COMMUNITY COMPOSITION, POPULATION DEMOGRAPHICS, AND TISSUE METAL CONCENTRATIONS IN BATS (CHIROPTERA) AT TAR CREEK SUPERFUND SITE: WITH FOCUS ON USE OF A BEHAVIORAL FLIGHT CAGE ASSAY

By

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Abstract:

My goals were to: 1) determine community structure and population demographics of Chiroptera within Tar Creek Superfund Site, an area contaminated with lead (Pb), zinc (Zn), and cadmium (Cd), 2) analyze liver, kidney, and hair for metal concentrations in bats from contaminated and reference sites, and 3) develop a behavioral assay to identify differences in flight ability in bats from the contaminated site and a reference site. I hypothesized that: 1) the bat community from the contaminated site would have lower diversity and evenness, 2) bats collected within the contaminated area would have higher levels of Pb, Zn, and Cd in tissues, and 3) lower maneuverability and willingness to fly compared to bats from reference sites. Mist netting occurred June-September 2012 and May-September 2013 at two sites within Tar Creek Superfund Site (TC, BC) and two reference sites within Oologah Wildlife Management Area (PLM, PAN). Both communities were dominated by Eastern Red Bats (*Lasiurus borealis*). Contaminated sites had lower, but not significantly different diversity (Simpson's *D*). Kidney Zn concentrations were significantly different for males from TC compared to BC males ($p = 0.02$), but were not significantly different from reference sites. Hair Zn concentrations in PAN males was significantly different compared to PLM males ($p = 0.04$), but not compared to contaminated sites. Finally, hair Pb concentration was significantly higher in females from BC than from TC ($p = 0.005$), and hair Pb concentrations in PAN males were significantly different compared to PLM males ($p = 0.0074$). Bats from PLM showed predictably strong relationships between flight time and obstacles dropped ($R^2 = 0.6945$), and flight time and movement between sections of the cage ($R^2 = 0.9758$), whereas bats from the contaminated area showed weaker relationships between flight time and obstacles dropped ($R^2 = 0.0004$) and flight time and movement between sections of the cage ($R^2 = 0.2422$). I demonstrated that a noninvasive behavioral assay can distinguish differences in flight ability in bats from a contaminated site compared to a reference site, showed differences in population demography, and provided tissue metal concentrations for bats from the Tar Creek Superfund Site.

PREFACE

The first chapter of this collective work provides a literature review of relevant history of the focus area, natural history of bats within this area, and the importance of bats as bioindicators. The second chapter is written in the format appropriate for submission to *Environmental Toxicology and Chemistry* and covers community structure, population demographics of *Lasiurus borealis,* and tissue metal concentrations. The third chapter is written in the format appropriate for submission to *Environmental Pollution* and describes the behavioral flight assay, relating results to tissue metal concentrations.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

BACKGROUND

Between the late 1800s and the 1970s, a 500 mi^2 area known as the Tri-State Mining Region, which includes portions of southeastern Kansas, southwestern Missouri, and northeastern Oklahoma, was heavily mined for lead (Pb) and zinc (Zn; USFWS 2000). In Oklahoma, Pb and Zn ore was extracted from within the Boone Formation (also known as the Boone Aquifer). This aquifer sits approximately 500 ft. above the Roubidoux Aquifer, which is the major source of dinking water in this area (USEPA 2005). Ore was removed via room-and-pillar mining techniques, meaning large underground cavities often 100 ft. high were cleared of ore leaving pillars to support the ceilings (USEPA 1994). At the peak of mining activities the annual extraction of Pb and Zn reached 130,410 tons and 749,254 tons, respectively. Because of the location of the mines within the aquifer, ground water had to be continually pumped out (USEPA 2005).

