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PRIMARY RESEARCH PAPER

The role of macroinvertebrates in the distribution of lead (Pb) within an urban marsh ecosystem

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Abstract Environmental risk from contaminated aquatic sediments requires an understanding of its spatial distribution, bioavailability and rate of transfer to resident aquatic and terrestrial biota. We hypothesized that macroinvertebrates play a role in the sequestration, distribution, and dispersal of lead from lead shot contaminated sediments. To assess this, we sampled the predominant aquatic macroinvertebrate, *Leptocerus americanus*, from sites within the La Crosse River Marsh (La Crosse, Wi) identified to contain high levels of lead contamination. We measured lead content in larval cases, larval tissues and emergent adult tissues. Lead concentrations within whole larvae correlated with levels of lead within sediments, and lead was differentially partitioned between larval tissue and their silk cases. Over 90% of the lead was retained in larval cases, while the rest was distributed to the body tissue, which was largely conserved during the process of metamorphosis. Our models support that *L. americanus* emerging from the marsh in the contaminated area transfer as much as 160 mgPb out of the aquatic habitat each year. Our work demonstrates that macroinvertebrates affect the mobilization and dispersal of contaminants within aquatic sediments, and this role should be evaluated when making management decisions regarding contaminated ecosystems.

Keywords Wetland · Trichoptera · Lead bioavailability · Lead shot · Contaminant

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Introduction

Marshes are biologically diverse and productive ecosystems whose shallow water and vegetative cover provides nursery habitats for larval insects, fish and amphibians that support higher trophic levels and fisheries. These wetlands also provide essential ecosystem services including retention and filtration of water, degradation of organic wastes, and sequestration of inorganic and organic contaminants in plants and sediments (Peltier et al., 2003). Organic

contaminants in wetlands can often be broken down into their constituent elements and rendered harmless by natural processes (Haarstad et al., 2011). Some materials such as lead shot, however, can remain toxic when broken down into their elemental form and can remain present for hundreds of years in marsh environments (Jørgensen & Willems, 1987).

Lead ammunition used for waterfowl hunting and trap shooting accumulates in aquatic sediments (Behan et al., 1979; Lund et al., 1991; Perroy et al., 2014) and can lead to the release of bioavailable lead within sediments (Eisler, 1988; Marr et al., 1998). The hydric soils of wetlands enable migration of dissolved lead particles into the surrounding sediments and the overlying water where it can be assimilated by resident wetland organisms (Hui, 2002; Peltier et al., 2003; Luo et al., 2013). Exposure to lead affects growth, development, reproduction, immune response, neuromuscular response and behavior in both aquatic vertebrates and invertebrates (Eisler, 1988; Vermeulen et al., 1991; de Bisthoven et al., 1992; Timmermans et al., 1992; Gerhardt 1994; Rademacher et al., 2003; Brix et al., 2011; Malaj et al., 2012). Animals can be exposed through direct ingestion of lead shot, lead-contaminated food or particulate matter, exposure to dissolved lead in the water, or adherence of lead to the organism by sorption (Mudge, 1983; Hare, 1992; Stansley et al., 1997). Even though lead poisoning of waterfowl prompted a ban on the use of lead shot over waterways in the U.S. in 1991 (Golden et al., 2016), because of its persistence within the environment and the spatial extent of lead deposition in North American wetlands, lead contamination continues to be a concern in wetland environments.

The fate and transport of metals within an ecosystem are governed by biological, geological, and chemical factors, as well as the site-specific food web (Eggleton & Thomas, 2004; Fritsch et al., 2011). Because movement of most metals within biota is governed by diet, macroinvertebrates play a particularly important role in the transfer of contaminants from sediment to organisms at higher trophic levels both within the system and to adjacent terrestrial ecosystems (Caussy et al., 2003; Gratton et al., 2008; Sullivan & Rodewald, 2012). The amount of lead transferred from an aquatic insect to a secondary consumer depends on the species and life stage at which it is consumed. Different species of insects partition lead to different tissues and organs in varying proportions (Hare et al., 1991; Timmermans et al., 1992). Additionally, the total body burden of lead can also be increased considerably by adsorbed accumulations of toxins on exoskeletons and casings (Krantzberg & Stokes, 1988). Insect larvae and juveniles frequently shed their exoskeletons or leave behind larval casings upon emergence that remain sequestered in the wetland sediment. Detritivores can then reintroduce the lead from these structures to the food chain with the remaining lead in the emergent insect being mobilized out of the wetland with the adult (Besser et al., 2001). This disperses the contamination to a wider environment and exposes the next trophic level within the terrestrial environment to contamination. Sites with extensive contamination and large numbers of emerging insects have substantial potential for transfer of contaminant materials from aquatic wetlands to terrestrial environments (Baxter et al., 2005; Runck, 2007).

Here, we focus on the role of an emerging, case-spinning caddis fly, *Leptocerus americanus* (Banks, 1899), in the sequestration, mobilization and transfer of lead within and out of a contaminated urban wetland system. We hypothesize that lead from contaminated sediments in the urban marsh are being assimilated by *L. americanus* in amounts proportional to the levels of contamination in the sediment. To further understand how lead may be accumulating in these organisms and whether the lead is exiting the system with emerging adults, we quantify how much lead is carried in the case versus the body of the larvae and track contamination into the body of the emergent adult. Using emerging insect biomass data of *L. americanus* from a marsh system and the known lead body burdens for the caddisfly we estimate the amount of lead that is potentially transferring from the contaminated aquatic environment into the surrounding terrestrial environment during emergence each year.

Materials and methods

Study site

The La Crosse River Marsh (LRM) is a 435 ha riverine wetland complex that bisects the City of La Crosse, Wisconsin (Fig. 1). During annual periods of high water it is hydrologically connected to the Mississippi River via the La Crosse River. Legacy trap shooting over the southeast end of the LRM from

