The influence of sediment and feeding on the elimination of polycyclic aromatic hydrocarbons in the freshwater amphipod, *Diporeia* spp.

Guilherme R. Lotufo, Peter F. Landrum *

Great Lakes Environmental Research Laboratory, 2205 Commonwealth Blvd, Ann Arbor, MI 48105, USA

Abstract

The elimination of non-polar organic contaminants from sediment dwelling aquatic invertebrates was thought to be dominated by fecal elimination. This was particularly thought to be the case for the amphipod, *Diporeia* spp. that encapsulates the fecal material in a peritrophic membrane. The elimination of selected PAH congeners by *Diporeia* spp. was determined in the presence of three solid substrates and under water only conditions. The elimination was generally enhanced by the presence of a solid substrate whether or not the organism employed the material as a food source. The greater the sorptive capacity of the substrate, the greater its influence on the elimination process. Elimination via the fecal route was generally insignificant except for the elimination of BaP in the presence of sediment. In this case, the fecal elimination accounted for up to 40% of the total elimination, and the extent of elimination via the fecal route increased with the amount of fecal material produced. Thus, it is clear from the above effort that the main mechanism for elimination of contaminants in the presence of a substrate is primarily via passive diffusion from the organism with subsequent sorption to the solid substrate, which maintains the chemical activity gradient between the organism and the water. This effort refutes the earlier hypothesis that fecal elimination is the dominant route of elimination for *Diporeia* spp., and that the peritrophic membrane plays any substantial role in the elimination process. Published by Elsevier Science Ireland Ltd.

1. Introduction

In aquatic toxicology, the focus of most mechanistic kinetics studies have been on the accumulation of contaminants and the processes that alter the bioavailability of the contaminants, while fewer studies have been done on the mechanisms driving elimination. Further, the studies available are primarily for fish and not invertebrates. In fish, the elimination route is dominated by the conditions under which elimination is determined. When the elimination is against uncontaminated water, elimination is generally driven by the loss of the compound (particularly neutral com-

* Corresponding author. Tel.: + 1-734-741-2276; fax: + 1-734-741-2055.

E-mail address: landrum@glerl.noaa.gov (P.F. Landrum).

pounds) across the gill (Hayton and Schultz, 1991). When the uptake is primarily via the diet, loss across the gill is also often observed (Hayton and Schultz, 1991). When the organism is at or near steady state with the surrounding water, the elimination is through the urine or the bile and subsequently through feces (Hayton and Schultz, 1991; Nichols et al., 1991; Gobas et al., 1989). Elimination through the urine and bile generally require that the compound undergo biotransformation. Mechanistic studies to establish the routes of elimination in fish have used larger fish that have been canulated or with fish where the respiratory function and the elimination functions are separated (e.g. Kleinow, 1991; McKim and Goeden, 1982; Black and McCarthy, 1988).

The elimination of contaminants by invertebrates is generally only studied by examination of the whole body rate processes. The rate of compound elimination decreases with increasing compound hydrophobicity, organism size, and organism lipid content (e.g. Landrum et al., 2001, 1998; Landrum, 1988; Landrum and Fisher, 1998). The route of elimination is not well studied and is thought to occur either across the respiratory membrane in the same manner as fish when the chemical activity in the organism exceeds that in the water (Landrum and Stubblefield, 1991), or via fecal elimination, when substrate (food) is ingested (Landrum, 1982, 1988). The importance of fecal elimination was hypothesized because the elimination rate was higher in the presence of sediment than in water-only studies with amphipods (Landrum and Scavia, 1983; Landrum, 1982) and oligochaetes (Kukkonen and Landrum, 1994). In addition, it was hypothesized that feeding and the formation of a peritrophic membrane was critical to this elimination process for the amphipod *Diporeia* spp. (Landrum, 1988), as was observed for mosquito larvae in the elimination of DDT (Abedi and Brown, 1961). Oligochaetes do not form such peritrophic membranes, and the enhanced elimination in sediment was thought to result from simple sorption to material passing through the gut. The sediment-egestion route for elimination was thought to be particularly relevant for *Diporeia* spp. since these amphipods do not measurably biotransform polycyclic aromatic

hydrocarbons or other non-polar contaminants (Landrum et al., 1994, 1984; Landrum, 1988) into forms that might be more water-soluble and thus more readily eliminated to the aqueous phase. It was hypothesized that the parent contaminant would sorb to the fecal material and the peritrophic membrane, enhancing its elimination (Landrum, 1988). The concept of elimination via sediment egestion was incorporated into a model for the accumulation of contaminants from sediments, which reasonably and accurately described the net accumulation from a sediment matrix (Landrum and Robbins, 1990). However, the influence of feeding and subsequent egestion on elimination had not been directly tested.

The objective of this study was to directly examine the role of fecal elimination and other factors that influence PAH elimination in *Diporeia* spp. by comparing elimination in the presence of differing substrates and in water with no substrate.

2. Material and methods

2.1. Experimental organisms

Diporeia spp. were collected from Lake Michigan approximately 3 miles southwest of Grand Haven, MI at a depth of 23–27 m in early April and late May 1997. Amphipods were placed in aquaria containing 2–3 cm of Lake Michigan sediment and 10 cm of overlying water and maintained at 4 °C for 3 weeks. Animals passing through a 2-mm mesh and retained on a 1-mm mesh were used in the experiments. Lipid content was measured prior to experimental use in 12 amphipods using a microgravimetric method (Gardner et al., 1985).

2.2. Radiolabeled chemicals

The chemicals used were ^{3}H-benzo[*a*]pyrene (BaP, 50 Ci mmol^{-1}), purchased from Amersham International (London, England), ^{3}H-chrysene (CHR, 0.34 Ci mmol^{-1}) from ChemSyn (Lenexa, KS), and ^{14}C-fluoranthene (FLU, 0.045 Ci mmol^{-1}), and ^{14}C-phenanthrene (PHE, 0.013 Ci

mmol^{-1}), purchased from Sigma Chemical (St. Louis, MO, USA). Radiopurity, determined using a combination of thin layer chromatography and liquid scintillation counting (LSC) (Landrum, 1988), was > 98% for all compounds.

