two additional metabolites, 7-methoxy-8-hydroxy-fluoranthene and acenaphthenone, were also identified by Kelley et al., but these were not commercially available and were not included in the present study. Percent recoveries were determined by spiking sediments followed by extraction, and detection limits were determined using standard curves with pure substance

Compound	Molecular weight	Ultraviolet λ_{max} (nm)	Percent recovery (mean ± standard deviation)	Detection limit (μg/ml ethyl acetate)
Fluoranthene	202	355, 340, 320, 282, 275, 234, 208	72 ± 4.8	0.31
Benzoic Acid	122	275, 230	49 ± 3.0	5.64
9-Fluorenone	180	298, 249	40 ± 2.3	0.49
Phenylacetic acid	136	260, 223	44 ± 4.7	5.6
9-Hydroxy-1-fluorene carboxylic acid	226	316, 271, 226	51 ± 9.2	20[a]
9-fluorenone-1-carboxylic acid	224	298, 260, 215	71 ± 5.5	0.48
9-Hydroxy-fluorene	182	271, 226	22 ± 4.4	0.52
2-Carboxy-benzaldehyde	150	275, 234	54 ± 2.9	6.20
Phthalic acid	166	279, 234	NR[b]	6.7
Adipic acid	146		NR	ND[b]

[a] Lowest concentration tested.
[b] NR = not recovered; ND = not detected.

Table 2. Metabolites derived from worm metabolism of fluoranthene. Ultraviolet maxima are shown in column 2 with the sample type given in parentheses. The values in columns 3 through 5 represent the times (minutes) at which the peaks appeared during the high-performance liquid chromatography sample runs. The most polar metabolites appeared early and the least polar metabolites appeared late in the runs. A dash indicates that the peak was not observed in any of the runs for that sample type

Peak code	Ultraviolet μ_{max} (nm)	Worm samples	Water samples	Sediment samples
1	358, 256, 219, 204 (sediment)	—	10.59	10.61
2	350, 253, 219 (worm)	11.46	11.99	11.67
3	279, 223 (worm)	13.49	13.27	13.53
4	354, 260, 204 (worm)	16.18	—	—
5	328, 249, 211 (worm)	16.73	—	16.64
6	354, 271, 223 (water)	—	16.74	16.80
7	301, 245, 223 (water)	—	20.12	20.46
8	358, 320, 279, 219 (sediment)	—	24.12	24.22
9	362, 350, 298, 238, 208 (worm)	23.86	—	—
10	332, 260, 204 (worm)	24.27	25.30	24.87
11	320, 230 (water)	—	26.00	26.01
12	313, 253, 223 (worm)	27.76	—	27.63
13	279, 230 (water)	—	27.99	27.99
14	271, 230, 208 (water)	28.12	28.75	—
15	278, 223 (sediment)	—	—	27.69
16	275, 227 (water)	—	29.36	—
17	346, 223 (worm)	31.23	—	—
18	271, 226 (sediment)	—	31.17	32.07
19	332, 275, 245, 215 (worm)	32.58	—	—
20	335, 260, 226, 208 (sediment)	—	33.59	33.39
21	286, 256, 230 (worm)	34.29	33.58	33.60
22	343, 286, 238, 223 (worm)	35.39	—	—
23	347, 275, 245, 219 (worm)	36.25	—	—
24	275, 226, 207 (water)	—	36.61	36.74
25	358, 286, 234 (worm)	37.44	—	37.68
26	358, 290, 230 (sediment)	—	38.38	38.59
27	347, 279, 238 (worm)	38.10	—	—
28	273, 226, 204 (sediment)	—	40.16	40.08
29	343, 286, 241, 215 (worm)	41.00	—	—
30	358, 268, 219 (sediment)	—	41.91	41.68
31	298, 253, 208 (worm)	41.66	—	—
32	343, 268, 219 (worm)	42.99	—	—
33	358, 324, 294, 234 (worm)	43.51	43.51	43.76
34	361, 320, 294, 241, 206 (worm)	44.92	44.80	44.76
35	362, 343, 331, 279, 238, 223 (worm)	45.04	—	—
36	290, 223 (worm)	46.71	—	—

found in worms at least during one of the sampling time periods and can be divided into three groups, which are metabolites appearing in worms at some but not all time periods and never in sediment or water (these may have been transformed into other products), metabolites that appear only in worms and persist throughout the duration of the experiment, and metabolites appearing initially in worms and then later in sediment or water. The most important of these metabolites are shown in Figure 2.