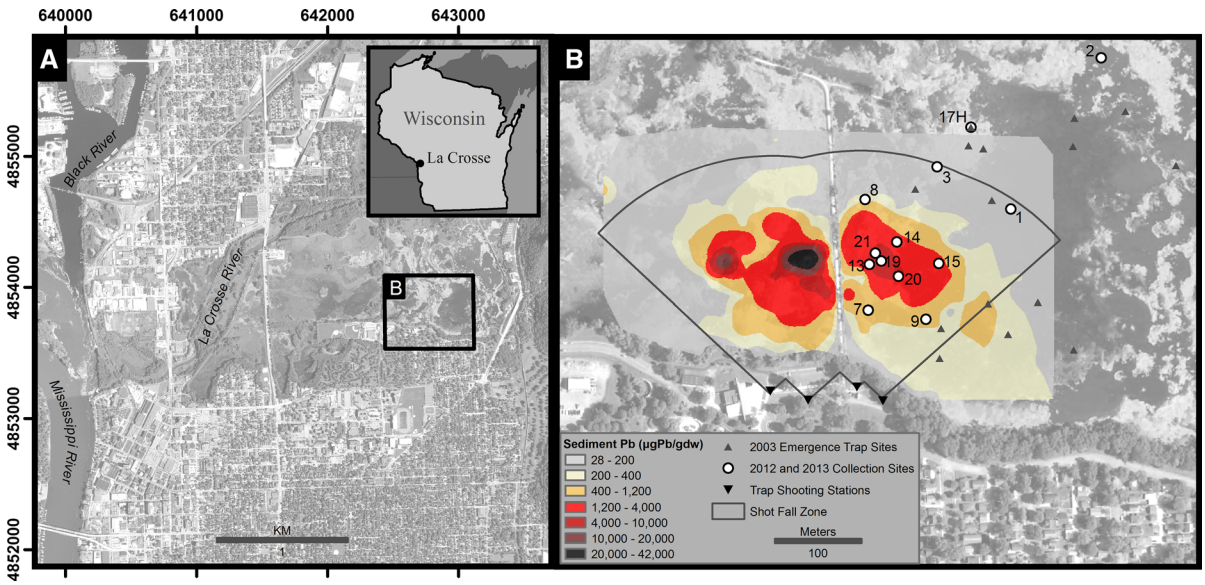

Fig. 1 **A** La Crosse River Marsh near the confluence of the La Crosse and Mississippi Rivers. **B** The 13.7 ha zone of surface sediment lead contamination in the La Crosse River Marsh (modified from Perroy et al., 2014) and location of collection sites. Each white circle shown is a site from which macroinvertebrate sampling occurred during 2012 and 2013 that contain background (sites 1, 2, 3), low (7, 8, 9), medium (13, 14, 15), or high (19, 20, 21) concentrations of lead within surface sediments. Each gray triangle indicates site locations where macroinvertebrate emergence traps were deployed in 2003

a 4-station range added lead shot to the system from 1929 to 1963, leaving behind lead shot and contaminated sediments within the shot-fall zone (Perroy et al., 2014). A recent three-dimensional analysis of the 13.7 ha shot-fall zone within the marsh (Perroy et al., 2014) found lead concentrations up to 26,700 µgPb/gdw in the surface sediments, far above the 400 µgPb/gdw level set by the U.S. Department of Health and Human Services (USDHHS) for lead in the soil of children's playgrounds (USDHHS, 2005). The probable effect concentration of 130 µgPb/gdw, above which adverse biological effects in freshwater systems are expected to be frequent, was exceeded in 8.9 ha of the marsh. Densities of lead pellets in the sediments reached up to 51,154 pellets/m^2, though the lead shot was typically located below 10–30 cm of the lead-contaminated flocculent sediment (Perroy et al., 2014).

Study organism: *Leptocerus americanus* Banks (1899)

The trichopteran, *Leptocerus americanus*, commonly referred to as the long-horned caddis fly (order Trichoptera, family Leptoceridae) has been found in waterbodies throughout the eastern and midwestern regions of North America with a range that extends north into Canada and south into Mexico (NatureServe, 2018). It is one of the most abundant macroinvertebrate species in the LRM composing up to 45% of emerging number of insects (Ogorek, 2003). This aquatic insect lives in a cone-shaped case spun from its own silk, projecting its upper body and legs forward out of the front of the case to swim or crawl. They measure up to 0.9 cm in length (including the case) and have long legs and setae that aid in swimming. *Leptocerus americanus* has a univoltine, holometabolous life cycle (Wiggins, 1977). They complete the larval stage in shallow waters of lakes and marshes (McGaha, 1952; Chilton, 1990). At the end of the larval stage, *L. americanus* attach their case to the stem or leaves of a submersed aquatic plant with silk, and they seal both ends of their case with a round silk cover. The larvae undergo pupation and the adult emerges as a terrestrial organism in late June or early July. Most species of Trichoptera of the family Leptoceridae are reported to be omnivorous as larvae (DeWalt et al., 2016). Several researchers have documented evidence of *L. americanus* feeding directly on the tissues of aquatic vascular plants

(McGaha, 1952; Balciunas & Minno, 1985), while Wiggins (1977) found the stomach contents of *L. americanus* contained mostly "fine particulate matter", a finding that would be consistent with a diet of periphyton found on macrophytes with which they associate (Balciunas, 1982).

L. americanus specimen collection and processing

Leptocerus americanus larvae were collected for lead analysis from the LRM during May and June, 2012, using standard D-dip nets with 250 µm mesh. Sites were selected for collection based on the interpolated model of surface lead concentrations in Perroy et al. (2014). Zones were selected to represent the range of lead concentrations within the LRM: background (0–200 mgPb/kg = Sites 1, 2 and 3), low (200–700 mgPb/kg = Sites 7, 8, and 9), medium (700–4000 mgPb/kg = Sites 13, 14 and 15), and high ($\geq$ 4000 mgPb/kg = Sites 19, 20 and 21) (Fig. 1). Within each concentration zone, sampling sites were randomly interspersed. But, because the contamination zones differ in size, the geographic distances between sites within each zone are shorter for the higher contamination zones and longer for the lower contamination zones (Fig. 1). Numbered flags were placed at each sampling location to simplify their relocation over time. The flag locations were georeferenced using a Trimble GeoXH 6000 differential GPS unit with sub-decimeter accuracy. Collections at each site were made from either a floating platform or a canoe, and sites were visited one to four times to collect sufficient mass for tissue analysis. To reduce the possibility of direct lead contamination from sediments, only organisms within the water column were targeted. During field collection efforts, organisms were separated as much as possible from macrophytes and other debris, and stored in marsh water until further processing was completed. Following collection, *L. americanus* specimens were brought into the laboratory alive and immediately sorted from other macroinvertebrates and macrophytes using the animal's phototactic behavior. A light was positioned to shine through one corner of a clear plastic collection container, drawing the specimens to the illuminated corner where they could be removed *en masse*. Additional individuals were picked from the plants and debris with stainless steel forceps. The *L. americanus* specimens were placed in Petri dishes of distilled water for 24 h to allow depuration. The dishes were cleared of all extraneous dirt and debris with the aid of a dissecting microscope, micropipette and stainless steel tweezers. For lead quantification at least 0.3 g of dried tissue is required, so samples were processed until at least 100 individuals (0.4 gdw) were acquired. After depuration, the specimens were removed from the Petri dishes, and stored frozen in aggregate in plastic 50 ml centrifuge tubes.

Collection of larval *L. americanus* was repeated in May of 2013 at one site within each level of contamination (sites 2, 9, 14, and 20) to analyze the partitioning of lead between the bodies and cases. Specimens were processed as described above to clean them and to allow gut clearance prior to lead analysis. A dissecting microscope, fine stainless steel tweezers and dissecting probes were used to separate the larvae from the cases and each partition was frozen in separate clean vials. Because larvae were partitioned more individuals were needed to provide sufficient biomass for lead analysis. Approximately 300 individuals from each lead contamination zone were separated into body and casing partitions and then aggregated as a single sample for lead analysis.