Under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) passed by Congress in 1980 the Environmental Protection Agency (EPA) has the authority to identify parties responsible for and enforce clean up of abandoned hazardous waste sites by placing sites on the National Priorities List (NPL). The addition of Tar Creek Superfund Site to the NPL was initially proposed in 1981, and formally occurred in 1983 (USFWS 2000). This 40 mi^2 area, within the Tri-State Mining District, is located in northeastern Oklahoma and impacts communities including Picher, Quapaw, Cardin, Commerce, and Miami (ATSDR 2004; USEPA 2000, 2010). In the 1970s, when mining operations ceased an estimated 100,000 acre-feet of underground cavities had been created. Exposed sulfide minerals oxidized while pumping occurred; however with the termination of mine operations came the cessation of water pumping. Ground water refilled the aquifer creating acid mine water, which continues to flow from the mines today (USEPA 2000, 2005). Chat currently is used commercially as construction aggregate. Records of chat sales between 1970 and 1980 show approximately 8.2 million tons of chat were sold from Ottawa Co., leaving behind 83 major piles and 33 minor piles in the area (OWRB 1983).

In 1994 the EPA was informed by the Indian Health Service that blood Pb levels were above CDC limits for children (10 ug/dL) in 65 of 192 children tested. Based on these findings the EPA began remediating residential sites with soil contamination levels exceeding 500 ug/g Pb and 100 ug/g cadmium (Cd) within 0-12 inches in depth, and within 1000 ug/g Pb and 100 ug/g Cd within 12-18 inches in depth. Public areas such as parks and schools were tested only to a depth of 12 inches (USEPA 2005). The Tar Creek Superfund Site area is not homogeneously contaminated, forcing the EPA to test multiple sites, and only remediate areas exceeding these levels. Of the locations tested 61% public sites and 65% residential sites exceeded these levels and contaminated soil was excavated and clean topsoil was filled in (USEPA 2005). In 2006 the EPA established the Lead Impacted Communities Relocation Assistance Trust (LICRAT) to conduct a voluntary buy out of properties within the center of the Tar Creek Superfund Site. To date several hundred properties have been purchased and remediated or demolished and occupants relocated (USEPA 2010), with finalized numbers likely reported in the fifth five-year review in 2015.

The EPA identified major sources of exposure for humans as including soil, mine tailings, ingestion of homegrown produce and tap water, airborne dust, and use of biota by Tar Creek area tribal populations (ATSDR 2004). Several of these pathways also have the potential to harm animal species living in this area. For example, although humans can avoid drinking from standing water in this area, most other species have no alternative, leading to a common source of contaminant exposure for wildlife. Pb, Zn, and Cd were identified as the primary contaminants, but Pb was the only chemical of concern to humans. Although the EPA was focused primarily on human risk within this area the U.S. Fish and Wildlife Service focused on a restoration plan for migratory birds, the endangered gray bat, and threatened Ozark cavefish and bald eagle. My thesis research also focuses on potential impacts to wildlife species.

LITERATURE REVIEW

Wildlife Toxicology and Tar Creek Superfund Site.—Wildlife toxicology, an important branch of ecotoxicology, focuses on reactions to contaminants in wildlife, rather than humans or laboratory models (Newman 2010; Rattner 2009). Unfortunately, research in this focus is primarily retroactive, trying to determine effects of anthropogenic sources of contamination on wildlife in a given area. Rattner (2009) reviews the history of wildlife toxicology beginning with documentation of Pb poisoning of waterfowl over 80 years ago due to ingestion of spent Pb shot (Phillips and Lincoln 1930).

Past research at Tar Creek Superfund Site has included studies of invertebrates, fish, birds, reptiles, rodents and deer (Beyer et al. 2004; Conder and Lanno 1999; Hays and McBee 2007; Phelps and McBee 2009, 2010; Schmitt et al. 2005, 2006); however, no research has yet investigated exposure or effects of metals on bat populations at Tar Creek Superfund Site.

Schmitt et al. (2005, 2006) conducted research in the Tri-State Mining District of northeastern Oklahoma to determine levels of contamination of fish and crayfish. They documented levels of Pb, Zn, and Cd contamination in species that had not been studied previously and used aminolevulinate dehydratase (ALA-d) activity as a biomarker for Pb exposure. Elevated blood Pb concentrations were detected in all fish samples. In Oklahoma, concentrations were highest in common carp (*Cyprinus carpio*) and lowest in crappie (*Pomoxis annularis*). Schmitt et al. (2005) noted that in the Tri-State Mining District, concentrations of Pb in blood of fish were higher than reference sites but lower than levels found in fish taken from Big River, a contaminated site in eastern Missouri.

