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A FIELD ASSESSMENT OF LONG-TERM LABORATORY SEDIMENT TOXICITY TESTS WITH THE AMPHIPOD *HYALELLA AZTECA*

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Abstract—Response of the amphipod *Hyalella azteca* exposed to contaminated sediments for 10 to 42 d in laboratory toxicity tests was compared to responses observed in controlled three-month invertebrate colonization exposures conducted in a pond. Sediments evaluated included a sediment spiked with dichlorodiphenyldichloroethane (DDD) or dilutions of a field sediment collected from the Grand Calumet River (GCR) in Indiana (USA) (contaminated with organic compounds and metals). Consistent effects were observed at the highest exposure concentrations (400 μg DDD/goc [DDD concentrations normalized to grams of organic carbon (goc) in sediment] or 4% GCR sediment) on survival, length, and reproduction of amphipods in the laboratory and on abundance of invertebrates colonizing sediments in the field. Effect concentrations for DDD observed for 10-d length and 42-d reproduction of amphipods (e.g., chronic value [ChV] of 66 μg DDD/goc and 25% inhibition concentration [IC25] of 68 μg DDD/goc for reproduction) were similar to the lowest effect concentrations for DDD measured on invertebrates colonizing sediment the field. Effect concentrations for GCR sediment on 28-d survival and length and 42-d reproduction and length of amphipods (i.e., ChVs of 0.20–0.66% GCR sediment) provided more conservative effect concentrations compared to 10-d survival or length of amphipods in the laboratory or the response of invertebrates colonizing sediment in the field (e.g., ChVs of 2.2% GCR sediment). Results of this study indicate that use of chronic laboratory toxicity tests with *H. azteca* and benthic colonization studies should be used to provide conservative estimates of impacts on benthic communities exposed to contaminated sediments. Bioaccumulation of DDD by oligochaetes colonizing the DDD-spiked sediment was similar to results of laboratory sediment tests previously conducted with the oligochaete *Lumbriculus variegates*, confirming that laboratory exposures can be used to estimate bioaccumulation by oligochaetes exposed in the field.

Keywords—Sediment toxicology *Hyalella azteca* Benthic community Polycyclic aromatic hydrocarbons Dichlorodiphenyldichloroethane

INTRODUCTION

Field validation is an important component of developing laboratory methods for evaluating the toxicity or bioaccumulation of sediment-associated contaminants [1–3]. Among concerns for extrapolating results of laboratory tests to the response of benthic invertebrates in the field include differences in sensitivity or behavior between native and laboratory-reared invertebrates, even of the same species; differences between native and laboratory environmental conditions; disturbance of sediment samples collected for laboratory testing, resulting in changes in bioavailability or distribution of contaminants; and the evaluation of effects on a select group of sensitive species versus the overall effects on many species in the field [4,5].

A limited number of studies have attempted to field validate laboratory sediment toxicity tests in freshwater with the amphipod *Hyalella azteca* or the midge *Chironomus dilutus* (formerly *C. tentans* [6]) or *C. riparius* by measuring the response of populations of benthic invertebrates in the field. Canfield et al. [7–9] evaluated the composition of invertebrate communities in sediments from a variety of freshwater locations, including the upper Clark Fork River in Montana, the Great Lakes, and the upper Mississippi River (USA) using the sediment quality triad (Triad) approach. The Triad approach integrates information from laboratory toxicity tests, sediment chemistry, and benthic community analyses to determine whether sediments are toxic. Results of these invertebrate community assessments were compared to sediment quality guidelines (SQGs) and 28-d sediment toxicity tests with *H. azteca*. Concordance was evident between measures of laboratory toxicity, SQGs, and invertebrate community composition in extremely contaminated samples. However, in moderately contaminated samples, less concordance was observed between the composition of the benthic community and either laboratory toxicity tests or SQGs. These differences could be due to the influence of other environmental factors such as alteration of habitat. Use of chronic laboratory toxicity tests better identified gradients in chemical contamination in sediments compared to many of the commonly used measures of invertebrate community structure. Therefore, use of longer-term toxicity tests in combination with SQGs was recommended to provide a more sensitive and protective measure of potential toxic effects of sediment contamination on benthic communities compared to the use of 10-d sediment toxicity tests (e.g., [8]). Similar agreement was also reported between results of chronic sediment toxicity tests with *H. azteca* and benthic community responses in the Anacostia River (Washington, DC, USA) [10] and in the Calcasieu estuary in Louisiana (USA) [11].

Similarly, a limited number of studies have attempted to

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field validate laboratory sediment toxicity tests with *C. dilutus*. Chironomids were not found in sediment samples collected from the field that decreased growth of *C. dilutus* by 30% or more in 10-d laboratory toxicity tests [12]. Wentsel et al. [13–15] also reported a correlation between effects on *C. dilutus* in laboratory tests and the abundance of *C. dilutus* in metal-contaminated sediments. Results from 10- to 28-d laboratory sediment toxicity tests with *C. dilutus* or *H. azteca* were compared to colonization of artificial substrates exposed in situ to Great Lakes sediment [16]. Survival or growth of midge or amphipods in these laboratory toxicity tests were negatively correlated to percent chironomids and percent tolerant taxa colonizing artificial substrates in the field.

The benefit of conducting Triad assessments is that direct measures of the effects of contaminants in sediment can be evaluated on invertebrates in situ under natural and realistic exposure conditions [17]. However, the trade-off is that numerous confounding factors are associated with these types of studies. For example, it is difficult to establish appropriate reference sites that encompass the sediment conditions observed across the gradient sampled. In addition, interpretation of observed benthic community effects is confounded by variation in physicochemical characteristics of sediments, overlying water quality, and habitats sampled (e.g., grain size, total organic carbon, salinity, depth, currents, hydrological conditions, latitude [17]). Therefore, investigators have evaluated the impacts of contaminated sediments on benthic communities using controlled colonization and mesocosm studies that can help account for some of these potential confounding factors.

Colonization studies have been used by ecologists to evaluate the factors controlling distributions and population dynamics of sediment-dwelling invertebrates (e.g., [18–23]). Such procedures typically involve use of either hard substrate samples (e.g., horizontal multiplate Hester–lDendy samplers [24] or vertical single-plate hard substrates [25]) or sediment trays deployed in specific locations of interest. Colonization of these substrates by invertebrates is then measured over a set period of time to evaluate processes such as recruitment or recovery from anthropogenic impacts. The assumption is made in these colonization studies that reduced abundance or diversity within a treatment (e.g., contaminated sediment) may better reflect what happens in situ compared to measures of benthic communities in the field that may be influenced by abiotic factors or by habitat differences in addition to effects associated with contaminants in sediment [17].

Methods for conducting colonization studies have been adapted to evaluate the impacts of contaminated sediments in the field (e.g., [26–48]). These studies typically have involved deploying a dilution series of contaminated sediments in trays (e.g., small trays about 0.5–1 L or larger trays about 4–12 L) placed in a variety of habitat types (e.g., ponds, lakes, estuaries). In addition to measuring changes in the invertebrates among treatments, concurrent measures of sediment toxicity in the laboratory and measures of sediment chemistry have been performed on splits or subsamples of sediment placed in the trays [26–28,39,46,47]. Sources of the contaminants have included sediments spiked with materials such as cadmium [34,35,42], zinc [36,39,40,47], copper [38,43], creosote [26], dibutyl phthalate [27], fenvalerate [28], drilling mud [30,48], antifouling paint [30], oil [31–33], or chlorpyrifos [40]. In contrast, Roach et al. [44] and Parrish et al. [45] evaluated contaminated sediment collected from the field placed into colonization trays. Importantly, these studies have been designed to attempt to control or account for sediment physical characteristics (e.g., grain size, organic carbon) or habitat characteristics (e.g., depth, lighting, current) on the response of benthic invertebrates to contaminated sediment.

A limitation to many of these previous colonization studies is substantial changes in concentrations or bioavailability of the chemicals over the duration of the colonization period (e.g., with metal-spiked sediments [34–36,39,41–43,47] or with chlorpyrifos-spiked sediment [40]). A second limitation to these previous colonization studies is the potential for invertebrates to be present in the spiked sediment at the start of the colonization period. To be effective in toxicity studies, trays containing uncontaminated sediment should exhibit species composition and abundance patterns similar to that in the surrounding environment [23,49]; however, no invertebrates should be present in the samples at the start of the colonization period (a potential problem with all the studies except for [26–28,36,41]).

In the present study, contaminants were selected that were expected to be relatively consistent over the duration of the colonization study in the field. Dichlorodiphenyldichloroethane (DDD) was chosen as the chemical to spike into sediment for the laboratory toxicity test and the invertebrate colonization study based on its persistence in sediment, high toxicity in water-only exposures, and relatively high water solubility. Although DDD has been produced as a pesticide [50], DDD and dichlorodiphenyldichloroethylene (DDE) are more commonly observed as breakdown products of DDT and are frequently reported as a chemical of concern in sediment [51]. Phipps et al. [52] and Hoke et al. [53,54] reported 10-d water-only median lethal concentrations (LC50s) for DDD of 0.19 μg/L for *H. azteca* and 0.18 to 0.42 μg/L for *C. dilutus* compared to 10-d water-only LC50s for DDE of 1.4 g/L for *H. azteca* and 3.0 μg/L for *C. dilutus*. In addition to DDD being more toxic than DDE to amphipods and midge, DDD is also more water soluble than DDE (160 μg DDD/L vs 1.3 μg DDE/L [55]). However, a wide range in the water solubility of DDD has been reported (2–160 μg/L [56]).

A second sediment evaluated in this study was a dilution series prepared from a highly contaminated sediment collected from the East Branch of the Grand Calumet River (GCR) located in northwestern Indiana (41°36′37.94″N, 87°25′ 14.82″W; near stations IH10 and IH11 described in Ingersoll et al. [57]). Contaminants of concern in sediment from the GCR include but are not limited to metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides. Sediment toxicity tests, invertebrate community assessments, and fish community surveys have been conducted with samples from various locations throughout the GCR area [58,59]. These studies have documented that sediments from the GCR area are among the most contaminated and toxic ever evaluated. Both DDD-spiked sediment and dilutions of GCR sediment were held for a period of at least 60 d to allow time for spiked contaminants to equilibrate with the sediment [1]. Previous evaluations demonstrated that no substantial changes in the toxicity or chemistry of DDD-spiked sediment occurred during a 120-d storage period [49].

