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Responses of *Hyalella azteca* and phytoplankton to a simulated agricultural runoff event in a managed backwater wetland

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ABSTRACT

We assessed the aqueous toxicity mitigation capacity of a hydrologically managed floodplain wetland following a synthetic runoff event amended with a mixture of sediments, nutrients (nitrogen and phosphorus), and pesticides (atrazine, *S*-metolachlor, and permethrin) using 48-h *Hyalella azteca* survival and phytoplankton pigment, chlorophyll *a*. The runoff event simulated a 1 h, 1.27 cm rainfall event from a 16 ha agricultural field. Water (1 L) was collected every 30 min within the first 4 h, every 4 h until 48 h, and on days 5, 7, 14, 21, and 28 post-amendment at distances of 0, 10, 40, 300 and 500 m from the amendment point for chlorophyll *a*, suspended sediment, nutrient, and pesticide analyses. *H. azteca* 48-h laboratory survival was assessed in water collected at each site at 0, 4, 24, 48 h, 5 d and 7 d. Greatest sediment, nutrient, and pesticide concentrations occurred within 3 h of amendment at 0 m, 10 m, 40 m, and 300 m downstream. Sediments and nutrients showed little variation at 500 m whereas pesticides peaked within 48 h but at <15% of upstream peak concentrations. After 28 d, all mixture components were near or below pre-amendment concentrations. *H. azteca* survival significantly decreased within 48 h of amendment up to 300 m in association with permethrin concentrations. Chlorophyll *a* decreased within the first 24 h of amendment up to 40 m primarily in conjunction with herbicide concentrations. Variations in chlorophyll *a* at 300 and 500 m were associated with nutrients. Managed floodplain wetlands can rapidly and effectively trap and process agricultural runoff during moderate rainfall events, mitigating impacts to aquatic invertebrates and algae in receiving aquatic systems.

1. Introduction

The lower Mississippi alluvial plain (i.e., the Delta) contains a relatively flat topography providing diverse aquatic habitats such as rivers and bayous, floodplain lakes, oxbow lakes, and backwater wetlands. The region provides conditions conducive to intensive row-crop agriculture such as highly productive soils, a long growing season, and wide availability of water (Locke et al., 2008). The Delta also provides habitat for a variety of weed and insect pests precluding the need for pesticides and nutrients to maximize productivity. In addition, Delta soils are primarily clay to sandy loam causing high sediment turbidity in runoff from cultivated fields into receiving aquatic systems (Reddy et al., 2006). For these reasons there is an increasing need to provide diverse management practices for mitigating impacts of pollutants in agricultural runoff on receiving waterways.

Backwater wetlands that occur adjacent to major river channels have important economic and ecological functions including providing habitat, natural buffers, and acting as filters for suspended sediment, nutrients and pesticides entering from upland agricultural fields (Lizotte et al., 2009; Shields and Pierce, 2010). Backwater wetlands may be hydrologically modified to more efficiently manage their natural filtering capabilities (Mitsch et al., 2002). Both natural and constructed wetlands are effective in removing contaminants in agricultural runoff (Knox et al., 2008; Moore et al., 2009). As a result, backwater wetlands, with modification, could be managed as an additional tool or management practice (BMP) for reducing pesticide loads from non-point sources and mitigating risk to aquatic biota in aquatic receiving systems. The purpose of this study was to assess the aqueous ecological effects of a mixture of nutrients (nitrogen and phosphorus) and three pesticides (atrazine, *S*-metolachlor, and permethrin) in a natural backwater wetland hydrologically modified to more efficiently manage natural filtering capabilities using *Hyalella azteca* (order: Amphipoda) 48 h whole effluent laboratory bioassays and *in situ* phytoplankton pigment, chlorophyll *a*.

2. Material and methods

2.1. Study area

The study area was adjacent to a reach of the Coldwater River about 20 km downstream from Arkabutla Lake Dam in northwest-

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ern Mississippi, USA. The backwater wetland site was part of a riverine isolated meander backwater in Tunica County about 2.5 km long and 20–40 m wide. Located inside the Coldwater River mainstem flood control levee, the backwater is the result of a 0.4 km cutoff constructed in 1941–1942. Land-use surrounding the backwater is in row-crop cultivation, with a buffer of natural vegetation 5–100 m wide on both banks. The backwater receives agricultural runoff from approximately 100 ha of cultivated lands, primarily through an intermittent slough hydraulically connected to a series of drainage ditches. The wetland backwater study area was managed using two water control weirs upstream (34°40′05.85″ N, 90°13′38.77″ W), and downstream (34°40′16.67″ N, 90°13′35.05″ W), creating a larger, deeper segment managed as a lake habitat and a smaller, shallower segment, 500-m long, 20-m wide, that was managed as a wetland (Fig. 1). The wetland supports a variety of plant species. Plant populations were dominated by grasses (*leersia* sp.), sedges (*cyperus* sp., *carex* sp.) and duckweed (*lemnaceae* sp.). Mature forest lined the banks of the old river channel that comprised the wetland cell, and woody species occasionally occurred in the wetland cell itself.