Effects of Pb on fish appear to vary by species, spanning from little or no effect to behavioral changes and neurological symptoms. Fathead minnows (*Pimephales promelas*) show correlations between feeding miscues and Pb brain concentrations (Weber et al. 1991), whereas rainbow trout (*Oncorhynchus mykiss*) may show signs of black tail, a neurological symptom that occurs at blood Pb levels of 1.7mg/L (Schmitt et al. 2005). Schmitt et al. (2006) focused on determining potential health hazards to Native Americans consuming fish and crayfish from contaminated areas. Based on recommendations set by the World Health Organization (WHO), Schmitt et al. (2006) determined that the concentrations of Pb and Cd from *C. carpio*, channel catfish (*Ictalurus punctatus*), and crayfish (*Orconectes spp.)* from sites in the Tri-State Mining District were high enough for potential harm to humans consuming these species (WHO 1992, 1995).

Phelps and McBee (2009, 2010) focused on impacts on community structure and population parameters of small mammals, determining that the community of small mammals at Tar Creek Superfund Site has lower diversity and evenness compared to reference sites. They also observed that the absence of some common species (e.g., fulvous harvest mouse, *Reithrodontomys fulvescens*) and the prevalence of certain other species (e.g., house mouse, *Mus musculus*) could be an indication of disruption or contamination.

Hays and McBee (2007) used flow cytometry to determine variation in nuclear DNA content in red-eared sliders (*Trachemys scripta*) at this contaminated site compared to populations at two reference sites. Although no significant difference in DNA content was found between the contamianted and reference sites, specimens captured at the contaminated site had a significantly higher frequency of aneuploidy than did those caught at reference sites.

Conder and Lanno (1999) analyzed metal levels in mandibles of white-tailed deer (*Odocoileus virginianus*) taken from hunters during a carcass check-in. They determined that Pb mandible levels were significantly higher within the Deer Kill Location Zones (DKLZ) containing Picher, OK and much of the Picher Mining District, compared to adjacent and other DKLZs. Zinc mandible levels decreased as distance increased from the core mining area; however, this difference was not significant.

Bats as Bioindicators.—Bats, (Order Chiroptera) are ideal bioindicators due to their near global range, dietary diversity, and variation in roosting sites. Their ability to fly has allowed them to fill a wide variety of niches, offering key insights to determining the health of ecosystems (Jones et al. 2009).

Insectivorous bats, specifically, can be used as bioindicators of environmental contamination due to several factors of their ecology (McBee and Bickham 1990). First, bats are known to accumulate toxicants in contaminated environments (Clark 1981; Geluso et al. 1976). Second, bats are long lived for their size and thus can give a longer view of the environment with which they are interacting. Third, insectivorous bats consume large amounts of arthropods each evening in order to maintain their high metabolism (Maina 2000; Voigt et al. 2010). Finally, bats may consume insects that are aquatic or that spend larval periods in the water or sediments; both types of arthropods are known to be sources of contamination for species that prey on them (Currie et al. 1997; McBee and Bickham 1990; Price et al. 1974). Furthermore, this last point illustrates that bats can also be bioindicators of water quality. Kalcounis-Rueppell et al. (2007) showed that insect community structure differed upstream compared to downstream from wastewater treatment plants, due to the high nutrient levels in the effluent. In turn, bats changed their foraging area to consume their preferred prey. By taking note of insects that benefit from increased nutrients, and the bats that consume them, pollution of a water system potentially may be recognized by observing which species of bats are foraging in an area (Kalcounis-Rueppell et al. 2007).

Bats and Metals.—Although there are fewer reported cases, Pb appears to have similar effects on bats as on birds, including landing accidents associated with difficulty flying and walking (De Francisco et al. 2003). Lead accumulation in bats has resulted from pesticide poisoning, vehicle pollution, and Pb paint in zoo enclosures (Clark 1979; Thies and Gregory 1994; Zook et al. 1970, 1972). Sutton (1987) observed uncoordinated bats, which were unable to fly and experienced muscle tremors due to Pb poisoning or plumbism most likely due to air pollution. Pb levels in liver and kidney from this group ranged from 12.1 to 47.1 ug/g and 21.7 to 30.8 ug/g, respectively (Sutton 1987).