The objective of this study was to determine if results of sediment toxicity tests conducted in the laboratory with *H. azteca* could be used to estimate results observed in a 12-week invertebrate colonization exposure in the field using splits of the same samples. Standard methods for conducting sediment

Table 1. Analytical results for dichlorodiphenyldichloroethane (DDD) measured in whole sediment or in pore water by storage day

	Day 0	Day 30	Day 60[a]	Day 150[b]
Nominal whole-sediment concn. (μg DDD/goc)[c]				
3	3.0	4.0	2.0	3.0
11	9.0	16	9.0	5.0
36	34	45	46	29
120	82	127	75	59
400	250	470	ND[d]	240
Predicted pore-water concn.[e] (μg/L)				
0.005	0.050	0.050	0.060	0.050
0.018	0.17	0.15	0.12	0.31
0.061	0.84	0.73	0.33	0.63
0.20	2.1	1.4	2.1	2.1
0.68	6.5	6.1	10	8.5

[a] Beginning of the definitive toxicity tests and invertebrate colonization study.
[b] End of the invertebrate colonization study.
[c] DDD concentrations normalized to grams of organic carbon (goc) in sediment.
[d] ND = not determined.
[e] Estimated by equilibrium partitioning [63].

toxicity tests have been developed for *H. azteca* [1,3,60]. A laboratory toxicity test with *C. dilutus* was conducted [49], but survival of midge in the controls did not meet test acceptability requirements outlined in the American Society for Testing and Materials and U.S. Environmental Protection Agency (U.S. EPA) methods [1,3]. Endpoints measured in sediment toxicity tests with *H. azteca* included 10- to 42-d survival, growth, and reproduction. A preliminary colonization study determined that 12 major taxa of invertebrates colonized Florissant soil placed in trays in the pond over a 12-week period [49]. Therefore, abundance of major taxonomic groups of invertebrates was the endpoint evaluated in the 12-week colonization study. Bioaccumulation of DDD by oligochaetes colonizing DDD-spiked sediment was also evaluated at the end of the 12-week colonization study.

MATERIALS AND METHODS

Preparation of sediment

Soil obtained from Florissant, Missouri, USA (primarily a mixture of silt and clay with 1% total organic carbon [49]) was selected to prepare DDD-spiked sediment or dilutions of the GCR sediment. The decision to use Florissant soil was based on a preliminary study that evaluated colonization of invertebrates in three different control sediments over 6- and 12-week periods. At week 12 in the pond, invertebrates colonizing the Florissant soil were most similar to the invertebrates in the surrounding environment collected using a Wildco Ekman grab sampler (Wildco, Buffalo, NY, USA) [49]. Previous studies have successfully used this material as a control sediment in toxicity tests (e.g., [61,62]).

Soil was dried and ground until it passed through a No. 20 U.S. standard sieve (850-μm opening; Wildco) before spiking. The chemical *p,p′*-DDD (98% pure) was purchased from Sigma Chemical (St. Louis, MO, USA). Two ''super stock''–spiked sediments were prepared by drenching a portion of sediment with a DDD-spiked acetone solution [49]. The stock used for the two highest concentrations consisted of 40,000 μg DDD/gram of organic carbon [goc] (412 g of clean sediment, spiked with 0.16 g of DDD), while the stock used for the three lowest concentrations had a concentration of 3,888 μg DDD/goc (459 g of clean sediment, spiked with 0.0178 g DDD). Wetted sediment was stirred every 30 min for about 4 h at 20°C to allow the acetone to evaporate. Heterogeneity of spiked sediment produced using this procedure was found to be <5% among subsamples. The super stock–spiked sediment was mixed with clean sediment in glass jars (10-L capacity with Teflon® lids) to achieve sediment concentrations ranging from 3 to 400 μg DDD/goc (Table 1). Two control treatments (a negative control and an acetone solvent control) were prepared in the same manner as the DDD-spiked sediment. The dry material was mixed by rolling the jars for 24 h. Sediments were then wetted using a ratio of 1 L of sediment to 1.5 L of well water (280 mg/L hardness as $CaCO_3$), rolled for 4 h at 13 rpm on a rolling mill, and stored in the dark at 4°C for 60 d before the start of the laboratory sediment toxicity test with *H. azteca* and the 12-week colonization study. Throughout the 60-d storage period, all sediments were rolled at 13 rpm for 1 h/week at 4°C.

Effect concentrations of chemicals in sediment have been correlated to pore-water concentrations, and effect concentrations in pore water are often similar to effect concentrations in water-only exposures [63]. Additionally, bioavailability of nonionic organic compounds such as DDD is often inversely correlated with the organic carbon content of sediment. Therefore, concentrations of DDD spiked into sediment were chosen to bracket effect concentrations for DDD in water-only exposures. The predicted concentrations of DDD in pore water (based on equilibrium partitioning [63]) ranged from 0.005 to 0.68 μg/L (Table 1). Whole-sediment samples were spiked with enough of the super stock of DDD to achieve nominal concentrations in whole sediment ranging from 3 to 400 μg DDD/goc (based on a log K_{oc} [organic carbon partitioning coefficient] of 5.9 for DDD [63,64]). The highest concentration of DDD tested in the present study was 400 μg DDD/goc (4,000 μg DDD/kg dry wt). Based on a database for contaminated sediments collected from freshwater habitats across North America [65,66], when DDD was detected in a sediment sample, the percentile concentrations of DDD were as follows: 50th: 13; 75th: 82; 90th: 13,000; 100th: 130,000 (μg/kg dry wt, $n = 243$). Hence, the high concentration of 4,000 μg DDD/kg tested in the present study was between the 75th and the 90th percentile for DDD in this freshwater database.

The procedure for preparing dilutions of GCR sediment was similar to the DDD spiking procedure, with the following

exceptions. Florissant soil was wetted to the same consistency as the field-collected sediment before the addition of the aliquots of GCR sediment. Dilutions were prepared by mixing GCR sediment with the control sediment (Florissant soil) to achieve 4.0, 1.2, 0.36, 0.11, and 0.03% dilutions of the original GCR sediment sample. The highest concentration of 4.0% GCR sediment was established on the basis of a preliminary 10-d range-finding toxicity study that determined that 4.0% GCR sediment was lethal to *H. azteca* and *C. dilutus*. The benefit of adding a maximum of only 4.0% GCR sediment to the control soil was that only a small difference (<0.4%) existed in organic carbon or particle size (both sediments were primarily silt-clay) across the dilution series of GCR sediment. Dilutions of GCR sediment were rolled at 13 rpm on a rolling mill for 4 h and stored in the dark at 4°C before the start of the laboratory sediment toxicity test with *H. azteca* and the 12-week colonization study (similar to DDD-spiked sediment). Throughout the 60-d storage period, all sediments were rolled at 4°C at 13 rpm for 1 h/week.

Chemical analyses of sediment

Samples of whole sediment and pore water were collected from the storage jars for DDD analyses on days 0, 30, and 60 after spiking and from the trays at the end of the 12-week colonization study. Pore-water samples were isolated by centrifugation at 5,200 rpm (7,000 *g*) for 15 min at 4°C [67]. Methods used to analyze concentrations of DDD in whole sediment and pore water are described in U.S. EPA [49]. Briefly, concentrations of DDD in pore-water samples were extracted by liquid/liquid partitioning with methylene chloride and analyzed by gas chromatography with electron capture detection (GC/ECD). Whole-sediment samples were dried, extracted with dichloromethane, extracted with high-pressure size exclusion chromatography, and analyzed by GC/ECD. Concentrations of PAHs or organochlorine compounds (including PCBs) in the 100% GCR sediment sample were determined by Mississippi State University Laboratory (Mississippi State, MS, USA) using capillary column, flame ionization gas chromatography, or gas chromatography and mass spectroscopy. In addition, total recoverable metals, acid-volatile sulfide (AVS), simultaneously extractable metals (SEM), and total metals in pore water were measured in the control and 4% GCR sediment [49].

Laboratory exposures

Mixed-age *H. azteca* were mass cultured in 80-L glass aquaria containing 50 L of water that received about six volume additions per day of well water (hardness 280 mg/L as $CaCO_3$) using procedures outlined in Ingersoll et al. [57]. The mean initial length of amphipods at the start of the sediment exposures was 1.4 mm (0.03 standard error; $n = 20$). Amphipod lengths were comparable with those of known-age 7- to 8-d-old amphipods previously used to start sediment tests (1.2–1.6 mm [62]). Methods for culturing and testing of midge are described in U.S. EPA [49] and are not reported here because of poor control survival of midge in the long-term toxicity tests (e.g., 10-d control survival <60% [49]).

The amphipod tests were started on May 16, 2000. Amphipods were exposed in 300-ml beakers containing 100 ml of sediment and 175 ml of overlying water [1,3,49]. Each sediment sample was thoroughly mixed using a stainless-steel spoon and bowl, visually inspected to judge homogeneity, and subsamples were then added to the exposure beakers the day before start of the sediment test (day − 1). The spoons and bowls were rinsed with acetone, well water, and deionized water between treatments. The amphipod exposures were conducted for 42 d at 23°C on a 16:8-h light:dark photoperiod at a light intensity of about 200 lux. The source of overlying water was well water, and two volume additions per day of water were added to each beaker using an automated system [68]. A total of 10 amphipods were exposed in each beaker and were fed 1.0 ml of yeast-cerophyl®-trout chow® (1,800 mg/L stock solution [1,3]; Ralston-Purina, St. Louis, MO, USA) every day.

A total of 16 replicates were evaluated with each sediment treatment in the amphipod toxicity tests (four replicates for 10-d growth and survival, four replicates for 28-d growth and survival, and eight replicates for 42-d reproduction, growth, and survival). On days 10 and 28 of the amphipod tests, sediment in each beaker was sieved through a No. 50 U.S. standard sieve (300-μm opening; Wildco). The debris and organisms remaining in the sieve were rinsed into a glass tray and searched for up to 20 min for organisms. Surviving amphipods from four replicates on day 10 or 28 were counted and preserved in 8% sugar formalin for later length measurements. Amphipods from eight replicates sampled on day 28 were placed into water-only beakers containing a thin layer of sand using the conditions outlined previously for the sediment exposures. The number of young amphipods produced in each of these beakers was determined on days 35 and 42. Length of amphipods sampled on days 10, 28, and 42 was measured along the dorsal surface from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface using a microscope and digitizing system [67].

Hardness, alkalinity, conductivity, dissolved oxygen, and pH were measured in overlying water at the beginning and end of each exposure. Conductivity and dissolved oxygen in overlying water were also measured every fourth day of the exposures. Overlying water quality characteristics were generally similar among all DDD treatments: hardness (290–320 mg/L as $CaCO_3$), alkalinity (230–260 mg/L as $CaCO_3$), conductivity (580–700 μmho/cm), and pH (8.0–8.3). Overlying water quality characteristics were also generally similar among all GCR treatments: hardness (270–310 mg/L as $CaCO_3$), alkalinity (230–280 mg/L as $CaCO_3$), conductivity (560–630 μmho/cm), and pH (8.1–8.3). Dissolved oxygen in overlying water was at or above the acceptable level of 2.5 mg/L [1,3] in all treatments throughout the study.

