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AN ASSESSMENT OF SEDIMENTS FROM THE UPPER MISSISSIPPI RIVER

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by

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ABSTRACT

The U.S. Geological Survey (USGS) has been monitoring the Upper Mississippi River (UMR) since 1987 to document the fate and transport of contaminats associated with sediments. The UMR is that part of the river upstream of the confluence with the Ohio River at Cairo, IL and consists of a series of 26 navigational pools created by a lock and dam system extending from Minneapolis, MN to St. Louis, MO. The navigational pools are shallow, lake-like areas which trap and store large quantities of fine-grained sediments during normal river flows. Concern with the redistribution of the river sediments arose after the flood of 1993. This project was designed to evaluate the current status of sediments in the UMR by: (1) measuring the concentrations of contaminants in sediments of the UMR, (2) evaluating the toxicity of sediments collected from the river, (3) determining the bioaccumulation of contaminants from UMR sediments using field-collected and laboratory exposed oligochaetes, and (4) determining the benthic community structure in fine-grain sediments within the river.

To conduct these assessments, sediment samples and benthic organisms were collected from 24 of the 26 navigational pools in the river and from one pool in the Saint Croix River. Two types of sediment samples were collected from the pools. One sediment sample was a composite of 15 to 20 sediment grabs along one to five transects across the downstream one-third of each pool (B samples). The other sediment sample was a composite of grabs from one station on one transect within each pool (C samples). The latter stations were selected based on historical chemistry data and the potential to collect oligochaetes. Samples were not collected from the main navigation channels. Chapter 1 of this report describes whole-sediment toxicity tests which were conducted for 28 days with the amphipod *Hyalella azteca*. Survival, growth and sexual maturation were the measurement endpoints. Toxicity tests were conducted with both the B and C sediment samples. Chapter 2 describes the bioaccumulation of contaminants from sediments using field-collected oligochaetes and 28-day bioaccumulation studies conducted in the laboratory with the oligochaete *Lumbriculus variegatus*. Bioaccumulation tests were conducted with 13 of the 24 C sediment samples. Chapter 3 assesses the benthic community in all 24 C samples. Using the Sediment Quality Triad approach, the status of UMR sediments was assessed by integrating sediment chemistry, laboratory toxicity tests and benthic community measurements.

In the toxicity tests, *Hyalella azteca* survival was significantly reduced in only one sediment sample (13B) relative to both a control and reference sediment. Growth of amphipods was also reduced in only one sediment sample (26C). Sexual maturation was not significantly reduced in any treatments. No correlations were observed between survival, growth or sexual maturation and any of the physical or chemical sediment characteristics. Using sediment chemistry and the Effect Range Median (ERM), 96% of the samples were classified as non-toxic (i.e. measured chemical concentrations rarely exceeded ERMs). Classifications using ERMs and sediment chemistry were consistent with the biological results from the *H. azteca* toxicity tests.

In the bioaccumulation tests, concentrations of contaminants were relatively low in native oligochaetes collected from the pools as well as in oligochaetes exposed to the sediments in the laboratory. Organochlorine pesticides were generally below detection in sediment and tissue samples. Only aliphatic and polycyclic aromatic hydrocarbons (PAHs) and total polychlorinated

biphenyls were frequently measured above detection limits in oligochaete tissue and sediment samples. Concentrations for a specific contaminant in laboratory-exposed and field-collected oligochaetes were similar within a station. About 90% of the paired PAH concentrations in laboratory-exposed and field-collected oligochaetes were within a factor of three of one another. With the detection limits used to analyze samples, contaminants were detected in tissue samples more often than in sediment samples. Concentrations of PAHs in oligochaetes collected from the pools or exposed in the laboratory to sediments from the UMR were up to 1000 times less than tissue concentrations measured in oligochaetes from highly-contaminated sites within the U.S. that our laboratory has previously studied.

The benthic community was dominated by oligochaetes and chironomids in 14 of the 23 sediment samples from the UMR and the one sediment sample from Saint Croix River. Fingernail clams comprised a large portion of the community in 3 of the samples and exceeded 1,000/m^2 in 5 of the samples. Total abundance values of invertebrates ranged from 250/m^2 (station 1C) to 22,389/m^2 (station 19C) and were comparable to previously reported values for the UMR. The frequency of chironomid mouthpart deformities was only 3% which is consistent with the incidence of mouthpart deformities from uncontaminated sediments. Correlations between benthic measures, sediment chemistry or other abiotic parameters exhibited few strong or significant correlations indicating benthic communities are most likely controlled by factors independent of contaminant concentrations.

The Sediment Quality Triad (Triad) is a weight-of-evidence approach used to assess the contamination of sediments by integrating sediment chemistry, laboratory toxicity testing and benthic community measures. Results from the Triad analysis indicated 88% of the samples were classified as not impacted based on sediment chemistry, laboratory toxicity and benthic measures. These results are consistent with the bioaccumulation study in which concentrations of contaminants in tissue were less than other U.S. sites that our laboratory has previously studied. In addition, pools in about the lower third of the river had lower sediment contaminant concentrations, less accumulation of contaminants in tissue, and greater taxa richness.

Sediments are often both a sink for water-borne contaminants and a source of contaminants to the overlying water. In addition, sediments may accumulate significant concentrations of contaminants even when water quality criteria are not exceeded. The results from the present study indicate that the UMR is not severely contaminated relative to other sites that have been studied in the U.S. Perturbations that may occur could be attributed to channelization, sedimentation from surface runoff or long term changes in the natural flow conditions of the river due to lock and dam construction. This study only conducted a partial assessment of the UMR sediments and included no assessment of river water. Further, this study was a one-time assessment that was conducted after a major flood event and does not evaluate temporal or spatial variability of sediment contamination within the pools. Future research on, or management of, the Upper Mississippi River should evaluate the limitations of this study.

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Introduction

The Mississippi River is the largest river system in the United States. Because of its location, the river receives contaminant inputs from a variety of industrialized and agricultural sources. The Upper Mississippi River (UMR), the stretch of river upstream from the confluence with the Ohio River at Cairo, IL, contains a series of 26 navigational pools created by a lock and dam system from St. Louis, MO to Minneapolis, MN (Rada *et al* 1990; Figure 1.1). These navigational pools are shallow lake-like areas which trap and store large quantities (1 to 4 cm/yr) of primarily fine-grained sediments during normal river flows (McHenry *et al* 1984; Nielsen *et al* 1984). Dredging activities, commercial navigation, recreational boating and natural resuspension processes can result in the remobilization of these sediments. Concern about the resuspension and transport of these sediments and the contaminants associated with them arose after the flood of 1993 (Moody and Meade 1995; Moody *et al* 1996).

The United States Geological Survey (USGS) has been monitoring the transport and degradation of pollutants in the UMR since the fall of 1987 (Moody and Meade 1995). Studies have monitored concentrations of contaminants in fish (Hora 1984; Wiener *et al* 1984), invertebrates (Beauvais *et al* 1995; Steingraeber and Wiener 1995), sediments (Bailey and Rada 1984; Wiener *et al* 1984; Rada *et al* 1990; Frazier *et al.* 1996; Ingersoll *et al* 1997) or a combination of the three (Peddicord *et al* 1980; Boyer 1984) in select pools in the UMR. However, little information was available on contaminant concentrations and toxicity in sediment samples throughout the entire pool system of the UMR.

Four studies were conducted to assess the nature and extent of sediment contamination in the navigational pools of the UMR: (1) contaminant concentrations were measured in sediments before and after the flood of 1993 (Moody *et al* 1996); (2) whole-sediment toxicity tests were conducted (this chapter); (3) whole-sediment bioaccumulation tests were conducted (i.e.; Chapter 2); and (4) benthic-community structure were evaluated (i.e.; Chapter 3). Sediment samples were collected from June 11th to July 5th, 1994 from pool 1 (near Minneapolis, MN) to pool 26 (near St. Louis, MO) of the UMR system (Figure 1.1). The objective of the study presented in this chapter was to assess the toxicity of sediments from navigational pools of the UMR system using 28-day toxicity tests with the amphipod *Hyalella azteca*, measuring for potential effects on survival, growth or sexual maturation.

Materials and Methods

Sample Collection, Handling, and Storage

Differential Global Positioning System (GPS) using a local reference was used to locate sampling stations in the upper pools (1-14) and the Saint Croix River. A differential GPS using the navigational beacon near St. Louis, MO., as the reference to locate sampling stations in the lower pools (15-26). A 3.5 composite sediment sample was collected from each of the 26 navigational pools (pool samples designated as "B" samples; Moody *et al* 1996). These composite samples of surface (upper 10 cm) sediments were collected using a van Veen grab from 15 to 20 stations along one to five transects (typically 3 to 5 stations/transect) from the downstream one-third of each navigation pool (except pool 17) in the UMR and from one site in the Saint Croix River (SC) just upstream from its confluence with the Mississippi River in Wisconsin (Figure 1.1; Moody *et al* 1996). Samples were not collected from the main navigation channel which was assumed to contain courser sediment that had been deposited for a short period of time. A 2-L subsample of the 3.5 L samples for toxicity testing and physical and chemical characterization were removed and placed in a 2-L high density polyethylene (HDPE) screw topped container. Samples were stored in a cooler at 4^{o}C for 7 to 14 days on the research ship Acadiana, then shipped on ice to the Environmental and Contaminants Research Center (ECRC - formerly the Midwest Science Center) in Columbia, MO. Two 125-mL subsamples from each B sample were collected at the start of the toxicity tests for physical (grain size and TOC) and chemical (organic and metal) characterization.

A second composite sediment sample was also collected from each pool at one station on one of the transects (station samples designated as "C" samples). The individual stations © samples) were selected based on historical chemistry data and the potential for the collection of large numbers of oligochaetes for bioaccumulation evaluations (Chapter 2). Station sediment samples (C samples) for toxicity and bioaccumulation (Chapter 2) testing were collected with a Ponar grab (529 cm^2 area). Each C sample was a composite sample collected from the upper 6 to 10 cm of the sediment surface within a 5-m radius area. A total of 35 to 80 L of sediment was collected from each C station. The sediment was then placed into a 120-L HDPE drum and homogenized on ship with a stainless steel auger on a hand-held power drill. Subsamples of these C samples were taken for (1) laboratory toxicity and laboratory bioaccumulation testing (10 L), (2) physical characterization (250 mL) and chemical characterization (250 mL for organics and 250 mL for metals) and (3) benthic invertebrate assessment (2 L). The remaining C sample was then sieved and native oligochaetes were collected for bioaccumulation analyses (Chapter 2). Sediment samples were stored in a cooler on the ship at 4^{o}C for 7 to 14 days, then shipped on ice to the ECRC in Columbia, MO. Once at the ECRC, sediment samples were stored in the dark at 4^{o}C until the start of the study. The control sediment (FLOR) used in the toxicity tests was a fine silt- and clay-particle size soil collected near St. Louis MO. This control sediment has been used in previous studies (Kemble *et al* 1994).

Culturing of Test Organisms

Amphipods were mass cultured at 23^{o}C with a luminance of about 800 lux according to procedures outlined in Tomasovic *et al* (1995) using 80-L glass aquaria containing 50 L of ECRC well water (hardness 283 mg/L as $CaCO_3$, alkalinity 255 as $CaCO_3$, pH 7.8). Artificial substrates were also placed in the amphipod culture aquaria (six 20-cm diameter sections/aquarium of "coiled web material"; 3M Corp., Saint Paul, MN). Known-age amphipods were obtained by isolating mixed-aged adults in a 5-mm mesh (#35 US Standard size sieve) sieve in a pan containing about 2 cm of well water. After 24 h, well water was sprinkled through the sieve, flushing <24-h-old amphipods into the pan below. These <24-h old amphipods were then placed into flow-through glass chambers for 10 d before the exposure began. Isolated amphipods were fed maple leaves and ground Tetramin® ad lib until the start of the test.

Toxicity Tests

Sediment Preparation: Sediment samples were re-homogenized in the laboratory using either a plastic spoon (for the B samples) or a hand-held power drill with a stainless steel auger (for the C samples). Subsamples were then collected for: (1) pore-water preparation, (2) physical and chemical characterizations, (3) toxicity testing, and (4) bioaccumulation testing © samples only; i.e., Chapter 2).

Water Quality: About 170 mL of pore water was isolated from each sample by centrifugation at 4°C for 15 min at 5200 rpm (7000 x G). A 50-mL subsample for total sulfide determination was removed from each sample and preserved with 0.1 mL of 2N zinc acetate solution (APHA 1985). Total dissolved sulfide was determined with an Orion EA940 Expandable ionAnalyzer, Orion 94-16 silver/sulfide electrode, and a Orion 90-02 double junction reference electrode. Dissolved oxygen (mg/L, with a YSI Model 54A oxygen meter and a YSI 5739 probe), temperature (°C) and conductivity (µs/cm @ 25°C with a Orion 140 S-C-T meter and a 014010 conductivity cell) were determined on the remaining volume. Subsamples of pore water were then removed for the following determinations: total ammonia (mg/L) with an Orion EA940, and Orion 95-12 ammonia electrode, alkalinity (mg/L, as $CaCO_3$) and pH with an Orion EA940 Expandable ionAnalyzer, Orion 917001 ATC probe, and Orion 8165BN combination pH probe, and total hardness as (mg/L, as $CaCO_3$) by EDTA titration. Unionized ammonia concentrations (mg/L, as NH_3) were calculated by adjusting total ammonia concentrations to pH and temperature using the formula presented in Thurston *et al* (1979). Hydrogen sulfide concentrations (mg/L) were calculated by adjusting the total dissolved sulfide concentrations to pH and temperature using the relationship presented in Broderius and Smith (1977).

Mean characteristics of porewater water quality (ranges in parentheses) are as follows: pH 7.45 (6.69 to 8.17); alkalinity 505 (244 to 852) mg/L; hardness 504 (148 to 852) mg/L; dissolved oxygen 5.04 (1.50 to 9.35) mg/L; conductivity 906 (380 to 1680) µs/cm @ 25^{0}C; total ammonia 5.320 (1.210 to 22.700) mg/L; unionized ammonia 0.007 (0.000 to 0.025) mg/L; total sulfide 0.055 (0.000 to 0.569) mg/L; and hydrogen sulfide 0.023 (0.000 to 0.569) mg/L (Appendix 1.1).

The following parameters were measured in overlying test water on Day -1 (the day before amphipods were placed into the beakers) and at the end of each toxicity test: dissolved oxygen, temperature, conductivity, pH, alkalinity, total hardness, and total ammonia. Methods used to characterize overlying water quality in the whole-sediment tests were similar to the methods described for characterization of pore water. Dissolved oxygen, pH, and conductivity were also measured weekly. Temperature in the water baths holding the exposure beakers was measured daily. Overlying water pH, alkalinity, total hardness, conductivity and total ammonia measurements were similar among all stations, the control, and the in flowing test water (Appendix 1.2). Dissolved oxygen measurements were at or above acceptable levels (>40% of saturation; ASTM 1995) in all treatments throughout the study (Appendix 1.2). Means (ranges in parentheses) of overlying water quality of each parameter are as follows: pH 8.07 (7.58 to 8.72); alkalinity 87 (59 to 151) mg/L; hardness 128 (111 to 160) mg/L; dissolved oxygen 6.70 (5.84 to 7.53) mg/L; conductivity 392 (359 to 428) µs/cm @25^0C; total ammonia 0.416 (0.090 to 1.520) mg/L; and unionized ammonia 0.003 (0.000 to 0.012) mg/L (Appendix 1.2).

Toxicity Tests: All sediment tests were started within three months of sample collection from the field. Due to the number of samples collected, half of the samples (i.e., half of the sites) were randomly selected for the initial testing. The second set of sediment samples was tested after completion of testing of the first set of samples. Sediment samples for the toxicity tests were homogenized the day before animals were added to exposure beakers (Day -1), using procedures previously described.

Toxicity tests were conducted with *Hyalella azteca* for 28 days. Effects of exposure to sediments on survival, length, and sexual maturation of amphipods were measured (USEPA 1994; ASTM 1995). Each 300-mL beaker contained 100 mL of sediment and 150 mL of overlying water. The photoperiod was 16:8 h (light:dark) at a light intensity of about 500 lux. Four replicate beakers/sample were placed in a ventilated water bath maintained at 23^{o}C. Each beaker received 1.0 volume additions/d of overlying water starting on Day -1 (Zumwalt *et al* 1994). The overlying water used in the sediment toxicity exposures was a reconstituted moderately hard water (hardness 95 mg/L as $CaCO_3$, alkalinity 65-70 mg/L as $CaCO_3$, pH 8.0-8.3; USEPA 1994). One diluter cycle delivered 50 mL of water to each beaker (diluters cycled every 8 h ± 15 min). Amphipods were acclimated to the test water over 6 h before exposures began by sequentially transferring animals at 2 h intervals into 50:50 and 25:75 mixtures of well water:test water, and then into 100% test water. Tests were started on Day 0 by placing 10 amphipods (10- to 11-d old) into each beaker. The water surface in each beaker was checked 15 min after organisms were placed in the beaker for floating organisms. Amphipods in each beaker were fed 3 mg of Purina Rabbit Pellets[R] in a water suspension three times a week for the first 7 days of the exposure, and 6 mg three times a week for the last 21 days of the exposure. If excessive mold (≥60% sediment surface) was observed on the sediment surface of any of the beakers in a treatment, feeding was withheld from all of the beakers for that treatment (the number of feedings withheld ranged from 0 to 5 depending on the treatment; USEPA 1994; ASTM 1996). Beakers were observed daily for the presence of animals, signs of animal activity (i.e., burrowing), and to monitor test conditions (i.e.; water clarity).

Amphipods were retrieved from each beaker at the end of exposures using procedures described in Kemble *et al* (1994). Surviving organism were combined into a scintillation vial and preserved in 8% sugar formalin for later measurement of length, and sexual maturation. A Zeiss® Interactive Digital Analysis System in combination with a Zeiss SV8 stereomicroscope at a magnification of 25x was used to measure amphipods following methods described in Kemble *et al* (1994). Amphipods were classified as either "mature male" or "not male" based on the presence of an enlarged second gnathopod (Kemble *et al* 1994). An enlarged second gnathopod of male amphipods was a consistent measure of sexual maturation (it is difficult to distinguish immature males from females at this age).

Chemical and Physical Characterization of Sediments

Acid-volatile Sulfides (AVS) and Simultaneously Extractable Metals (SEM): Subsamples of sediments were measured for acid-volatile sulfides (AVS) and simultaneously extractable metals (SEM) immediately after homogenization. Station samples (C samples) were collected on the boat and stored at 4ºC until shipment to the laboratory. Pool samples (B samples) were collected in the laboratory immediately after sediment homogenization before the start of toxicity tests. Concentrations of AVS in sediment samples were determined using a silver/sulfide electrode following methods described in Brumbaugh *et al* (1994). Concentrations of SEM were determined using atomic spectroscopy following methods described in Brumbaugh *et al* (1994).

Percentage recoveries for inorganics from both blank and sediment extracts averaged 96%. The average range was from a low of 78% for antimony (spiked as sodium sulfide) in the sediment extract to a high of 110% for Zn in the sediment extract. The average duplicate coefficient of variation was 1.7% (6 compounds, n=2). Average duplicate coefficient of variation ranged from 0.2% for both Pb samples to 5.1% for S in one of the duplicate samples.

Organochlorine Pesticides (OCPs), Polychlorinated Biphenyls (PCBs), and Aliphatic and Polycyclic Aromatic Hydrocarbons (PAHs): Sediment samples © samples) were prepared for the analyses of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and aliphatic and polycyclic aromatic hydrocarbons (PAHs) by extracting twenty grams of sediment with acetone, followed by petroleum ether. A final acetone/petroleum ether extraction was done and the extracts combined, centrifuged and transferred to a separatory funnel containing sufficient water to facilitate partitioning of residues into petroleum ether portion. The petroleum ether was washed twice with water and concentrated by Kuderna-Danish to appropriate volume.

Organochlorine determination was conducted by transferring an aliquot of concentrated extract to a 1.6 g Florisil mini-column topped with 1.6 g sodium sulfate. Residues were eluted from the column in two elution fractions. The first fraction consisted of 12 mL of hexane followed by 12 mL of 1% methanol in hexane; the second fraction consisted of an additional 24 mL of 1% methanol in hexane. Quantification of residues in the two Florisil fractions and three silicic acid fractions was performed using a packed or megabore column and electron capture gas chromatography.

Hydrocarbon determination was conducted by transferring a second aliquot of the concentrated extract to a 20 g 1% deactivated silica gel column, topped with 5-g neutral alumina. Aliphatic and polynuclear aromatic hydrocarbon residues were fractioned by eluting aliphatics from the column with 100-mL petroleum ether (Fraction 1) followed by elution of aromatics using, 100-mL 40% methylene chloride/60% petroleum ether, followed by 50-mL methylene chloride (combined eluates, Fraction 2). Quantification of fraction 1 by capillary column, flame ionization gas chromatography was performed once the fraction was concentrated to appropriate volume. The silica gel (fraction 2) containing aromatic hydrocarbons was concentrated, reconstituted in methylene chloride and quantified by gas chromatography and mass spectrometry.

Average percent spike recovery for eighteen OCPs was 103% (n=2). The smallest average spike recovery was 68% for HCB while o,p’-DDE had the greatest average spike recovery

(120%). Individual OCP concentrations were below minimum detection limits so duplicate analyses were not evaluated. Average percent spike recovery for PAH compounds was 98% (29 compounds, n=2). Naphthalene (84%) had the smallest average percent recovery while fluoranthene had the greatest average spike recovery (110%). The average duplicate coefficient of variation was 12.6% (13 compounds, n=2). Average duplicate coefficient of variation ranged from 0% for multiple PAHs in both duplicate samples to 61% for benzo(a)pyrene in one of the samples.

Methods for the analyses of the B samples, detection limits and quality control are described in Moody *et al* (1996). Quality control of B sediment samples analyzed for PAHs included: (1) estimates of accuracy determined from the standard deviation of the percent recovery of deuterated compounds added to the extracts and calculated based on absolute area counts and external calibration, and (2) precision, based on the relative standard deviation of the absolute area of multiple analyses of a surrogate compound (Moody *et al* 1996). A list of all the PAHs and OCPs analyzed for in both sets of sediment samples (B and C) are listed in Appendix 1.3.

Physical Characterization of Sediments

Physical characterization of sediments included: (1) percentage water (Kemble *et al* 1993), (2) particle size using a hydrometer (Forth *et al* 1982; Gee and Bauder 1986; Kemble *et al* 1993), and (3) total organic carbon using a coulometric titration (Cahill *et al* 1987; Kemble *et al* 1993). All physical characterizations included analysis of duplicate samples. Differences in percentage water for duplicate samples ranged from 0% in treatments 2B, 7B, 13C, 14B and 18B to 7% in treatment 10C. Duplicate samples of control sediment, sucrose standards and blanks were analyzed when determining sediment total organic. Precision and accuracy of the coulometric technique used was tested against National Bureau of Standards and Standard Reference Materials (NBS-SRM) with an error of less than 0.03% of the excepted values (Cahill *et al* 1987). Differences between duplicates ranged from 0% in treatments 3B, 11B, 12B, 13C, 14C, 15C, 18C, 20C, 22C, 22B, 24C and 26C to 0.9% in treatments 5C, 9C and 26B.

Data Analysis and Statistics

Toxicity Tests: Before statistical analyses were performed, data for survival and maturation were arcsin transformed. Comparisons of mean survival and percentage sexual maturation were made using a one-way nested analysis of variance (ANOVA) with mean separation by Fisher's protected least significant difference test at alpha = 0.05 (Snedecor and Cochran 1982). Data for length had a normal distribution and were not transformed before statistical analysis. Comparison of mean body length was made using a one-way ANOVA with mean separation by Fisher's protected least significant difference test at alpha = 0.05 (Snedecor and Cochran 1982). A sample was designated as toxic when survival, growth, or sexual maturation were significantly reduced relative to the control and reference sediments. Sediments from pools 6 and 11 were chosen as reference sediment based on low concentrations of contaminants. Simple linear regression was used to compare physical and chemical sediment characteristics to amphipod survival, length or sexual maturation. All statistical analyses were performed with Statistical

Analysis System (SAS) programs (SAS 1994).

Effects Range Median: Chemistry concentrations and toxicity endpoints were evaluated using 28-day *Hyalella azteca* Effect Range Medians (ERMs) reported by Ingersoll *et al* (1996) and Smith *et al* (1996). An ERM is defined as the concentration of a chemical in sediment above which effects are frequently or always observed or predicted for most species (Long *et al* 1995). The total number of individual ERMs exceeded with each sample was plotted against the sum ERM quotient (SERM-Q; where Q is equal to the concentration of each chemical in the sediment sample divided by the ERM for that chemical), similar to the toxic unit described by Canfield *et al* (1996), Ingersoll *et al* (1996) and Swartz *et al* (1997). We chose to evaluate sediment toxicity relative to nine ERMs which correctly classified >70% of the samples in Ingersoll *et al* (1996). These 9 individual ERMs tended to minimize Type I (false positive) and Type II (false negative) errors relative to other SECs reported by Ingersoll *et al* (1996). Due to insufficient chemistry data for chromium and total PCBs, only 7 of the 9 individual ERMs were used in this evaluation. These ERMs included: cadmium, lead, nickel, zinc, chrysene, benzo(a)pyrene, and benzo(g,h,i)perylene.

Results and Discussion

Toxicity Tests

Survival of amphipods was significantly reduced relative to the control and reference sediments only in the 13B treatment (Table 1.1). Body length of amphipods was significantly reduced relative to the control and reference sediments in only the 26C treatment (Table 1.1; Appendices 1.4 and 1.5). Sexual maturation was not significantly reduced in any treatments when compared to the control and reference sediments (Table 1.1; Appendices 1.6 and 1.7).

Indigenous organisms recovered at the end of amphipod exposures included oligochaetes, ostracods, clams, and a snail. Clam shells were present in many of the sediments; however, only a few live clams were retrieved at the end of the exposure. Pairs of amphipods were observed in amplexus in the control, 1-B, 2-B, 5-B, 6-C, 8-B, 8-C, 9-B, 10-B, 11-B, 14-C, 15-B, 18-C, 24-B, 24-C, and 26-B treatments, and gravid females were observed in the control, 11-B, 16-C, and 24-B treatments.

Although significant differences in survival of amphipods relative to the control and reference sediments were only observed in sample 13B, there was a relatively wide range in survival among the treatments. For example survival was below 70% in 13 of the 51 treatments (Table 1.1). Survival of amphipods in the control was acceptable (≥80%), however, survival in two of the four reference treatments (11C and 6B) was below 80%. Subsequent studies have found that the reconstituted water described in USEPA (1994) that was used to conduct this study does not consistently support adequate survival and growth of *Hyalella azteca* in 28-day exposures (McNulty 1995; Kemble *et al* 1996). Ingersoll *et al* (1997) retested sediment samples 4C, 11C, 14C, and 24C using well water as an overlying water and observed a mean survival of >90% in all of the samples with no substantial effects on growth, or reproduction of *H. azteca*. Survival of amphipods in these same sediments ranged from 48% to 63% in the present chapter (Table

1.1). Similarly, Benoit *et al* (1997) tested Station samples (7C, 9C, 13C, 22C, and 24C) in chronic toxicity tests with midge *Chironomus tentans* using a natural overlying water and did not observe effects on survival, growth, emergence, or reproduction. Additional studies are ongoing to evaluate 28-day *Hyalella azteca* exposures using reconstituted waters.

Physical and Chemical Characteristics of Sediments

Physical and chemical characteristics of sediment samples are listed in Table 1.2. Sediment organic carbon content ranged from 0.2% for the sediment samples from Stations 6B and 20B to 5.2% for Station 10C. Organic carbon content in the control sediment was 1.2%. Percentage solids ranged from 21% in the sediment sample from stations 4C and 10C to 84% for the sediment sample from Station 20B. Classification of the sediment samples for grain size varied from pool to pool (i.e., loam (11C), sandy-loam (8B), silty-clay-loam (25 C and 22C)) while the control sediment was a silty-clay-loam (Table 1.2). Acid volatile sulfide levels ranged from 0.005 µmoles/g in the 1C sample to 63.0 µmoles/g in the 10C sample (Table 1.2).

Concentrations of simultaneously extracted metals in sediment samples are listed in Appendix 1.8. Sediment from sample 4C had the highest concentrations of extractable SEM Cd, Cu, Ni, and Pb. Sample 12C had the highest concentration of SEM Zn (Appendix 1.8). The sum SEM/AVS molar ratio in the present study was typically less than 1 (except the two samples from pool 1). This indicates the concentration of divalent metals listed in Appendix 1.8 were probably not high enough to result in toxicity of the samples (DiToro *et al* 1990). Concentrations of SEM Cd, Cu, Ni and Pb were highest in sediment samples from treatment 4C (Appendix 1.8). However, concentrations of SEM Cu and Pb were still below the ERMs reported by Ingersoll *et al* (1996; Figures 1.2 and 1.3).

Significant positive correlations were observed between SEM metals vs. TOC (Cu > Zn > Cd>Pb>Ni), SEM metals vs. percentage clay (Zn>Ni>Pb>Cu>Cd) and between SEM metals vs. percentage silt (Ni>Cu>Pb>Zn>Cd) when tested by Spearman's rho coefficient of rank correlation (Table 1.3). The significant negative correlation with sand and the positive correlation with clay and silt indicates that metals were concentrated in the finer sediment particles.

Concentrations of organochlorine pesticides (OCPs) in sediment samples are listed in Appendix 1.9. Concentrations of OCPs were below detection limits (0.01 µg/g) in all of the C samples except the 2C and SCC samples which had detectable concentrations of DDE and DDD (Appendix 1.9). Amphipod survival in the 2C sediment sample was 75%. However, despite having concentrations which were similar for both chemicals, survival of amphipods in the SCC sample was 90%. This indicates that the levels of DDE and DDD detected in these samples was not the sole cause of the lower survival observed in the 2C sediment sample. Concentrations of OCPs in the B samples were at or below detection limits for 10 of the 15 individual pesticides evaluated (Appendix 1.9). Concentrations for all 5 OCPs detected in the B samples were $\leq$0.079 µg/g dry weight and were below calculated ERMs (Smith *et al* 1996; Appendix 1.9).

Concentrations of polycyclic aromatic hydrocarbons (PAHs) in sediment samples are listed in Appendix 1.10. The highest concentrations were observed at Pool 1 and were generally lower in the downstream pools. Concentrations of PAHs in river sediments exceeded the Method Lower

Limit of Quantitation (MLLQ; 0.03 µg/g) in at least one sediment sample for every PAH evaluated (except for 1-methylnaphthalene; Appendix 1.10). Concentrations of 4 of the 11 PAHs measured exceed at least one calculated ERM (Ingersoll *et al* 1996; Figures 1.4 and 1.5). Elevated PAH concentrations in sediment samples were associated with sediment collected from pools near Minneapolis, MN. Concentrations of PAHs below pool 4 were similar in the remaining pools. Concentrations of fluoranthene exceeded the calculated ERM (0.175 µg/g) in 9 of the sediment samples from the Upper Mississippi River. Amphipod survival in these samples was above 75% in all but one of the samples (sample 4C which had a survival of 63%; Table 1.1). This would indicate that concentrations of fluoranthene in these samples had little or no effect on amphipod survival.

Comparisons of Sediment Characteristics to Toxicity Responses

Relationships of physical or chemical characteristics of sediments to toxicity were evaluated using rank correlation (Table 1.4). No significant correlations were observed between survival, growth or maturation and the measured physical or chemical characteristics of the sediment samples (Table 1.4). Additionally, no significant correlation was observed between the toxicity endpoints and concentrations of PAHs or OCPs normalized to total organic carbon concentrations (Table 1.5). Sediments from Pool 1 had the highest percent sand (>88%), but amphipod length and maturation were not reduced with exposure to 1B or 1C sediments relative to the control and reference sediments (Table 1.1). Similarly, the control sediment had the highest percent silt and clay relative to the other samples. Ingersoll and Nelson (1990), Kemble *et al* (1994), and Ingersoll *et al* (1997) also reported sediment particle size did not affect the response of *Hyalella azteca* in 28-d sediment exposures.

None of the 49 sediment samples exceeded any of the 7 individual ERMs. Use of these 7 ERMs correctly classified 47 of the 49 (96%) sediment samples from the UMR as non-toxic. The two samples incorrectly classified were both type II errors (false negative; toxic sample that does not exceed an ERM). This again may indicate something other than contaminants or contaminants not measured were the cause of the relatively wide range in survival among the treatments.

Additional ERMs for individual chemicals listed in Ingersoll *et al* (1996) and Smith *et al* (1996) were also evaluated. About 20% of the sediment samples exceeded at least one of these ERMs. However, use of these additional ERMs to classify samples as toxic or non-toxic resulted in increased Type I error (false positive; non-toxic sample that exceeds an ERM). As was the case when using only the seven ERMs, chemical concentrations from the two samples classified as toxic did not exceed any of the additional ERMs.

The prediction of sediment toxicity was also evaluated using a toxic quotient approach. A toxic quotient was calculated for each sample by first dividing the concentration of individual chemicals by their respective ERM and then summing each of the individual values (Canfield *et al* 1996; Ingersoll *et al.* 1996). In the present study, quotients for the seven chemicals listed above were used to calculate a toxic quotient for each sample (Table 1.2). Figure 1.6 plots the relationship between the frequency of ERM exceedances and the sum of the ERM toxic quotient. In the present study, the ERM toxic quotient was $\leq$ 2.6 and individual ERMs were not exceeded indicating the sediment samples from the UMR were relatively non-contaminated compared to sediments from areas of known contamination in the United States (Kemble *et al* 1994; Ingersoll *et al* 1996). A toxic quotient approach was also used in Chapter 3 using a quadrant frequency analysis to evaluate the benthic community of the pools in the UMR system.

Summary

Toxicity tests using amphipods identified only two of the 49 sediment samples from the Upper Mississippi River system as toxic (a significant reduction in survival, growth or sexual maturation compared to the control and reference sediments). However, there was a relatively wide range in survival among the treatments. The overlying water used in this test was the reconstituted water described in USEPA (1994), which McNulty (1995) and Kemble *et al* (1996) have reported does not consistently support adequate survival of *Hyalella azteca* in 28-d sediment exposures. Survival of amphipods and midge was >90% in subsequent studies with sediments from the present study when natural water was used as the overlying test water (Benoit *et al* 1997; Ingersoll *et al* 1997). This would indicate that the reconstituted test water was a significant factor in the wide range of survival observed in the present study.

Effect Range Medians (ERMs) were used to evaluate the toxicity of contaminants associated with field collected sediments. ERMs correctly classified 96% of the UMR sediment samples as non-toxic. The two samples incorrectly classified were type II errors (false negatives). Again this indicates that factors other than contaminants or unmeasured contaminants may have been responsible for the variation in amphipod survival that was observed.

Concentrations of contaminants in sediments from the UMR were typically 10 to 100 times less than concentrations of contaminants in sediments previously associated with toxicity (Kemble *et al* 1994; Ingersoll *et al* 1996; Figure 1.7). This would indicate that the sediment samples from the UMR were relatively non-contaminated compared to other areas of know contamination across the United States.

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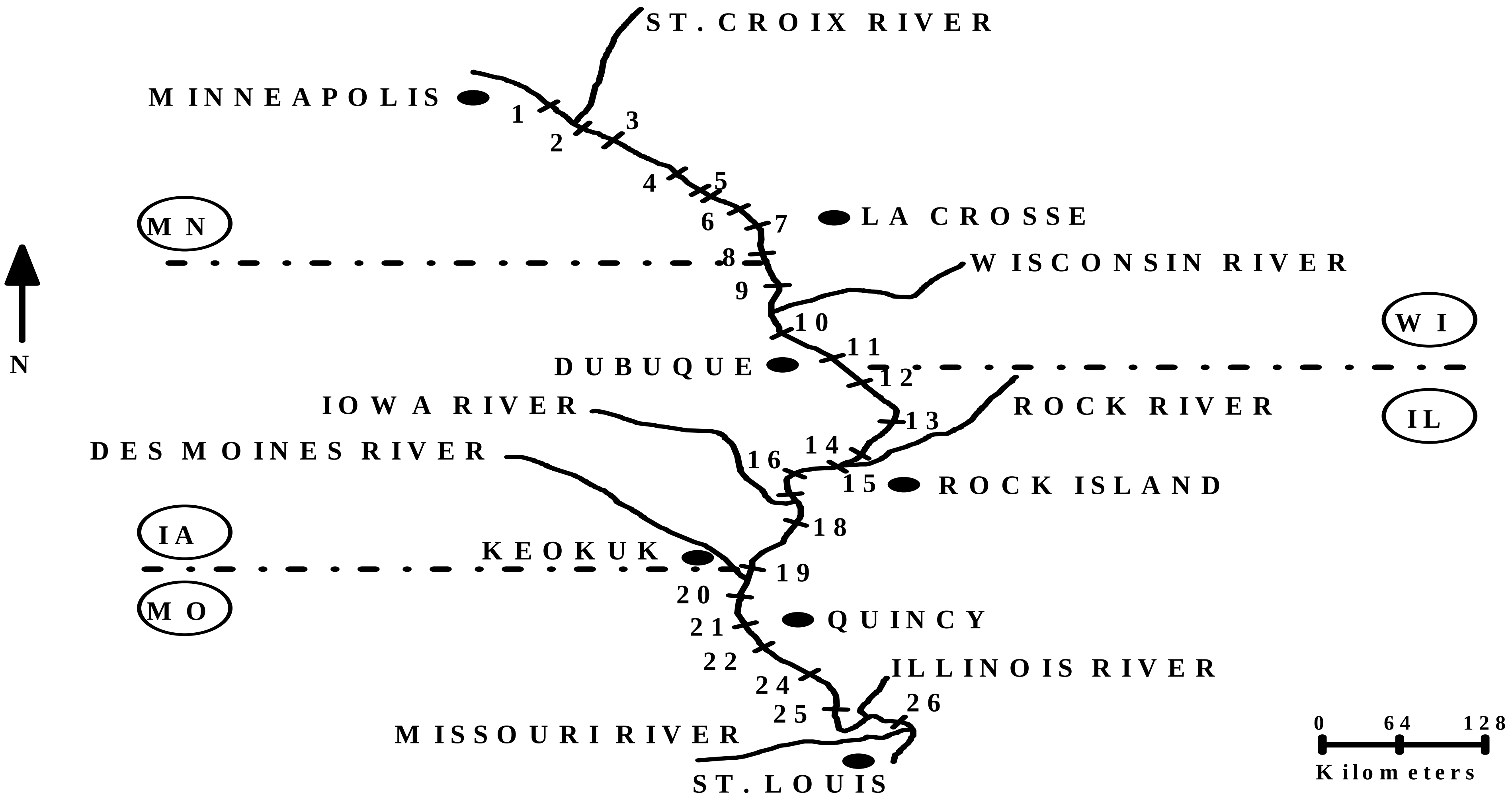

Fig. 1.1. Map of the Upper Mississippi River (UMR) from Minneapolis, MN to Saint Louis, MO.