2.3. Uptake exposure

For each experiment, 150 amphipods were exposed to aqueous solutions containing select PAH for bioaccumulation. Exposure water was prepared by adding trace amounts of binary mixtures of PAH congeners to filtered Lake Michigan water. FLU and BaP or PHE and CHR were added to 1-l of water in 0.2 ml of acetone. To determine the compound concentrations, 1 ml of exposure water was transferred to 12 ml of scintillation cocktail (3a70b, Research Products International, IL, USA) and simultaneously assayed for ^{14}C and ^{3}H activities by LSC on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR, Packard Instrument, Meridien, CT, USA). Final compound concentrations were 0.0002 μM (11.91 μCi l^{-1}) for ^{3}H-BaP, 0.57 μM (2.57 μCi l^{-1}) for ^{14}C-FLU, 0.69 μM (22.34 μCi l^{-1}) for ^{3}H-CHR, and 0.31 μM (4.06 μCi l^{-1}) for ^{14}C-PHE. Spiked water and amphipods were transferred to a glass bottle closed with a glass stopper and maintained at 4 °C. The bottle was filled to the top to eliminate any air space between the water and the glass stopper, therefore, preventing amphipods from getting caught in the surface tension of the water. After 72 h, amphipods were retrieved from the spiked water by sieving and transferred to clean (uncontaminated) media for elimination. Immediately following exposure, 20 amphipods were individually weighed and transferred to scintillation cocktail and, after allowing for compound extraction by the scintillation cocktail and subsidence of chemical luminescence (24 h), were assayed for ^{14}C and ^{3}H activities by LSC. Remaining amphipods were transferred to elimination chambers.

2.4. Elimination experiments

The PAH-exposed amphipods were maintained in uncontaminated media for the elimination of the bioaccumulated compounds for a period of 15 days (BaP/FLU experiment) or 10 days (CHR/ PHE experiment). The elimination of PAH was examined using four different substrate treatments: sediment, clay, silica, or no substrate (water treatment). Elimination chambers were prepared in 50-ml glass centrifuge tubes containing 35 ml of filtered Lake Michigan water. For the sediment treatment, Lake Michigan sediment was collected from a 45-m deep station (43.03′N, 86.37′W) and press sieved to yield a < 20 μm fraction. For the clay treatment, Lake Michigan red clay (Wickham et al., 1978) was collected from a 207-m deep station (44.77′N, 86.84′W) and was used without any manipulation. The total organic carbon content, determined on a model 2400 CHN Elemental Analyzer (Perkin-Elmer Corp., Norwalk, CT, USA) after acidification to remove carbonates, was 1.37% for the < 20 μm sediment and 0.21% for the clay. The volume-to-dry weight ratio of sediment and clay was determined, and a wet amount corresponding to 1 g dry weight was added with minimal disturbance to replicate chambers. For the silica treatment, replicate chambers received 1 g of adsorbosil silica (10 μm diameter, Alltech, Dearfield, IL, USA). Chambers for the water treatment did not receive any substrate.

Elimination chambers were placed in plastic racks and stored for 48 h at 4 °C to allow for the substrate to settle and temperature equilibration before the amphipods were added. Amphipods were transferred to elimination chambers following their exposure to aqueous solutions of PAH. Each chamber received a single amphipod, and 20 chambers were used for each substrate treatment for each PAH binary combination. Chambers were left uncapped and maintained in an environmental chamber with no illumination at a constant temperature of 4 °C for the duration of the elimination period. The overlying water was not renewed, and no external food was provided.

At termination of the elimination period, the contents of the chambers were retrieved. An aliquot of the overlying water (1 ml) was sampled for radioactivity determination. All the remaining water was removed using a transfer pipette, with minimal disturbance of the underlying substrate, and its volume determined. To remove the sub-

strate, 30 ml of clean water was added to each chamber and mixed with the substrate by vigorous shaking. The volume of the sediment slurry was determined, and a 1-ml aliquot was assayed for radioactivity determination. The remaining slurry was poured over a sieve (63-μm mesh) that retained the amphipod and their fecal pellets. Each recovered amphipod was rinsed in clean water, blotted dry, weighed and assayed for radioactivity. Fecal pellets retained in the sieve were transferred to a petri dish and transferred to a pre-weighed Nytex mesh placed on paper towel that absorbed the excess water. After assuring that the mesh pores did not contain any water, the mesh and fecal pellets were weighed and assayed for radioactivity.

2.5. Calculations

At initiation of the elimination period, all the radiolabel in each chamber was in the amphipod tissue. The amount of radiolabel in amphipods at initiation of the elimination experiment could not be measured directly because amphipods must be sacrificed for LSC analysis. Therefore, the initial amount of radiolabel was estimated for each amphipod by multiplying its biomass (g wet weight), measured at experiment termination, by the mean initial radiolabel concentration (dpm g^{-1}) measured using a subgroup of PAH-exposed amphipods. The difference between the estimated initial amphipod radioactivity and the final amphipod radioactivity, measured at termination of the elimination period, corresponded to the estimated amount of radiolabel eliminated. The fraction eliminated (percent elimination) was estimated for each amphipod as follows:

$$\text{Percent elimination} = \frac{\text{estimated initial radioactivity (dpm)} - \text{measured final radioactivity (dpm)}}{\text{estimated initial radioactivity (dpm)}} \times 100 \quad (1)$$

During the elimination period, a portion of the bioaccumulated radiolabel was eliminated by the amphipods across the integument and via feces, when feeding occurred. At experiment termination, non-eliminated radiolabel was present in the amphipods, and eliminated radiolabel was present in the surrounding media (sediment and overlying water) and in the fecal pellets. The sum of the total radioactivity in the water, substrate, and in the fecal pellets, measured for each chamber, corresponded to the recovered eliminated radiolabel. The fraction of recovered eliminated radiolabel plus the final amount of radiolabel in amphipods (non-eliminated radiolabel) comprised the total recovered radiolabel. Since the overlying water interfaced with the atmosphere, volatilization of dissolved, eliminated radiolabel was expected, but was not measured. This loss to the atmosphere was estimated from the difference between the estimated total radiolabel in the amphipod at experiment initiation and the total recovered radiolabel.

