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Annemette Palmqvist
Roskilde University, Denmark, apalm@ruc.dk

Lene Juel Rasmussen
Roskilde University, lenera@sund.ku.dk

Valery E. Forbes
University of Nebraska-Lincoln, vforbes3@unl.edu

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Influence of Biotransformation on Trophic Transfer of the PAH, Fluoranthene

Annemette Palmqvist,[1] Lene Juel Rasmussen,[2] and Valery E. Forbes [1]

1. Department of Environmental, Social and Spatial Change, Roskilde University, Roskilde, Denmark
2. Department of Science, Systems and Models, Roskilde University, Roskilde, Denmark

Corresponding author – A. Palmqvist, email apalm@ruc.dk

Abstract

The persistence of polycyclic aromatic hydrocarbons (PAHs) in marine sediments may be influenced by benthic invertebrate bioturbation. Through processes such as deposit-feeding and enhancement of microbial metabolic activity PAHs may be remobilized from the sediment compartment, and either transferred to organisms at higher trophic levels or to the overlying water column, both processes inevitably changing the bioavailability of the PAH. Accumulation of contaminants from one level in the food chain to the next depends on feeding rate and assimilation efficiency, two factors that basically vary with food quality and contaminant type. Though it is generally believed that pre-consumptive biotransformation will reduce bioavailability due to the more polar nature of the metabolites compared to the unchanged parent compound, theoretically the decrease in lipophilicity will increase the sediment/food desorption rate in the intestine, and some metabolites will still be lipophilic enough to be absorbed by passive diffusion. We examined the trophic transfer of the PAH, fluoranthene from two closely related polychaete species (i.e., *Capitella* sp. I and *Capitella* sp. S), differing in their biotransformation ability, to the predatory polychaete, *Nereis virens*. We found that *N. virens* fed the biotransforming species, *Capitella* sp. I, accumulated significantly more Flu equivalents compared to worms fed *Capitella* sp. S, which have a very limited biotransformation ability. The dose-specific increase in *N. virens* intestinal Flu concentration was approximately twice as high in worms fed *Capitella* sp. I (equation: gut content = 7.3 × dose - 3.9) compared to worms fed *Capitella* sp. S (equation: gut content = 3.2 × dose + 0.6). In addition, we measured DNA damage, using the comet assay, in *N. virens* intestinal cells after feeding with the two prey species. We did not detect DNA damage above 'background' levels for worms fed either of the two *Capitella* species, possibly due to relatively low intestinal Flu concentrations in *N. virens*. Our results indicate that accumulation of PAHs by infaunal organisms may play an important role in the transfer of this type of contaminant to higher trophic levels. Moreover, we observed differences in transfer potential between parent compounds and their respective metabolites, which may influence the fate of these compounds in marine ecosystems. However, from the present study it cannot be concluded whether differences in biotransformation ability among prey species can lead to different effects in their predators.

Keywords: *Capitella* species, *Nereis virens,* DNA damage, Metabolites, Sediment contaminants

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds in the marine environment, originating primarily from anthropogenic sources. Due to their hydrophobic nature they tend to adsorb to organic particles in the sediment where they are inherently persistent (Neff, 1979). PAHs as a group are considered to be among the most hazardous pollutants, and consequently several of them are classified as priority chemicals by the U.S. Environmental Protection Agency (http://www.epa.gov). Generally, the high molecular weight PAHs are found to possess mutagenic, carcinogenic and/or teratogenic characteristics (Kanaly and Harayama, 2000; Babson et al., 1986), whereas the low molecular weight PAHs are known to exert acutely toxic effects such as narcosis (e.g. Swarts et al., 1990). There is also limited evidence indicating that at least some PAHs may show hormone disrupting properties (Fertuck et al., 2001; Kennedy and Farrell, 2005). While the acutely toxic effect of the low molecular weight PAHs is typically caused by the untransformed parent compound, the genotoxic effects of the high molecular weight PAHs rely on metabolic transformation (biotransformation) of the PAH. The biotransformation process generally involves both cytochrome P-450 mixed-function oxygenase, a step potentially producing reactive intermediate metabolites, and one or more subsequent en-

zymatic conjugation processes, rendering the metabolite water-soluble and thereby more easily excretable (Lee and Singer, 1980). Large differences in biotransformation ability exist among different organisms (McElroy et al., 2000), and even closely related species can differ considerably in the degree to which they are capable of biotransforming PAHs (Bach et al., 2005).

The persistence of PAHs in marine sediments may be influenced by benthic invertebrate bioturbation (Christensen et al., 2002; Ciarelli et al., 1999; McElroy et al., 1990). Through processes such as deposit-feeding and enhancement of microbial metabolic activity PAHs may be remobilized from the sediment compartment, and either transferred to organisms at higher trophic levels or to the overlying water column, both processes inevitably changing the bioavailability of the PAH. A large number of studies has been performed on the bioavailability and toxicity of PAHs to sediment dwelling organisms, and most of these have involved primary exposure either through deposit-feeding or through passive diffusion over the body wall (Connolly, 1991; Rand, 1995; Gunnarson et al., 1996; Selck et al., 2003b). Only a few studies have examined trophic transfer of PAHs in the marine benthic environment (McElroy and Sisson, 1989; Clements et al., 1994). Biotransformation of a PAH may change its bioavailability, and the transfer of PAHs between prey and predator may depend on the biotransformation capability of the prey species (McElroy and Sisson, 1989). However, since biotransformation may also markedly change the genotoxicity of the accumulated PAHs, risk to predators from the trophic transfer of PAHs, may be influenced by the biotransformation capability of their prey.

Fluoranthene (Flu) is a four-ringed PAH that is one of 16 priority PAHs defined by the U.S. Environmental Protection Agency (EPA). It is one of the most common PAHs in marine sediments, where it has been found in concentrations of tens to hundreds of g/g dry weight sediment (Shiaris and Jambard-Sweet, 1986; Gao et al., 1998). Flu can be readily biotransformed by some marine organisms (Forbes et al., 1996, 2001; Selck et al., 2003a; Kane Driscoll et al., 1997, 1998), and consequently may exert both acutely toxic and genotoxic effects in marine organisms (Swartz et al., 1990; Sepic et al., 2003; Palmqvist et al., 2003).