Thies and Gregory (1994) sampled Mexican Free-tailed Bats (*Tadarida brasiliensis*) at Carlsbad Caverns in New Mexico and Vickery Cave in Oklahoma for traces of Pb and Cd in liver tissues. They found measurable levels of Pb (0.74 - 49.44 µg/g WW) in all individuals examined. Sixteen of 48 animals had levels above those associated with bovine chronic toxicity. Liver Cd levels ranged from below detection limits in males from Oklahoma to 1.98 µg/g WW in females from Oklahoma.

Clark (1979) compared Pb levels in bats and small terrestrial mammals collected near a major highway in Baltimore, Maryland. He found Pb levels in wild caught Big Brown Bats (*Eptesicus fuscus)* and Little Brown Bats (*Myotis lucifugus)* to be greater than most terrestrial small mammals, other than shrews. Levels of Pb (µg/g WW) from frozen carcasses of *E. fuscus* were greater in males than females and both sexes of *E. fuscus* had greater levels of Pb than the pooled sexes for *M. lucifugus*. Clark (1979) suggested this might be due to the primarily insectivorous diet and high metabolism of both bats and shrews, requiring consumption of food at a higher rate, thus increasing the rate of Pb ingestion.

Clark et al. (1986) studied effects of metal contamination from a battery salvage plant in Jackson Co. FL. Livers and kidneys were collected for analysis of Pb, Zn, Cd, and Chromium (Cr) from Southeastern Bats (*Myotis austroriparius*) roosting in Judges Cave, an important maternity colony of the endangered Gray Bat (*Myotis grisescens)*, and *M. austroriparius* roosting under a highway bridge in Gainesville, FL for use as reference bats. Liver levels of Zn, and Cd from bats in Judges Cave were 1.1 and 2.4 times higher, respectively, than levels in bats from Gainesville. Kidney Cd levels were 3.3 times higher in bats from Judges Cave compared to bats from Gainesville. Pb was found in only 5 Gainesville bats and 4 Judges Cave bats. Gainesville bats had a higher mean Pb levels (0.318 ug/g WW) compared to Judges Cave bats (0.195 ug/g WW), which may be due to heavy traffic on the bridge.

All three of the metals of interest for this study, Pb, Zn, and Cd, can be identified within soft tissues of an organism, including liver, kidney, pectoral muscle, and hair (Hickey et al. 2001; Pikula et al. 2008). Hickey et al. (2001) detected mercury (Hg), Zn, selenium (Se), Pb, aluminum (Al), and iron (Fe) in the hair of Canadian bats. They used hair samples to observe variation in metal levels among and within species. They detected Hg and Zn most frequently, with concentrations varying between species. Levels of Zn ranged from below detection limits to 900 mg/kg among several species including *M. lucifugus*, Northern Long-eared Bat (*Myotis septentrionalis)*, *E. fuscus*, and Eastern Small-footed Myotis (*Myotis leibii*). Hg and Zn were detected in 98 and 96% of samples, respectively, ranging from 2.0–7.6 mg/kg and 130–200 mg/kg, respectively, whereas Se and Pb were detected at a much lower frequency, in 12 and 8% of samples, ranging from 9.5–69 mg/kg and 1.6–8.8 mg/kg, respectively.

The use of hair tissue means animals do not need to be killed to determine levels of metals in tissues; however the relationship between levels present in hair and levels present in internal tissues needs to be established before researchers can rely on determining levels only in hair. Nam et al. (2012) found a significant positive correlation between Hg levels in fur of bats at a contaminated site in Virginia and those in liver and brain: however, these relationships have not been determined for most other metals or species. Once this relationship is better understood in many species, hair samples may provide a non-lethal method for determining levels of metal in wildlife.

Natural History.—Bats have a unique natural history that has allowed for great evolutionary success demonstrated by a near cosmopolitan distribution, and their position as the second most specious order of mammals (Altringham 1996). Bats have avoided competition in two important ways: by developing the ability to fly, and by being nocturnal. These adaptations also make bats difficult to study (Clark and Hothem 1991; Kunz et al. 2011). In the last several decades the amount of information known about bats has increased dramatically, increasing public interest in bats as more is discovered about the crucial ecosystem services they provide.

