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Effects of an Atrazine, Metolachlor and Fipronil Mixture on *Hyalella azteca* (Saussure) in a Modified Backwater Wetland

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Abstract We examined the toxicity mitigation efficiency of a hydrologically modified backwater wetland amended with a pesticide mixture of atrazine, metolachlor, and fipronil, using 96 h survival bioassays with *Hyalella azteca*. Significant *H. azteca* 96 h mortality occurred within the first 2 h of amendment at the upstream amendment site but not at any time at the downstream site. *H. azteca* survival varied spatially and temporally in conjunction with measured pesticide mixture concentrations. *Hyalella azteca* 96 h survival pesticide mixture effects concentrations ranges were 10.214–11.997, 5.822–6.658, 0.650–0.817, and 0.030–0.048 μg L^{-1} for atrazine, metolachlor, fipronil, and fipronil-sulfone, respectively.

Keywords Floodplain wetland · Pesticide mixture

The lower Mississippi River alluvial plain (e.g. the Delta), a region of intensive agricultural use, contains numerous oxbow lakes and backwater wetlands along river channels. These backwater wetlands have important economic and ecological functions such as providing habitat, natural buffers, and filters for suspended sediment, nutrients and pesticides entering as runoff from adjacent agricultural fields. In this study, a natural backwater wetland along the Coldwater River in Tunica County, Mississippi (MS), was modified for hydrologic control by adding weirs at both upstream and downstream ends to more efficiently utilize natural filtering capabilities.

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In 2007, corn (*Zea mays*) subsidies for biofuel production increased and as a result an increase in corn production was predicted. With this expected increased corn acreage was an expected increase in corn-related pesticide use. Herbicides such as atrazine and metolachlor are commonly used in mixtures for corn crops for weed control (Young 2006). The phenylpyrazole insecticide, fipronil has been marketed for corn crops to replace organophosphate insecticides such as chlorpyrifos and methyl parathion because of fipronil's effectiveness at low field application rates against insect pests that have become resistant to organophosphates (Bobe et al. 1997). According to USDA NASS (2008), in 2003 ~24.3 million kg of atrazine, 2.9 million kg of metolachlor, and 63,000 kg of fipronil were used on corn crops in the US for pest management. As a result, the current study targeted these pesticides in a watershed within the Mississippi Delta region. The purpose of this study was to examine the efficiency of this modified backwater wetland in mitigating the toxicity of an amended pesticide mixture including, atrazine, metolachlor, and fipronil, using 96 h bioassays with *Hyalella azteca*.

Materials and Methods

The study site was a severed compound meander bend backwater in Tunica County, Mississippi about 2.5 km long and 40 m wide along the Coldwater River (Fig. 1). Land-use is row-crop cultivation, both inside and outside the bend, but there was a buffer of natural vegetation 5–100 m wide on both banks. The modified backwater study site had two water control weirs (34°40′04.93″N, 90°13′38.09″W, and 34°40′15.15″N, 90°13′35.36″W) creating a larger, deeper area managed as a lake-type aquatic habitat and a smaller, shallower area, 700 m long, 25 m

Fig. 1 Aerial photograph of the Coldwater River modified backwater wetland (*white lines* represent weirs) in Tunica County, Mississippi, USA

wide, that supports wetland and terrestrial plants managed as a wetland. A mean water depth of 28 cm was measured in the wetland cell when water was present. Both weirs were designed with adjustable crest drainage structures. Weirs were protected with riprap to allow for overflow in either direction. A pesticide mixture consisting of atrazine (6,600 mg a. i.) and metolachlor (5,220 mg a. i.) as Bicep II Magnum® (33.0% a. i. atrazine and 26.1% a. i. metolachlor), and fipronil (630.4 mg a. i.) as Regent 4SC® (39.4% a. i. fipronil) was injected into the backwater wetland at the upstream (lake) weir crest drainage structure for 1.3 h. Water was released from the upstream lake area of the backwater into the modified wetland area over a 4 h period during a 730 m^3 simulated runoff event, representing a 1.27 cm rainfall. This amount of runoff was equivalent to a 0.1% pesticide loss in runoff from a 16 ha agricultural field in a 1.27 cm rainfall event. Water samples were collected at upstream and downstream sites ~5 m from respective weirs 48 h prior and 1, 2, 4, 6, and 24 h post-amendment. Samples were placed on ice, transported to the USDA-ARS National Sedimentation Laboratory, Oxford, MS (NSL) for bioassay and pesticide analyses.