The biomass of fecal pellets from the sediment and clay treatments was used in the calculation of amphipod egestion rate (mg g^{-1} per day) as follows:

$$\text{Egestion rate} = \frac{\text{fecal pellets biomass (mg)/amphipod biomass (g)}}{\text{Experiment duration (day)}} \quad (2)$$

Radiolabel elimination was assumed to be a first-order decay process for the calculation of elimination rate constant (k_e) estimates (h^{-1}) for each compound using the mean initial and the mean measured final radioactivity:

$$k_e = \frac{\ln[\text{initial radioactivity concentration (dpm g}^{-1}\text{)}] - \ln[\text{final radioactivity concentration (g)}]}{\text{Elimination period (h)}} \quad (3)$$

2.6. Statistical analysis

All measurements are expressed as mean ± S.D. One-way analysis of variance (ANOVA) was used to compare means. Tukey's honestly significant difference test was used for pairwise comparisons among means. Untransformed data met the requirement for homogeneity of variances. Linear or non-linear regressions were performed to establish the relationship between two continuous variables. The significance level (α) was set at 0.05. Analyses were performed using Sigma Stat Ver-

sion 3.0 and Sigma Plot Version 5.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Estimated initial radiolabel concentrations and lipid contents

In both experiments, amphipod biomass was relatively uniform, as indicated by the low coefficient of variation (CV) (15–17%) for wet weight (Table 1). Mean amphipod biomass was significantly higher in the CHR/PHE experiment than in the BaP/FLU experiment (Table 1). The initial radiolabel concentration (dpm mg^{-1}), determined simultaneously for ^{3}H and ^{14}C, was relatively uniform among individuals for all radiolabeled compounds, as indicated by the low CV (11–30%) (Table 1). The mean lipid content in amphipods at experiment initiation was significantly higher in the CHR/PHE experiment (0.194 ± 0.058 g lipids g^{-1} dry weight, $n = 12$) than in the BaP/FLU experiment (0.100 ± 0.038 g lipids g^{-1} dry weight, $n = 12$). Amphipods used in the CHR/PHE experiment were collected later in the spring, when lipid content in *Diporeia* spp. from Lake Michigan is expected to increase following a seasonal diatom bloom (Cavaletto et al., 1996).

3.2. Radiolabel elimination

Radiolabel elimination was estimated as the difference between the amount of radiolabel mea-

sured in amphipods at experiment termination and the estimated amount in the amphipod calculated using the mean initial radiolabel concentration Eq. (1). Since earlier experiments demonstrated little or no measurable capability for *Diporeia* spp. to biotransform PAH out to an exposure of 56 days (Landrum et al., 1994, 1984; Landrum, 1988), all chemicals in the amphipods, water, and solid substrates were assumed to be parent compound. Further, single point elimination assumes a first order elimination process. This is a reasonable assumption for *Diporeia* based on multiple studies of elimination by this organism (reviewed in Landrum and Nalepa, 1998) that have all demonstrated first order elimination. The measured amount at experiment termination was higher than the estimated initial amount in some replicates in the water treatments for CHR. Thus, the calculated average percent elimination of CHR was negative for *Diporeia* spp. in the water only elimination. However, some radioactivity associated with CHR was measured in the water in all replicates, indicating that CHR elimination occurred in all replicates where the calculated elimination was negative. The requirement of using a mean value to represent the pre-elimination condition can result in an underestimate of the actual bioaccumulation for some organisms, which is particularly evident where the elimination is very low. Percent elimination in the CHR water treatment calculated using the difference between the total recovered radioactivity and the measured final radioactivity in the amphipods was 5.6%.

Table 1
Average (Avg), standard deviation (Std), and coefficient of variation (CV) of biomass and radioactivity concentration in amphipods at initiation of the elimination exposure

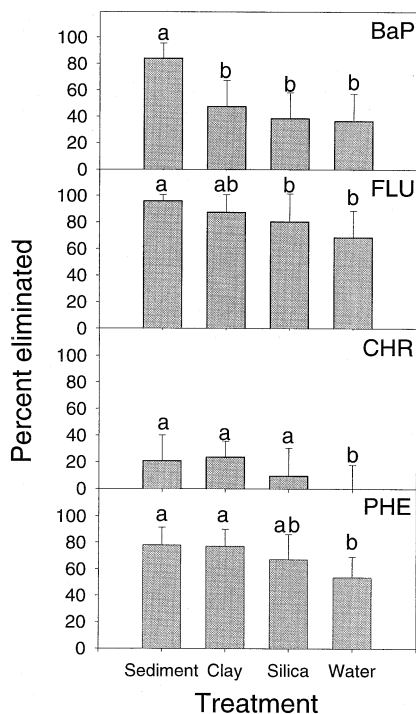| Experiment		Wet weight (mg)	Radioactivity concentration (dpm mg^{-1})		Ratio
			^{3}H	^{14}C	^{3}H/^{14}C
^{3}H-BaP/^{14}C-FLU	Avg	3.11	56 907	13 495	4.3
	Std	0.54	5993	2.377	
	CV	17%	11%	18%	
^{3}H-CHR/^{14}C-PHE	Avg	4.64	10 550	2887	3.9
	Std	0.69	2418	852	
	CV	15%	23%	30%	

Fig. 1. *Diporeia* spp. elimination experiments. Percent of the estimated initial amount of radiolabel that was eliminated in different substrate treatments. BaP, benzo(*a*)pyrene; FLU, fluoranthene; CHR, chrysene; PHE, phenanthrene. Different letters indicate significant differences among treatments.

