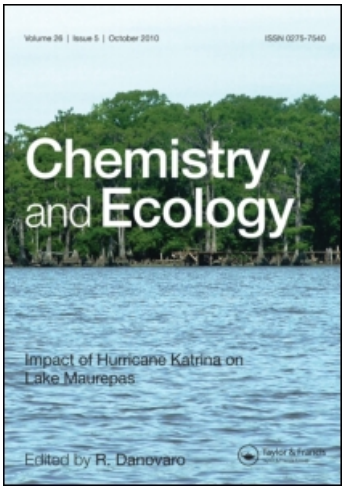

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Sediment quality assessment of Beasley Lake: bioaccumulation and effects of pesticides in *Hyalella azteca*

Richard E. Lizotte Jr[a]; Scott S. Knight[a]; Charles T. Bryant[a]
[a] USDA-ARS National Sedimentation Laboratory, Oxford, MS, USA

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Sediment quality assessment of Beasley Lake: bioaccumulation and effects of pesticides in *Hyalella azteca*

Richard E. Lizotte, Jr*, Scott S. Knight and Charles T. Bryant

USDA-ARS National Sedimentation Laboratory, Oxford, MS, USA

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Beasley Lake is a Conservation Effects Assessment Program (CEAP) watershed in the intensively cultivated Mississippi Delta, USA. Lake sediment quality at three sites was evaluated in 2004 and 2008 for biological impairment and uptake (animal tissue pesticide residues) from 14 pesticides and three metabolites using *Hyalella azteca* (Saussure). Eleven pesticides and three metabolites were detected in sediment among the three sites in 2004 and all 17 compounds examined were detected among the three sites in 2008, with the herbicide atrazine having the greatest concentrations. Twenty-eight-day *H. azteca* survival and growth (mg w/w) indicated no survival effects at any site for either year, but growth impairment occurred in *H. azteca* exposed to sediments in 2004, whereas growth enhancement occurred in *H. azteca* exposed to sediments at one site in 2008. Pesticides observed in animal tissue pesticide residues occurred more frequently and in greater concentrations in 2004 compared with 2008. Thirteen pesticides were detected in animal tissue pesticide residues in 2004, with chlorpyrifos occurring in the greatest concentrations, and six pesticides were detected in 2008, with p,p'-DDT occurring in the greatest concentrations. *H. azteca* tissue pesticide residues of seven pesticides, two herbicides, three insecticides, one insecticide metabolite, and p,p'-DDT, were associated with growth.

Keywords: agriculture; land management; pesticides; *Hyalella azteca* tissue pesticide residues

1. Introduction

Assessing sediment quality in aquatic habitats is an increasingly important issue internationally. Poor sediment quality resulting from chemical contaminants in sediment can be potentially costly from both an economic and an ecological perspective, especially for navigation dredging projects, river or lake restoration programmes and fisheries management [1]. Within agricultural watersheds, pesticides are the most likely source of chemical contamination of sediments leading to economic and ecological degradation of aquatic ecosystem services [2]. Although agricultural pesticide use to control fungus, weed and insect pests provides significant benefits in the protection of crop yields [3], these chemical compounds can be transported via storm-event runoff

*Corresponding author. Email: richard.lizotte@ars.usda.gov

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with suspended sediment and deposited in sediments of receiving aquatic systems. The resulting accumulation of pesticides in sediment potentially provides a long-term source of toxicity and/or accumulation in benthic organisms, transferring to higher trophic organisms within the food web [4].

The Mississippi River watershed is the single largest drainage basin in North America, encompassing ~2.9 million km^2, and includes some of the most intensively cultivated regions of the continent [5]. The lower Mississippi River alluvial plain, commonly referred to as 'the Delta', is the region comprising the southern portion of the Mississippi River basin. The Delta extends over 1100 km from southeastern Missouri to Louisiana at the Gulf of Mexico, encompassing 18,130 km^2, and is intensively agricultural and rural [6]. The Delta has numerous bayous, sloughs and oxbow lakes, water bodies that have been physically isolated from their respective main river channels [7]. In addition, the Delta has a long growing season and average annual rainfall amounts between 114 and 152 $cm \cdot year^{-1}$ [8], conditions conducive to the proliferation of insect and weed pests and requiring frequent pesticide use for control [9]. Associated with this use is a concomitant potential for transport into nearby water bodies such as lakes, rivers and streams, which additionally receive frequent pulses of pesticide-laden effluent from agricultural fields, primarily during storm events [6,10]. Mississippi Delta oxbow lakes were historically valued for their productivity and recreational use but due to agricultural practices, many lakes have become impaired from sediment, nutrient and pesticide contamination [6,10]. As a result, environmental quality and ecological diversity in the Delta have declined, and recreational popularity has decreased. Despite these conditions, the Delta remains a region with a high diversity of warm-water fish such as sunfish (*Lepomis* sp.) and catfish (*Ictalurus* sp.) that feed on benthic and epibenthic aquatic invertebrates [11]. The Delta is also part of the Mississippi flyway, a major route for migratory waterfowl that also feed on fish and aquatic invertebrates while wintering [12]. For these reasons, there is a possibility of trophic transfer of pesticides from contaminated Mississippi Delta oxbow lake sediments to benthic and epibenthic aquatic invertebrates to fish and waterfowl.