In the first group, metabolite 4 (Fig. 2a) was detected in 100% of the worm samples after 1 and 3 h and in a third of the worm samples up to 24 h; metabolites 9 (Fig. 2b) and 27 (Fig. 2i) were detected in worm tissue during the first 6 h only; metabolites 2, 5, 14, 19, and 32 were detected in worm tissues only sporadically. Metabolites 17 (Fig. 2d), 22 (Fig. 2f), 23 (Fig. 2g), 29 (Fig. 2j), 31 (Fig. 2k), 35 (Fig. 2l), and 36 (Fig. 2m) belonged to the second group and were found in most or all of worm samples but never in sediment or water throughout the course of the experiment. In the third group, metabolites 3 and 10 were only detected sporadically. Metabolite 12 (Fig. 2c) appeared in worms and sediment (but not in water) during the first 3 h only; the concentration in worms was about a factor of eight higher than the concentration in sediment. Metabolite 21 (Fig. 2e) was detected in worms throughout the course of the experiment but only in sediment and water sporadically during the first 6 h; the concentration in worms was a factor of 200 greater than that in water and a factor of 75 greater than in sediment. Metabolite 25 (Fig. 2h) was detected in worms and sediment (but not in water) throughout the course of the experiment; the concentration in worms was a factor of 190 greater than in sediment. Metabolite 33 (Fig. 3a) was detected in worms and sediment throughout the experiment and in water starting at 24 h; there was a factor of 30 more of this metabolite in worms than in sediment and a factor of 100 more in worms than in water. Metabolite 34 (Fig. 3b) was detected in worms during the first 3 h, in water between 6 and 72 h, and in sediment between 3 and 24 h; the concentration in worms was a factor of 30 greater than that in sediment and a factor of 150 greater than in water.

Whereas we could be confident that metabolites found in worms at one or more of the sample collection times derived from worm metabolism, metabolites found in sediment and/or water but not in worms could possibly have arisen from other sources. In principle, it is possible that some of these peaks (particularly those found in the first few hours of the experiment) represent very polar metabolites that were rapidly excreted and therefore not detected in worm tissues. Fluoranthene itself was detected in worm tissues during the first 24 h of the experiment but was not found thereafter.