In 2013 adult *L. americanus* were captured for lead analysis using emergence traps placed at collection sites 1, 2, 7, 9, 14, 15, 19, and 20 with two sites located in each lead concentration zone: background, low, medium, and high. A ninth trap was placed at a background level lead site overlapping a collection location that was also sampled for emergence in 2003 (Site 17H, Fig. 1) outside the area of contamination. The emergence traps were four-sided pyramid-shaped frames constructed of 2.5 cm diameter PVC pipe measuring 1 m on a side (covering an area of 1 m^2 at the base). The traps were covered with a fiberglass window screen on all four sides and fitted with floats. An open section of PVC pipe (5 cm diameter) was fitted vertically at the apex of the pyramid with a small collection jar placed upright in the external end of this pipe. Traps were deployed and anchored in place for a period of 4 weeks, during which time the traps were emptied three times a week. Adult *L. americanus* were collected from the jars and the sides of the trap with a battery powered vacuum suction or forceps and transported back to the laboratory in glass jars. At the lab, adult *L. americanus* were counted, dried, ground and weighed with the same protocol used for the *L. americanus* larvae. Only emergence traps from

sites 3, 9, 15, 20 and 17 H captured enough individuals (approximately 200) to provide sufficient biomass for lead quantification.

Macroinvertebrate estimations

The numbers of non-*Leptocerus americanus* taxa captured during D-net sampling and emergence trapping were recorded and all specimens collected were identified to the nearest order. D-net sampling focused on pelagic and macrophyte sweeps and was not standardized for a specific surface area or time. Therefore, data are presented as relative abundances and were arcsine square root transformed prior to statistical evaluation using a two-way ANOVA in R (2013). Emergence traps captured all emerging insects during 24 h sampling periods and were sampled three times per week for a 4-week period (during the annual peak emergence time) during the summer of 2013.

Lead analysis

Surface sediment (0–5 cm) samples were collected at 412 sites throughout the potential shot fall zone and analyzed for lead via X-ray florescence by Perroy et al. (2014). A 1 m resolution raster model of surface lead concentrations was interpolated from the georeferenced sediment samples using the natural neighbor tool within ArcGIS. The extract values to points tool within ArcGIS was then used to estimate the surface sediment lead concentration for each macroinvertebrate sampling site from the flag's GPS coordinate and the raster model.

All samples of *L. americanus*: (a) whole larvae, (b) larval casings, (c) larval bodies and (d) adult bodies were processed as aggregates of collected individuals. Frozen specimens were thawed and dried at 35°C for 48 h. Samples were homogenized using a ceramic mortar and pestle, weighed and placed in 60 ml plastic centrifuge tubes. To avoid cross-contamination during preparation, the samples were processed in order from the least to most contaminated sites and the mortar and pestle were thoroughly cleaned between samples. Lead analyses for larval and adult samples were conducted at the Wisconsin State Lab of Hygiene Ultra Trace Elements and Metals Testing Facility (Madison, Wisconsin) using Krynitsky's nitric acid digestion protocol (Krynitsky, 1987) for recovery of metals from organic tissue. Whole larvae samples were analyzed in October, 2012. Samples of larval bodies, larval cases and adult bodies were analyzed in February, 2013. Methods adhere to EPA SW846-Method 6010B (https://www.epa.gov/sites/production/files/documents/6010b.pdf). Samples were housed at 4°C until processed. Dried tissue (500 mg) was dissolved in concentrated nitric acid and 30% hydrogen peroxide and digested following the EHD METALS Method 750.1 (WSLH). Lead analysis was completed on a Perkin Elmer 5300 Dual View Inductively Coupled Plasma Atomic Emission Spectrometer (WSLH ICP EHD Metal Methods 400.2, 2013). Quality assurance and quality control procedures for the WSLH are outlined in WSLH SOP ICP 400.2 (2013). Quality control included blank, duplicate, metal spike detection and certified reference material analyses. Duplicate tissue samples had a relative standard deviation (rsd) of 2% and 4% in 2012 and 2013, respectively. Spike recovery of standard reference material was 92.2% and 95.9% in 2012 and 2013, respectively. Calibration blanks were analyzed immediately following calibration and after every ten samples. All calibrations fell within ± one-half the limit of detection (LOD for Pb = 0.2 µg/g) and QA/QC was verified for all samples confirming a limit of quantification (LOQ) of 0.6 µg/g. All sample measurements exceeded both the LOD and LOQ limits.

Estimation of lead flux out of the LRM in emerging adult *L. americanus*

Adult *L. americanus* emergence throughout the LRM had previously been thoroughly quantified (Ogorek, 2003) at 15 locations across a gradient of water depths (0.18–0.82 m) and submerged aquatic vegetation levels. Those previously unpublished data, presented here, provide the necessary information to develop a whole marsh lead flux model. Since the La Crosse River stage and water levels are managed to minimize flood risk and limited or no modification has taken place in the La Crosse River Marsh over the last 20 years the measured emergence values from 2003 are used to model lead transfer values that likely encompass transfer rates for the past and present marsh environment. The 2003 emergence traps were similar in design to the 2013 traps, but measured 0.5 m on each side and covered an area of 0.25 m^2. To capture and immediately preserve the emerging adult insects, each animal was funneled into a jar at the top

of the trap that was filled with 70% ethanol. Traps were placed on the water for 37 days in June and July 2003, covering the complete 32 day emergence period in 2003. Emerging adult *L. americanus* were retrieved daily from the traps by transferring the ethanol and insects into a separate clean jar. The collection jars were then rinsed, refilled with ethanol and reattached to the top of the trap. Following collection, the captured insects were counted, dried and weighed.

Daily measurements of male and female *L. americanus* emerging from the 15 traps sampled over the complete 32 day emergence period in 2003 were used to generate cumulative estimates of emergence throughout the lead shot affected area. One thousand samples with $n = 15$ (i.e., 15 traps) were generated for each day of the emergence period by randomly resampling (with replacement) the original 2003 daily emergence data in a bootstrap simulation (R, 2013). Modeled values represent low (25%), intermediate (50%) and high (75%) quartiles of the measured potential for emergence of *L. americanus*. Bootstrapped daily values were used to generate cumulative mean and standard deviation estimates for each quartile of the number of *L. americanus* emerging per square meter for the full summer emergence period. Cumulative count estimates were converted to grams dry weight. To be conservative, we used a measured dry weight of 0.00044 g dry weight per individual, which represents the lower 25th quartile of adult weight for this species within the marsh. The exponent equation best fit to the relationship between sediment and larval contamination was applied to each 1 m cell within the 13.7 ha marsh area of contamination using the raster calculator tool within ArcGIS (Fig. 1, Perroy et al., 2014). The mean and standard deviation of lead content transferring to larvae in the marsh was calculated (ESRI, 2016). By multiplying the mean sediment to larval transfer observed in marsh sediments with the ratio between larval and emerging adults we calculated the mean lead transfer from sediments to emerging adults taking place within the 13.7 ha LRM study area. Lead flux out of the marsh in emerging *L. americanus* equals the total adult emergent biomass estimated for each quartile multiplied by the transfer estimate.