Beasley Lake, an agriculturally impacted oxbow lake located in the Delta, was chosen as a Conservation Effects Assessment Program (CEAP) watershed. Reasons for this were an extensive long-term database (~8 years) available on the contributions and understanding of long-term changes in multiple integrated farm best management practices (BMPs) effectiveness on watershed scales [13,14]. Recently, Locke et al. [15] reported some success in restoring productivity and recreational value in Beasley Lake. Lake improvements included decreased sediment, nutrient and pesticide loads, with resultant increased water clarity, primary productivity and fisheries productivity from 1995 to 2005 [13,15]. Although water quality issues have been assessed, there is a need to assess sediment quality in Beasley Lake over time. This study examined the influence of BMPs implemented over several years on Beasley Lake sediment quality by focusing on four aims: (i) to determine the degree of pesticide contamination in Beasley Lake sediments with BMPs implemented over time; (ii) to assess biological effects (i.e. survival and growth) of sediment-bound pesticides over time through 28-day exposure of an epibenthic detritivore, *Hyalella azteca* (Amphipoda); (iii) to determine the mobility and bioaccumulation of Beasley Lake sediment pesticides over time to *H. azteca* via tissue pesticide residues; and (iv) to ascertain associations between biological effects and tissue pesticide residues in *H. azteca*.

2. Materials and methods

2.1. *Study area and sample collection*

Beasley Lake watershed (Sunflower County, MS, USA: latitude 33°24′15″ N, longitude 90° 40′05″ W) was selected as a CEAP watershed beginning in 2003 (Figure 1). The watershed drainage area

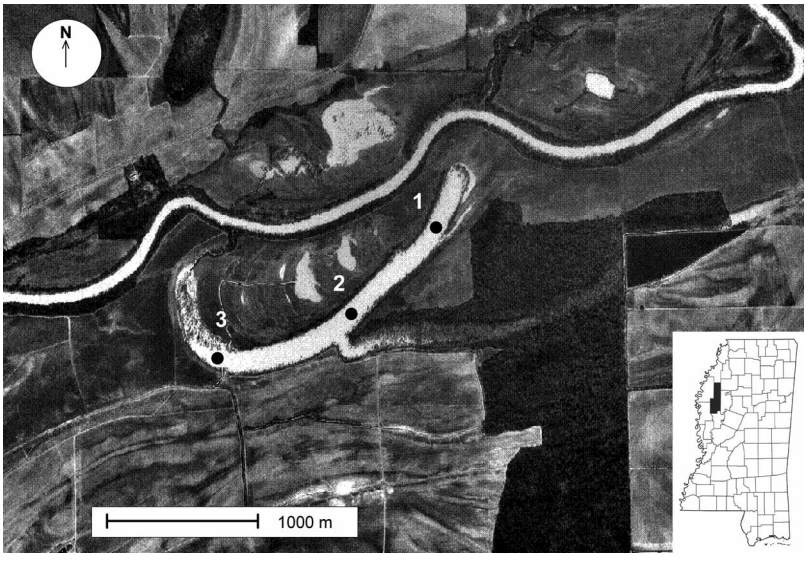

Figure 1. Aerial photograph showing three sampling locations for sediment quality assessment of Beasley Lake, Sunflower County, MS, USA: latitude 33°24′15″ N, longitude 90° 40′05″ W.

is ∼915 ha and the lake has a surface area of ∼25–30 ha. An unusual feature of the watershed is a 5.5-m change in elevation from the highest point in the watershed to the lake, whereas most Delta oxbow lake watersheds have a <3 m change [16]. Approximately 150 ha of the watershed is non-arable wetland with hardwood forest and herbacious riparian vegetation. The 722 ha of arable land in the watershed has been primarily farmed in cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybeans (*Glycine max* [L.] Merr.) and occasionally milo (*Sorghum bicolor* L.). Cropping patterns within the watershed have varied over the 14-year observation period (Figure 2). From 1995 to 2001, an average 508 ha cotton, 117 ha soybeans and 62 ha corn were planted. Agricultural land use from 2002 to 2008 in the watershed included 70 ha cotton, 376 ha soybean and 33 ha corn. Beginning in 1995, only structural BMPs (e.g. grassed buffers, slotted pipes) were in-place in the watershed. These BMPs were followed with the implementation of reduced tillage in specific regions of the watershed in 2001, reduced tillage throughout the entire watershed in 2003, and most recently, CRP enrolment in 2003 consisting of 91 ha planted with hardwood cottonwood (*Populus deltoides* Bartr. ex. Marsh.) trees [15].

Within Beasley Lake, replicate (two) surface bulk sediment samples (top 5 cm) from each of three longitudinal sites, inflow (upstream), middle, and outflow (downstream) in the profundal zone (Figure 1) were collected in June 2004 and again in July 2008, coinciding with peak pesticide applications and ensuing runoff in spring and early summer. Sites 1 and 3 were ∼800 m equidistant from site 2 (Figure 1). Approximately 1 L (∼2 kg) whole-bulk wet sediment samples were obtained in replicate using an acetone-washed 196-B15 model Ekman Grab sampler (15 × 15 × 15 cm) and transferred to 1 L acetone/hexane triple-washed amber-coloured glass jars fitted with a Teflon™-lined screw cap. Sample jars were preserved on wet ice and transported to the USDA-ARS National Sedimentation Laboratory (NSL), Oxford, MS, USA for analysis.

2.2. *Sediment characterisation and pesticide analysis*

Upon arrival at the NSL, replicate bulk sediment samples from each site were thoroughly homogenised and an aliquot (∼200 g w/w) was sub-sampled and air-dried for 48 h for sediment characterisation and pesticide analysis. Sediment sand, silt and clay fraction distribution