The species complex, *Capitella capitata* consists of a large number of morphologically similar sibling species that are all infaunal, deposit-feeding polychaetes. Generally, they thrive in organically enriched sediments, where they can reach densities of more than 200,000 individuals/m^2 (Sanders et al., 1980; Tsutsumi, 1987, 1990). Recently, interspecies variability with regard to tolerance to contaminants has been found within the species complex (Linke-Gamenick et al., 2000), which is associated with differences in biotransformation ability (Bach et al., 2005). The most opportunistic of the species so far examined, *Capitella* sp. I is significantly more tolerant to Flu exposure than the slightly smaller *Capitella* sp. S (Linke-Gamenick et al., 2000), and this species is also a much more efficient biotransformer of accumulated Flu (Selck et al., 2003a; Bach et al., 2005). Though the former species may encounter genotoxic metabolites during the process of biotransformation, it is able to cope with the adverse effects of these by inducing DNA repair (Palmqvist et al., 2003). Due to the large densities in which these worms are found, they may be important prey species for demersal fish and predatory invertebrates, such as the polychaete *Nereis virens*. *N. virens* is a large, benthic omnivorous polychaete, which is capable of several different feeding strategies (i.e., predation, deposit-feeding, scavenging and filter-feeding), though adults are almost exclusively carnivorous (to the extent that food is available) (Tita et al., 2000). This polychaete is an efficient biotransformer of PAHs (McElroy, 1990; Christensen et al., 2002), and has been shown to be relatively tolerant to organic contaminants (Reish and Gerlinger, 1997).

The objective of this study was to examine how pre-consumptive biotransformation influences the trophic transfer and genotoxicity of the PAH, Flu. Two sibling species, *Capitella* sp. I and *Capitella* sp. S, which were chosen on the basis of their different biotransformation capabilities, were exposed to Flu-contaminated sediment and subsequently fed to the predatory polychaete *N. virens*. Biotransformation efficiency was estimated in the two prey species, and additionally Flu bioaccumulation was measured in both prey species (whole body burden) and consumer species (intestinal tissue) after exposure and feeding, respectively. The comet assay was used to measure DNA damage in *N. virens* intestinal cells after feeding with exposed or control worms of either of the two *Capitella* species.

2. Materials and methods

2.1. Culturing and acclimatization of worms

Surface sediment was collected at St. Havelse, Roskilde Fjord (Denmark; 55°54′ N, 12°02′ E), sieved and frozen to -20 °C for at least 1 week to kill meiofauna before use.

Capitella sp. I were originally collected from Setauket Harbor, Long Island Sound (New York, New York, USA) in 1984 and identified by J.P. Grassle. *Capitella* sp. S were originally collected from an intertidal flat of the Island of Sylt (Germany) and identified by Gamenick and Giere (1994). Laboratory cultures of both worm species were reared at 20-24 °C in aquaria containing ca. 3 cm of azoic prefrozen sediment (<250 μm) and filtered (<0.2 μm), aerated seawater (31‰ salinity). The sediment was regularly supplemented with a mixture (1:1:1) of ground fish food (Tetramin©, Tetra Werke, Melle, Germany), dried spinach and baby cereal (Milpo©, Milupa, Hørsholm, Denmark).

Nereis virens were obtained from a local bait shop as live bait imported from a bait farm in The Netherlands. The worms were transferred to 12 l aquaria (8-10 worms in each) containing ca. 10 cm of prefrozen sediment (<1,000 μm) and filtered (<0.2 μm), aerated seawater (18‰ salinity). The worms were acclimatized to laboratory conditions for 3 weeks before use, and during that period they were fed cooked shrimp meat.

2.2. Overall experimental design

The experiment was divided into two parts. The first part consisted of the exposure of *Capitella* sp. I and sp. S to Flu-contaminated sediment, and the subsequent measurements of to-

tal Flu accumulation and the percent distribution of parent Flu and Flu metabolites in whole worm tissue. In the second part of the experiment (the feeding experiment) contaminated and control *Capitella* spp. tissue homogenates from the first part of the experiment were fed to *N. virens*, and Flu accumulation and DNA damage were measured in *N. virens* gut tissue.

2.3. Preparation of sediment

For all *Capitella* exposures, sieved sediment (<125 µm) with a water content of 55.6 ± 0.26% was used. Stock solutions of Flu in acetone (10 mg crystalline Flu [98% GC grade, Sigma-Aldrich, Copenhagen, Denmark] pr. ml acetone) and radiolabelled Flu (fluoranthene-3-14C, Sigma-Aldrich, Copenhagen, Denmark) in methanol (0.1 µCi/µl methanol) were prepared. Flu-contaminated sediment was made by adding known volumes of Flu-and radiolabelled Flu stock solutions to a 2 l glass container, and control sediment was prepared by adding equivalent volumes of acetone and methanol to another glass container. Both containers were left in a shaking water-bath (~20 °C) in the dark for ca. 90 min until the solvents had evaporated. Known, equivalent volumes of sediment were added to the two containers and left in the shaking water-bath for another 48 h at 20 °C in the dark. The sediments were then frozen (-20 °C) until use.

The initial nominal Flu concentration was 100 µg Flu/g dry wt. sediment. Radiolabelled Flu was used to determine the concentration of Flu equivalents (i.e., parent Flu together with Flu metabolites) in sediment, *Capitella* tissue and *N. virens* gut tissue. It was assumed that radiolabelled Flu and non-radiolabelled Flu behaved identically. The concentration of Flu equivalents could be calculated from the measured concentration of radiolabelled Flu according to the ratio of non-radiolabelled Flu to radiolabelled Flu, which was 375 µg Flu/µCi.

2.4. Capitella exposure

Prior to exposure, contaminated and control sediments were thawed and homogenized. Four samples were taken out for measurement of the actual sediment Flu concentration. Wet sediments were added to 250 ml glass beakers in amounts equivalent to 30 g dry weight sediment/beaker, and frozen until use. One day prior to exposure, sediments were thawed, 150 ml of filtered seawater (31‰) was added to each beaker and the beakers were left in the dark overnight. Likewise, worms of both species were sieved out of the cultures 1 day prior to exposure. They were kept in the dark in clean, filtered seawater overnight to empty their guts. The exposure period was started by changing 125 ml of the overlying water with new filtered and aerated seawater, and transferring either 100 worms of *Capitella* sp. I or 150 worms of *Capitella* sp. S to each of the glass beakers with either contaminated or control sediments. Each beaker was aerated through a syringe, and all syringes were connected via silicone tubes to an air-pump. The beakers were covered with parafilm to minimize water loss by evaporation. The worms were exposed for 4 days in the dark, after which they were sieved out of the sediment and left in clean, filtered seawater for ca. 2 h to empty their guts. Worms from each beaker were divided among three Eppendorf tubes: one tube (two worms) for measurement of the total concentration of Flu equivalents in the worm tissue, another tube (four worms) for measuring the biotransformation ability and a third tube (rest of the worms) to use in the feeding experiment. Prior to transfer to the tubes, the worms used for measurement of total Flu were videotaped for volume measurements. All tubes were immediately frozen at -20 °C until further analysis. Additionally, sediment samples and samples of the overlying water were taken from the beakers prior to harvest of the worms.