The taxonomical order Chiroptera is separated into two suborders, Yinpterochiroptera and Yangochiroptera, with Yinpterochiroptera containing all members of the previously recognized suborder Megachiroptera and some families of the suborder Microchiroptera and Yangochiroptera containing the remaining families of the previously recognized suborder Microchiroptera (Teeling et al. 2002). Bats vary in size, from the smallest living mammal, the bumblebee bat (1.5–2.0 g) to the largest bats, the flying foxes, weighing 1 kg (Altringham 1996). To maintain a near cosmopolitan distribution, bats have become highly adapted to their environment in several different ways.

One major adaptation found in bats is an enormous variety in the food items that are consumed, including fruit, pollen, nectar, insects, spiders, scorpions, blood, fish, birds, mammals, leaves and more (Kunz et al. 2011). Major ecosystem services are provided by interactions with their food sources (Kunz et al. 2011). Nectivores pollinate the plants from which they feed, and help a large number of plants continue to propagate successfully, as well as disperse seeds for colonization. Frugivores travel far distances within a single evening in search of fruit. By ingesting the seeds within the fruit they eat they are major contributors to seed dispersal for these plants. Insectivores help regulate population sizes of insects.

Insectivorous Bats & Ecological and Economic Services.— Due to the ubiquity of insects, insectivorous bats can be found in most places across the globe. Although insect numbers far exceed numbers of insectivorous bats, there must be some specialization occurring among bat species to reduce competition. Bats can specialize in multiple different ways, including diet, foraging time, foraging strategy, habitat, morphology, and prey size (Fenton 1982; Gaisler et al. 1998; Whitaker 1995; Zhang et al. 2005). These types of specializations allow for multiple species to coexist in the same general area.

A healthy ecosystem regulates itself by processes termed ecosystem services, which provide various natural checks and balances (Kunz et al. 2011). These processes are required for healthy growth and development of the natural environment on which humans rely for survival. If these services are not provided the production of certain items may cease; a loss to which a monetary figure can be placed. Likewise, any service provided by a healthy ecosystem, which increases the production of an item, can also be given value. Insectivorous bats provide ecosystem services in the form of nutrient cycling via dispersal of guano and insect suppression (Federico et al. 2008; Jones et al. 2009; Kunz et al. 1995, 2011; Lee and McCracken 2005; Whitaker 1995).

Cucumber beetles and corn rootworm beetles cost farmers in the U.S. over one billion dollars in losses annually (Whitaker 1995). Mexican Free-tailed Bats (*Tadarida brasiliensis*) tend to prefer beetles and true bugs, making these bats the cheapest pesticide available to farmers (Whitaker 1995). Although they do not completely eliminate agricultural pests, these bats have a major impact on the amount of crops lost to pests (Lee and McCracken 2005). For example, Kunz et al. (1995) found that a typical maternity colony of approximately 100 million *T. brasiliensis* could consume approximately four billion corn earworm-sized insects in a given night.

As natural pest suppressors, bats are largely impacted by the heavy and ever increasing use of pesticides. With such high-energy demands, insectivorous bats must consume a large number of prey items each night or use less energy. It is energetically efficient for these bats to forage in areas of high insect density, thus areas with high insect abundance should also have high bat activity (Wickramasinghe et al. 2003, 2004). Modern agricultural practices have affected bats in two ways. First is the removal of the natural habitat, which may have provided shelter and prey, and second is the use of pesticides to remove a large density of prey items that would have otherwise been in the area (Naylor and Ehrlich 1997; Wickramasinghe et al. 2003, 2004). The benefits of insectivorous bats consuming agricultural pests can be observed as avoided costs, which save local and regional farmers money. A study conducted in southwest Texas, USA determined that about 1.5 million bats feeding over economically important agricultural fields in the Winter Gardens region prevented one, potentially two, pesticide sprayings per year, an annual savings to individual farmers of about $200,000 (Cleveland et al. 2006).

Organic contaminant exposure is a major contributing factor in bat death and population decline (Bayat et al. 2014; Clark 1981). Exposure to these toxicants can be direct, where the bat comes into contact with the pesticide, or secondarily through diet or across the placental membrane. Worldwide, insectivorous bats foraging near agricultural fields are especially susceptible to chronic, or long-term contamination of pesticides through consumption of contaminated prey and water. Effects of chronic exposure often result in cancerous tumors, reproductive failure, endocrine disruption, susceptibility to disease, or behavioral changes that lower survival (Berny 2007; Kannan et al. 2010). These low level effects are difficult to measure, but can have lasting negative effects on populations. Many studies have been conducted on the impact of pesticide contamination of bats (Bayat et al. 2014; Clark 1981); however, only a few studies have discussed metal contamination in bats. Routes of metal exposure for bats within the Tar Creek Superfund Site area most likely include ingestion through diet, water, and grooming (Schmidt and Tyrell 2001).