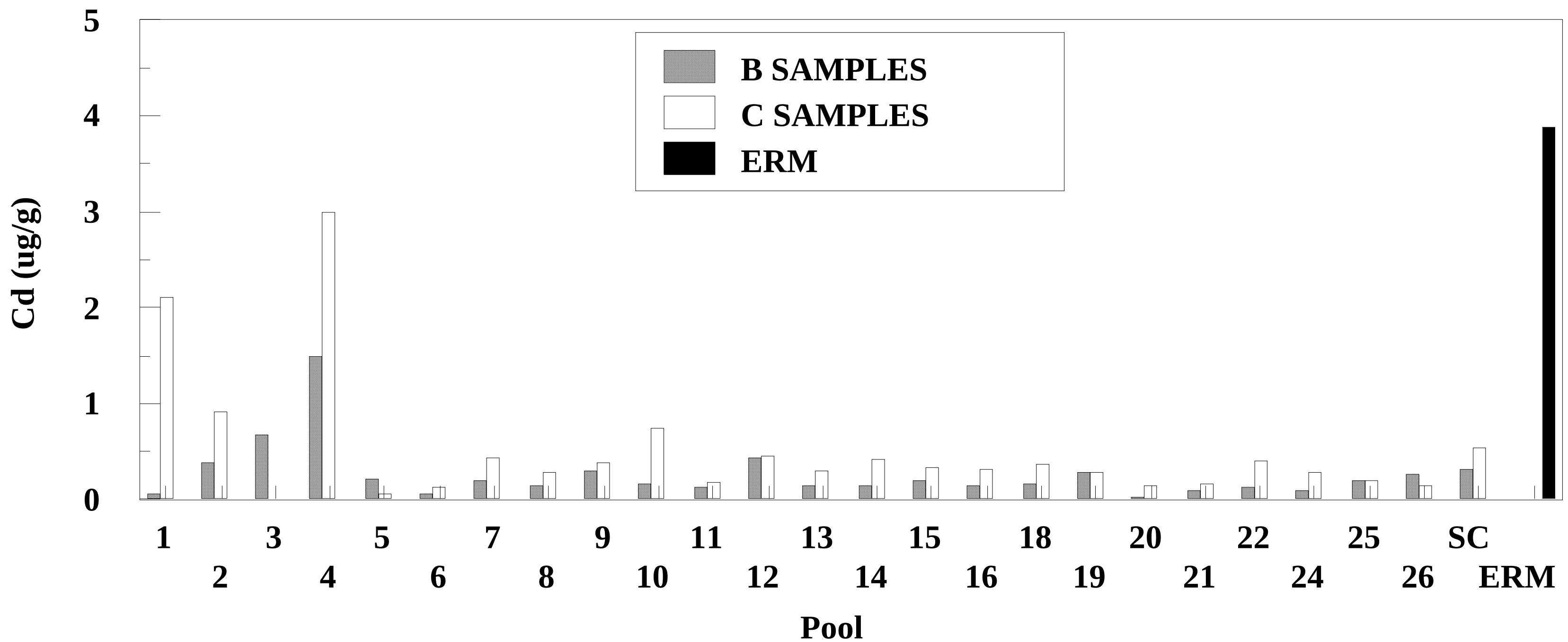

Fig. 1.2. Concentrations of Simultaneously Extracted Metal (SEM) Cd in UMR sediment samples compared to a Effect Range Median (ERM) for Cd.

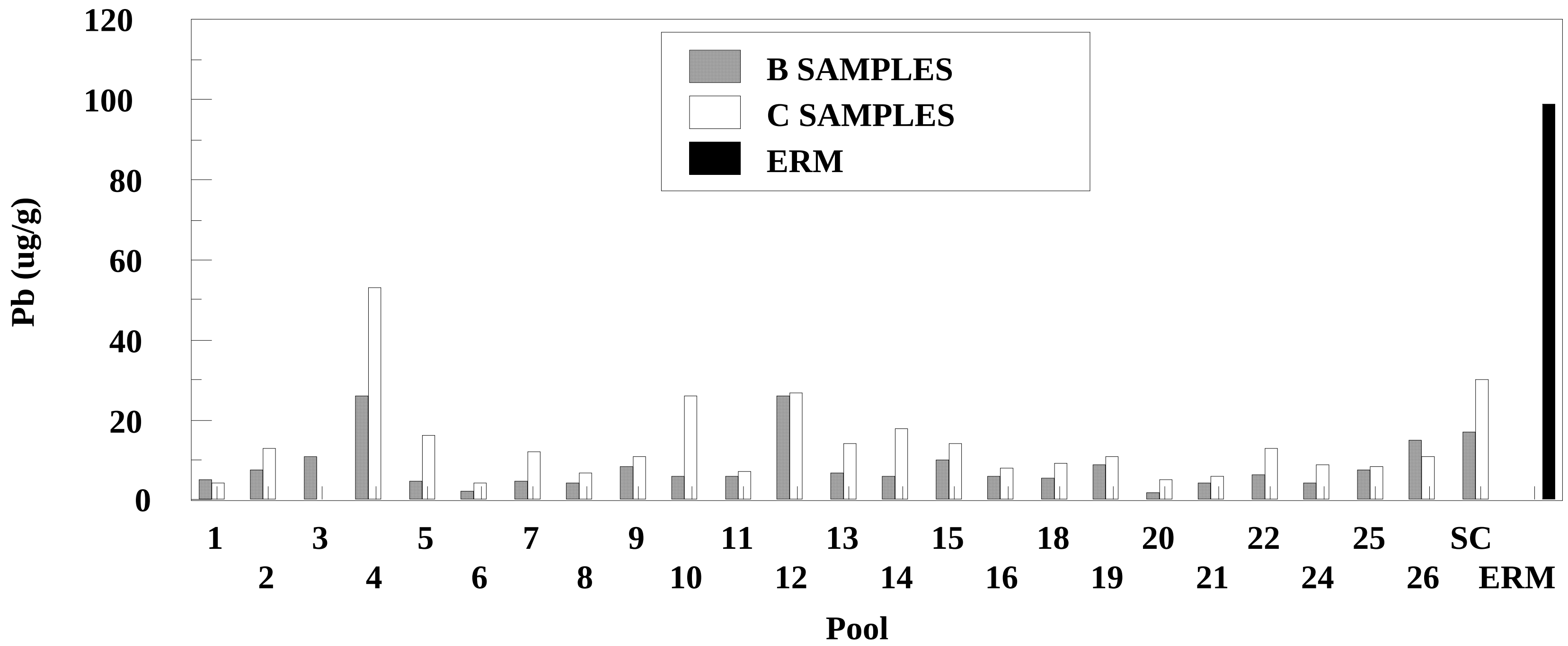

Fig. 1.3. Concentrations of SEM Pb in UMR sediment samples compared to a ERM for Pb.

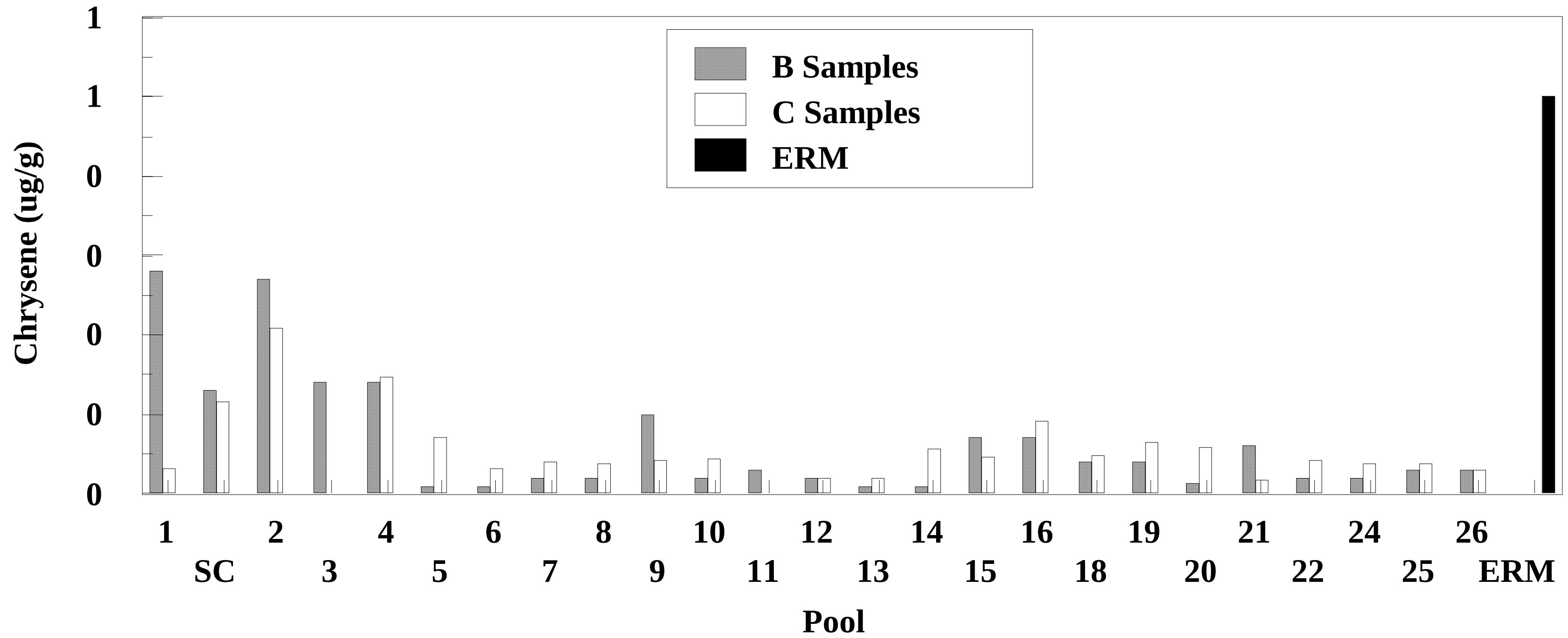

Fig. 1.4. Concentrations of Chrysene in UMR sediment samples compared to a ERM for Chrysene.

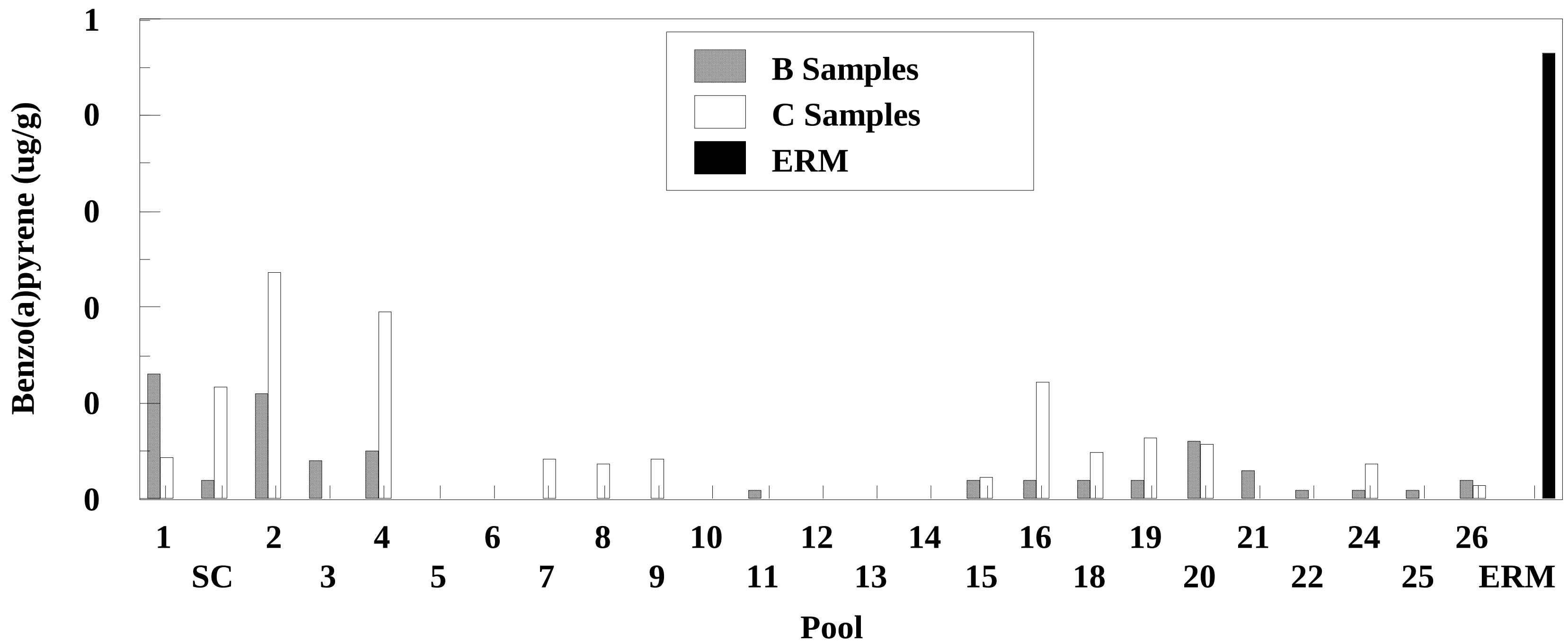

Fig. 1.5. Concentrations of Benzo(a)pyrene (BAP) in UMR sediment samples compared to a ERM for BAP.

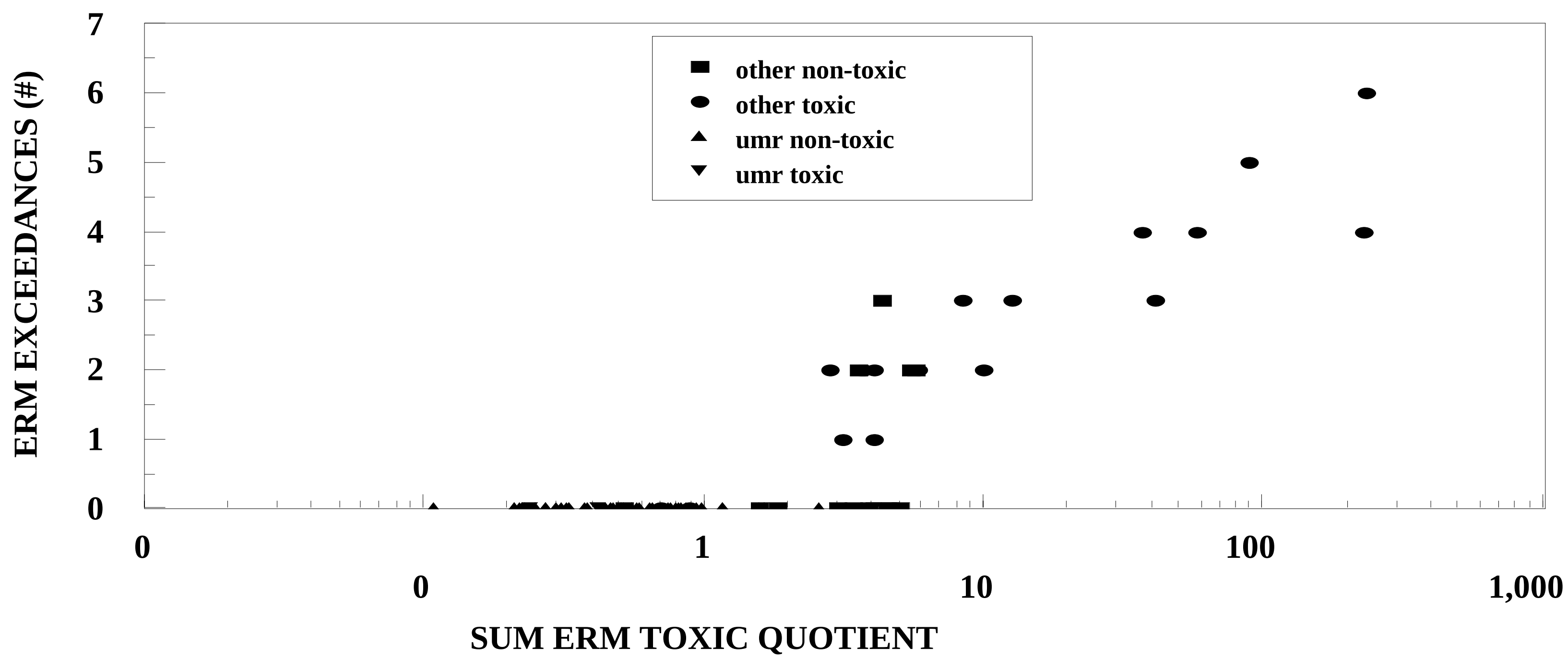

Fig. 1.6: Number of ERM exceedances for the 7 chemicals that correctly classified 70% of the samples compared to sum ERM toxic quotient.

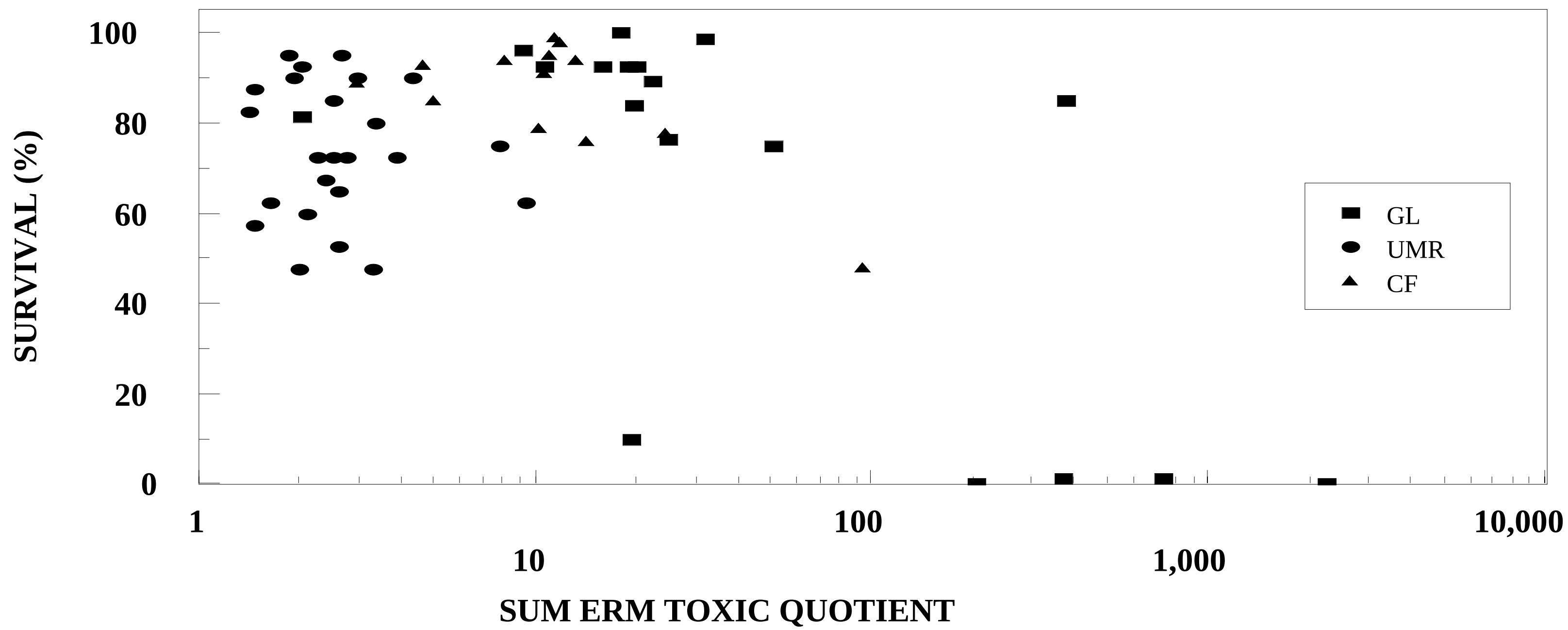

Fig. 1.7. Survival vs Sum ERM toxic quotients of sediment samples from the UMR compared to survival vs. sum ERM toxic quotients of sediment samples from the Great Lakes and the Clark Fork River and Milltown Reservoir MT.

Table 1.1. Results of the Upper Mississippi River sediment tests with *Hyalella azteca*. Means (Standard error of the means in parentheses) within a column and within a set of sample are significantly different ($p < 0.05$; n=4) from the control and reference sediment and are designated with an asterix.

Sample	Survival (%)	Length (mm)[1]	Mature Males (%)
<u>1st set of samples</u>			
Control	80.0 (4.08)	3.39 (0.16)	36.7 (8.91)
1B	92.5 (4.79)	3.66 (0.11)	39.1 (5.71)
1C	65.0 (5.00)	3.17 (0.11)	16.9 (6.90)
3B	95.0 (5.00)	4.27 (0.08)	44.9 (8.43)
5B	80.0 (7.07)	4.23 (0.06)	44.8 (10.30)
5C	80.0 (7.07)	4.06 (0.10)	21.6 (4.23)
8B	97.5 (2.50)	3.69 (0.09)	40.5 (7.72)
8C	92.5 (2.50)	4.09 (0.11)	32.3 (7.68)
10B	92.5 (7.50)	4.28 (0.09)	39.5 (18.49)
10C	72.5 (13.15)	3.86 (0.08)	34.4 (6.88)
11B (reference)	87.5 (2.50)	4.31 (0.07)	43.3 (11.57)
11C (reference)	57.5 (8.54)	3.61 (0.07)	32.8 (15.79)
12B	72.5 (9.46)	3.48 (0.07)	34.5 (3.00)
12C	85.0 (6.45)	3.78 (0.07)	32.4 (5.85)
15B	90.0 (4.08)	3.74 (0.08)	51.3 (11.46)
15C	72.5 (2.50)	3.59 (0.09)	34.0 (8.64)
16B	70.0 (9.13)	3.72 (0.08)	40.6 (6.56)
16C	90.0 (7.07)	3.83 (0.07)	30.0 (10.13)
21B	95.0 (2.89)	3.46 (0.06)	52.2 (6.08)
21C	87.5 (4.79)	3.87 (0.09)	51.4 (5.29)
25B	62.5 (13.15)	3.60 (0.11)	23.8 (10.51)
25C	62.5 (15.48)	3.63 (0.08)	29.6 (8.34)
26B	92.5 (4.79)	3.51 (0.09)	42.0 (6.82)
26C	90.0 (7.07)	2.88 (0.01) *	48.8 (11.30)
<u>2nd Set of samples</u>			
Control	97.5 (2.50)	2.59 (0.08)	5.9 (3.42)
2B	75.0 (8.66)	4.07 (0.11)	31.3 (6.25)
2C	75.0 (10.41)	3.47 (0.10)	43.8 (8.08)
4B	85.0 (6.45)	3.39 (0.10)	36.7 (13.72)
4C	62.5 (21.75)	3.35 (0.09)	12.1 (5.22)
6B (reference)	67.5 (17.02)	3.53 (0.09)	26.9 (9.21)
6C (reference)	82.5 (2.50)	4.08 (0.10)	54.5 (2.97)
7B	100.0 (0.00)	3.66 (0.06)	42.5 (10.31)
7C	95.0 (2.89)	3.70 (0.07)	35.5 (3.41)
9B	75.0 (10.41)	3.72 (0.09)	43.6 (6.47)
9C	67.5 (13.77)	3.65 (0.08)	32.8 (11.24)
13B	32.5 (7.50) *	3.87 (0.19)	18.8 (11.97)
13C	47.5 (10.31)	3.56 (0.11)	50.0 (9.64)
14B	65.0 (5.00)	3.85 (0.12)	31.6 (7.36)

Table 1.1. (continued)

Sample	Survival (%)	Length (mm)[1]	Mature Males (%)
14C	47.5 (7.50)	3.50 (0.12)	43.8 (15.72)
18B	77.5 (7.50)	3.57 (0.12)	50.0 (18.89)
18C	72.5 (17.97)	3.52 (0.09)	20.8 (7.50)
19B	85.0 (6.45)	3.31 (0.07)	40.2 (7.50)
19C	72.5 (7.50)	3.44 (0.07)	32.3 (15.91)
20B	82.5 (8.54)	3.43 (0.08)	11.9 (5.14)
20C	95.0 (2.89)	3.30 (0.06)	27.2 (10.74)
22B	85.0 (6.45)	3.79 (0.10)	24.4 (3.00)
22C	52.5 (10.31)	3.64 (0.11)	39.9 (14.20)
24B	87.5 (2.50)	3.61 (0.08)	34.4 (4.65)
24C	60.0 (8.16)	3.78 (0.12)	66.9 (14.19)
SCB	75.0 (10.41)	3.42 (0.10)	11.9 (7.89)
SCC	90.0 (4.08)	3.03 (0.06)	31.7 (5.60)

[1]starting body length of amphipods in the 1st set of samples was 1.05 mm (0.02 SE, n=11) and was 1.17 mm (0.04 SE, n=10) in the 2nd set of samples.

Table 1.2. Physical and chemical characteristics of sediments from the Upper Mississippi River at the start of whole-sediment tests. The sum ERM-quotient are also calculated for each sample.

Sample	Total Organic Carbon (%)	Solids (%)	Particle Size (%) sand	clay	silt	Sum ERM Quotient	Sediment Class
1B	0.3	76.5	88.6	9.3	2.1	1.17	Sand/Loamy Sand
1C	0.5	77.9	88.8	10.1	1.1	0.80	Sand/Loamy Sand
2B	3.6	61.3	53.5	25.5	21.0	1.66	Sandy Clay Loam
2C	3.3	45.0	15.4	43.1	41.5	1.58	Silty Clay
3B	2.7	53.2	27.5	23.5	49.0	1.00	Loam
4B	4.8	26.2	11.6	49.0	39.5	1.68	Clay
4C	5.0	20.8	33.4	39.8	26.9	2.60	Clay Loam
5B	1.6	61.5	53.6	19.4	26.4	0.22	Sandy Loam
5C	5.1	27.7	31.6	31.0	37.5	0.81	Clay Loam
6B	0.2	77.3	84.6	12.4	3.0	0.11	Loamy Sand
6C	0.7	70.2	78.1	13.6	8.3	0.28	Sandy Loam
7B	1.0	47.7	17.1	32.1	50.7	0.27	Silty Clay Loam
7C	2.3	62.1	56.5	16.8	26.7	0.65	Sandy Loam
8B	1.3	57.5	58.0	18.8	23.2	0.21	Sandy Loam
8C	2.2	55.5	11.5	37.0	51.5	0.47	Silty Clay Loam
9B	2.0	56.3	27.6	21.5	50.9	0.52	Silt Loam
9C	2.9	48.0	9.3	29.4	61.3	0.60	Silty Clay Loam
10B	1.2	55.2	59.6	36.9	3.5	0.25	Sandy Clay
10C	5.2	20.7	24.3	41.7	34.0	0.94	Clay
11B	1.3	59.8	46.1	18.8	35.1	0.28	Loam
11C	1.8	64.7	46.2	21.6	31.3	0.31	Loam
12B	2.0	54.2	20.0	20.9	59.1	0.77	Silt Loam
12C	2.3	54.9	15.3	21.4	63.3	0.84	Silt Loam
13B	1.8	65.4	33.2	23.1	43.7	0.24	Loam
13C	1.8	52.1	14.6	22.0	63.4	0.50	Silt Loam
14B	0.6	35.8	4.0	42.5	53.5	0.23	Silty Clay
14C	3.0	61.0	58.7	18.4	22.9	0.70	Sandy Loam
15B	1.4	46.9	0.0	23.0	77.0	0.48	Silt Loam
15C	1.9	59.0	41.5	20.5	38.0	0.59	Loam

Table 1.2. (continued).

Sample	Total Organic Carbon (%)	Solids (%)	Particle Size (%) sand	clay	silt	Sum ERM Quotient	Sediment Class
16B	1.2	67.0	53.7	18.9	27.4	0.39	Sandy Loam
16C	2.8	67.4	51.3	21.9	26.8	0.76	Sandy Clay Loam
18B	0.7	69.1	64.0	19.5	16.5	0.33	Sandy Loam
18C	1.7	62.6	21.8	23.8	54.5	0.56	Silt Loam
19B	1.9	54.9	33.8	29.4	36.9	0.47	Clay Loam
19C	2.3	49.2	7.6	34.0	58.4	0.66	Silty Clay Loam
20B	0.2	84.1	81.4	11.7	6.8	0.23	Loamy Sand
20C	0.8	73.5	52.1	22.0	26.0	0.45	Sandy Clay Loam
21B	0.5	69.9	64.0	23.5	12.5	0.32	Sandy Clay Loam
21C	1.1	59.0	44.4	25.8	29.8	0.30	Loam
22B	0.5	73.3	62.1	23.4	14.5	0.25	Sandy Clay Loam
22C	2.4	44.4	0.3	40.3	59.4	0.59	Silt Clay Loam
24B	0.7	74.6	57.5	23.0	19.5	0.21	Sandy Clay Loam
24C	1.7	57.1	30.7	22.0	47.4	0.50	Loam
25B	1.4	63.3	33.2	30.7	36.1	0.33	Clay Loam
25C	1.1	56.2	16.6	28.0	55.4	0.38	Silty Clay Loam
26B	2.0	54.5	24.1	33.5	42.5	0.51	Clay Loam
26C	0.7	72.6	43.5	27.0	29.5	0.42	Clay Loam
SCB	3.0	34.0	53.4	24.8	21.9	0.88	Sandy Clay Loam
SCC	4.3	26.6	36.1	25.5	38.5	1.17	Loam
FLORB	1.2	32.0	12.3	26.5	61.3	1.04	Silty Clay Loam
FLORC	1.2	32.0	12.3	26.5	61.3	1.04	Silty Clay Loam

Table 1.3. Spearman rank correlation for SEM Cd, Cu, Ni, Pb, and Zn with TOC, percent Sand, percent Silt, and percent Clay for Upper Mississippi River sediments (excluding the control sediment). All of the correlations listed below were significant ($p \leq 0.05$).

Element	TOC%	%Sand	%Silt	%Clay
Cd	0.826	-0.449	0.341	0.394
Cu	0.868	-0.556	0.563	0.468
Ni	0.808	-0.634	0.594	0.553
Pb	0.823	-0.583	0.434	0.549
Zn	0.854	-0.589	0.385	0.570

Table 1.4. Linear regression (r^2) of amphipod survival, length, or sexual maturation to sediment physical and chemical characteristics. None of the regression were significant ($p<0.05$).

	Survival	Length	Sexual Maturation
PW Total Ammonia	0.11	0.07	0.17
PW Unionized Ammonia	0.01	0.06	0.03
PW Total Sulfide	0.05	0.05	0.04
PW Hydrogen Sulfide	< 0.01	0.03	0.09
PW Alkalinity	< 0.01	0.09	0.08
PW Hardness	0.01	0.16	0.13
PW pH	0.02	< 0.01	< 0.01
PW DO	< 0.01	0.04	0.01
PW conductivity	0.01	0.13	0.03
AVS	0.11	< 0.01	0.02
Total Organic Carbon	0.02	< 0.01	0.02
Percent Sand	0.02	< 0.01	< 0.01
Percent Clay	0.01	< 0.01	0.01
Percent Silt	0.05	< 0.01	0.05
Percent Fines[1]	0.02	< 0.01	< 0.01
Percent Water	< 0.01	0.02	0.02
SEM Cd	0.03	0.04	0.11
SEM Cu	0.01	0.03	0.03
SEM Ni	< 0.01	< 0.01	< 0.01
SEM Pb	0.02	0.05	0.05
SEM Zn	0.02	0.01	0.02
Toxaphene	0.01	0.02	< 0.01
Mirex	0.05	0.04	< 0.01
DDD	< 0.01	0.10	< 0.01
DDT	0.05	0.04	< 0.01
DDE	< 0.01	0.12	0.00
Endrin	0.05	0.04	< 0.01
Dieldrin	0.05	0.04	< 0.01
Heptachlor epoxide	0.05	0.04	< 0.01
Lindane	0.05	0.04	< 0.01
Naphthalene	0.04	0.04	0.01
Acenaphthalene	0.04	< 0.01	< 0.01
Acenaphthene	< 0.00	0.02	0.07
Phenanthrene	0.02	0.01	0.01
Anthracene	< 0.01	< 0.01	< 0.01

Table 1.4. (Continued)

	Survival	Length	Sexual Maturation
Fluorene	0.01	0.01	< 0.01
Fluoranthene	0.04	< 0.01	< 0.01
Chrysene	0.02	< 0.01	< 0.01
Pyrene	0.03	< 0.01	< 0.01
Benzo(b)fluoranthene	0.04	< 0.01	< 0.01
Benzo(k)fluoranthene	0.01	0.07	0.03
Benzo(a)pyrene	0.01	0.05	0.04
Indeo(1,2,3,-cd)pyrene	< 0.01	< 0.01	0.01
Benzo(g,h,j,i)perylene	< 0.01	0.01	0.01

[1]Silt and Clay combined

Table 1.5. Linear regression (r^2) of amphipod survival, length, sexual maturation to sediment chemical characteristics normalized to organic carbon. None of the regressions were significant ($p<0.05$).

	Survival	Length	Sexual Maturation
Toxaphene	0.03	0.01	0.05
Mirex	0.02	0.04	< 0.01
DDD	0.01	0.10	0.01
DDT	0.01	0.02	0.01
DDE	< 0.01	0.03	< 0.01
Endrin	0.01	0.05	< 0.01
Dieldrin	< 0.01	0.07	< 0.01
Heptachlor epoxide	0.01	0.07	< 0.01
Lindane	0.02	0.05	< 0.01
Naphthalene	0.03	< 0.01	< 0.01
Acenaphthalene	< 0.01	< 0.01	< 0.01
Acenaphthene	0.02	0.02	0.02
Phenanthrene	0.01	< 0.01	< 0.01
Anthracene	< 0.01	0.06	< 0.01
Fluorene	< 0.01	< 0.01	0.01
Fluoranthene	0.04	< 0.01	< 0.01
Chrysene	0.14	0.07	< 0.01
Pyrene	0.05	0.06	< 0.01
Benzo(b)fluoranthene	0.06	0.02	< 0.01
Benzo(k)fluoranthene	0.01	0.03	< 0.01
Benzo(a)pyrene	0.04	0.07	< 0.01
Indeo(1,2,3,-cd)pyrene	< 0.01	0.05	0.01
Benzo(g,h,j,i)perylene	< 0.01	0.05	0.01

Appendix 1.1. Pore water quality for the whole-sediment tests with Upper Mississippi river samples.