2.5. Measurements in sediment and overlying water

Small aliquots of the sediments sampled prior to and at the end of the exposure period were transferred to pre-weighed scintillation vials, the vials were reweighed and 750 µl of tissue solubilizer (Solulyte, J.T. Baker Chemicals, Deventer, Holland) was added to each vial. After 24 h, 15 ml of scintillation cocktail (Packard Ultima Gold XR scintillation cocktail, Packard, Denmark) was added to each vial, and the radioactivity was quantified by liquid scintillation counting (LSC) on a Wallac 1409-012 liquid scintillation counter (Wallac, Gaithersburg, Maryland, USA). The measured radioactivity was corrected for quench by the external standards ratio method after subtracting background, and converted into Flu equivalents using the fluoranthene/radioactivity ratio of 375 µg Flu/µCi. From each of the water samples (overlying water) 2 ml was transferred to a scintillation vial, 15 ml of scintillation cocktail was added and, as for sediment samples, radioactivity was quantified by LSC and converted to Flu equivalents.

2.6. Measurements in Capitella

2.6.1. Extraction of fluoranthene and its metabolites

Biotransformation capability in the two *Capitella* species was quantified by extracting Flu equivalents from the worm tissue and fractionating them into four phases; i.e. untransformed parent compound, polar metabolites, aqueous metabolites (conjugates) and residual Flu bound to cellular constituents (hereafter referred to as tissue Flu-residues) according to the method described by Selck et al. (2003a). The frozen worm samples were transferred to preweighed Pyrex centrifuge tubes (10 ml), thawed, weighed and homogenized in 0.8 ml of milliQ water (2 min) using a micro-homogenizer (Jencons, Bedfordshire, England). One milliliter of chloroform and 2 ml of methanol were added to each sample, the samples were mixed (on a whirlmixer) for 30 s after which they were exposed to ultrasonic treatment for 20 min. Subsequently, 1 ml of chloroform and 1 ml of milliQ water were added to each sample, and again the samples were mixed for 30 s, followed by 5 min centrifugation at 250 × *g*. After centrifugation the solution had separated into a water/methanol phase (aqueous metabolites), a chloroform phase (parent Flu and polar metabolites), and a tissue pellet (tissue Flu-residues). The two phases were transferred separately to new glass tubes, and the extraction procedure repeated twice from the pelleted tissue with half volumes of the solvents. After extraction, 1 ml of tissue solubilizer was added to each pellet and the samples were left overnight. The solution was transferred to a scin-

tillation vial, and 15 ml scintillation cocktail was added. The water/methanol phase was carefully mixed, and from each replicate 2 ml was transferred to a scintillation vial and 15 ml scintillation cocktail was added. In order to separate parent Flu and polar metabolites the chloroform phase was further fractionated. The chloroform phase was evaporated (with N_2) to almost dryness and redissolved in 600 µl DMSO (HPLC grade, purity 99%), 250 µl MilliQ water and 150 µl KOH (1 M). The solution was mixed (30 s), 2 ml of hexane was added and the solution was mixed for another 30 s. The solution was centrifuged for 5 min at 250 × *g*, and had now separated into a hexane phase (parent Flu) and a DMSO phase (polar metabolites). The hexane phase was transferred to a new glass tube, and the procedure repeated twice from the addition of hexane. After completion of the extraction procedure, 2 ml of the hexane phase and 600 µl of the DMSO phase were transferred to separate scintillation vials, and 15 ml of scintillation cocktail was added to each vial. The radioactivity in all four phases was quantified by LSC, corrected for quench and converted into Flu equivalents by using the fluoranthene/radioactivity ratio of 375 µg Flu/µCi.

2.6.2. Measurement of total fluoranthene in Capitella

The total content of Flu and Flu equivalents in *Capitella* species was measured by transferring two worms from each replicate to a scintillation vial and adding 1 ml of tissue solubilizer (solulyte, J.T. Baker Chemicals, Deventer, Holland). The worms were treated with the solubilizer for 24 h. Fifteen milliliter of scintillation cocktail was then added to the solution, radioactivity was quantified by LSC, the measured radioactivity was corrected for quench and converted into Flu equivalents (ratio of 375 µg Flu/µCi).

Individual worm body volumes (BV, mm^3) were estimated from the video sequences obtained prior to freezing worms. Area (A, mm^2) and length (L, mm) were estimated using Sigma Scan Pro (SPSS Science, Chicago, Illinois, USA) and BV was calculated according to: $BV = (\pi \times A^2)(4 \times L)^{-1}$ (Self and Jumars, 1978). The estimated BVs were converted to dry weights following Bach et al. (2005): $\log_{10} DW = 2.26 + 0.86 \times \log_{10} BV$, where DW is worm dry weight (µg) and BV is body volume (mm^3). These calculations were used to convert the total Flu equivalents to weight-specific concentrations (µg Flu/g dry wt. worm tissue).

2.7. *Nereis feeding experiment*

2.7.1. Food preparation

Four food types were produced from the frozen, exposed and control worms of the two *Capitella* species (i.e., Flu exposed and control *Capitella* sp. I were called I-F and I-C, respectively, and Flu exposed and control *Capitella* sp. S were called S-F and S-C, respectively). All capitellid worms (replicates) of the same species that had undergone the same treatment were pooled. The pooled samples were homogenized using a microhomogenizer, and small subsamples (ca. 10 mg) of the homogenized tissue were mixed with sodium alginate. The alginate solution was prepared by dissolving 159 mg alginic acid sodium salt (Sigma-Aldrich, Brøndby, Denmark) in 10 ml filtered seawater (18‰ salinity), heating the solution until the alginate had completely dissolved, and then cooling the solution to <35 °C. Approximately, 10 mg of homogenized tissue was added to the top of a small droplet of alginate solution (the precise amount was determined by weighing) and another droplet of alginate solution was added on top of the tissue. Then, a 2% CaCl solution was carefully poured onto the alginate-tissue sample, which caused the alginate to gel, producing a small alginate-tissue disc (hereafter referred to as a food disc). Flu concentrations in the food discs were measured by homogenizing excess food discs (three for *Capitella* sp. S and two for *Capitella* sp. I), separately, in 750 µl tissue solubilizer and leaving them on a shaking table overnight to fully dissolve. Fifteen milliliter of scintillation cocktail was then added, the radioactivity quantified by LSC, and the measured radioactivity corrected for quench and converted into Flu equivalents using the ratio of 375 µg Flu/µCi. Mean values of the resulting food disc Flu concentrations were used to calculate the Flu concentrations in the food discs used in the feeding experiment.