Habitat Preferences.—Bats may require multiple types of habitats throughout the year including winter hibernacula, summer maternity roosting site, day roosting sites, and night roosting sites. The purposes of each of these sites are different and therefore require different characteristics. Winter hibernacula are vital in maintaining an appropriate ambient temperature for these heterothermic mammals. Maternity colonies may be comprised of a few individuals or several thousand individuals, usually all females, of potentially different species roosting in one location to rear their pups. Day roosts tend to be sheltered from any extreme elements, allowing the individual to enter torpor, to conserve energy throughout the day. The night roost generally serves the purpose of allowing bats to remain in a given foraging area between bouts of foraging throughout the evening, before returning to a day roost for the daylight hours.

Some species use caves as hibernacula because certain spots in caves can be much warmer than the overall ambient temperature. Often bats will roost in multispecies colonies of hundreds to thousands to help increase the temperature in that roost during winter (Altringham 1996). Foliage roosts are often used primarily by species that migrate to warmer locations during the temperate winter months, rather than hibernating during the winter (Altringham 1996), although tree bats have been found hibernating in leaf piles on banks (Boyles et al. 2003; Dunbar and Tomasi 2006; Moorman et al. 1999; Saugey et al. 1989, 1998).

Habitat preferences vary amongst species, due to different environmental and physical characteristics, such as food availability, size of the animal, presence and quality of roosts, and amount of water. For many species, habitat will be determined based on presence of their food source in a given area. As expected, nectivores will be found in areas containing high nectar-producing plants, while sanguivores can be found near livestock or large groups of medium to large sized animals, which provide ample sources of blood. Some species forage strictly in a particular type of area, while others may combine a few areas. Examples include the Little Brown Bat (*Myotis lucifugus)*, which is known to forage in riparian areas, consuming aquatic insects as a major portion of it's diet, and the Eastern Red Bat which forages along streams, forest edges, open areas, around street lights, and occasionally in the presence of other species (Barbour and Davis 1969; Shump and Shump 1982).

Differences in maneuverability also can allow for multiple species of bats to share an area by foraging in different types of habitats, without competition. Bats with lower wing loading often will be found foraging in areas that have high clutter, such as within the forest and amongst branches of a tree. Bats with higher wing loading and aspect ratio forage in more open areas, such as above the canopy or over water. Bats with low aspect ratio and wing loadings have longer, thinner wings allowing them to fly at high speeds, but with limited maneuverability (Norberg and Rayner, 1987). By categorizing bats into three different groups: slow agile fliers, fast strong fliers with little maneuverability, and a group that lies in the middle, researchers are able to determine where to look for species in each category and what they might be eating.

Many studies show that edges, the areas where two discrete types of habitats meet, tend to have more bat activity than either of the habitats that compose the edge (Jantzen and Fenton 2013; Lesinski et al. 2011; Limpens and Kapteyn 1991; Morris et al. 2010; Wolcott and Vulinec 2012). Reasons behind more activity in these areas may include a higher abundance of insects, more space to maneuver, the use of edges as commuting highways to other foraging areas, more efficient flight due to wind protection, and protection from predators (Jantzen and Fenton 2013; Morris et al. 2010; Verboom and Spoelstra 1999). It seems intuitive that bats will fly in certain areas based on their wing shape and flight categories; however, of the species studied, most of them, regardless of category, seem to have higher activity levels in edge habitats (Jantzen and Fenton 2013; Wolcott and Vulinec 2012).

Insectivorous bats require a high level of maneuverability in order to locate and reach prey, water sources, and roosts, as well as avoid predators. Due to the high-energy requirements associated with flight, bats have a high metabolism, requiring them to consume close to their body weight in insects each evening (Kunz et al. 1995). Decreased maneuverability, for any reason, impacts foraging ability, which in turn can have a real effect on survival and fitness. Even low levels of exposure to a contaminant with neurological effects can potentially affect a bat's maneuverability. Bats within the Tar Creek Superfund Site have great potential for exposure to Pb, a neurotoxin, which may impact their maneuverability and willingness to fly (De Francisco et al. 2003; Sutton 1987).