Field exposures

The invertebrate colonization study was started on May 23, 2000, and was conducted until August 18, 2000. A total of 13 treatments were evaluated (five DDD concentrations and a solvent control and negative control, five GCR dilutions and a negative control) with a total of seven replicate trays/treatment [49]. Each 0.9-L glass Pyrex tray (14 × 19-cm surface area, 3.5 cm deep) was filled to the top with sediment, and a Styrofoam bobber was attached to the outside of each tray to aid in the retrieval of the trays at the end of the 12-week colonization study. In addition, four trays per DDD treatment were placed in the pond for the bioaccumulation study, and one tray per treatment was placed in the pond for chemical analysis of DDD in whole sediment and pore water at the end of the colonization study.

The pond used for the colonization study had a surface area of 0.1 ha with a clay liner (pond 37 at the Columbia Envi-

ronmental Research Center, Columbia, MO, USA [69]). The water level of the pond was raised and lowered using a kettle at the deeper end of the pond. The pond contained well water for over one year before the start of the study, and the water quality typically ranged in hardness between 150 and 180 mg/L as $CaCO_3$. The study area was a 1 × 3-m area in the center of the pond. To control growth of submersed vegetation (primarily *Chara* and *Naja*), a 30 × 30-m, 95% shade cover was placed over the center of the pond. Mean water depth was 2.5 m in the study area in the pond during the colonization period. The grid was 12 trays by 11 trays, with about one tray-length space between each tray [49]. Because of concerns about organisms potentially having difficulty reaching the inner portion of the grid, trays in the inner portion of the grid were filled with sediment for analyses of DDD in whole sediment and pore water. The outer four rings of the grid contained the randomly placed trays for the colonization (DDD and GCR dilution) and oligochaete bioaccumulation study (DDD only). In order to minimize disturbance of the sediment in the pond, a scaffold was built with angle iron around the perimeter of the 1 × 3-m area in the pond. The water depth in the pond was lowered to about 0.5 m, and wooden planks were placed on the scaffold when trays were placed into the pond or retrieved from the pond.

In addition to invertebrates sampled using sediment placed in colonization trays, an Ekman grab sampler was used to collect sediment and invertebrates from the surrounding area of the pond at the end of the 12-week colonization study. The purpose of collecting these Ekman grab samples was to determine if differences existed between invertebrates colonizing control sediment in the trays compared to the surrounding sediment. The Ekman grab sampler was 15 × 15 cm (surface area 225 cm^2) and sampled to a depth of about 10 cm. Seven replicates grabs were collected using the Ekman grab sampler from both inside and outside the 30 × 30-m shade cover area. Significantly fewer chironomids and other Diptera (Chaoboridae and Ceratopogonidae) were observed in the surrounding sediment under the covered section of the pond relative to the abundance of these taxa colonizing the control trays [49]. Abundance of nematodes and other Diptera in sediment collected using the Ekman grab sampler were significantly higher inside the covered area compared to outside the covered area of the pond. In contrast, abundance of mayflies in sediment was significantly higher outside the covered area compared to inside the covered area of the pond [49]. Differences between organisms inside the covered area compared to outside the covered area of the pond were likely due to the large amount of vegetation present outside the shade cover by the end of the 12-week colonization study. Analyses were also performed to determine if differences existed in abundance of taxa due to placement position in the pond under the shade cover [49]. For these analyses, the study area was divided into four quadrants or into a series of three rings and an inner area. No significant difference was observed in the abundance of major taxa of invertebrates colonizing trays among these various locations within the shade cover area [49].

After collection of the Ekman grab samples, the depth of the water in the pond was lowered, and each tray was covered with a plastic lid before removal from the pond. The contents of each tray or Ekman grab sample was washed through a 500-μm mesh sieve within 30 min of collection and preserved in 4% formalin containing 250 mg/L Rose Bengal [23]. After 48 h, samples were rinsed and preserved in 80% ethanol. Organisms in each sample were sorted using a microscope into major taxonomic groups of invertebrates (a 5% recheck of the samples indicated that 96% of the organisms had been successfully removed). Number of invertebrates per sample was converted to density (abundance of invertebrates/m^2) using the surface area of either the tray (0.0266 m^2) or the Ekman grab sampler (0.0225 m^2). The major taxonomic groups of invertebrates in the trays and in samples collected using the Ekman grab sampler included nematodes (Nematoda), oligochaetes (Annelida: Oligochaeta), chironomids (Diptera: Chironomidae), other Diptera (both Chaoboridae and Ceratopogonidae), and mollusks (both Gastropoda and Pelecypoda). Nematodes were not sorted from the samples in the preliminary study in 1999 [49]. Total abundance of major taxa in each sample was calculated by summing the number of individual taxa from each of these groups. Only a limited number of mayflies (Ephemeroptera), caddisflies (Tricoptera), leeches (Hirudinea), dragonflies and damselflies (Odonata), dobsonflies (Megaloptera), true bugs (Hemiptera), and beetles (Coleoptera) were found in the samples (e.g., only one to two organisms in some of the replicate samples), so these data were not included in the analyses.

Oligochaetes collected from the bioaccumulation trays were analyzed for DDD after a 12-week colonization period. Oligochaetes were isolated from bioaccumulation trays by sieving the sediment and removing the remaining debris. Oligochaetes were depurated overnight in 1-L glass containers with a brown paper towel. Oligochaete samples were then separated from remaining debris and frozen at −20°C until chemical analyses were performed using the same procedure used to analyze DDD in the whole-sediment samples [49].

Data analyses

Effect concentrations were estimated from laboratory toxicity data and field data by hypothesis testing and by linear interpolation [1,3,70]. The differences in the endpoints in the solvent control and negative control were compared using a *t* test of whether the data were normally distributed (Shapiro–Wilk's test) and variance of treatment groups were homogeneous (Bartlett's test). If the assumptions of the *t* test were not met, Wilcoxon rank sum test was used. If no significant difference was observed between solvent and negative controls, the two control groups were pooled [1,3]. The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) for each endpoint were determined using Williams's test if the data were normally distributed and variance of treatment groups were homogeneous. Otherwise, Wilcoxon rank sum test with Bonferroni adjustment was used. The level of statistical significance was set at $\alpha = 0.05$. The ChV was calculated as the geometric mean of the NOEC and LOEC. Concentrations above the NOEC for survival were not included in the calculation of the NOEC or LOEC for length or reproduction [70]. A linear interpolation procedure [71] was used to estimate concentrations causing 25 and 50% inhibition of test endpoints (IC25 and IC50). Lower inhibition concentrations were not included because of the higher variance associated with these estimates (e.g., IC10 and IC20). Inhibition concentrations were not reported if a lack of a consistent concentration–response relationship was observed for a particular endpoint. All data analyses were performed using Toxstat [72].

RESULTS

Physical and chemical characterization of sediments

Nominal and measured concentrations of DDD in whole sediment were similar at the start of the laboratory toxicity

Table 2. Concentrations of polycyclic aromatic hydrocarbons (PAHs) and total polychlorinated biphenyls (PCBs) measured in the 100% Grand Calumet River (IN, USA) (GCR), sediment (GCR 100) and concentrations of total metals measured in the 4% dilution of GCR sediment (GCR4.0). Quotients for individual compounds and means for groups of compounds are estimated for the 4% dilution of GCR sediment based on sediment quality guidelines (SQGs; probable effect concentrations [65] or probable effect levels [92])

Compound	SQG	Unit	Source	GCR100	GCR4.0	4.0-Q
Low-molecular-weight PAHs						
Naphthalene	561	μg/kg	PEC[a]	5,581	231	0.41
2-Methylnaphthalene	201	μg/kg	PEL[b]	83,721	3,349	16.66
Fluorene	536	μg/kg	PEC	97,674	3,912	7.30
Phenanthrene	1,170	μg/kg	PEC	141,860	5,679	4.85
Anthracene	845	μg/kg	PEC	32,558	1,307	1.55
Acenaphthene	88.9	μg/kg	PEL	151,163	6,051	68.07
Acenaphthylene	128	μg/kg	PEL	9,070	368	2.87
High-molecular-weight PAHs						
Fluoranthene	2,230	μg/kg	PEC	88,372	3,540	1.59
Pyrene	1,520	μg/kg	PEC	62,791	2,516	1.66
Benzo[*a*]anthracene	1,050	μg/kg	PEC	30,233	1,214	1.16
Chrysene	1,290	μg/kg	PEC	19,535	786	0.61
Benzo[*a*]pyrene	1,450	μg/kg	PEC	46,512	1,865	1.29
Total PAHs	22,800	μg/kg	PEC	769,070	30,818	1.35
Total PCBs	676	μg/kg	PEC	160,000	6,426	9.51
As	33	μg/g	PEC		20	0.59
Cd	4.98	μg/g	PEC		0.79	0.16
Cr	111	μg/g	PEC		129	1.16
Cu	149	μg/g	PEC		49	0.33
Pb	128	μg/g	PEC		79	0.62
Ni	48.6	μg/g	PEC		39	0.81
Zn	459	μg/g	PEC		277	0.60
Total PAH-Q						1.35
Total PCB-Q						9.51
Mean metals-Q						0.61
Mean probable effect concentration (PEC)-Q						3.82

[a] PEC = probable effect concentration.
[b] PEL = probable effects level.

tests and at the start of the field colonization study (day 60 in Table 1). However, concentrations of DDD in the whole-sediment samples collected from the pond at the end of the colonization study exhibited about a 33 to 38% decline at the two highest exposure concentrations compared to the average of the samples analyzed from days 0 to 60 of the sediment storage study (Table 1). All subsequent calculations of NOECs, LOECs, IC25s, and IC50s are based on nominal DDD concentrations. Concentrations of DDD in pore water remained relatively consistent between days 0 and 60 of the storage study and after the 12-week period in the pond (day 150 after spiking; Table 1). However, concentrations of DDD measured in pore water tended to be about 10-fold higher than predicted concentrations (Table 1). Higher-than-expected concentrations of DDD in pore water may have been due to DDD associated with particulate or dissolved organic carbon remaining in the pore water after centrifugation. Samples were cloudy after centrifugation, and the organic carbon concentration (particulate and dissolved) in a sample of pore water isolated from the control sediment was 30 mg/L [49]. Calculations of predicted pore-water concentrations based on 30 mg/L organic carbon in pore water were within about twofold of the measured pore-water concentrations in Table 1 [49].