2.7.2. Feeding of N. virens

For the feeding experiment 40 *N. virens* were transferred from the acclimatization aquaria to separate 1,000 ml glass beakers each containing 700 ml of filtered seawater (18‰ salinity). The beakers were covered with aluminum-foil to limit evaporation. The worms were starved for 20 days, at 15 °C in the dark to ensure that they consumed the administered food discs shortly after addition. Water in the beakers was changed twice a week during the starvation period, the last water change 1 day prior to the start of the experiment. To ensure an even distribution of worm sizes in the groups the 40 worms were divided into four groups by visual inspection. Group 1 was fed S-F food discs, group 2 was fed I-F food discs, group 3 was fed S-C food discs and group 4 was fed I-C food discs. On day 1, all worms had one food disc each of the appropriate type. On both day 2 and day 3, three worms (replicates) from each group were sampled and dissected for measurements, and the rest of the worms were fed one food disc each of the appropriate type. On day 4, three replicates from each group were sampled and dissected for measurements. On each dissection day, water samples were taken for Flu analysis.

2.7.3. Dissection of N. virens

The intestine was dissected out of the worms by pinning them to a prefrozen, wetted Styrofoam block with a pin a few segments behind the peristomium, cutting the dorsal wall with scissors along the dorsal blood vessel to open the coelom to the gut. Approximately, the first half of the intestine (without the esophagus) was cut free, and transferred to an Eppendorph tube and kept on ice. The gut tissue was washed twice with ice-cold Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS) (Gibco-BRL, Life Technologies, Taastrup, Denmark) to remove blood and coelomic fluids from the tissue. The tissue was homogenized on ice by cutting it with scissors, and a small sample was transferred to a new Eppendorf tube on ice in the dark for use in the comet assay. The rest of the gut tissue was immediately frozen to -20 °C to be used in the measurements of gut Flu contents.

2.8. Measurements in N. virens

2.8.1. Preparation of worm gut cells for comet assay

A suspension of single cells was prepared by placing the gut tissue in an Eppendorf tube and treating the tissue with 0.5 ml of trypsin/EDTA (GibcoBRL, Life Technologies, Taastrup, Denmark) for 5 min. The tissue was then centrifuged at 180 × *g* and 4 °C for 2 min, the trypsin/EDTA solution replaced by 0.2 ml of PBS and the cells dispersed by carefully pipetting the solution up and down. From each cell suspension (replicate) two subsamples (pseudo-replicates) were taken for further analysis as recommended by Tice et al. (2000). The comet assay was carried out immediately after preparation of the cell suspension and cells were kept in the dark on ice throughout the entire experiment to prevent further DNA damage or repair of DNA damage occurring following the sampling. Positive controls, in which worm cells were treated with a mutagen known to produce strand breaks, were made in order to validate the assay at each run. For the positive controls 50 µl was taken from each of the treatment controls, transferred to new Eppendorf tubes and centrifuged at 180 × *g* and 4 °C for 2 min. The supernatant was removed and the pellet was resuspended in a 50 µM H_2O_2 solution in PBS. The cells were treated with the H_2O_2 solution for 10 min., centrifuged at 180 × *g* and 4 °C for 2 min and the supernatant replaced with PBS.

2.8.2. Performance of comet assay

We used a modified version (McNamee et al., 2000) of the single cell gel electrophoresis assay originally described by Singh et al. (1988). Twenty-five microliters of the prepared cell suspensions was mixed with 225 µl molten (37 °C) LMP agarose (GibcoBRL, Life Technologies, Taastrup, Denmark), and 180 l of the resulting mixture was immediately cast on the hydrophilic side of 85 mm × 100 mm GelBond film (Medinova Scientific, Hellerup, Denmark) using chambers from Lab-Tek II Chamber Slides (Nunc, Life Technologies, Taastrup, Denmark) as moulds. The gels were cooled for approximately 10 min at 4 °C in the dark and the Lab-Tek II chambers removed. The gels were submersed in cold lysis buffer (pH 10) for 60 min, carefully washed three times with millipore water and submersed in electrophoresis buffer (pH 13.2) for 40 min before being electrophoresed for 20 min at ≈300 mA and 25 V. After electrophoresis, samples were neutralized for 2 × 5 min and washed once with millipore water before they were fixed in 96% ethanol for approximately 90 min. The gels were air-dried overnight, dyed with SYBR Gold (Bie & Berntsen, Rødovre, Denmark), and analyzed under a fluorescence microscope (Dialux, 50× oil immersion objective) using the software Comet assay III (Perceptive Instruments, Suffolk, UK). In each subsample 50 randomly selected cells were examined (i.e., 2 subsamples × 50 cells/subsample), and the level of DNA damage in a replicate expressed as mean tail moment (tail length × percentage of DNA in the tail).

2.8.3. Measurement of gut Flu content

The frozen tissue samples were transferred to preweighed scintillation vials and reweighed. To each sample 750 µl of tissue solubilizer was added, and the samples were shaken on a shaking table for 40 h. Subsequently, 15 ml of scintillation cocktail was added, the radioactivity quantified by LSC and the measured radioactivity corrected for quench and converted into Flu equivalents using the ratio of 375 µg Flu/µCi.

2.9. Statistical analysis

Assumptions of normality and homogeneity of variances were checked by visual graphical inspection of the data prior to analysis. Single classification analysis of variance (ANOVA) was used to test for differences between pre- and post-exposure sediment Flu concentrations in the *Capitella* exposure experiments. The Student's *t*-test was used to test for differences in post-exposure Flu concentrations in overlying water and total Flu accumulation (worm body burdens) between the two polychaete species, *Capitella* sp. I and *Capitella* sp. S. Differences in the percent distribution of parent Flu and Flu metabolites between *Capitella* sp. I and *Capitella* sp. S were tested by the RxC Test of independence using the G-test (Sokal and Rohlf, 1981), and using the mean of each fraction. Differences in Flu concentration of the surrounding water in the *N. virens* feeding experiment were tested by two-way ANOVA with food-type and number of feedings as factors. For the DNA damage, differences in tail extent moment at each sampling time were analyzed by two-way ANOVA with species (*Capitella* I or S) and treatment (*Capitella* fed control or Flu-exposed sediment) as factors, for each sampling time separately. In cases where the two-way ANOVAs showed a non-significant time × treatment interaction effect, a reduced model was run in which the interaction term was omitted. Where significant main effects were found Tukey HSD tests were performed post hoc on the individual main effects. Analysis of covariance (ANCOVA) was used to test for differences in both dose-specific Flu accumulation in *Nereis virens* gut tissue and body burden-specific DNA damage. Significance of the dose-specific accumulation rate in *N. virens* gut tissue was tested by linear regression for both *N. virens* fed *Capitella* sp. I and *N. virens* fed *Capitella* sp. S. A significance level of $p \leq 0.05$ was used throughout. With the exception of the RxC test (performed by hand), all statistical analyses were performed using the software Systat Version 10 (Systat software GmbH, Erkrath, Germany).