This Study.—I wanted to gain a better understanding of how the bat community is impacted by contaminants within Tar Creek Superfund Site. To do this I took a three-way approach. First, mist netting techniques were employed to test the hypotheses that community structure and population demographics of bats inhabiting sites within the contaminated area will be significantly different compared to uncontaminated reference sites. Second, maneuverability and willingness to fly were measured for each bat using an obstacle course within a flight cage. Flight trials were conducted to test the hypothesis that bats inhabiting contaminated sites will have significantly lower maneuverability/willingness to fly compared to bats inhabiting uncontaminated reference sites. Third, tissue samples, including hair, liver, and kidneys, were collected to test the hypothesis that metal levels in bats inhabiting contaminated sites would be significantly higher compared to bats inhabiting uncontaminated reference sites. Hair, liver, and kidney metal levels were used for comparison with results from flight cage assays.

Data from this study are compared to that for other species from Tar Creek Superfund Site to provide a better understanding of effects of mining and metals on multiple species that may occupy different trophic levels, be exposed to metals through different routes, and live in/on different substrates in an ecosystem. I am interested in moving towards a non-lethal method for metals analysis in small mammals. Previous studies have relied on liver and kidney samples; however, by including hair samples along with liver and kidney I should be able to see how internal and external metal levels are related, so that in future studies accurately interpreted metal levels can be taken from hair only.

This study provides three interrelated types of data. First, as far as we know this is the only assay to date measuring behavioral impacts of metals for chiropterans. Three variables were measured, # of obstacles dropped, # of movements throughout the flight cage, and time in flight (s), using an obstacle course within a flight cage. Second, tissues were collected from all bats tested in the flight cage and flight scores were then compared to metal levels from all three tissues. Third, this study collected the first population data and the first voucher specimens of bats within Tar Creek Superfund Site, adding to the little information on bats from within the area to date.

REFERENCES

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Report to Congress, Tar Creek Superfund Site, Ottawa County, Oklahoma. Centers for Disease Control and Prevention, Atlanta, GA. 35 pp.

Altringham, J.D. 1996. Bats: Biology and Behavior. Oxford University Press, Oxford, New York, NY.

Barbour, R.W., and W.H. Davis. 1969. Bats of America. The University of Kentucky Press. Lexington, KY.

Bayat, S., F. Geiser, P. Kristiansen, and S.C. Wilson. 2014. Organic contaminants in bats: Trends and new issues. Environment International 63:40–52.

Berny, P. 2007. Pesticides and the intoxication of wild animals. Journal of Veterinary Pharmacology and Therapeutics 30:93–100.

Beyer, W.N., J. Dalgarn, S. Dudding, J.B. French, R. Mateo, J. Miesner, L. Sileo, and J. Spann. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology 48:108–117.

Boyles, J.G., J.C. Timpone, and L.W. Robbins. 2003. Late-winter observations of red bats, *Lasiurus borealis*, and evening bats, *Nycticeius humeralis* in Missouri. Bat Research News 44:59–61.

Clark, D.R., Jr. 1979. Lead concentrations: bats vs. terrestrial small mammals collected near a major highway. Environmental Science and Technology 13:338–341.

Clark, D.R., Jr. 1981. Bats and environmental contaminants: a review. United States Department of the Interior, Fish and Wildlife Service. Wildlife No. 235.

Clark, D.R., Jr., and R.L. Hothem. 1991. Mammal mortality at Arizona, California, and Nevada gold mines using cyanide extraction. California Fish and Game 77:61–69.

Clark, D.R., Jr., A.S. Wenner, and J.F. Moore. 1986. Metal residues in bat colonies, Jackson County, Florida, 1981-1983. Florida Field Naturalist 14:38–51.

Cleveland, C.J., M. Betke, P. Federico, J.D. Frank, T.G. Hallam, J. Horn, J.D. Lopéz Jr., G.F. McCracken, R.A. Medellín, A. Moreno-Valdez, C.G. Sansone, J.K. Westbrook, and T.H. Kunz. 2006. Economic value of the pest control service provided by Brazilian free-tailed bats in southcentral Texas. Frontiers in Ecology and the Environment 4:238–243.