Concentrations of PAHs and PCBs measured in the 100% GCR sediment sample and concentrations of metals measured in the 4% GCR sediment sample are listed in Table 2. Concentrations of all the organochlorine pesticides in the 100% GCR sediment sample were <0.02 μg/g dry weight. Concentrations of PAHs and PCBs in each of sediment dilution were estimated on the basis of the concentrations of these compounds measured in the 100% GCR sample, and concentrations of metals in each sediment dilution were estimated on the basis of concentrations of metals measured in the 4% GCR sediment sample. The sum of the micromolar SEM concentrations of Cd, Cu, Pb, Ni, and Zn (ΣSEM)–AVS measured in the 4% GCR sediment sample is reported in U.S. EPA [49]. The ΣSEM–AVS was evaluated instead of the SEM/AVS ratio as recommended by Brumbaugh and Arms [73] because of low concentration of AVS in the samples. The ΣSEM was not substantially in excess of AVS, indicating that metals would not be expected to substantially contribute to toxicity. While concentrations of several metals measured in pore water were elevated, these concentrations were analyzed as total metals and may have been associated with particulate material in the pore water, which may reduce the bioavailability of these metals [49].

Laboratory toxicity tests

Hyalella azteca *toxicity test with DDD-spiked sediment.* Mean survival, length, or reproduction of amphipods in the solvent control and in the negative control were not significantly different; therefore, these control data were pooled for data analyses (Table 3 [1,3]). Mean survival in the solvent control and in the negative control was greater than 94% across the exposure periods at days 10, 28, and 42 and exceeded the acceptability criterion of 80% survival [1,3]. Only survival in the highest concentration (400 μg DDD/goc) was significantly reduced relative to the controls across the exposure periods (Table 3). The ChVs for survival were 220 μg DDD/goc across the exposure periods, and the IC25s for survival ranged from

Table 3. *Hyalella azteca* mean survival and length at days 10 and 28 and survival, length, and reproduction at day 42 in dichlorodiphenyldichloroethane (DDD)-spiked sediments (standard deviations in parentheses). Chronic value (ChV; geometric mean of the no-observed-effect concentration and lowest-observed-effect concentration) and 25% and 50% inhibition concentrations (IC25, IC50, with 95% confidence intervals [CI]) are presented for each endpoint[a]

	Day 10		Day 28		Day 42		
Treatment (μg DDD/goc)	Survival (%; $n = 4$)	Length (mm; $n = 4$)	Survival (%; $n = 12$)	Length (mm; $n = 4$)	Survival (%; $n = 8$)	Length (mm; $n = 8$)	Reproduction ($n = 8$)
Control	100 (0)[b]	2.57 (0.11)[b]	98 (5.1)[c]	3.99 (0.20)[b]	94 (12)[d]	4.31 (0.24)[d]	31 (15)[d]
3.0	100 (0)	2.47 (0.12)	93 (15)	3.57 (1.07)	96 (5.2)	4.08 (0.22)*	26 (13)
11	98 (5.0)	2.46 (0.11)	97 (4.5)	3.65 (0.22)	95 (7.6)	3.99 (0.13)*	28 (8.0)
36	100 (0)	2.52 (0.10)	98 (3.8)	3.76 (0.31)	98 (4.6)	4.03 (0.23)*	32 (14)
120	98 (5.0)	2.26 (0.15)*	91 (12)	3.44 (0.60)	90 (11)	4.14 (0.23)*	15 (15)*
400	0 (0)*	—	0 (0)*	—	1.3 (3.5)*	—	—
ChV	220	66	220	220	220	<3.0	66
IC25 (CI)	190 (170–190)	160 (140–170)	170 (150–190)	160 (100–180)	180 (150–190)	180 (170–180)	68 (29–140)
IC50 (CI)	260 (250–260)	240 (230–250)	250 (240–260)	240 (210–250)	250 (240–260)	250 (250–260)	120 (77–230)

[a] Asterisks indicate significant reduction relative to controls within a column ($p < 0.05$).
[b] $n = 8$ for pooled controls.
[c] $n = 24$ for pooled controls.
[d] $n = 16$ for pooled controls.

170 to 190 μg DDD/goc; the IC50s for survival ranged from 250 to 260 μg DDD/goc (Table 3).

Length of amphipods was not consistently affected across the exposure periods. Length was significantly reduced relative to the controls at 120 μg DDD/goc at day 10 and was significantly reduced at all the exposure concentrations at day 42 but was not significantly reduced at day 28 at any of the exposure concentrations (Table 3). The ChV for length was 66 μg DDD/goc at day 10, 220 μg DDD/goc at day 28, and <3 μg DDD/goc at day 42. The IC25s and the IC50s for length were more consistent across the exposure periods (IC25s of 160–180 μg DDD/goc and IC50s of 240–250 μg DDD/goc; note that these IC25 and IC50 estimates incorporate effects on both survival and length). Reproduction was significantly reduced relative to the controls above 36 μg DDD/goc with a ChV of 66 μg DDD/goc, an IC25 of 68 μg DDD/goc, and an IC50 of 120 μg DDD/goc (Table 3).

Hyalella azteca *toxicity test with dilutions of the GCR sediment.* Mean survival of amphipods in the control was greater than 95% across the exposure periods at days 10, 28, and 42 (Table 4). Survival in the highest dilution of GCR sediment (4%) was consistently reduced relative to the control across the exposure periods. Survival at the four lowest dilutions of GCR sediment was not consistently reduced relative to the control across the exposure periods. The ChVs for survival ranged from 0.66 to 2.2% GCR sediment across the exposure periods. The IC25s for survival decreased from 1.6% GCR sediment at day 10 to 0.87% GCR sediment at day 28 and 0.70% GCR sediment at day 42. Similarly, the IC50s for survival decreased from 3.2 and 3.3% GCR sediment at days 10 and 28 to 1.1% GCR sediment at day 42.

At day 10, no significant effects on length of amphipods relative to the controls were observed (Table 4). However, length at days 28 and 42 was significantly reduced at 0.36 or 1.2% GCR sediment relative to the control. The ChVs for length was 2.2% GCR sediment at day 10 and 0.20% at days 28 and 42. The IC25s and the IC50s for length were more consistent across the exposure periods (IC25s ranging from 1.6 to 1.9% GCR sediment and IC50s ranging from 2.7 to 3.6% GCR sediment). Reproduction of amphipod was significantly reduced relative to the control above 0.36% GCR sediment with a ChV of 0.66%, an IC25 of 0.24%, and an IC50 of 0.71% GCR sediment (Table 4).

Invertebrate colonization

Colonization of DDD-spiked sediment. No significant differences were observed between the abundance of invertebrates colonizing trays containing solvent control sediment compared to trays containing negative control sediment; therefore, these data were pooled for data analyses (Table 5). Total abundance of major taxa and abundance of nematodes and other Diptera colonizing the trays in the highest DDD concentration (400 μg DDD/goc) were significantly reduced relative to the controls. Additionally, the abundance of chironomids was significantly reduced at 120 and 400 μg DDD/goc relative to the controls. The abundance of oligochaetes and mollusks colonizing the trays was not significantly reduced at any of the exposure concentrations relative to the controls. For treatments where significant effects were observed, the ChVs ranged from 66 μg DDD/goc (abundance of chironomids) to 220 μg DDD/goc (total abundance of major taxa or abundance of nematodes or other Diptera). The IC25s ranged from 47 μg DDD/goc (abundance of chironomids) to 270 μg DDD/goc

Table 4. *Hyalella azteca* mean survival and length at days 10 and 28 and survival, length, and reproduction at day 42 in dilutions of Grand Calumet River (GCR) sediment (standard deviations in parentheses). Chronic value (ChV; geometric mean of no-observed-effect concentration and lowest-observed-effect concentration) and 25% and 50% inhibition concentrations (IC25, IC50, with 95% confidence intervals [CI]) are presented for each endpoint[a]

	Day 10		Day 28		Day 42		
Treatment (%)	Survival (%; $n = 4$)	Length (mm; $n = 4$)	Survival (%; $n = 12$)	Length (mm; $n = 4$)	Survival (%; $n = 8$)	Length (mm; $n = 8$)	Reproduction ($n = 8$)
Control	100 (0)	2.28 (0.18)	98 (5.7)	3.92 (0.22)	95 (7.6)	4.11 (0.26)	27 (14)
0.03	90 (8.2)	2.14 (0.21)	93 (6.5)	3.69 (0.13)	90 (12)	4.20 (0.40)	27 (17)
0.11	90 (8.2)	2.15 (0.37)	98 (5.0)	3.62 (0.32)	94 (12)	3.87 (0.18)	23 (15)
0.36	80 (8.2)*	1.90 (0.46)	91 (10)	3.38 (0.58)*	91 (8.4)	3.48 (0.67)*	18 (3.8)
1.2	80 (14)	1.89 (0.36)	63 (44)*	*3.72 (0.58)*	43 (47)	3.60 (0.47)*[b]	5.6 (4.4)*[c]
4.0	40 (26)*	*0.53 (0.085)*	44 (23)*	*1.04 (0.47)*	43 (23)*	*1.83 (0.86)*	*8.6 (7.2)*
ChV	2.2	2.2	0.66	0.20	2.2	0.20	0.66
IC25 (CI)	1.6 (0.36–2.1)	1.6 (0.32–1.9)	0.87 (0.62–2.0)	1.9 (1.5–2.1)	0.70 (0.58–1.4)	1.9 (1.2–2.3)	0.24 (0.05–0.48)
IC50 (CI)	3.3 (2.4–4.0)	2.7 (2.3–2.9)	3.2 (1.1–3.9)	3.0 (2.7–3.3)	1.1 (0.91–3.6)	3.6 (3.0–4.0)	0.71 (0.11–0.95)

[a] Asterisks indicate significant reduction relative to control within a column ($p < 0.05$). Length or reproduction data in treatments with significant reductions in survival (numbers in italics) were excluded from the statistical analyses.
[b] $n = 5$.
[c] $n = 4$.