3. Results

3.1. Capitella exposure

3.1.1. Measured sediment and water Flu concentration

The sediment Flu concentration was measured prior to exposure and after 4 days of exposure (Table 1). The measured concentration prior to exposure of 67.1 ± 31.1 µg Flu/g dry wt. sediment was lower than the nominal concentration of 100 µg Flu/g dry wt. sediment. However, the sediment concentrations prior to exposure and after exposure were not significantly different for any of the species meaning that neither of the worm species depleted the sediment during the exposure period (single classification

Table 1. *Capitella* spp. Exposure

Measure	*Capitella* sp. I	*Capitella* sp. S
Sediment Flu concentration		
Pre-exposure	67.1 ± 31.1 (4)	67.1 ± 31.1 (4)
Post-exposure	92.3 ± 92.6 (4)	58.1 ± 32.7 (4)
Water Flu concentration	39.6 ± 4.3 (3)	30.1 ± 5.4 (4)
Worm body burden		
Per gram wet weight	138.4 ± 23.7 (4)	125.2 ± 15.7 (4)
Per gram dry weight	504.1 ± 101.4 (2)	581.5 ± 319.7 (2)

Sediment Flu concentrations (μg/g dry wt. sediment) prior to and after exposure, overlying water Flu concentrations (μg/l) after exposure and total Flu accumulation (worm body burden in μg/g) after a 4-day exposure period of *Capitella* sp. I and sp. S. All numbers are given as: mean ± SD (*n*).

ANOVA, $p = 0.711$). The Flu concentration in the overlying water after 4 days of exposure was low for both species (Table 1) but differences between beakers inhabited by the two *Capitella* species bordered on significance (Student's *t*-test: $p = 0.054$ for pooled variances, and $p = 0.048$ for separate variances).

3.1.2. Measurements of Flu and Flu metabolite accumulation in Capitella sp. I and sp. S

The total body burden of Flu equivalents after 4 days of exposure (Table 1), was not significantly different between the two *Capitella* species (Student's *t*-test: $p = 0.390$). However, the degree to which the accumulated Flu was biotransformed differed considerably between *Capitella* sp. I and sp. S (*G*-test; $p < 0.001$) (Figure 1). After the 4-day exposure period approximately 70% of the accumulated Flu was biotransformed into aqueous and polar metabolites in *Capitella* sp. I compared to no more than 5% in *Capitella* sp. S. Therefore, only 26% of the accumulated Flu was in the form of parent compound in *Capitella* sp. I compared to 93% in *Capitella* sp. S.

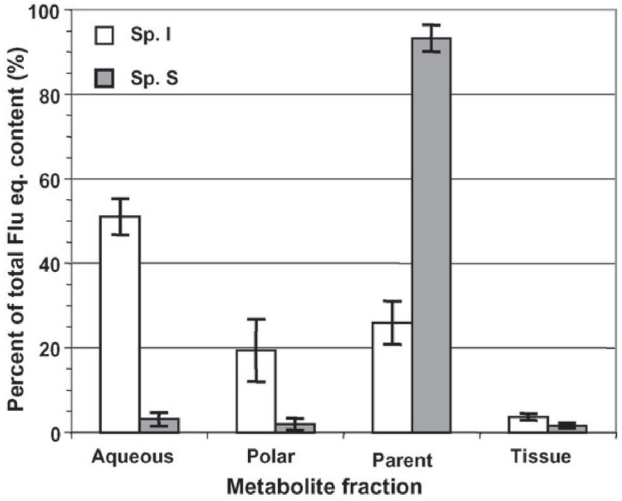

Figure 1. Biotransformation of Flu by the two *Capitella* species, *Capitella* sp. I (white bars) and *Capitella* sp. S (gray bars) after the 4-day exposure period. The bars represent the percentage of the total content of Flu equivalents in the different fractions, i.e., aqueous-and polar metabolites (phase I and phase II metabolites, respectively), parent compound and unextractable tissue residues (see text for method).

3.2. Nereis feeding experiment

3.2.1. Measured food and water Flu concentration

The mean Flu concentrations in the food discs are shown in Table 2. The amount of homogenized tissue varied slightly in the individual food discs, but the exact weight of each food disc was measured and taken into account in the calculation of total dose administered to *N. virens*. The Flu equivalent concentration in the 700 ml surrounding water (Table 3) increased with the number of feedings for *N. virens* fed both *Capitella* sp. I and *Capitella* sp. S. However, the increase in the surrounding water content was higher for *N. virens* fed *Capitella* sp. I compared to worms fed *Capitella* sp. S (two-way ANOVA; food type × number of feedings, $p = 0.035$).

Table 2. *Nereis virens* feeding experiment

Treatment (food-type)	*N. virens* wet weight (g)	Flu content in food (μg/g wet wt.)	Homogenate in food discs (mg)
I-F	4.20 ± 1.13 (9)	360.9 (2)	10.45 ± 0.78 (21)
S-F	4.07 ± 1.01 (9)	358.9 (3)	10.64 ± 0.71 (21)
I-C	3.98 ± 0.98 (9)	–	10.08 ± 0.81 (18)
S-C	3.87 ± 0.91 (9)	–	10.28 ± 0.84 (21)

Mean worm wet wt. ± SD (*n*) in the different treatments, mean Flu equivalent concentration of the administered food (*n*) and mean *Capitella* spp. homogenate content in the food discs ± SD (*n*).

Table 3. *Nereis virens* feeding experiment

Food	Mean water Flu concentration (ng/ml)			Percent of total dose		
	1 feeding	2 feedings	3 feedings	1 feeding	2 feedings	3 feedings
I-F	0.19 ± 0.21	0.74 ± 0.13	1.33 ± 0.11	3.44 ± 3.80	6.75 ± 1.39	8.41 ± 0.73
S-F	0.08 ± 0.11	0.42 ± 0.15	0.87 ± 0.08	1.37 ± 2.00	3.91 ± 1.55	5.34 ± 0.58

Worms were fed either *Capitella* sp. I (I-F) or *Capitella* sp. S (S-F) once, twice or three times during the experiment (with a 24 h interval between feeding occasions) and sacrificed 24 h after feeding. Mean water Flu equivalent concentrations ± SD ($n = 3$) in μg/l 24 h after the last feeding (i.e., at the time of sacrifice) and the total water Flu content as a percentage of the total administered dose.

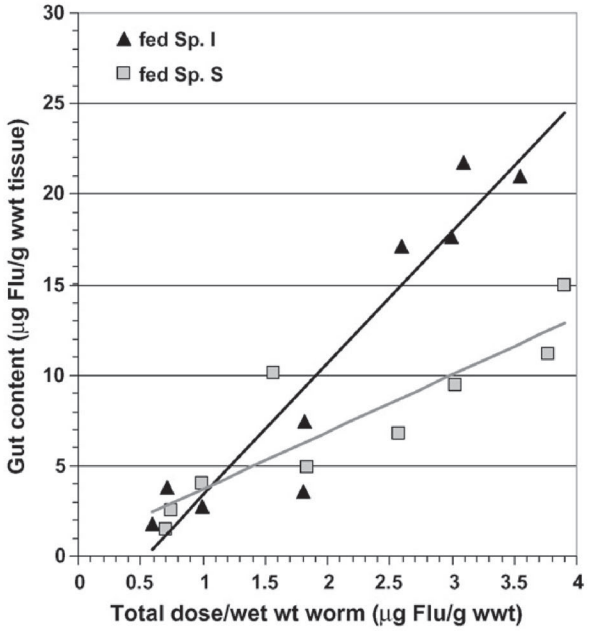

Figure 2. Bioaccumulation of Flu in the intestinal tissue of *Nereis virens* fed either of the two *Capitella* species after they had been pre-exposed to Flu for 4 days. There was a significant interaction in the bioaccumulation between dose and food species (ANCOVA; dose × food species, $p = 0.002$).