Statistical analysis

Lead concentrations in sediments and aggregate whole larvae samples were compared statistically between sediment contamination zones designated as background, low, medium and high using ANOVA on values that were normalized using a natural log transformation (R, 2013). Within contamination zones, lead levels were highly variable, especially in areas of high contamination. Therefore, we also analyzed the linear relationship between sediment and larval concentrations using linear regression (R, 2013). Prior to statistical analyses the data were transformed using a natural log transformation. Paired *t* tests were run in R (2013) to test for significant differences in lead concentrations between (1) cases and caseless larval bodies and (2) caseless larval bodies and emergent adult bodies of *L americanus*.

Results

Leptocerus americanus was the most abundant aquatic insect captured in all pelagic and macrophyte sweeps, and the only species that differed significantly in relative abundance from the other taxa (Fig. 2, main effect of taxa $F = 138.22$, $P_{df = 5} < 0.0001$; Tukey's $P < 0.0001$ for all *L. americanus* comparisons). *Caenis* (Ephemeroptera), *Neoplea* (Heteroptera) and Chironomidae (Diptera) were the next most abundant taxa but the numbers captured varied (even within contamination zones) and abundances were an order of magnitude less than *L. americanus*. Diptera and *L. americanus* were captured in higher abundances than all other taxa in emergence traps (Fig. 3). Diptera emergence was an order of magnitude higher than all other taxa and *L. americanus* emergence was an order of magnitude higher than all other non-Diptera taxa. Diptera abundances were very high, but the individuals captured were very small in size (approximately 0.5–3 mm).

The average sediment lead concentration measured in the selected sites of each contamination zone differed significantly ($F = 81.1$, $P_{df = 3} < 0.0001$, Tukey's HSD < 0.03 for all comparisons). Larval *L. americanus* lead concentrations were significantly lower in the background zone than in either the medium or high contamination zones ($F = 8.809$, $P_{df = 3} = 0.0129$, Tukey's < 0.05), but did not differ

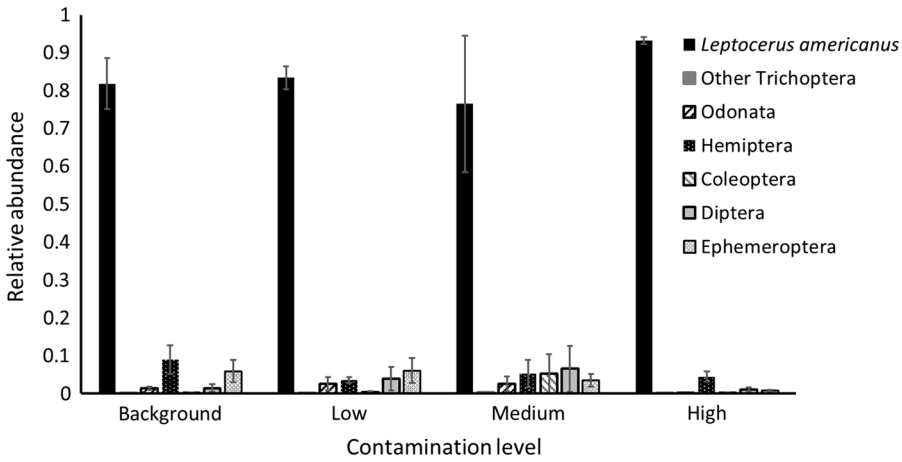

Fig. 2 Relative abundance (± SE) of *L. americanus* compared to all other major orders of macroinvertebrates captured in pelagic and vegetative D-net sweeps within the La Crosse River Marsh across contamination zones: background ($N = 3$), low ($N = 2$), medium ($N = 3$) and high ($N = 3$)

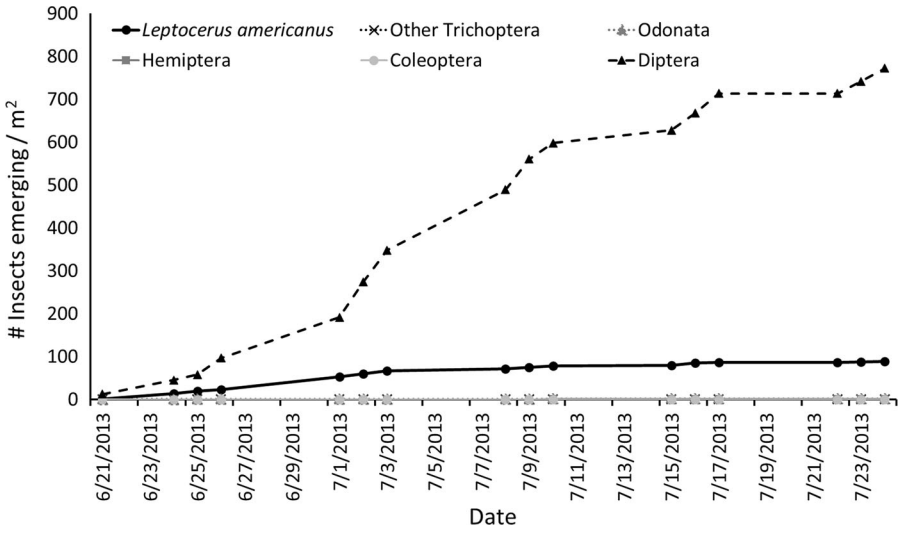

Fig. 3 Emergence of *L. americanus* compared with all other major orders of macroinvertebrates ($N = 9$ emergence traps). Each line represents the cumulative numbers captured/square meter in periodic (3 days/week) sampling intervals throughout the summer of 2013

significantly from larval concentrations measured in the low contamination zone (Fig. 4A). There is a significant positive relationship between the calculated concentrations of lead in the sediment and the measured concentration of lead in the whole larvae (bodies and cases) of *L. americanus* ($R^2 = 0.7593$, $P_{df = 8} = 0.001$) (Fig. 4B). Lead is being assimilated by this macroinvertebrate population at levels directly proportional to the concentration of lead in the underlying sediment.

Lead was partitioned unequally between the cases and bodies (Fig. 5). Concentrations of lead in larval cases of *L. americanus* were 11.93 ± 2.26 (SE) times higher than in the larval bodies extracted from those cases (paired $t = -3.124$, $P_{df = 3} = 0.052$). The concentration of lead in larval bodies of *L. americanus* collected within each zone of contamination did not significantly differ from lead concentrations found in emerging adults from the same contamination zones (Fig. 6) (paired $t = 0.253$, $P_{df = 4} = 0.813$). The ratio of lead in the larval bodies versus the emerging adults is 1.19 ± 0.18 (SE), indicating that 100% of lead is transferring from larval bodies to emergent adult bodies during metamorphosis.