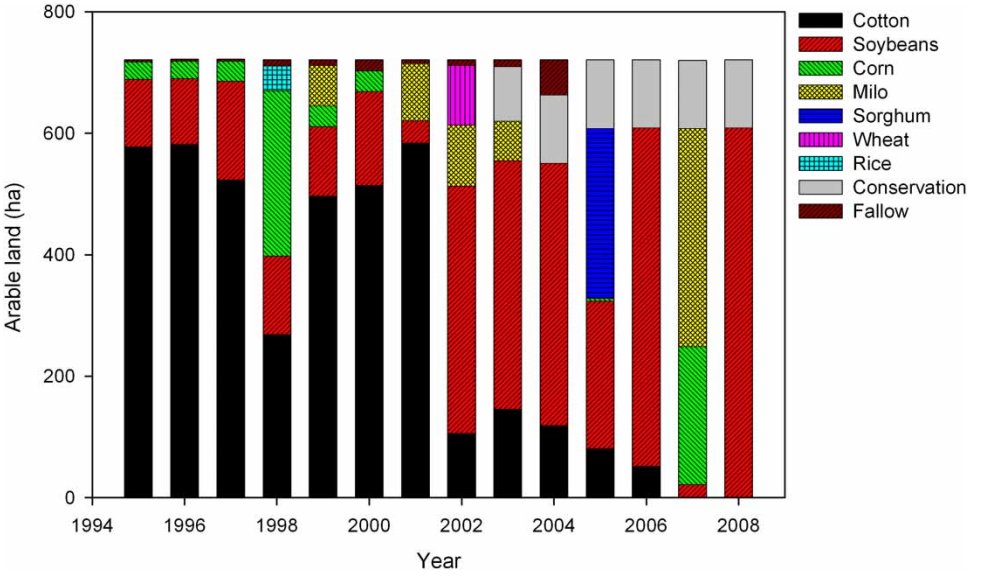

Figure 2. Land-use and crops planted from 1995 to 2008 in Beasley Lake watershed.

was determined using a Horiba model LA–90 laser-scattering particle size distribution analyser according to methods reported by Schaff et al. [17]. Analysis of sediment total organic carbon (TOC) was conducted via dry combustion using a LECO CN2000 carbon/nitrogen analyser at 1300–1350 °C as described by Shields et al. [18].

A total of 14 pesticides and 3 metabolites were targeted based upon past and present pesticide usage in the Delta [10]. Pesticide analysis was conducted with an Agilent Model 7890A gas chromatograph (GC) equipped with dual Agilent 7683B series autoinjectors, dual split-splitless inlets, dual capillary columns, an Agilent ChemStation, with autoinjection set at 1.0 μL injection volume. The Agilent 7890A GC was equipped with two micro-electron capture detectors (μECDs) [16,19] to determine concentrations of 12 current-use pesticides, 2 legacy pesticides, p,p'-DDT and dieldrin, and 3 metabolites, fipronil sulfone, p,p'-DDD and p,p'-DDE (Table 1). Dry sediment samples (15 g) were ground, placed in 50 mL centrifuge tubes and 20 mL of ethyl acetate was added. The mixture was sonicated for 1 min and centrifuged (2000–2500 *g*) for 5 min during the extraction process. The solvent layer was transferred into a second 50 mL centrifuge tube, an additional 10 mL of ethyl acetate was added, and the extraction process was repeated. The extract was concentrated to near dryness using a nitrogen evaporator and solvent exchanged into 10 mL hexane/acetone 90:10 (azeotrope). Extracted samples were subjected to silica gel clean-up prior to analysis. Extraction efficiencies of all fortified samples analysed using quality assurance/quality control protocols were ≥90%. Level of quantification and detection for sediment analysis was $0.01 \mu g \cdot kg^{-1}$.

Similar to sediment pesticide analysis, *Hyalella azteca* tissue pesticide residue analysis was performed at the conclusion of the 28-day bioassay period. Surviving animals were counted, weighed (w/w) and placed in 5-mL pesticide-grade ethyl acetate for extraction and pesticide analysis using a method similar to that reported by Smith et al. [20] with modifications. Animals from each of four replicate exposures per site were pooled to increase biomass for extraction and pesticide analysis. The mixture was sonicated for 1 min and centrifuged at 2000–2500 *g*, and the extract was concentrated to 1-mL volume using a high purity nitrogen evaporator. The 1-mL extract was subjected to silica gel column clean-up and re-concentrated to 1 mL under dry nitrogen for GC analysis. Extracts were analysed by GC as previously described for sediments.

Table 1. Characteristics (%) and pesticide concentrations ($\mu g \cdot kg^{-1}$) of Beasley Lake sediments during 2004 and 2008.

Characteristic or pesticide	Pesticide class	2004				2008			
		Control	Site 1	Site 2	Site 3	Control	Site 1	Site 2	Site 3
Sand		98.3	12.0	0.8	4.1	100	35.0	8.3	18.6
Silt		1.7	83.9	91.2	92.0	0.0	61.1	87.7	77.0
Clay		0.0	4.1	8.0	3.9	0.0	3.9	4.0	4.4
TOC		0.2	2.3	2.3	1.8	0.3	2.2	2.0	1.7
Trifluralin	Herbicide	B	B	B	B	B	0.50	2.93	B
Pendimethalin	Herbicide	B	B	B	B	B	0.62	0.89	1.19
Atrazine	Herbicide	B	B	5.22	52.91	B	96.02	227.63	43.47
Cyanazine	Herbicide	B	B	B	1.02	B	2.81	23.69	3.50
Alachlor	Herbicide	B	0.14	0.04	0.18	B	2.46	2.47	1.97
Metolachlor	Herbicide	B	1.80	2.37	10.17	B	3.20	11.87	1.83
Methyl Parathion	Insecticide	B	6.41	6.37	13.10	B	9.58	9.86	9.31
Chlorpyrifos	Insecticide	B	4.09	B	6.24	B	1.28	7.19	0.38
Chlorfenapyr	Insecticide	B	0.91	0.66	1.08	B	1.05	0.58	0.86
Bifenthrin	Insecticide	B	B	B	B	B	0.35	0.13	0.28
λ-Cyhalothrin	Insecticide	B	B	1.55	0.71	B	B	B	0.78
Fipronil	Insecticide	B	B	B	0.39	B	0.79	0.66	0.64
Fipronil Sulfone	Metabolite	B	0.64	0.69	0.91	B	0.77	0.49	1.53
Dieldrin	Legacy	B	0.10	0.16	0.28	0.26	0.40	0.46	0.45
p,p′-DDT	Legacy	2.57	6.80	2.94	9.83	2.77	13.47	5.86	14.96
p,p′-DDD	Legacy	B	1.75	1.17	3.73	B	1.22	1.61	1.93
p,p′-DDE	Legacy	0.12	2.30	0.65	7.35	B	3.03	4.22	3.59

Note: B, below detection limit of $0.01\,\mu g \cdot kg^{-1}$.