Table 5. Mean abundance of major taxa (abundance/m^2) colonizing trays across dichlorodiphenyldichloroethane (DDD)-spiked sediments (standard deviations in parentheses). Chronic value (ChV; geometric mean of no-observed-effect concentration and lowest-observed-effect concentration) and 25% and 50% inhibition concentrations (IC25, IC50, with 95% confidence intervals [CI]) are presented for each endpoint[a]

Treatment (μg DDD/goc)	No. of replicates	Total	Nematodes	Oligochaetes	Chironomids	Other diptera	Mollusks
Control	14	12,000 (3,900)	6,700 (4,100)	3,300 (1,500)	300 (250)	1,200 (500)	130 (160)
3.0	7	7,400 (3,500)	4,100 (3,100)	2,000 (1,400)	110 (82)	1,100 (350)	59 (43)
11	7	12,000 (3,400)	7,900 (3,800)	2,900 (1,800)	240 (330)	1,300 (420)	21 (20)
36	7	14,000 (9,100)	9,700 (9,600)	3,100 (1,800)	380 (500)	1,100 (410)	120 (120)
120	7	11,000 (8,300)	7,300 (8,800)	2,200 (1,690)	80 (59)*	870 (421)	120 (120)
400	7	7,200 (2,600)*	3,500 (1,541)*	2,800 (2,670)	91 (94)*	610 (370)*	120 (98)
ChV		220	220	>400	66	220	>400
IC25 (CI)		270 (61–360)	260 (31–310)	>400	47 (18–77)	100 (23–270)	NR[b]
IC50 (CI)		>400	400 (110–400)	>400	85 (2–100)	>400	NR

[a] Asterisks indicate significant reduction relative to controls within a column ($p < 0.05$).
[b] NR = not reported due to a lack of a consistent concentration–response relationship.

Table 6. Mean abundance of major taxa (abundance/m^2) colonizing trays across dilutions of Grand Calumet River (GCR) sediments (standard deviations in parentheses). Chronic value (ChV; geometric mean of no-observed-effect concentration and lowest-observed-effect concentration) is presented for each endpoint[a]

Treatment (%)	No. of replicates	Total	Nematodes	Oligochaetes	Chironomids	Other diptera	Mollusks
Control	7	11,000 (5,100)	6,300 (4,100)	2,700 (3,100)	150 (260)	1,700 (720)	110 (100)
0.03	7	7,100 (3,800)	3,000 (2,800)	3,100 (1,100)	86 (140)	900 (540)	70 (95)
0.11	7	7,700 (4,300)	4,900 (5,000)	1,000 (830)	86 (47)	1,600 (730)	38 (38)
0.36	7	9,400 (5,000)	4,300 (4,000)	2,900 (2,900)	110 (91)	2,000 (750)	48 (67)
1.2	7	7,300 (3,100)	3,000 (2,300)	2,500 (1,300)	80 (40)	1,700 (1,100)	53 (68)
4.0	6	5,000 (3,000)*	1,600 (1,300)*	1,700 (2,400)	81 (80)	1,500 (600)	69 (69)
ChV		2.2	2.2	>4.0	>4.0	>4.0	>4.0

[a] Asterisks indicate significant reduction relative to control within a column ($p < 0.05$); 25% and 50% inhibition concentrations not reported because of a lack of consistent concentration–response relationships.

Table 7. Measured concentration of dichlorodiphenyldichloroethane (DDD) and biota-sediment accumulation factors (BSAFs) for DDD in oligochaetes collected from DDD bioaccumulation trays placed in a pond for 12 weeks

Nominal whole-sediment concn. (μg DDD/goc)[a]	Oligochaete tissue (μg DDD/g lipid)	BSAF
3	0.50	0.17
11	5.5	0.50
36	19	0.53
120	180	1.5
400	1,500	3.8

[a] DDD concentrations normalized to grams of organic carbon (goc) in sediment.

(total abundance of major taxa), and the IC50s ranged from 85 to >400 DDD/goc. However, confidence intervals for the IC25s and IC50s were quite broad (Table 5). The IC25 and IC50 were not reported for abundance of mollusks because of a lack of consistent concentration–response relationship.

Colonization of dilutions of GCR sediment. Only total abundance of major taxa and abundance of nematodes colonizing the trays in the highest dilution of GCR sediment (4%) were significantly reduced relative to the control (Table 6). The ChV was 2.2% GCR sediment for total abundance of major taxa and for abundance of nematodes. The IC25s and IC50s were not reported because of a lack of consistent concentration–response relationships.

DDD bioaccumulation by oligochaetes. Oligochaetes were the only taxa that colonized the trays in enough mass for chemical analysis of DDD. Oligochaetes from all four trays per treatment were composited to obtain enough sample mass for the analysis (0.83–1.35 g/treatment, wet wt). Biota-sediment accumulation factors (BSAFs) were calculated using the ratio of the μg DDD/goc tissue lipid divided by the μg DDD/goc in whole sediment [2]. A lipid concentration of 0.5% (wet wt) was used in this calculation based on a previous laboratory study with the oligochaete *Lumbriculus variegatus* [74]. Concentrations of DDD measured in the oligochaetes at the end of the colonization study ranged from 0.5 to 1,500 μg DDD/g lipid, corresponding to BSAFs ranging from 0.17 to 3.8 (Table 7).

Laboratory-to-field comparisons

DDD-spiked sediment. Consistent effects were observed at the highest DDD exposure concentration (400 μg DDD/goc) on survival, length, and reproduction of amphipods in the laboratory and on total abundance of major taxa and on abundance of nematodes, chironomids, and other Diptera in the field (Fig. 1A). Higher effect concentrations were observed for 10-d survival of amphipods (e.g., ChV of 220 μg DDD/goc and IC25 of 190 μg DDD/goc) compared to effect concentrations observed for abundance of chironomids (ChV of 66 μg DDD/goc and IC25 of 47 μg DDD/goc) or abundance of other Diptera (IC25 of 100 μg DDD/goc) colonizing sediment in the field (Fig. 2A). Effect concentrations observed for 10-d length and 42-d reproduction of amphipods (e.g., ChVs of 66 μg DDD/goc and IC25 of 68 μg DDD/goc for reproduction) were similar to the lowest effect concentrations measured in the field on abundance of chironomids or other Diptera (Fig. 2A). However, effect concentrations for length were not consistent across all three time periods (Table 3). A similar pattern was

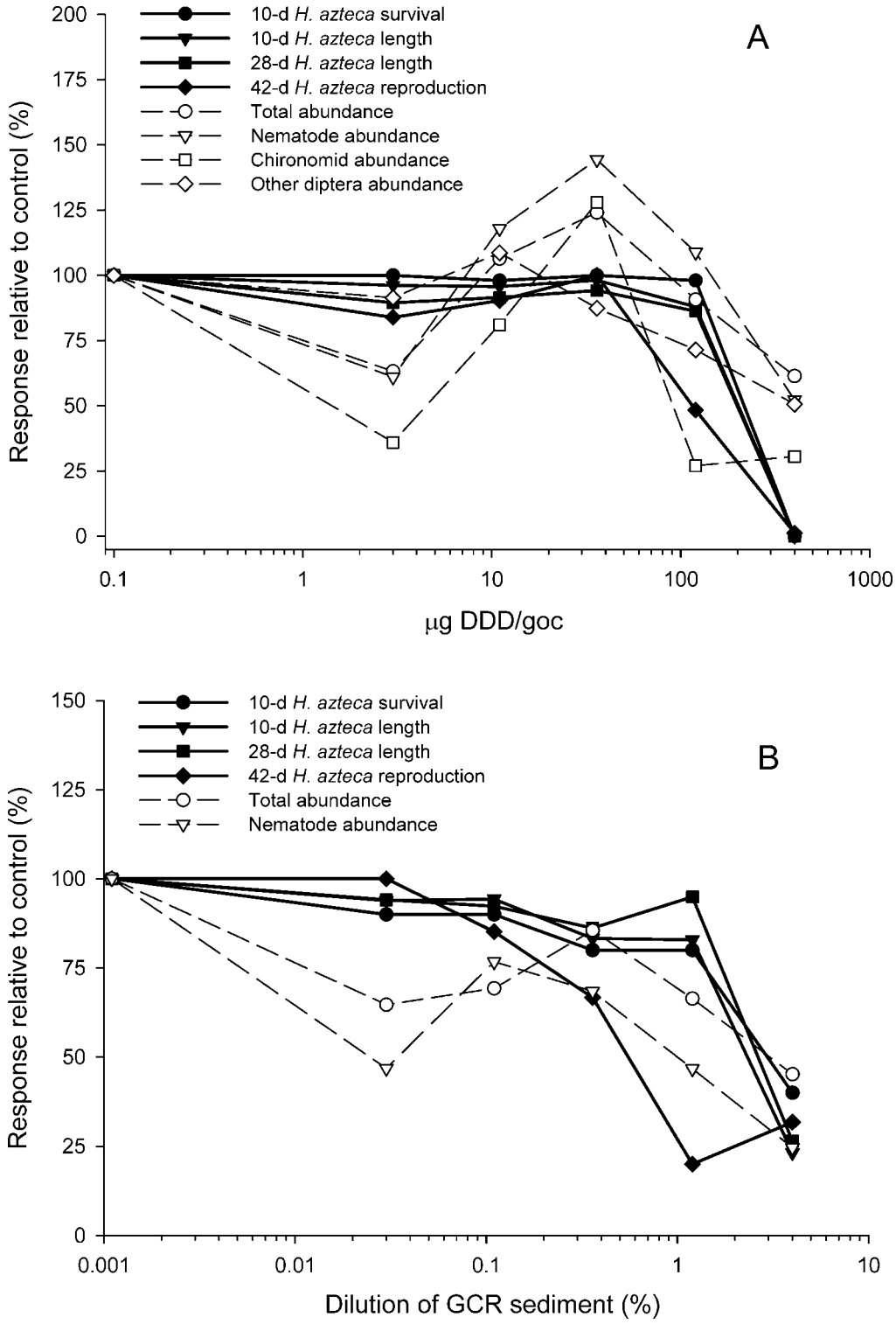

Fig. 1. Responses of *Hyalella azteca* in laboratory exposures compared to responses of taxa in colonizing trays containing a gradient of dichlorodiphenyldichloroethane (DDD) spiked in sediment (**A**) or dilutions of Grand Calumet River (GCR) sediment (**B**). Only field endpoints with significant treatment effects and select endpoints for the *H. azteca* tests are included.

evident between the response of amphipods in the laboratory and the response of invertebrates in the field when IC50s were considered (e.g., reproduction of amphipods was affected at concentrations reducing abundance of chironomids colonizing the sediment; Fig. 2A).