Cumulative *L. americanus* emergence across trap locations in 2003 ranged from 108 to 9048 individuals/m^2 (Fig. 7A). In trap locations that showed highest cumulative emergence values an early burst of

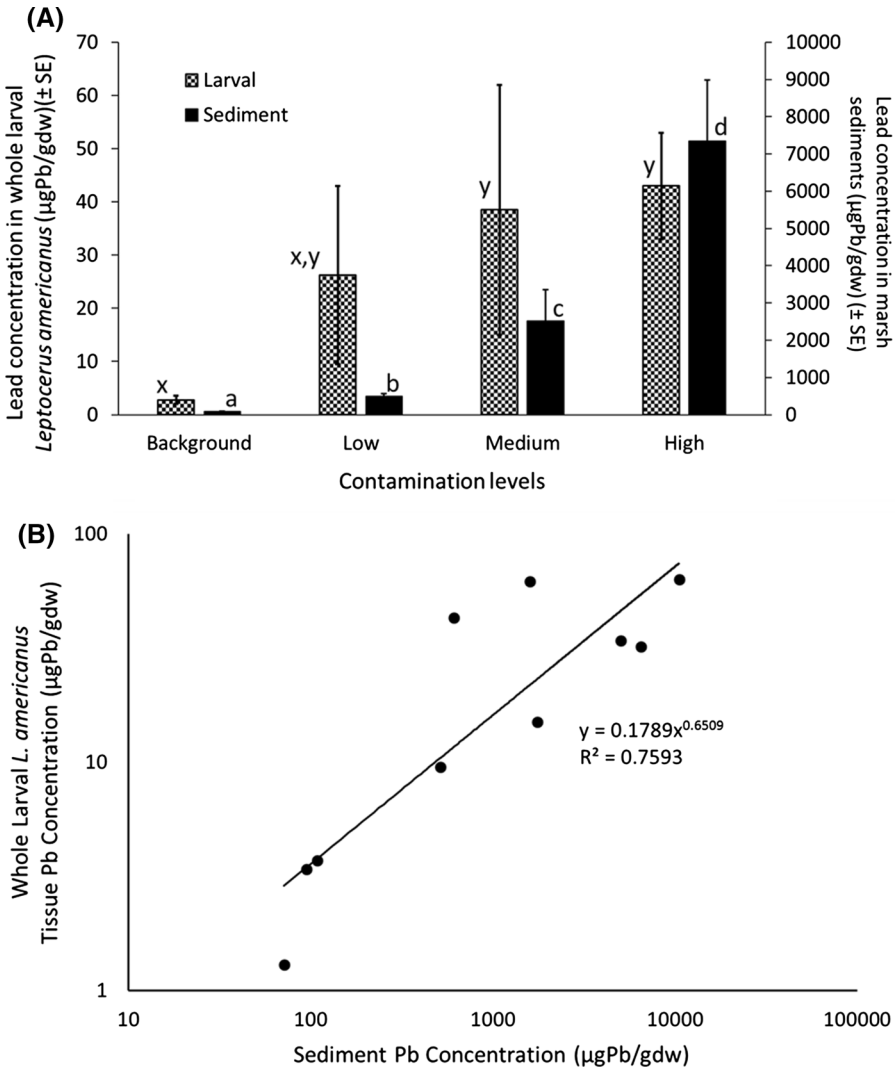

Fig. 4 **A** Average concentrations of lead (± SE) in the sediments and dried tissues of aggregated whole larval (bodies + cases) *L. americanus* within each sediment lead concentration zone (background [*N* = 3], low [*N* = 2], medium [*N* = 2] and high [*N* = 3]). Significant differences are designated by assigned letters (*x, y* or *a, b, c, d*); **B** linear regression of the measured sediment lead concentration at each site by the lead concentration of an aggregate sample of all larval *L. americanus* taken at each site throughout all zones of contamination (background—high)

emergence was observed between June 13th and June 26th, 2003 (calendar days 164–178). A second burst of emergence was observed across most sites from June 29th to July 8th, 2003 (calendar days 181–191) (Fig. 7A). The first burst of emergence helps explain the large cumulative differences observed between trap locations. Males and females were captured in relatively equal number, but adult female biomass (0.57 mg dwt ± 0.01, n = 183) was significantly higher than adult male biomass (0.47 mg dwt ± 0.01, n = 173) (Fig. 8).

Bootstrap analysis yielded clear asymptotic accumulations of emergent adults with the greatest number of emerging adults observed from calendar days 165–185 (Fig. 7B), matching expectations based on measured values from the emergence traps in 2003 (Fig. 7A). The cumulative mean endpoints for the 25th, 50th, and 75th quartiles (415, 1204, and 3369 individuals/m^2, respectively) were then converted to grams dry weight (gdw) using the 0.00044 gdw/individual conversion (Table 1). When multiplied by the 13.7 hectare surface area where lead contamination is a concern, 25,106–203,777 gdw of *L. americanus* adult tissue emerges from the region each year (Table 1). The potential for lead transfer from LRM sediments ranged from minimum predicted larval concentrations of 0.00166 mgPb/gdw in low contamination zones to 0.18171 mgPb/gdw in highly contaminated regions. When averaged across the overall area of concern in the LRM, the mean lead

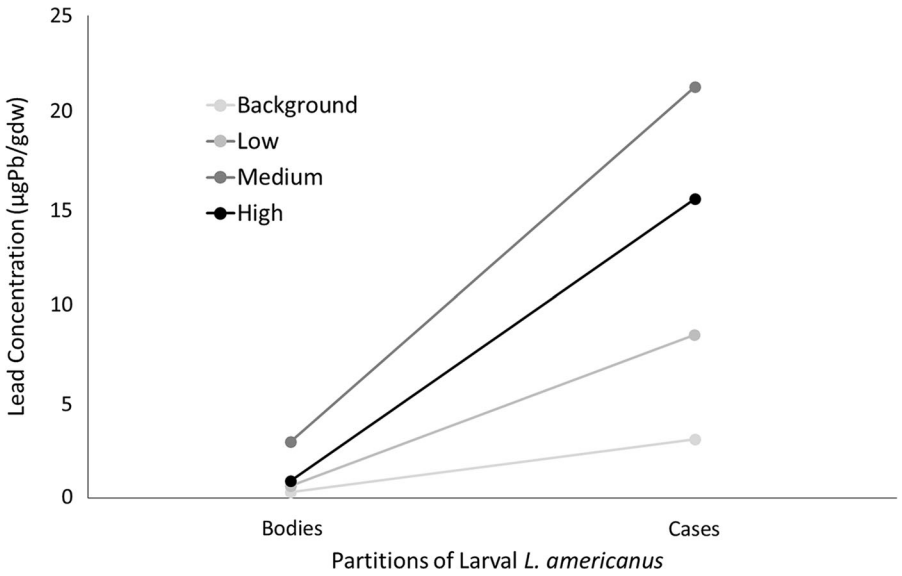

Fig. 5 Concentration of lead in caseless larval bodies and their separated larval cases of *L. americanus*; each data point represents the lead concentration measured in a single aggregated sample of > 200 dried caseless larval bodies or cases collected from each zone of contamination (background—high)