Extraction efficiencies of all fortified samples analysed using quality assurance/quality control protocols were ≥90%. Level of quantification and detection for body residue analysis was $0.01\,mg \cdot kg^{-1}$.

2.3. *Sediment bioassays*

Static non-renewal bulk whole-sediment bioassays lasting 28 days and using 4–5-day-old *H. azteca* were conducted according to USEPA [21] protocols, with the following modifications. Sediment exposures were initiated within 24 h of sediment sample collection. In brief, four replicate exposures consisted of 40 g wet weight Beasley Lake sediment sample or control sediment from the University of Mississippi Field Station (UMFS) with 160 mL overlying hardness adjusted water (free from priority pollutants) from the UMFS [22] placed in exposure chambers (250 mL borosilicate glass beakers). Overlying water hardness was adjusted by the addition of 100 mg $CaCl_2$ and 100 mg $NaHCO_3$ per L of UMFS water used. Six *H. azteca*, obtained from laboratory cultures maintained at the NSL according to standard procedures [21], were placed in each exposure chamber along with two, 6-mm diameter maple leaf discs as substrate and food. Aeration occurred for 30 min every other day to maintain oxygen levels and UMFS water was added to replace evaporated water. Additional feeding of 0.1, 0.5, 0.5, 0.5 mL of a 1:1 suspension rabbit chow/Tetramin® flake food at 2, 2, 4 and $10\,g \cdot L^{-1}$ occurred every 2 days during week 1, 2, 3 and 4, respectively. Toxicity tests were conducted in a Powers Scientific, Inc. Animal Growth Chamber with a 16:8 h (light/dark) photoperiod under wide-spectrum fluorescent lights having an illuminance of ∼500–600 lux and a temperature of 23 ± 1 °C. Standard physical and chemical water characteristics for sediment bioassays (temperature, pH, dissolved oxygen, conductivity, hardness, alkalinity, ammonium-N, nitrate-N, and nitrite-N) were measured according to Eaton et al. [23]. Bioassay endpoints measured were survival (%) and growth (mg w/w). Bioassay acceptability was assessed as average *H. azteca* survival of ≥80% at the end of the bioassay in control sediment.

2.4. *Data analysis*

Data were analysed using SigmaStat® v.2.03 statistical software [24]. For each year, *H. azteca* survival data were analysed using a Kruskal–Wallis analysis of variance (ANOVA) on ranks. Similarly, animal growth (mg w/w) data were analysed using one-way ANOVA. If significant differences ($p < 0.05$) were observed among median and/or mean endpoint variables a Dunnet's multiple-range test vs. controls was conducted. In addition, a two-way ANOVA was conducted to assess the effects of Beasley Lake sediment quality by site location (1, 2 and 3) and year (2004 and 2008) on *H. azteca* growth (mg w/w). Associations between *H. azteca* pesticide tissue residues and growth (mg w/w) were analysed using Pearson's product moment correlation with Bonferroni adjusted $\alpha = 0.0033$ [25].

3. Results

3.1. *Sediment characteristics and pesticide loads*

Sediment in Beasley Lake is characterised as a silty loam (80–90% silt, 4–5% clay) with ~2% TOC. Control sediment was predominantly sandy loam (80–95% sand, <1% clay) with <1% TOC. Pesticide analysis revealed variations in sediment pesticide concentrations among sites and between 2004 and 2008 (Table 1). All lake sediments examined had detectable concentrations of at least 7 of the 14 pesticides and all 3 metabolites examined. Sediment pesticide concentrations were often observed to be lower in 2004 than in 2008. Herbicides trifluralin and pendimethalin were not detected at any site in 2004, whereas trifluralin concentrations ranged from undetected to 2.93 $\mu g \cdot kg^{-1}$ and pendimethalin ranged from 0.62 to 1.19 $\mu g \cdot kg^{-1}$ in 2008. Sediment concentrations of atrazine (96.02–227.63 $\mu g \cdot kg^{-1}$), cyanazine (2.81–23.69 $\mu g \cdot kg^{-1}$), alachlor (2.46–2.47 $\mu g \cdot kg^{-1}$) and metolachlor (3.20–11.87 $\mu g \cdot kg^{-1}$) were all much greater at sites 1 and 2 in 2008 than in 2004 (Table 1). Insecticides methyl parathion, bifenthrin and fipronil had similar sediment concentration patterns. Concentrations of methyl parathion at sites 1 and 2 during 2008 (9.58–9.86 $\mu g \cdot kg^{-1}$) were greater than during 2004 (Table 1). Bifenthrin was undetected at any site in 2004 but ranged from 0.13 to 0.35 $\mu g \cdot kg^{-1}$ in 2008. Fipronil ranged from undetected to 0.39 $\mu g \cdot kg^{-1}$ in 2004, but ranged from 0.64 to 0.79 $\mu g \cdot kg^{-1}$ in 2008. Sediment concentrations of legacy pesticides dieldrin and p,p'-DDT were lower in 2004 (0.10–0.28 and 2.94–9.83 $\mu g \cdot kg^{-1}$, respectively) than in 2008 (0.40–0.46 and 5.86–14.96 $\mu g \cdot kg^{-1}$, respectively) at all three sites (Table 1). Sediments collected in 2004 had pesticides and metabolites detected most frequently and in the greatest concentrations at site 3 located adjacent to two major drainage ditch structures (Figure 1), especially atrazine (52.91 $\mu g \cdot kg^{-1}$), methyl parathion (13.10 $\mu g \cdot kg^{-1}$) and p, p'-DDT (9.83 $\mu g \cdot kg^{-1}$). By contrast, site 1 had the fewest pesticides detected and did not show peak concentrations for any pesticide or metabolite detected for that year. In 2008, pesticide and metabolite detections were more equally distributed among all three sites with no clear pattern of peak concentrations observed.