3.2.2. Flu accumulation in N. virens intestinal tissue

The concentration of Flu equivalents increased significantly in *N. virens* intestinal tissues with increasing weight-specific dose for both worms fed contaminated *Capitella* sp. I (linear regression; $n = 9$, equation: gut content = 7.3 × dose - 3.9, where gut content is in µg Flu/g wet wt. tissue and dose is in µg Flu/g wet wt. worm, $p < 0.001$) and worms fed contaminated *Capitella* sp. S (linear regression; $n = 9$, equation: gut content = 3.2 × dose + 0.6, $p = 0.002$) (Figure 2). However, the accumulation rate was significantly higher in *N. virens* fed *Capitella* sp. I than in worms fed *Capitella* sp. S (ANCOVA; dose × food species, $n = 18$, $p = 0.002$). The highest Flu concentration obtained in *N. virens* gut tissue during the experiment was 21.75 and 14.95 µg/g wet wt. gut tissue for *N. virens* fed *Capitella* sp. I and sp. S, respectively.

3.2.3. DNA damage in N. virens intestinal cells

An ANOVA with treatment (i.e., three treatments: control food, exposed food and positive control) as a factor and a subsequent Tukey HSD test showed that, as expected, DNA damage was significantly higher in the positive controls than in control treatments (Table 4). There was no interaction in tail extent moment between species and treatment for either of the sampling times (two-way ANOVA; species × treatment, after one feeding, $p = 0.768$, after two feedings, $p = 0.450$ and after three feedings, $p = 0.890$). A reduced model (i.e., species × treatment term omitted) showed that the measured tail extent moment did not differ significantly in intestinal cells of *N. virens* fed *Capitella* sp. I compared to *Capitella* sp. S for any of the sampling times ($p = 0.821$, 0.979 and 0.142 for 1, 2 and 3 feedings, respectively). In addition, the reduced model showed that treatment had an effect on tail extent moment for all three sampling times ($p = 0.006$, 0.002 and 0.010 for 1, 2 and 3 feedings, respectively). However, this was only due to the significantly higher tail extent moments of the positive controls (i.e., there were no differences between *N. virens* fed control food and *N. virens* fed Flu-contaminated food).

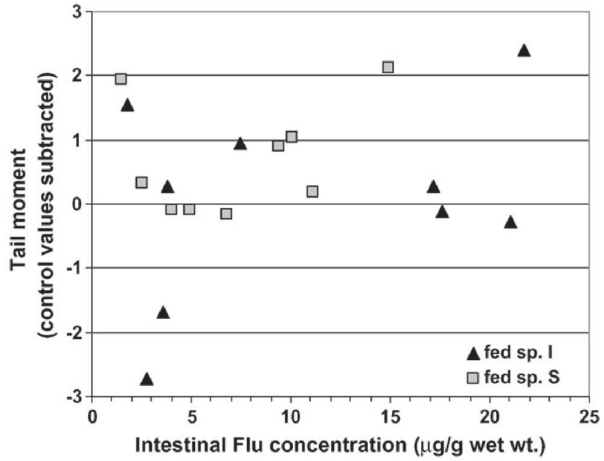

Figure 3. DNA damage (i.e., tail extent moment) in *Nereis virens* intestinal cells as a function of the intestinal concentration of Flu resulting from feeding with pre-exposed *Capitella* sp.Ior *Capitella* sp. S. Control values from *N. virens* fed uncontaminated *Capitella* sp. I or sp. S were subtracted. See text for statistics.

Table 4. *Nereis virens* feeding experiment

Food-type	Tail extent moment after		
	1 feeding	2 feedings	3 feedings
Species I			
Flu exposed	4.30 ± 2.20	3.88 ± 1.36	2.28 ± 1.50
Control	4.60 ± 1.86	4.03 ± 1.47	1.61 ± 0.17
Positive control	9.00 ± 3.04	8.00 ± 1.80	3.88 ± 0.41
Species S			
Flu exposed	3.80 ± 1.08	2.97 ± 0.68	3.25 ± 0.98
Control	3.09 ± 1.87	2.71 ± 0.79	2.18 ± 0.45
Positive control	10.06 ± 5.82	10.14 ± 5.39	5.24 ± 2.87

DNA damage measured as tail extent moment in *N. virens* gut cells after feeding with either control or pre-exposed *Capitella* sp. I or sp. S, and measured DNA damage in the positive controls (hydrogen peroxide treated *N. virens* gut cells, see Section 2.8.1 in text for further explanation). Mean tail extent moment ± SD, $n = 3$ for all numbers.

Relating the net DNA damage (i.e., DNA damage in worms fed exposed *Capitella* subtracted by mean DNA damage in worms fed control *Capitella*, for each *Capitella* species separately) to the measured intestinal Flu concentration (dose at target site) (Figure 3), we found no significant differences in DNA damage between *Nereis* worms fed *Capitella* sp. I and worms fed *Capitella* sp. S and no increase in DNA damage with increasing intestinal Flu concentration for either group (ANCOVA; dose at target site × food species, $p = 0.954$).

4. Discussion

4.1. Capitella exposure

The primary aim of this study was to determine whether biotransformation ability of prey species measurably influences