2.2. Simulated runoff event and sampling

To simulate the rainfall event, the release of approximately 720 m^3 of water from the upstream lake segment of the backwater into the managed wetland segment occurred over a 4 h period on 24 June 2009. A hydrograph for the artificial event (Fig. 1, inset) was designed by scaling an observed hydrograph from the tributary slough (Shields and Pierce, 2010) so that the peak flow was equal to the maximum discharge that could be obtained by releasing water from the lake cell into the wetland through the drainage structure (90 L s^{-1}). During the event, the hydrograph was generated by removing and replacing flashboards from the drainage structure at set times. Flow rates were continuously recorded by measuring the depth of flow over the weir and converting flow depth to discharge using a rating curve provided by the manufacturer. Flow rates were verified using acoustic and electromagnetic devices in the discharge channel. Outflow from the wetland was monitored throughout the experiment using a logging pressure transducer to record the depth of flow over the weir structure. No outflow occurred during simulated event, and no outflow occurred during the period following the event.

A mixture comprising suspended sediment, nitrogen (34% NH_4NO_3), phosphorus (42% triple super phosphate P_2O_5), atrazine, *S*-metolachlor, and permethrin was amended for 1 h simulating first flush agricultural runoff (Bertrand-Krajewski et al., 1998) during a 1.27 cm rainfall event from a 16 ha cultivated field. To simulate first flush, 25 min after initiating flow through the upstream weir drainage structure (10:25 AM; see insert hydrograph Fig. 1) the mixture was delivered directly into the drainage structure (allowing for complete mixing). Amendment was pumped from two 20 L stock solutions through Teflon® tubes using two FMI piston metering pumps (Fluid Metering, Inc., Syosset, Long Island, New York, USA) calibrated at 333 mL min^{-1} for 1 h (until 11:25 AM) into the drainage structure coinciding with peak flow (see insert hydrograph Fig. 1). A total of 270.8 kg sediment, 6.1 kg NH_4NO_3, 3.6 kg P_2O_5, 72.7 g active ingredient (ai) atrazine with 54.5 g ai *S*-metolachlor (Bicep II Magnum®), and 11.4 g ai permethrin (Hi-Yield 38®) were amended at the upstream weir.

Water samples (1 L) were collected every 30 min during the first 4 h, at 5 h, 8 h, and then every 4 h thereafter until 48 h. Additional collections occurred on days 5, 7, 14, 21, and 28. This sample design allowed for assessment of both: (a) acute direct contaminant toxicity of the simulated first flush occurring within hours; and (b) longer-term post-runoff ecological responses occurring within days to weeks after the event. Samples were collected at distances from upstream weir inflow of 0, 10, 40, 300, and 500 m (adjacent to downstream weir, Fig. 1). Samples obtained within the first 48 h were collected using an automated pumping sampler (ISCO Model 3700) modified from Smith (1993). Remaining

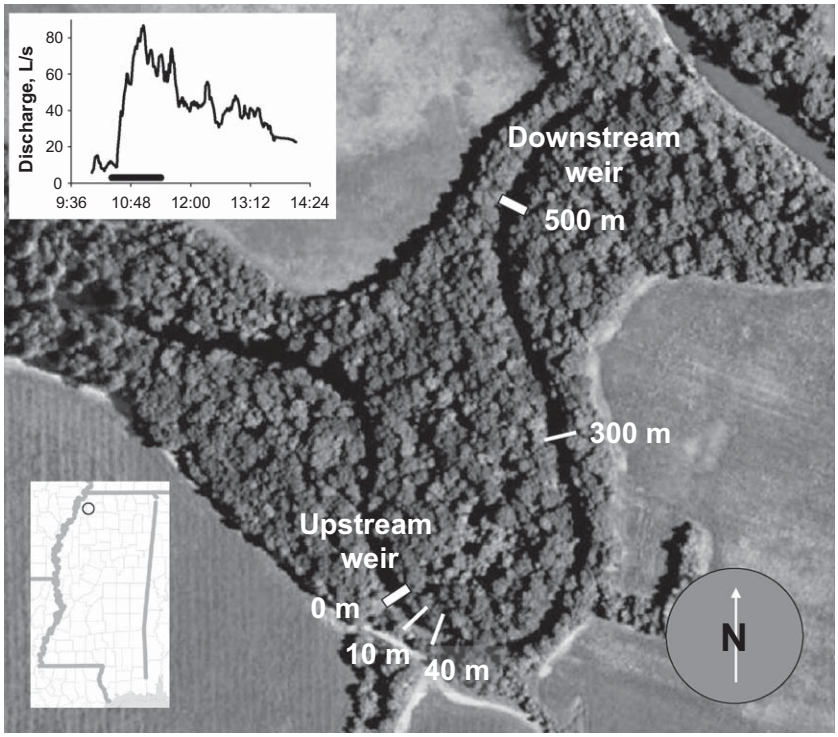

Fig. 1. Aerial photograph of the location and configuration of the Coldwater River managed backwater wetland in Tunica County, Mississippi, with both upstream and downstream weirs and sampling locations. Inset of hydrograph during simulated agricultural runoff event on June 24, 2009.

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samples were collected by hand. Sample containers were 1 L polyethylene plastic bottles fitted with a Teflon-lined screw cap. All samples were placed on wet ice, transported to the USDA-ARS National Sedimentation Laboratory, and stored at 4 °C (typically <24 h) for sediment, nutrient, and pesticide analysis.

2.3. Sample analysis

Water samples were analyzed for total suspended sediment (TSS), total orthophosphate (TOP), dissolved inorganic nitrogen (DIN; NH_4^+, NO_3^-, and NO_2^-), and total nitrogen (TN). TSS was assessed via the difference in measurement of total solids dried at 105 °C and total dissolved solids dried at 180 °C (APHA, 2005). TOP was determined using the persulfate oxidation digestion procedure followed by the ascorbic acid colorimetric method (APHA, 2005). The cadmium reduction method was used to analyze NO_3^- and NO_2^-, whereas NH_4^+ was analyzed using the phenate colorimetric method (APHA, 2005) and the sum of all three constituents reported as DIN. TN was determined as the sum of NO_3^-, NO_2^- and total Kjeldahl N (using block digestion and flow injection analysis; APHA, 2005). All colorimetric analyses were performed using a ThermoSpectronic Genesys™ 10 ultraviolet (UV) spectrophotometer (Spectronic Instruments, Inc., Rochester, New York, USA).