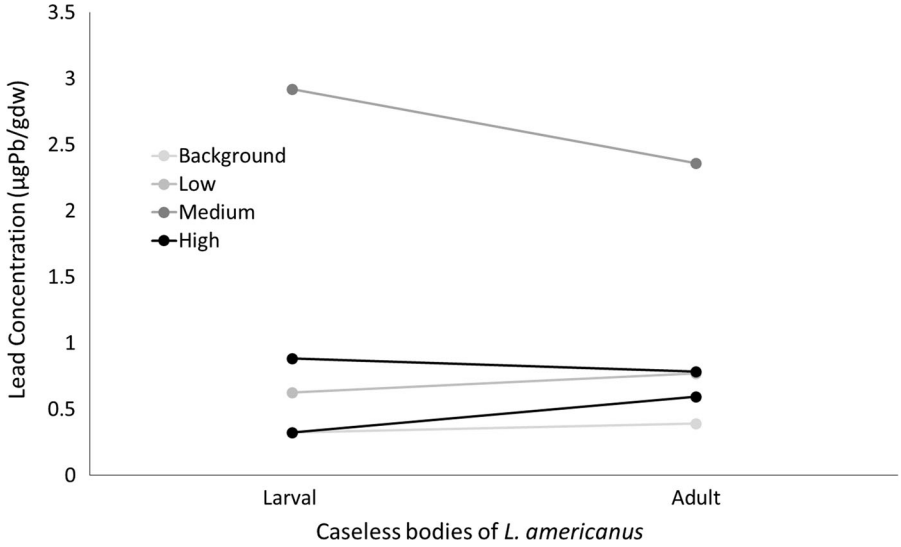

Fig. 6 Concentration of lead in the tissues of caseless larval bodies and the tissues of emerging adults of *L. americanus*; each data point represents the lead concentration measured in a single aggregated sample of > 200 dried larval or adult bodies collected from each zone of contamination (background—high)

transfer from sediments to larvae is 0.01037 mgPb/gdw larval *L. americanus*. Only 7.7% of the lead in larvae is retained in the emerging adult (Fig. 5). When combined, these numbers support that adult *L. americanus* from the LRM carry an average of 0.0008 mgPb/gdw out of the aquatic habitat upon emergence. Conservatively, the total flux of lead out of the LRM surface is predicted to range from 20 to 163 mg annually (Fig. 9, Table 1).

Discussion

Understanding the mechanisms of contaminant mobilization through urban wetlands is crucial to ensure wise management choices when conserving these natural areas. The degradation of lead shot and the bioavailability of lead to biota in a wetland environment is dependent on temperature, pH, hardness, salinity, redox potential, and the presence of organic matter (USEPA, 1984; Hui, 2002; Malaj et al., 2012).

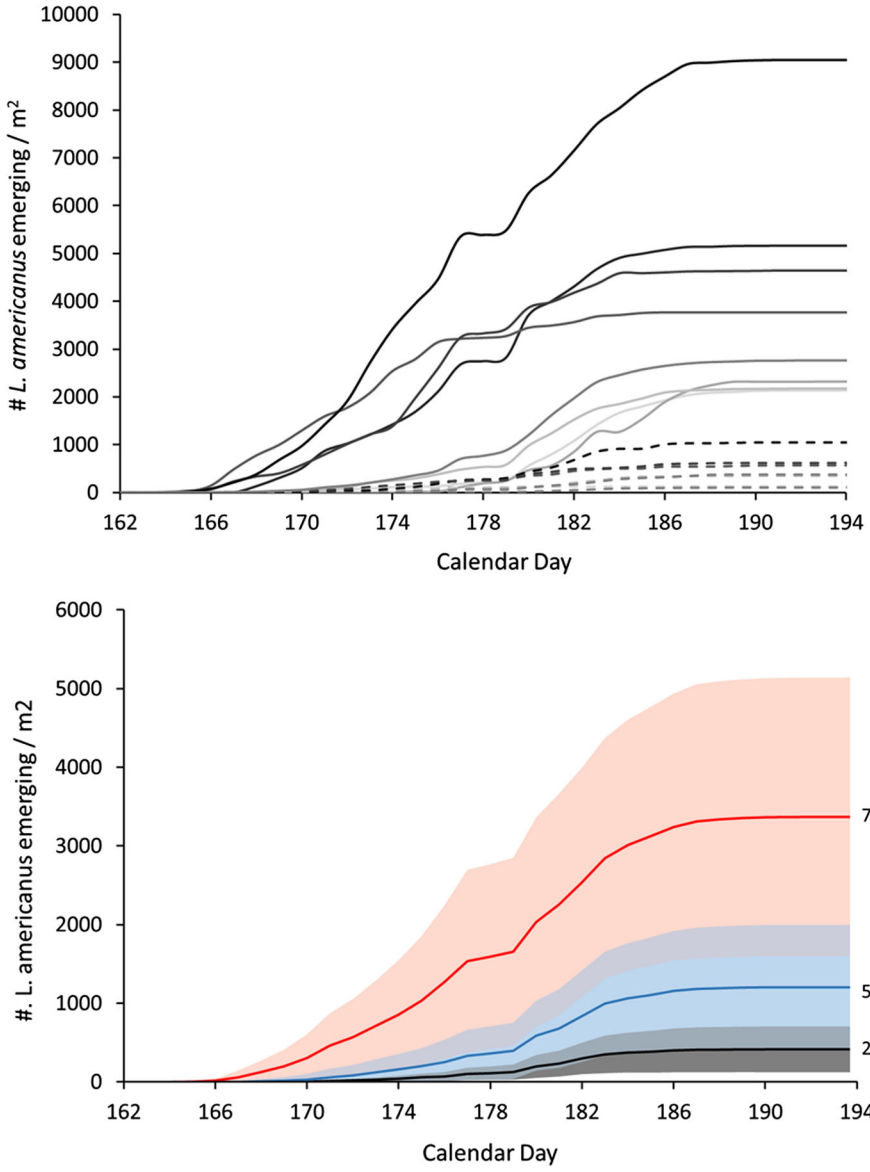

Fig. 7 **A** Total *L. americanus* cumulative emergence from 15 independent trap locations throughout the La Crosse River Marsh habitat over a 32 day period from mid-June to mid-July 2003. **B** Simulated accumulation of daily emergence from bootstrap resampling of daily values that represent 25th quartile, 50th quartile, and 75th quartile emergence estimates for the marsh. Lines represent average predicted emergence for each quartile ± SD (shaded areas)

Because dissolved metals are the most bioavailable (Sola & Prat, 2006), contaminated aquatic habitats with lower pH and lower salinity are likely to have more bioavailable lead (Li et al., 2013). One aspect that has not been extensively considered is the role of macroinvertebrates in the mobilization and distribution of lead from contaminated sediments. Our work demonstrates that macroinvertebrates play a crucial role in the distribution and biotic transfer of lead from contaminated sediments, both within the system and likely to adjacent terrestrial ecosystems.