A total of 12 1 L aqueous samples were analyzed for atrazine, metolachlor, fipronil, and fipronil-sulfone (common metabolite). Pesticide analysis was conducted using a modified gas chromatography (GC) method similar to one described by Smith et al. (2006). In brief, pesticides were extracted by phase partitioning into 100 mL pesticide-grade ethyl acetate after sediments were deflocculated with reagent-grade KCl and sonication, dried over anhydrous Na_2SO_4 and concentrated to near dryness by rotary evaporation. Next, the extract was subjected to silica gel column chromatography cleanup, and concentration to 1 mL volume under high purity dry nitrogen for GC analysis. Two Agilent HP model 6890 gas chromatographs were used for all targeted pesticide analyses. Both gas chromatographs were equipped with dual Agilent HP 7683 ALS autoinjectors, dual split-splitless inlets, dual capillary columns, and an Agilent HP Kayak XA Chemstation. Autoinjectors were set at 1.0 µL injection volume fast mode. Pesticide recoveries and extraction efficiencies, based on fortified samples, were ≥90% for targeted pesticides (Smith et al. 2006).

Because no known published data are currently available for aqueous effects concentrations of *Hyalella azteca* exposed to fipronil, we conducted an acute (96 h) laboratory bioassay pilot study to elucidate fipronil effects on this organism. A series of four individual toxicity tests, each with a range of 5–6 fipronil concentrations and a non-treated control, were completed (Table 1). Twenty-seven

Table 1 Pilot 96 h aqueous fipronil bioassay nominal and measured concentrations (ug L^{-1}), LC50 values (ug L^{-1}), and confidence intervals (CI) using *Hyalella azteca* (BD = below detection limit 0.025 ug L^{-1})

Nominal concentration	Measured concentration						
	Test 1	Test 2	Test 3	Test 4	Mean	SD	R^a
Control	BD	BD	BD	BD	NA[b]	NA	NA
0.125	0.15	0.05	0.10	0.11	0.10	0.04	82.5
0.25	0.22	0.12	0.16	0.17	0.17	0.04	66.5
0.5	0.50	0.22	0.27	0.35	0.34	0.12	67.1
1	0.63	0.53	0.56	0.65	0.59	0.06	59.3
2	1.94	0.89	1.12	1.34	1.32	0.45	66.0
4	NA	1.99	2.39	2.62	2.33	0.32	58.3
Response							
LC50	0.76	0.52	0.39	0.48	0.54	0.16	NA
CI	0.61–0.94	0.43–0.63	0.30–0.50	0.39–0.59	NA	NA	NA

[a] *R* Fipronil recovery (%)