For pesticide analysis, atrazine, *S*-metolachlor, and permethrin were extracted using pesticide-grade ethyl acetate, 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 concentrated to 1 mL volume under high purity dry nitrogen for GC analysis. Pesticide analysis was conducted using an Agilent Model 7890A gas chromatograph (Agilent Technologies, Inc., Waldbronn, Germany) equipped with dual Agilent 7683B series autoinjectors, dual split–splitless inlets, dual capillary columns, an Agilent ChemStation, and the autoinjector set at 1.0 μL injection volume were used according to Smith and Cooper (2004) and Moore et al. (2009). The Agilent 7890A GC was equipped with two micro-electron capture detectors (μECDs), and the analytical column was an Agilent HP 1MS capillary column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness. Column oven temperatures for atrazine and *S*-metolachlor were: inlet at 75 °C for 1 min; ramp at 10 °C to 175 °C and hold for 15 min; ramp at 10 °C to 225 °C and hold for 15 min. Retention times were 14.79 and 22.70 min for atrazine and *S*-metolachlor, respectively. Column oven temperatures for permethrin were: inlet at 75 °C for 1 min; ramp at 35 °C to 230 °C and hold for 15 min. Retention times were 15.43 min for cis-permethrin and 15.89 min for trans-permethrin. Carrier gas used was ultra-high purity (UHP) helium at 28 mL min^{-1} and inlet temperature at 250 °C. The μECD temperature was 325 °C with a constant make-up gas flow of 60 mL min^{-1} UHP nitrogen. Analytical detection limits for all pesticides were 0.1 μg L^{-1}, and recoveries were ⩾95% based on fortified samples.

2.4. Biological responses

Wetland water (1 L) was collected at each distance during 0, 4, 24, 48 h, 5 d, and 7 d, hardness adjusted to about 100 mg $CaCO_3$ L^{-1} using $CaCl_2$ and $NaHCO_3$, and transported to the USDA-ARS NSL for whole effluent toxicity analysis. For bioassays, animals were obtained via passing through a 600 μm stainless steel mesh sieve but retained by a 425 μm stainless steel mesh sieve (approximately 1–2 weeks old) (Suedel et al., 1997; Moore et al., 2004). Bioassays were 48-h static non-renewed aqueous exposures assessing *H. azteca* survival, in five serial dilutions with four replicates each, according to modified USEPA (2000) protocol for *H. azteca* reference toxicity tests. Five *H. azteca* were placed in each replicate 88 mL polypropylene plastic test chamber with one 2 cm × 2 cm square sterile cotton gauze as substrate. Bioassays were conducted in a Powers Scientific incubator (Powers Scientific, Inc., Pipersville, Pennsylvania, USA) at 23 ± 1 °C with a photoperiod of 16:8 light:-dark. Aqueous exposures consisted of 75 mL hardness adjusted (~100 mg L^{-1} as $CaCO_3$) sample and/or control/dilution water with five serial dilutions at 0.25 dilution factor. Control and dilution water, free from priority pollutants, were from a natural 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 (μS 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^+ (μg L^{-1}), 0–136; NO_3^- (μg L^{-1}), 42–170; NO_2^- (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}.

Phytoplankton photosynthetic pigment chlorophyll *a* was analyzed using the trichromatic method (pigment extraction and spectrophotometric determination; APHA, 2005). Water samples of 100 mL were filtered through a 0.45 μm filter. Filters were collected and stored at −15 °C for at least 12 h. After 12 h, pigments were extracted using 90% buffered (10% $MgCO_3$) acetone at 4 °C for 2 h. Extracted samples were vortexed for 10 s and centrifuged at 2500 rpm for 10 min. Chlorophyll *a* was calculated based upon corrected optical densities from equations reported in standard methods for the examination of water and wastewater (APHA, 2005).

2.5. Data analysis

H. azteca 48-h survival statistical analysis was conducted using ToxCalc v5.0.32 (ToxCalc, 2008; McKinleyville, CA, USA). Survival data were arcsin square root transformed and Shapiro–Wilk's test for normality and Bartlett's test for homogeneity of variance were performed. Point estimates of no-observed effects effluent dilution fractions (NOECs) were determined by analysis of variance (ANOVA) with Dunnett's multiple range test or Steel's Many-One Rank test when appropriate. NOECs were based on the lack of statistically significant differences ($p > 0.05$) relative to controls. Estimated 10% and 50% lethal effluent dilution fraction effects, LC10s (%), LC50s (%) and their 95% confidence intervals were determined using probit analysis or logit analytical methods, when appropriate (Kerr and Meador, 1996; USEPA, 2000; ToxCalc, 2008). Acute (96-h) toxicity units (TUs), the measured concentration divided by the median lethal concentration (LC50) described by Pape-Lindstrom and Lydy (1997) were calculated for DIN and pesticide components of the mixture. *H. azteca* TUs for atrazine, 1500 μg L^{-1} (Ralston-Hooper et al., 2009), *S*-metolachlor, 6000 μg L^{-1} (Wan et al., 2006), permethrin, 0.037 μg L^{-1} (Wheelock et al., 2005), and NH_4^+, 39.8 mg L^{-1} (Ankley et al., 1995) were calculated. TUs for NO_3^-, 62.5 mg L^{-1} (Camargo et al., 2005) and NO_2^-, 2.09 mg L^{-1} (Camargo and Alonso, 2006) were from amphipods *Echinogammarus echinosetosus* and *Eulimnogammarus toletanus*, respectively. These were used in lieu of *H. azteca* due to the lack of published effects concentrations. Sum of NH_4^+, NO_3^-, and NO_2^- TUs in the mixture provided the DIN TU. In addition, because there was no available published data on the acute toxicity of TOP on crustaceans, phosphorus was not included in the model. Non-linear logistic regression was performed on TUs versus *H. azteca* survival to elucidate likeliest sources of observed toxicity using SigmaStat v2.03 statistical software (Chicago, IL, USA).