3.2. *Sediment bioassay responses*

Bioassay overlying water quality parameters were within acceptable limits for 28-day sediment bioassays according to USEPA protocol [16]. Mean ± SD water quality data were as follows: temperature, 20.0–20.2 °C; pH 7.9–8; dissolved oxygen, 7.56–7.70 $mg \cdot L^{-1}$; conductivity, 227–300 $\mu S \cdot cm^{-1}$; alkalinity, 34.2–74.1 $mg \cdot L^{-1}$ as $CaCO_3$; hardness, 62.7–79.8 $mg \cdot L^{-1}$ as $CaCO_3$; ammonia-N, 1.8–4.2 $mg \cdot L^{-1}$; nitrate-N, 0.0–0.0 $mg \cdot L^{-1}$; nitrite-N, 0.0–0.0 $mg \cdot L^{-1}$. *Hyalella azteca* 28-day bioassay mean control survival ranged from 96 to 100% for both years.

Table 2. Mean ± SD ($n = 4$) 28-day *Hyalella azteca* survival and growth exposed to Beasley Lake surface sediments during summer 2004 and 2008.

Year	Site	Survival (%)	Growth (mg w/w)
2004	Control	100.0 ± 0.0	2.1 ± 0.1
	1	100.0 ± 0.0	1.2 ± 0.1*
	2	87.5 ± 16.0	0.6 ± 0.3*
	3	87.5 ± 25.0	0.6 ± 0.3*
2008	Control	96.9 ± 6.3	1.7 ± 0.3
	1	93.8 ± 12.5	2.3 ± 0.4*
	2	87.5 ± 17.7	1.6 ± 0.2
	3	87.5 ± 17.7	1.5 ± 0.2

Note: *Significantly different from controls $p < 0.05$.

Survival of *H. azteca* exposed to lake sediments for 28 days ranged from 87 to 100% in 2004 and from 87 to 93% in 2008. As a result, there were no statistically significant differences in animal survival among lake sites or among years (Table 2).

In contrast with survival, differences in growth patterns were observed. Twenty-eight-day *H. azteca* growth, relative to controls, was significantly ($p < 0.05$) impaired in 2004 with a 43, 71 and 71% decrease in growth at sites 1, 2 and 3, respectively (Table 2). However, in 2008, 28-day animal growth, relative to controls, was not significantly different at sites 2 and 3, but significant ($p < 0.05$) growth enhancement of 35% was observed at site 1 (Table 2). Comparisons of growth patterns among sites and years was made using relative growth ratios accounting for variations in control animal growth for both years. Relative growth ratios were significantly different ($p < 0.05$) among sites within both 2004 and 2008 (Figure 3). In 2004, sites 1 and 2 had greater relative growth ratios than site 3. In 2008, site 1 had greater relative growth ratios than both sites 2 and 3. Between years, the relative growth ratio for 2008 was greater at each site than

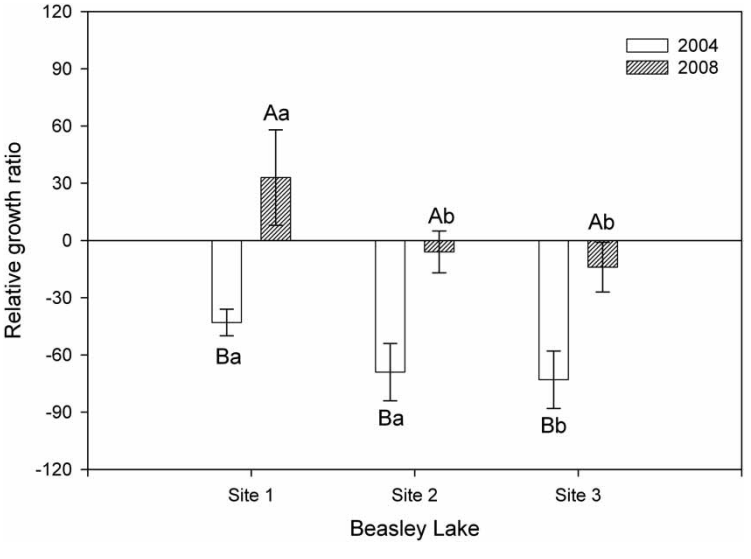

Figure 3. Twenty-eight-day relative growth ratios (% relative to controls) for *Hyalella azteca* exposed to Beasley Lake sediments from 2004 and 2008. Values with different upper case letters denote statistically significant differences ($p < 0.05$) among years within site. Values with different lower case letters denote statistically significant differences ($p < 0.05$) among locations within year.

2004 (Figure 3). No interaction effects were observed between years or sites (df = 2, $F = 0.716$, $p = 0.502$) suggesting observed growth patterns were year and site specific.