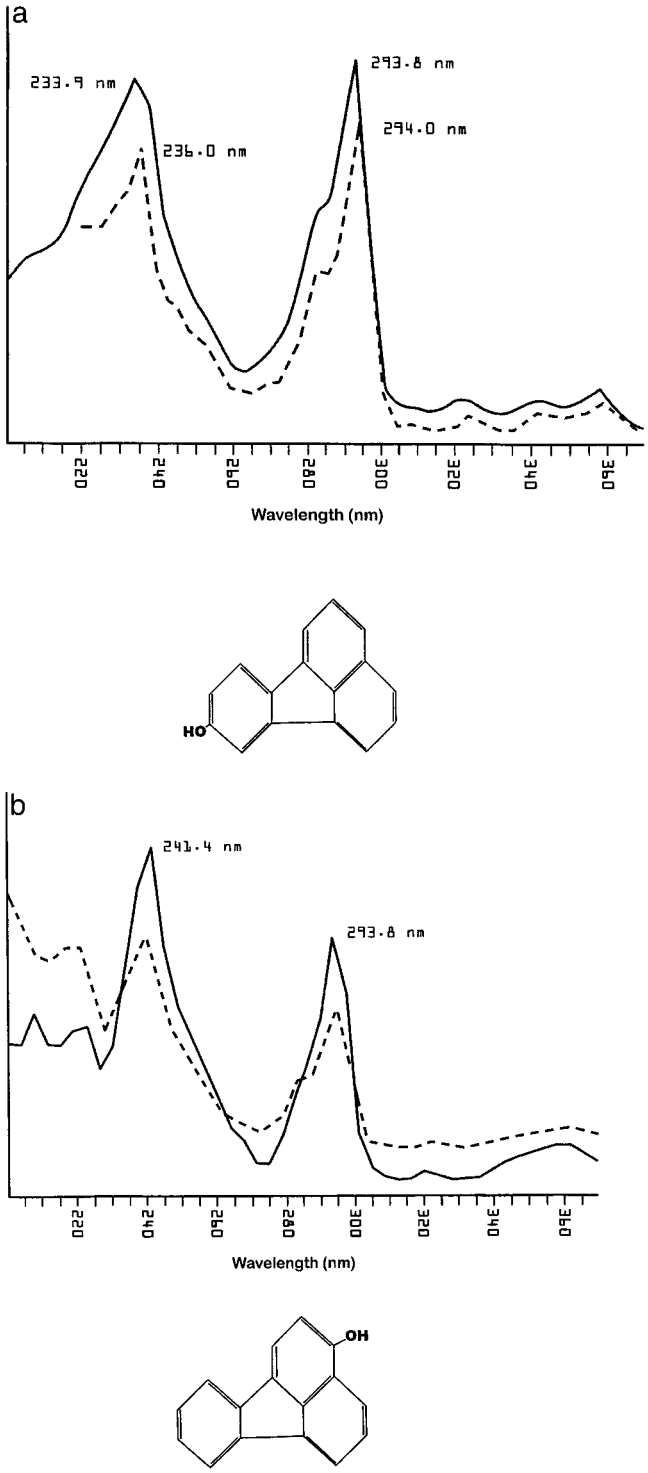

Fig. 3. Ultraviolet spectra of our results (solid lines) and selected fluoranthene metabolites identified in rat liver (dashed lines) [4,23]. The *y*-axis shows absorbance and is the same scale for both figures. (**a**) Our peak 33 compared with 8-hydroxyfluoranthene; (**b**) our peak 34 compared with 3-hydroxyfluoranthene.

DISCUSSION

Polychaetes have not been well investigated with respect to cytochrome P450 mixed-function oxygenase, and studies of induction of cytochrome P450 in this taxonomic group by exposure to PAH have provided conflicting results [31]. Differences among polychaete species in ability to metabolize PAH can be substantial, leading to large differences in bioaccumulation rates with potentially important consequences for trophic transfer [32]. Several authors have shown that the presence of *C. capitata* results in an increased disappearance of fluoranthene from contaminated sediment [13,33,34]. One hypothesis to explain this result is enhanced microbial degradation in response to the burrowing and feeding of worms, which acts to add both oxygen and labile organic matter (e.g., mucus) to the sediment. Our results suggest that, although worms may stimulate microbial activity, an important process by which they enhance the disappearance of fluoranthene from contaminated sediment is by their own metabolism of this toxicant. *Capitella* sp. I appears to have a very effective enzyme system for PAH metabolism. Although we could not

detect fluoranthene in worm tissues after the worms had been in clean sediment for 24 h, we detected a large number of intermediate products associated with fluoranthene metabolism. In comparison, elimination rates of the PAH benzo[*a*]pyrene by the polychaetes *Leitoscoloplos fragilis* (half-life = 10.3 d), *Marenzellaria viridis* (half-life = 5.1 d), and *Nereis diversicolor* (half-life = 3.7 d) were somewhat slower [32].

One of our objectives was to determine whether metabolism by bacteria living in association with *Capitella* sp. I (either in the digestive tract or on the body surface) were primarily responsible for the decline in worm body burden of fluoranthene that we observed in the present and previous [11] experiments. Although some variability among bacterial types in the formation of specific metabolites has been reported, in general, bacteria appear to employ similar PAH metabolic pathways (e.g., starting with an initial step involving dioxygenase) [35–39]. Assuming that bacteria living in association with worms would utilize similar metabolic pathways as those described by Kelley et al. [25], it is therefore of importance that we could detect none of the metabolites shown in Table 1 in any of our samples. This was also the case for the contaminated sediment incubated for a week without worms. Kelly et al.'s studies were performed using concentrated cultures of bacteria whereas ours were conducted using field-collected sediments having their natural assemblage of associated bacteria. Thus, in natural marine sediments containing large populations of infauna such as *Capitella* sp. I (that can reach densities up to ~500,000/m^2), bacterial biotransformation of PAHs may be less important relative to infaunal metabolism than suggested by the results of bacterial slurry studies.