Forward stepwise linear regressions were conducted to assess multiple independent variables that could influence observed changes in chlorophyll *a* concentrations according to Berenson

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Table 1
Mean (range) concentrations of sediments (mg L^{-1}), nutrients (mg L^{-1}), and pesticides (μg L^{-1}) from a simulated agricultural runoff in a managed backwater wetland.

Contaminant	Distance (m)				
	0	10	40	300	500
TSS	361 (8–2289)	574 (28–5136)	400 (45–4722)	458 (27–3052)	92 (20–178)
TOP	1.80 (0.65–5.54)	2.21 (0.65–4.08)	2.09 (1.12–4.07)	2.27 (1.00–3.79)	1.77 (1.16–2.88)
DIN	0.16 (0.01–0.56)	0.12 (0.05–0.49)	0.11 (U[a]–0.46)	0.24 (0.09–1.40)	0.16 (0.06–1.24)
TN	3.21 (1.24–12.00)	3.19 (1.34–10.57)	3.34 (1.35–9.30)	3.50 (1.13–7.09)	2.90 (1.30–4.00)
Atrazine	19.2 (U–71.7)	19.0 (U–104.4)	21.7 (U–96.3)	25.1 (0.2–128.2)	4.0 (U–14.8)
S-metolachlor	12.0 (U–47.9)	10.7 (U–67.7)	14.0 (U–71.9)	16.6 (U–100.1)	2.5 (U–9.8)
Permethrin	0.6 (U–3.3)	1.4 (0.2–6.4)	1.3 (U–10.2)	0.6 (U–4.5)	0.2 (U–0.5)

[a] U: below detection limit.

et al. (1983). Log_{10}-transformed independent variables of amended agrichemicals (TSS, nutrients, and pesticides; Table 1) were used to predict the dependent variable, chlorophyll *a* at distances of 10, 40, 300, and 500 m from inflow within 24, 48, 168, 336, and 672 h post-amendment to examine spatial and temporal changes in concentration–responses using SigmaStat v2.03 statistical software. Independent variables were added or removed using *F*-to-enter of 4.0 and *F*-to-remove of 3.9. Standardized dimensionless regression coefficients and coefficients of determination were calculated and reported for each variable.

3. Results

3.1. Sediment, nutrient and pesticide concentrations

Concentrations of TSS ranged from 8 to 5136 mg L^{-1} within the managed wetland during the study (Table 1). TSS concentrations decreased spatially from upstream (0 m) to downstream (500 m). Temporally, rising TSS concentrations occurred between 0.5 and 1 h of the study (1623–4722 mg L^{-1}), and again at 24 h (795–5136 mg L^{-1}), falling to lows of TSS after 5–7 d (8–46 mg L^{-1}). Backwater wetland water TOP concentrations ranged from 0.65 to 5.54 mg L^{-1} during the 28-d study period (Table S1, Supplemental Material). TOP decreased spatially from upstream to downstream within the first 2 h of the study with concentrations of 5.54 mg L^{-1} at 0 m and 2.31 mg L^{-1} at 500 m. Temporally, increasing TOP occurred between 0.5 and 1.5 h of the study (2.31–5.54 mg L^{-1}), rapidly decreasing within 36–48 h to 1.14–1.67 mg L^{-1}. Lowest TOP concentrations (0.85–1.72 mg L^{-1}) were observed after 14–28 d. Dissolved inorganic nitrogen concentrations ranged from <0.01 to 1.40 mg L^{-1} within the backwater wetland during the study (Table S1). Within first 4 h of the simulated runoff event, DIN concentrations spatially and temporally peaked and declined from 0 to 300 m ranging from 0.01 to 0.56 mg L^{-1}. An unrelated influx of DIN, 1.40 and 1.24 mg L^{-1}, occurred 21 d after amendment at 300 m and 500 m downstream, respectively, likely due to localized runoff from adjacent fields after a 19 mm of rainfall event occurred 1–2 d prior to the 21-d sampling period. Concentrations of TN during the study period ranged from 1.13 to 12.01 mg L^{-1} (Table 1). TN concentrations spatially and temporally peaked and declined from 0 to 300 m within first 4 h of the simulated runoff event, mirroring DIN concentrations (Table S1).