Dilutions of GCR sediment. Consistent effects were observed in the highest dilution of GCR sediment (4%) on survival, length, and reproduction of amphipods in the laboratory and on total abundance of major taxa and on abundance of nematodes in the field (Fig. 1B). The same effect concentrations were observed for 10-d survival or length of amphipods in the laboratory compared to effect concentrations observed for total abundance of major taxa or abundance of nematodes in the field (ChVs of 2.2% GCR sediment; Tables 4 and 6 and

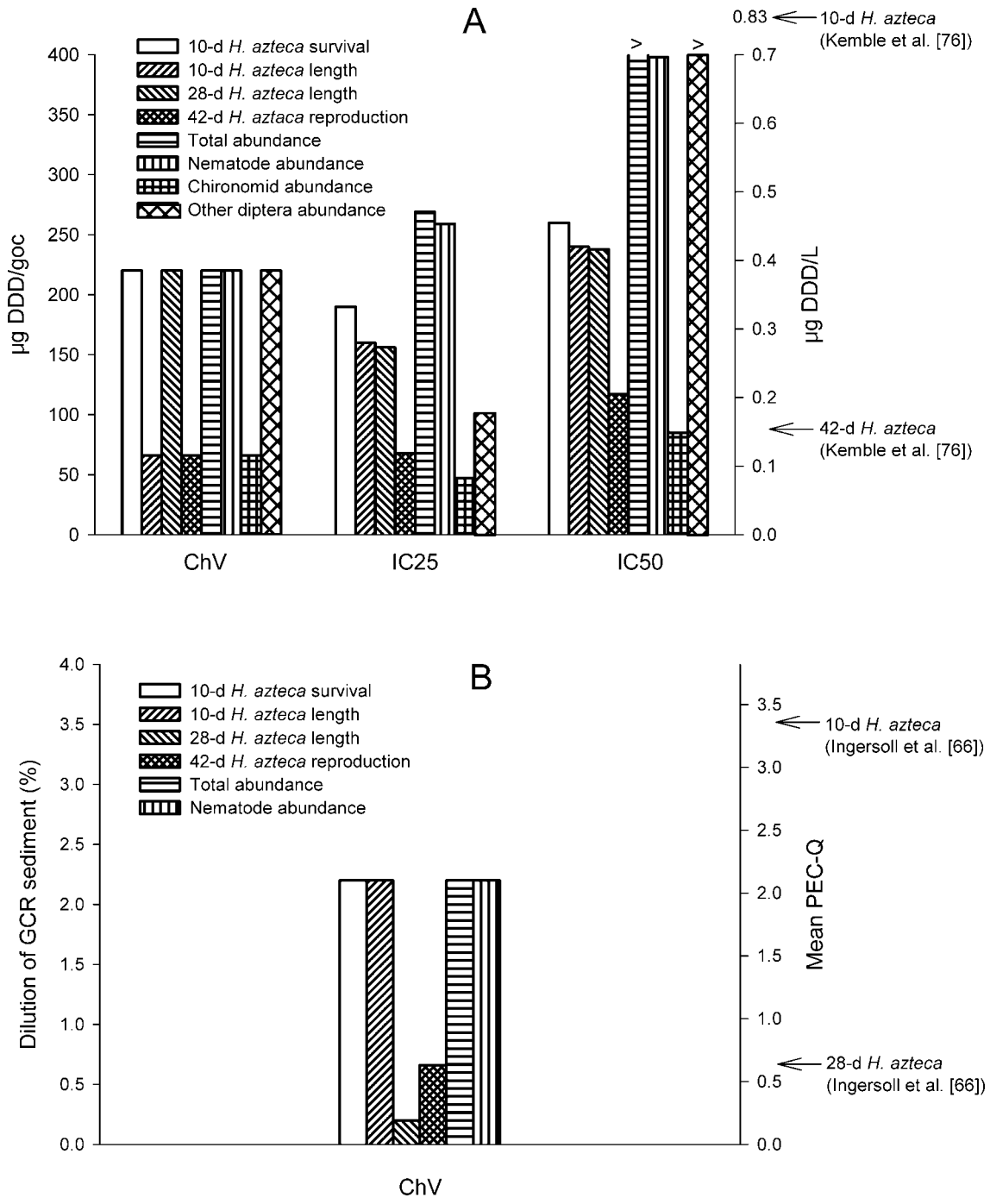

Fig. 2. Effect concentrations for *Hyalella azteca* in laboratory exposures compared to abundance of taxa in colonizing trays containing a gradient of dichlorodiphenyldichloroethane (DDD) spiked in sediment (**A**) or dilutions of Grand Calumet River (GCR) sediment (**B**). Only field endpoints with significant treatment effects and select endpoints for the *H. azteca* tests are included. Predicted pore-water DDD toxicity effect concentrations for *H. azteca* [76] and predicted mean probable effect concentration quotient (PEC-Q) toxicity effect concentrations for *H. azteca* [66] are also designated. ChV = chronic value; IC25 = 25% inhibition concentration; IC50 = 50% inhibition concentration.

Fig. 2B). However, 28-d survival and length and 42-d reproduction and length provided more conservative effect concentrations compared to the response of benthic invertebrates in the colonization study (ChVs of 0.20–0.66% GCR sediment; Fig. 2B).

DISCUSSION

Effects in laboratory toxicity tests

The most sensitive endpoints measured in the laboratory toxicity test with amphipods were length or reproduction in the exposure to DDD-spiked sediment and 28- to 42-d survival, length, or reproduction in the exposure to dilutions of GCR sediment. The least sensitive endpoint was typically 10-d survival of amphipods. However, variance associated with reproduction (e.g., standard deviation of the mean or confidence intervals associated with the IC25s or IC50s) was generally larger than the variance associated with the survival or length (Tables 3 and 4), and length was not consistently affected across all the time periods in the exposure to DDD-spiked sediment (Table 3). Measurement of sublethal endpoints in

exposures with *H. azteca* have been previously reported to provide a more sensitive measure of effects in sediment toxicity tests with *H. azteca* [66,75] and in water-only toxicity tests with *H. azteca* exposed to DDD, fluoranthene, or cadmium [76]. Effect concentrations for *H. azteca* based on predicted concentrations of DDD in pore water (estimated by equilibrium partitioning) would correspond to an IC25 of 0.12 μg DDD/L for 42-d reproduction and an IC50 for 10-d survival of 0.43 μg DDD/L. Predicted pore-water concentrations were used in these calculations because the measured pore-water concentrations were elevated, likely because of DDD associated with particulate or dissolved organic carbon in the pore water (Table 1). These predicted pore-water effect concentrations are similar to 10-d water-only effect concentrations reported for *H. azteca* of 0.19 μg DDD/L [52,53]. Kemble et al. [76] reported LC50s of 0.83 μg/L for 10-d survival and an IC25 of <0.16 μg/L for 42-d reproduction in water-only exposure with *H. azteca* that are similar to the effect concentrations for *H. azteca* observed in the present study (Fig. 2A). Results of these analyses indicate that the toxic effects observed on *H. azteca* in whole-sediment exposures could be predicted with a reasonable level of certainty based on equilibrium partitioning and predicted pore-water concentrations.

Control survival of midge in the long-term toxicity test conducted with DDD-spiked sediment was not acceptable (e.g., 10-d control survival <60% [49]). However, in a preliminary sediment storage study, 10-d IC50s for *C. dilutus* survival ranged from 77 to 210 μg DDD/goc [49]. The predicted pore-water 10-d IC50s for *C. dilutus* would range from 0.13 to 0.36 μg DDD/L, which are similar to 10-d LC50s reported for water-only exposures with *C. dilutus* (0.18–0.42 μg DDD/L reported by Phipps et al. [52] and Hoke et al. [53] and 0.63 μg/L reported by Kemble et al. [76]). While results of the long-term midge toxicity test with DDD-spiked sediment were not considered acceptable because of low survival of organisms in the controls, a consistent effect on midge was observed between 36 and 120 μg DDD/goc [49], which corresponds to predicted pore-water concentration of 0.061 to 0.20 μg DDD/L (Table 1). This range of effects, based on predicted pore-water concentration, is similar to an IC25 of <0.11 μg DDD/L for emergence of *C. dilutus* in water-only exposures [76]. Results of these analyses indicate that the toxic effects observed on *C. dilutus* in whole-sediment exposures could also be predicted on the basis of equilibrium partitioning and predicted pore-water concentrations (within the limited quality of the data from the long-term midge exposures [49]).

MacDonald et al. [65] published a probable effect concentration (PEC) for DDD of 28 ng/g (dry wt) based on matching toxicity and chemistry data for field-collected freshwater sediments. The PECs are effect-based sediment quality guidelines that were established as concentrations of individual chemicals above which adverse effects in sediments are expected to frequently occur in field-collected sediments. The database used to develop the PECs consisted primarily of toxicity tests conducted with *H. azteca* (10–14-d or 28–42-d exposures) and *C. dilutus* or *C. riparius* (10–14-d exposures [65,66]). Unfortunately, data were limited for DDD in the database developed by MacDonald et al. [65] to determine if the PEC for DDD could reliably predict toxicity in freshwater sediments. If a sediment contained 1% total organic carbon (as was the case for the present study), the PEC for DDD normalized to organic carbon would be 2.8 μg DDD/goc, which is similar to the lowest concentration of DDD observed to significantly affect amphipod length (3 μg DDD/goc). However, the IC25 for amphipod reproduction (68 μg DDD/goc) and the IC25 for chironomid abundance (47 μg DDD/goc) were considerably higher than 2.8 μg DDD/goc. This difference suggests that the PEC for DDD is too low to reliably predict toxicity due to DDD alone in sediments based on results of the laboratory toxicity tests and the colonization study. However, the PEC for DDD may be useful in determining the toxicity of DDD associated with complex mixtures of contaminants in sediment that may be associated with DDD in the field (including DDT, DDE, other organic contaminants, and metals [17]).

Effect concentrations for dilutions of GCR sediment were also calculated on the basis of PEC quotients (PEC-Qs) and on concentrations of metals, total PCBs, and total PAHs in the sediment dilutions (Table 2). A PEC-Q is calculated by dividing the concentration of a chemical by the PEC for that chemical. A mean quotient can then be calculated by summing the individual quotients (e.g., quotient for total PAHs, quotient for PCBs, and average quotient for metals) and dividing this sum by 3 (Table 2 [66]). The IC25s for amphipods in the present study would be a mean PEC-Q of 1.5 for 10-d survival (based on an IC25 of 1.6% GCR sediment) and mean PEC-Q of 0.60 for 42-d survival (based on an IC25 of 0.70% GCR sediment; Tables 2 and 4). These mean PEC-Q effect concentrations are similar to mean PEC-Q effect concentrations observed in a database for 10- to 28-d *H. azteca* toxicity tests with field-collected sediments (Fig. 2B [66]). Specifically, a 50% incidence of toxicity was observed at a mean PEC-Q of 3.4 in 10-d tests when survival of *H. azteca* was measured and at a mean PEC-Q of 0.63 in 28-d tests when survival or growth of *H. azteca* was measured [66] (a 50% incidence of toxicity corresponds to about a 25% reduction in survival [11]). In the present study, an IC25 for the 42-d reproduction was estimated to occur at a mean PEC-Q of 0.21 (based on the IC25 of 0.24% GCR). The database contained insufficient reproduction data to evaluate the ability of mean PEC-Qs to estimate reproductive effects in sediment toxicity tests with *H. azteca* [66,75].