toxicant accumulation by the species that consume them. Although we found no significant difference in the total Flu equivalent body burden between the two *Capitella* (prey) species during 4 days of exposure to 67 μg Flu/g dry wt. sediment, there were marked species-specific differences in the distribution of parent compound and metabolites. This result is consistent with an earlier study in which the two species were exposed to a nominal Flu concentration of 30 μg/g dry wt. sediment for 15 days (Bach et al., 2005). In this earlier study, differences between species in accumulation of Flu equivalents first became significant after 10 days of exposure and were due to a higher accumulation of Flu metabolites in *Capitella* sp. I compared to *Capitella* sp. S. The present study has shown that even at Flu concentrations as high as 67 μg/g dry wt. sediment *Capitella* sp. I were able to maintain their Flu biotransformation at a high level, so that after 4 days of exposure only 26% of the total Flu equivalents were in the form of parent compound. This biotransformation efficiency is similar to that found in earlier studies in which parent Flu constituted 26% of the total Flu equivalent body burden after 5 days of exposure (Selck et al., 2003a) and 38% after 15 days (Bach et al., 2005) of exposure in *Capitella* sp. I exposed to nominal concentrations of 30 μg Flu/g dry weight sediment for both studies. Consistent with Bach et al. (2005) the present study also showed that *Capitella* sp. S had a very limited ability to biotransform Flu, and more than 90% of the total accumulated Flu equivalents was in the form of parent compound after 4 days of exposure. This obvious difference in biotransformation ability between the two *Capitella* species is probably an important underlying mechanism for the differences in Flu tolerance between the two species (Linke-Gamenick et al., 2000). Biotransformation of PAHs is a means of avoiding narcotic effects that could be caused by the hydrophobic parent compounds. In addition, Palmqvist et al. (2003) found that DNA damage caused by exposure to 30 μg Flu/g dry wt. sediment in *Capitella* sp. I was transient, due to an adaptive response of the DNA repair system. So, despite the fact that reactive intermediates formed during the biotransformation process may cause genotoxic effects in the biotransforming *Capitella* sp. I, it seems that biotransformation is an effective means of detoxification in this species as opposed to in *Capitella* sp. S. The present study also showed that *Capitella* sp. I accumulated high concentrations of Flu equivalents, despite its ability to biotransform Flu to more easily excretable aqueous metabolites. Thus, *Capitella* sp. I may survive high sediment concentrations of Flu, with a relatively high body burden of Flu equivalents, and therefore has the potential to remobilize Flu from the sediment, and pass the accumulated Flu, in whatever form, on to organisms at higher trophic levels. Although *Capitella* sp. S accumulated the same amount of Flu, this was primarily in the form of parent compound, which could potentially be readily accumulated by consumer species. However, due to the lower Flu tolerance of this species, its survival at high sediment Flu concentrations will be lower than that of its sibling species *Capitella* sp. I.

The high standard deviations (Table 1) of the measured sediment Flu concentrations, suggest that the sediment may not have been entirely homogeneous. However, due to the mobility of the worms, this was fortunately not reflected in the Flu accumulation in the worms.

4.2. Feeding experiment

In the feeding experiment, individuals of the polychaete species *N. virens* were fed food discs made from one of the two *Capitella* species that had been exposed to either control or Flu-contaminated sediment. The most important result of this experiment, was that *N. virens* fed *Capitella* sp. I accumulated significantly more Flu equivalents in their gut tissue compared to *N. virens* fed *Capitella* sp. S. This implies that in *N. virens* the Flu metabolites produced by *Capitella* sp. I are more easily taken up than the parent compound. The results of the present study differ from those of McElroy and Sisson (1989). These authors examined trophic transfer of benzo[*a*]pyrene (BaP) between *N. virens* (prey) and the winter flounder, *Pseudopleuronectes americanus* (consumer). Single oral doses of either BaP mixed into a *N. virens* homogenate or a homogenate of *N. virens* that had been exposed to BaP (i.e., containing the BaP metabolites naturally produced by the worms) were fed to flounder, and the accumulation of total BaP in flounder liver, bile and intestine was measured. McElroy and Sisson (1989) found significantly higher accumulation of BaP equivalents in fish fed parent BaP compared to fish fed the naturally produced BaP metabolites (McElroy and Sisson, 1989). However, potential differences in bioavailability between a compound mixed into a homogenate (adsorbed to) and a compound incorporated into cells (absorbed in) of worm homogenate (i.e., the two different treatments) were not taken into account. In another study on trophic transfer between *N. virens* and *P. americanus*, McElroy et al. (1991) found that the BaP-metabolite, benzo[*a*]pyrene-7,8-dihydrodiol (7,8-D) was accumulated to the same or greater degree as parent BaP depending on the tissue or body fluid in question (i.e., bile, liver, intestine and muscle). In this study, both compounds were given as single oral doses mixed into *N. virens* homogenate. This result shows that at least some polar metabolites (products of phase I detoxification) may be taken up over intestinal epithelia in predators preying on organisms containing BaP metabolites. In the human colon carcinoma cell line Caco-2, Cavret and Feidt (2005) found that transepithelial transport of the PAHs BaP, pyrene and phenanthrene was significantly enhanced by the biotransformation of the PAHs in these intestinal epithelial cells. In fact, the transcellular transport of BaP was completely inhibited by inhibiting BaP biotransformation in the cells. In the present study, for worms fed *Capitella* sp. S, which contains >90% parent compound, parent Flu alone must account for an appreciable amount of the Flu taken up by *Nereis*. However, whether the parent compound or Flu metabolites alone could account for the Flu taken up in *N. virens* fed *Capitella* sp. I, or whether a mixture of parent Flu and Flu metabolites must have been taken up to account for the *N. virens* total Flu body burden, could be estimated from a total mass balance of the administered Flu. However, since our primary aim was to make a qualitative evaluation of whether Flu metabolites could be transferred from the prey to the predator, rather than a quantitative measure of trophic transfer, we did not measure the Flu content in whole *N. virens*. Due to the lack of knowledge about accumulation rates of different compounds from intestinal tissues to other tissues, it is

Table 5. Content of Flu and metabolites in *Nereis virens* after three feedings with *Capitella* sp. I or sp. S

	After three feedings for *N. virens* fed	
	Capitella sp. S	*Capitella* sp. I
Total dose (Flu eq.) administered (measured) (µg)	11.41	11.08
Total aqueous metabolites administered	0.35	5.66
Total polar metabolites administered	0.23	2.14
Total parent compound administered	10.65	2.87
Total tissue residues administered	0.18	0.41
Total Flu eq. accumulated in 1/2 gut (measured) (µg)	0.70	1.30
Total Flu eq. in surrounding water (measured) (µg)	0.61	0.93

All values are mean values. The top part of the table is related to administered dose, i.e., total dose of Flu equivalents and the dose of different metabolites calculated according to the percentages shown in Figure 1. The bottom part of the table is the measured intestinal content of Flu equivalents, i.e., the total content of Flu equivalents in the proximal half of the intestine and the total Flu content in all 700 ml of surrounding water.