Herbicide concentrations of atrazine and *S*-metolachlor ranged from <0.1 μg L^{-1} (below detection limits) to 128.2 μg L^{-1} (Table 1). Dissimilar to other contaminants, herbicide concentrations increased downstream to 300 m within 2 h of amendment before greatly decreasing at 500 m. Temporally, greatest herbicide concentrations occurred within 1–2 h post-amendment from 0 to 300 m and 48 h post-amendment at 500 m. Thereafter, herbicide concentrations slowly decreased temporally until 672 h (28 d) when concentrations were at pre-amendment levels. Permethrin insecticide concentrations increased spatially up to 40 m within 2 h of amendment and decreased downstream thereafter (Table S1). As with herbicides, peak temporal permethrin concentrations occurred within 1–2 h post-amendment within 300 m and 32 h post-amendment at 500 m. Thereafter, permethrin concentrations rapidly dissipated from the water column, returning to pre-amendment levels within 48–120 h (2–5 d).

3.2. Hyalella azteca responses

Measured water quality was within acceptable parameters for acute aqueous bioassays according to USEPA (2000) protocol for *H. azteca* reference toxicity tests. Ranges of water quality for the toxicity tests with field site water were as follows: temperature (°C), 22.9–24.0; dissolved oxygen (mg L^{-1}), 4.6–8.3; pH, 7.3–8.6; alkalinity (mg L^{-1} as $CaCO_3$), 51.3–102.1; hardness (mg L^{-1} as $CaCO_3$), 85.5–273.6; conductivity (μS cm^{-1}), 282.0–717.0. *H. azteca* survival in all pre-treatment samples (0 h) and controls (0% effluent) was ⩾90% for all 48 h bioassays (Table 2). Within the first 4 h of amendment, animal survival significantly decreased in effluent samples at 0, 10, 40, and 300 m, but not 500 m. Greatest toxicity occurred within 4 h post-amendment at 10 and 40 m with LC50s of 4.1% and 3.9%, respectively (Table 2). Four-hour survival NOECs and LC10s ranged from 0.4 to 25% and 0.1–>100%, respectively. Wetland effluent toxicity rapidly dissipated within 24–48 h at all affected sites with survival NOECs and LC10s ranging from 1.6 to 100% and 1.1–>100%, respectively. By day 5, toxicity was no longer observed at any site (Table 2).

Hyalella azteca survival was associated with pesticides permethrin, and *S*-metolachlor, while observed DIN and atrazine was not associated with animal survival (Fig. 2). While it is understood that association does not imply causation, an attempt to determine likely causes of observed toxicity was done using a TU model approach as described previously to examine mixture toxicity. Non-linear logistic regression models constructed showed that permethrin TUs explained 73% of *H. azteca* survival variation followed by *S*-metolachlor (34.6%) and atrazine (22.7%; Fig. 2). Examination of calculated TUs presented in these models showed permethrin to be the primary toxicant, with both *S*-metolachlor and atrazine well below toxic concentrations.

3.3. Phytoplankton chlorophyll a responses

Variations in phytoplankton chlorophyll *a* concentrations within the managed floodplain wetland during the experiment are shown in Fig. 3. Chlorophyll *a* was reduced by 72–74% at 10 m and 40 m, respectively, within 1 h post-amendment and by an additional 13% at both sites within 20 h post-amendment (Fig. 3). Thereafter, chlorophyll *a* increased, with fluctuations, at 10 m and 40 m through day 28. Chlorophyll *a* concentrations at 300 m and 500 m fluctuated from ~40 to 110 μg L^{-1} and ~100–400 μg L^{-1}, respectively, throughout the observation period (Fig. 3).

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Table 2
Hyalella azteca 48-h survival NOECs, LC10s (%) (95% confidence intervals), and LC50s (%) (95% confidence intervals) exposed to simulated agricultural runoff in a managed backwater wetland ($n = 4$); (NC = value could not be determined).

Distance (m)	Endpoint	Time					
		0 h	4 h	24 h	48 h	5 d	7 d
0	NOEC	100	25	1.6	6.3	100	100
	LC10	>100	8.6 (0.8–27.5)	1.7 (0.3–4.0)	12.6 (2.9–23.1)	>100	>100
	LC50	>100	>100	42.8 (19.9–159.4)	110.4 (74.4–221.3)	>100	>100
10	NOEC	100	0.4	25	100	100	100
	LC10	>100	0.4 (0.1–1.1)	17.3 (7.4–26.7)	>100	>100	>100
	LC50	>100	4.1 (1.8–7.8)	104.1 (76.2–169.4)	>100	>100	>100
40	NOEC	100	0.4	100	1.6	100	100
	LC10	>100	0.4 (0.1–0.8)	>100	1.1 (0.2–2.8)	>100	>100
	LC50	>100	3.9 (2.2–6.9)	>100	27.1 (13.4–77.8)	>100	>100
300	NOEC	100	0.4	100	25	100	100
	LC10	>100	0.1 (<0.1–0.6)	>100	(NC)	>100	>100
	LC50	>100	11.5 (4.5–38.3)	>100	(NC)	>100	>100
500	NOEC	100	100	100	100	100	100
	LC10	>100	>100	>100	>100	>100	>100
	LC50	>100	>100	>100	>100	>100	>100

Forward stepwise linear regression analysis revealed components of the sediment–nutrient–pesticide mixture amended to the managed floodplain wetland that most influenced phytoplankton chlorophyll *a* responses spatially and temporally (Table 3). At the 10 m site, the herbicide atrazine was the most influential variable up to 336 h post-amendment followed by *S*-metolachlor by 672 h post-amendment. TSS and TN, while significant, were less influential. Similarly at 40 m, atrazine was the most influential variable up to 336 h post-amendment followed by *S*-metolachlor by 672 h whereas TSS, TOP, and TN were less influential. Results at these two upstream sites (within 40 m) show herbicides inhibiting chlorophyll *a* after peak pulse mixture concentrations $\geqslant$96 μg atrazine L^{-1} and $\geqslant$67 μg S-metolachlor L^{-1} (Table S1). At 300 m downstream, nitrogen as TN was the most influential variable up to 672 h post-amendment with DIN less influential. At the 500 m site, phosphorus as TP was the most influential variable on chlorophyll *a* throughout the study period with TSS less influential.