With the HPLC methods used in the present study, definitive identification of individual peaks is only possible for compounds for which known standards can be compared. We were able to compare our unknown metabolites with fluoranthene and nine bacterial metabolites previously described by Kelley et al. [25]. Although we were able to detect 36 peaks that we attribute to worm-derived metabolites of fluoranthene, none of these corresponded to the nine standards that we were able to obtain. Unfortunately, many of these compounds were recovered in such small quantities that further analyses to determine their identities (e.g., using nuclear magnetic resonance techniques or gas chromatography-mass spectrometry) were not possible. Of the 36 peaks detected, the spectra of 7 of them closely resemble various hydroxyfluoranthenes that have been identified in rat liver [4,30] in terms of relative elution times, UV maxima, relative peak height, and shape. All seven peaks lie toward the less polar end of the metabolite distribution, appearing toward the end (between 35 and 45 min) of the sample run. Of these, peaks 22, 29, and 31 were detected only in worms during the course of the 96-h experiment, whereas peaks 25, 26 (which appeared infrequently and which we suspect may be identical to peak 25), 33, and 34 appeared initially in worms but also in water and/or sediment at later time intervals. In particular, we propose that peak 34 represents 3-hydroxyfluoranthene and that peak 33 represents 8-hydroxyfluoranthene. Although we did not have standards against which to run these metabolites, their spectra (i.e., UV maxima and relative peak heights) closely resemble spectra found by Babson et al. using the same methanol:water gradient as used here [30] (Fig. 3a and b). The metabolites identified by Babson et al. (by comparison of chromatographic properties and UV spectra with synthetic standards) are products of phase I metabolism and are consistent with observations of P450 enzyme activity in *Capitella* species [24].

Although very few PAHs have been tested as substrates for polychaete mixed-function oxygenase [31], examination of benzo[*a*]pyrene metabolism by the polychaete *Nereis virens* also found hydroxy derivatives to be the dominant metabolites produced. In this study, 3-hydroxybenzo[*a*]pyrene was found to be the major metabolite, with small amounts of 7-hydroxy and 9-hydroxy derivatives and two diols, trans-4,5-dihydrodiol benzo[*a*]pyrene and trans-7,8-dihydrodiolbenzo[*a*]pyrene, also produced by this species [40].

Whereas the addition of a single OH-group to the PAH molecule to form hydroxy derivatives seems to increase the availability of the parent PAH for further metabolism, the addition of two OH-groups to form diols is believed to be an important factor controlling the mutagenic activity of PAH such as fluoranthene [30]. The exact position of groups attached to the fluoranthene molecule is critical for determining whether a given metabolite leads to detoxification or mutagenicity. Hydroxyfluroanthenes, while not mutagenic themselves, may be oxidized to diols that are potentially mutagenic. The degree of mutagenicity can vary markedly among diols depending on the position at which the oxidation occurs [30]. Determining whether *Capitella* sp. I produces diol metabolites during fluoranthene metabolism will require targeted investigations and synthesis of appropriate standards. In the meantime, direct assays to compare the mutagenic activity of fluoranthene with *Capitella* sp. I metabolite extracts are underway in our laboratory.

In conclusion, our results show that *Capitella* species I is able to metabolize fluoranthene ingested from contaminated sediment to more hydrophilic metabolites, some of which are readily excreted to water and/or sediment but most of which are retained by worms to some degree. Compared with other aquatic invertebrates, *Capitella* sp. I appears to be very effective at metabolizing PAHs, which is consistent with observations of its dominance in oil-contaminated sediments. Definitive identification of the metabolites produced and determination of their biological activity are necessary to evaluate the extent to which PAH metabolism by *Capitella* sp. I increases or decreases the hazard of these toxicants to benthic marine ecosystems.

Acknowledgement—We are grateful to E. Lauridsen and E. Karlsen for assistance with the HPLC analyses, to I. Linke-Gamenick for help in culturing *Capitella,* and to B. Larsen, J. P. Nielsen, and A.-L. Gudmundsson for additional technical assistance. Comments from three anonymous reviewers are gratefully acknowledged.

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