Pool	pH	Alkalinity (mg/L)	Hardness (mg/L)	DO (mg/L)	Conductivity (µmho @25ºC)	Total ammonia (mg/L)	unionized ammonia (mg/L)	Total Sulfide (mg/L)	Hydrogen Sulfide (mg/L)
01B	7.33	376	852	4.60	758	7.240	0.008	0.019	0.006
01C	7.69	292	280	7.60	541	1.540	0.004	0.000	0.000
02B	7.38	732	758	2.38	1354	5.900	0.007	0.011	0.003
02C	7.47	560	552	4.80	969	3.310	0.005	0.005	0.001
03B	7.50	624	664	6.60	1131	3.980	0.006	0.006	0.001
04B	7.59	ND	ND	4.23	779	2.745	0.005	0.302	0.056
04C	7.84	ND	ND	7.90	747	1.370	0.005	ND	0.000
05B	7.61	455	460	4.30	832	3.310	0.007	ND	0.000
05C	7.40	374	360	3.50	700	3.580	0.004	0.011	0.003
06B	7.82	432	404	6.20	830	3.470	0.011	0.099	0.012
06C	7.46	548	580	5.45	1031	11.400	0.016	0.040	0.009
07B	7.50	636	652	1.50	1186	5.640	0.009	0.000	0.000
07C	7.29	396	ND	4.35	765	6.150	0.006	0.009	0.003
08B	7.62	522	519	5.10	961	3.420	0.007	0.000	0.000
08C	7.45	596	600	5.60	1055	6.480	0.009	0.011	0.003
09B	7.43	835	750	6.15	1462	10.500	0.014	0.037	0.009
09C	7.41	ND	ND	4.00	1262	10.400	0.013	0.124	0.032
10B	7.48	488	480	4.05	912	4.730	0.007	0.002	0.000
10C	7.26	452	468	5.45	785	3.550	0.003	0.039	0.013
11B	7.50	ND	ND	5.60	930	4.120	0.006	0.000	0.000
11C	7.45	415	418	5.90	786	4.440	0.006	0.007	0.002
12B	7.40	568	620	6.00	1013	4.900	0.006	0.037	0.010
12C	7.20	710	600	4.60	1163	6.350	0.005	0.018	0.006
13B	7.24	852	808	4.60	1680	22.700	0.020	0.013	0.004
13C	7.41	ND	ND	4.75	897	8.070	0.010	0.011	0.003
14B	7.43	436	440	2.55	846	4.540	0.006	0.029	0.007
14C	7.53	ND	ND	4.35	636	4.290	0.007	0.465	0.096
15B	8.17	ND	ND	5.80	847	3.440	0.025	0.023	0.001
15C	7.47	364	360	7.20	671	2.360	0.003	0.012	0.003
16B	7.50	464	484	3.50	892	6.190	0.010	0.003	0.001
16C	7.40	ND	ND	5.40	998	6.970	0.009	0.031	0.008
18B	7.37	420	408	5.30	835	4.690	0.005	0.025	0.007

Appendix 1.1. Pore water quality for the whole-sediment tests with Upper Mississippi river samples (continued).

18C	7.44	340	348	2.95	652	3.180	0.004	0.007	0.002
19B	7.49	573	505	3.00	1027	5.440	0.008	ND	0.000
19C	7.33	ND	ND	3.90	1077	6.840	0.007	0.202	0.059
20B	0.00	ND	ND	ND	ND	2.750	0.000	0.569	0.569
20C	7.78	ND	ND	6.80	643	2.650	0.008	0.007	0.001
21B	7.45	ND	ND	5.30	1019	9.030	0.013	0.001	0.000
21C	7.31	ND	540	2.50	945	8.730	0.009	0.041	0.012
22B	7.41	495	475	5.10	934	6.370	0.008	0.000	0.000
22C	7.20	ND	ND	4.50	1109	12.300	0.010	0.159	0.057
24B	7.65	ND	ND	6.75	568	2.780	0.006	0.003	0.000
24C	7.45	ND	ND	6.40	708	ND	0.000	0.052	0.012
25B	7.34	ND	ND	1.50	869	3.260	0.004	ND	0.000
25C	7.42	440	352	5.60	768	2.270	0.003	0.008	0.002
26B	7.47	528	528	4.95	1003	5.520	0.008	0.007	0.002
26C	7.45	480	484	5.70	891	3.800	0.005	0.001	0.000
SCB	7.27	244	216	6.95	380	1.370	0.001	0.038	0.012
SCC	7.19	ND	148	6.50	386	1.210	0.001	0.057	0.021
FLOR	6.69	ND	ND	9.35	1176	1.410	0.000	0.001	0.000
Mean	7.45	505	504	5.04	906	5.320	0.007	0.055	0.023
Max.	8.17	852	852	9.35	1680	22.700	0.025	0.569	0.569
Min.	6.69	244	148	1.50	380	1.210	0.000	0.000	0.000
Std	0.21	141	159	1.60	246	3.649	0.005	0.114	0.084
Median	7.45	480	484	5.10	892	4.440	0.007	0.012	0.003

Appendix 1.2. Mean measured overlying water quality for the whole-sediment tests with Upper Mississippi river samples. Water quality was conducted on Days 0, 7,14,21, and 27.

Pool	pH	Alkalinity (mg/L)	Hardness (mg/L)	DO (mg/L)	Conductivity (µmho @25ºC)	Total ammonia (mg/L)	unionized ammonia (mg/L)
01B	7.77	78	115	7.20	375	0.303	0.001
01C	7.87	72	112	7.44	367	0.045	0.000
02B	8.14	102	139	6.32	419	0.634	0.004
02C	8.72	151	160	6.52	408	0.473	0.012
03B	8.16	84	128	6.50	402	0.190	0.001
04B	7.96	83	129	6.04	396	0.312	0.001
04C	8.09	76	125	7.04	386	0.171	0.001
05B	8.10	78	121	7.18	388	0.183	0.001
05C	8.36	79	122	7.20	363	0.308	0.004
06B	7.97	81	130	7.02	391	0.331	0.002
06C	8.01	122	124	6.05	396	0.981	0.005
07B	8.10	115	144	7.06	391	0.610	0.004
07C	8.00	80	126	6.80	378	0.684	0.003
08B	7.95	79	120	7.04	386	0.218	0.001
08C	8.04	88	127	6.36	400	0.453	0.002
09B	8.17	111	148	6.44	413	0.902	0.007
09C	8.08	93	148	6.40	402	0.857	0.005
10B	8.15	82	122	7.04	396	0.285	0.002
10C	8.01	83	124	6.86	392	0.268	0.001
11B	8.11	78	119	6.94	386	0.310	0.002
11C	8.16	75	120	7.02	398	0.228	0.002
12B	8.30	83	125	7.05	395	0.228	0.002
12C	8.28	84	125	6.74	394	0.292	0.003
13B	8.06	129	154	5.84	428	1.520	0.009
13C	8.09	101	135	6.24	398	0.808	0.005
14B	8.02	94	135	6.21	403	0.570	0.003
14C	8.09	87	136	6.24	403	0.397	0.002
15B	8.20	78	117	6.88	400	0.160	0.001
15C	7.92	80	122	6.50	385	0.157	0.001
16B	8.15	81	126	6.76	412	0.324	0.002
16C	8.18	85	127	6.72	399	0.350	0.003

Appendix 1.2. Mean measured overlying water quality for the whole-sediment tests with Upper Mississippi river samples (continued).

18B	7.97	86	129	6.00	399	0.480	0.002
18C	8.19	83	144	6.40	391	0.354	0.003
19B	8.10	98	140	6.26	411	0.842	0.005
19C	8.07	94	140	5.92	411	0.593	0.003
20B	8.08	79	127	7.06	388	0.090	0.001
20C	7.97	78	119	7.02	374	0.369	0.002
21B	8.14	85	113	7.04	387	0.346	0.002
21C	8.10	83	124	6.26	395	0.423	0.003
22B	8.13	104	141	6.37	404	1.214	0.008
22C	8.09	94	140	6.24	410	0.587	0.004
24B	8.15	87	133	7.08	381	0.194	0.001
24C	8.14	96	136	6.76	397	0.377	0.003
25B	8.04	74	114	7.02	388	0.129	0.001
25C	8.05	76	116	6.94	385	0.220	0.001
26B	8.06	78	120	6.50	394	0.511	0.003
26C	8.03	76	121	6.54	383	0.254	0.001
SCB	7.74	60	113	6.88	364	0.199	0.001
SCC	7.86	66	111	7.04	359	0.199	0.001
FLOR B	7.71	59	115	7.53	373	0.220	0.001
FLOR C	7.58	71	116	7.24	369	0.091	0.000
Mean	8.07	87	128	6.70	392	0.416	0.003
Max.	8.72	151	160	7.53	428	1.520	0.012
Min.	7.58	59	111	5.84	359	0.090	0.000
Std	0.17	16	11	0.41	14	0.294	0.002
Median	8.09	83	125	6.76	394	0.324	0.002

Appendix 1.3. List of polycyclic aromatic hydrocarbons (PAHs) and organochlorines (OCs) analyzed for in the sediment samples from the Upper Mississippi River.

Polycyclic aromatic hydrocarbons

1. Naphthalene
2. 1-methylnaphthalene
3. 2,6-dimethylnaphthalene
4. Acenaphthene
5. Fluorene
6. Phenathrene
7. 1,-methylphenanthrene
8. Pyrene
9. Chrysene
10. 1,2-Benzanthracene
11. Perylene
12. Indeno(1,2,3-cd)pyrene
13. Benzo(g,h,i)perylene
14. C1-fluorenes
15. C2-fluorenes
16. C3-fluorenes
17. C1-phenanthrenes
18. C2-phenanthrenes
19. C3-phenanthrenes
20. C4-phenanthrenes
21. C1-fluoranthenes+C1-pyrene
22. C2-dibenzothiophenes
23. 2-methylnaphthalene
24. Biphenyl
25. Acenaphthalene
26. 2,3,5-trimethylnaphthalene
27. Dibenzothiophene
28. Anthracene
29. Fluoranthene
30. Benzo(b)fluoranthene
31. Benzo(k)fluoranthene
32. Benzo(e)pyrene
33. Benzo(a)pyrene
34. 1,2,5,6-dibenzanthracene
35. C1-naphthalenes
36. C2-naphthalenes
37. C3-naphthalenes
38. C4-naphthalenes
39. C1-dibenzothiophenes
40. C3-dibenzothiophenes
41. C1-chrysenes
42. C2-chrysenes
43. C3-chrysenes
44. C4-chrysenes

Organochlorines

1. Lindane
2. Heptachlor
3. Aldrin
4. Heptachlor epoxide
5. Chlordane
6. Endo
7. Dieldrin
8. DDE
9. Endrin
10. Perthane
11. DDD
12. DDT
13. Methoxychlor
14. Mirex
15. Toxaphene
16. o.p' DDE

15. HCB
16. alpha BHC
17. beta BHC
18. delta BHC
19. Oxychlordane
20. gamma Chlordane
21. trans-nonachlor
22. PCB 1242
23. PCB 1248
24. PCB 1254
25. PCB 1260
26. alpha Chlordane
27. o.p' DDD
28. cis-nonchlor
29. o,p' DDT

Appendix 1.4. Amphipod length data for the 1st set of sediment samples. Replication (Rep), Animal (individual animal number), and length (mean length for individual animal; n=2 measurements).

Sample	Animal	Rep	Length
ARCH	1	NA	0.946
ARCH	2	NA	1.077
ARCH	3	NA	1.134
ARCH	4	NA	1.092
ARCH	5	NA	0.973
ARCH	6	NA	1.024
ARCH	7	NA	1.122
ARCH	8	NA	1.086
ARCH	9	NA	1.000
ARCH	10	NA	1.051
ARCH	11	NA	1.086
1B	1	A	3.373
1B	2	A	2.522
1B	3	A	3.048
1B	4	A	3.084
1B	5	A	3.610
1B	6	A	3.090
1B	7	A	3.655
1B	8	A	3.265
1B	1	B	3.843
1B	2	B	3.666
1B	3	B	4.348
1B	4	B	3.630
1B	5	B	3.783
1B	6	B	3.765
1B	7	B	4.207
1B	8	B	3.556
1B	9	B	3.846
1B	10	B	3.332
1B	1	C	4.398
1B	2	C	4.889
1B	3	C	3.864
1B	4	C	4.646
1B	5	C	3.409
1B	6	C	4.942
1B	7	C	4.073
1B	8	C	4.883
1B	9	C	4.222
1B	1	D	3.173
1B	2	D	2.925
1B	3	D	2.474
1B	4	D	4.282
1B	5	D	2.752
1B	6	D	3.179
1B	7	D	3.164
1B	8	D	3.472
1B	9	D	3.215
1C	1	A	3.170
1C	2	A	3.610
1C	3	A	3.475
1C	4	A	3.783
1C	5	A	3.669
1C	1	B	2.794
1C	2	B	3.616
1C	3	B	3.102
1C	4	B	4.112
1C	5	B	3.078
1C	6	B	2.946
1C	1	C	2.157
1C	2	C	2.656
1C	3	C	2.943
1C	4	C	2.271
1C	5	C	3.445
1C	6	C	2.695
1C	7	C	2.531
1C	1	D	2.725
1C	2	D	3.433
1C	3	D	4.076
1C	4	D	3.616
1C	5	D	3.454
1C	6	D	2.656
3B	1	A	4.595
3B	2	A	4.456
3B	3	A	4.441
3B	4	A	3.690
3B	1	B	5.497
3B	2	B	4.119
3B	3	B	4.885
3B	4	B	4.985
3B	5	B	4.388
3B	6	B	4.077
3B	7	B	4.607
3B	8	B	4.030
3B	1	C	4.296
3B	2	C	4.118
3B	3	C	4.335
3B	4	C	4.036
3B	5	C	4.935
3B	6	C	4.068
3B	7	C	4.027
3B	8	C	3.879
3B	9	C	3.891
3B	10	C	3.891
3B	11	C	3.923
3B	1	D	4.089
3B	2	D	4.476

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
3B	3	D	4.053	5C	5	B	3.388
3B	4	D	4.181	5C	6	B	3.099
3B	5	D	3.805	5C	1	C	3.822
3B	6	D	3.778	5C	2	C	3.825
3B	7	D	4.867	5C	3	C	3.762
3B	8	D	3.920	5C	4	C	3.750
5B	1	A	3.974	5C	5	C	4.046
5B	2	A	3.825	5C	6	C	3.944
5B	3	A	4.037	5C	7	C	3.995
5B	4	A	4.103	5C	8	C	4.700
5B	5	A	4.754	5C	9	C	3.553
5B	6	A	4.249	5C	1	D	4.667
5B	7	A	4.357	5C	2	D	4.617
5B	8	A	3.669	5C	3	D	4.569
5B	9	A	4.112	5C	4	D	4.327
5B	1	B	4.467	5C	5	D	5.295
5B	2	B	3.965	5C	6	D	4.019
5B	3	B	4.198	5C	7	D	4.431
5B	4	B	3.834	5C	8	D	3.801
5B	5	B	4.524	5C	9	D	4.482
5B	6	B	4.404	8B	1	A	4.443
5B	7	B	4.216	8B	2	A	3.867
5B	8	B	4.070	8B	3	A	3.887
5B	9	B	3.971	8B	4	A	3.517
5B	1	C	4.682	8B	5	A	3.858
5B	2	C	4.088	8B	6	A	4.094
5B	3	C	4.387	8B	7	A	4.046
5B	4	C	4.987	8B	8	A	5.017
5B	5	C	4.216	8B	9	A	4.826
5B	6	C	5.265	8B	10	A	5.098
5B	7	C	3.732	8B	1	B	4.159
5B	1	D	4.422	8B	2	B	3.777
5B	2	D	4.088	8B	3	B	3.622
5B	3	D	4.159	8B	4	B	3.834
5B	4	D	4.261	8B	5	B	4.270
5B	5	D	4.091	8B	6	B	3.669
5B	6	D	4.162	8B	7	B	3.580
5C	1	A	3.251	8B	8	B	3.490
5C	2	A	4.216	8B	9	B	3.242
5C	3	A	4.073	8B	10	B	2.253
5C	4	A	3.230	8B	1	C	3.054
5C	5	A	4.512	8B	2	C	3.654
5C	6	A	5.157	8B	3	C	3.389
5C	7	A	3.765	8B	4	C	3.816
5C	1	B	4.192	8B	5	C	3.455
5C	2	B	3.696	8B	6	C	3.066
5C	3	B	3.974	8B	7	C	3.081
5C	4	B	3.672	8B	8	C	3.837

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
8B	1	D	3.352	10B	5	A	4.694
8B	2	D	3.675	10B	6	A	4.288
8B	3	D	3.151	10B	7	A	3.702
8B	4	D	4.045	10B	8	A	4.733
8B	5	D	3.557	10B	9	A	4.403
8B	6	D	3.374	10B	10	A	3.995
8B	7	D	3.560	10B	1	B	4.440
8B	8	D	2.940	10B	2	B	3.738
8B	9	D	3.922	10B	3	B	3.741
8B	10	D	3.075	10B	4	B	4.415
8B	11	D	3.373	10B	5	B	4.781
8C	1	A	4.283	10B	6	B	3.675
8C	2	A	4.238	10B	7	B	4.161
8C	3	A	3.581	10B	8	B	4.252
8C	4	A	4.027	10B	9	B	5.477
8C	5	A	3.916	10B	1	C	4.025
8C	6	A	2.807	10B	2	C	4.724
8C	7	A	3.367	10B	3	C	3.922
8C	8	A	2.458	10B	4	C	3.665
8C	9	A	4.142	10B	5	C	3.687
8C	1	B	4.241	10B	6	C	4.636
8C	2	B	4.253	10B	7	C	3.696
8C	3	B	3.858	10B	1	D	4.412
8C	4	B	5.099	10B	2	D	6.042
8C	5	B	3.831	10B	3	D	4.155
8C	6	B	3.678	10B	4	D	3.892
8C	1	C	4.136	10B	5	D	4.781
8C	2	C	4.184	10B	6	D	4.512
8C	3	C	4.127	10B	7	D	3.959
8C	4	C	3.467	10B	8	D	4.276
8C	5	C	4.524	10B	9	D	4.739
8C	6	C	3.876	10B	10	D	4.001
8C	7	C	3.461	10C	1	A	3.641
8C	8	C	3.587	10C	2	A	3.829
8C	9	C	4.425	10C	3	A	4.573
8C	1	D	4.460	10C	4	A	4.052
8C	2	D	4.346	10C	5	A	3.989
8C	3	D	4.322	10C	6	A	3.396
8C	4	D	3.654	10C	1	B	3.321
8C	5	D	4.747	10C	2	B	3.944
8C	6	D	4.383	10C	3	B	4.086
8C	7	D	5.024	10C	4	B	3.411
8C	8	D	5.575	10C	5	B	3.647
8C	9	D	5.015	10C	6	B	3.844
10B	1	A	4.594	10C	1	C	3.826
10B	2	A	4.104	10C	2	C	3.575
10B	3	A	3.959	10C	3	C	4.122
10B	4	A	3.632	10C	4	C	4.815

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
10C	5	C	4.691	11C	1	A	3.813
10C	6	C	3.947	11C	2	A	3.095
10C	7	C	3.620	11C	3	A	3.628
10C	8	C	3.638	11C	4	A	3.357
10C	9	C	4.270	11C	5	A	3.741
10C	1	D	3.807	11C	6	A	3.143
10C	2	D	4.255	11C	7	A	3.732
10C	3	D	3.650	11C	1	B	3.741
10C	4	D	3.811	11C	2	B	4.179
10C	5	D	2.858	11C	3	B	3.325
10C	6	D	3.623	11C	4	B	4.107
10C	7	D	4.180	11C	5	B	3.497
10C	8	D	3.547	11C	1	C	3.280
11B	1	A	4.589	11C	2	C	3.664
11B	2	A	4.051	11C	3	C	3.571
11B	3	A	4.333	11C	4	C	4.146
11B	4	A	4.164	11C	5	C	4.122
11B	5	A	4.152	11C	6	C	3.652
11B	6	A	4.262	11C	1	D	3.315
11B	7	A	4.066	11C	2	D	3.717
11B	8	A	3.950	11C	3	D	3.688
11B	1	B	3.828	11C	4	D	3.574
11B	2	B	4.220	11C	5	D	3.057
11B	3	B	5.000	12B	1	A	4.003
11B	4	B	3.768	12B	2	A	4.275
11B	5	B	4.119	12B	3	A	4.095
11B	6	B	4.244	12B	4	A	3.586
11B	7	B	3.408	12B	5	A	3.414
11B	8	B	3.661	12B	6	A	3.837
11B	9	B	4.235	12B	7	A	2.515
11B	1	C	4.357	12B	8	A	3.154
11B	2	C	4.057	12B	9	A	3.870
11B	3	C	4.878	12B	1	B	3.210
11B	4	C	4.351	12B	2	B	2.669
11B	5	C	5.122	12B	3	B	3.447
11B	6	C	4.408	12B	4	B	3.678
11B	7	C	4.351	12B	5	B	2.964
11B	8	C	5.006	12B	6	B	3.039
11B	9	C	5.116	12B	7	B	3.873
11B	1	D	3.479	12B	8	B	3.769
11B	2	D	4.116	12B	9	B	3.876
11B	3	D	4.661	12B	1	C	3.453
11B	4	D	4.432	12B	2	C	3.755
11B	5	D	4.577	12B	3	C	3.293
11B	6	D	4.360	12B	4	C	3.036
11B	7	D	5.027	12B	5	C	3.494
11B	8	D	4.137	12B	6	C	3.335
11B	9	D	4.217	12B	7	C	3.512

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
12B	1	D	3.663	15B	5	A	3.293
12B	2	D	3.352	15B	6	A	3.801
12B	3	D	2.808	15B	7	A	3.341
12B	4	D	3.565	15B	8	A	3.968
12B	5	D	3.988	15B	9	A	4.216
12B	6	D	3.293	15B	1	B	3.873
12B	7	D	3.518	15B	2	B	4.736
12C	1	A	4.236	15B	3	B	3.813
12C	2	A	4.233	15B	4	B	3.699
12C	3	A	4.209	15B	5	B	3.995
12C	4	A	3.249	15B	6	B	4.401
12C	5	A	4.518	15B	7	B	4.533
12C	6	A	4.155	15B	8	B	4.073
12C	7	A	3.051	15B	1	C	3.364
12C	8	A	3.738	15B	2	C	3.811
12C	9	A	4.224	15B	3	C	3.485
12C	1	B	4.026	15B	4	C	4.006
12C	2	B	3.477	15B	5	C	4.003
12C	3	B	3.813	15B	6	C	2.624
12C	4	B	4.353	15B	7	C	3.926
12C	5	B	3.669	15B	8	C	4.036
12C	6	B	4.242	15B	9	C	3.953
12C	7	B	3.711	15B	1	D	3.281
12C	8	B	4.506	15B	2	D	3.355
12C	9	B	3.033	15B	3	D	3.444
12C	10	B	3.594	15B	4	D	3.447
12C	1	C	3.960	15B	5	D	3.267
12C	2	C	3.615	15B	6	D	3.314
12C	3	C	4.062	15B	7	D	3.550
12C	4	C	3.798	15B	8	D	3.494
12C	5	C	3.972	15C	1	A	3.169
12C	6	C	4.110	15C	2	A	2.908
12C	7	C	2.748	15C	3	A	3.033
12C	8	C	4.062	15C	4	A	4.287
12C	1	D	3.639	15C	1	B	4.240
12C	2	D	3.705	15C	2	B	3.278
12C	3	D	3.408	15C	3	B	3.497
12C	4	D	3.327	15C	4	B	3.391
12C	5	D	3.249	15C	5	B	3.796
12C	6	D	3.831	15C	6	B	4.219
12C	7	D	3.240	15C	7	B	4.459
12C	8	D	4.083	15C	1	C	4.932
12C	9	D	3.444	15C	2	C	4.764
12C	10	D	3.681	15C	3	C	3.698
15B	1	A	3.801	15C	4	C	3.352
15B	2	A	4.273	15C	5	C	3.538
15B	3	A	3.870	15C	6	C	3.355
15B	4	A	3.054	15C	7	C	3.113

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length
15C	8	C	4.077
15C	1	D	3.234
15C	2	D	3.589
15C	3	D	3.059
15C	4	D	3.391
15C	5	D	3.793
15C	6	D	3.166
15C	7	D	3.722
15C	8	D	3.056
15C	9	D	3.204
16B	1	A	3.196
16B	2	A	3.507
16B	3	A	3.547
16B	4	A	4.435
16B	5	A	3.302
16B	6	A	3.485
16B	7	A	3.581
16B	8	A	3.245
16B	9	A	2.920
16B	1	B	3.097
16B	2	B	3.838
16B	3	B	3.941
16B	4	B	4.510
16B	5	B	3.072
16B	1	C	4.078
16B	2	C	3.889
16B	3	C	3.805
16B	4	C	3.625
16B	5	C	4.134
16B	6	C	3.917
16B	1	D	4.280
16B	2	D	4.230
16B	3	D	3.699
16B	4	D	3.733
16B	5	D	3.764
16B	6	D	3.864
16C	1	A	4.261
16C	2	A	3.494
16C	3	A	4.469
16C	4	A	3.901
16C	5	A	3.929
16C	6	A	2.770
16C	1	B	4.581
16C	2	B	3.327
16C	3	B	3.777
16C	4	B	3.907
16C	5	B	3.578
16C	6	B	4.009
16C	7	B	3.612
16C	8	B	3.755
16C	9	B	3.590
16C	1	C	3.497
16C	2	C	3.503
16C	3	C	3.308
16C	4	C	4.084
16C	5	C	3.330
16C	6	C	3.637
16C	7	C	3.991
16C	8	C	3.376
16C	9	C	3.951
16C	1	C	3.376
16C	1	D	3.851
16C	2	D	3.680
16C	3	D	3.730
16C	4	D	3.541
16C	5	D	4.438
16C	6	D	4.364
16C	7	D	4.659
16C	8	D	4.165
16C	9	D	4.308
16C	10	D	4.395
16C	11	D	3.826
21B	1	A	3.280
21B	2	A	3.224
21B	3	A	3.301
21B	4	A	3.916
21B	5	A	3.200
21B	6	A	3.999
21B	7	A	3.107
21B	8	A	3.483
21B	9	A	2.872
21B	10	A	3.265
21B	1	B	3.283
21B	2	B	3.319
21B	3	B	3.030
21B	4	B	3.781
21B	5	B	4.160
21B	6	B	4.178
21B	7	B	2.809
21B	8	B	3.775
21B	9	B	3.856
21B	1	C	3.480
21B	2	C	3.161
21B	3	C	3.808
21B	4	C	3.579
21B	5	C	4.056

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
21B	6	C	3.501	25B	1	A	2.579
21B	7	C	3.960	25B	2	A	4.374
21B	8	C	3.579	25B	3	A	3.433
21B	1	D	3.611	25B	4	A	3.347
21B	2	D	2.887	25B	5	A	3.735
21B	3	D	3.140	25B	6	A	2.955
21B	4	D	3.632	25B	1	B	3.777
21B	5	D	2.565	25B	2	B	3.726
21B	6	D	3.543	25B	3	B	3.840
21B	7	D	3.811	25B	4	B	3.735
21B	8	D	3.012	25B	5	B	4.225
21B	9	D	3.537	25B	6	B	4.237
21B	10	D	3.254	25B	7	B	4.129
21C	1	A	4.595	25B	1	C	3.816
21C	2	A	3.895	25B	2	C	2.854
21C	3	A	4.476	25B	3	C	3.675
21C	4	A	3.397	25B	4	C	4.094
21C	5	A	4.512	25B	5	C	3.923
21C	6	A	3.343	25B	1	D	3.837
21C	7	A	4.509	25B	2	D	2.561
21C	8	A	3.850	25B	3	D	3.565
21C	9	A	3.808	25B	4	D	3.430
21C	1	B	3.069	25B	5	D	2.976
21C	2	B	3.358	25C	1	A	2.952
21C	3	B	2.863	25C	2	A	3.120
21C	4	B	3.295	25C	3	A	3.953
21C	5	B	3.069	25C	4	A	3.831
21C	6	B	3.671	25C	5	A	4.052
21C	7	B	3.110	25C	6	A	3.834
21C	8	B	3.376	25C	7	A	3.729
21C	1	C	3.865	25C	8	A	3.093
21C	2	C	4.539	25C	1	B	5.053
21C	3	C	4.109	25C	2	B	3.920
21C	4	C	4.366	25C	1	C	3.547
21C	5	C	4.921	25C	2	C	3.487
21C	6	C	2.920	25C	3	C	3.469
21C	7	C	3.901	25C	4	C	3.299
21C	8	C	3.620	25C	5	C	3.215
21C	1	D	4.047	25C	6	C	3.681
21C	2	D	4.273	25C	7	C	3.356
21C	3	D	3.373	25C	8	C	3.108
21C	4	D	4.545	25C	9	C	3.571
21C	5	D	3.987	25C	10	C	3.448
21C	6	D	3.957	25C	1	D	3.645
21C	7	D	4.643	25C	2	D	4.494
21C	8	D	4.094	25C	3	D	3.344
21C	9	D	4.050	25C	4	D	3.538
21C	10	D	4.088	25C	5	D	3.729

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
25C	6	D	3.266	26C	10	A	2.829
25C	7	D	3.648	26C	1	B	2.764
25C	8	D	3.887	26C	2	B	3.267
25C	9	D	3.953	26C	3	B	3.084
26B	1	A	3.090	26C	4	B	2.713
26B	2	A	3.837	26C	5	B	2.875
26B	3	A	3.750	26C	6	B	3.171
26B	4	A	3.642	26C	7	B	3.006
26B	5	A	4.744	26C	1	C	2.761
26B	6	A	4.735	26C	2	C	2.686
26B	7	A	4.163	26C	3	C	2.731
26B	8	A	3.319	26C	4	C	2.883
26B	9	A	4.072	26C	5	C	2.632
26B	10	A	4.603	26C	6	C	2.680
26B	1	B	3.883	26C	1	D	3.204
26B	2	B	4.253	26C	2	D	3.012
26B	3	B	2.313	26C	3	D	2.665
26B	4	B	3.762	26C	4	D	2.620
26B	5	B	3.139	26C	5	D	2.593
26B	6	B	3.536	26C	6	D	2.764
26B	7	B	3.825	26C	7	D	2.964
26B	8	B	4.184	26C	8	D	3.054
26B	1	C	3.072	26C	9	D	2.958
26B	2	C	2.958	26C	10	D	2.255
26B	3	C	3.293	FLOR	1	A	2.734
26B	4	C	2.922	FLOR	2	A	4.479
26B	5	C	3.338	FLOR	3	A	3.796
26B	6	C	3.039	FLOR	4	A	3.362
26B	7	C	3.533	FLOR	5	A	4.323
26B	8	C	2.961	FLOR	6	A	3.401
26B	9	C	3.003	FLOR	7	A	3.826
26B	1	D	2.946	FLOR	1	B	4.156
26B	2	D	3.740	FLOR	2	B	3.087
26B	3	D	3.434	FLOR	3	B	3.237
26B	4	D	3.219	FLOR	4	B	3.278
26B	5	D	3.084	FLOR	5	B	4.587
26B	6	D	2.931	FLOR	6	B	2.455
26B	7	D	2.925	FLOR	7	B	3.332
26B	8	D	3.344	FLOR	8	B	3.434
26C	1	A	2.701	FLOR	1	C	2.731
26C	2	A	3.018	FLOR	2	C	1.338
26C	3	A	3.039	FLOR	3	C	1.976
26C	4	A	2.832	FLOR	4	C	3.683
26C	5	A	2.659	FLOR	5	C	3.668
26C	6	A	3.341	FLOR	6	C	2.973
26C	7	A	3.063	FLOR	7	C	1.868
26C	8	A	3.012	FLOR	1	D	4.341
26C	9	A	3.126	FLOR	2	D	3.790

Appendix 1.4. Amphipod length data for the 1st set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
FLOR	3	D	3.644	FLOR	7	D	3.976
FLOR	4	D	4.072	FLOR	8	D	4.231
FLOR	5	D	2.578	FLOR	9	D	1.967
FLOR	6	D	4.760				

Appendix 1.5. Amphipod length data for the 2nd set of samples. Replication (Rep), Animal (individual animal number), and length (mean length for individual animal).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
ARCH	1	NA	1.339	2C	6	B	3.066
ARCH	2	NA	1.369	2C	7	B	3.475
ARCH	3	NA	1.193	2C	8	B	3.200
ARCH	4	NA	1.178	2C	9	B	2.755
ARCH	5	NA	1.101	2C	10	B	3.956
ARCH	6	NA	1.107	2C	1	C	2.848
ARCH	7	NA	1.021	2C	2	C	3.938
ARCH	8	NA	1.056	2C	3	C	4.109
ARCH	9	NA	1.134	2C	4	C	3.884
ARCH	10	NA	1.196	2C	5	C	3.514
2B	1	A	4.177	2C	6	C	3.804
2B	2	A	3.556	2C	7	C	4.046
2B	3	A	3.890	2C	1	D	4.593
2B	4	A	4.572	2C	2	D	3.093
2B	5	A	4.001	2C	3	D	2.352
2B	6	A	3.920	2C	4	D	4.001
2B	7	A	4.467	2C	5	D	3.968
2B	8	A	4.195	2C	6	D	4.443
2B	1	B	4.461	2C	7	D	3.117
2B	2	B	4.491	2C	8	D	4.234
2B	3	B	3.729	2C	9	D	4.243
2B	4	B	4.246	4B	1	A	3.616
2B	5	B	4.622	4B	2	A	4.485
2B	6	B	3.502	4B	3	A	4.467
2B	7	B	4.718	4B	4	A	3.174
2B	8	B	3.421	4B	1	B	3.337
2B	1	C	5.184	4B	2	B	3.243
2B	2	C	3.980	4B	3	B	3.295
2B	3	C	5.558	4B	4	B	2.579
2B	4	C	4.213	4B	5	B	3.391
2B	1	D	2.874	4B	6	B	3.343
2B	2	D	4.548	4B	7	B	4.148
2B	3	D	3.027	4B	8	B	2.841
2B	4	D	3.711	4B	1	C	3.415
2B	5	D	3.568	4B	2	C	3.457
2B	6	D	4.040	4B	3	C	3.270
2B	7	D	3.777	4B	4	C	3.682
2B	8	D	3.436	4B	5	C	3.944
2C	1	A	3.108	4B	6	C	2.291
2C	2	A	2.898	4B	7	C	3.433
2C	3	A	2.737	4B	8	C	3.388
2C	4	A	2.880	4B	9	C	4.413
2C	5	A	3.102	4B	1	D	2.600
2C	1	B	3.096	4B	2	D	2.444
2C	2	B	3.442	4B	3	D	3.860
2C	3	B	3.359	4B	4	D	4.205
2C	4	B	3.403	4B	5	D	3.240
2C	5	B	2.764	4B	6	D	2.850

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
4B	7	D	3.502	6B	6	C	4.360
4B	8	D	3.213	6B	7	C	3.045
4B	9	D	2.714	6B	8	C	3.200
4B	10	D	3.821	6B	9	C	3.242
4B	11	D	3.478	6B	10	C	3.831
4B	12	D	2.868	6B	1	D	2.943
4B	13	D	2.922	6B	2	D	3.311
4B	14	D	3.785	6B	3	D	3.935
4C	1	B	3.024	6B	4	D	4.634
4C	2	B	2.744	6B	5	D	3.553
4C	3	B	2.594	6B	6	D	2.994
4C	4	B	3.661	6B	7	D	3.335
4C	5	B	2.802	6B	8	D	3.777
4C	6	B	3.195	6C	1	A	3.568
4C	7	B	2.934	6C	2	A	3.819
4C	8	B	3.556	6C	3	A	2.943
4C	9	B	3.562	6C	4	A	4.204
4C	10	B	3.234	6C	5	A	4.336
4C	11	B	3.439	6C	6	A	5.462
4C	1	C	3.093	6C	7	A	3.027
4C	2	C	3.030	6C	8	A	4.608
4C	3	C	4.269	6C	9	A	4.019
4C	4	C	3.036	6C	1	B	4.790
4C	5	C	3.039	6C	2	B	4.775
4C	6	C	3.986	6C	3	B	4.183
4C	7	C	3.830	6C	4	B	3.870
4C	8	C	3.144	6C	5	B	4.688
4C	1	D	3.493	6C	6	B	4.760
4C	2	D	3.758	6C	7	B	4.617
4C	3	D	4.323	6C	8	B	3.855
4C	4	D	3.721	6C	1	C	3.583
4C	5	D	3.427	6C	2	C	4.682
4C	6	D	3.195	6C	3	C	3.439
4C	7	D	3.090	6C	4	C	3.317
6B	1	A	3.317	6C	5	C	4.969
6B	2	A	3.508	6C	6	C	3.601
6B	3	A	3.499	6C	7	C	3.589
6B	4	A	3.326	6C	8	C	4.115
6B	5	A	3.132	6C	1	D	3.765
6B	6	A	3.493	6C	2	D	3.911
6B	7	A	2.949	6C	3	D	3.622
6B	1	B	3.024	6C	4	D	3.941
6B	2	B	3.908	6C	5	D	4.318
6B	1	C	3.132	6C	6	D	4.464
6B	2	C	3.636	6C	7	D	4.085
6B	3	C	4.013	6C	8	D	3.657
6B	4	C	4.443	7B	1	A	3.886
6B	5	C	3.741	7B	2	A	3.844