While results of the long-term sediment exposures with dilutions of GCR sediment were not considered acceptable because of low control survival of midge, a consistent effect was observed on midge between 0.36 and 1.2% in this long-term exposure [49]. These dilutions of GCR sediment correspond to mean PEC-Qs of 0.42 to 1.2 (Table 2) that were considerably lower than the mean PEC-Q of 3.5 that was reported to result in a 50% incidence in toxicity in 10-d tests with midge (exposures with *C. dilutus* or *C. riparius* started with second- to third-instar larvae [66]). Hence, a mean PEC-Q of 3.5 may not be adequately protective of effects observed in long-term sediment toxicity tests started with midge <24 h old. However, poor performance of midge in the exposures with dilutions of GCR sediment (and with DDD-spiked sediment) limits the confidence of these evaluations [49].

Effects in the colonization study

A primary goal of the colonization study was to evaluate effects of contaminated sediments on invertebrates exposed in the field under controlled conditions. Past studies have attempted to evaluate effects of contaminated sediment on invertebrates in the field by synoptically collecting invertebrates and sediments across concentration gradients (e.g., sediment quality triad [8]). However, the influence of physicochemical characteristics of sediments, conditions in the overlying water,

and variation in the habitats sampled (e.g., grain size, total organic carbon, salinity, depth, currents, hydrological conditions, latitude) have confounded the interpretation of data generated from these types of studies. Controlled colonization studies with spiked sediments have been conducted to help account for these types of potential confounding factors (see the *Introduction* for a summary of these studies).

A limitation of many of these studies conducted with spiked sediments has been changes in concentrations or bioavailability of the chemicals during the colonization period. For example, Liber et al. [39] observed concentrations of zinc in pore water to decrease with increasing concentrations of acid volatile sulfides during a one-year colonization study. Watzin et al. [36] and Watzin and Roscigno [41] reported zinc concentrations in sediment to decrease by over 50% during a 7- to 9-d colonization study. Similarly, concentrations of cadmium [34,35,42] or copper [43] decreased in surface sediments in colonization studies conducted up to one year. The reason for these decreases in metal concentrations may be the relatively short holding time (typically only a few days) between spiking of sediment and the start of the colonization studies. In the present study, these problems were minimized by selection of DDD or a dilution of a highly contaminated field-collected sediment for testing in the laboratory exposures and in the 12-week colonization study. The DDD-spiked sediment or the organic contaminants in GCR sediment would likely exhibit less temporal or spatial variability compared to metals spiked into sediment. Additionally, sediments in the present study were held after spiking for at least 60 d before the start of the laboratory toxicity or field exposures. The ASTM [1] and U.S. EPA [3] recommend that sediment spiked with organic compounds should be held for at least one month before the start of an exposure to allow the chemicals to better equilibrate with the sediment, but two months or more may be necessary for chemicals with high K_{ow} (octanol–water partitioning coefficient). For metals, shorter holding times (e.g., one to two weeks) may be sufficient [1,3].

Other colonization studies have been conducted using field-collected sediments sampled across gradients of contamination (e.g., [44–46]) or by placing trays with a clean formulated sediment into contaminated sites (e.g., [29]). While contaminants in field-collected samples may be well equilibrated with sediment, concentrations of contaminants in sediment often covary with the physicochemical characteristics (e.g., grain size and total organic carbon) of sediment. Hence, interpreting the cause of the effects observed on distributions of invertebrates in these studies is difficult. Additionally, invertebrates should not be present in sediment at the start of the colonization period (a potential problem with all these colonization studies except for studies conducted with formulated sediment [26–29,36,41]). Investigators have frozen field-collected samples before the start of the colonization studies in an attempt to eliminate indigenous invertebrates (e.g., [30,32,37,40,43–45]). While freezing may help eliminate indigenous invertebrates from a sediment sample, freezing can also alter the bioavailability of contaminants and is not recommended for toxicity or bioaccumulation testing with sediments [1,3]. Other investigators (e.g., [39,42,47]) have spiked chemicals into subsurface sediments, but these sediments may also contain indigenous invertebrates [23]. In the present study, spiking DDD or mixing a small amount of highly contaminated GCR sediment into the dry Florissant soil avoided the problem of covarying physicochemical characteristics across the sediment treatments and eliminated indigenous invertebrates in sediment samples at the start of the colonization study.

Colonization studies with contaminated sediments have been conducted in trays ranging from about 0.5 to 12 L placed either in a frame above the sediment surface [36,41,42] or in direct contact with sediment. While placement of trays in a frame above the sediment surface is useful in evaluating pelagic recruitment of invertebrates, a primary goal of the present study was to evaluate colonization of sediment by both sediment-dwelling invertebrates (e.g., oligochaetes, nematodes) and by pelagic invertebrates (e.g., Chaoboridae [phantom midge]). Smaller trays were also selected for use in the present study to increase the number of replicates tested [49]. Unfortunately, increasing the number of replicate trays from three to seven did not reduce the variance observed within treatments.

Effects observed on invertebrates colonizing sediments placed in trays may result from direct toxicity on invertebrates inhabiting the substrate or from indirect effects such as substrate avoidance [77]. In marine or estuarine studies, reduced total abundance of major taxa or abundance of individual taxa colonizing sediment have been observed with sediments spiked with creosote [26], dibutyl phthalate [27], zinc [36,41], copper [43], chlorpyrifos [40], or oil [31,32,37] or with field-collected sediments contaminated with complex mixtures of metals and organic compounds [44]. While total abundance of major taxa colonizing estuarine or marine sediments has not typically been a sensitive endpoint, changes in diversity of invertebrates colonizing trays have been observed in some studies. For example, specific families of polychaetes, copepods, and ostracods were most sensitive to zinc-spiked sediment in 7- to 9-d colonization studies [41], and individual species of polychaetes, mollusks, and echinoderms were most sensitive to copper-spiked sediment in seven-month colonization studies [43].

In freshwater studies, less dramatic effects have been reported on abundance or diversity of taxa colonizing sediments. The colonization studies conducted in freshwater have tested either cadmium [34,35,42] or zinc [39,47] spiked into sediment at low to moderate concentrations. In contrast, the studies in estuarine or marine systems described here have been conducted with more severely contaminated sediments. Hare et al. [34,35] and Warren et al. [42] evaluated the colonization of invertebrates in cadmium-spiked sediments after placement in a lake for up to 14 months. Chironomids were the major taxa colonizing trays along with oligochaetes and other Diptera. Abundance of most major taxa was not affected, and the abundance of only one species of chironomid decreased with increasing concentrations of cadmium tested [34]. Warren et al. [42] observed that population densities of oligochaetes declined with increasing cadmium concentrations. Liber et al. [39] evaluated the colonization of invertebrates in zinc-spiked sediments after placement in a lake for up to one year. Chironomids were the major taxa colonizing the trays along with oligochaetes, bivalves, and nematodes. No substantial effect on total abundance of major taxa or abundance of individual taxa were observed across concentrations of zinc tested; however, a significant reduction was observed in some of the families of oligochaetes in the highest concentration of zinc tested on two of the five sampling dates. Burton et al. [47] observed toxic effects on a variety of benthic indices when concentrations of zinc were in excess of acid volatile sulfide. Lack of effects observed in the study by Hare et al. [34] was attributed

in part to the insensitivity of invertebrates colonizing the sediments; however, toxicity and bioavailability of cadmium or zinc in these studies was likely mediated by acid volatile sulfides in the sediments.

In the present study, major taxa colonizing sediments included nematodes (about 55%), oligochaetes (about 30%), and to a lesser degree Diptera (about 10%, primarily Chaoboridae and chironomids; Tables 5 and 6). In contrast, chironomids were the major taxa colonizing sediments in the studies conducted by Hare et al. [34], Liber et al. [39], and Warren et al. [42]. It is not clear why such a difference existed in major taxa colonizing sediments between these previous studies and the present study. Procedures used to quantify nematodes may have contributed to these differences. Perhaps the smaller pond used in the present study (0.1-ha surface area and relatively oligotrophic [69]) compared to the larger systems used by Hare et al. (105-ha lake [34]), Hare et al. (60-ha lake [35]), Liber et al. ([2-ha pond [39]), or Warren et al. (500-ha lake [42]) also contributed to differences in major taxa colonizing the sediments.

In the present study, significant reductions in total abundance of major taxa and abundance of nematodes and other Diptera were observed at the highest DDD or GCR exposure concentrations, and a significant reduction in abundance of chironomids was observed at the two highest exposure concentrations in the DDD exposure (Tables 5 and 6). More subtle effects may have been observed if additional taxonomic resolution were used. Roach et al. [44] evaluated the influence of taxonomic resolution on the invertebrates colonizing trays filled with contaminated marine sediments and reported benthic community composition to vary on the basis of the degree of contamination. However, smaller differences were observed among treatments with decreasing taxonomic resolution (at the phylum level [44]). While a higher level of taxonomic identification was sufficient to identify major trends, taxonomic identification to only the phylum level resulted in the fewest significant differences among treatments. Therefore, Roach et al. [44] recommended that family-level taxonomy at a minimum should be used in colonization studies.

In the present study, the abundance of other Diptera (including phantom midge [Chaoboridae]) was reduced with increasing concentrations of DDD in sediment (IC25 100 μg DDD/goc; Table 5). Larvae of phantom midge are mobile and migrate daily between the water column (where they feed on zooplankton at night) and the sediment (where they remain at the sediment–water interface during the day [23]). Abundance of phantom midge was not influenced by concentrations of cadmium in a sediment colonization study [34]. Perhaps phantom midge might be able to avoid DDD but not cadmium spiked into sediment.

Wogram and Liess [78] evaluated data in the U.S. EPA Acquire database to compare relative sensitivity of invertebrates to organic compounds. Taxa relevant to the present study listed in order of sensitivity were amphipods > mayflies > Diptera > Odonates > Oligochaeta. Amphipods (the primary organism used in laboratory toxicity tests) were the most sensitive to organic compounds [78] but were not observed in sediments in the Ekman grab samples or in the colonization samples in the present study. Mayflies were the second most sensitive taxa but did not consistently colonize the trays. Diptera were the third most sensitive taxa, and similar effect concentrations were observed for *C. dilutus* in laboratory [49] and for abundance of chironomids or other Diptera exposed in the field to DDD-spiked sediment. Abundance of nematodes was significantly reduced in both DDD-spiked sediment and dilutions of GCR sediment. Unfortunately, data regarding the relative sensitivity of nematodes to other taxa were not summarized by Wogram and Liess [78]. The least sensitive taxa were oligochaetes (the second most abundant taxa colonizing the trays), and no significant effects were observed on abundance of oligochaetes in DDD-spiked sediment or in dilutions of GCR sediment.