3.3. *Animal pesticide tissue residues and endpoint associations*

H. azteca 28-day animal tissue pesticide residue analysis revealed no measurable quantities of pesticides in animals exposed to control sediments (Table 3). *Hyaella azteca* exposed to lake sediments during 2004 showed animal tissue pesticide residues containing 9–11 pesticides and 1–2 metabolites. Animal tissue herbicide residue concentrations in 2004 were greatest for alachlor (3.05–5.29 $mg \cdot kg^{-1}$) and metolachlor (3.54–7.10 $mg \cdot kg^{-1}$) at all three sites. Overall, methyl parathion (1.76–6.90 $mg \cdot kg^{-1}$) and chlorpyrifos (2.92–8.82 $mg \cdot kg^{-1}$) had greater animal tissue insecticide residue concentrations in 2004 relative to other insecticides examined. Animal tissue legacy pesticide residue concentrations in 2004 were greatest for p,p'-DDT (3.48–6.54 $mg \cdot kg^{-1}$) at all sites (Table 3). As with sediments collected in 2004, animal tissue pesticide residue concentrations were greatest at site 3 located adjacent to two major drainage ditch structures (Figure 1), especially metolachlor (7.10 $mg \cdot kg^{-1}$), chlorpyrifos (8.82 $mg \cdot kg^{-1}$) and p,p'-DDT (6.54 $mg \cdot kg^{-1}$). *H. azteca* exposed to lake sediments during 2008 showed animal tissue pesticide residues containing 2–3 pesticides and all 3 metabolites. No animal tissue herbicide residues were detected in 2008. The insecticide fipronil (0.32 mg kg^{-1} at site 2), the legacy insecticide p,p'-DDT (0.55–0.67 $mg \cdot kg^{-1}$) and the metabolite p,p'-DDD (0.30–0.44 $mg \cdot kg^{-1}$) had the greatest animal tissue pesticide residue concentrations during 2008 (Table 3). In general, *H. azteca* tissue residues of herbicides and insecticides were detected more frequently and in greater concentrations in 2004 than 2008. Animal tissue residues of metabolites were detected more frequently in 2008 than in 2004 and legacy insecticides occurred with equal frequency but in greater concentrations in 2004 than in 2008. Biologically relevant associations were observed between 28-day *H. azteca* growth and animal tissue pesticide and metabolite residues. Pearson product moment correlation analysis after Bonferroni adjustment ($\alpha = 0.0033$) showed animal tissue residues of two herbicides,

Table 3. Twenty-eight-day *Hyalella azteca* tissue pesticide residue concentrations ($mg \cdot kg^{-1}$ w/w) exposed to Beasley Lake sediments during 2004 and 2008.

		2004				2008			
Pesticide	Class	Control	Site 1	Site 2	Site 3	Control	Site 1	Site 2	Site 3
Trifluralin	Herbicide	B	B	B	B	B	B	B	B
Pendimethalin	Herbicide	B	B	B	B	B	B	B	B
Atrazine	Herbicide	B	2.86	1.10	0.98	B	B	B	B
Cyanazine	Herbicide	B	0.20	0.43	0.46	B	B	B	B
Alachlor	Herbicide	B	3.05	3.36	5.29	B	B	B	B
Metolachlor	Herbicide	B	3.54	5.91	7.10	B	B	B	B
Methyl Parathion	Insecticide	B	1.76	5.82	6.90	B	B	B	B
Chlorpyrifos	Insecticide	B	2.92	7.70	8.82	B	B	B	B
Chlorfenapyr	Insecticide	B	0.37	0.43	0.52	B	0.03	0.02	0.03
Bifenthrin	Insecticide	B	0.02	B	B	B	B	B	B
λ-Cyhalothrin	Insecticide	B	2.21	1.05	0.95	B	B	B	B
Fipronil	Insecticide	B	B	B	B	B	B	0.32	B
Fipronil Sulfone	Metabolite	B	0.36	0.42	0.52	B	0.05	0.05	0.04
Dieldrin	Legacy	B	0.16	B	B	B	B	B	B
p,p'-DDT	Legacy	B	3.48	5.71	6.54	B	0.67	0.55	0.60
p,p'-DDD	Legacy	B	0.16	B	B	B	0.44	0.30	0.43
p,p'-DDE	Legacy	B	B	B	B	B	0.02	0.02	0.04

Note: B, below detection limit of 0.01 $mg \cdot kg^{-1}$

Table 4. Pearson product moment correlation coefficients (r) between 28-day growth (mg w/w) and 28-day animal tissue pesticide residues of *Hyalella azteca* exposed to Beasley Lake sediments during 2004 and 2008.

Pesticide	Class	N	r	p	Significant
Atrazine	Herbicide	8	−0.8840	0.0036	No
Cyanazine	Herbicide	8	−0.9044	0.0020	Yes
Alachlor	Herbicide	8	−0.8781	0.0041	No
Metolachlor	Herbicide	8	−0.9032	0.0021	Yes
Methyl Parathion	Insecticide	8	−0.8877	0.0032	Yes
Chlorpyrifos	Insecticide	8	−0.8971	0.0025	Yes
Chlorfenapyr	Insecticide	8	−0.8892	0.0031	Yes
Bifenthrin	Insecticide	8	−0.1636	0.6987	No
λ-Cyhalothrin	Insecticide	8	−0.6279	0.0956	No
Fipronil	Insecticide	8	0.0995	0.8147	No
Fipronil Sulfone	Metabolite	8	−0.8903	0.0030	Yes
Dieldrin	Legacy	8	−0.1639	0.6981	No
p,p'-DDT	Legacy	8	−0.9051	0.0020	Yes
p,p'-DDD	Legacy	8	0.0102	0.9809	No
p,p'-DDE	Legacy	8	0.2770	0.5066	No

cyanazine and metolachlor, three insecticides methyl parathion, chlorpyrifos, chlorfenapyr, the metabolite, fipronil sulfone, and a legacy insecticide, p,p'-DDT were significantly related to *H. azteca* growth (Table 4).