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
7B	3	A	3.605	7C	2	B	3.593
7B	4	A	3.725	7C	3	B	3.889
7B	5	A	3.635	7C	4	B	3.859
7B	6	A	3.695	7C	5	B	3.546
7B	7	A	3.361	7C	6	B	4.172
7B	8	A	4.294	7C	7	B	4.232
7B	9	A	3.537	7C	8	B	3.304
7B	10	A	4.127	7C	9	B	3.895
7B	1	B	3.263	7C	10	B	4.348
7B	2	B	3.185	7C	1	C	3.507
7B	3	B	3.835	7C	2	C	3.450
7B	4	B	3.447	7C	3	C	4.578
7B	5	B	3.263	7C	4	C	3.725
7B	6	B	3.587	7C	5	C	3.701
7B	7	B	4.262	7C	6	C	3.435
7B	8	B	3.832	7C	7	C	3.656
7B	9	B	3.158	7C	8	C	3.534
7B	10	B	3.641	7C	9	C	3.898
7B	1	C	3.549	7C	1	D	3.602
7B	2	C	3.531	7C	2	D	3.743
7B	3	C	3.531	7C	3	D	4.005
7B	4	C	3.140	7C	4	D	3.925
7B	5	C	3.087	7C	5	D	3.811
7B	6	C	3.084	7C	6	D	3.671
7B	7	C	3.486	7C	7	D	3.352
7B	8	C	3.110	7C	8	D	3.307
7B	9	C	4.411	7C	9	D	3.477
7B	10	C	3.570	7C	10	D	3.632
7B	1	D	4.470	7C	11	D	1.813
7B	2	D	4.136	9B	1	A	4.027
7B	3	D	4.238	9B	2	A	4.675
7B	4	D	4.050	9B	3	A	4.033
7B	5	D	3.543	9B	4	A	3.801
7B	6	D	3.707	9B	5	A	3.178
7B	7	D	3.638	9B	6	A	3.587
7B	8	D	3.948	9B	7	A	3.443
7B	9	D	3.543	9B	1	B	3.301
7B	10	D	3.590	9B	2	B	3.398
7C	1	A	4.405	9B	3	B	5.358
7C	2	A	4.160	9B	4	B	4.527
7C	3	A	3.283	9B	5	B	3.346
7C	4	A	3.659	9B	1	C	3.214
7C	5	A	3.468	9B	2	C	3.455
7C	6	A	3.090	9B	3	C	3.705
7C	7	A	4.005	9B	4	C	3.844
7C	8	A	3.361	9B	5	C	3.870
7C	9	A	3.987	9B	6	C	3.654
7C	1	B	4.178	9B	7	C	3.843

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
9B	8	C	3.772	13B	1	C	4.581
9B	9	C	3.352	13B	2	C	3.388
9B	10	C	3.566	13B	3	C	4.079
9B	11	C	3.461	13B	4	C	3.308
9B	1	D	3.662	13B	5	D	3.006
9B	2	D	3.759	13B	1	D	2.766
9B	3	D	2.822	13B	2	D	3.970
9B	4	D	3.304	13B	3	D	3.635
9B	5	D	4.416	13B	4	D	3.701
9B	6	D	3.753	13C	1	A	3.968
9B	7	D	3.699	13C	2	A	3.553
9B	8	D	3.361	13C	3	A	3.460
9C	1	A	2.913	13C	1	B	3.364
9C	2	A	2.976	13C	2	B	3.290
9C	3	A	3.666	13C	3	B	3.905
9C	4	A	4.283	13C	4	B	4.138
9C	1	B	4.093	13C	5	B	3.759
9C	2	B	3.843	13C	6	B	3.977
9C	3	B	3.374	13C	1	C	2.940
9C	4	B	4.202	13C	2	C	3.448
9C	5	B	4.081	13C	3	C	2.851
9C	6	B	3.708	13C	4	C	4.643
9C	7	B	4.280	13C	5	C	3.565
9C	8	B	4.018	13C	6	C	2.737
9C	1	C	3.072	13C	1	D	3.478
9C	2	C	3.229	13C	2	D	3.565
9C	3	C	3.334	13C	3	D	3.451
9C	4	C	3.982	14B	1	A	3.092
9C	5	C	3.681	14B	2	A	3.693
9C	6	C	3.509	14B	3	A	3.503
9C	7	C	3.178	14B	4	A	3.235
9C	8	C	3.340	14B	5	A	4.714
9C	9	C	3.566	14B	6	A	3.271
9C	10	C	3.692	14B	7	A	3.024
9C	11	C	3.162	14B	1	B	4.211
9C	12	C	2.825	14B	2	B	3.429
9C	13	C	3.289	14B	3	B	4.274
9C	14	C	3.656	14B	4	B	4.482
9C	15	C	4.075	14B	5	B	3.786
9C	16	C	4.256	14B	6	B	4.092
9C	1	D	4.100	14B	7	B	5.723
9C	2	D	3.991	14B	1	C	3.542
9C	3	D	3.256	14B	2	C	3.408
9C	4	D	4.142	14B	3	C	3.780
13B	1	A	5.304	14B	4	C	2.872
13B	2	A	4.062	14B	5	C	4.452
13B	1	B	4.652	14B	6	C	4.036
13B	2	B	3.870	14B	1	D	3.934

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
14B	2	D	3.173	18B	3	D	3.534
14B	3	D	3.756	18B	4	D	3.618
14B	4	D	3.863	18B	5	D	3.693
14B	5	D	4.821	18C	1	A	3.608
14B	6	D	4.036	18C	2	A	3.310
14B	7	D	3.863	18C	3	A	3.841
14C	1	A	3.434	18C	4	A	2.696
14C	2	A	3.066	18C	5	A	3.412
14C	3	A	4.009	18C	6	A	4.169
14C	4	A	3.268	18C	7	A	3.793
14C	5	A	4.053	18C	8	A	3.552
14C	6	A	3.426	18C	9	A	2.767
14C	1	B	2.568	18C	1	B	2.857
14C	2	B	3.009	18C	2	B	3.823
14C	3	B	2.640	18C	3	B	4.601
14C	4	B	2.631	18C	4	B	3.626
14C	5	B	3.765	18C	5	B	3.304
14C	6	B	4.054	18C	1	C	3.444
14C	1	C	3.527	18C	2	C	3.578
14C	2	C	4.161	18C	3	C	4.178
14C	3	C	3.134	18C	4	C	2.994
14C	4	C	3.946	18C	5	C	3.414
14C	1	D	3.934	18C	6	C	3.868
14C	2	D	3.845	18C	7	C	2.896
14C	3	D	4.077	18C	8	C	3.728
18B	1	A	3.816	18C	9	C	3.155
18B	2	A	3.375	18C	10	C	3.364
18B	3	A	2.355	18C	1	D	3.904
18B	4	A	4.167	19B	1	A	4.054
18B	5	A	3.978	19B	2	A	3.348
18B	6	A	3.000	19B	3	A	3.872
18B	7	A	2.559	19B	4	A	3.339
18B	1	B	4.803	19B	5	A	3.304
18B	2	B	3.636	19B	6	A	3.167
18B	3	B	2.898	19B	7	A	3.146
18B	4	B	3.681	19B	1	B	3.348
18B	5	B	3.738	19B	2	B	4.214
18B	6	B	4.503	19B	3	B	2.450
18B	7	B	4.020	19B	4	B	3.455
18B	1	C	3.618	19B	5	B	2.938
18B	2	C	2.664	19B	6	B	3.923
18B	3	C	3.711	19B	7	B	3.506
18B	4	C	3.579	19B	8	B	3.726
18B	5	C	3.570	19B	9	B	3.173
18B	6	C	2.946	19B	1	C	3.774
18B	7	C	2.928	19B	2	C	3.851
18B	1	D	4.065	19B	3	C	3.378
18B	2	D	4.434	19B	4	C	3.295

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length
19B	5	C	2.818
19B	6	C	3.711
19B	7	C	2.970
19B	8	C	3.205
19B	1	D	3.009
19B	2	D	3.304
19B	3	D	3.319
19B	4	D	3.015
19B	5	D	2.774
19B	6	D	3.089
19B	7	D	3.545
19B	8	D	2.616
19B	9	D	2.836
19B	10	D	2.917
19C	1	A	3.893
19C	2	A	3.792
19C	3	A	3.238
19C	4	A	2.929
19C	5	A	3.581
19C	6	A	2.515
19C	7	A	3.512
19C	8	A	4.137
19C	1	B	3.631
19C	2	B	3.777
19C	3	B	2.997
19C	4	B	3.176
19C	5	B	3.875
19C	6	B	2.979
19C	1	C	3.646
19C	2	C	3.256
19C	3	C	3.435
19C	4	C	3.955
19C	5	C	3.438
19C	6	C	3.307
19C	7	C	3.149
19C	8	C	3.720
19C	1	D	2.920
19C	2	D	3.845
19C	3	D	3.366
19C	4	D	3.494
19C	5	D	3.173
19C	6	D	3.485
20B	1	A	3.630
20B	2	A	3.580
20B	3	A	4.004
20B	4	A	3.711
20B	5	A	3.834
20B	6	A	3.884
20B	7	A	3.454
20B	8	A	2.940
20B	1	B	3.433
20B	2	B	3.663
20B	3	B	3.577
20B	4	B	3.837
20B	5	B	3.269
20B	6	B	3.317
20B	7	B	3.768
20B	8	B	3.281
20B	9	B	4.132
20B	10	B	3.995
20B	1	C	3.122
20B	2	C	2.510
20B	3	C	2.854
20B	4	C	4.183
20B	5	C	3.155
20B	6	C	3.442
20B	7	C	3.380
20B	8	C	3.353
20B	1	D	2.582
20B	2	D	2.872
20B	3	D	2.883
20B	4	D	3.433
20B	5	D	3.738
20B	6	D	3.030
20C	1	A	3.669
20C	2	A	3.807
20C	3	A	3.454
20C	4	A	4.138
20C	5	A	3.403
20C	6	A	3.750
20C	7	A	3.634
20C	8	A	3.030
20C	9	A	3.466
20C	1	B	3.370
20C	2	B	3.379
20C	3	B	2.794
20C	4	B	3.466
20C	5	B	3.364
20C	6	B	3.547
20C	7	B	2.749
20C	8	B	3.484
20C	9	B	3.457
20C	1	C	3.472
20C	2	C	2.994
20C	3	C	3.374
20C	4	C	3.320

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
20C 5	C	3.350		22B 6	D	3.873	
20C 6	C	3.418		22C 1	A	3.914	
20C 7	C	3.143		22C 2	A	4.025	
20C 8	C	3.021		22C 3	A	4.679	
20C 9	C	3.780		22C 1	B	3.335	
20C 1	D	2.979		22C 2	B	3.813	
20C 2	D	2.964		22C 3	B	3.126	
20C 3	D	3.230		22C 4	B	3.063	
20C 4	D	3.185		22C 5	B	3.598	
20C 5	D	3.388		22C 6	B	3.601	
20C 6	D	2.522		22C 7	B	2.674	
20C 7	D	3.182		22C 8	B	3.935	
20C 8	D	3.015		22C 1	C	3.457	
20C 9	D	3.221		22C 2	C	3.998	
20C 10	D	2.564		22C 3	C	3.427	
22B 1	A	3.586		22C 4	C	2.755	
22B 2	A	3.708		22C 5	C	3.391	
22B 3	A	3.786		22C 1	D	3.610	
22B 4	A	4.004		22C 2	D	3.227	
22B 5	A	3.063		22C 3	D	4.144	
22B 6	A	3.550		22C 4	D	3.436	
22B 7	A	3.350		22C 5	D	4.856	
22B 8	A	4.121		22C 6	D	3.311	
22B 9	A	3.415		22C 7	D	4.252	
22B 10	A	4.778		24B 1	A	3.925	
22B 1	B	5.253		24B 2	A	2.958	
22B 2	B	4.351		24B 3	A	3.886	
22B 3	B	3.666		24B 4	A	3.617	
22B 4	B	3.732		24B 5	A	3.533	
22B 5	B	3.281		24B 6	A	3.781	
22B 6	B	3.173		24B 7	A	3.434	
22B 7	B	3.885		24B 8	A	4.302	
22B 8	B	3.290		24B 1	B	2.263	
22B 9	B	2.848		24B 2	B	3.165	
22B 1	C	3.188		24B 3	B	3.820	
22B 2	C	3.639		24B 4	B	4.159	
22B 3	C	4.760		24B 5	B	4.108	
22B 4	C	3.726		24B 6	B	3.536	
22B 5	C	4.527		24B 7	B	4.054	
22B 6	C	4.258		24B 8	B	3.599	
22B 7	C	4.978		24B 9	B	3.329	
22B 8	C	2.934		24B 1	C	3.293	
22B 9	C	3.729		24B 2	C	4.045	
22B 1	D	3.595		24B 3	C	4.362	
22B 2	D	3.753		24B 4	C	2.931	
22B 3	D	3.556		24B 5	C	3.012	
22B 4	D	3.917		24B 6	C	3.707	
22B 5	D	3.636		24B 7	C	3.922	

Appendix 1.5. Amphipod length data for the 2nd set of sediment samples (continued).

Sample	Animal	Rep	Length	Sample	Animal	Rep	Length
24B 8	C	3.189		SCB 5	C	2.784	
24B 9	C	3.257		SCB 6	C	2.651	
24B 1	D	3.862		SCB 7	C	3.661	
24B 2	D	3.877		SCB 1	D	3.605	
24B 3	D	3.563		SCB 2	D	3.708	
24B 4	D	3.931		SCB 3	D	4.795	
24B 5	D	3.054		SCB 4	D	3.343	
24B 6	D	3.060		SCB 5	D	3.352	
24B 7	D	3.653		SCB 6	D	2.955	
24B 8	D	3.722		SCC 1	A	3.373	
24B 9	D	4.311		SCC 2	A	2.286	
24C 1	A	3.877		SCC 3	A	2.506	
24C 2	A	3.750		SCC 4	A	2.973	
24C 3	A	4.007		SCC 5	A	2.678	
24C 4	A	3.490		SCC 6	A	3.051	
24C 5	A	2.985		SCC 7	A	3.331	
24C 6	A	3.414		SCC 8	A	3.238	
24C 7	A	3.481		SCC 1	B	3.259	
24C 8	A	3.980		SCC 2	B	2.955	
24C 1	B	2.822		SCC 3	B	3.346	
24C 2	B	3.820		SCC 4	B	3.563	
24C 3	B	3.841		SCC 5	B	3.518	
24C 4	B	4.542		SCC 6	B	2.952	
24C 5	B	3.611		SCC 7	B	3.111	
24C 6	B	4.836		SCC 8	B	2.961	
24C 1	C	3.157		SCC 1	C	2.789	
24C 2	C	3.859		SCC 2	C	2.584	
24C 3	C	3.505		SCC 3	C	2.732	
24C 4	C	3.832		SCC 4	C	2.753	
24C 5	C	3.756		SCC 5	C	2.937	
24C 6	C	3.139		SCC 6	C	2.861	
24C 1	D	4.001		SCC 7	C	2.855	
24C 2	D	5.571		SCC 8	C	2.922	
24C 3	D	3.387		SCC 9	C	3.352	
24C 4	D	4.083		SCC 10	C	2.404	
SCB 1	A	3.794		SCC 1	D	2.725	
SCB 2	A	3.490		SCC 2	D	3.275	
SCB 3	A	3.758		SCC 3	D	3.359	
SCB 4	A	3.171		SCC 4	D	3.741	
SCB 1	B	3.748		SCC 5	D	3.648	
SCB 2	B	3.736		SCC 6	D	2.537	
SCB 3	B	2.994		SCC 7	D	2.523	
SCB 4	B	3.239		SCC 8	D	3.143	
SCB 5	B	3.393		SCC 9	D	3.648	
SCB 1	C	3.325		FLOR1	A	2.334	
SCB 2	C	2.933		FLOR2	A	2.522	
SCB 3	C	3.319		FLOR3	A	2.256	
SCB 4	C	3.583		FLOR4	A	2.561	

FLOR	5	A	2.624
FLOR	6	A	2.546
FLOR	7	A	1.743
FLOR	8	A	1.960
FLOR	9	A	4.070
FLOR	1	B	2.394
FLOR	2	B	1.978
FLOR	3	B	2.913
FLOR	4	B	2.949
FLOR	5	B	2.650
FLOR	6	B	2.850
FLOR	7	B	2.901
FLOR	8	B	3.436
FLOR	9	B	2.268
FLOR	10	B	2.531
FLOR	1	C	3.320
FLOR	2	C	2.328
FLOR	3	C	3.230
FLOR	4	C	3.606
FLOR	5	C	2.444
FLOR	6	C	2.916
FLOR	7	C	2.866
FLOR	8	C	2.761
FLOR	1	D	2.113
FLOR	2	D	1.909
FLOR	3	D	2.576
FLOR	4	D	1.790
FLOR	5	D	2.283
FLOR	6	D	2.360
FLOR	7	D	2.522
FLOR	8	D	3.347
FLOR	9	D	2.668
FLOR	10	D	2.059
FLOR	11	D	1.981

Appendix 1.6. Amphipod maturation and survival data for the 1st set of samples. Replication (Rep), number of amphipods recovered (Recov), and number of males recovered (Males).

Sample	Rep	Recov	Males	Sample	Rep	Recov	Males
01B	1	8	3	12C	1	9	4
01B	2	10	3	12C	2	10	2
01B	3	9	5	12C	3	8	2
01B	4	9	3	12C	4	10	4
01C	1	5	1	15B	1	9	3
01C	2	6	0	15B	2	8	6
01C	3	7	1	15B	3	9	6
01C	4	6	2	15B	4	10	3
03B	1	4	1	15C	1	7	1
03B	2	8	3	15C	2	7	2
03B	3	11	6	15C	3	8	3
03B	4	8	5	15C	4	9	5
05B	1	9	2	16B	1	9	2
05B	2	9	6	16B	2	5	2
05B	3	7	4	16B	3	6	3
05B	4	6	2	16B	4	6	3
05C	1	7	1	16C	1	6	1
05C	2	6	1	16C	2	9	1
05C	3	9	3	16C	3	9	5
05C	4	9	2	16C	4	11	4
08B	1	10	5	21B	1	10	5
08B	2	10	2	21B	2	9	4
08B	3	8	3	21B	3	9	4
08B	4	11	6	21B	4	10	7
08C	1	9	3	21C	1	9	5
08C	2	6	3	21C	2	8	3
08C	3	8	1	21C	3	8	5
08C	4	9	3	21C	4	10	5
10B	1	10	5	25B	1	6	1
10B	2	9	2	25B	2	7	2
10B	3	7	6	25B	3	5	0
10B	4	10	0	25B	4	4	2
10C	1	6	3	25C	1	8	2
10C	2	6	1	25C	2	2	1
10C	3	9	3	25C	3	10	1
10C	4	8	3	25C	4	9	3
11B	1	7	2	26B	1	10	5
11B	2	9	3	26B	2	8	2
11B	3	9	3	26B	3	9	5
11B	4	9	7	26B	4	8	3
11C	1	7	1	26C	1	10	7
11C	2	5	4	26C	2	7	2
11C	3	6	1	26C	3	6	4
11C	4	5	1	26C	4	10	3
12B	1	9	3	FLB	1	6	2
12B	2	9	3	FLB	2	8	5
12B	3	7	2	FLB	3	7	2
12B	4	7	3	FLB	4	9	2

Appendix 1.7. Amphipod maturation and survival data for the 2nd set of samples. Replication (Rep), number of amphipods recovered (Recov), and number of males recovered (Males).

Sample	Rep	Recov	Males	Sample	Rep	Recov	Males
02B	1	8	4	14B	1	7	1
02B	2	8	2	14B	2	7	2
02B	3	4	1	14B	3	6	3
02B	4	8	2	14B	4	6	2
02C	1	5	2	14C	1	6	1
02C	2	10	4	14C	2	6	1
02C	3	7	2	14C	3	4	3
02C	4	9	6	14C	4	3	2
04B	1	4	3	18B	1	7	2
04B	2	8	2	18B	2	7	4
04B	3	9	1	18B	3	7	1
04B	4	14	5	18B	4	5	5
04C	1	0	0	18C	1	9	3
04C	2	11	1	18C	2	5	1
04C	3	8	2	18C	3	10	3
04C	4	7	1	18C	4	1	0
06B	1	7	1	19B	1	7	3
06B	2	2	1	19B	2	9	7
06B	3	10	1	19B	3	8	0
06B	4	6	2	19B	4	10	4
06C	1	9	5	19C	1	8	3
06C	2	8	4	19C	2	6	3
06C	3	8	4	19C	3	8	2
06C	4	8	5	19C	4	6	1
07B	1	10	2	20B	1	8	2
07B	2	10	7	20B	2	10	1
07B	3	10	4	20B	3	8	1
07B	4	10	4	20B	4	6	0
07C	1	9	3	20C	1	9	4
07C	2	10	3	20C	2	9	0
07C	3	9	3	20C	3	9	4
07C	4	11	5	20C	4	10	2
09B	1	7	4	22B	1	10	2
09B	2	5	2	22B	2	9	2
09B	3	11	3	22B	3	9	2
09B	4	8	4	22B	4	6	2
09C	1	4	2	22C	1	3	2
09C	2	8	3	22C	2	8	4
09C	3	16	7	22C	3	5	0
09C	4	4	0	22C	4	7	3
13B	1	2	0	24B	1	8	3
13B	2	2	1	24B	2	9	2
13B	3	5	0	24B	3	9	4
13B	4	4	1	24B	4	9	3
13C	1	3	2	24C	1	8	3
13C	2	6	2	24C	2	5	4
13C	3	6	2	24C	3	6	6
13C	4	3	2	24C	4	4	2

Appendix 1.7. (Continued)

Sample	Rep	Recov	Males	Sample	Rep	Recov	Males
SCB	1	4	0	FLC	1	9	1
SCB	2	5	0	FLC	2	10	0
SCB	3	7	1	FLC	3	8	1
SCB	4	6	2	FLC	4	11	0
SCC	1	8	2				
SCC	2	8	3				
SCC	3	10	2				
SCC	4	9	4				

Appendix 1.8. Concentrations of acid volatile sulfide (µmoles/g) and simultaneously extracted metals (SEM in ug/g dry weight) and the sum of the molar concentration of SEMs and the sum of the molar concentration of SEM divided by the molar concentration of AVS for the Upper Mississippi River sediment samples.

Sample	AVS	Cd	Cu	Ni	Pb	Zn	∑SEM	∑SEM/AVS
1B	0.19	0.058	1.1	1.2	5.2	11	0.232	1.22
1C	0.005	2.1	0.67	0.76	4.3	7	0.170	17.00
2B	1.9	0.39	4.7	4.6	7.7	26	0.591	0.31
2C	3.1	0.91	8.4	7.6	13.0	47	1.051	0.34
3B	2.1	0.68	7.3	6.1	11.0	35	0.813	0.39
4B	9.4	1.5	13.0	9.9	26.0	73	1.629	0.17
4C	16.0	3.0	20.0	15.0	53.0	118	2.658	0.17
5B	1.9	0.21	3.4	3.0	4.8	15	0.359	0.19
5C	16.0	0.63	6.3	9.4	16.0	45	1.030	0.06
6B	0.8	0.062	0.85	1.5	2.1	7.1	0.158	0.20
6C	2.9	0.12	2.0	2.7	4.2	12	0.282	0.10
7B	1.4	0.2	2.7	4.6	4.6	15	0.374	0.27
7C	8.7	0.43	5.3	6.6	12.0	35	0.793	0.09
8B	0.6	0.14	2.4	2.6	4.1	12	0.287	0.48
8C	2.2	0.28	4.1	4.6	6.9	20	0.485	0.22
9B	3.8	0.3	4.4	4.9	8.4	24	0.563	0.15
9C	3.8	0.38	5.2	5.4	11.0	31	0.705	0.19
10B	4.8	0.17	3.7	3.2	6.0	18	0.419	0.09
10C	63.0	0.74	11.0	10.0	26.0	68	1.516	0.02
11B	1.1	0.13	2.5	3.2	5.8	16	0.368	0.33
11C	6.5	0.18	2.8	3.8	7.1	17	0.405	0.06
12B	3.8	0.43	4.8	4.8	26.0	137	2.382	0.63
12C	5.9	0.45	4.5	4.1	27.0	143	2.462	0.42
13B	3.1	0.14	2.3	3.2	6.8	22	0.461	0.15
13C	5.2	0.3	4.4	4.5	14.0	48	0.950	0.18
14B	3.5	0.14	2.8	3.3	6.1	19	0.422	0.12
14C	18.0	0.41	6.8	6.6	18.0	51	1.090	0.06
15B	3.6	0.2	4.0	3.9	9.9	31	0.653	0.18
15C	3.7	0.34	5.3	4.5	14.0	44	0.904	0.24
16B	2.0	0.14	2.5	3.1	5.8	20	0.427	0.21
16C	3.5	0.31	2.7	2.9	8.0	30	0.592	0.17

Appendix 1.8. (continued)

Sample	AVS	Cd	Cu	Ni	Pb	Zn	∑SEM	∑SEM/AVS
18B	2.8	0.17	2.6	3.1	5.6	20	0.428	0.15
18C	7.1	0.36	3.6	3.4	9.2	33	0.667	0.09
19B	4.1	0.28	4.3	5.0	8.8	27	0.611	0.15
19C	5.1	0.28	5.4	5.3	11.0	30	0.690	0.14
20B	0.1	0.028	0.49	1.4	1.7	5.6	0.126	0.79
20C	1.8	0.15	2.4	2.6	5.3	15	0.338	0.19
21B	1.0	0.095	2.1	2.2	4.2	12	0.275	0.28
21C	2.4	0.16	3.6	3.6	5.9	16	0.393	0.16
22B	1.8	0.12	2.8	2.8	6.3	16	0.368	0.20
22C	10.0	0.4	4.9	5.1	13.0	38	0.812	0.08
24B	0.81	0.091	2.5	2.8	4.3	11	0.277	0.34
24C	3.2	0.28	4.3	4.4	8.9	26	0.586	0.18
25B	1.2	0.19	4.6	4.0	7.7	18	0.455	0.38
25C	2.9	0.19	3.8	3.8	8.3	17	0.426	0.15
26B	2.4	0.27	7.1	6.0	15.0	25	0.671	0.28
26C	1.7	0.15	3.4	5.0	11.0	19	0.484	0.28
SCB	1.7	0.31	9.0	3.4	17.0	35	0.820	0.48
SCC	5.5	0.54	14.0	4.9	30.0	53	1.264	0.23

Appendix 1.9. Concentrations (µg/g dry weight) of organochlorine pesticides (OCs) in Upper Mississippi River sediments.

POOL	Chlordane	Dieldrin	DDE	DDD	DDT
1B	0.001	ND[1]	0.0004	0.0005	ND
1C	ND	ND	ND	ND	ND
2B	0.001	0.0003	ND	0.0016	0.0002
2C	ND	ND	0.0520	0.0790	ND
3B	ND	0.0003	0.0011	0.0038	0.0002
4B	0.002	0.0005	0.0010	0.0019	ND
4C	ND	ND	ND	ND	ND
5B	ND	ND	0.0001	0.0001	ND
5C	ND	ND	ND	ND	ND
6B	ND	ND	0.0001	0.0003	ND
6C	ND	ND	ND	ND	ND
7B	ND	ND	0.0003	0.0010	0.0001
7C	ND	ND	ND	ND	ND
8B	ND	ND	0.0002	0.0004	ND
8C	ND	ND	ND	ND	ND
9B	ND	ND	0.0003	0.0010	0.0001
9C	ND	ND	ND	ND	ND
10B	ND	ND	0.0002	0.0001	ND
10C	ND	ND	ND	ND	ND
11B	ND	ND	0.0002	0.0004	ND
11C	ND	ND	ND	ND	ND
12B	ND	ND	0.0003	0.0006	ND
12C	ND	ND	ND	ND	ND
13B	ND	ND	0.0002	0.0004	ND
13C	ND	ND	ND	ND	ND
14B	ND	0.0001	0.0001	0.0002	ND
14C	ND	ND	ND	ND	ND
15B	0.001	0.0002	0.0004	0.0005	0.0018
15C	ND	ND	ND	ND	ND
16B	ND	0.0002	0.0004	0.0004	ND
16C	ND	ND	ND	ND	ND
18B	ND	0.0002	0.0003	0.0006	ND
18C	ND	ND	ND	ND	ND
19B	ND	0.0003	0.0001	0.0002	ND
19C	ND	ND	ND	ND	ND
20B	ND	ND	0.0001	0.0002	ND
20C	ND	ND	ND	ND	ND
21B	0.002	0.0004	0.0004	0.0008	0.0003
21C	ND	ND	ND	ND	ND

Appendix 1.9. Concentrations of organochlorine pesticides (OCs) in Upper Mississippi River sediments (cont.).

POOL	Chlordane	Dieldrin	DDE	DDD	DDT
22B	ND	0.0003	0.0001	0.0001	ND
22C	ND	ND	ND	ND	ND
24B	0.0010	0.0004	0.0001	0.0001	ND
24C	ND	ND	ND	ND	ND
25B	0.0010	0.0006	0.0005	0.0005	ND
25C	ND	ND	ND	ND	ND
26B	ND	0.0007	0.0005	0.001	ND
26C	ND	ND	ND	ND	ND
SCB	ND	ND	0.0007	0.0004	0.0001
SCC	ND	ND	0.0780	0.0780	ND

ND = Not detected

Appendix 1.10. Concentrations (µg/g dry weight) of polycyclic aromatic hydrocarbons (PAHs) in Upper Mississippi River sediments. (ND = Not Detected)

Pool	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1B	1.080	ND	ND	0.230	0.040	0.200	0.300	0.040	ND	0.600	0.580	0.190	0.280	0.390	ND	ND	0.130
1C	0.013	0.013	ND	ND	ND	ND	0.013	ND	ND	0.031	0.025	0.031	0.031	0.038	0.038	0.031	0.044
2B	0.030	ND	ND	0.020	0.020	0.020	0.230	0.020	ND	0.450	0.470	0.140	0.270	0.330	ND	ND	0.110
2C	0.052	0.052	0.026	ND	ND	ND	0.157	0.026	0.052	0.340	0.314	0.157	0.209	0.157	0.183	0.131	0.236
3B	1.030	ND	ND	0.380	ND	0.050	0.090	0.010	ND	0.210	0.190	0.060	0.140	0.210	ND	ND	0.040
4B	ND	ND	ND	0.590	ND	ND	0.050	0.010	ND	0.350	0.420	0.050	0.140	0.230	ND	ND	0.050
4C	0.049	0.049	ND	ND	ND	ND	0.049	ND	ND	0.196	0.245	0.098	0.147	0.147	0.147	0.147	0.196
5B	ND	ND	ND	0.050	ND	ND	0.010	ND	ND	0.030	0.030	ND	0.010	0.020	ND	ND	ND
5C	0.036	0.036	ND	ND	ND	ND	ND	ND	ND	0.036	0.036	ND	0.071	0.036	ND	ND	ND
6B	ND	ND	ND	0.010	ND	ND	0.010	ND	ND	0.090	0.100	ND	0.010	0.010	ND	ND	ND
6C	0.016	0.016	ND	ND	ND	ND	ND	ND	ND	0.031	0.031	ND	0.031	0.016	ND	ND	ND
7B	ND	ND	ND	0.010	ND	ND	ND	ND	ND	0.110	0.130	ND	0.020	0.010	ND	ND	ND
7C	0.020	ND	ND	ND	ND	ND	0.020	ND	ND	0.041	0.041	0.020	0.041	0.041	0.041	0.041	0.041
8B	ND	ND	ND	0.040	ND	ND	0.020	ND	ND	0.040	0.030	0.010	0.020	0.020	ND	ND	ND
8C	0.019	0.019	ND	ND	ND	ND	0.019	ND	ND	0.037	0.019	0.019	0.037	0.019	0.019	0.019	0.037
9B	0.010	ND	ND	0.010	ND	0.010	0.040	ND	ND	0.080	0.070	ND	0.100	0.100	ND	ND	ND
9C	0.021	0.021	ND	ND	ND	ND	ND	ND	ND	0.042	0.021	0.021	0.042	0.021	0.042	0.021	0.042
10B	ND	ND	ND	0.010	ND	ND	0.010	ND	ND	0.020	0.020	0.010	0.020	0.020	ND	ND	ND
10C	0.044	0.044	ND	ND	ND	ND	ND	ND	ND	0.044	0.044	ND	0.044	ND	ND	ND	ND

PAH-1 = Naphthalene
PAH-2 = 2-methylnaphthalene
PAH-3 = 1-methylnaphthalene
PAH-4 = Acenaphthalene
PAH-5 = Acenaphthene
PAH-6 = Fluorene
PAH-7 = Phenathrene
PAH-8 = Anthracene
PAH-9 =1,-methylphenanthrene
PAH-10 =Fluoranthene
PAH-11 = Pyrene
PAH-12 = 1,2-Benzanthracene
PAH-13 = Chrysene
PAH-14 = Benzo(b)fluoranthene
PAH-15 = Benzo(k)fluoranthene
PAH-16 = Benzo(e)pyrene
PAH-17 - Benzo(a)pyrene

Appendix 1.10. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in Upper Mississippi River sediments (cont.).

Pool	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1B	ND	0.080	0.010	0.060	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1C	ND	ND	ND	ND	0.013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2B	ND	0.210	0.060	0.150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2C	0.209	ND	ND	ND	0.079	ND	0.052	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3B	ND	0.050	0.010	0.040	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4B	ND	0120	0.020	0.080	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4C	2.304	ND	ND	ND	0.049	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5C	1.286	ND	ND	ND	0.014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6C	0.094	ND	ND	ND	0.027	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7C	1.660	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8C	0.242	ND	ND	ND	0.022	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9C	0.565	ND	ND	ND	0.019	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10C	3.289	ND	ND	ND	0.013	0.026	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

PAH-18 = Perylene
PAH-19 = Indeno(1,2,3-cd)pyrene
PAH-20 = 1,2,5,6-dibenzanthracene
PAH-21 = Benzo(g,h,i)perylene
PAH-22 = C1-naphthalenes
PAH-23 = C2-naphthalenes
PAH-24 = C1-phenanthrenes
PAH-25 = C2-phenanthrenes
PAH-26 = C3-phenanthrenes
PAH-27 = C4-phenanthrenes
PAH-28 = C1-dibenzothiophenes
PAH-29 = C2-dibenzothiophenes
PAH-30 = C3-dibenzothiophenes
PAH-31 = C1-fluoranthenes+C1-pyrene
PAH 32 - C2-chrysenes
PAH-33 = C3-chrysenes
PAH-34 = C4-chrysenes

Appendix 1.10. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in Upper Mississippi River sediments (cont.).

Pool	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
11B	ND	ND	ND	0.230	ND	ND	0.020	ND	ND	0.090	0.080	0.010	0.030	0.040	ND	ND	0.010
11C	0.016	0.016	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12B	ND	ND	ND	ND	ND	ND	0.010	ND	ND	0.060	0.050	0.010	0.020	0.020	ND	ND	ND
12C	0.019	0.019	ND	ND	ND	ND	ND	ND	ND	0.019	0.019	ND	0.019	0.019	0.019	ND	ND
13B	ND	ND	ND	0.020	ND	ND	0.010	ND	ND	0.030	0.020	0.010	0.010	0.020	ND	ND	ND
13C	0.020	0.020	ND	ND	ND	ND	ND	ND	ND	0.020	0.020	ND	0.020	ND	ND	ND	ND
14B	ND	ND	ND	0.030	ND	0.040	0.020	ND	ND	0.030	0.030	0.010	0.010	0.020	ND	ND	ND
14C	0.028	0.028	ND	ND	ND	ND	0.028	ND	ND	0.085	0.085	0.028	0.057	0.028	ND	ND	ND
15B	ND	ND	ND	0.140	ND	ND	0.070	ND	ND	0.210	0.170	0.040	0.070	0.100	0.020	ND	0.020
15C	0.023	0.023	ND	ND	ND	ND	0.023	ND	ND	0.046	0.046	0.046	0.046	0.023	0.023	0.023	0.023
16B	ND	ND	ND	0.050	ND	0.050	0.070	0.070	ND	0.140	0.120	0.030	0.070	0.090	ND	ND	0.020
16C	0.015	0.015	0.015	ND	ND	ND	0.046	ND	ND	0.091	0.107	0.091	0.091	0.061	0.076	0.061	0.122
18B	ND	ND	ND	0.330	ND	ND	0.020	ND	ND	0.080	0.090	0.020	0.040	0.050	ND	ND	0.020
18C	0.016	0.016	ND	ND	ND	ND	0.033	ND	ND	0.049	0.049	0.033	0.049	0.033	0.049	0.033	0.049
19B	ND	ND	ND	0.180	ND	0.010	0.030	0.010	ND	0.060	0.070	0.02	0.040	0.060	ND	ND	0.020
19C	0.021	0.021	ND	ND	ND	ND	0.021	ND	ND	0.064	0.064	0.043	0.064	0.043	0.043	0.021	0.064
20B	ND	ND	ND	0.034	0.004	0.004	0.002	0.003	ND	0.033	0.030	0.012	0.010	0.001	ND	ND	0.061
20C	0.014	0.014	ND	ND	ND	ND	0.021	ND	ND	0.043	0.043	0.043	0.058	0.043	0.029	0.029	0.058

PAH-1 = Naphthalene
PAH-2 = 2-methylnaphthalene
PAH-3 = 1-methylnaphthalene
PAH-4 = Acenaphthalene
PAH-5 = Acenaphthene
PAH-6 = Fluorene
PAH-7 = Phenathrene
PAH-8 = Anthracene
PAH-9 =1,-methylphenanthrene
PAH-10 =Fluoranthene
PAH-11 = Pyrene
PAH-12 = 1,2-Benzanthracene
PAH-13 = Chrysene
PAH-14 = Benzo(b)fluoranthene
PAH-15 = Benzo(k)fluoranthene
PAH-16 = Benzo(e)pyrene
PAH-17= Benzo(a)pyrene

Appendix 1.10. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in Upper Mississippi River sediments (cont.).