4. Discussion

Because agricultural runoff often contains a wide variety of contaminants including high suspended sediment loads, excessive nutrients, and mixtures of residual pesticides, aquatic organisms inhabiting wetlands anthropogenically designed or altered to intercept and process these contaminants are at risk of exposure to these complex mixtures. Although numerous studies have been conducted to assess how managed wetlands treat and remove a variety of influent contaminants (Detenbeck et al., 1996; Huddleston et al., 2000; Moore et al., 2000, 2001, 2009; Schulz and Peall, 2001; Vymazal, 2008), currently, there is a paucity of information regarding the mitigation capacity of aqueous-phase agricultural effluent toxicity in managed wetlands (Schulz and Peall, 2001; Hunt et al., 2008). Rapid dissipation of contaminant mixture constituents (TSS, nutrients, and permethrin) within 7–14 d of amendment is supported by results of several previous reports examining dissipation of these constituents from the aqueous phase in managed wetlands (Jordan et al., 2003; Hunt et al., 2008; Knox et al., 2008). Pyrethroids such as permethrin can be rapidly removed from the aqueous phase via adsorption to organic material (e.g., plants, algae, detritus) and sediment. This can result in decreased bioavailability and concomitant toxicity to aquatic animals such as *H. azteca* (Barry et al., 1995; Maund et al., 2002; Maul et al., 2008; Lizotte et al., 2011). Herbicides atrazine and metolachlor dissipated more slowly within 21–28 d after amendment, although the dissipation rate for these contaminants was comparable to those of other studies (Detenbeck et al., 1996; Moore et al., 2000; Moore et al., 2001).

Toxicity of the amended agricultural contaminant effluent mixture to *H. azteca* after 48-h laboratory exposures provided clear spatial and temporal patterns within the managed wetland up to 48 h post-amendment. Although an increasing number of studies have focused on responses of non-target aquatic invertebrates to contaminated agricultural runoff influent entering managed wetlands (Schulz and Peall, 2001; Hunt et al., 2008), most studies assessed responses to insecticide contaminants and did not include other contaminants present (i.e., DIN). As a result, direct effects of DIN on aquatic invertebrates, in particular crustaceans, potentially inhabiting managed wetlands have not been well assessed (Spieles and Mitsch, 2000; Huddleston et al., 2000; Camargo and Alonso, 2006). The current study showed amended DIN within the backwater wetland had little to no effect on observed animal toxicity since greatest TUs were <0.04 and were not correlated with observed animal survival patterns. Associations between observed toxicity to *H. azteca* occurred only for pesticides (permethrin, *S*-metolachlor, and atrazine) and the TU model showed permethrin to be the primary contaminant eliciting animal toxicity in the current study. These results are in agreement with those of Hunt et al. (2008) using a similar approach to assess observed laboratory toxicity in effluent from managed on-farm vegetated treatment ponds receiving agricultural runoff. Wetland amended herbicides atrazine and *S*-metolachlor appeared to have no effect on animal toxicity with atrazine and *S*-metolachlor TUs less than 0.05 and 0.01, respectively. These results are in agreement with known acute toxicity (96 h) of these compounds to *H. azteca* (Wan et al., 2006; Ralston-Hooper et al., 2009) despite TU values being significantly correlated with animal survival. *H. azteca* aqueous toxicity of the wetland amended permethrin showed the transient nature of pyrethroids in the aqueous phase due to their physico-chemical properties (Shamim et al., 2008), with no acute toxicity observed 5 d post-amendment. Resulting risk of pulsed permethrin exposure to sensitive aquatic biota (specifically crustaceans) in the 500 m managed backwater wetland would be brief (>2 and <5 d) and would only occur within the first 300 m of the system.

In contrast to responses of aquatic invertebrates, freshwater phytoplankton can have varied responses to complex mixtures found in agricultural runoff. It is well established that freshwater algae respond to changes in nutrient loads (Bellinger and Sigee, 2010), suspended sediment (Bilotta and Brazier, 2008), and herbi-