Percent elimination was generally higher in substrate treatments than in the water treatment for all PAH congeners (Fig. 1). Radiolabel elimination was significantly higher in the sediment treatment (1.36% organic carbon) compared with the water treatment for all compounds, but for the clay treatment (0.21% organic carbon) only CHR and PHE elimination was significantly greater than the only water treatment. Elimination in the silica treatment was only significantly higher than in the water treatment for CHR. Elimination in the sediment treatment was only significantly higher than in the clay treatment for BaP. The fraction of eliminated radiolabel was greatest for FLU (67–96%), followed by PHE (54–78%) and BaP (37–84%), and was lowest for CHR (6–28%) (Fig. 1). Wide ranges in the fraction eliminated are observed because of the large variation among the treatments. Since the duration of the elimination period for the CHR/PHE experiment (10 days) was different than the duration of the BaP/FLU experiment (15 days), comparisons of the overall mass of compound eliminated can be made within experiments and not across experiments. Elimination rate constant estimates, which account for the elimination period, were highest for FLU ($0.0032–0.0089$ h^{-1}), followed by PHE ($0.0047–0.0063$ h^{-1}) and BaP ($0.0013–0.0051$ h^{-1}), and lowest for CHR ($0.0002–0.0013$ h^{-1}) (Table 2). The rates of elimination were similar to earlier studies that used much larger systems (Landrum, 1995); thus, there was likely little recycling of the eliminated compound.

3.3. Radiolabel mass balance

The amount of total recovered radiolabel was typically lower than the estimated amount of initial radiolabel, suggesting that a fraction of the eliminated radiolabel was lost by volatilization. The percent radiolabel recovery (total recovered

Table 2
Elimination rate constants (h^{-1}) estimated for each substrate treatment in the elimination experiment and the octanol-water partitioning coefficient for different PAH congeners

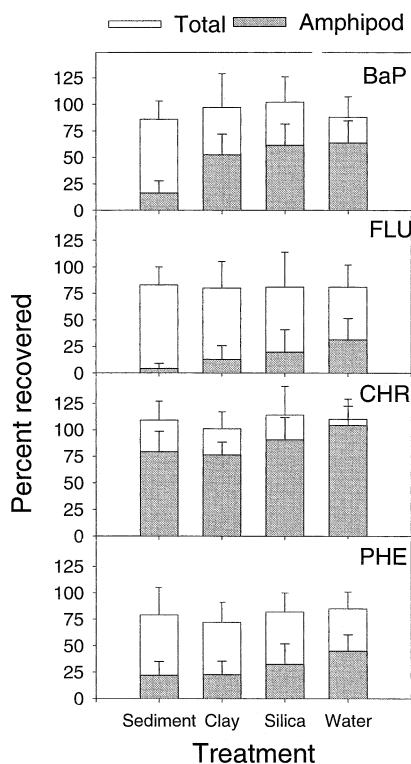| Compound	Substrate treatment				Log Kow[a]
	Sediment	Clay	Silica	Water	
Benzo[*a*]pyrene	0.0051	0.0018	0.0014	0.0013	5.98
Chrysene	0.0013	0.0012	0.0009	0.0002	5.79
Fluoranthene	0.0089	0.0057	0.0044	0.0032	5.2
Phenanthrene	0.0063	0.0062	0.0047	0.0032	4.57

[a] Miller et al., 1985.

Fig. 2. *Diporeia* spp. elimination experiments. Percent of the estimated initial amount of radiolabel recovered at termination of the elimination experiments from the surrounding media (solid substrate and water), fecal pellets and amphipods (total) and in amphipods alone (amphipod) in different substrates treatments. BaP, benzo(a)pyrene; FLU, fluoranthene; CHR, chrysene; PHE, phenanthrene.

radiolabel/estimated initial radiolabel × 100) was calculated for each replicate. The mean recovery ranged from 86 to 102% for BaP, 101 to 114% for CHR, 80 to 83% for FLU and 72 to 85% for PHE (Fig. 2). Therefore, the fraction of the eliminated radiolabel that was lost by volatilization (100% − mean percent radiolabel recovery) was highest for FLU and PHE (Fig. 2). The mean recovery of the radiolabel was greater than 100% for the clay treatment with BaP and all treatments with CHR. This indicates that the initial radiolabel concentration, estimated in *Diporeia* from a subset of exposed amphipods when initiating the elimination, may have been lower than the actual mean for the subset used for the elimination study. No significant difference in radiolabel re-

covery was observed among substrate treatments for any PAH congener. At the end of the elimination period, most of the radiolabel was in the surrounding media and fecal pellets in all treatments for FLU, in the sediment treatment for BaP, and in all treatments except for water for PHE (Fig. 2). For CHR, most of the radiolabel remained in the amphipods in all treatments (Fig. 2).

While most of the recovered eliminated radiolabel was present in the media surrounding the amphipod, i.e., substrate and overlying water, relatively smaller amounts were present in fecal pellets (Fig. 3). The fate of eliminated compounds was examined by comparing the fraction of radio-
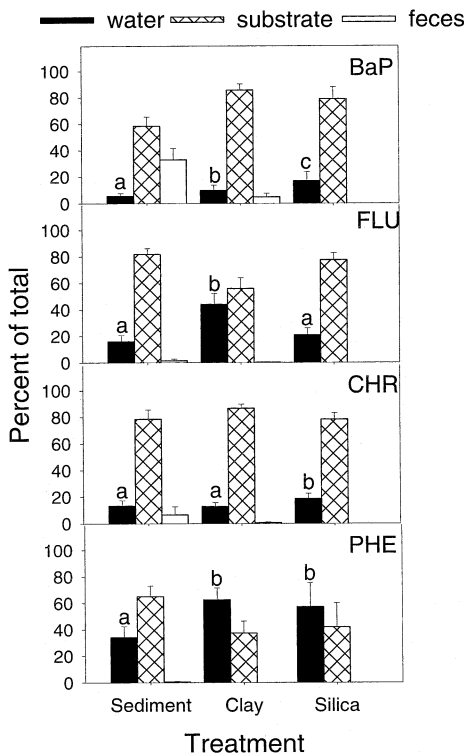