Pool	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
11B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11C	0.639	ND	ND	ND	0.016	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12C	0.599	ND	ND	ND	0.019	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13C	0.357	ND	ND	ND	0.020	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14C	1.420	ND	ND	ND	0.028	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15C	0.616	ND	ND	ND	0.023	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16C	0.213	ND	ND	ND	0.030	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18C	0.492	ND	ND	ND	0.016	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19B	ND	0.010	0.010	0.010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19C	0.596	ND	ND	ND	0.021	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20C	0.261	ND	ND	ND	0.014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

PAH-18 = Perylene
PAH-19 = Indeno(1,2,3-cd)pyrene
PAH-20 = 1,2,5,6-dibenzanthracene
PAH-21 = Benzo(g,h,i)perylene
PAH-22 = C1-naphthalenes
PAH-23 = C2-naphthalenes
PAH-24 = C1-phenanthrenes
PAH-25 = C2-phenanthrenes
PAH-26 = C3-phenanthrenes
PAH-27 = C4-phenanthrenes
PAH-28 = C1-dibenzothiophenes
PAH-29 = C2-dibenzothiophenes
PAH-30 = C3-dibenzothiophenes
PAH-31 = C1-fluoranthenes+C1-pyrene
PAH 32 - C2-chrysenes
PAH-33 = C3-chrysenes
PAH-34 = C4-chrysenes

Appendix 1.10. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in Upper Mississippi River sediments (cont.).

POOL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
21B	ND	ND	ND	0.030	ND	ND	0.070	0.010	ND	0.220	0.190	0.040	0.060	0.090	ND	ND	0.030
21C	0.017	0.017	ND	ND	ND	ND	ND	ND	ND	0.017	ND	ND	0.017	ND	ND	ND	ND
22B	ND	ND	ND	0.260	ND	ND	0.020	ND	ND	0.070	0.080	0.010	0.020	0.030	ND	ND	0.010
22C	0.021	0.021	ND	ND	ND	ND	0.021	ND	ND	0.063	0.042	0.042	0.042	0.021	0.042	0.021	ND
24B	ND	ND	ND	ND	ND	ND	0.010	ND	ND	0.060	0.070	0.010	0.020	0.020	ND	ND	0.010
24C	0.019	0.019	ND	ND	ND	ND	0.019	ND	ND	0.037	0.037	0.019	0.037	0.019	0.019	0.019	0.037
25B	ND	ND	ND	0.010	ND	ND	0.020	ND	ND	0.050	0.050	0.020	0.030	0.040	ND	ND	0.010
25C	0.018	0.018	ND	ND	ND	ND	ND	ND	ND	0.018	0.018	ND	0.037	ND	ND	ND	ND
26B	ND	ND	ND	0.010	ND	ND	0.020	ND	ND	0.050	0.050	0.020	0.030	0.050	ND	ND	0.020
26C	0.014	0.014	ND	ND	ND	ND	ND	ND	ND	0.036	0.029	0.014	0.029	0.014	0.014	0.014	0.014
SCB	ND	ND	ND	0.610	0.070	ND	0.050	0.010	ND	0.230	0.210	0.040	0.130	0.140	ND	ND	0.020
SCC	ND	0.039	ND	ND	ND	ND	ND	ND	ND	0.156	0.156	0.078	0.117	0.117	0.078	0.078	0.117

PAH-1 = Naphthalene
PAH-2 = 2-methylnaphthalene
PAH-3 = 1-methylnaphthalene
PAH-4 = Acenaphthalene
PAH-5 = Acenaphthene
PAH-6 = Fluorene
PAH-7 = Phenathrene
PAH-8 = Anthracene
PAH-9 =1,-methylphenanthrene
PAH-10 =Fluoranthene
PAH-11 = Pyrene
PAH-12 = 1,2-Benzanthracene
PAH-13 = Chrysene
PAH-14 = Benzo(b)fluoranthene
PAH-15 = Benzo(k)fluoranthene
PAH-16 = Benzo(e)pyrene
PAH-17= Benzo(a)pyrene

Appendix 1.10. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in Upper Mississippi River sediments (cont.).

POOL	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
21B	ND	ND	ND	ND	ND	ND	0.06	ND	ND	ND	0.03	ND	ND	ND	ND	0.07	0.01
21C	0.087	ND	ND	ND	0.017	ND	0.017	ND	ND	ND	ND	0.087	ND	ND	0.017	ND	ND
22B	ND	ND	ND	ND	ND	ND	0.02	0.03	ND	ND	0.01	0.26	ND	ND	ND	0.02	ND
22C	0.549	ND	ND	ND	0.021	ND	0.042	0.021	0.042	0.021	ND	0.549	ND	ND	0.021	0.021	ND
24B	ND	ND	ND	ND	ND	ND	ND	0.02	ND	ND	ND	ND	ND	ND	ND	0.01	ND
24C	0.595	ND	ND	ND	0.019	ND	ND	0.019	0.019	0.019	0.037	ND	ND	ND	ND	0.019	ND
25B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	ND
25C	0.349	ND	ND	ND	0.018	ND	ND	0.018	ND	ND	ND	ND	ND	ND	ND	ND	ND
26B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.01	ND	ND	ND	0.02	ND
26C	0.180	ND	ND	ND	0.014	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SCB	ND	0.080	0.020	ND	0.050	ND	ND	ND	ND	ND	ND	0.61	0.08	0.02	0.05	0.05	0.01
SCC	5.469	ND	ND	ND	0.039	ND	ND	ND	0.078	0.078	0.117	ND	ND	ND	ND	ND	ND

PAH-18 = Perylene
PAH-19 = Indeno(1,2,3-cd)pyrene
PAH-20 = 1,2,5,6-dibenzanthracene
PAH-21 = Benzo(g,h,i)perylene
PAH-22 = C1-naphthalenes
PAH-23 = C2-naphthalenes
PAH-24 = C1-phenanthrenes
PAH-25 = C2-phenanthrenes
PAH-26 = C3-phenanthrenes
PAH-27 = C4-phenanthrenes
PAH-28 = C1-dibenzothiophenes
PAH-29 = C2-dibenzothiophenes
PAH-30 = C3-dibenzothiophenes
PAH-31 = C1-fluoranthenes+C1-pyrene
PAH 32 - C2-chrysenes
PAH-33 = C3-chrysenes
PAH-34 = C4-chrysenes

Chapter 2: An Evaluation of Bioaccumulation of Contaminants from Sediments from the Upper Mississippi River Using Field-collected Oligochaetes and Laboratory-exposed *Lumbriculus variegatus*

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Introduction

Over the past 10 years, a variety of methods have been described for evaluating the toxicity of sediment-associated contaminants with benthic invertebrates. However, only a limited number of methods are currently available for assessing bioaccumulation of contaminants from field-collected or laboratory spiked sediments (Ingersoll et al 1995). Standard guides have been published for conducting 28-d bioaccumulation tests with the oligochaete *Lumbriculus variegatus* including determination of bioaccumulation kinetics for different compound classes (USEPA, 1994; ASTM 1996). *Lumbriculus variegatus* was selected for use in sediment bioaccumulation testing in the present study of upper Mississippi River (UMR) for six reasons: (1) ease of culture and handling, (2) known chemical exposure history, (3) adequate tissue mass for chemical analyses, (4) tolerance of a wide range of sediment physico-chemical characteristics, (5) low sensitivity to contaminants associated with sediment, and (6) amenability to long-term exposures without feeding. Other organisms do not meet many of these selection criteria including mollusks (valve closure), midges (short-life cycle), mayflies (difficult to culture), amphipods (small tissue mass, too sensitive), cladocerans and fish (not in direct contact with sediment).

Several investigators have conducted bioaccumulation studies in the laboratory with *L. variegatus* using either field-collected or laboratory-spiked sediments (Schuytema *et al.* 1988; Nebeker *et al.* 1989; Ankley *et al.* 1991; Call *et al.*, 1991; Carlson *et al.* 1991; Ankley *et al.* 1993; Kukkonen and Landrum 1994). However, only one previous study has compared results of laboratory bioaccumulation studies conducted with *L. variegatus* to residues from synoptically-collected field populations of oligochaetes (Ankley *et al.* 1992). The author reported good agreement between concentrations of polychlorinated biphenyls in the laboratory and field organisms, particularly for PCB congeners with K_{ow} values <7. This suggests that laboratory exposures longer than 28 d may be required to reach equilibrium for super-hydrophobic chemicals.

The United States Geological Survey (USGS) has been monitoring the Upper Mississippi River since 1987 to document the fate and transport of contaminated sediments (Moody and Meade 1995). Concern with the redistribution of these contaminated sediments arose after the flood of 1993. This project is designed to evaluate the current status of sediments in the UMR and is one chapter in a series designed to assess the extent of sediment contamination in navigational pools of the river. The overall project consists of the following assessments: (1) measuring concentrations of contaminants in sediments of the UMR (Moody *et al.* 1996), (2) toxicity testing with sediments collected from the river (Chapter 1), (3) analysis of benthic community structure (Chapter 3), and (4) bioaccumulation of sediment associated contaminants (the present chapter). The present study had two objectives: (1) to assess the bioaccumulation of contaminants from UMR sediments using *L. variegatus* and (2) to compare bioaccumulation in these laboratory-exposed oligochaetes to oligochaetes collected from the field.

Materials and Methods

Sample Collection

Sediment samples and native oligochaetes were collected from 23 navigational pools on the UMR and from the Saint Croix River ("C" samples described in Chapter 1). Sample stations were selected based on the potential of oligochaetes or fine grained sediment. For each C sample, 35- to 80-L of sediment (6 to 25 grabs) were collected with a stainless steel Ponar grab sampler (Wildlife Supply Company, Saginaw, MI). All grabs from a station within a pool were collected within a 5-meter radius and combined in a 114-L high-density polyethylene (HDPE) container. The composited sample was homogenized on board the research ship Acadiana using an electric drill and a stainless steel auger. Once homogenized, the following subsamples were removed: (1) three separate 250 ml subsamples for organic chemistry, metals/acid-volatile sulfides, and total organic carbon/particle size (Chapter 1), (2) one 2-L subsample for benthic invertebrates (Chapter 3), and (3) one 10-L subsample for laboratory toxicity (Chapter 1) and bioaccumulation testing. Sediment samples were stored at 4^o C until used in laboratory exposures or physical/chemical analysis.

The remainder of the composited C sample of sediment was rinsed on ship through a Wildco wash bucket (U.S. Standard sieve size #30, 600 µm opening). The material captured by the wash bucket was transferred to a HDPE tub along with river water. After all the sediment was sieved, native oligochaetes were isolated from the detritus. These oligochaetes from each sample were placed in a HDPE jar containing aerated river water and held for 24 hours to depurate gut contents. After the 24-hour elimination period, dead oligochaetes were discarded. The remaining oligochaetes were rinsed, blotted dry, weighed, transferred to clean glass jars, and frozen at -22^{o}C until analyzed for chemical contaminants. Weights of native oligochaete samples selected for analysis ranged from 0.34g (Pool 4) to 9.8g (Pool 9)

Laboratory Testing

Lumbriculus variegatus were exposed in the laboratory to sediment following methods described in USEPA (1994) and ASTM (1996). Sediment from 13 of the 23 sampled pools were used in these laboratory exposures. Samples were chosen for testing on the basis of sufficient mass of field-collected oligochaetes for chemical analyses (or the previously documented presence of PCBs for pool 4 in lower Lake Pepin; e.g. Rostad *et al.*, 1996). Oligochaetes were mass cultured in the laboratory following methods similar to those described in USEPA (1994) using 75-L glass aquarium containing 50 L of well water (hardness 290 mg/L as $CaCO_3$, alkalinity 255 mg/L as $CaCO_3$, pH 7.8). Each aquaria received about 27 volume additions (about 1.5 L/minute) of well water daily. The culture water was aerated and maintained at 23^{o}C. Pre-soaked, shredded brown paper towels were used as substrate. Cultures were fed Tetramin flake fish food twice weekly *ad libitum*.

Exposures of oligochaetes in the laboratory were conducted for 28 days in 4-L glass Pyrex beakers containing 1 L of sediment and 3 L of overlying water. Four replicate chambers were tested for each of the thirteen sediment samples. Reconstituted fresh water (hardness 90 to 96 mg/L as $CaCO_3$, alkalinity 60 to 70 mg/L as $CaCO_3$; USEPA 1994) was used as the overlying water. Each beaker was calibrated to 4-L using a glass standpipe that exited through the beaker wall and was held in place with a silicon stopper. Test chambers received 2 volume additions (6 L $\pm$ 10%) of overlying water per day. Water was delivered using a modified Mount and Brungs diluter system (Ingersoll and Nelson 1990). An in-line flow splitter was attached to each delivery line to split the water flow evenly to each of four test chambers. The splitters were constructed of 1/4 inch PVC pipe with four silicone stoppers and 14-gauge stainless steel hypodermic needles with the points and connector ends cut off the needles (Figure 2.1). Glass stands were used to support the splitters keeping them level to maintain a constant volume delivery to each exposure chamber. Chambers were held in a temperature-controlled waterbath (23$\pm$1^{o}C) on a 16:8 light:dark photoperiod at about 500 lux. Oligochaetes were not fed during the sediment exposure.

Sediment and overlying water were placed in the chambers the day before adding organisms (Day -1). Sediments were first homogenized with a hand-held electric drill and stainless steel auger before being placed into the test beakers. One-L of sediment was transferred into each chamber using a plastic spoon. Overlying water was poured into the beakers through a piece of fine-mesh Nitex® material to minimize suspension of the sediment. Water delivery started after chambers were placed in the waterbath.

Twenty-four hours before stocking the test (Day -1) oligochaetes were removed from the culture with a fine-mesh nylon aquarium net, placed in beakers containing well water, and rinsed to remove excess toweling and debris. Beakers containing the oligochaetes were then placed in a waterbath and aerated. With substrate absent, the *L. variegatus* formed tight masses or clumps in the beakers which was helpful during transfer of organisms into the exposure chambers.

Oligochaetes were acclimated to the test water by removing half of the water in each beaker and replacing it with temperature-acclimated test water. Two hours later this process was repeated. After another two hours, the *L. variegatus* were combined into a glass pan and rinsed with well water to break up the masses of worms and remove any remaining debris. With the mass of worms disturbed, oligochaetes were grouped together with a stainless steel dental pick and allowed to form small clumps of about 1 g. The clumps of oligochaetes were removed from the pan with the dental pick, touched against the rim of the pan to remove excess water, and placed on a tared weigh boat. About 2.6 g unblotted oligochaetes were transferred to each test chamber containing sediment and overlying water . Using this approach, the 2.6 g of unblotted oligochaetes represents about 2 g of blotted oligochaetes or about 200 organisms.

General conditions of the exposure system and behavior were evaluated daily. Dissolved oxygen and conductivity of the overlying water were measured weekly in all chambers. Total hardness (as $CaCO_3$), pH, alkalinity (as $CaCO_3$), and total ammonia of overlying water were measured at the beginning and end of the test. Overlying water pH, alkalinity, total hardness, conductivity and total ammonia measurements were similar among all stations and inflowing test water (Appendix 2.1). Dissolved oxygen measurements were at or above acceptable levels (>40% of saturation; ASTM 1996) in all treatments throughout the study (Appendix 2.1). Ranges of mean water quality for each parameter were as follows: pH 7.7 to 7.9; alkalinity as $CaCO_3$ 61 to 67 mg/L; total hardness as $CaCO_3$ 104 to 110 mg/L; conductivity 342 to 350 µS @25°C; total ammonia 0.1 to 0.4 mg/L; and calculated unionized ammonia 0.0028 to 0.0094 mg/L.

On Day 28 of the exposure, *L. variegatus* were isolated from each test chamber by washing the sediment through No. 18 (1.0 mm opening) followed by No. 50 (300 µm opening) U.S. standard stainless steel sieves. The contents of each sieve was rinsed into several clear glass pans and all oligochaetes were removed. *Lumbriculus variegatus* were separated from native oligochaetes based on behavior (native oligochaetes tended to form a tight, spring-like coil, whereas *L. variegatus* would not (USEPA 1994)). Once isolated, all *L. variegatus* from a chamber were cleaned of any remaining debris and held for 24 h in 1-L water-only chambers to allow them to clear their gut contents. The *L. variegatus* were then isolated, cleaned of any remaining debris, and transferred to a tared weigh boat. Samples were then blotted, weighed, placed in glass jars, and stored at -22 °C pending chemical analysis for contaminants. Weights of laboratory-exposed oligochaete samples ranged from 1.3g to 3.0g.

Chemical Analyses

Sediment physical characteristics included the following: (1) sediment particle size, (2) total organic carbon, (3) inorganic carbon and (4) percent water. Sediment chemical parameters included: (1) organochlorine pesticides (OCs), (2) polychlorinated biphenyls (PCB), (3) select aliphatic and polynuclear aromatic hydrocarbons (PAH), (4) simultaneously extracted metals (SEM), (5) acid volatile sulfide (AVS), and (6) total metals. See Chapter 1 for additional information on methods and results of chemical and physical characterizations of the sediments.

Concentrations of metals and organochlorines in sediment samples were low (Chapter 1). Therefore, replicate tissue samples from the laboratory exposures were combined for organochlorine pesticide/PCB analyses and metals were not analyzed because of limited sample mass. Tissues were analyzed by Geochemical and Environmental Research Group at Texas A&M University, College Station, Texas for the following: (1) organochlorine pesticides (OCs), (2) polychlorinated biphenyls (PCBs), (3) select aliphatic and polynuclear aromatic hydrocarbons (PAHs), and (4) percent lipid. Prior to analysis, tissue samples were homogenized and extracted using a Teckmar Tissumizer, sodium sulfate, and methylene chloride (MacLeod *et al.* 1985; Wade *et al.* 1988; Brooks *et al.* 1989). Tissue extracts were split into two fractions: one fraction was used to measure percent lipid and the second fraction was used for measuring PAHs, OCs, and PCBs. Extracts for chemical analyses were purified using absorption chromatography to isolate the aliphatic fraction and the PAH/OC/PCB fraction. Lipid interference in the PAH/OC/PCB fraction was eliminated with further purification using HPLC. The quantitative analyses were performed by capillary gas chromatography (CGC) with electron capture detector for OCs and PCBs and a mass spectrometer detector in the SIM mode for PAHs (Wade et al., 1988). Percent lipids were calculated on a wet-weight basis. A 20-ml aliquot of the total extract was filtered, concentrated to 1 ml, and weighed. A 100-ul subsample was then removed, evaporated to dryness, and weighed. Percent lipid was calculated using the weight of the dried subsample and the concentrated sample. Tissue residue data are presented in Appendix 2.2 and Appendix 2.3. Sediment data are shown in Table 1.1, and Tables 1.3 to 1.5 in Chapter 1.

Average percent spike recovery for twenty-two OCs and was 88% (n=4). Beta BHC had the smallest average spike recovery (53%) while oxychlordane had the greatest average spike recovery (104%). Individual OC concentrations were often below minimum detectable limits so duplicate analyses were evaluated only for total PCBs. The average duplicate coefficient of variation was 26% (range 0.7 to 61%, n=4). Average percent spike recovery for PAH compounds was 96% (25 compounds, n=4). L123(c,d)pyrene had the smallest average percent recovery (81%) while 1-methylnaphthalene had the greatest average percent recovery (110%). The average duplicate coefficient of variation was 21% (34 possible compounds, n=1-4). Average duplicate coefficient of variation ranged from 1% for c1-phenanthracene to 79% for benzo-a-pyrene.

In addition to the laboratory-exposed and field-collected oligochaetes, three samples of oligochaetes from laboratory cultures were collected at the beginning of the exposure for analysis contaminants. Two of the three samples had detectable concentrations of PAHs and total PCBs however, the concentrations were generally less than those of oligochaetes exposed to or collected from the UMR sediments. For some unexplained reason, total PCB (1.3 µg/g dry wt) and some PAH concentrations (up to 0.25 µg/g dry wt.) in one of those three samples was similar to oligochaetes exposed during the test.

Results and Discussion

General Trends

Individual organochlorine pesticides (OC) were generally below the detection limits (ranging from 0.0007 to.0.0217 µg/g wet weight) for oligochaetes from both field-collection and laboratory-exposed animals (Appendix 2.2). For the 13 field collected samples and 22 OCs measured, individual OCs were identified a total of 6 times. The greatest individual OC concentration was 0.009 µg/g (wet weight) for dieldrin from oligochaetes collected from Pool 22. As was the case with the field-collected oligochaetes, tissue concentrations of individual OCs were often below the detection limit for many of the laboratory-exposed oligochaetes. All oligochaete samples had at least one OC concentration above background (Pool 13 and Pool 16; 4,4'DDE); however, no sample had more than 6 OCs detected (Pool 11 and 14; gamma-chlordane, alpha-chlordane, aldrin, dieldrin, 4,4'DDE, 4,4'DDD). The greatest individual OC concentration was 0.013 µg/g (wet weight) for 4,4 DDE for oligochaetes exposed in the laboratory to sediment collected from Pool 4. Also, 4,4 DDE was the most frequently measured OC (12 samples) with concentrations ranging from 0.0021 to 0.013 µg/g (wet weight).

Total PCBs were the only chlorinated organic compound detected in all field-collected and laboratory-exposed oligochaetes. Concentrations ranged from 0.045 µg/g (wet weight - pool 13) to 0.697 µg/g (wet weight - Pool 4). The geometric mean for total PCBs measured in oligochaetes exposed to the sediment samples was 0.129 µg/g

Field-collected and laboratory-exposed oligochaete samples were analyzed for 44 PAH isomers. Field collected oligochaetes from Pool 4 had the fewest number of PAHs (14) while Pool 19 had the most (36). Only 16 PAH isomers (about 40% of those analyzed for) had detectable concentrations (detection limits from 0.0217 to 0.0024 µg/g wet weight) in 7 of the 13 Pools for both the field-collected and laboratory-exposed oligochaetes (for the laboratory exposures, 2 of the 4 replicates had to exceed the detectable limit in order to be included in this analysis). Table 2.1 lists all compounds measured in tissues that met these selection criteria. Figures 2.2 and 2.3 depict accumulation of total PAH in samples from laboratory-exposed or field-collected oligochaetes for each UMR pool evaluated. Concentrations of the 16 PAH isomers were converted to molar units, normalized to percent lipid, and summed. Total PAH from field-collected and laboratory-exposed oligochaetes, show a trend of decreasing concentrations in the down river Pools (14 to 22). Field-collected oligochaetes from Pool 7 were more contaminated than oligochaetes from the other pools. For the laboratory exposures, oligochaetes exposed to sediments from Pool 4 were more contaminated than oligochaetes exposed to sediments from the other pools. In general, perylene had the highest concentration of any PAH from field-collected and laboratory-exposed oligochaetes. This trend was greater for laboratory exposed oligochaetes than for those collected from the field. Perylene concentrations ranged from 0.056 to 0.53 µg/g (wet weight) in field collected oligochaetes and from 0.052 to 0.84 µg/g (wet weight) in oligochaetes from laboratory exposures.

Sediments and oligochaetes from the UMR are relatively uncontaminated compared to other locations we have evaluated using sediment toxicity tests (Ingersoll *et al.* 1996) or bioaccumulation tests (sediments from Little Scioto River in Ohio, unpublished data). Ingersoll *et al* (1996) calculated sediment effect concentrations including Effects Range Medians (ERMs) from 28-day sediment exposures with *Hyalella azteca*. An ERM is defined as that concentration of a material in sediment above which toxic effects are frequently or always observed or predicted. In the current study, tissue concentrations of PAHs were generally greatest in samples from Pool 4. Two low molecular weight (LMW) PAHs (naphthalene and phenanthrene) and two high molecular weight (HMW) PAHs (pyrene and chrysene) were generally the PAHs of highest concentration in tissue samples from pool 4. The calculated sediment ERM concentrations (ug/g dry weight) for those PAHs are; naphthalene - 0.097 , phenanthrene - 0.345, pyrene - 0.347, and chrysene - 0.500. The sediment concentrations (ug/g dry weight) from Pool 4 were; naphthalene - 0.049, phenanthrene- 0.049, pyrene - 0.245, and chrysene - 0.147. The sediment ERMs are 1.4 to 7 times greater than the highest concentrations of these PAHs in sediments from the current study. ERMs are not directly applicable to contaminant concentrations in tissues; however, tissue concentrations in UMR Pool 4 were more than two orders of magnitude less than tissue concentrations of oligochaetes exposed to sediments from the Little Scioto River. Collectively, this information would indicate that sediment and biota from the UMR is relatively uncontaminated when compared to known contaminated sites previously evaluated by our laboratory.

Detection of Compounds in Tissue vs. Sediment

Detection limits for tissue and sediment are usually different which creates difficulties in interpreting bioaccumulation potential from relatively uncontaminated sediments. In the UMR, concentrations of PAHs and PCBs were detected in both sediments and tissue samples 79% of the time for the laboratory-exposed oligochaetes and 58% of the time for the field-collected oligochaetes. PAHs and PCBs were not detected in the sediments but were detected in laboratory-exposed oligochaetes in 17% of the samples and in field-collected oligochaetes in 41% of the samples. PAHs and PCBs were detected in sediment samples but not in 3% of the samples from laboratory-exposed oligochaetes and 1% of the samples of field-collected oligochaetes. Although the detection limits for sediments and tissues met established guidelines (USEPA 1984), detection limits for sediments may need to be decreased in order to better represent potentially bioavailable compounds.

Laboratory to Field Comparisons

Tissue concentrations of naphthalenes were generally higher in field-collected oligochaetes than in laboratory exposed oligochaetes (Figure 2.4). Naphthalenes are LMW PAHs with log K_{ow} values less than 4.5. PAHs with similar concentrations in both the laboratory-exposed and field-collected oligochaetes included a similar number of HMW and LMW compounds (biphenyl, fluorene, 1-methylphenanthrene, pyrene, fluoranthene, chrysene, and benzo(e)pyrene). Most of these compounds are intermediate in molecular weight and log K_{ow} (except for benzo(e)pyrene which has the highest molecular weight and log Kow of all compounds included in Figure 2.4). PAHs typically higher in the laboratory-exposed than in field-collected oligochaetes were primarily HMW compounds (benzo(a)anthracene, benzo[b(k)]fluoranthene, and perylene) with log K_{ow}s greater than 5.1 (Figure 2.4 and 2.5).

The ratio of tissue concentrations in laboratory-exposed oligochaetes to concentrations in field-collected oligochaetes were generally similar (Figure 2.5). About 90% of the corresponding concentrations were within a factor of three between the laboratory-exposed and field collected oligochaetes (represented by the crosshatched region in Figure 2.5). However, there appears to be a shift from field>lab to lab>field as the molecular weight of PAHs increases. Concentrations that differed by more than a factor of three were primarily LMW PAHs (naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, fluorene, 1,6,7-trimethylnaphthalene, phenanthrene, and 1-methylphenanthrene) and were usually elevated in the field-collected oligochaetes compared to the laboratory-exposed oligochaetes. Ratios >3 in the laboratory-exposed or field-collected oligochaetes were most frequently associated with a small group of pools (Field > 3x lab in Pools 4, 12, 22; lab >3x field in Pool 7).

Differences between tissue concentrations in the laboratory-exposed and field-collected oligochaetes may have resulted from LMW PAHs being lost during the sampling of sediments. A second possibility for differences between the laboratory and field-exposed may be spatial heterogeneity of contaminants in the sediments in the field. Other possible explanations could include the rout of exposure. Exposure to contaminants in the field may occur through sediment, food and overlying water while the route of exposure to oligochaetes in the laboratory was sediment. Species-specific differences in exposure between *Lumbriculus variegatus* and the native oligochaetes may also contribute to the differential accumulation. For example, concentrations of metals reportedly differ among taxa inhabiting the same locations (Cain *et al.* 1992).

Biota-sediment Accumulation Factors

Biota-sediment accumulation factors (BSAFs) were calculated by dividing the lipid-normalized tissue concentrations by the organic-carbon normalized sediment concentrations (USEPA 1994). Mean BSAFs for this study were only listed for compounds in which BSAF could be calculated for both laboratory-exposed and field-collected oligochaetes in at least seven of 13 pools (Table 2.2). For laboratory-exposed oligochaetes, mean BSAFs ranged from 1.1 for benzo(a)anthracene to 5.3 for naphthalene. Mean BSAFs for field-collected oligochaetes, mean BSAFs ranged from 0.5 for benzo(a)anthracene to 8.8 for naphthalene. Individual sample BSAFs for naphthalene ranged from 1.6 to 10.1 in laboratory-exposed oligochaetes and 2.5 to 26.6 in field-collected oligochaetes. BSAFs for pyrene, benzo(a)anthracene, and benzo(b,k)fluoranthene were typically greater than BSAFs reported for marine organisms (Lee 1992). BSAFs were also calculated using PCB homolog data reported in Ankley *et al.* (1992) for laboratory-exposed *L. variegatus* and field-collected oligochaetes (Figure 2.6). BSAFs were similar between laboratory-exposed and field-collected oligochaetes in both Ankley *et al.* (1992) and in the present study; however, BSAFs in the present study were typically greater (0.5 to 8.8) than those from Ankley *et al.* (1992; 0.17 to 2.26).

A theoretical value of 1.7 for BSAFs has been estimated based on partitioning of non-ionic organic compounds between sediment carbon and tissue lipids (McFarland and Clarke 1986). A BSAF of less than 1.7 indicates less partitioning into lipids than predicted and a value greater than 1.7 indicates more uptake than can be explained by partitioning theory alone (Lee 1992). The majority of the BSAFs in Table 2.2 were within a range of about 0.5 to 2.6 suggesting the theoretical BSAF value of 1.7 could be used to predict these mean BSAFs with a fair amount of certainty. However, mean BSAFs for naphthalene (8.8) and 2-methyl naphthalene (6.7) in the field-collected oligochaetes were elevated relative to a theoretical BSAF of 1.7. Moreover, BSAFs for individual pools were as high as 10.1 for laboratory-exposed oligochaetes and 26.6 for field-collected oligochaetes. The higher BSAFs in the field-collected oligochaetes may be the result of (1) exposure to contaminants in the overlying water, (2) spatial differences in sediment contamination (i.e., sediments were not sampled from a depth representative of the habitat of the oligochaetes), (3) increased error in chemical determinations due to low concentration of contaminants in sediments, or (4) taxonomic-specific differences in exposure. BSAFs substantially different from the theoretical value of 1.7 may also result when the system has not reached steady state (i.e., depletion or release of contaminants in pore water).

Summary

Contaminant concentrations were relatively low in sediments and tissues from the 13 UMR pools evaluated. Only PAHs and total PCBs were frequently measured above detection limits. Most of the concentrations of PAHs in UMR sediment were similar to concentrations in sediments identified as non-toxic in amphipod toxicity tests from these previous studies. PAH concentrations in tissues of oligochaetes tested with highly contaminated samples from previous studies were up to 1000 times greater than tissue concentrations measured in the present study. Concentrations in laboratory exposed and field-collected oligochaetes for a compound from a specific pool in the UMR were generally similar. About 90% of the paired PAH concentrations in laboratory-exposed and field-collected oligochaetes were within a factor of three of one another. With the detection limits used to analyze samples in the present study, contaminants were detected in tissue samples more often than in the associated sediment samples.

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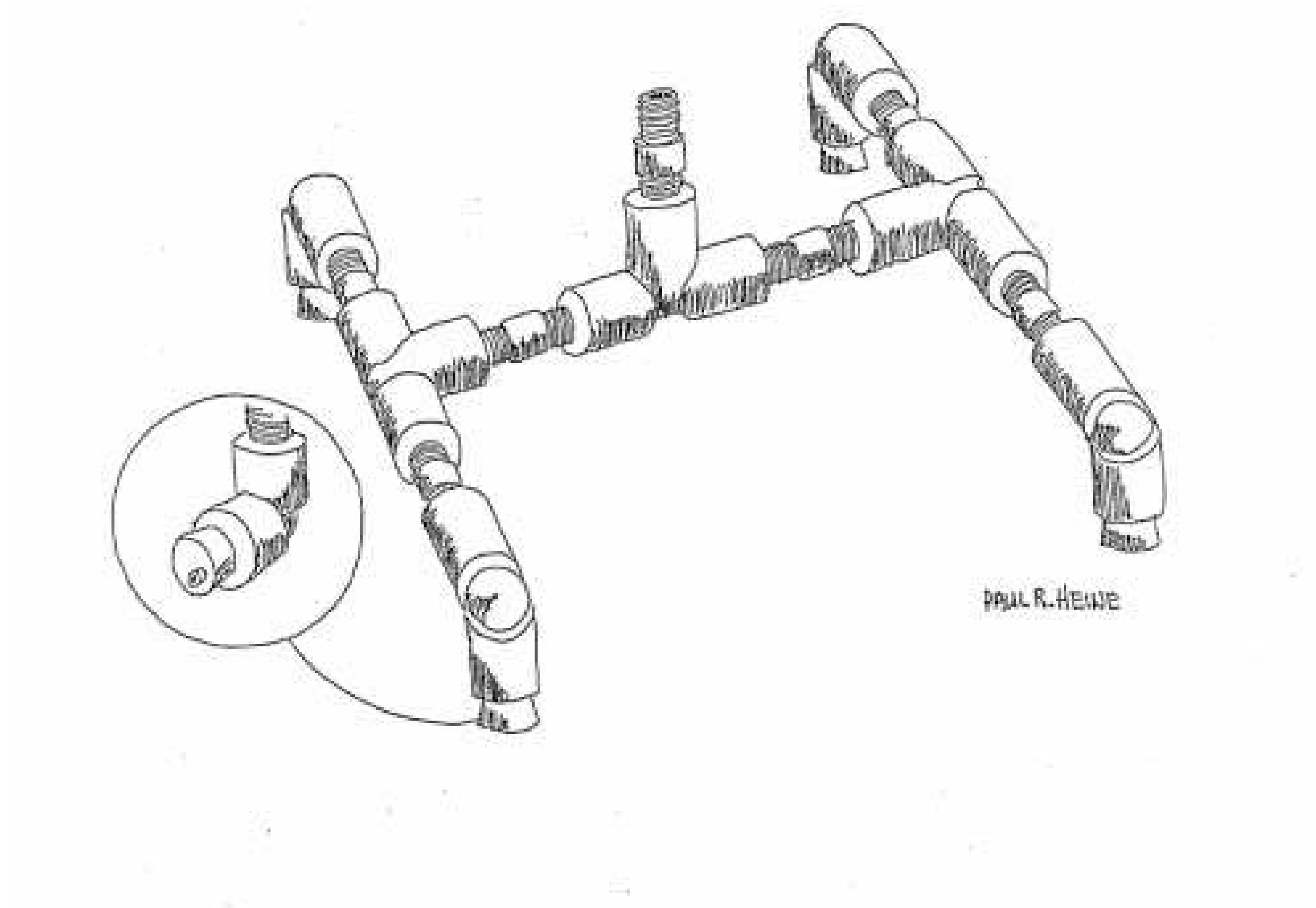

Fig. 2.1. Diagram of in-line flow splitter used to deliver overlying water.