4. Discussion

4.1. *Beasley Lake sediment pesticide loads*

Sediment quality assessment begins with the examination of sediment chemistry and contamination [1]. However, Stewart and Conwell [26] note the importance of examining long-term temporal trends (years) in assessing sediment contamination and toxicity to determine changes in sediment remediation. Currently there is a paucity of information regarding long-term temporal studies of pesticide-contaminated sediments in freshwater systems, with most focusing on the distribution of persistent organochlorine pesticides such as p,p'-DDT and dieldrin [27,28]. By contrast, the current study provides information about long-term changes in sediment contamination from multiple current-use and legacy pesticides, thus providing additional insight into a neglected aspect of sediment quality assessments [26]. As a result, comparisons of long-term temporal changes in sediment pesticide contamination in relation to changes in land-use practices and agricultural BMPs must be obtained from previous studies of agricultural watersheds, similar to the current study watershed Beasley Lake. Several studies have examined sediment contamination of a broad suite of pesticides in sediments within a variety of Mississippi Delta watersheds with and without BMPs [9,16,29,30,31]. Moore et al. [16] examined the same group of pesticides within Deep Hollow Lake watershed having BMPs and during a cotton/soybean cropping system in 2000. Atrazine (1.3–27.4 $\mu g \cdot kg^{-1}$) and metolachlor (8.7–10.6 $\mu g \cdot kg^{-1}$) contamination in Deep Hollow Lake sediments under these conditions was very similar to Beasley Lake with a similar cropping system in 2004 [16] (Table 1). Likewise, Thighman Lake watershed having reduced tillage BMPs during a corn cropping system in 2000, had similar concentrations of current-use herbicides metolachlor (2.5–8.5 $\mu g \cdot kg^{-1}$), alachlor (1.6–3.0 $\mu g \cdot kg^{-1}$) and pendimethalin (2.1–2.5 $\mu g \cdot kg^{-1}$) as Beasley Lake with a corn/soybean cropping system in 2007–2008 (Table 1). Knight et al. [31] in 2004 also studied the same suite of pesticides within the sediments of two agricultural Delta lake watersheds with no BMPs and primarily cotton cropping systems, Roebuck Lake and Bee Lake. These two watersheds had greater concentrations of atrazine (67.7–465.6 $\mu g \cdot kg^{-1}$), metolachlor

(6.2–51.2 $\mu g \cdot kg^{-1}$), chlorpyrifos (11.4-15.6 $\mu g \cdot kg^{-1}$) and bifenthrin (0.8-1.4 $\mu g \cdot kg^{-1}$) than Beasley Lake in either 2004 or 2008 (Table 1).

Specifically within Beasley Lake, Moore et al. [16] examined the same group of pesticides and metabolites at the same locations as the present study, and reported widespread pesticide contamination in Beasley Lake sediment during 2000. Up to 2000, relatively few BMPs were in place such as vegetated buffers (initiated in 1994) and slotted board risers, and slotted inlet pipes at lower elevations for selected field drainage (initiated in 1994) [15]. Agricultural land-use between 1999 and 2000 was predominantly cotton with some soybeans (Figure 2). As a result, Moore et al. [16] observed peak herbicide, insecticide, legacy insecticide and metabolite concentrations of 6.3–22.5, 26–36.5, 9.4–17 and 21.9–78 $\mu g \cdot kg^{-1}$, respectively. In comparison, the current study in 2004 measured lower pesticide loads and more localised (site 3) contamination (Table 1). This can be attributed to additional BMPs such as reduced tillage practices (initiated in 2001) and enrolment in CRP (initiated in 2003) [15] in addition to changes in agricultural land-use from 2003 to 2004 when soybeans became the dominant crop over cotton (Figure 2). By 2008, although the BMPs discussed previously were still in place, additional land-use changes in 2007 with the planting of primarily corn and milo (Figure 2), affected patterns of pesticide contamination in watershed sediments. According to the US National Agricultural Statistics Service (NASS), US corn production, among all crops planted within Beasley watershed, utilises the greatest number and volume of current-use pesticides [32] (excluding dieldrin, p,p'-DDT and metabolites) assessed in the current study (9 of 14). In comparison, soybeans are intermediate (6 of 12) and milo the lowest (4 of 12) [32] pesticide-intensive crops in the Beasley Lake watershed. This includes herbicides such as alachlor and atrazine, and insecticides such as bifenthrin and fipronil. This coincides with the measured increase in pesticide sediment levels in the current study from 2004 to 2008. Results of this study suggest that land-use patterns such as crop type and rotation could significantly influence year-to-year variation in lake sediment contamination of current-use pesticides regardless of the effectiveness of BMPs.

4.2. *Assessment of Beasley Lake sediment toxicity*

Sediment quality assessment also requires additional tools such as the characterisation of biological responses concomitant with contamination [1]. In the current study, survival and growth of the epibenthic detritivore, *H. azteca*, were the biological responses measured to assess surface sediment toxicity. Although an increasing number of studies have attempted to assess surface sediment toxicity in the Mississippi Delta for such a wide range of agricultural pesticides [9,16,30,33], few have attempted such an assessment within any agricultural region of the USA over a period of several years [34] or in conjunction with changes in agricultural land-use practices. Spatial sediment toxicity assessments in agricultural regions using acute (10-day) and chronic (28-day) *H. azteca* bioassays are the most common, and numerous studies have been conducted assessing the geographic range of pesticide contamination and pollution [9,16,30,31,33–35]. In comparison, the current study observed limited significant chronic (28-day) sediment toxicity to *H. azteca* both spatially and temporally within the Mississippi Delta oxbow lake (Beasley Lake) examined. Results of this study indicate that *H. azteca* survival was not affected by observed pesticide concentrations in sediment in either 2004 (87.5–100%) or 2008 (87.5–93.8 %) and our results are similar to numerous studies within agricultural watersheds within the Mississippi River drainage basin where survival was typically >80% [9,16,30,31,33,36,37]. However, 28-day animal growth was significantly affected by pesticides in sediment with average growth ranging from 0.6 to 1.2 mg w/w in 2004 and 1.5 to 2.3 mg w/w in 2008. Our growth measurements in 2004 were comparable with agricultural watersheds without BMPs (e.g. Roebuck Lake and Bee Lake) [31,33] showing

significantly impaired growth, whereas growth in 2008 was more similar to agricultural watersheds utilising BMPs (e.g. Deep Hollow Lake and Thighman Lake) where growth had moderate to no impairment [31].