Fig. 3. *Diporeia* spp. elimination experiments. Percent of the amount of eliminated radiolabel that was recovered at termination of the elimination experiments in different compartments in different substrate treatments. BaP, benzo(*a*)pyrene; FLU, fluoranthene; CHR, chrysene; PHE, phenanthrene. Different letters indicate significant differences in fraction present in the water compartment among treatments.

label recovered from the solid phase (sediment including porewater + fecal pellets) with that found in the overlying water. It was possible to statistically compare the relative amount of radiolabel in each phase because the amount of water and substrate was the same in all replicates. The fraction of eliminated radiolabel present in the water was significantly lower than the fraction present in the solid phase in all solid substrate treatments for BaP, FLU, and CHR and the sediment treatment for PHE (Fig. 3). In contrast, the fraction of eliminated radiolabel was higher in the water than in the solid phase for the PHE clay and silica treatments (Fig. 3). The fraction of eliminated radiolabel present in the overlying water was also compared among substrate treatments for each compound. The fraction present in the water was significantly lower in the sediment treatment than in the clay treatment for all compounds except for CHR. The fraction present in the water was significantly lower in the sediment treatment than in the silica treatment for all compounds except for FLU. Within experiments (BaP/FLU or CHR/FLU), the relative amount of radioactivity in the water was significantly higher for FLU than for BaP and for PHE than for CHR in all solid phase substrate treatments (Fig. 3), reflecting the greater water solubility of FLU and PHE compared with BaP and CHR, respectively, (Mackay et al., 1992).

Fecal pellets were present in the sediment and clay treatments but not in the silica treatment in both experiments, indicating that amphipods ingested sediment and clay but not silica. Overall, the fraction of the estimated initial amount of compound eliminated via fecal pellets was larger for the sediment (0.4–32.9%) than for the clay treatment (0–4.9%) across PAH congeners (Fig. 3). The relative contribution of fecal pellets to the total recovered eliminated radioactivity was highest for BaP (32.9 and 4.9% for sediment and clay, respectively) followed by CHR (6.5 and 0.6% for sediment and clay, respectively), FLU (1.5 and 0.2% for sediment and clay, respectively), and PHE (0.4 and 0% for sediment and clay, respectively) (Fig. 3). It was assumed that the radiolabel measured in the fecal pellets at termination of the exposure corresponded to the amount of radiolabel eliminated via egestion, i.e. the loss of PAHs from the egested fecal pellets upon egestion was negligible. This assumption is based on the characteristics of the fecal pellets, which are encased in a peritrophic membrane and appeared to remain intact during the study. While the loss of PAH from fecal pellets was not measured, desorption loss from the fecal pellets of the marine species *Capitella* was reported to be low 4–5% in 60 days (Horng and Taghon, 2001). Thus, the compaction of particles in fecal pellets appears to reduce the exchange with water. The loss rates from *Diporeia* fecal pellets are not expected to be greater than those for *Capitella*. Thus, desorption should have minimal impact on the results. However, if desorption loss occurred, the estimates for the contribution of fecal egestion to the total elimination would be underestimated.

3.4. Effects of feeding rate on radiolabel elimination

The relationships between egestion rate, a surrogate for feeding rate, and radiolabel elimination were examined for the sediment and clay treatments. For sediment, the mean egestion rates in the BaP/FLU experiment (51.3 ± 38.2 mg g^{-1} per day) and in the CHR/PHE (67.0 ± 78.1 mg g^{-1} per day) were not significantly different. The egestion rate in clay treatments was lower than in sediment treatments and was significantly higher in the CHR/PHE experiment (22.9 ± 9.1 mg g^{-1} per day) than in the BaP/FLU experiment (14.0 ± 8.4 mg g^{-1} per day).

The relationship between feeding rate and compound elimination was examined in sediment treatments. The fraction of the estimated initial radiolabel that was eliminated via fecal pellets (fecal pellets radioactivity/estimated initial radioactivity) increased exponentially to a plateau with increasing egestion rate for BaP (Fig. 4, $r^2 = 0.78$) and increased linearly for the other PAH congeners (FLU, $r^2 = 0.48$; CHR, $r^2 = 0.87$; PHE, $r^2 = 0.86$, data not shown), indicating that the contribution of the egestion route for PAH elimination was proportional to the feeding rate. The likely reason for the difference in models
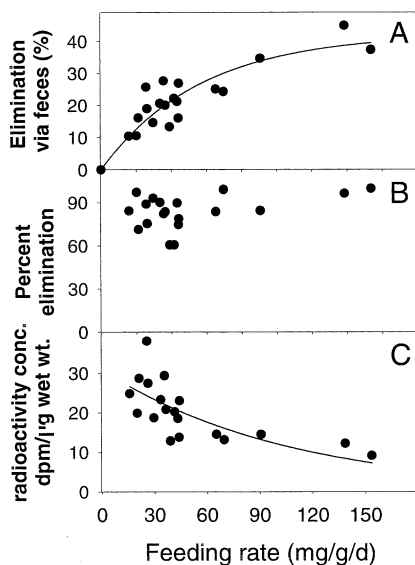
Fig. 4. *Diporeia* spp. benzo(*a*)pyrene elimination experiment. Fraction of estimated total radiolabel eliminated via fecal pellets (A); percent of the estimated initial amount of radiolabel that was eliminated (B); and radioactivity concentration in fecal pellets (C) as a function of feeding rate in the sediment treatment.

between BaP and the other PAH is that the fraction eliminated via the fecal route was small for all PAH except BaP. It is expected that there should be a plateau in the fraction eliminated through the fecal route for all compounds but for the compounds where the fraction is small the relationship still appears linear. However, when the fraction becomes larger, the route likely becomes saturated from the combination of rate of fecal material throughput and the rate of movement of the compound for sorption to the fecal material. Despite the large fraction of BaP eliminated through feces, the feeding rate did not influence overall compound elimination, as the regression of percent elimination versus egestion rate was not significant for BaP (Fig. 4, $P = 0.08$) or for the other PAH congeners ($P > 0.08$) where the fecal elimination was of lower importance. The radiolabel concentration in the fecal pellets decreased exponentially with feeding rate for BaP (Fig. 4, $r^2 = 0.50$), but not for the other PAH congeners ($P > 0.07$) where the fraction eliminated was much smaller.