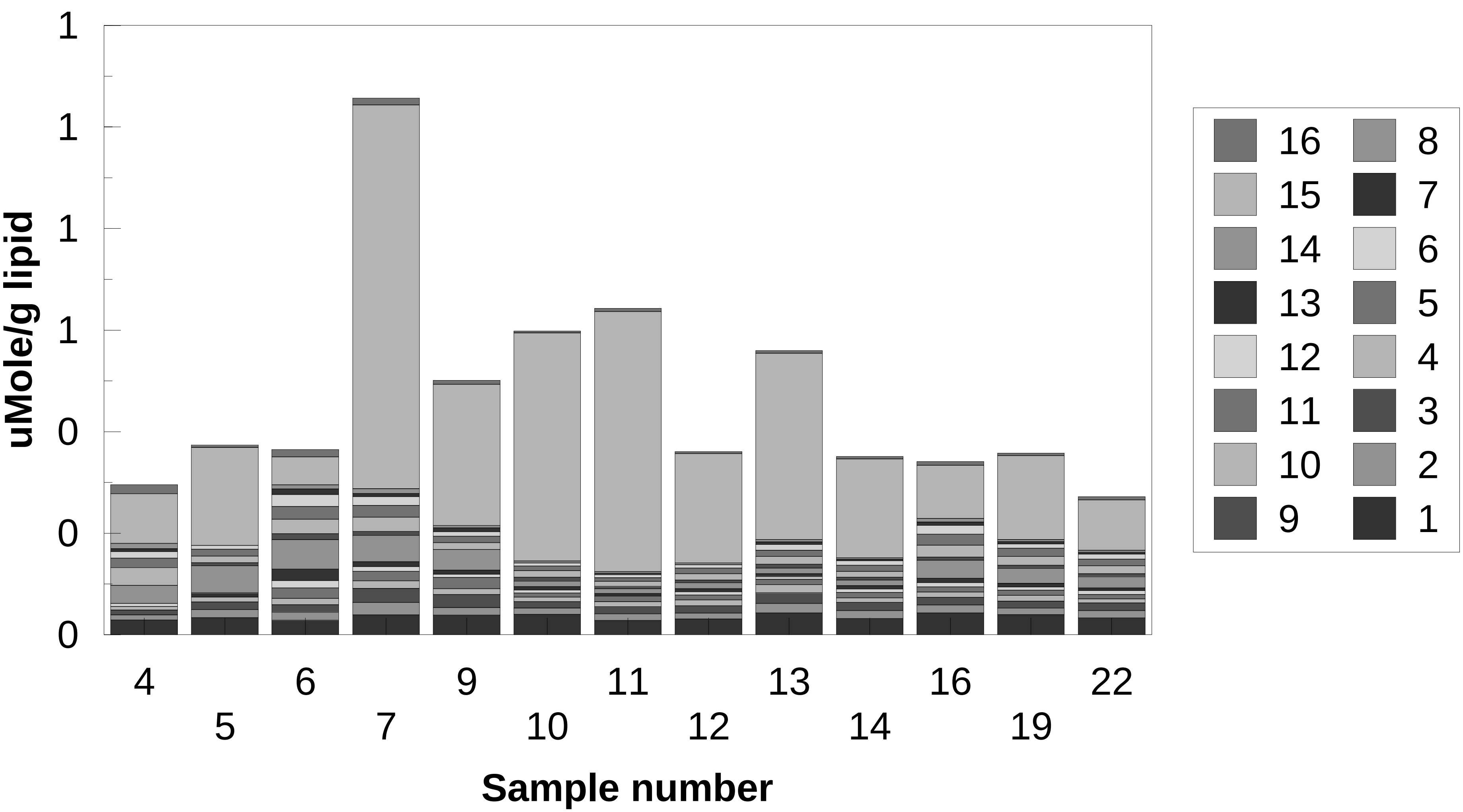

Fig. 2.2. Total accumulation of polycyclic aromatic hydrocarbons (µMole/g lipid) by *Lumbriculus variegatus* exposed in the laboratory to sediments from the Upper Mississippi River. Chemical numbers correspond to the following chemicals: (1) naphthalene, (2) 1-methylnaphthalene, (3) 2-methylnaphthalene, (4) biphenyl, (5) 2,6-dimethylnaphthalene, (6) fluorene, (7) 1,6,7-trimethylnaphthalene, (8) phenanthrene, (9) 1-methylphenanthrene, (10) pyrene, (11) fluoranthene, (12) chrysene, (13) benzo(a)anthracene, (14) benzo[b(k)]fluoranthene, (15) perylene, and (16) benzo(e)pyrene.

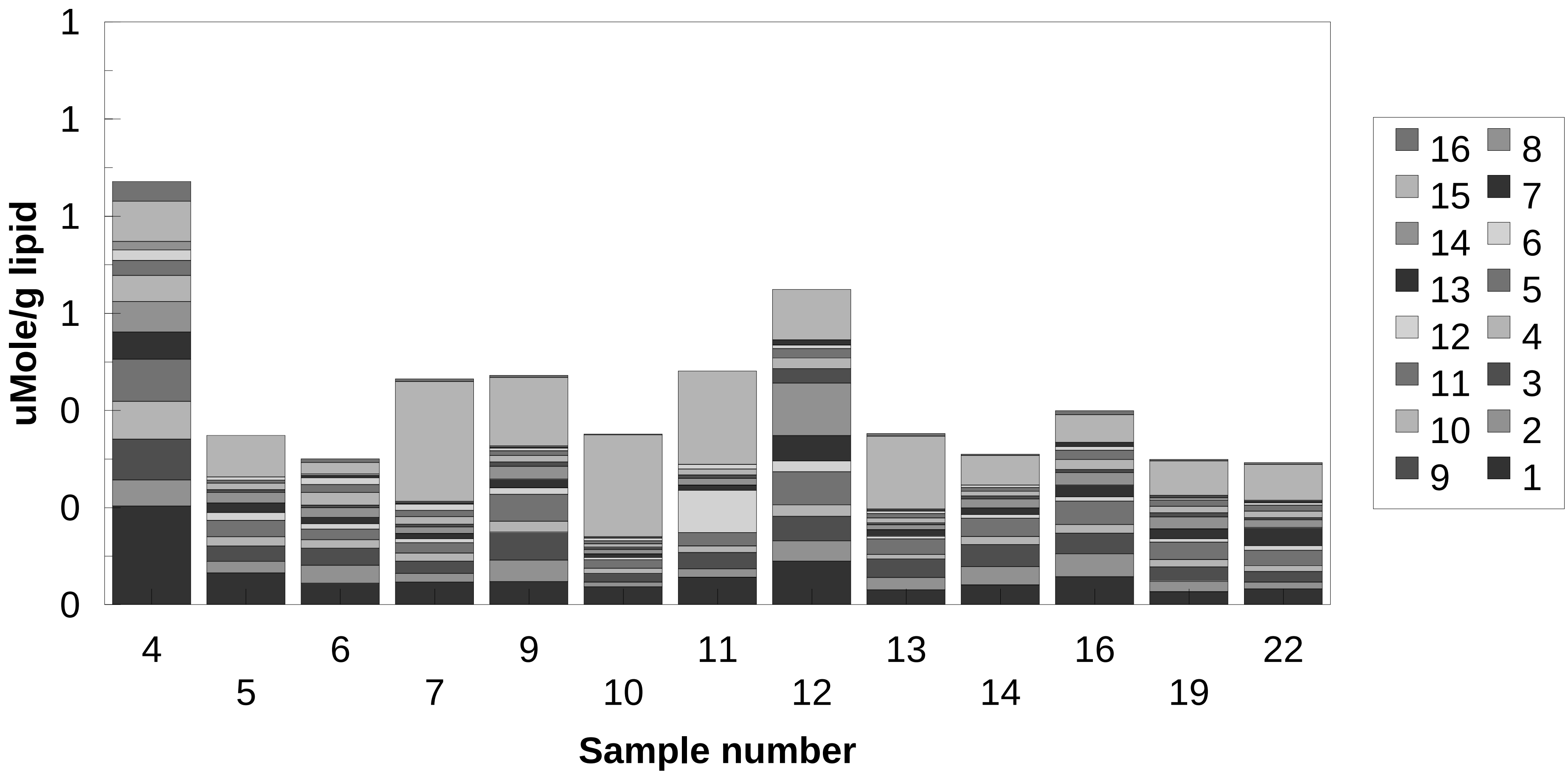

Fig. 2.3. Total accumulation of polycyclic aromatic hydrocarbons (µMole/g lipid) by oligochaetes collected from select pools of the Upper Mississippi River. Chemical numbers correspond to those listed for Figure 2.2.

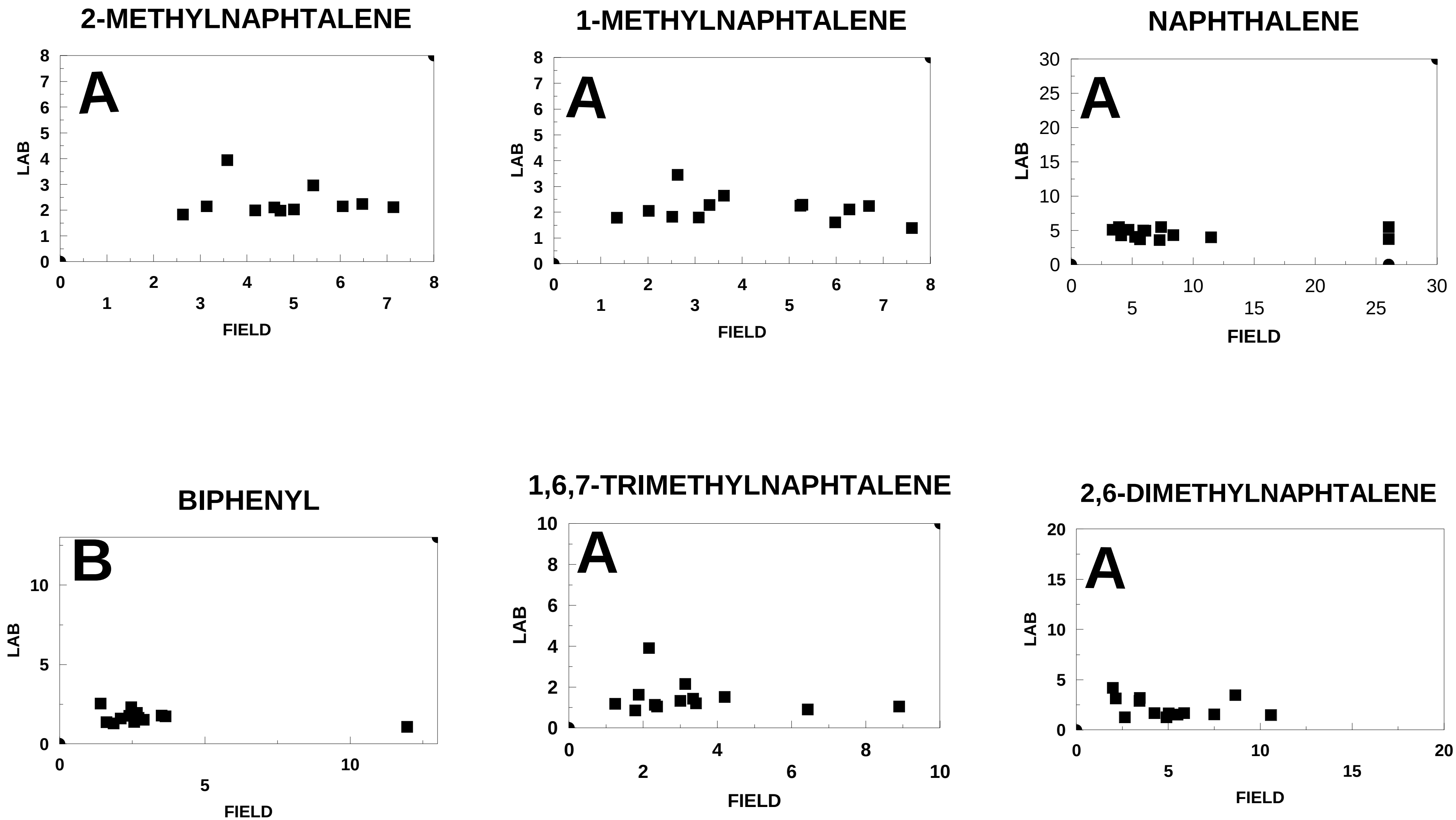

Fig 2.4. Comparison of tissue concentrations in laboratory-exposed *L. variegatus* verses field-collected oligochaetes. An "A" indicates field > lab, "B" indicates laboratory = field, and "C" indicates laboratory > field.

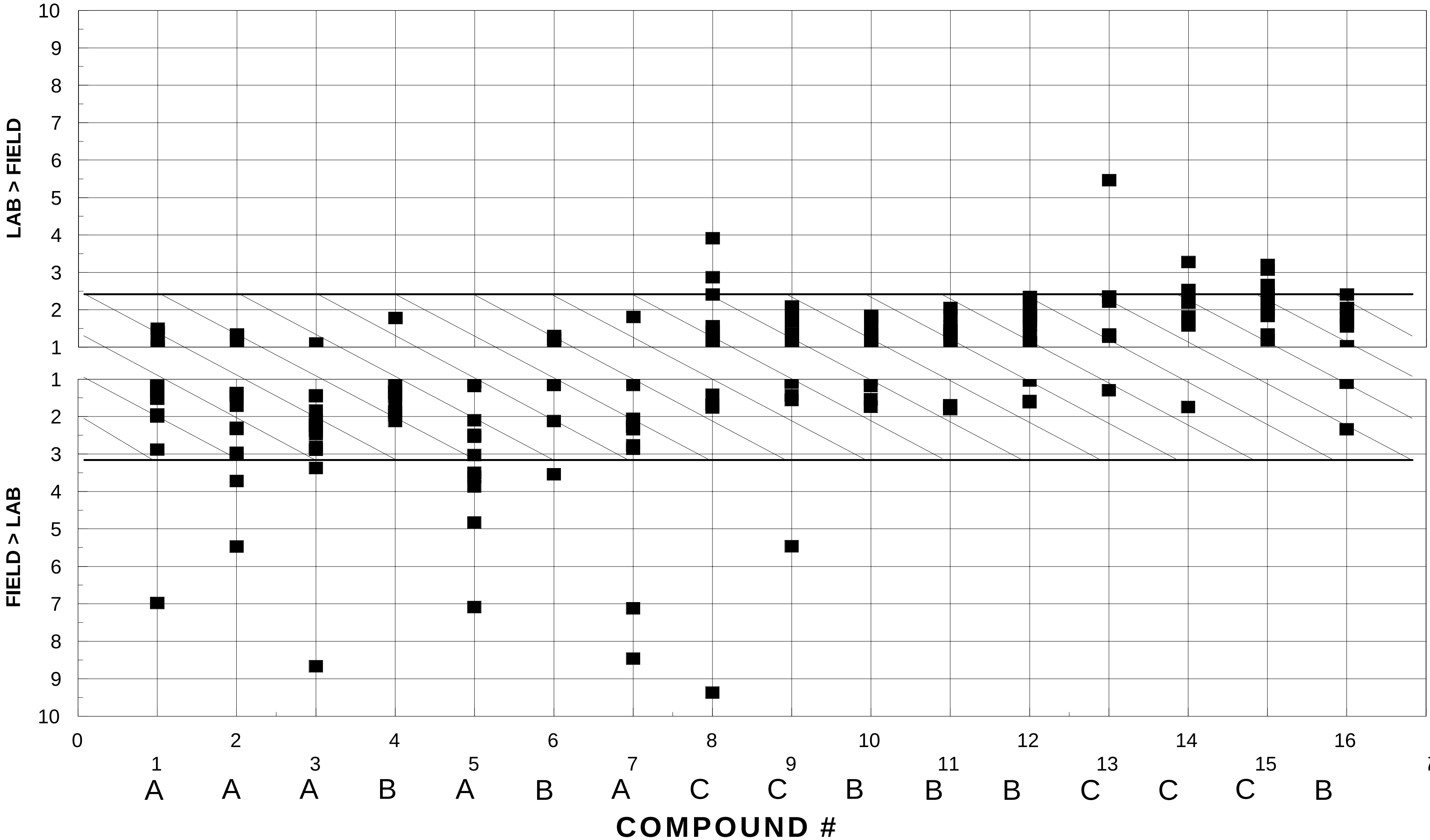

Fig. 2.5. Ratio of tissue concentrations in laboratory-exposed or field-collected oligochaetes for select PAHs. See the legend to Figure. 2.2 for a listing of the specific compounds by number. An "A" indicates field > laboratory, "B" indicates laboratory similar to field, and "C" indicates laboratory > field. Compounds are plotted in order of molecular weight with molecular weight increasing from left to right. If the laboratory concentration of a compound for a pool is higher than the corresponding field concentration, then the laboratory/field ratio is plotted on the upper half of the plot. If the field concentration of a compound for a pool is higher than the corresponding laboratory concentration, then the field/laboratory ratio is plotted on the lower half of the plot (see Appendix 2.4 for a list of ratio values).

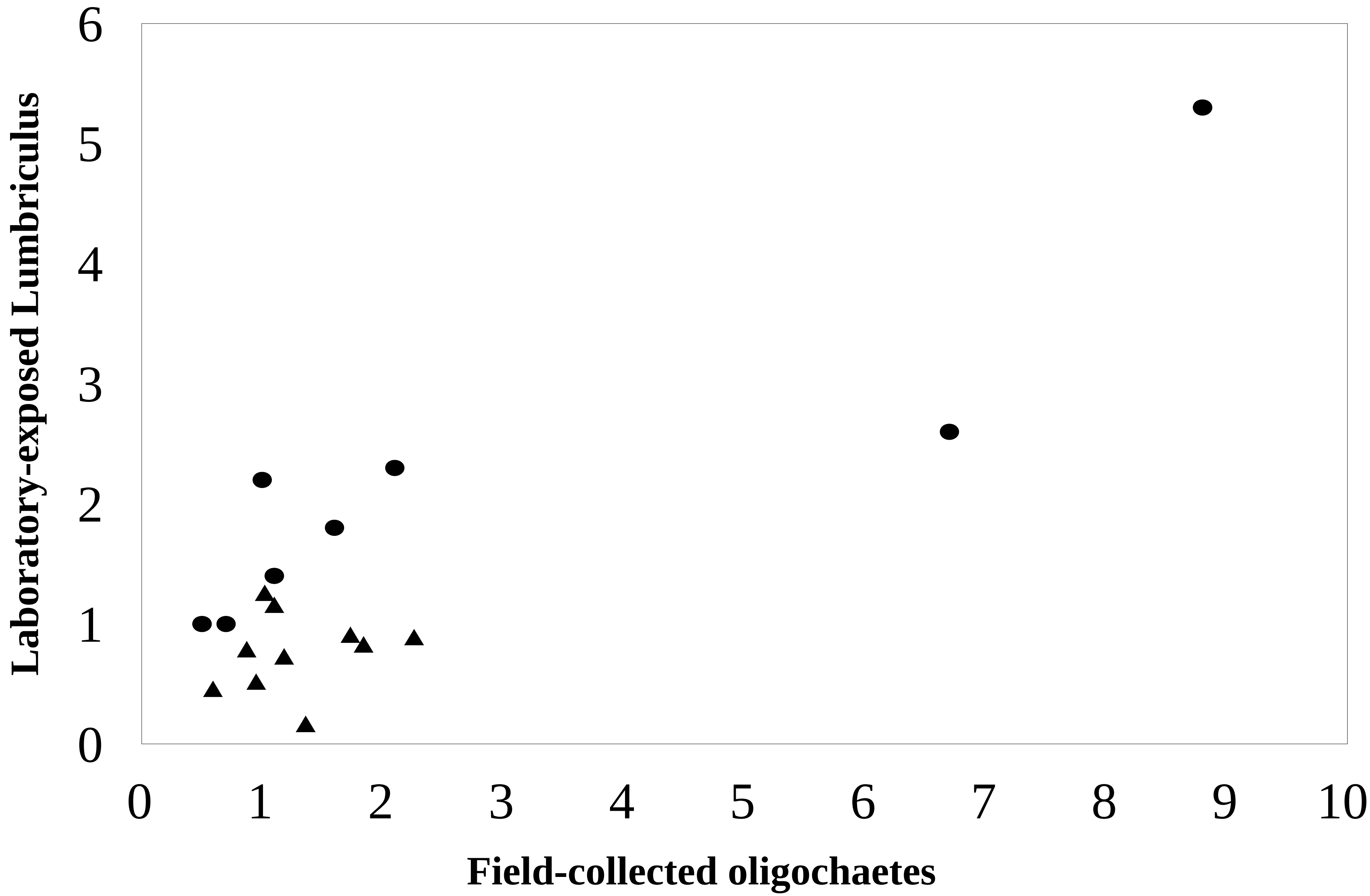

Fig 2.6. Biota-sediment accumulation factors (BSAFs) for laboratory-exposed *Lumbriculus variegatus* and field-collected oligochaetes for PAHs in the present study (circles) and calculated from PCB homolog data reported in (Ankley *et al.* 1992;triangles).

Table 2.1. List of chemicals that met our criteria for laboratory to field comparisons of tissue concentrations and their associated molecular weight and log $K_{ow.}$

Chemical No.	Low molecular-weight PAHs	Molecular Weight	Log Kow	Plot Pattern
1	NAPHTHALENE	128.17	3.35	A
2	1-METHYLNAPHTHALENE	142.20	3.87	A
3	2-METHYLNAPHTHALENE	142.20	4.00	A
4	BIPHENYL	154.21	3.90	B
5	2,6-DIMETHYLNAPHTHALENE	156.23	4.31	A
6	FLUORENE	166.22	4.38	B
7	1,6,7-TRIMETHYLNAPHTHALENE	170.25		A
8	PHENANTHRENE	178.23	4.57	C
9	1-METHYLPHENANTHRENE	192.26	5.14	B
	High Molecular-weight PAHs			
10	PYRENE	202.26	5.18	B
11	FLUORANTHENE	202.26	5.22	B
12	CHRYSENE	228.29	5.86	B
13	BENZOaANTHRACENE	228.29	5.91	C
14	BENZO[b(k)]FLUORANTHENE	252.32	5.78, 6.20	C
15	PERYLENE	252.32	6.25	C
16	BENZOePYRENE	252.32	6.44	B

Table 2.2. Biota-sediment accumulation factors reported by Lee (1992) and in the present study. The meas BSAFs for the present study are listed where there was matching detection of a particular compound in both sediment and tissue in a least seven on 13 pools for laboratory-exposed (lab) or field collected (field) oligochaetes. The range in BSAFs in the present study are reported for as values for samples from the individual pools of the upper Mississippi River.

COMPOUND	Lee (1992)	RANGE	LAB	RANGE	FIELD	RANGE
Naphthalene			5.3	1.6-10.1	8.8	2.5-26.6
2-methylNaph.			2.6	0.9-5.1	6.7	2.2-12.2
Pyrene	0.4	0.18-0.5	2.3	0.8-3.9	2.2	0.7-5.6
Fluoranthene			1.8	0.9-3.9	1.6	0.6-4.9
Chrysene			1.5	0.7-2.4	1.1	0.3-2.0
Benzo(a)anthracene	0.4	0.2-0.6	1.1	0.4-2.5	0.5	0.4-0.7
Benzo[b,(k)]fluoran.	0.4	0.2-1.0				
Perelene			2.24	0.5-4.7	1.02	0.3-1.9

Appendix 2.1. Mean measured overlying water quality for the laboratory bioaccumulation test with Upper Mississippi River samples.

Pool	Dissolved Oxygen (mg/L)	Temperature C	Conductivity (uS)	pH	Alkalinity (mg/L CaCO3)	Hardness (mg/L CaCO3)	Total NH_3 (mg/L)	Unionized NH_3 (mg/L)
4	6.8	22.4	344.2	7.9	63.0	109.5	0.1	0.0047
5	6.3	22.3	346.2	7.8	62.0	104.0	0.1	0.0028
6	6.2	22.3	347.0	7.8	65.5	105.0	0.3	0.0071
7	6.7	22.3	341.6	7.8	64.0	105.5	0.2	0.0054
9	6.8	22.1	348.2	7.9	65.0	109.0	0.2	0.0055
10	6.5	22.3	348.8	7.8	63.0	107.5	0.2	0.0059
11	6.4	22.5	349.2	7.8	61.0	106.5	0.2	0.006
12	5.8	22.5	347.0	7.7	66.5	105.0	0.2	0.0046
13	6.5	22.3	344.2	7.8	64.0	106.5	0.2	0.0051
14	6.9	22.4	345.2	7.8	65.0	107.0	0.1	0.004
16	6.0	22.5	350.0	7.8	66.0	109.5	0.2	0.0064
19	6.2	22.5	347.2	7.8	67.0	107.0	0.2	0.0053
22	6.0	22.3	350.3	7.7	65.0	110.0	0.4	0.0094
Mean	6.4	22.4	346.9	7.8	64.4	107.1	0.2	0.0055
Max.	6.9	22.5	350.3	7.9	67.0	110.0	0.4	0.0094
Min.	5.8	22.1	341.6	7.7	61.0	104.0	0.1	0.0028
Std.	0.4	0.1	2.5	0.1	1.8	1.9	0.1	0.0016
Median	6.4	22.3	347.0	7.8	65.0	107.0	0.2	0.0054

Appendix 2.2. Tissue concentrations of organochlorine compounds measured in laboratory-exposed and field-collected oligochaetes. All concentrations are on a wet-weight basis (µg/g). Appendix 2.2 data can be obtained electronically from:

anonymous ftp - ftp://ftp.msc.nbs.gov/pub/umr/umr.zip

world wide web - http://www.msc.nbs.gov/pubs/umr.html

For problems with access to the above addresses please e-mail the Webmaster, Chris Henke, at chenke@msc.nbs.gov or call 573-875-5399.

Appendix 2.3. Tissue concentrations of PAHs measured in laboratory-exposed and field-collected oligochaetes. All concentrations are on a wet-weight basis (µg/g). Appendix 2.3 data can be obtained electronically from:

anonymous ftp - ftp://ftp.msc.nbs.gov/pub/umr/umr.zip

world wide web - http://www.msc.nbs.gov/pubs/umr.html

For problems with access to the above addresses please e-mail the Webmaster, Chris Henke, at chenke@msc.nbs.gov or call 573-875-5399.

Appendix 2.4 Total accumulation (µMole/g lipid) of PAHs in Laboratory exposed (LMML) and Field Collected (FMML) oligochaetes. Lipid-normalized concentrations (µg/g lipid) are given for laboratory-exposed (LLCONC) and field-collected (FLCONC) oligochaetes. Chemical numbers (CHEM) correspond to those listed in Figure 2.3.

OBS	POOL	LLCONC	FLCONC	MOLEWT	CHEM	LMML	FMML
1	4	3.7278	26.0370	128.17	1	0.02908	0.20314
2	4	1.3910	7.6111	142.20	2	0.00978	0.05352
3	4	1.3835	12.0000	142.20	3	0.00973	0.08439
4	4	1.0671	11.9630	154.21	4	0.00692	0.07758
5	4	0.0000	13.5741	156.23	5	0.00000	0.08689
6	4	0.9915	0.0000	166.22	6	0.00597	0.00000
7	4	0.0000	9.4630	170.25	7	0.00000	0.05558
8	4	6.3817	11.2222	178.23	8	0.03581	0.06296
9	4	7.0939	10.9074	202.26	10	0.03507	0.05393
10	4	3.6973	6.2778	202.26	11	0.01828	0.03104
11	4	3.0205	4.9074	228.29	12	0.01323	0.02150
12	4	1.2794	0.0000	228.29	13	0.00560	0.00000
13	4	2.5340	4.4444	252.32	14	0.01004	0.01761
14	4	24.7897	20.7963	252.32	15	0.09825	0.08242
15	4	4.4511	10.4074	252.32	16	0.01764	0.04125
16	5	4.2986	8.3673	128.17	1	0.03354	0.06528
17	5	2.2823	3.3061	142.20	2	0.01605	0.02325
18	5	2.0964	4.5918	142.20	3	0.01474	0.03229
19	5	1.5249	2.8980	154.21	4	0.00989	0.01879
20	5	0.0000	5.3061	156.23	5	0.00000	0.03396
21	5	0.0000	2.6735	166.22	6	0.00000	0.01608
22	5	1.4302	3.3469	170.25	7	0.00840	0.01966
23	5	9.4287	3.9184	178.23	8	0.05290	0.02198
24	5	1.1943	1.0204	192.26	9	0.00621	0.00531
25	5	2.7115	2.6735	202.26	10	0.01341	0.01322
26	5	2.7236	1.3265	202.26	11	0.01347	0.00656
27	5	1.6731	1.5306	228.29	12	0.00733	0.00670
28	5	48.6282	21.6735	252.32	15	0.19272	0.08590
29	5	1.2731	0.0000	252.32	16	0.00505	0.00000
30	6	3.6883	5.6421	128.17	1	0.02878	0.04402
31	6	2.2485	5.2421	142.20	2	0.01581	0.03686
32	6	2.0274	5.0105	142.20	3	0.01426	0.03524
33	6	1.9501	2.6632	154.21	4	0.01265	0.01727
34	6	3.1882	3.4526	156.23	5	0.02041	0.02210
35	6	2.4097	1.8526	166.22	6	0.01450	0.01115
36	6	3.9054	2.1579	170.25	7	0.02294	0.01267
37	6	10.3210	3.6000	178.23	8	0.05791	0.02020
38	6	2.1980	1.0526	192.26	9	0.01143	0.00548
39	6	5.8612	5.3158	202.26	10	0.02898	0.02628
40	6	5.0932	3.2842	202.26	11	0.02518	0.01624
41	6	5.2747	3.0947	228.29	12	0.02311	0.01356
42	6	2.5364	1.0737	228.29	13	0.01111	0.00470

43	6	2.0751	0.8211	252.32	14	0.00822	0.00325
44	6	13.9559	5.9053	252.32	15	0.05531	0.02340
45	6	3.5580	1.9474	252.32	16	0.01410	0.00772
46	7	5.014	5.9123	128.17	1	0.03912	0.04613
47	7	3.454	2.6316	142.20	2	0.02429	0.01851
48	7	3.939	3.5789	142.20	3	0.02770	0.02517
49	7	2.312	2.4737	154.21	4	0.01500	0.01604
50	7	2.896	3.4386	156.23	5	0.01853	0.02201
51	7	1.600	1.3158	166.22	6	0.00962	0.00792
52	7	1.622	1.8772	170.25	7	0.00953	0.01103
53	7	9.197	2.3509	178.23	8	0.05160	0.01319
54	7	1.527	1.1228	192.26	9	0.00794	0.00584
55	7	5.646	3.1053	202.26	10	0.02791	0.01535
56	7	4.740	2.5789	202.26	11	0.02344	0.01275
57	7	3.972	3.0175	228.29	12	0.01740	0.01322
58	7	1.362	0.6140	228.29	13	0.00597	0.00269
59	7	2.357	0.7193	252.32	14	0.00934	0.00285
60	7	190.789	62.2456	252.32	15	0.75614	0.24669
61	7	3.386	1.4035	252.32	16	0.01342	0.00556
62	9	4.952	6.0877	128.17	1	0.03864	0.04750
63	9	2.112	6.2807	142.20	2	0.01485	0.04417
64	9	3.648	8.1579	142.20	3	0.02566	0.05737
65	9	1.782	3.5088	154.21	4	0.01156	0.02275
66	9	3.469	8.6491	156.23	5	0.02220	0.05536
67	9	1.057	2.2456	166.22	6	0.00636	0.01351
68	9	1.321	3.0000	170.25	7	0.00776	0.01762
69	9	7.274	4.6667	178.23	8	0.04081	0.02618
70	9	0.000	1.8070	192.26	9	0.00000	0.00940
71	9	2.744	2.7719	202.26	10	0.01356	0.01370
72	9	2.532	1.8947	202.26	11	0.01252	0.00937
73	9	2.018	1.2807	228.29	12	0.00884	0.00561
74	9	1.819	0.3333	228.29	13	0.00797	0.00146
75	9	1.052	0.6667	252.32	14	0.00417	0.00264
76	9	70.149	35.6667	252.32	15	0.27802	0.14135
77	9	2.052	1.1579	252.32	16	0.00813	0.00459
78	10	5.103	4.6900	128.17	1	0.03981	0.03659
79	10	1.794	1.3400	142.20	2	0.01261	0.00942
80	10	1.835	2.6300	142.20	3	0.01290	0.01850
81	10	1.360	1.6200	154.21	4	0.00882	0.01051
82	10	1.261	2.6500	156.23	5	0.00807	0.01696
83	10	0.971	0.9100	166.22	6	0.00584	0.00547
84	10	1.175	1.2500	170.25	7	0.00690	0.00734
85	10	1.916	1.6300	178.23	8	0.01075	0.00915
86	10	1.466	0.8700	192.26	9	0.00762	0.00453
87	10	2.581	1.4000	202.26	10	0.01276	0.00692
88	10	1.785	1.2300	202.26	11	0.00883	0.00608
89	10	1.412	1.2500	228.29	12	0.00618	0.00548
90	10	0.000	0.1800	228.29	13	0.00000	0.00079
91	10	1.042	0.4700	252.32	14	0.00413	0.00186

92	10	113.346	52.9900	252.32	15	0.44922	0.21001
93	10	1.006	0.4900	252.32	16	0.00399	0.00194
94	11	3.610	7.2371	128.17	1	0.02817	0.05646
95	11	1.830	2.5155	142.20	2	0.01287	0.01769
96	11	1.981	4.7216	142.20	3	0.01393	0.03320
97	11	1.585	2.1031	154.21	4	0.01028	0.01364
98	11	1.673	4.2474	156.23	5	0.01071	0.02719
99	11	0.000	14.5464	166.22	6	0.00000	0.08751
100	11	0.858	1.7938	170.25	7	0.00504	0.01054
101	11	1.735	2.4639	178.23	8	0.00974	0.01382
102	11	0.855	1.3299	192.26	9	0.00445	0.00692
103	11	2.051	2.4433	202.26	10	0.01014	0.01208
104	11	1.421	0.0000	202.26	11	0.00702	0.00000
105	11	1.413	2.2474	228.29	12	0.00619	0.00984
106	11	0.613	0.0000	228.29	13	0.00268	0.00000
107	11	0.709	0.0000	252.32	14	0.00281	0.00000
108	11	129.307	48.6082	252.32	15	0.51247	0.19265
109	11	1.672	0.0000	252.32	16	0.00663	0.00000
110	12	3.986	11.4694	128.17	1	0.03110	0.08949
111	12	1.608	5.9796	142.20	2	0.01131	0.04205
112	12	2.121	7.1429	142.20	3	0.01491	0.05023
113	12	1.727	3.6531	154.21	4	0.01120	0.02369
114	12	1.493	10.5918	156.23	5	0.00956	0.06780
115	12	1.071	3.7959	166.22	6	0.00645	0.02284
116	12	1.052	8.8980	170.25	7	0.00618	0.05226
117	12	2.053	19.2245	178.23	8	0.01152	0.10786
118	12	1.043	5.6939	192.26	9	0.00542	0.02962
119	12	2.587	4.5102	202.26	10	0.01279	0.02230
120	12	2.167	3.9184	202.26	11	0.01071	0.01937
121	12	1.548	1.6122	228.29	12	0.00678	0.00706
122	12	0.000	2.4490	228.29	13	0.00000	0.01073
123	12	0.876	0.0000	252.32	14	0.00347	0.00000
124	12	54.336	26.2653	252.32	15	0.21535	0.10410
125	12	1.057	0.0000	252.32	16	0.00419	0.00000
126	13	5.511	3.9242	128.17	1	0.04300	0.03062
127	13	2.642	3.6212	142.20	2	0.01858	0.02547
128	13	2.956	5.4242	142.20	3	0.02079	0.03815
129	13	2.530	1.4242	154.21	4	0.01641	0.00924
130	13	1.658	5.0303	156.23	5	0.01061	0.03220
131	13	0.805	0.8333	166.22	6	0.00484	0.00501
132	13	1.055	2.3788	170.25	7	0.00619	0.01397
133	13	1.927	1.7727	178.23	8	0.01081	0.00995
134	13	1.420	0.7121	192.26	9	0.00739	0.00370
135	13	3.165	2.0455	202.26	10	0.01565	0.01011
136	13	2.4696	1.8182	202.26	11	0.01221	0.00899
137	13	2.6575	1.1364	228.29	12	0.01164	0.00498
138	13	1.1395	0.4848	228.29	14	0.00499	0.00212
139	13	1.0265	0.5606	252.32	14	0.00407	0.00222
140	13	92.5926	37.9545	252.32	15	0.36696	0.15042

141	13	1.4223	1.3788	252.32	16	0.00564	0.00546
142	14	4.0636	5.2432	128.17	1	0.03170	0.04091
143	14	2.2940	5.2793	142.20	2	0.01613	0.03713
144	14	2.2365	6.4685	142.20	3	0.01573	0.04549
145	14	1.3810	2.5676	154.21	4	0.00896	0.01665
146	14	1.6780	5.8739	156.23	5	0.01074	0.03760
147	14	1.1280	1.2973	166.22	6	0.00679	0.00780
148	14	1.1299	2.3243	170.25	7	0.00664	0.01365
149	14	1.9380	3.2793	178.23	8	0.01087	0.01840
150	14	1.0663	1.1622	192.26	9	0.00555	0.00604
151	14	2.3709	1.9459	202.26	10	0.01172	0.00962
152	14	2.4181	1.6577	202.26	11	0.01196	0.00820
153	14	2.0901	1.1351	228.29	12	0.00916	0.00497
154	14	0.5499	0.0000	228.29	13	0.00241	0.00000
155	14	0.7160	0.0000	252.32	14	0.00284	0.00000
156	14	49.2031	15.3694	252.32	15	0.19500	0.06091
157	14	1.2293	0.6036	252.32	16	0.00487	0.00239
158	16	5.4776	7.3816	128.17	1	0.04274	0.05759
159	16	2.2395	6.6974	142.20	2	0.01575	0.04710
160	16	2.1533	6.0526	142.20	3	0.01514	0.04256
161	16	1.6495	2.7237	154.21	4	0.01070	0.01766
162	16	1.5549	7.5132	156.23	5	0.00995	0.04809
163	16	1.3456	1.4868	166.22	6	0.00810	0.00895
164	16	1.5136	4.1974	170.25	7	0.00889	0.02465
165	16	6.3437	4.5263	178.23	8	0.03559	0.02540
166	16	1.0846	1.1579	192.26	9	0.00564	0.00602
167	16	4.8371	4.2237	202.26	10	0.02392	0.02088
168	16	4.3327	3.8684	202.26	11	0.02142	0.01913
169	16	3.9894	1.7500	228.29	12	0.01748	0.00767
170	16	1.4460	1.8816	228.29	13	0.00633	0.00824
171	16	1.8866	0.0000	252.32	14	0.00748	0.00000
172	16	26.3365	14.3816	252.32	15	0.10438	0.05700
173	16	1.9889	2.1974	252.32	16	0.00788	0.00871
174	19	5.0755	3.4023	128.17	1	0.03960	0.02655
175	19	1.8034	3.0805	142.20	2	0.01268	0.02166
176	19	1.9948	4.1839	142.20	3	0.01403	0.02942
177	19	1.7565	2.4023	154.21	4	0.01139	0.01558
178	19	1.5249	5.5057	156.23	5	0.00976	0.03524
179	19	1.1007	1.2529	166.22	6	0.00662	0.00754
180	19	1.1964	3.4253	170.25	7	0.00703	0.02012

OBS	POOL	LLCONC	FLCONC	MOLEWT	CHEM	LMML	FMML
181	19	5.2950	4.3793	178.23	8	0.02971	0.024571
182	19	1.1791	1.6897	192.26	9	0.00613	0.008788
183	19	3.5176	2.6322	202.26	10	0.01739	0.013014
184	19	3.1881	2.5747	202.26	11	0.01576	0.012730
185	19	1.9302	0.9425	228.29	12	0.00846	0.004129
186	19	1.0474	0.7816	228.29	13	0.00459	0.003424
187	19	1.1345	0.5172	252.32	14	0.00450	0.002050
188	19	41.6354	18.0115	252.32	15	0.16501	0.071384
189	19	1.2650	0.8161	252.32	16	0.00501	0.003234
190	22	4.2526	4.1146	128.17	1	0.03318	0.032103
191	22	2.0454	2.0208	142.20	2	0.01438	0.014211
192	22	2.1461	3.1354	142.20	3	0.01509	0.022049
193	22	1.2772	1.8646	154.21	4	0.00828	0.012091
194	22	1.2666	4.8958	156.23	5	0.00811	0.031337
195	22	1.3352	1.5521	166.22	6	0.00803	0.009338
196	22	0.9046	6.4375	170.25	7	0.00531	0.037812
197	22	3.9079	2.7708	178.23	8	0.02193	0.015546
198	22	1.1377	0.8542	192.26	9	0.00592	0.004443
199	22	3.1845	2.6979	202.26	10	0.01574	0.013339
200	22	2.5594	2.4896	202.26	11	0.01265	0.012309
201	22	2.3395	1.2188	228.29	12	0.01025	0.005339
202	22	0.8239	0.6458	228.29	13	0.00361	0.002829
203	22	0.9537	0.5625	252.32	14	0.00378	0.002229
204	22	25.0994	18.6667	252.32	15	0.09947	0.073980
205	22	1.5719	0.9583	252.32	16	0.00623	0.003798

Appendix 2.5. Ratios of laboratory to field (L:F) and field to laboratory (F:L) tissue concentrations. Ratios were calculated using lipid-normalized tissue concentrations. Lipid-normalized concentrations (µg/g lipid) are listed for laboratory-exposed (LLCONC) and field-collected (FLCONC) oligochaetes.