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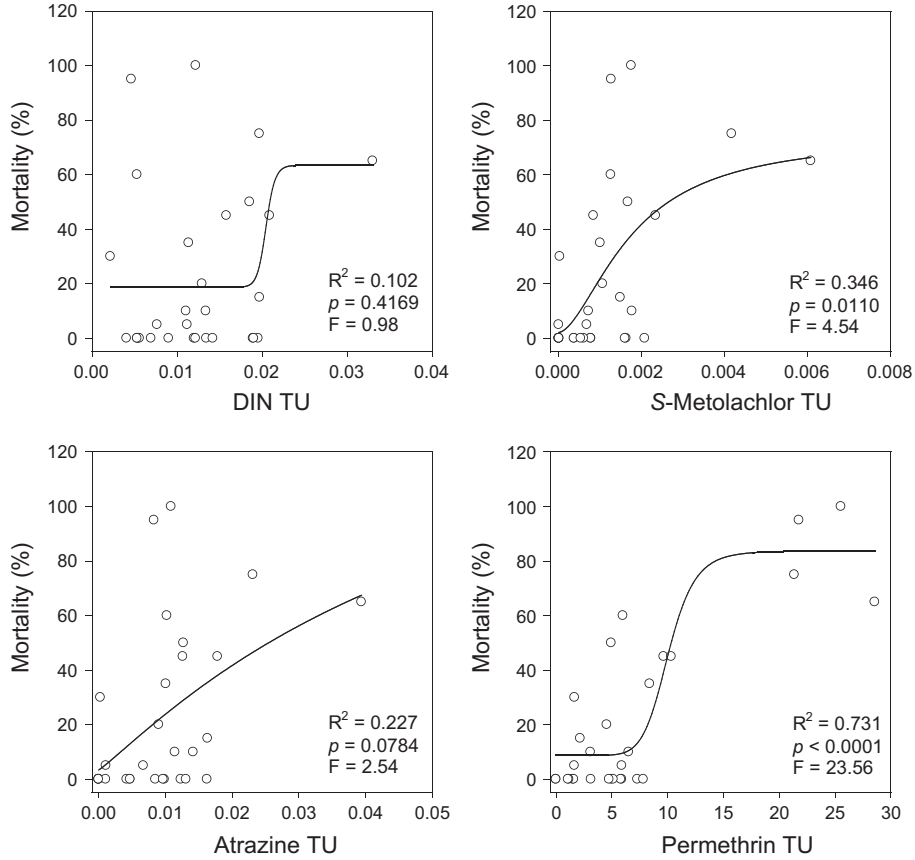

Fig. 2. Non-linear logistic regression relationships ($N = 30$) between *Hyalella azteca* 48-h survival, pesticide TUs, and DIN (dissolved inorganic nitrogen) TUs in a managed backwater wetland.

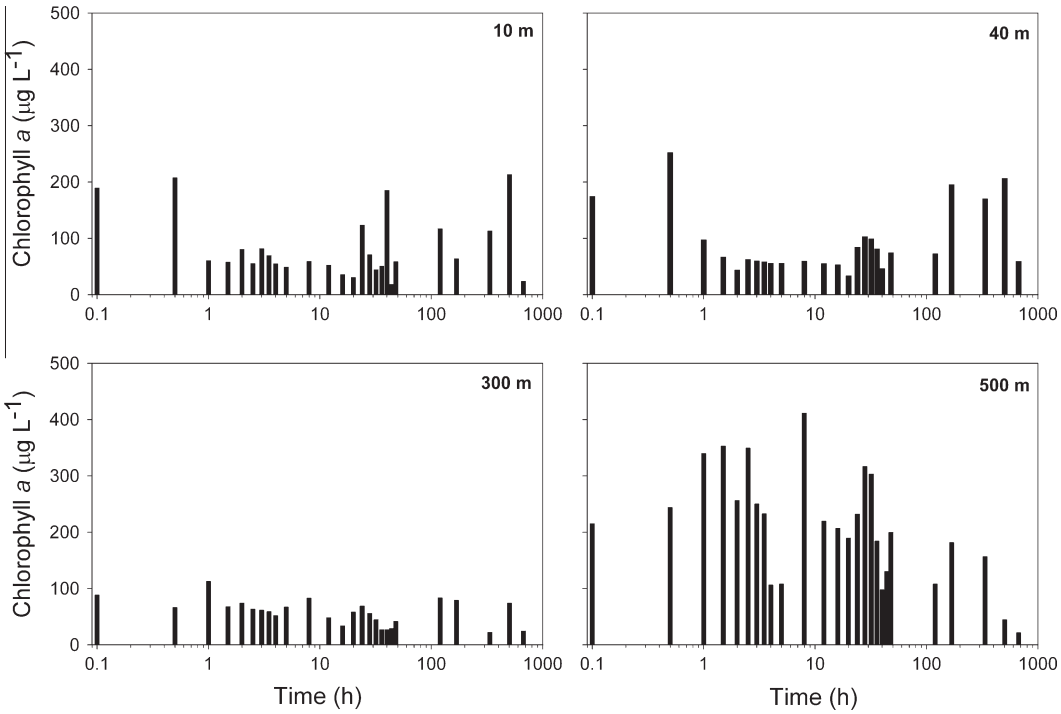

Fig. 3. Chlorophyll *a* concentrations over time at 10, 40, 300, and 500 m in a managed backwater wetland.

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Table 3
Standardized dimensionless regression coefficients and coefficients of determination for forward stepwise linear regressions computed using chlorophyll *a* as the dependent variable and amended agrichemicals (log_{10}-transformed) as independent variables at distances of 10, 40, 300, and 500 m from inflow within 24, 48, 168, 336, and 672 h post-amendment. Bold font indicates the largest standardized coefficient in each regression and blank cells indicate variables were dropped from stepwise regression due to a lack of significance.