4. Discussion

Different routes are expected to be involved in the elimination of unmetabolized PAHs by invertebrates. Loss via the respiratory membranes (Landrum and Stubblefield, 1991) and fecal pellet egestion (Landrum, 1982, 1988) are thought to occur. While contaminants have earlier been measured in the fecal pellets of *Diporeia* spp. (Lydy and Landrum, 1993; Harkey et al., 1994), the measurements were made as a part of bioaccumulation experiments and could not be specifically attributed to elimination. The present study provides the first direct measurement of eliminated compound in fecal pellets for *Diporeia* spp.

We employed a mass balance approach to examine the fate of eliminated PAH by *Diporeia* spp. using radioactivity as the surrogate for chemical concentration. The experimental design allowed eliminated PAH to be recovered and quantified in the surrounding media and fecal pellets. The material in the water and on the substrate particles were assumed to be eliminated across the respiratory membranes while the material associated with the fecal pellets was assumed to be eliminated through the egestion process. Therefore, by comparing the amount of compound eliminated via each route, direct examination of the relative importance of egestion in the elimination process could be determined for *Diporeia* spp.

4.1. Fate of eliminated compounds

Recovery of the radioactivity based on the initial amount in the amphipods ranged from 72 to 114% (Fig. 2). Most of the radiolabel eliminated by *Diporeia* spp. was present in the media surrounding the amphipods, water and non-ingested sediment particles, at the end of the elimination period. The fraction of the initial compound, which was eliminated and lost to the atmosphere did not exceed 20% among all the PAH congeners. Compound volatilization was considerably higher for PHE and FLU, as expected due to their lower sorptive characteristics as represented by the lower log Kow values and higher vapor pressure relative to BaP and CHR (Mackay et al., 1992).

In treatments where substrates were present, most of the eliminated radiolabel was sequestered on the solid phase compared with the overlying water (Fig. 3). The compound concentration was much higher in the solid phase than in the overlying water in most solid substrate treatments except for phenanthrene where the sorption capacity of the clay and silica was low enough that the amount in the water was about equal with that on the substrate. Thus, the solid substrate is the major repository for eliminated hydrophobic compounds. This was particularly the case for sediment. The fractions found in the various substrates were likely not influenced by the system characteristics since the capacity of the system was generally far greater than amount of compound added via the organisms. The minimal capacity of the water alone was 5-fold greater than the amount of CHR added. However, this is not to say that the exact proportions in the distribution between water and sediment observed in these will be the same in the natural environment. The critical point is that the solid phase assists in maintaining a gradient for the elimination that exceeds what was observed in the water-only case. Since *Diporeia* reside in the sediment and rarely venture into the water column, the volume of water available for elimination may well be lower than exists in these experiments and the role of sediment sorption may be greater. However, it is clear that the elimination rates in the presence of sediment reasonably represent the elimination in the natural environment since the values measured here are similar to those measured earlier and used to obtain reasonable predictions of *Diporeia* concentrations in the natural environment (Landrum et al., 1992).

4.2. Differences among PAH congeners

Percent elimination was higher for FLU than BaP and for PHE than for CHR (Fig. 1). Due to the different durations of the elimination experiments, comparisons among all PAH congeners are best assessed using elimination rate constants (Table 2) rather than total percent elimination, which increases with time. The elimination rate constants determined in the presence of sediment

for this experiment are similar to those found for the same compounds in earlier experiments (Landrum, 1988) and the very low values in the water only elimination are also similar to earlier findings (Landrum, 1982). Since the media accumulated compound over the course of the study some re-absorption of the compound could have occurred resulting in lower overall rates. This might have been particularly true for the water-only exposure. However, this design still allows comparison between treatments even if the absolute rates are somewhat reduced. Overall, elimination rates were highest for the PHE and FLU, the congeners with the lowest log K_{ow} values. This is expected since elimination rates have been shown to be inversely proportional to the compound hydrophobicity in experiments where several lipophilic compounds were investigated (Landrum, 1988; Lydy et al., 1992).

4.3. Influence of substrate on PAH elimination

The influence of substrate quality on PAH elimination was compound-specific (Fig. 1). Elimination in the three solid substrate treatments was similar for all PAH congeners, except for BaP. Sediment substantially promoted higher BaP elimination relative to the other substrates. Such higher positive impact of sediment relative to the other substrates is related to the presence of elevated amounts of BaP in fecal pellets in the sediment treatment, which was not observed with the other congeners.

The elimination of the four different PAH congeners in *Diporeia* spp. was significantly higher in the presence of sediment than in water only. Increased PAH elimination in the presence of sediment has been earlier observed for *Diporeia* spp. (Landrum, 1982), and the amphipod *Hyalella azteca* (Landrum and Scavia, 1983), the insect larvae *Chironomus riparius* (Leversee et al., 1982) and the oligochaete *Lumbriculus variegatus* (Kukkonen and Landrum, 1994). Similar enhancement of elimination by the presence of sediment was also observed for other lipophilic compounds using *C. riparius* (Lydy et al., 1992). Lake Michigan red clay had a lesser impact on the PAH elimination than Lake Michigan sediment.

Relative to water, the influence of clay on elimination was significant only for chrysene and phenanthrene. Further, the elimination of BaP in clay was significantly lower than in sediment and not different from water. The relative difference between the influence of the sediment and the clay reflects the difference in the sorption capacity of the two substrates due largely to the organic carbon content, which is much greater in the sediment. To further exaggerate the difference between the two materials, *Diporeia* spp. are known selective feeders and preferably ingest fine particles that are high in organic carbon (Harkey et al., 1994) thus enhancing the sorptive capacity of the ingested material. The influence of the sediment organic carbon content is confirmed by studies with the midge where the elimination of lipophilic organic compounds increased with the increasing organic carbon content of different sediments (Lydy et al., 1992). The presence of an inorganic substrate (silica gel) promoted significantly higher elimination relative to water for CHR but not for the other three PAH. Therefore, surrounding substrates that lack of organic carbon and are not used as a food source may also enhance PAH elimination. The sorption capacity of the solid substrates around the amphipods likely enhanced the passive diffusion of PAH across respiratory surfaces of *Diporeia* spp. by sequestering the eliminated material and keeping the dissolved concentration very low forcing a chemical activity gradient between the water and the organism. Thus, the greater the sorption capacity of the surrounding media the better it will serve to enhance elimination. Overall, before considering elimination through the egestion route, the more sorptive sediment substrate with the high organic carbon content best promotes the elimination of PAH even without egestion by sequestering the eliminated compound.