OBS	CHEMICAL	CHEM #	POOL	LLCONC	FLCONC	F:L	L:F
1	1,6,7-TRIMETHNAPH	7	5	1.43018	3.3469	2.34022	0.42731
2	1,6,7-TRIMETHNAPH	7	6	3.90541	2.1579	0.55254	1.80982
3	1,6,7-TRIMETHNAPH	7	7	1.62179	1.8772	1.15748	0.86395
4	1,6,7-TRIMETHNAPH	7	9	1.32051	3.0000	2.27184	0.44017
5	1,6,7-TRIMETHNAPH	7	10	1.17534	1.2500	1.06352	0.94027
6	1,6,7-TRIMETHNAPH	7	11	0.85849	1.7938	2.08951	0.47858
7	1,6,7-TRIMETHNAPH	7	12	1.05160	8.8980	8.46136	0.11818
8	1,6,7-TRIMETHNAPH	7	13	1.05454	2.3788	2.25577	0.44331
9	1,6,7-TRIMETHNAPH	7	14	1.12992	2.3243	2.05706	0.48613
10	1,6,7-TRIMETHNAPH	7	16	1.51357	4.1974	2.77316	0.36060
11	1,6,7-TRIMETHNAPH	7	19	1.19635	3.4253	2.86311	0.34927
12	1,6,7-TRIMETHNAPH	7	22	0.90463	6.4375	7.11619	0.14052
13	1-METHYLNAPH	2	4	1.39105	7.6111	5.47149	0.18277
14	1-METHYLNAPH	2	5	2.28229	3.3061	1.44860	0.69032
15	1-METHYLNAPH	2	6	2.24855	5.2421	2.33133	0.42894
16	1-METHYLNAPH	2	7	3.45378	2.6316	0.76194	1.31244
17	1-METHYLNAPH	2	9	2.11157	6.2807	2.97442	0.33620
18	1-METHYLNAPH	2	10	1.79360	1.3400	0.74710	1.33851
19	1-METHYLNAPH	2	11	1.82974	2.5155	1.37477	0.72740
20	1-METHYLNAPH	2	12	1.60816	5.9796	3.71828	0.26894
21	1-METHYLNAPH	2	13	2.64189	3.6212	1.37069	0.72956
22	1-METHYLNAPH	2	14	2.29404	5.2793	2.30131	0.43454
23	1-METHYLNAPH	2	16	2.23951	6.6974	2.99055	0.33439
24	1-METHYLNAPH	2	19	1.80344	3.0805	1.70810	0.58545

25	1-METHYLNAPH	2	22	2.04537	2.0208	0.98801	1.01214
26	1-METHYLPHEN	9	5	1.19430	1.0204	0.85440	1.17041
27	1-METHYLPHEN	9	6	2.19802	1.0526	0.47890	2.08812
28	1-METHYLPHEN	9	7	1.52692	1.1228	0.73534	1.35992
29	1-METHYLPHEN	9	10	1.46592	0.8700	0.59348	1.68497
30	1-METHYLPHEN	9	11	0.85487	1.3299	1.55566	0.64281
31	1-METHYLPHEN	9	12	1.04266	5.6939	5.46091	0.18312
32	1-METHYLPHEN	9	13	1.42040	0.7121	0.50135	1.99461
33	1-METHYLPHEN	9	14	1.06633	1.1622	1.08987	0.91754
34	1-METHYLPHEN	9	16	1.08460	1.1579	1.06758	0.93670
35	1-METHYLPHEN	9	19	1.17914	1.6897	1.43296	0.69786
36	1-METHYLPHEN	9	22	1.13769	0.8542	0.75079	1.33193
37	2,6-DIMETHNAPH	5	6	3.18818	3.4526	1.08295	0.92341
38	2,6-DIMETHNAPH	5	7	2.89561	3.4386	1.18752	0.84209
39	2,6-DIMETHNAPH	5	9	3.46864	8.6491	2.49352	0.40104
40	2,6-DIMETHNAPH	5	10	1.26107	2.6500	2.10139	0.47588
41	2,6-DIMETHNAPH	5	11	1.67319	4.2474	2.53851	0.39393
42	2,6-DIMETHNAPH	5	12	1.49311	10.5918	7.09381	0.14097
43	2,6-DIMETHNAPH	5	13	1.65836	5.0303	3.03330	0.32967
44	2,6-DIMETHNAPH	5	14	1.67797	5.8739	3.50059	0.28567
45	2,6-DIMETHNAPH	5	16	1.55492	7.5132	4.83186	0.20696
46	2,6-DIMETHNAPH	5	19	1.52495	5.5057	3.61045	0.27697
47	2,6-DIMETHNAPH	5	22	1.26658	4.8958	3.86540	0.25871
48	2-METHYLNAPH	3	4	1.38352	12.0000	8.67355	0.11529
49	2-METHYLNAPH	3	5	2.09642	4.5918	2.19032	0.45655
50	2-METHYLNAPH	3	6	2.02741	5.0105	2.47140	0.40463
51	2-METHYLNAPH	3	7	3.93896	3.5789	0.90860	1.10059
52	2-METHYLNAPH	3	9	3.64825	8.1579	2.23611	0.44720
53	2-METHYLNAPH	3	10	1.83465	2.6300	1.43351	0.69759
54	2-METHYLNAPH	3	11	1.98082	4.7216	2.38369	0.41952

55	2-METHYLNAPH	3	12	2.12090	7.1429	3.36784	0.29693
56	2-METHYLNAPH	3	13	2.95584	5.4242	1.83509	0.54493
57	2-METHYLNAPH	3	14	2.23649	6.4685	2.89224	0.34575
58	2-METHYLNAPH	3	16	2.15334	6.0526	2.81082	0.35577
59	2-METHYLNAPH	3	19	1.99482	4.1839	2.09739	0.47678
60	2-METHYLNAPH	3	22	2.14610	3.1354	1.46098	0.68447
61	ACENAPHTHENE		10	0.80778	0.3800	0.47042	2.12574
62	ACENAPHTHENE		12	1.00739	1.3265	1.31680	0.75942
63	ACENAPHTHENE		13	0.69608	0.5758	0.82714	1.20898
64	ACENAPHTHENE		14	0.90012	0.6667	0.74064	1.35018
65	ACENAPHTHENE		16	1.13831	1.0132	0.89005	1.12353
66	BENaANTHRACENE	13	6	2.53638	1.0737	0.42331	2.36231
67	BENaANTHRACENE	13	7	1.36242	0.6140	0.45069	2.21880
68	BENaANTHRACENE	13	9	1.81888	0.3333	0.18326	5.45664
69	BENaANTHRACENE	13	13	1.13952	0.4848	0.42549	2.35025
70	BENaANTHRACENE	13	16	1.44604	1.8816	1.30119	0.76853
71	BENaANTHRACENE	13	19	1.04739	0.7816	0.74624	1.34005
72	BENaANTHRACENE	13	22	0.82395	0.6458	0.78383	1.27579
73	BENbkFLUORAN	14	4	5.06801	8.8889	1.75392	0.57015
74	BENbkFLUORAN	14	6	4.15012	1.6421	0.39568	2.52732
75	BENbkFLUORAN	14	7	4.71436	1.4386	0.30515	3.27705
76	BENbkFLUORAN	14	9	2.10390	1.3333	0.63374	1.57792
77	BENbkFLUORAN	14	10	2.08379	0.9400	0.45110	2.21680
78	BENbkFLUORAN	14	13	2.05292	1.1212	0.54615	1.83099
79	BENbkFLUORAN	14	19	2.26898	1.0345	0.45593	2.19334
80	BENbkFLUORAN	14	22	1.90736	1.1250	0.58982	1.69543
81	BENePYRENE	16	4	4.45106	10.4074	2.33819	0.42768
82	BENePYRENE	16	6	3.55795	1.9474	0.54733	1.82706
83	BENePYRENE	16	7	3.38590	1.4035	0.41452	2.41246
84	BENePYRENE	16	9	2.05173	1.1579	0.56435	1.77195

85	BENePYRENE	16	10	1.00561	0.4900	0.48727	2.05226
86	BENePYRENE	16	13	1.42232	1.3788	0.96939	1.03157
87	BENePYRENE	16	14	1.22928	0.6036	0.49102	2.03656
88	BENePYRENE	16	16	1.98890	2.1974	1.10481	0.90513
89	BENePYRENE	16	19	1.26503	0.8161	0.64512	1.55010
90	BENePYRENE	16	22	1.57189	0.9583	0.60967	1.64024
91	BIPHENYL	4	4	1.06709	11.9630	11.2109	0.08920
92	BIPHENYL	4	5	1.52488	2.8980	1.9004	0.52619
93	BIPHENYL	4	6	1.95013	2.6632	1.3656	0.73226
94	BIPHENYL	4	7	2.31239	2.4737	1.0698	0.93480
95	BIPHENYL	4	9	1.78193	3.5088	1.9691	0.50785
96	BIPHENYL	4	10	1.35955	1.6200	1.1916	0.83923
97	BIPHENYL	4	11	1.58544	2.1031	1.3265	0.75386
98	BIPHENYL	4	12	1.72667	3.6531	2.1157	0.47266
99	BIPHENYL	4	13	2.52996	1.4242	0.5629	1.77636
100	BIPHENYL	4	14	1.38104	2.5676	1.8592	0.53788
101	BIPHENYL	4	16	1.64953	2.7237	1.6512	0.60563
102	BIPHENYL	4	19	1.75647	2.4023	1.3677	0.73116
103	BIPHENYL	4	22	1.27716	1.8646	1.4599	0.68496
104	C1-NAPHTHALENES		4	2.77457	19.6111	7.0682	0.14148
105	C1-NAPHTHALENES		5	3.58325	7.8980	2.2041	0.45369
106	C1-NAPHTHALENES		6	4.28271	10.2421	2.3915	0.41815
107	C1-NAPHTHALENES		7	7.39486	6.2105	0.8398	1.19070
108	C1-NAPHTHALENES		9	5.75268	14.4386	2.5099	0.39842
109	C1-NAPHTHALENES		10	3.63483	3.9700	1.0922	0.91557
110	C1-NAPHTHALENES		11	3.81527	7.2371	1.8969	0.52718
111	C1-NAPHTHALENES		12	3.72231	13.1224	3.5254	0.28366
112	C1-NAPHTHALENES		13	5.59283	9.0455	1.6173	0.61830
113	C1-NAPHTHALENES		14	4.52395	11.7477	2.5968	0.38509
114	C1-NAPHTHALENES		16	4.39785	12.7500	2.8991	0.34493

115	C1-NAPHTHALENES		19	3.80030	7.2644	1.9115	0.52314
116	C1-NAPHTHALENES		22	4.18440	5.1563	1.2323	0.81152
117	CHRYSENE	12	4	3.02049	4.9074	1.6247	0.61550
118	CHRYSENE	12	5	1.67312	1.5306	0.9148	1.09311
119	CHRYSENE	12	6	5.27471	3.0947	0.5867	1.70441
120	CHRYSENE	12	7	3.97181	3.0175	0.7597	1.31624
121	CHRYSENE	12	9	2.01778	1.2807	0.6347	1.57553
122	CHRYSENE	12	10	1.41153	1.2500	0.8856	1.12922
123	CHRYSENE	12	11	1.41262	2.2474	1.5910	0.62855
124	CHRYSENE	12	12	1.54802	1.6122	1.0415	0.96016
125	CHRYSENE	12	13	2.65747	1.1364	0.4276	2.33857
126	CHRYSENE	12	14	2.09014	1.1351	0.5431	1.84131
127	CHRYSENE	12	16	3.98941	1.7500	0.4387	2.27966
128	CHRYSENE	12	19	1.93023	0.9425	0.4883	2.04792
129	CHRYSENE	12	22	2.33951	1.2188	0.5209	1.91960
130	FLUORANTHENE	11	4	3.69733	6.2778	1.6979	0.58896
131	FLUORANTHENE	11	5	2.72360	1.3265	0.4871	2.05317
132	FLUORANTHENE	11	6	5.09321	3.2842	0.6448	1.55082
133	FLUORANTHENE	11	7	4.74009	2.5789	0.5441	1.83799
134	FLUORANTHENE	11	9	2.53183	1.8947	0.7484	1.33624
135	FLUORANTHENE	11	10	1.78517	1.2300	0.6890	1.45136
136	FLUORANTHENE	11	12	2.167	3.9184	1.80861	0.55291
137	FLUORANTHENE	11	13	2.470	1.8182	0.73624	1.35826
138	FLUORANTHENE	11	14	2.418	1.6577	0.68552	1.45875
139	FLUORANTHENE	11	16	4.333	3.8684	0.89285	1.12001
140	FLUORANTHENE	11	19	3.188	2.5747	0.80760	1.23823
141	FLUORANTHENE	11	22	2.559	2.4896	0.97273	1.02804
142	FLUORENE	6	6	2.410	1.8526	0.76882	1.30069
143	FLUORENE	6	7	1.600	1.3158	0.82251	1.21578
144	FLUORENE	6	9	1.057	2.2456	2.12404	0.47080

145	FLUORENE	6	10	0.971	0.9100	0.93722	1.06699
146	FLUORENE	6	12	1.071	3.7959	3.54323	0.28223
147	FLUORENE	6	13	0.805	0.8333	1.03481	0.96636
148	FLUORENE	6	14	1.128	1.2973	1.15012	0.86947
149	FLUORENE	6	16	1.346	1.4868	1.10498	0.90499
150	FLUORENE	6	19	1.101	1.2529	1.13824	0.87855
151	FLUORENE	6	22	1.335	1.5521	1.16242	0.86027
152	NAPHTHALENE	1	4	3.728	26.0370	6.98461	0.14317
153	NAPHTHALENE	1	5	4.299	8.3673	1.94651	0.51374
154	NAPHTHALENE	1	6	3.688	5.6421	1.52973	0.65371
155	NAPHTHALENE	1	7	5.014	5.9123	1.17919	0.84804
156	NAPHTHALENE	1	9	4.952	6.0877	1.22923	0.81351
157	NAPHTHALENE	1	10	5.103	4.6900	0.91909	1.08804
158	NAPHTHALENE	1	11	3.610	7.2371	2.00455	0.49887
159	NAPHTHALENE	1	12	3.986	11.4694	2.87759	0.34751
160	NAPHTHALENE	1	13	5.511	3.9242	0.71211	1.40429
161	NAPHTHALENE	1	14	4.064	5.2432	1.29029	0.77502
162	NAPHTHALENE	1	16	5.478	7.3816	1.34760	0.74206
163	NAPHTHALENE	1	19	5.075	3.4023	0.67034	1.49178
164	NAPHTHALENE	1	22	4.253	4.1146	0.96754	1.03355
165	PERYLENE	15	4	24.790	20.7963	0.83891	1.19202
166	PERYLENE	15	5	48.628	21.6735	0.44570	2.24367
167	PERYLENE	15	6	13.956	5.9053	0.42314	2.36330
168	PERYLENE	15	7	190.789	62.2456	0.32625	3.06510
169	PERYLENE	15	9	70.149	35.6667	0.50844	1.96680
170	PERYLENE	15	10	113.346	52.9900	0.46751	2.13901
171	PERYLENE	15	11	129.307	48.6082	0.37591	2.66019
172	PERYLENE	15	12	54.336	26.2653	0.48339	2.06873
173	PERYLENE	15	13	92.593	37.9545	0.40991	2.43956
174	PERYLENE	15	14	49.203	15.3694	0.31237	3.20138

175	PERYLENE	15	16	26.337	14.3816	0.54607	1.83127
176	PERYLENE	15	19	41.635	18.0115	0.43260	2.31160
177	PERYLENE	15	22	25.099	18.6667	0.74371	1.34461
178	PHENANTHRENE	8	4	6.382	11.2222	1.75850	0.56867
179	PHENANTHRENE	8	5	9.429	3.9184	0.41558	2.40627
180	PHENANTHRENE	8	6	10.321	3.6000	0.34880	2.86693
181	PHENANTHRENE	8	7	9.197	2.351	0.25562	3.91206
182	PHENANTHRENE	8	9	7.274	4.667	0.64156	1.55870
183	PHENANTHRENE	8	10	1.916	1.630	0.85076	1.17542
184	PHENANTHRENE	8	11	1.735	2.464	1.41975	0.70435
185	PHENANTHRENE	8	12	2.053	19.224	9.36506	0.10678
186	PHENANTHRENE	8	13	1.927	1.773	0.92014	1.08679
187	PHENANTHRENE	8	14	1.938	3.279	1.69212	0.59098
188	PHENANTHRENE	8	16	6.344	4.526	0.71351	1.40152
189	PHENANTHRENE	8	19	5.295	4.379	0.82707	1.20909
190	PHENANTHRENE	8	22	3.908	2.771	0.70903	1.41037
191	PYRENE	10	4	7.094	10.907	1.53757	0.65038
192	PYRENE	10	5	2.711	2.673	0.98599	1.01421
193	PYRENE	10	6	5.861	5.316	0.90694	1.10261
194	PYRENE	10	7	5.646	3.105	0.54999	1.81823
195	PYRENE	10	9	2.744	2.772	1.01032	0.98978
196	PYRENE	10	10	2.581	1.400	0.54242	1.84359
197	PYRENE	10	11	2.051	2.443	1.19119	0.83949
198	PYRENE	10	12	2.587	4.510	1.74316	0.57367
199	PYRENE	10	13	3.165	2.045	0.64631	1.54726
200	PYRENE	10	14	2.371	1.946	0.82078	1.21836
201	PYRENE	10	16	4.837	4.224	0.87318	1.14524
202	PYRENE	10	19	3.518	2.632	0.74829	1.33639
203	PYRENE	10	22	3.185	2.698	0.84720	1.18036
204	TOTAL PCBs		4	133.321	149.278	1.11969	0.89310

205	TOTAL PCBs	5	26.355	66.061	2.50663	0.39894
206	TOTAL PCBs	6	28.644	13.768	0.48067	2.08043
207	TOTAL PCBs	7	14.275	99.316	6.95756	0.14373
208	TOTAL PCBs	9	15.253	17.000	1.11453	0.89724
209	TOTAL PCBs	10	23.962	14.350	0.59887	1.66982
210	TOTAL PCBs	11	39.490	19.979	0.50593	1.97656
211	TOTAL PCBs	12	25.261	21.204	0.83940	1.19132
212	TOTAL PCBs	13	10.182	8.197	0.80506	1.24214
213	TOTAL PCBs	14	14.400	27.045	1.87813	0.53245
214	TOTAL PCBs	16	58.854	25.026	0.42523	2.35168
215	TOTAL PCBs	19	50.689	6.310	0.12449	8.03273
216	TOTAL PCBs	22	25.622	5.813	0.22686	4.40802

Chapter 3: Assessing Sediment Toxicity from Upper Mississippi River Navigational Pools Using a Benthic Invertebrate Community Evaluation and the Sediment Quality Triad Approach

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Introduction

The Mississippi River is the central catchment for a majority of the water runoff between the west side of the Appalachian mountain range to the east side of the Rocky mountain range. This makes the Mississippi River system the largest in the United States, the third largest drainage worldwide, and the seventh largest average discharge worldwide (Van der Leeden, Troise and Todd 1990). The river receives inputs from municipal, agricultural, and industrial sources. Previous studies have examined the concentrations of organic and inorganic contaminants in the sediments from select pools in the Upper Mississippi River (Wiebe 1927; Bailey and Rada 1984; Weiner et al. 1984; Rada et al. 1990). Recently, some studies have reported a decline in the levels of contaminants in the sediments of the Upper Mississippi River (Rada et al. 1990).

The Upper Mississippi River (UMR), that part of the river north of the confluence with the Ohio River at Cairo, IL, is divided into a series of large runs and pools by 26 locks and dams constructed for navigational purposes (Rada et al. 1990). This lock and dam system, which runs from Minneapolis, MN to St. Louis, MO, provides areas for deposition of large quantities of fine grained sediments during normal and low flows (Nielson et al. 1984). Contaminants are often associated with fine-grained sediments and settle along with these sediments.(Forstner and Wittmann 1979, Hassett et al. 1980) Sediments often serve as a sink for an array of organic and inorganic contaminants when the water to sediment gradient is high, and these sediments can act as a source of contamination when the water to sediment gradient is low (Shimp et al. 1971; Oschwald 1972; Medine and McCutcheon 1989).

Benthic macroinvertebrates inhabiting the sediments are presumably exposed continuously to any contaminants contained in the sediments. Benthic macroinvertebrate abundance, community structure, and ecological function have long been used to characterize water quality in freshwater ecosystems (Davis and Lathrop 1992). Numerous studies have documented potential changes in benthic invertebrate community structure associated with the impacts of contaminants (Cook and Johnson 1974; Rosenberg and Wiens 1976; Hilsenhoff 1982, 1987; Waterhouse and Farrell 1985; Clements et al. 1992). Most studies in lotic environments have examined the responses of benthic macroinvertebrate communities in riffle areas due to ease of collection and observed higher taxa richness. However, only a limited number of assessments have been conducted in depositional soft-sediments (Canfield et al. 1996).

The spatial and temporal distribution of resident organisms may reflect the degree to which chemicals in the sediments are bioavailable and toxic. Field surveys of invertebrates can provide an important component of biological assessments of toxicity associated with contaminated sediments for several reasons: (1) macroinvertebrates are abundant, relatively sedentary, easy to collect, and ubiquitous across a broad array of sediment types; (2) many indigenous benthic organisms complete all or most of their life cycles in the aquatic environment and may serve as continuous monitors of sediment quality; and (3) results of an assessment of indigenous populations may be useful for quantifying resource damage (Cook 1976, Pratt and Coler 1976, Davis and Lathrop 1989).

The United States Geological Survey (USGS) has been monitoring the UMR since 1987 to document the fate, transport, and distribution of contaminated sediments (Moody and Meade 1995). Concern with regard to the fate of contaminated sediments in the UMR arose after the flood of 1993, because of the potential for re-exposure of deeply buried, potentially highly contaminated sediments. Further, the flood inundated numerous riparian areas known to contain both diffuse and concentrated (i.e. fuel tanks, warehouses) sources of contaminants. This study was designed to evaluate the current status of sediments in the UMR and is one chapter in a series designed to assess the extent of sediment contamination in the navigational pools of the river. The overall study consisted of the following components: (1) monitoring concentrations of contaminants in the Mississippi River sediments (Moody et al. 1996); (2) toxicity testing with whole-sediments collected from the river (Chapter 1); (3) bioaccumulation tests with whole-sediments collected from the river (Chapter 2); and (4) analysis of benthic invertebrate community structure. The objective of this portion of the study was three-fold: (1) describe distributions and abundances of benthic invertebrates in soft-sediments from selected locations in pools of the UMR; (2) evaluate impacts of contaminants associated with these sediments using measures of benthic invertebrate community structure; and (3) evaluate the concordance of benthic invertebrate assessments to sediment toxicity and sediment chemistry using the sediment quality triad approach.

Materials and Methods

Sampling Locations

Stations were selected for assessment of sediment toxicity, sediment chemistry and benthic macroinvertebrate communities based on historical chemistry data (Moody et al. 1996) and the availability of soft sediments (Chapter 1). Upper Mississippi River pools were sampled from June 11 to July 5, 1994. Stations were located in 23 of 26 pools in the UMR from pool 1 near Hastings, MN to pool 26 near St. Louis, MO (Figure 1.1, Chapter 1). A complete description of the sampling locations in each pool is described in Kemble et al. (Chapter 1) and bioaccumulation data is contained in Brunson et al. (Chapter 2).

Sediment Collection, Handling, and Storage

Locations of stations for field sampling were determined with a Global Positioning System. A stainless steel standard Ponar grab (23 x 23 cm, 529 cm^2 area) was used to collect bulk sediments from about the upper 6 to 10 cm of the sediment for chemistry analyses, laboratory toxicity assessments and benthic invertebrates assessments at one station per pool (Chapter 1). Each sample was a composite of 35 to 80 L of sediment/station (identified as C samples in Chapter 1). Sediments were placed in a 120-L high density polyethylene drum and homogenized with a hand-held power drill and a stainless steel auger. A 2.5 liter subsample of sediment for evaluations of benthos was obtained taken from the composite C sample before subsamples were obtained for chemistry and laboratory analyses (Chapter 1). To isolate the benthos, these 2.5 liter subsamples were sieved through an ASTM No.30 (533 μm) and an ASTM No. 60 (250 μm) bucket connected in series using screened river water for rinsing. Material containing benthos retained by the sieves was combined and transferred into 1L high density polyethylene jars, preserved with 10% buffered formalin, and transported to the laboratory. Subsamples for use in toxicity and bioaccumulation testing (10L), for chemical characterization (250 ml for metals, 250 ml for organics), and for physical characterization (250 ml) were taken and stored in high-density polyethylene containers or amber glass I-CHEM bottles (chemical characterizations only). All samples were stored at 4 ^{o}C in the dark (Chapter 1).

Taxonomic Identification

The preserved samples of benthos were placed in a sieve (250 μm) and rinsed thoroughly with tap water in the laboratory to remove formalin and excess silt or mud before sorting. The samples were drained of excess water, returned to the original jars, filled with 95% ethanol and allowed to soak for at least 24 h to facilitate extraction of volatile compounds. Aliquots of the sample were sequentially removed from the jar to sort benthic invertebrates until the entire sample had been sorted.

A binocular dissecting microscope (4x to 12x power) was used to sort and pick the entire sample. Invertebrates were initially sorted and enumerated into the following orders or families: Oligochaeta, Chironomidae, Bivalvia, Gastropoda, Ephemeroptera, Odonata, Plecoptera, Hemiptera, Megaloptera, Trichoptera, Coleoptera, Diptera, Hirudinea, and Amphipoda. Taxa were identified to the lowest practical level using appropriate taxonomic keys (Wiederholm 1983; Merritt and Cummins 1984; Pennak 1989; Thorp and Covich 1991). The following benthic macroinvertebrate metrics were calculated: macroinvertebrate abundance (number/m^2), species composition, and taxa richness (Appendix 3.1, 3.2, 3.3). All taxa were either identified or verified by personnel at the Aquatic Resources Center in Franklin, TN.

Chironomid larvae (midge) were examined for deformities in mouthpart structures. These deformities, which included various types of asymmetry, missing teeth, extra teeth, fusion among various teeth, and labial separation, have been described by several investigators (Saether 1970; Hamilton and Saether 1971; Hare and Carter 1976; Warwick et al. 1987; Warwick 1989). Individual midge were mounted on slides and their mouthparts were examined for deformities in the mentum and ligula (Tanypodinae only). Occurrence of deformities was expressed as a proportion of the total number of midges at each station.

Physical and Chemical Characterizations of Sediment

Sediment physical characteristics included the following: (1) sediment particle size, (2) total organic carbon, (3) inorganic carbon and (4) percent water. Sediment chemical parameters included the following: (1) chlorinated pesticides, (2) polychlorinated biphenyls (PCB), (3) select aliphatic and polynuclear aromatic hydrocarbons (PAH), (4) simultaneously extracted metals (SEM), (5) acid volatile sulfide (AVS), and (6) total metals. See Chapter 1 for additional information on chemical and physical characteristics of the sediments.

Statistical Analyses

Statistical analysis was performed with the Statistical Analysis System (SAS, Statistical Analysis System 1994). Comparisons between benthic invertebrate abundance and physical and chemical data were made with a Spearman Rank correlation and multivariate regression. If not reported, statements of statistical significance indicate $p \leq 0.05$.

Sediment Quality Triad Assessments

The sediment quality triad (Triad) approach was used as an effects based approach to integrate data from chemical and physical analyses (e.g., PAH's, metals, grain size), sediment laboratory toxicity exposures (e.g., *Hyalella azteca* survival and growth) and benthic community structure (e.g. biotic index, taxa richness, midge mouth-part deformities) in order to evaluate the level of concordance between these three measures and the degree of contaminant-induced degradation in aquatic communities in soft-sediment depositional areas (Chapman et al. 1992). Toxicity, benthos, and chemistry data were scored using procedures developed by Kreis (1988) and data were plotted using procedures described by Canfield et al. (1994, 1996). Values for each individual variable for all samples were scaled proportionally between 1 and 100 (e.g., 1 is indicative of the lowest concentration or least impacted, and 100 is the greatest concentration or most impacted). Scaling data retains proportional differences between measurements and results in an identical range for all variables. Typically, more than one variable is determined for a particular Triad component (e.g. *Hyalella azteca* survival and growth). In these instances Kreis (1988) recommends: (1) scaling each individual variable among samples, (2) summing the scaled values for each variable, and then (3) re-scaling the sums for all samples. This results in scaled scores (e.g., toxicity, benthos, or chemistry) between 1 and 100 for each Triad component which can be compared graphically or in tabular form.

The high and low values used to establish scores for each of the sets of information for benthos, chemistry, and laboratory toxicity were previously reported in Canfield et al. (1996). In order to evaluate the extent of contamination of the UMR in the context of other areas of concern in North America, we used data from three Great Lakes Areas of Concern (Canfield et al. 1996) and data from a study of the upper Clark Fork River, including Milltown Reservoir, in Montana (Canfield et al. 1994). Inclusion of these data sets provided a larger number of stations with a broad range in levels of contamination that could be used in the analyses of the relative responses of benthic communities in select sampling locations in the UMR and evaluate the relative contamination of sediments in the UMR sediments when compared to other areas. Six benthic invertebrate indices were used to evaluate the extent of sediment contamination in the UMR system: (1) total taxa richness, (2) chironomid genera richness, (3) chironomid mouthpart deformities, (4) chironomid biotic index, (5) chironomid/oligochaete ratio, and (6) oligochaete biotic index. The Hilsenhoff index of Biotic Integrity was used to calculate the biotic indices for both the midges and oligochaetes. Species sensitivity within a genera was obtained primarily from those assigned by Hilsenhoff (1982, 1987) and secondarily by Lenat (1993).

To evaluate the chemistry portion of the Triad, we used Effect-Range Median (ERM) concentrations calculated by Ingersoll et al. (1996). An ERM is defined as the concentration of a chemical in sediment above which effects are frequently or always observed (Long et al. 1995). We used seven ERM values which correctly classify laboratory toxicity >70% of the time in *Hyalella azteca* 28-d tests (Ingersoll et al. 1996). These seven ERMs would more closely identify cause and effect toxicity rather than correlative toxicity (Canfield et al. 1996). These ERMs included: cadmium, nickel, lead, zinc, chrysene, benzo(a)pyrene, and benzo(g,h,i)perylene.

To evaluate the laboratory toxicity portion of the Triad we used amphipod (*Hyalella azteca*) 28-d growth and survival to score laboratory toxicity (Chapter 1). Amphipods are sensitive to contaminated sediments and frequently exhibit reduced survival and growth following exposures to contaminated sediments (Burton et al. 1996, Ingersoll et al. 1996). Each sample was designated as toxic when either survival or growth of Hyalella were significantly reduced relative to the control or reference sediment (Chapter 1; Kemble et al. 1994; Ingersoll et al. 1996; USEPA 1993). Associations between benthic indices or laboratory toxicity tests and sediment chemistry were evaluated by plotting the scores of either the benthic indices or laboratory toxicity data against the sum of the ERM quotient (SERM-Q: ERM-Q=concentration of a chemical in sediment sample / ERM for that chemical) for all seven chemicals in a sample. This approach is similar to a toxic unit approach.

Well defined guidelines have not been developed for distinguishing impacts of contaminant effects on benthos found in soft sediments in either lakes, streams, or rivers. Canfield et al. (1996) incorporated data plots (partitioned by using quadrants defined by no effect concentration data for plotting) and frequency analysis to identify the distribution of the data points to identify relations between sediment chemistry, laboratory toxicity and benthic invertebrate distributions. This quadrant frequency analysis (essentially a frequency analysis to identify correct classification and Type I and Type II error) was conducted in order to evaluate which benthic indices were most sensitive to elevated contaminant concentrations. In this analysis, scores for benthic indices are plotted against scores for chemical contamination in sediments. Quadrants were then defined which identified one of four possible conditions: (1) low chemical concentration and benthos not adversely impacted, (2) elevated chemical concentration and benthos adversely impacted, (3) low chemical concentration and benthos adversely impacted (Type I error, false positive), and (4) elevated chemical concentration and benthos not adversely impacted (Type II error, false negative). Various combinations of benthic indices were evaluated by adding the individual scores and re-scoring. These analyses were conducted for all possible combinations of the six scored benthic indices listed above.

Sediment toxicity studies were conducted on sediments from all of the UMR pools (except pools 3 and 17, Chapter 1). The results of the tests on the UMR sediments were combined with data from 19 Great Lakes sediment samples (Ingersoll et al. 1996) and 13 Clark Fork River/Milltown Reservoir samples (Kemble et al. 1994) in order to evaluate the toxicity of the UMR sediments in the context of other samples previously evaluated. Based on previous plots of toxicity scores (Canfield et al. 1996), the vertical quadrant line above which no non-toxic samples were observed and above which chemical contamination was considered toxic was sum of the ERM quotient of 39 (Figure 3.1). The horizontal quadrant line depicting laboratory toxicity was a score of 30, which corresponded to the greatest laboratory score above which no non-toxic samples were observed. This selection procedure for establishing quadrant lines may be less environmentally protective since some of the samples that had a sum of the ERM quotient score less than 39 were toxic to *Hyalella azteca* in the laboratory studies (Chapter 1; Kemble et al. 1994; Ingersoll et al. 1996).

Scores for each of the benthic indices and all combinations of scores were plotted against the sum of the ERM quotient. The position of quadrant lines for benthic indices were determined in 3 steps: (1) plotting the data, (2) drawing the vertical quadrant line at 39 for the Sum of the ERM quotient, (3) by evaluating the distribution of the data and selecting a benthic score (horizontal quadrant line) which maximized the number of points in quadrants which would be considered "correctly classified" and minimized the number of samples with "Type I, false positive" and "Type II, false negative" error results.

Results and Discussion

Benthic Invertebrate Assessments

Abundance:

Benthic invertebrates from the UMR exhibited a wide range of abundance values. Benthic invertebrate abundance (number/m^2) in samples ranged from 250/m^2 in sample 1C to a maximum of 22,389/m^2 in sample 19C (Table 3.1). Total abundance values were less than 8,000/m^2 in 21 of 24 samples with the remaining 3 samples having abundance values two-fold greater than any of the other samples. Oligochaetes were numerically dominant in 12 of 24 samples. Midge comprised the majority of the community in 8 of 24 samples with the bivalves (2), mayflies (1) and nematodes (1) comprising the majority of the community in 4 of 24 samples (Table 3.1; Appendix 3.1, 3.2, 3.3).

Oligochaete abundance ranged from 63/m^2 in sample 5C to 12,111/m^2 in sample 19C (Table 3.1). Across the pools there were order of magnitude differences in abundance values. In general, oligochaete abundance is lowest in samples from the upper pools (1 to 7) and higher in the lower pools (Table 3.1). We expected these differences to be explained by organic carbon and grain size although no significant correlations were observed in the correlation analysis (Table 3.2) evident with this data set.