A concern for colonization studies is whether steady state is reached in populations of invertebrates colonizing the trays compared to sediments in the surrounding environment. Costello and Thrush [79] concluded that the optimum time for sampling colonization of substrates by benthic invertebrates may be as soon as steady state in the number of taxa is reached, which may maximize diversity and minimize interspecific interactions. At the start, rates of immigration and extinction would probably be linear because neither of these rates depend on population density (a noninteractive model with regard to other species [80]). Once populations of invertebrates in the trays become large enough for competition and predation to become important factors (interactive species or community equilibrium), successful immigration depends on the resistance of species already present [80,81]. The preliminary colonization study conducted in 1999 [49] attempted to determine if various taxa would reach steady state between the trays and the surrounding environment in 6 to 12 weeks. At 12 weeks, no significant differences were observed between taxa present in the trays and in the surrounding environment collected using an Ekman grab sampler [49].

Contaminants in sediment may change the time to reach steady state with invertebrates in the surrounding habitat. During the first stage of colonization, survival of or avoidance by colonizing invertebrates would depend primarily on the conditions of the unestablished area (e.g., before interactions among populations occurred). Contaminated sediment may then present adverse conditions, resulting in a change in the colonization period and thereby a change in the time at which population interactions become important [81]. If contaminated sediments were to take a longer time to reach a community steady state, this delay should be considered a toxic effect. Interestingly, Berge [32] observed that the time needed to reach 90% of steady state in distribution of invertebrates colonizing sediment trays was actually shorter in oil-contaminated sediments (259 d) compared to control sediments (459 d). Moreover, effects were first observed on filter- and surface-deposit feeders, followed by effects on subsurface-deposit feeders [32]. Future studies should consider the time course of colonization of contaminated sediments by invertebrates [34,35,39].

Laboratory-to-field comparisons

Laboratory sediment toxicity tests provide rapid information about toxicity of contaminants to benthic invertebrates [1,3] but have been criticized as being too simple to represent responses of benthic invertebrates in the field (e.g., [44,47]). In the present study, sublethal effects observed in laboratory toxicity tests occurred at similar or lower concentrations observed to adversely affect colonization by several major taxa in the field. Effect concentrations observed for DDD on length or reproduction of *H. azteca* in laboratory toxicity tests (and possibly toxicity tests with *C. dilutus*) were similar to or lower than effect concentrations observed on abundance of major

taxa or on abundance of nematodes, chironomids, or other Diptera colonizing DDD-spiked sediment in the field (Fig. 2A). Effect concentrations for dilutions of GCR sediment on survival, length, and reproduction of *H. azteca* in 28- to 42-d exposures tended to be lower than effect concentrations observed on abundance of major taxa or on abundance of nematodes colonizing dilutions of GCR sediment in the field (Fig. 2B). While significant effects were observed on survival *H. azteca* in 10-d exposures and on invertebrates colonizing sediments at the highest exposure concentrations of DDD or dilutions of GCR sediment (Tables 3–6), measurement of sublethal endpoints was needed to provide a more sensitive and protective measure of effects on invertebrates exposed to contaminated sediments in the field. Therefore, results of this study indicate that sublethal endpoints in chronic toxicity tests, in combination with measures of sediment chemistry, should be used to estimate effects of contaminated sediments on invertebrates in the field.

Only a limited number of studies have evaluated toxicity in laboratory tests with splits of contaminated sediments used to evaluate colonization by invertebrates in the field. Liber et al. [39] did not observe toxicity in 10-d toxicity tests conducted with *H. azteca* or *C. dilutus*, and they observed marginal toxic effects on colonization using sediment spiked with zinc. Changes in the bioavailability of zinc in sediment during the one-year colonization study likely contributed to this lack of toxicity [39]. Similarly, Parrish et al. [45] did not observe effects in field colonization studies, and they observed marginal toxicity in laboratory tests conducted with marine dredge materials. Moore et al. [46] reported acute and chronic marine laboratory tests provided a more sensitive measure of effects compared to a field colonization study. Effects on invertebrates colonizing creosote-contaminated sediment [26] or dibutyl phthalate–contaminated sediment [27] were similar in trays held in the laboratory receiving a natural water compared to trays placed in a estuary during an eight-week colonization study. In contrast, sediment spiked with fenvalerate in a colonization study was less toxic in the field compared to the laboratory [28].

Canfield et al. [7–9] used the sediment quality triad approach to compare responses of invertebrates collected from the field to responses of *H. azteca*, *C. dilutus*, or *C. riparius* in laboratory toxicity tests. In extremely contaminated samples, concordance was observed among measures of sediment chemistry and effects observed in the laboratory and in the field. However, these relationships did not hold as well for moderately contaminated samples, where the variability in responses of invertebrate in the field was attributed to noncontaminant factors (e.g., depth, vegetation, sediment organic carbon, or grain size). In the present study, controlling for habitat or physicochemical variables in the field resulted in a more consistent concentration–response relationship between the laboratory and the field across the range of exposure concentrations in DDD-spiked sediments and in dilutions of GCR sediment. However, even under these controlled conditions, variability within treatments was still quite high in the field compared to the laboratory (Tables 3–6).

Effect concentrations observed in the colonization study with dilutions of GCR sediment in the present study were generally above effect concentrations reported for surveys of estuarine sediment contamination and the distributions of invertebrates by Hyland et al. [82,83] and Van Dolah et al. [84]. Specifically, synoptic surveys of contaminated sediments and benthic community structure were conducted in estuaries in the southeastern United States and were used to evaluate the predictive ability of SQG quotients [82,83]. At mean effect-range median quotients (ERM-Qs) >0.36 and at mean probable effect level quotients (PEL-Qs) >0.78, 74 to 77% of stations were classified as having degraded benthic communities [82,83]. A similar incidence of toxicity in 10-d estuarine and marine laboratory tests with amphipods has been observed at higher levels of sediment contamination (mean ERM-Qs >1.5 and mean PEL-Qs >2.3 [85]). The effect concentrations for benthic communities reported by Hyland et al. [82,83] are lower than effect concentrations observed in the present study for total abundance of major taxa or abundance of nematodes (e.g., ChV based on mean PEC-Q of 2.1; Fig. 1B). The lower effect concentrations for benthic community responses reported by Hyland et al. [82,83] may result from both chemical toxicity and natural confounding factors (e.g., grain size or total organic carbon) influencing distributions of benthic communities in the field [85].

Results of the present study also suggest that effects on benthic communities may occur at lower chemical concentrations compared to concentrations that are lethal in 10-d laboratory tests with *H. azteca*. Moreover, use of chronic laboratory toxicity tests with *H. azteca* and colonization studies that help control for potential confounding factors may be needed to provide conservative estimates of impacts observed on benthic communities exposed to contaminated sediments compared to estimating impacts observed using synoptic surveys of benthic communities [85].

Bioaccumulation of DDD by oligochaetes colonizing DDD-spiked sediment was consistent with a previous laboratory bioaccumulation study conducted with DDD-contaminated sediments collected from the field [74]. Specifically, a 56-d laboratory bioaccumulation study was conducted with the oligochaete *Lumbriculus variegatus* using a field-collected sediment containing 220 μg DDD/goc (along with elevated concentrations of DDT and DDE [74]). Concentrations of DDD in the oligochaetes in this laboratory exposure reached steady state after about 14 d, with BSAFs ranging from about 2 to 4. Concentrations of DDD measured in native oligochaetes collected at the time and location that sediment was collected for laboratory testing were similar to steady-state concentrations estimated from the 56-d laboratory exposures with *L. variegatus* [74]. In the present study, oligochaetes collected from the sediment containing the two highest DDD exposure concentrations at the end of the 12-week colonization study had BSAFs of 1.5 and 3.8 (Table 7). Results of this previous study indicate that the oligochaetes collected from DDD-spiked sediment at the end of the 12-week colonization study were likely at steady state with DDD in sediment. Moreover, results of the present study confirm that BSAFs measured in laboratory sediment exposures with *L. variegatus* can be used to estimate bioaccumulation of contaminants in sediment by oligochaetes exposed in the field.

Recommendations for future studies

The present study may have benefited from more detailed taxonomic identification of invertebrates colonizing the trays (e.g., to the lowest practical taxonomic level, such as species or family [39,43,44]). However, the decision on the level of taxonomic identification depends on the objectives of the study and resources available to conduct the study [86,87]. A narrower dilution series of chemicals spiked into sediment could

be tested to improve the estimates of effect concentrations (e.g., a 30–50% dilution compared to the 70% dilution used in the present study).

Additional studies are needed to determine optimum size of the colonization trays and optimum number of replicate trays that should be tested in the field. In the present study, even with seven replicates, high variability still existed within treatments. Sediments in trays should be sampled to evaluate potential changes in sediment chemistry over time across all the treatments evaluated [34,35,39]. Time to reach steady state in invertebrates colonizing sediments in trays should be evaluated (e.g., sampling at weeks 2, 4, 8, and 12). Different habitat types for placement of colonization trays should be considered (e.g., use of locations with a higher abundance and diversity of invertebrates within lakes, streams, or rivers [32,41,43]).

In situ toxicity tests should be conducted in combination with laboratory toxicity tests and colonization study (e.g., placing test organisms into chambers containing contaminated sediment in the same habitat where the colonization study is conducted [88]). Sediment avoidance should also be further evaluated in laboratory and field studies (e.g., based on the apparent avoidance by Chaoboridae of DDD-spiked sediment).

Finally, additional comparisons between colonization of sediments in the field and chronic laboratory toxicity tests with *C. dilutus* are needed. *Chironomus dilutus* seemed more sensitive than *H. azteca*, but poor performance of midge in the long-term toxicity tests compromised these comparisons [49]. The reason for the poor performance of midge in the toxicity tests is unknown. Throughout the period that the midge sediment tests were conducted, our laboratory successfully conducted 60-d water-only toxicity tests with *C. dilutus* [76]. Midge larvae <24 h old are difficult to handle, and this may have contributed to the poor performance. Alternatively, changes may have been occurring in sediments over the storage period or in the quality of organisms in laboratory cultures that affected midge performance (e.g., no successful midge tests were conducted after day 60 of the sediment storage study [49]). Importantly, other investigators have successfully conducted chronic sediment toxicity tests using the methods used in the present study [1,3,89–91]. Therefore, the problem with poor performance of midge in the present study should be correctable.

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