4.4. Influence of feeding on PAH elimination

The feeding rates, as determined by rates of egestion, of *Diporeia* spp. in Lake Michigan sediment were similar to those earlier observed in similar sediment (Harkey et al., 1994; Kukkonen and Landrum, 1995). Feeding rates in red clay for

Diporeia spp. were lower than those for sediment. This is not unexpected since *Diporeia* spp. selectively ingests particles organic carbon rich particles (Harkey et al., 1994). Thus, it is not surprising that *Diporeia spp*. did not ingest the silica gel, a non-naturally occurring inorganic substrate.

The influence of the egestion on the overall elimination was low. The greatest extent of elimination via the fecal route was about 40% of the eliminated material in the sediment substrate for BaP (Fig. 4). In the case of BaP, increases in the feeding rate increase the fraction of compound eliminated via egestion up to a plateau (Fig. 4) but did not influence the overall elimination of BaP. This appears to occur because the concentration on the egested sediment declines with increasing feeding rate. This is likely the result of a shorter residence time in the gut as the feeding rate increases. Thus, for the highly sorbed BaP, fecal elimination is an important elimination route although it accounts for generally less than 30% of the overall elimination. In all other cases, the percentage eliminated via the fecal route is so low as to be only a minimal influence on the overall elimination process. Thus, earlier hypothesis that the egestion route was the dominant route of elimination for organic contaminants from *Diporeia* spp. (e.g. Landrum, 1982) is not accurate. This work clearly demonstrates that the influence of the solid substrate on the elimination process is dominated by maintaining a chemical activity gradient between the organism and the water by acting as a partitioning media. In the absence of such a mechanism, the water concentration around the organism would be much greater. The capacity of the water is substantially lower for hydrophobic compounds, thus the rate of elimination would be substantially reduced as is evident when comparing the elimination in water only versus that in the presence of substrate. This work also demonstrates that the role of the peritrophic membrane is minimal in the elimination of non-polar organic contaminants for *Diporeia* spp., as the compound was not largely associated with the fecal material. It may be that the peritrophic membrane does contribute to the elimination of the highly sorptive BaP in a man-

ner similar to the elimination of DDT by the mosquito larvae (Abedi and Brown, 1961) since both compounds have similar large log Kow values. But apparently, it requires that the compound be very hydrophobic for this route to reach significant proportions in the elimination process.

The question can be asked about whether these same elimination processes will hold when the substrate is contaminated and serves as a source of the contaminant to the benthic invertebrate. This experiment was not designed to address that issue because the experiment was designed to test the earlier hypothesis that the egestion route was dominant. It may be possible that the egestion route will have more influence in the case where the sediment is a dominant source because the water concentration may be higher due to desorption off particles thus reducing the chemical activity gradient between the organism and the media. However, the sorption capacity of particles is generally large, and desorption rates are slow relative to the rate of adsorption so that the overall elimination processes may well remain the same. Additional work will be required to confirm that the route of elimination remains essentially passive diffusion in a contaminated environment, but this is likely the case.

References

Abedi, Z.H., Brown, A.W.A., 1961. Peritrophic membrane as a vehicle for DDT and DDE excretion in *Ades aegypti* larvae. Ann. Ent. Soc. Am. 54, 539–542.

Black, M.C., McCarthy, J.F., 1988. Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants in rainbow trout (*Salmo gairdneri*). Environ. Toxicol. Chem. 7, 593–600.

Cavaletto, J.F., Nalepa, T.F., Dermott, R., Gardner, W.S., Quigley, M.A., Lang, G.L., 1996. Seasonal variation of lipid composition, weight and length in juvenile *Diporeia* spp. (Amphipoda) from Lakes Michigan and Ontario. Can. J. Fish Aquat. Sci. 53, 2044–2051.

Gardner, W.S., Frez, W.A., Chichocki, E.A., Parish, C.C., 1985. Micromethod for lipids in aquatic invertebrates. Limnol. Oceanogr. 30, 1099–1105.

Gobas, F.A.P.C., Clark, K.E., Shiu, W.Y., Mackay, D., 1989. Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: role of bioavailability and elimination into feces. Environ. Toxicol. Chem. 8, 231–245.

Harkey, G.A., Lydy, M.J., Kukkonen, J., Landrum, P.F., 1994. Feeding selectivity and assimilation of PAH and PCB in *Diporeia* spp. Environ. Toxicol. Chem. 13, 1445–1456.

Hayton, W.L., Schultz, I.R., 1991. Scaling bioconcentration and pharmacokinetic parameters for body size and environmental variables in fish. In: Mayes, M.A., Barron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment: Fourteenth Volume. American Society for Testing and Materials, Philadelphia, pp. 149–165 ASTM STP 1124.

Horng, C.-Y., Taghon, G.L., 2001. Loss rates for polycyclic hydrocarbons from sediment and deposit-feeder fecal pellets. Environ. Toxicol. Chem. 20, 1465–1473.

Kleinow, K.M., 1991. Experimental techniques for pharmacokinetic data collection in free swimming fish. In: Mayes, M.A., Barron, M.G. (Eds.), Aquatic Toxicology and Risk Assessment: Fourteenth Volume. American Society for Testing and Materials, Philadelphia, pp. 131–138 ASTM STP 1124.