Chironomid abundance ranged from zero in samples from station 7C to 8,889/m^2 in samples from station 15C (Table 3.1). Distribution of midge was fairly even across this range. These values for chironomid abundances were generally higher than those reported from contaminated sediments in Milltown Reservoir/Clark Fork River (Canfield et al. 1994) or the Great Lakes (Canfield et al. 1996).

Community Composition:

Samples from the UMR had a fairly diverse benthic invertebrate community (Table 3.1). Overall taxa richness was greater in samples from the lower two-thirds of the river than in the upper 8 pools. Oligochaete abundance accounted for 5 to 90% of the community in all samples. Combined oligochaete and midge abundance accounted for 8 to 100% of the total benthic invertebrate community in all samples, with the remainder of the benthic community abundance coming primarily from the Bivalvia and Ephemeroptera.

The oligochaete community was comprised of 2 families, 5 genera and 9 species (Appendix 3.1). Samples from 20C and 11C had the highest number of species, while samples from 1C, 4C and 5C each had only one species. Except for 10C, the oligochaete community was made up entirely of the family Tubificidae. *Limnodrilus spp*., generally considered tolerant of organic and metal contamination (Kennedy 1965, Brinkhurst et al. 1972, Burt et al. 1991), was the most common genera occurring in samples from the UMR.

The midge community was comprised of 4 subfamilies (Chironomini, Tanipodinae, Tanytarsini, Orthocladinae) and 18 genera (Appendix 3.2). The sample from station 10C had the highest number of genera present (8), while sample from station 7C had no genera present. *Chironomus spp*. was the most abundant genera present in 17 of 24 samples, with *Procladius spp*. the most abundant in 3 of the remaining samples.

The Bivalvia (clams) and aquatic insects (excluding midge) comprised a large part (>20%) of the community collected in 11 of 24 samples (Appendix 3.3). Bivalvia abundance ranged from zero in 7 samples to 16,722/m^2 in sample 9C (Table 3.1). The Bivalvia were present in 17 of 24 samples. Bivalvia abundance was greater than or equal to 1,000/m^2 in 5 of 24 samples. Bivalvia abundance of 16,722/m^2 in sample 9C is 1 to 2 orders of magnitude greater than all other samples collected and comprises 77% of the overall community abundance (Table 3.1) . The Bivalvia community was made up almost entirely of *Musculium transversum.*

The Ephemeroptera (mayflies) were present in 16 of 24 samples. Ephemeroptera abundance ranged from absent in 8 samples to 3,278/m^2 in sample 19C (Table 3.1). Ephemeroptera abundance was greater than or equal to 500/m^2 in 6 of 24 samples, but were entirely absent in 8 of 24 samples. The Ephemeroptera community was comprised of 2 families, 2 genera and 3 species. The majority of the insect community (chironomidae excluded) was comprised of *Hexagenia sp.*

The estimated abundance values of benthic invertebrates collected in this study are comparable with the values of invertebrates collected in previous studies of the UMR (Eckblad et al. 1977; Butts and Sparks 1982; Neuswanger, Taylor and Reynolds 1982; Eckblad 1986; Jahn and Anderson 1986; Hornbach et al. 1989). Although there is some variation among studies, abundances were within the same range regardless of the study. In 1991, the Environmental Management Program, Long Term Resource Monitoring Program (LTRMP) issued an observation bulletin (LTRMP Observational Bulletin NO. 1, Eckblad 1991) which reported on the observed decline of macroinvertebrate communities (primarily the fingernail clams) in the UMR. Data from our study does not support the trends reported in the LTRMP report. Benthos abundances were above the low level warnings issued in the LTRMP report. Differences in abundances may be due to natural spatial or temporal variation in the invertebrate communities or conditions in the river and sediments have changed between the time when the LTRMP report was issued and when we conducted our study.

Deformities in chironomids

The frequency of mouth part deformities in the midge community ranged from a low of zero in samples from 11 stations to a maximum of 13% in sample 20C (Figure 3.2). Deformities were present in 13 out of 24 samples in the UMR, although only 4 of 24 samples had deformities which could be considered above the identified background levels of 3 to 4% (Dickman, Brindle and Benson 1992).

Different genera of midge exhibit different levels of susceptibility or tolerance to contaminants (Hamilton and Saether 1971; Hare and Carter 1976; Warwick 1985, 1988; Wiederholm 1984). Some genera are quite intolerant and are eliminated from locations with relatively low levels of contaminants, while other genera such as *Procladius* spp., *Chironomus* spp. and *Cryptochironomus* spp. are more tolerant and may persist in contaminated locations (Warwick 1985; Bode 1988). An association between increased contamination and the presence of midge deformities has been observed by several investigators (Hamilton and Saether 1971; Warwick 1985; Tennessen and Gottfried 1983; Cushman 1984; Wiederholm 1984; Diggins and Stewart 1993). Deformities reported in these studies include thickening of the exoskeleton, enlargement and darkening of the head capsule, asymmetry in mouth parts, missing or fused lateral teeth, and antennal deformities. None of the specimens examined in the present study exhibited antennal deformities. Deformities observed in this study occurred only in *Procladius* spp. and *Chironomus* spp.

The occurrence of midge deformities is reportedly less than 1% in non-impacted or pre-industrialization communities (Wiederholm 1984; Warwick et al. 1987). Background levels have been estimated at 3% to 4% (Dickman, Brindle and Benson 1992), and investigators have suggested that frequency of deformities in the range of 5 to 25% or greater are generally associated with moderate to severe contamination (Wiederholm 1984; Warwick et al. 1987). Based on these criteria, deformities in midge from the UMR indicate that sediments from only 4 samples would be classified as “moderately contaminated” (Figure 3.2). Deformities of midges in samples from the UMR were considerably lower than those from contaminated sediments in studies from either the Milltown Reservoir/Clark Fork River (Canfield et al. 1994) or the Great Lakes (Canfield et al.1996). These data indicate that overall the sediments in the UMR are uncontaminated relative to other locations with documented occurrences of deformities in midges.

Correlation Data

Spearman rank correlations were used to compare associations of physical and chemical measures to benthic responses because of non-normal distribution of data (Snedecor and Cochran 1982). Few significant correlations were detected between benthic parameters and either contaminants or abiotic factors evaluated (Table 3.2). Significant negative correlations were observed between total Ephemeroptera abundance (r=-0.43) and total abundance (r=-0.54) with percent sand. Conversely, significant positive correlations were observed between clay and total numbers (r=0.59), bivalve abundance (r=0.49), chironomid abundance (r=0.48, Ephemeroptera abundance (r=0.47), number of chironomid genera (r=0.46), and number of chironomid taxa (r=0.46). Positive correlations with clay and negative correlations with sand imply that hydrological factors such as current velocity may have been determinants of benthic distributions. For example, clay dominated areas may support greater benthic nymphs due to increased stability of physical habitat and increased deposition of organic matter compared to sandy areas. However, abiotic causality is difficult to infer without additional, manipulative studies.

Significant correlations with measures of chemical contamination were sporadic, observed comparing the measures of total abundance (TOTAL) with zinc (Zn), number of oligochaete taxa (OTAXA) with cadmium (Cd), the oligochaete biotic index (OLBI) with chrysene (CHRYS) and cadmium, and total taxa richness (TXRICH) with chrysene, benzo(a)pyrene (BAP), cadmium and nickel (Ni, Table 3.2). Although these correlations were significant, they still explained no more than 35% of the total variability. Further, the number of positive and negative correlations varied within a particular chemical. This makes interpretation difficult, but may not be unexpected given that the measured chemicals in almost all the sediments were extremely low compared to sediments in other locations in the United States and the relative weakness of non-parametric correlations as statistical tools.

Sediment Quality Triad

Spearman rank correlations described above were used to make initial comparisons between measures of the benthic invertebrate community to measures of sediment chemistry or overlying water quality at the sampling stations. While rank correlation analysis can be used to demonstrate association among variables, this ranking of data eliminates proportional relationships among variables by re-ranking data to simple rank-order (e.g. 1,2,3,). Thus, this ranking can not be used to adequately evaluate dose response relationships. For these reasons, we evaluated benthic community and laboratory toxicity data using a quadrant classification approach described below (see also Canfield et al. 1996).

Results of toxicity and chemistry evaluations of UMR sediments presented in Chapter 1 indicate these sediment samples were relatively uncontaminated compared to other locations in the United States (Kemble et al. 1994; Ingersoll et al 1996). We used the sediment quality triad approach in order to evaluate how benthic communities sampled from the UMR compared to other locations in the U.S. we previously have evaluated (Canfield et al. 1994, 1996).

Scores for various benthic indices relating benthic alterations to contaminant levels were previously identified using data sets from the

Great Lakes (Canfield et al. 1996) and the Clark Fork River in Montana (Canfield et al. 1994). The scores were used to evaluate the scores for samples from the current study. In the present study, benthos samples were not classified as impacted or non-impacted *a priori*, but rather samples were considered to be classified as “incorrect” only if the scores were in the false positive or false negative error quadrants as established in Canfield et al. (1996) (Figure 3.1).

Four benthic indices (midge biotic index, midge richness, percent midge deformities, and taxa richness) were previously found to provide some degree of discrimination among samples from the Great Lakes with differing degrees of contamination (Canfield et al. 1996). In the present study, midge deformities (19%) had the smallest combined false positive and false negative error rate relative to the sum of the ERM quotient score (Table 3.3). Midge oligochaete ratio, midge biotic index, midge taxa richness, and total taxa richness had a combined false positive and false negative error rate of 34% to 35%. A benthos score required to obtain this degree of discrimination was always $\geq$ 75%.

In addition to assessments using single indices, the combined scores of benthic indices were evaluated which provided the best classification (smallest combined false positive and false negative error). Quadrant classification using the sum of the ERM quotient score of two to three combined benthic indices reduced the false positive and false negative error rate to 19 to 24% , which is less than all individually scored benthic indices except midge deformities (Table 3.3). The various combinations of four to all six benthic indices were not included in or discussed since the accuracy of classification did not increase with combinations of more than three benthic indices (Table 3.3). The combinations were restricted so that the benthos score required to minimize false positive and false negative error was a score no greater than 80 to 81. We were unable to identify a combined score of less than 80 which minimized both false positive and false negative error. The combined metric of midge oligochaete ratio, midge taxa richness and total taxa richness provided the combination which had the lowest false positive and false negative error of 19% (Table 3.3).

Table 3.4 summarizes the classification of sediment samples based on exceedances of scores for toxicity, chemistry, or benthos by quadrant analyses as described in Canfield et al. (1996). Twenty-one of 24 samples (88%) showed good agreement (i.e. all "minuses") among all three measures of the Triad, which indicated that no contaminant induced degradation was observed (Table 3.4). None of the samples were scored with all pluses (i.e. evidence of contaminant induced degradation). In one of the 24 (4%) samples laboratory toxicity and sediment chemistry measures were in agreement, however the benthic component was not in accordance. Similarly in one of the samples sediment chemistry and benthos response are in agreement, yet toxicity did not occur. High concordance among laboratory toxicity, chemistry, and benthos is evidence that these sediment samples from the UMR were relatively low in contamination or toxic effects compared to other locations we have previously evaluated (Canfield et al. 1994, 1996).

Summary

Benthic invertebrate abundance values in sediment samples from the UMR were comparable to values reported from relatively uncontaminated sediments. The percent composition of the benthic invertebrate community also indicates a relatively healthy community compared to more contaminated locations. Oligochaetes and chironomids constituted over 90% of the benthic invertebrate communities collected in 10 of 24 samples from the UMR, which is expected given the pre-dominance of soft sediments. However, most of the UMR pools had a relatively high diversity of representatives from orders other than the oligochaetes and chironomids , which is different from observations from other highly contaminated areas (Canfield et al. 1994, 1996). Further benthic community indices were only weakly correlated with sediment contaminants.

The occurrence of midge deformities ranged from 0 to 13% in the UMR pool samples, which were relatively low compared to those chironomids from more highly contaminated sediments. Sediment Quality Triad analyses classified a high percentage of the samples (88%) to be not impacted. These data indicate that these sediment samples were relatively uncontaminated.

Additional studies are needed to evaluate specific contaminant, biotic, and abiotic factors controlling benthic communities in soft sediments associated with backwater areas of both lotic and lentic environments. Studies designed to evaluate benthic distributions in relation to factors influencing variation on a local microhabitat scale are necessary in order to reduce the variation in the relations between sediment chemistry, habitat, and measures of benthic invertebrate communities. These studies should greatly expand our ability to evaluate environmental quality of ecosystems such as the

UMR.

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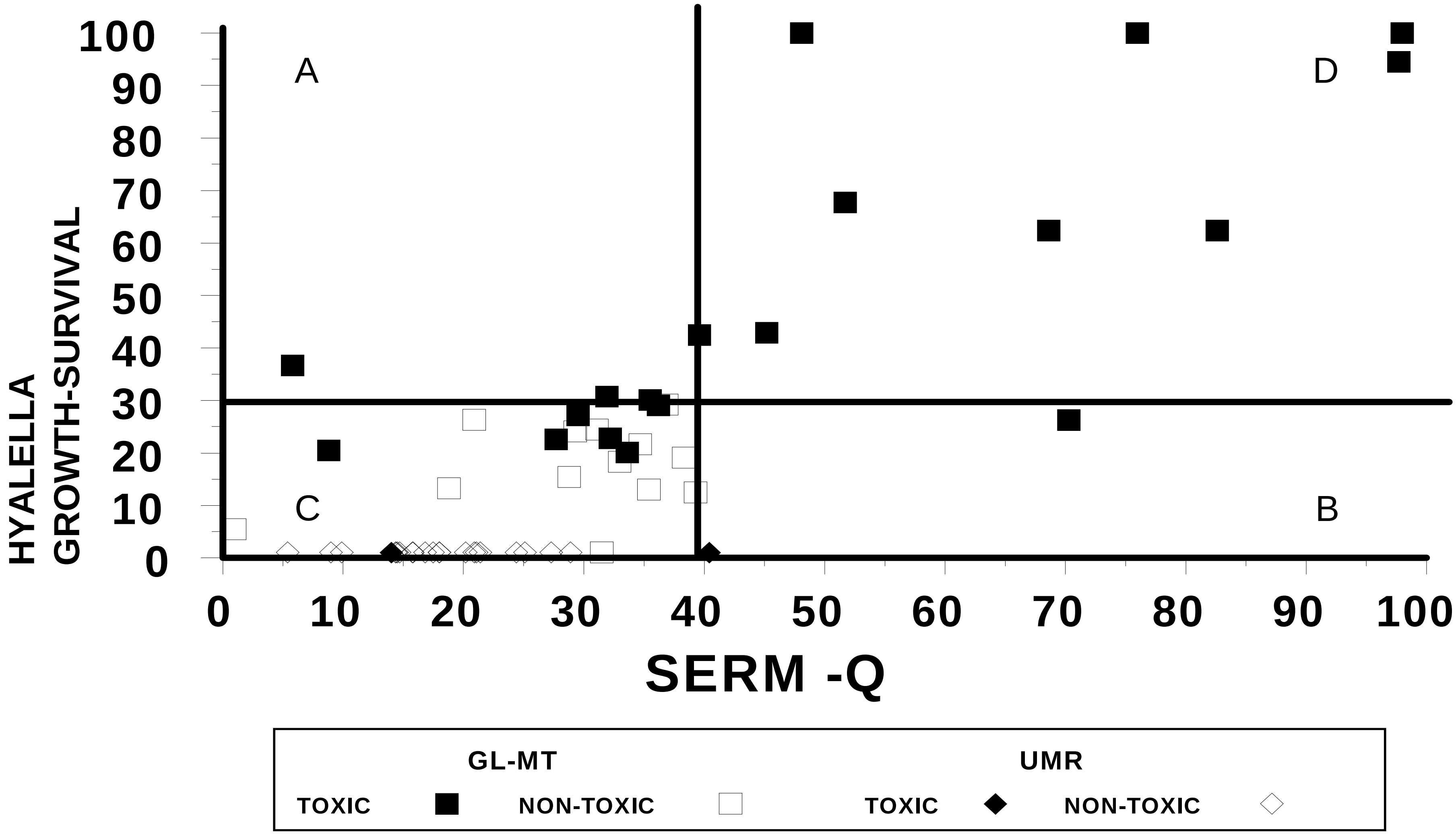

Fig. 3.1. Quadrant analysis with the *Hyalella azteca* toxicity score and the SERM-Q. Quadrants are labeled : A: false positive (Type II error; high toxicity, low chemistry), B: false negative (Type II error; low toxicity, high chemistry), C: non-impacted (low toxicity, low chemistry), D: impacted (high toxicity, high chemistry). A dark square or diamond indicates toxic samples and an open square or triangle indicates non-toxic samples in *Hyalella azteca* toxicity tests. A square (either dark or open) indicates data from samples collected in the Great Lakes and Milltown Reservoir/Clark Fork River, Montana, and a diamond (either dark or open) indicates data from samples collected in the Upper Mississippi River.

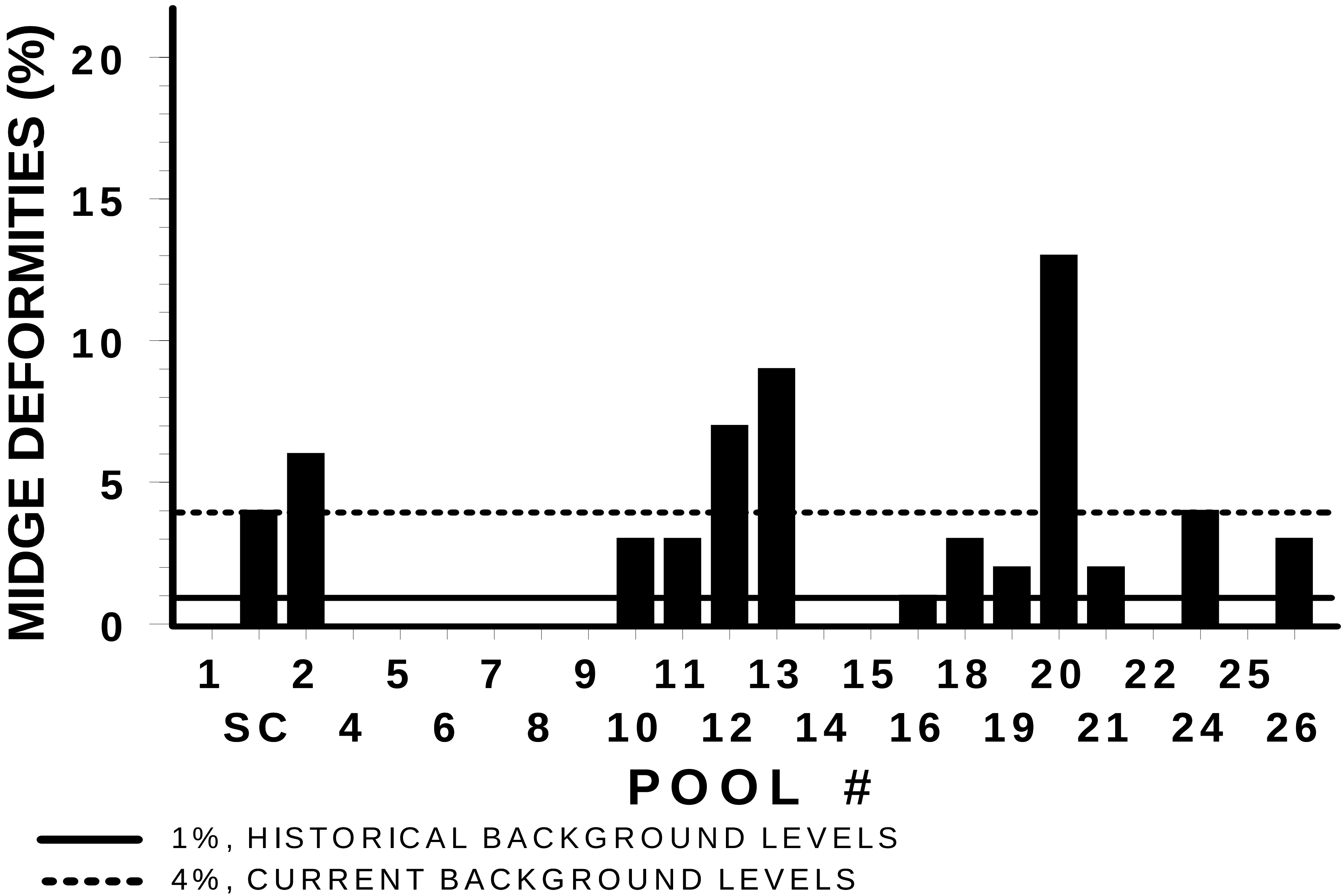

Fig. 3.2. Comparison of chironomid deformities (%) for samples from the UMR samples. Solid line (—) represents the 1% historical background level of deformities (based on analysis of core samples of pre-industrialization sediments) and the dashed line (------) represents the 4% current background levels from modern day studies of uncontaminated sediments.

Table 3.1. Percent contribution of each taxa to the overall total abundance estimates. Values for total abundance are actual number of taxa estimated in each sample. OL- Oligochaeta; CH- Chironomidae; BIVL- Bivalvia; EPHM- Ephemeroptera; ODON- Odonata; HEM- Hemiptera; TRI- Trichoptera; DPT- Diptera; HR- Hirudinea; AMP- Amphipoda; NEMA- Nematoda; HDRNID- Hydrachnida. Sample numbers designate the pools in the Upper Mississippi River where the samples were taken. The sample number SCC corresponds to the sampling site in the Saint Croix River.

	TAXA												
SAMPLE	**OL**	**CH**	**BIVL**	**EPHM**	**ODON**	**HEM**	**TRI**	**DPT**	**HR**	**AMP**	**NEMA**	**HDRNID**	**TOTAL**
SCC	32	63	2	0	0	0	0	0	0	0	2	0	2,733
1C	33	67	0	0	0	0	0	0	0	0	0	0	250
2C	43	36	0	20	0	0	0	0	0	0	0	0	2,933
4C	18	3	0	3	0	0	0	0	0	0	75	1	3,381
5C	11	33	22	22	0	0	11	0	0	0	0	0	563
6C	53	26	3	5	0	2	0	7	0	2	2	0	1,611
7C	12	0	62	22	0	0	1	0	0	1	1	0	4,222
8C	43	35	0	18	1	0	0	2	0	0	0	0	4,000
9C	5	3	77	9	0	0	1	0	2	4	0	0	21,611
10C	46	51	2	0	0	0	0	0	0	0	0	0	4,071
11C	63	29	1	5	0	0	1	0	0	0	1	0	2,581
12C	7	30	19	35	0	1	0	2	1	1	4	0	7,611
13C	39	27	14	6	0	0	0	0	10	5	0	0	7,294
14C	75	20	2	0	0	0	0	2	0	0	0	0	5,500
15C	53	44	1	1	0	0	0	1	0	0	1	0	20,222
16C	46	53	0	0	1	0	0	0	0	0	0	0	3,909
18C	28	69	3	0	0	0	0	0	0	0	1	0	5,583
19C	54	25	6	15	0	0	0	0	0	0	0	0	22,389
20C	90	6	1	2	1	0	1	1	0	0	0	0	1,702
21C	16	60	0	18	3	0	0	3	0	0	0	0	2,429
22C	67	30	2	0	0	0	2	0	0	0	0	0	3,389

24C	13	75	2	6	0	0	0	4	0	0	0	0	2,000
25C	78	16	3	3	0	0	0	0	0	0	0	0	4,808
26C	55	45	0	0	0	0	0	0	0	0	0	0	2,286

Table 3.2. Spearman rank correlation for whole sediment measured naphthalene (NAPH), chrysene (CHRYS), benzo(a)pyrene (BAP), cadmium (Cd), nickel (Ni), lead (Pb), zinc (Zn), percent water (%H2O), percent sand (%SAND), percent silt (%SILT), percent clay (%CLAY), total organic carbon (TOC) with oligochaete abundance (OL), chironomid midge abundance (CH), bivalve abundance (BIVL), mayfly abundance (EPHM), total abundance (TOTAL), number of oligochaete taxa (OTAXA), number of chironomid midge taxa (MTAXA), oligochaete biotic index (OLBI), chironomid midge biotic index, ratio of oligochaetes to chironomid midge (MGOLRAT), chironomid midge mouthpart deformities (MGDFRM), chironomid midge genera richness (MGRICH), and total taxa richness (TXRICH). Significant correlations are designated with an asterisk (*; P≤ 0.05).

METRIC	NAPH	CHRYS	BAP	Cd	Ni	Pb	Zn	%H2O	%SAND	%SILT	PCLAY	TOC
OL	0.21	0.15	-0.09	-0.23	0.07	0.11	0.13	-0.07	-0.32	0.17	0.29	0.09
CH	0.04	0.03	-0.01	-0.06	-0.08	0.24	0.32	-0.17	-0.39	0.07	0.48*	0.11
BIVL	0.28	-0.04	-0.25	0.09	0.21	0.38	0.37	-0.24	-0.33	-0.17	0.49*	0.19
EPHM	0.34	-0.21	-0.01	-0.10	0.16	0.0	0.04	-0.22	-0.43*	0.09	0.47*	0.03
TOTAL	0.39	0.08	0.0	0.10	0.26	0.39	0.46*	-0.31	-0.54*	0.09	0.59*	0.29
OTAXA	-0.20	-0.14	-0.21	-0.60*	-0.33	-0.33	-0.29	0.34	0.0	-0.04	0.03	-0.24
MTAXA	0.08	-0.33	-0.24	-0.23	-0.20	-0.02	0.07	-0.12	-0.34	0.03	0.46*	-0.12
OLBI	0.06	0.45*	0.30	0.42*	0.16	0.12	0.16	-0.12	0.16	0.0	-0.31	0.33
MGBI	0.17	0.22	0.0	0.05	0.29	0.29	0.25	0.02	0.22	-0.08	-0.23	0.24
MGOLRAT	-0.17	-0.12	-0.02	0.16	-0.19	0.03	0.08	-0.07	-0.09	0.0	0.19	0.0
MGDFRM	-0.25	-0.06	0.14	-0.15	-0.19	0.09	0.14	0.0	-0.14	0.05	0.25	-0.09
MGRICH	0.08	-0.33	-0.24	-0.23	-0.19	-0.02	0.07	-0.12	-0.35	0.03	0.46*	-0.12
TXRICH	-0.08	-0.46*	-0.41*	-0.54*	-0.41*	-0.26	-0.16	0.16	-0.09	-0.23	0.23	0.27

Table 3.3. Summary of quadrant analysis for scores of individual benthic measures and combined benthic metric for the sum ERM-Quotients (SERM-Q: ERM-Q=concentration of a chemical in sediment sample/ERM for that chemical) as a score of chemical contamination. Benthos quadrant score is the score which maximized the number of points in quadrants which would be considered "correctly classified" and minimized the incidence of false negative error (low chemical concentrations and benthos adversely impacted) and false positive error (high chemical concentrations and benthos not adversely impacted as established in Canfield et al. 1996).

Number of Benthic Indices in Score	Benthos Score	Error-SERM-Q (%)	Olig. Biotic Index	Midge-Olig Ratio	Midge Biotic Index	Midge Deformities	Midge Taxa Richness	Total Taxa Richness
1	80	59	X					
1	80	35		X				
1	80	35			X			
1	75	19				X		
1	81	34					X	
1	75	34						X
2	80	28			X			X
3	81	18		X	X			X
3	81	15		X			X	X
3	81	24			X		X	X
3	80	19		X	X		X	
Total			1	4	5	1	4	5

Table 3.4. Summary of Sediment Quality Triad data. A plus (+) for chemistry indicates a concentration of contaminants that exceed a SERM-Q score of >39. A plus (+) for laboratory toxicity is based on a Hyalella azteca 28-d growth and survival score of >30. A plus (+) for benthos is based on a combined metric of midge-oligochaete ratio, midge taxa richness and total taxa richness score of >81.

CHEMISTRY	**TOXICITY**	**BENTHOS**	**POOL NUMBER**	**% OF SAMPLE**	**POSSIBLE CONCLUSIONS**
-					
+	+	+	None	0	Evidence of contaminant induced degradation
-	-	-	All pools except 4, 7 and 26	88	No evidence of contaminant induced degradation
+	-	-	None	0	Contaminants not bioavailable
-	+	-	26	4	Chemicals not measured or conditions exist with potential to cause degradation
-	-	+	7	4	Benthos response not due to contaminants
+	+	-	None	0	Contaminants may be stressing system
-	+	+	None	0	Unmeasured chemicals or other conditions causing degredation
+	-	+	4	4	Chemicals not bioavailable or response not due to chemistry

Appendix 3.1. Mean abundance data (number/m^2) for Oligochaeta taxa. DDIG - *Dero digitata*; BSWRE - *Branchiura sowerbyi*; PMOLD - *Potamothrix moldaviensis*; LHOFF - *Limnodrilus hoffmeisteri*; LUDEK - *L. udekemianus*; LCER - *L. cervix;* LCLAP - *L. claparedeianus*; LMAUM - *L. maumeensis*; IBIFID - Immature with bifid setae; VFULL - *Varichaetadrilus fulleri*; IHRPCNS - Immature with hair/pectinate (normal setae); IHRPCES - Immature with hair/pectinate (enlarged setae); TOTAL - total number of individuals; NTAXA - total number of taxa.

	SAMPLE																							
TAXA	SCC	1c	2c	4c	5c	6c	7c	8c	9c	10c	11c	12c	13c	14c	15c	16c	18c	19c	20c	21c	22c	24c	25c	26c
DDIG	0	0	0	0	0	0	0	0	0	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BSWRE	0	0	67	0	0	0	167	48	56	71	163	444	1,412	125	56	0	83	278	36	0	333	42	77	0
PMOLD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
LHOFF	67	0	0	0	0	194	0	190	167	429	140	0	471	375	0	152	0	2,222	274	0	444	42	115	71
LUDEK	0	83	0	0	0	28	0	0	0	0	23	0	59	0	167	212	0	0	71	107	0	0	0	107
LCER	0	0	133	0	0	0	0	95	0	0	140	0	0	250	0	30	0	0	167	0	111	0	231	0
LCLAP	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LMAUM	0	0	0	143	0	0	0	143	0	0	0	0	0	250	0	212	0	0	12	0	0	0	0	0
IBIFID	733	0	1,067	476	0	639	333	952	778	1,286	512	111	882	2,938	5,611	1,152	1,375	9,556	369	107	1,167	83	2,038	1,000
VFULL	0	0	0	0	0	0	0	0	0	0	628	0	0	0	0	0	0	0	583	179	0	83	1,115	71
IHRPCNS	0	0	0	0	63	0	0	190	0	0	23	0	0	0	2500	0	83	0	0	0	56	0	0	0
IHRPCES	0	0	0	0	0	0	0	95	0	0	0	0	0	188	2444	30	0	56	0	0	167	0	154	0
TOTAL	867	83	1,267	619	63	861	500	1,714	1,000	1,857	1,628	556	2,824	4,125	10,778	1,788	1,542	12,111	1,524	393	2,278	250	3,731	1,250
NTAXA	2	1	2	1	1	2	2	5	2	3	6	2	3	5	4	5	3	3	7	3	4	3	5	3

Appendix 3.2. Mean abundance data (number/m^2) for Chironomidae taxa. ABL - *Ablabesmyia*; COLTP - *Coelotanypus;* PRO - *Procladius*; TP - *Tanypus*; CRI - *Cricotopus*; EPCLD - *Epoicocladius*; CRNI - *Chironomini*; CHI - *Chironomus*; CRYP - *Cryptochironomus*; ENCHI - *Endochironomus*; GLPTP - *Glyptotendipes*; HARN - *Harnischia*; LIPNL - *Lipinella*; MICHI - *Microchironomus*; PARCHI - *Parachironomus*; PLUTB - *Paralaurterborniella*; POLY - *Polypedilum*; TRIB - *Tribelos*; STEMP - *Stempellinella*; TOTAL - total number of individuals; NTAXA - total number of taxa.

	SAMPLE																							
TAXA	SCC	1c	2c	4c	5c	6c	7c	8c	9c	10c	11c	12c	13c	14c	15c	16c	18c	19c	20c	21c	22c	24c	25c	26c
ABL	0	0	267	0	0	28	0	48	0	0	23	444	118	0	0	0	0	1,444	12	71	111	0	154	36
COLTP	0	0	0	0	0	0	0	0	333	0	0	0	235	0	0	0	0	222	0	36	0	125	115	0
PRO	533	0	533	0	125	139	0	333	278	71	23	56	59	0	500	0	42	278	0	0	0	42	0	36
TP	0	0	0	0	0	0	0	0	0	143	0	0	0	0	0	0	0	0	0	0	0	167	0	0
CRI	0	0	0	0	0	0	0	0	0	929	0	0	0	0	111	0	0	0	0	0	0	0	0	0
EPCLD	0	0	0	0	0	0	0	0	0	0	0	167	235	0	0	0	0	0	0	0	0	0	0	0
CRNI	0	0	0	0	0	0	0	0	56	214	0	111	0	0	111	30	42	0	0	0	0	0	0	0
CHI	1,067	0	0	95	63	167	0	857	0	143	628	1,111	1,118	1,125	8,056	2,000	3,625	3,611	0	1,286	889	1,083	462	964
CRYP	0	83	0	0	0	28	0	0	56	0	0	0	235	0	56	30	83	111	83	36	0	42	0	0
ENCHI	0	0	0	0	0	0	0	0	0	429	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLPTP	0	0	0	0	0	0	0	0	0	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HARN	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0
LIPNL	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MICHI	0	0	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PARCHI	0	0	0	0	0	0	0	0	0	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLUTB	0	0	133	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
POLY	0	0	67	0	0	0	0	0	0	0	23	389	0	0	56	30	42	0	0	36	0	42	38	0
TRIB	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STEMP	0	83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	1,733	167	1,067	95	188	417	0	1,381	722	2,071	744	2,278	2,000	1,125	8,889	2,091	3,833	5,667	95	1,464	1,000	1,500	769	1,036
NTAXA	3	2	5	1	2	5	0	4	4	8	5	6	6	1	6	4	5	5	2	5	2	6	4	3

Appendix 3.3. Mean abundance data (number/m^2) for benthic invertebrate taxa (excluding oligochaetes and chironomids). HIRUDINEA: HSTAG - Helobdella stagnalis; AMPHIPODA: GPSEUD - Gammarus pseudolimnaeus; HYDRACHNIDA: UNICOLA - Unionicola; EPHEMEROPTERA: BRACH - Brachycercus; HBILIM - Hexagenia bilineata\limbata; ODONATA: DSPIN - Dromogomphus spinosus; HEMIPTERA: CORIX - Corixidae; TRICHOPTERA: HBDNS - Hydropsyche bidens; PFLVA - Potomyia flava; LEPTOCERIDAE: NCTO - Nectopsyche; OCTS - Oecetis; TRICP - Trichoptera Pupae; DIPTERA: CRATO - Ceratopogon; PALPO - Palpomyia; PROBEZ - Probezzia; HEMRO - Hemerodromia; BIVALVIA: SPHAE - Sphaeriidae; MTRANS - Musculium transversum; TOTAL - total number of individuals; NTAXA - total number of taxa.

	SAMPLE																							
TAXA	SCC	1c	2c	4c	5c	6c	7c	8c	9c	10c	11c	12c	13c	14c	15c	16c	18c	19c	20c	21c	22c	24c	25c	26c
HSTAG	0	0	0	0	0	0	0	0	333	0	0	56	706	0	0	0	0	0	0	0	0	0	0	0
GPSEUD	0	0	0	0	0	28	56	0	833	0	0	56	353	0	0	0	0	0	0	0	0	0	0	0
UNICOLA	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRACH	0	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	83	0	0	0	0
HBILIM	0	0	600	95	125	28	944	0	1,889	0	140	2,667	412	0	222	0	0	3,278	36	917	0	125	154	0
DSPIN	0	0	0	0	0	0	0	48	0	0	0	0	0	0	0	30	0	0	12	167	0	0	0	0
CORIX	0	0	0	0	0	28	0	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0
HBDNS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	56	0	0	0
PFLVA	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	12	0	0	0	0	0
NCTO	0	0	0	0	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OCTS	0	0	0	0	63	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TRICP	0	0	0	0	0	0	56	0	0	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CRATO	0	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	83	0	83	0	0
PALPO	0	0	0	0	0	0	0	0	0	0	0	0	0	63	0	0	0	0	0	0	0	0	0	0
PROBEZ	0	0	0	0	0	56	0	95	0	0	0	167	0	63	111	0	0	0	0	83	0	0	0	0
HEMRO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0
SPHAE	67	0	0	0	125	0	0	0	0	71	23	0	0	125	0	0	0	0	12	0	56	42	0	0
MTRANS	0	0	0	0	0	0	2,611	0	16,667	0	0	1,667	1,000	0	167	0	167	1,333	0	0	0	0	154	0
TOTAL	133	0	600	2,667	313	278	3,722	905	19,833	143	209	5,000	2,471	250	556	30	208	4,611	83	1,333	111	250	308	0
NTAXA	2	0	1	3	3	7	5	4	6	2	4	7	4	3	4	1	2	2	5	5	2	3	2	0