[b] NA not applicable

0.5 L aqueous samples were analyzed for fipronil, as previously described, and reported as measured exposure concentrations. Bioassays using wetland samples were 96 h static non-renewed aqueous exposures assessing *H. azteca* survival within five serial dilutions of four replicates each according to modified USEPA (2000) protocol for *H. azteca* reference toxicity tests. All bioassays were conducted in a Powers Scientific Inc. incubator at 23 ± 1°C with a photoperiod of 16:8 light:dark at the USDA-ARS National Sedimentation Laboratory (NSL), Oxford, Mississippi, USA. Animals passing a 600 μm stainless steel mesh sieve but retained by a 425 μm stainless steel mesh sieve (~1–2 weeks old) were collected for the bioassays. Six *H. azteca* were placed in each replicate 120 mL polypropylene plastic test chamber with one 2 cm × 2 cm square sterile cotton gauze as substrate. Aqueous exposures consisted of 100 mL hardness adjusted (~100 mg/L as $CaCO_3$) sample and/or control/dilution water with five serial dilutions at 0.5 dilution factor. Control and dilution water, free from priority pollutants, were from a naturally spring-fed pond located at the University of Mississippi Field Station (UMFS) and having the following ranges of measured water quality parameters: dissolved oxygen (mg L^{-1}), 4.5–12.6; pH, 5.9–7.3; alkalinity (mg L^{-1} as $CaCO_3$), 8–16; hardness (mg L^{-1} as $CaCO_3$), 10–30; conductivity (μmhos cm^{-1}), 20.3–25.7; turbidity (NTU), 8.3–25.1; dissolved solids (mg L^{-1}), 9–86; suspended solids (mg L^{-1}), 0–28; total phosphorus (μg L^{-1}), 0–81; NH_4–N (μg L^{-1}), 0–136; NO_3–N (μg L^{-1}), 42–170; NO_2–N (μg L^{-1}), 1–15; chlorophyll *a* (μg L^{-1}), 0–23. Because of the very soft nature of the control and dilution water, hardness and alkalinity were adjusted using $CaCl_2$ and $NaCO_3$ at 100 mg L^{-1}. Animals were fed 0.1 mL of a 1:1 suspension rabbit chow:Tetramin® flake food at 2 g/L at test initiation. After 96 h, survival was determined by the number of organisms not responding when gently prodded with forceps. Standard bioassay water quality parameters of temperature, pH, dissolved oxygen, alkalinity, hardness, and conductivity were measured according to APHA (1998). All *H. azteca* were cultured at the NSL culturing facility according to the procedures of de March (1981). For the fipronil pilot study exposures, *H. azteca* survival data were analyzed using the trimmed Spearman–Karber method (Hamilton et al. 1977) to obtain estimated LC50 values and corresponding confidence intervals. For wetland sample exposures, animal 96 h survival data were analyzed with Sigma Stat® statistical software (SPSS 1997) to determine no observed effects dilutions (NEDil) and lowest observed effects dilutions (LEDil) using analysis of variance (ANOVA) or Kruskal–Wallis (ANOVA on ranks) with Dunnett's multiple range test when appropriate. NEDil values were based on lack of significant differences ($p < 0.05$) relative to controls and LEDil values were lowest dilutions that provided significant differences ($p < 0.05$) relative to controls. Survival data were also analyzed using the trimmed Spearman–Karber method (Hamilton et al. 1977) to obtain dilution LDil50s (percent dilution resulting in 50% animal mortality) estimates and 95% confidence intervals according to Sundberg et al. (2006).

Results and Discussion

In the fipronil bioassay pilot study, average measured concentrations ranged from 58.3 to 82.5% of nominal target concentrations (Table 1). Pilot study water quality data were within parameters for acute aqueous bioassays. Parameters ranged as follows: temperature (°C), 22.7–23.6; dissolved oxygen (mg L^{-1}), 6.9–8.2; pH, 6.9–7.7; alkalinity (mg L^{-1} as $CaCO_3$), 34.2–51.3; hardness (mg L^{-1} as $CaCO_3$), 34.2–68.4; conductivity (μmhos cm^{-1}), 69.5–144.0. Animal survival in all controls was ≥95% for all 96 h pilot bioassays. Measured *H. azteca* LC50 values ranged from 0.39 to 0.76 μg L^{-1} and averaged 0.54 μg L^{-1} (Table 1). These LC50 concentrations are comparable to LC50 values of aquatic insect species such as midges (0.42 μg L^{-1}) and mosquitos (*Culex* sp., 0.35–0.87 μg L^{-1}) but are greater than tenfold below reported freshwater crustacean (i.e. *Ceriodaphnia dubia*, *Daphnia pulex*, *Procambarus clarkii*, *Procambarus zonangulus*) acute (48–96 h) LC50s for this compound (10.3–19.5 μg L^{-1}) (Gunasekara et al. 2007).

Greatest pesticide mixture concentrations were located within the upstream site of the backwater wetland within 24 h of amendment (Table 2). Only low concentrations of atrazine (0.352–0.056 μg L^{-1}) and the metabolite fipronil

Table 2 Aqueous pesticide concentrations (μg L^{-1}) in the modified backwater wetland (BD = below detection limit of 0.001 μg L^{-1})

Location	Time (h)	Atrazine	Metolachlor	Fipronil	Fipronil-Sulfone
Upstream	−48	BD	BD	BD	BD
	1	10.214	5.822	0.650	0.030
	2	11.997	6.658	0.817	0.048
	4	1.116	0.645	0.088	BD
	6	3.781	1.937	0.207	0.010
	24	4.409	3.248	0.316	0.013
Downstream	−48	BD	BD	BD	BD
	1	0.265	BD	BD	BD
	2	0.352	BD	BD	BD
	4	BD	BD	BD	BD
	6	BD	BD	BD	BD
	24	0.058	BD	BD	0.009

sulfone (0.009) were detected at the downstream site of the backwater wetland within 24 h of amendment (Table 2). The atrazine, metolachlor, and fipronil pesticide mixture was efficiently trapped within the 700 m long modified backwater wetland studied. After 24 h, only low concentrations of atrazine and the metabolite fipronil-sulfone were detected at the 700 m downstream site. Moore et al. (2000, 2001) has estimated travel distances of 100–400 m are necessary to effectively mitigate atrazine and metolachlor laden runoff within smaller constructed wetlands. Less is known about the necessary distance required to mitigate fipronil, however, during the current study, fipronil was not detected at the 700 m downstream site. As a result, <700 m × 25 m was required to mitigate fipronil runoff.