not possible to extrapolate directly from the measured intestinal concentration of Flu equivalents to a Flu equivalent concentration in whole worms. The high turnover of intestinal cells further complicates such an extrapolation, since it may limit the accumulation of Flu equivalents in systemic tissues compared to what could be expected. In addition, we did not measure the Flu content in the feces, due to difficulties of collecting the fecal pellets that were typically entangled in worm mucus and often adhered to the worms themselves. Therefore, it was not possible to make full Flu mass balances for the worms fed different food types, and impossible to determine the Flu assimilation efficiency of the worms. However, all the information available from the present study, which can be used to make a partial Flu mass balance for worms fed *Capitella* sp. I and sp. S, is summarized in Table 5. The measurements in Table 5 can show whether it is plausible that either the metabolites or the parent compound alone account for the accumulation of Flu equivalents in *N. virens*.For *N. virens* fed *Capitella* sp. I the 1.3 µg Flu equivalents in the proximal half of the intestine constitute ca. 45% of the administered parent compound (2.87 µg Flu). McElroy et al. (1991) found a total absorption efficiency of orally administered BaP in bile, liver, intestine and muscle tissue of 27.8%, 24 h after dosing the flounder, *P. americanus*. So even ignoring the content of Flu equivalents in the other half of the intestine as well as in the rest of the worm body, an assimilation efficiency of 45% of the parent compound is very high. It is, therefore, not likely that the Flu is transferred as parent compound alone from *Capitella* sp.I to *N. virens*. In *Capitella* sp. I, the largest part of the Flu equivalents is in the form of aqueous metabolites. But even for this group of metabolites, the 1.3 µg Flu equivalents in just the proximal half of the intestine constitutes ca. 23% of the administered dose. From the present results, it is obvious that the Flu equivalents taken up by *N. virens* consisted of a mixture of metabolites and untransformed parent compound, at least for worms fed *Capitella* sp. I. For *Capitella* sp. S the total Flu content of 0.7 µg in the proximal half intestine accounts for more than 100% of the administered dose of both polar and aqueous metabolites together, but only for ca. 7% of the parent compound. So for *N. virens* fed *Capitella* sp. S, it is likely that a larger part of the Flu equivalents transferred were in the form of parent compound compared to worms fed *Capitella* sp. I. One could argue that we should have measured the body burdens of the respective parent Flu/metabolite groups in *N. virens*. However, this was not feasible due to much too low levels of accumulated radioactivity in *N. virens*. In addition, such a measure may not have contributed to quantifying the relative transfer of parent Flu versus metabolites from prey to predator, because *N. virens* itself is an efficient biotransformer.

The difference in Flu concentration in the water surrounding *N. virens*, fed the two different food-types, could occur from differences in leakage from the food discs prior to exposure, differences in leakage from the fecal pellets or increased excretion of Flu metabolites by *N. virens* fed *Capitella* sp. I compared to worms fed *Capitella* sp. S. Since, in a pilot experiment without worms added, the food discs did not seem to disintegrate during several days of submersion in 18‰ seawater, and the worms in the present study consumed the food discs within a few hours, we assume that the water content of Flu equivalents originates from either leakage from fecal pellets or from *N. virens* excretion of Flu metabolites, or from a combination of these. Whatever the source, the results suggest that surrounding water concentrations of Flu metabolites will be higher if *Nereis* feeds on *Capitella* sp. I than on *Capitella* sp. S, which ultimately means that *Capitella* sp. I remobilizes Flu from the sediment compartment to a higher degree than *Capitella* sp. S.

The present study shows that trophic transfer from *Capitella* spp. to the polychaete *N. virens* can occur. From a study examining the relative importance of BaP uptake from water and trophic transfer of BaP from the chironomid, *Chironomus riparius* to the bluegill, *Lepomis macrochirus*, Clements et al. (1994) concluded that food was an important BaP source for *L. macrochirus*, though direct contact with sediment and/or uptake from water were also significant. In the same study, Clements et al. (1994) also fed Flu and BaP exposed *C. riparius* directly to juvenile *L. macrochirus*, and found that for chironomids with similar PAH concentrations Flu was accumulated in the fish and could be measured 24 h after feeding, whereas BaP could not be measured in the fish after 24 h.

Since Flu metabolites that are potentially genotoxic are transferred from *Capitella* sp. I to *N. virens* through trophic transfer there is the potential for increased DNA damage to

occur in *N. virens* as a result. However, we observed no significant increase in DNA damage in *N. virens* fed up to three meals of Flu-contaminated *Capitella* sp. I. The highest intestinal concentration of total Flu equivalents measured in *N. virens* was 21.75 µg/g wet wt. gut tissue. In Palmqvist et al. (2003), DNA damage in *Capitella* sp. I exposed to 30 µg/g dry wt. sed. was significantly higher than control values at a whole worm Flu eq. body burden of approximately 130 µg/g dry wt. tissue. Assuming a dry weight/wet weight relationship in *Capitella* sp. I of ca. 15% (which is within values that have been found in different pilot experiments in our laboratory), a back of the envelope calculation shows that the maximal intestinal Flu burden found in the present study, only just reached the whole body Flu concentration in which DNA damage was found in the study on *Capitella* sp. I (ca. 20 µg/g wet wt. tissue), and only for the *N. virens* fed *Capitella* sp. I. However, another explanation for the lack of a response in DNA damage could be that in *N. virens* DNA repair is rapidly induced already at a relatively low Flu body burden, and as a consequence DNA damage above background levels could not be detected. In a study by De Boeck and Kirsch-Volders (1997), in which *N. virens* was exposed to different water concentrations of BaP and the resulting DNA damage measured by the comet assay, the authors concluded that *N. virens* was not an adequately sensitive species for use in environmental monitoring. This conclusion was based on the fact that DNA damage was only induced in *N. virens* at the highest exposure concentration (45 mg/ml), suggesting that the lack of DNA damage response in the present study is not likely to be due to induction of repair mechanisms. In order to determine whether intestinal Flu concentrations were too low to induce DNA damage in the present study, we suggest that continued feeding over more than 3 days or feeding with food containing higher Flu concentrations is needed in order to study the potential genotoxic effects on *N. virens*. The reasons why we did not use higher sediment Flu concentrations for *Capitella* exposures in the present study is that *Capitella* sp. S does not survive well above concentrations around 100 µg/g dry wt sed, and in addition concentrations above this level are not very environmentally realistic.

4.3. Conclusion

The present study has shown that *Capitella* sp. I was significantly better at biotransforming Flu than its sibling species *Capitella* sp. S. In addition, the study showed that Flu incorporated in prey items is bioavailable and accumulates in the gut tissue of *N. virens*, and that biotransformed Flu is taken up more easily than untransformed Flu (resulting gut tissue concentration was approximately three times the administered dose in worms fed *Capitella* sp. S and seven times the administered dose in worms fed *Capitella* sp. I). At the resulting gut tissue concentrations, DNA damage in Flu exposed *N. virens* was not measurably higher than in control *N. virens* for any of the treatments, nor were there differences in DNA damage in worms fed *Capitella* sp. I compared to worms fed *Capitella* sp. S. Therefore, from the present study it cannot be concluded whether differences in biotransformation ability among prey species can lead to different effects in their predators.

However, evidence was provided that accumulation of PAHs by infaunal organisms may play an important role in the transfer of this type of contaminant to higher trophic levels. Moreover, we observed differences in transfer potential between parent compounds and their respective metabolites, which may influence the fate of these compounds in marine ecosystems.

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