Concentrations of lead found in *L. americanus* larvae correlated with the amount of lead within the sediments where they were collected ($R^2 = 0.76$) similar to other reports that represent a wide range of habitats and taxonomic groups (Table 2). Lead is a divalent metal and replaces calcium in basic cellular processes. This association could explain the similar transfer ratios observed across groups of animals in which these processes are conserved. Amyot et al. (1994) and Tessier et al. (1984) found strong correlations between the tissue lead concentration of mollusks and the concentration of lead found within

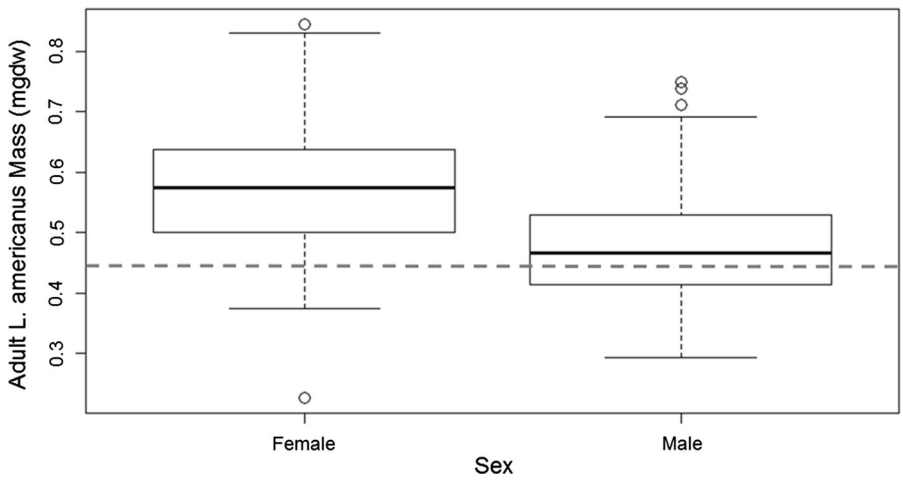

Fig. 8 Box plots of adult dry weights for both sexes of *L. americanus* emerging from the La Crosse River Marsh in 2003. The dashed line represents the 25th quartile weight value for all (♀ + ♂) sampled adults (n = 356)

Table 1 Total emergence and flux estimations (± SD) for the entire 13.7 ha region of contamination concern in the La Crosse River Marsh

	Multiplier	Quartile		
		25th	50th	75th
Contaminant area (m^2)	137,453.00			
Estimated adult *L. americanus* emerging (gdw/m^2/year)		0.18 ± 0.13	0.53 ± 0.35	1.48 ± 0.78
Total emerging adult *L. americanus* biomass (gdw/year)		25,105.79 ± 17,868.89	72,803.36 ± 48,108.55	203,776.82 ± 107,213.34
Lead transfer from sediments to emerging adults (mgPb/gdw)	0.00080			
Total lead flux emerging in adult *L. americanus* (mgPb/year)		20.05 ± 14.30	58.13 ± 38.49	162.71 ± 85.77

certain chemical fractions of the sediment. Axtmann & Luoma (1991) successfully correlated the lead concentration of fine-grained sediment (< 60 µm) of the Clark Fork River in Montana with lead tissue concentrations of resident *Hydropsyche*. Despite the fact that they represent studies of various organisms and environments, a common range for the coefficients of the correlation between concentrations of lead within sediments and tissue falls between 0.54 and 0.73. Differences in bioavailability may result from variations in the size and organic content of the sediments (Timmermans et al., 1989; Van Hattum et al., 1991; Bervoets et al., 1997) or the result of the extent of the contamination (Dukowska et al., 2012), differences in correlations could also be caused by variations in where the lead is held within in the organism.

Trichopterans that spin their own cases such as *L. americanus* have both the case and body as potential surfaces for the adsorption of lead. The proportion of lead in the casings of our specimens was approximately eleven times higher than in the larval bodies, a proportion considerably higher than those reported elsewhere. In the wild, a portion of the total lead body burden in the aquatic insect is present in the content of the gut prior to digestion. To determine the actual amount of lead assimilated into the body tissues it is necessary to clear the gut contents of feces and undigested food before analysis for lead. Sola & Prat (2006) found that at least 71% of the total lead in the body was lost after 24 h of gut clearance and

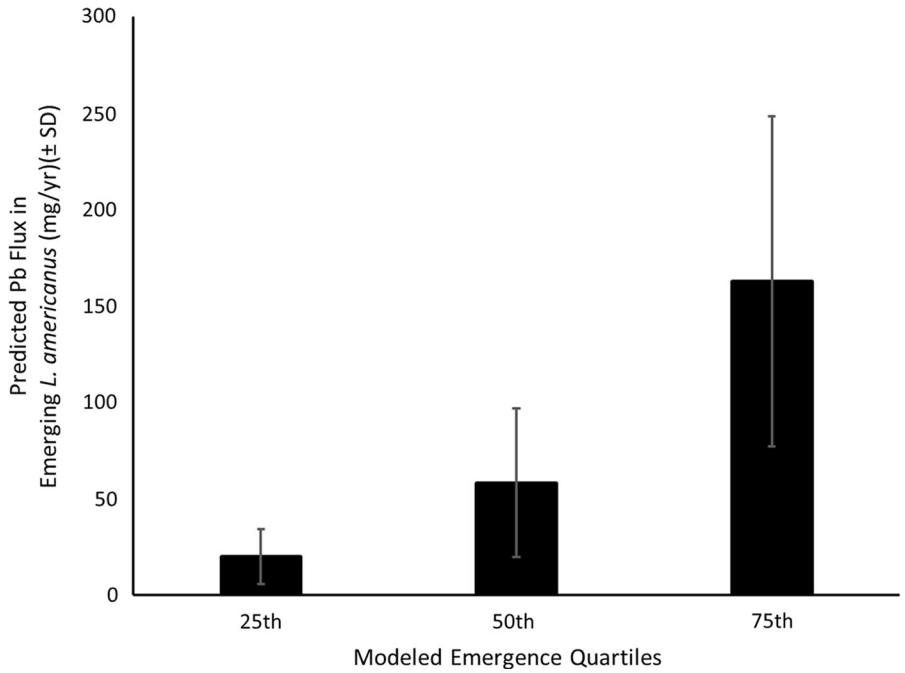

Fig. 9 Mean (± 1 SD) predicted total annual lead flux in emerging adult *L. americanus* in predicted low, medium and high emergence years