Water quality data for the toxicity tests with field site water were as follows: temperature (°C), 23.3–23.6; dissolved oxygen (mg L^{-1}), 6.9–7.5; pH, 7.8–7.9; alkalinity (mg L^{-1} as $CaCO_3$), 38.5–47.0; hardness (mg L^{-1} as $CaCO_3$), 89.8–119.7; conductivity (μmhos cm^{-1}), 258.3–305.0. Animal survival in all controls was ≥90% for all 96 h bioassays. Prior to pesticide amendment (−48 h), no significant *H. azteca* mortality was observed at either wetland site (Table 3). Within the first 2 h of pesticide amendment, 100% influent sample from the upstream site elicited 100% *H. azteca* mortality after 96 h exposures and 50% influent dilutions caused 50–75% mortality. As a result, 1 and 2 h dilution NEDils and LEDils were both 50 and 25%, respectively (Table 3). Observed 1 and 2 h dilution LDil50s were 59.46 and 49.27%, respectively. Upstream samples collected 4, 6, and 24 h post pesticide amendment elicited no significant *H. azteca* 96 h survival effects. No samples collected at the 700 m downstream site affected *H. azteca* 96 h survival at any time during the study (Table 3). Based upon patterns of observed *H. azteca* 96 h survival (Table 3), pesticide mixture effects concentrations ranges were 10.214–11.997, 5.822–6.658, 0.650–0.817, and 0.030–0.048 μg L^{-1} for atrazine, metolachlor, fipronil, and fipronil-sulfone, respectively (Table 1). Greatest observed measured pesticide mixture NOEC was 4.409, 3.248, 0.316, and 0.013 μg L^{-1} for atrazine, metolachlor, fipronil, and fipronil sulfone, respectively.

Table 3 *Hyalella azteca* 96 h aqueous survival effects dilutions (%) in the modified backwater wetland

Location	Time (h)	NEDil	LEDil	LDil50 (95% CI)
Upstream	−48	100	>100	>100
	1	25	50	59.46 (52.60–67.21)
	2	25	50	49.27 (42.45–57.19)
	4	100	>100	>100
	6	100	>100	>100
	24	100	>100	>100
Downstream	−48	100	>100	>100
	1	100	>100	>100
	2	100	>100	>100
	4	100	>100	>100
	6	100	>100	>100
	24	100	>100	>100

Mixture toxicity of atrazine with other insecticides, including organophosphates (OP) and fipronil in crustaceans has been examined (Trimble and Lydy 2006; Key et al. 2007). Although Trimble and Lydy (2006) observed greater than additive toxicity with atrazine and OP in *H. azteca*, Key et al. (2007) observed no increase in toxicity with atrazine and fipronil in *Palaemonetes pugio*. In our study, observed mixture toxicity to *H. azteca* appeared primarily due to fipronil and possibly fipronil-sulfone based upon previous results from the fipronil pilot study. Fipronil-sulfone was observed to have similar or greater toxicity to crustaceans as the parent compound. Reported fipronil-sulfone effects concentrations for freshwater crustaceans ranged from 4.5 μg L^{-1} (*Daphnia magna* 21 d EC50) to 11.2 μg L^{-1} (*Procambarus clarkii* 96 h LC50) (Schlenk et al. 2001; Gunasekara et al. 2007). In the current study, atrazine and metolachlor concentrations were well below reported effects concentrations for crustaceans (14,700 μg atrazine L^{-1} and 25,100 μg metolachlor L^{-1}) (Munn and Gilliom 2001). As a result, observed peak herbicide concentrations in our study were not considered great enough to significantly add to observed insecticide toxicity.

Mitigation efficiency of this modified backwater wetland amended with atrazine, metolachlor, and fipronil was shown and is relevant to observed peak concentrations of these contaminants in runoff in Mississippi rivers and streams during storm events (Smith et al. 2006). Our results suggest that riverine backwater wetlands can be modified to mitigate pesticide runoff and concomitant toxicity prior to entering rivers and streams in Mississippi.

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