Distance (m)	Time (h)	*n*	Standardized dimensionless regression coefficients							R^2	*p*-Value
			TSS	TOP	DIN	TN	Atrazine	*S*-metolachlor	Permethrin		
10	0–24	15				0.409	**−0.798**		0.316	0.835	0.0001
	0–48	21	0.448				**−0.474**			0.543	0.0009
	0–168	23	0.460				**−0.471**			0.537	0.0005
	0–336	24	0.451				**−0.487**			0.542	0.0003
	0–672	26				0.457		**−0.464**		0.402	0.0027
40	0–24	15	0.352				**−2.528**	1.998		0.972	<0.0001
	0–48	20	0.469				**−0.717**			0.867	<0.0001
	0–168	22		0.474			**−0.637**			0.747	<0.0001
	0–336	23		0.342			**−0.703**			0.662	<0.0001
	0–672	25				0.470		**−0.701**		0.619	<0.0001
300	0–24	15			−0.826	**1.248**				0.749	0.0005
	0–48	21			−0.601	**1.185**				0.832	<0.0001
	0–168	23			−0.511	**1.016**				0.616	0.0001
	0–336	24			−0.515	**0.973**				0.663	<0.0001
	0–672	26				**0.714**				0.510	<0.0001
500	0–24	15	0.541	**0.556**						0.743	0.0003
	0–48	21		**0.669**						0.448	0.0009
	0–168	23		**0.688**						0.473	0.0003
	0–336	24		**0.683**						0.466	0.0002
	0–672	26		**0.726**						0.528	<0.0001

cides such as atrazine and *S*-metolachlor (Tang et al., 1997; Fairchild et al., 1998) as individual contaminants or in simple mixtures (e.g., nitrogen and phosphorus). By way of comparison, few studies have assessed such complexity of mixtures and their effects on phytoplankton under controlled conditions (via amendment) as the current study (Waiser and Robarts, 1997; Roessink et al., 2008). Photosynthetic pigments such as chlorophyll *a* may be used as an indirect estimate of algal biomass (Bellinger and Sigee, 2010). Within 24 h of amendment, backwater wetland chlorophyll *a* concentrations at 10 m and 40 m downstream from amendment were inhibited by the herbicide mixture of atrazine and *S*-metolachlor. Effects of both atrazine and *S*-metolachlor have been reported to occur rapidly (within 24 h) in green algal species (Vallotton et al., 2008; Liu and Xiong, 2009). Vallotton et al. (2008) reported a 44% reduction in growth rate of *Scenedesmus vacuolatus* after 24 h pulsed exposure to 125 $\mu g\ L^{-1}$ atrazine and Liu and Xiong (2009) reported a 24 h EC50 of 116 $\mu g\ L^{-1}$ *S*-metolachlor for *Chlorella pyrenoidosa*. Although peak herbicide concentrations in this study were below reported acute (24–48 h) median effects concentrations for green algae (Vallotton et al., 2008; Liu and Xiong, 2009), herbicide concentration addition mixture toxicity would have ranged from 0.4 to 1.4 TUs at 10 m, 40 m, and 300 m for several hours during the pulse amendment exposure (Table S1). Similar to the transient response of *H. azteca* to pyrethroid exposure, chlorophyll *a* concentrations at 10 and 40 m began recovering from the pulsed herbicide exposures within 24–48 h post-amendment (Fig. 3) and recovered to near pre-treatment levels at 7 and 21 d, respectively. Rapid recoveries from atrazine exposures have been previously reported (Vallotton et al., 2008) where *S. vacuolatus* began recovering from a single pulsed dose within 5 h. While herbicide concentrations at 300 m appeared to be great enough to elicit decreases in chlorophyll *a* (Table S1), no clear pattern was observed (Fig. 3) suggesting algae at this site were unaffected by these herbicides. Guasch and Sabater (1998) observed decreased sensitivity of algae to atrazine under shaded (lower light) conditions and such shaded canopy conditions occurred at 300 m of the backwater wetland. Chronic effects on chlorophyll *a* concentrations of herbicide exposure were less apparent, as shown by decreased but significant standardized dimensionless regression coefficients for atrazine and *S*-metolachlor by 28 d (Table 3), indicating longer term recovery from pulsed exposure as herbicides dissipated from the water column over time.

Nutrient effects on phytoplankton chlorophyll responses after amendment in the wetland were more complex than herbicides. Although several significant standardized dimensionless regression coefficients occurred between TOP, TN and chlorophyll *a* during the 672 h study period at all sites (Table 3), initial relatively high wetland chlorophyll *a* levels (Fig. 3) would indicate high algal densities limiting rapid growth rates for phytoplankton, and these communities would not have time to respond (doubling of populations) in less than 24 h of increased nutrient loads (Scheffer, 2004). As a result, observed associations between chlorophyll *a* concentrations and organic nutrient levels (TOP, TN) are more likely to be due to preexisting algal populations and resuspension of organic material(s) during the 4-h flow event (Scheffer, 2004) rather than an algal growth response to increasing nutrient loads. In contrast, effects of available nutrients on chlorophyll *a* by 672 h post-amendment were mitigated where herbicide effects occurred (10, 40 m) and more pronounced where chlorophyll *a* was unaffected by herbicides and nutrient levels were greater than pre-amendment levels (300 m). These results are supported by those of Waiser and Robarts (1997) where phytoplankton chlorophyll *a* concentrations increased in the presence of nutrients only, but were mitigated in the presence of a mixture of herbicide and nutrients.

5. Conclusions

The current study showed managed floodplain wetlands can rapidly and effectively trap and process agricultural runoff during moderate rainfall events, mitigating impacts to aquatic invertebrates and algae in receiving aquatic systems. Mitigation of insecticide permethrin toxicity to the aquatic crustacean, *H. azteca*, in the 500 m managed backwater wetland occurred rapidly (2–5 d) and at distances >300 m within the system. Similarly, mitigation

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of herbicides atrazine and *S*-metolachlor effects on phytoplankton chlorophyll *a* concentrations occurred rapidly (1–7 d) and at distances >40 m and nutrients at distances of ⩾300 m within the wetland. When properly managed, such naturally occurring floodplain wetlands have the potential to be used as an agricultural conservation practice to intercept and process agricultural runoff and improve downstream water quality.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.12.058.

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