Associations of surface sediment pesticide contamination with *H. azteca* responses have been attempted by researchers in the Mississippi Delta [31,33,36] with limited results. More often, numerical sediment quality guidelines (SQGs) have been employed in monitoring programmes involving both water quality and sediment quality assessments using threshold effects concentrations (TECs) and probable effects concentrations (PECs) in North America for a variety of organic and inorganic contaminants [38]. SQGs are limited, however, as they are currently lacking for the many current-use pesticides that are commonly used in US agriculture. In addition, unlike water quality assessments where there is a large database of pesticide effects concentrations available, relatively few pesticide effects concentrations exist for sediment matrixes. Limited sediment pesticide effects concentrations exist for only 6 of the 12 current-use pesticides assessed in this study: atrazine and metolachlor [39], chlorfenapyr [40], bifenthrin and λ-cyhalothrin [41] and fipronil [42]. As a result, data gaps in the SQGs of current-use pesticides limit its usefulness for this study.

Another potentially confounding factor is the significance of mixture toxicity. Because all Beasley Lake sediments showed significant, but varying, degrees of mixed current-use pesticide contamination, there was a concern of mixture toxicity effects. While the assumption with mixtures in SQGs is additive toxicity, several studies have observed synergistic effects on aquatic invertebrates with certain classes of current-use pesticides. Anderson and Lydy [43] and Trimble and Lydy [44] noted synergistic toxicity of the triazine herbicides atrazine and cyanazine mixed with organophosphate insecticides chlorpyrifos and methyl parathion in *H. azteca*. Other possible synergistic mixtures have been suggested by Bouldin et al. [45] with atrazine and the pyrethroid λ-cyhalothrin in the midge (*Chironomus tentans*). Because all of these current-use pesticides occurred in various Beasley Lake sediments, in potentially synergistic mixtures, SQGs may not be a suitable tool for assessing sediment quality for this study. That both mixtures were observed in body residues from 2004 exposures and the same mixtures were not measured in animal body residues following sediment exposures in 2008 may explain the significant growth impairment in 2004 and lack of impairment in 2008.

4.3. *Animal tissue pesticide residues and growth*

Another important aspect of sediment quality assessment is the bioaccumulation of sediment-associated contaminants and their effects on benthic and epibenthic organisms [1]. Relatively few studies have attempted to assess animal tissue pesticide residues of *H. azteca* [46–48]. Such studies exclusively examined animal body residues of orgaonchlorine insecticides and metabolites such as p,p'-DDT, p,p'-DDD and p,p'-DDE. Studies by Smith et al. [20] and Lizotte et al. [30] examined a greater range of current-use and legacy animal tissue pesticide residues in *H. azteca* exposed to Mississippi Delta sediments. However, both these studies focused exclusively on spatial variation in sediment exposures among agricultural watersheds and not temporal ones within the same watershed(s). The current study had animal tissue current-use pesticide residues of atrazine (0.98–2.86 $mg \cdot kg^{-1}$ w/w) and λ-cyhalothrin (0.95–2.21 $mg \cdot kg^{-1}$ w/w) during 2004 that were comparable with animal tissue pesticide residues found in animals exposed to sediments from non-BMP agricultural watersheds during the growing season (summer) [20]. By contrast, the limited observation of animal tissue current-use pesticides observed in 2008, such as chlorfenapyr (0.02–0.03 $mg \cdot kg^{-1}$ w/w) and fipronil (0.32 $mg \cdot kg^{-1}$ w/w), were more similar to animal tissue current-use pesticide residues seen in animals exposed to sediments from agricultural watersheds during a non-growing season (autumn) [30]. Because crustaceans such as *H. azteca* are sometimes prey items of higher trophic organisms such as catfish [49],

H. azteca tissue pesticide residues would be incorporated into tissues of these predators. Cooper [50] observed the bioaccumulation of current-use insecticides such as the pyrethroid permethrin (0.7–3.81 $\mu g \cdot kg^{-1}$) and the organophosphate methyl parathion (1.81–17.8 $\mu g \cdot kg^{-1}$) in tissues of benthic feeding catfish (*Ictalurus* sp.) from Moon Lake, a Delta agricultural watershed, showing the potential for trophic transfer of these pesticides to predators.

The importance of assessing biologically relevant relationships between animal tissue pesticide residues and effects is an important aspect of sediment quality assessments [1]. Because of the limited nature of animal tissue pesticide residue effects data [1], this study provides additional information relevant in assessing the potential effects of bioaccumulation. Previous studies by Smith et al. [20] and Lizotte et al. [30] observed only persistent organochlorine contaminants in animal tissue pesticide residues related to impaired growth in *H. azteca* exposed to Mississippi Delta sediments. By contrast, this study noted significant associations between several currently used pesticides in animal tissue pesticide residues (two herbicides and three insecticides) and growth in *H. azteca* exposed to Beasley Lake sediments collected during 2004 and 2008, and suggests that these pesticides are bioavailable pollutants. Despite the comparatively rapid degradation and transformation of currently used pesticides vs. persistent organochlorine compounds observed in Beasley Lake, current-use pesticides have the potential to impact ecosystem components of intensively cultivated Mississippi Delta watersheds.

5. Conclusions

The results of this study were limited in their ability to show that implementation of agricultural BMPs has conclusively decreased sediment contamination and improved sediment quality in Beasley Lake. Although BMPs in Beasley Lake watershed have reduced aqueous sediment, nutrient and pesticide loads in aquatic systems over time [10], other land-use patterns such as crop type and rotation could significantly influence year-to-year variation in lake sediment contamination. Further examination of the role of BMPs in sediment quality is needed. More frequent, targeted sediment assessments are needed to better understand associations between land-use, BMPs and contamination in agricultural watersheds. In addition, more detailed examination of factors affecting uptake of pesticides from sediments into animal tissues such as sediment and detrital organic carbon matrix and food webs need to be addressed.

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