Kukkonen, J., Landrum, P.F., 1994. Toxicokinetics and toxicity of sediment-associated pyrene to *Lumbriculus variegatus* (Oligochaeta). Environ. Toxicol. Chem. 13, 1457–1468.

Kukkonen, J., Landrum, P.F., 1995. Measuring assimilation efficiencies for sediment-bound PAH and PCB congeners by benthic organisms. Aquat. Toxicol. 32, 75–92.

Landrum, P.F., 1982. Uptake, depuration and biotransformation of anthracene by the scud, *Pontoporeia hoyi*. Chemosphere 11, 1049–1057.

Landrum, P.F., 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. Aquat. Toxicol. 12, 245–271.

Landrum, P.F., 1995. Toxicokinetics from aqueous and sediment exposures for *Diporeia* spp. NOAA Technical Memorandum ERL GLERL-87, Great Lakes Environmental Research Laboratory, Ann Arbor, MI.

Landrum, P.F., Scavia, D., 1983. Influence of sediment on anthracene uptake, elimination, and biotransformation by the amphipod, *Hyalella azteca*. Can. J. Fish Aquat. Sci. 40, 731–743.

Landrum, P.F., Robbins, J.A., 1990. Bioavalibility of sediment-associated contaminants to benthic invertebrates. In: Bando, R., Giesy, J.P. Jr, Muntau, H. (Eds.), Sediments: Chemistry And Toxicity of In-place Pollutants. Lewis Publishers, Inc, Ann Arbor, pp. 237–263.

Landrum, P.F., Stubblefield, C.R., 1991. Role of respiration in the accumulation of organic xenobiotics by the amphipod, *Diporeia* sp. Environ. Toxicol. Chem. 10, 1019–1028.

Landrum, P.F., Fisher, S.W., 1998. Influence of lipids on the bioaccumulation and trophic transfer of organic contaminants in aquatic organisms. In: Arts, M., Wainman, B. (Eds.), Lipids in Freshwater Ecosystems. Springer, New York, pp. 203–234.

Landrum, P.F., Nalepa, T.F., 1998. A review of the factors affecting the ecotoxicology of *Diporeia* spp. J. Great Lakes Res. 24, 889–904.

Landrum, P.F., Eadie, B.J., Faust, W.R., Morehead, N.R., McCormick, M.J., 1984. Role of sediment in the bioaccu-

mulation of benzo[a]pyrene by the amphipod *Pontoporeia hoyi*. In: Cooke, M.W., Dennis, A.J. (Eds.), Polynuclear Aromatic Hydrocarbons: Eighth International Symposium on Mechanisms, Methods and Metabolism. Battelle, Columbus, OH, pp. 799–812.

Landrum, P.F., Fontaine, T.D., Faust, W.R., Eadie, B.J., Lang, G.A., 1992. Modeling the accumulation of polycyclic aromatic hydrocarbons by the amphipod *Diporeia* spp. In: Gobas, F.A.C., McCorquodale, A. (Eds.), Chemical Dynamics in Freshwater Ecosystems. Lewis Publishers, Chelsea, MI, pp. 111–128.

Landrum, P.F., Dupuis, W.S., Kukkonen, J., 1994. Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia* spp.: examination of equilibrium-partitioning theory and residue-based effects for assessing hazard. Environ. Toxicol. Chem. 13, 1769–1780.

Landrum, P.F., Kane Driscoll, S., Tigue, E.A., Gossiaux, D., Gedeon, M.L., Adler, M., 1998. Toxicokinetics of polychlorinated biphenyl congeners by *Diporeia* spp.: Effects of temperature and organism size. NOAA technical Memorandum ERL GLERL-106, Great Lakes Environmental Research Laboratory, Ann Arbor, MI.

Landrum, P.F., Kane Driscoll, S., Tigue, E.A., Gossiaux, D., Gedeon, M.L., Adler, M., 2001. Toxicokinetics of polychlorinated biphenyl congeners by *Diporeia* spp.: effects of temperature and organism size. J. Great Lakes Res. 27, 117–133.

Leversee, G.L., Giesy, J.P., Landrum, P.F., Gerould, S., Fannin, T.E., Haddock, J.D., Bartell, S.M., 1982. Kinetics and biotransformation of benzo(a)pyrene in *Chironomus riparius*. Arch. Environ. Contam. Toxicol. 11, 25–31.

Lydy, M.J., Landrum, P.F., 1993. Assimilation efficiency for sediment sorbed benzo(a)pyrene by *Diporeia* spp. Aquat. Toxicol. 26, 209–224.

Lydy, M.J., Oris, J.T., Baumann, P.C., Fisher, S.W., 1992. Effects of sediment organic carbon content on the elimination rates of neutral lipophilic compounds in the midge (*Chironomus riparius*). Environ. Toxicol. Chem. 11, 347–356.

Mackay, D., Shiu, W.Y., Ma, K.C., 1992. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals, Volume II Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins and Dibenzoburans. Lewis Publishers, Boca Raton, FL, p. 597.

McKim, J.M., Goeden, H.M., 1982. A direct measure of the uptake efficiency of a xenobiotic chemical across the gills of brook trout (*Salvelinus fontinalis*) under normoxic and hypoxic conditions. Comp. Biochem. Physiol. 72C, 65–74.

Miller, M.M., Wasik, S.P., Huang, G.-L., Shiu, W.-Y., Mackay, D., 1985. Relationships between octanol-water partition coefficient and aqueous solubility. Environ. Sci. Technol. 19, 522–529.

Nichols, J.W., McKim, J.M., Lien, G.J., Hoffman, A.D., Bertelsen, S.L., 1991. Physiologically based toxicokinetic modeling of three waterborne chloroethanes in rainbow trout (*Oncorhynchius mykiss*). Toxicol. Appl. Pharm. 110, 374–389.

Wickham, J.T., Gross, D.L., Lineback, J.A., Thomas, R.L., 1978. Lake Quaternary Sediments of Lake Michigan. Studies of Lake Michigan Bottom Sediments, Number 13. Environmental Geological Notes Number 83. Illinois State Geological Survey, Urbana, IL.