Table 2 Reported linear regressions between lead in the sediment and lead in the tissues of macroinvertebrates for studies of lead accumulation by macroinvertebrates in contaminated aquatic environments

Study	System	Organisms	R^2	*P* value
Sola & Prat (2006)	River	Trichopteran Hydropsyche	0.6	< 0.05
Farag et al. (2007)	Stream	Aquatic insects	0.67	< 0.05
Tessier et al. (1984)	Lakes	Bivalve	0.72	*P* not given
Amyot et al. (1994)	Fluvial lakes	Mollusks	0.73	
Luo et al. (2013)	Wetlands, streams	Fish	0.61	
	Ponds	Snails	0.29	
Taylor & Maher (2014)	Laboratory w/estuarine sediments	Bivalves	0.72	< 0.0001
Axtmann & Luoma (1991)	river	Hydropsyche	0.54	< 0.005

Tochimoto et al. (2003) estimated that 82–91% of the lead in the body of collected macroinvertebrates was in the gut contents. Since our specimens were depurated for at least 24–48 h, and therefore, presumably devoid of gut contents, larval *L. americanus* in the marsh could potentially be passing a lead burden on to predators that is approximately 70–90% higher than the concentration in the processed specimens. The proportion of lead adsorbed onto or incorporated into the tissues of the exoskeleton or casing of an aquatic insect is an important indicator of the amount of lead that will remain in the aquatic system following molting or metamorphosis. This proportion varies widely between species (Hare et al., 1991). In laboratory experiments of *Chironomus riparius* (Meigen, 1804) exposed to 10 mgPb/l, Timmermans et al. (1992) estimated that the amount of lead adhered to the exoskeleton is insignificant compared to the total

amount of lead found in the body. Hare et al. (1991) collected and analyzed the shed exuvia of *Hexagenia limbata* (Serville, 1829) and found that the lead burden in the exuvium was about the same as in the body (excluding the gut and organs) of the insect. In field studies of *C. riparius*, Krantzberg & Stokes (1988) attributed as much as 75% of the body burden of lead to surface adsorption. When comparing taxa, Hare et al. (1991) found that Ephemeroptera, Odonata, and Diptera had relatively larger proportions of lead adsorbed externally than other orders. *L. americanus* uses the casing as a pupa, which means that 90% of the total body burden of lead is left behind in the organic component of wetland sediments. Detritivores can then reintroduce the lead from these structures to the food chain (Besser et al., 2001). This could partially explain why surface sediments within the marsh contain significant concentrations of lead despite the fact that most of the lead shot remains buried in deeper sediments.

Aquatic insects occupy multiple intermediate trophic levels in wetlands and are essential prey for larval and adult fish, amphibians, birds and some small mammals. Heavy metals, including lead, are passed to higher trophic levels via aquatic insects and other macroinvertebrates (Luo et al., 2013). *Leptocerus americanus* is the most abundant and likely highest biomass invertebrate in the pelagic region of the La Cross River Marsh. It carries a proportionally larger lead burden in its manufactured casing, which in the pelagic food web is likely to be consumed when *L. americanus* is preyed on by fish, amphibian or birds that feed underwater. Experimental tests are needed to directly measure how *L. americanus* bodies and casings facilitate the transfer of lead through the pelagic food web. Even though *L. americanus* is consistently the most abundant species captured in the pelagic sweeps, densities are variable across LRM habitats (Fig. 7A) and density differences may be attributable to habitat factors such as macrophyte cover and depth (Ogorek, 2003). The benthic food web likely includes an entirely different suite of dominant macroinvertebrates such as Chironomidae (abundant in Fig. 3) and mussels that more closely interact with the highly contaminated sediments. While we have clearly outlined lead contamination levels in multiple life stages, and aquatic to aerial transfer of lead for *L. americanus*, many additional habitat variables and organisms must be considered to model lead trophic transfer for the full ecosystem of the LRM.

There is an increasing number of studies demonstrating the transfer of nutrient subsidies from aquatic to terrestrial ecosystems (Sullivan & Rodewald, 2012). Terrestrial areas adjacent to lakes, wetlands, rivers and streams can benefit significantly from nutrient subsidies from emerged aquatic insects. Emerging aquatic insects account for as much as 25–100% of the food consumed by riparian populations of bats, birds, lizards or spiders (Baxter et al., 2005). The emerging adults of *L. americanus* present in the LRM carry a lead burden of up to 0.00436 µg each. Our model (Fig. 7, Table 1) shows that this one species over one season of emergence could possibly transfer about 163 mg of lead to adjacent systems. While this value is relatively low compared to the amount of lead available in the sediments, we counted at least sixteen other species of emergent aquatic insects residing in this zone that could also be carrying a lead burden away from the marsh (Ryan, 2015). It is unlikely that this level of transfer would cause a drastic immediate impact to regions surrounding the LRM, but emerging insects have the potential to move large portions of nutrients and contaminants from aquatic systems. In studies in Iceland, Gratton et al. (2008) found that chironomid midges emerging from Lake Myvatn contributed a median of 2500 kgdw ha^{-1} $year^{-1}$ of biomass to the adjacent terrestrial ecosystem. Along with the subsidies come the contaminants that the insects carry (Runck, 2007; Walters et al., 2008; Raikow et al., 2011). A diet consisting primarily of aquatic insects with an average lead concentration of 2.3 µg g^{-1} was associated with lead transfer from contaminated sediments to tree swallows sampled along contaminated reaches of the Arkansas River in Colorado (Custer et al., 2003). In quantifying the amount of mercury exported to the terrestrial environment, Runck (2007) estimated that emerging chironomids removed 4.1 g of inorganic mercury per year from a 2.1 km section of a contaminated stream in Tennessee. Raikow et al. (2011) estimated that adult chironomids emerging from a lake exported 41 ± 29 g of PCB per year to its riparian zone. Taken together, this suggests that over many generations the cumulative transfer could be a substantial dispersion of lead away from contaminated sediments to surrounding habitat.

Water purification, removal and sequestration of contaminants are commonly listed as wetland ecosystem functions. Indeed, many wetlands are constructed for those express purposes. Sequestration implies that a contaminant is static, but it is clear that the lead contamination in the La Crosse River Marsh is dynamic, and while most of the lead being mobilized from the sediment is temporarily sequestered within the sediment and in the tissues of resident organisms, some of it is finding its way beyond the aquatic habitat and into the terrestrial ecosystem. Further studies should be done to determine if the lead exposure is detrimental to consumers like birds and bats that depend on emerging insects for food.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

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