Conder, J.M., and R.P. Lanno. 1999. Heavy metal concentrations in mandibles of white-tailed deer living in the Picher Mining District. Environmental Contamination and Toxicology 63:80–86.

Currie, R.S., W.L. Fairchild, and D.C.G. Muir. 1997. Remobilization and export of cadmium from lake sediments by emerging insects. Environmental Toxicology and Chemistry 16:2333–2338.

De Francisco, N., J.D. Ruiz Troya, and E.I. Agüera. 2003. Lead and lead toxicity in domestic and free living birds. Avian Pathology 32:2–13.

Dunbar, M.B., and T.E. Tomasi. 2006. Arousal patterns, metabolic rate, and an energy budget of eastern red bats (*Lasiurus borealis*) in winter. Journal of Mammalogy 87:1096–1102.

Federico, P., T.G. Hallam, G.F. McCracken, S.T. Purucker, W.E. Grant, A.N. Correa-Sandoval, J.K. Westbrook, R.A. Medellín, C.J. Cleveland, C.G. Sansone, J.D. López Jr., M. Betke, A. Moreno-Valdez, and T.H. Kunz. 2008. Brazilian free-tailed bats as insect pest regulators in transgenic and conventional cotton crops. Ecological Applications 18:826–837.

Fenton, M.B. 1982. Ecology of bats. Plenum Press, New York, NY.

Gaisler, J., J. Zukal, Z. Rehak, and M. Homolka. 1998. Habitat preference and flight activity of bats in a city. Journal of Zoology 244:439–445.

Geluso, K.N., J.S. Altenbach, and D.E. Wilson. 1976. Bat mortality: pesticide poisoning and migratory stress. Science 194:184–186.

Hays, K.A., and K. McBee. 2007. Flow cytometric analysis of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Ecotoxicology 16:353–361.

Hickey, M.B.C., M.B. Fenton, K.C. MacDonald, and C. Souliere. 2001. Trace elements in the fur of bats (Chiroptera; Vespertilionidae) from Ontario and Quebec, Canada. Bulletin of Environmental Contamination and Toxicology 66:699–706.

Jantzen, M.K., and M.B. Fenton. 2013. The depth of edge influence among insectivorous bats at forest-field interfaces. Canadian Journal of Zoology 91:281–292.

Jones, G., D.S. Jacobs, T.H. Kunz, M.R. Willig, and P.A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8:93–115.

Kalcounis-Rueppell, M.C., V.H. Payne, S.R. Huff, and A.L. Boyko. 2007. Effects of wastewater treatment plant effluent on bat foraging ecology in an urban stream system. Biological Conservation 138:120–130.

Kannan, K., S.H. Yun, R.J. Rudd, and M. Behr. 2010. High concentrations of persistent organic pollutants including PCBs, DDT, PBDEs, and PFOs in little brown bats with white-nose syndrome in New York, USA. Chemosphere 80:613–618.

Kunz, T.H., J.O. Whitaker Jr., and M.D. Wadanoli. 1995. Dietary energetics of the insectivorous Mexican free-tailed bat (*Tadarida brasiliensis*) during pregnancy and lactation. Oecologia 101:407–415.

Kunz, T.H., E. Braun de Torrez, D. Bauer, T. Lobova, and T.H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences 1223:1–38.

Lee, Y., and G.F. McCracken. 2005. Dietary variation of brazilian free-tailed bats links to migratory populations of pest insects. Journal of Mammalogy 86:67–76.

Lesinski, G., A. Olszewski, and B. Popczyk. 2011. Forest roads used by commuting and foraging bats in edge and interior zones. Polish Journal of Ecology 59:611–616.

Limpens, H.J.G.A., and K. Kapteyn. 1991. Bats, their behavior and linear landscape elements. Bat Research News 29:39–47.

Maina, J.N. 2000. What it takes to fly: the structural and functional respiratory refinements in birds and bats. Journal of Experimental Biology 203:3045–3064.

McBee, K., and J.W. Bickham. 1990. Mammals as bioindicators of environmental toxicity. Current Mammalogy 2:37–88.

Moorman, C.E., K.R. Russell, M.A. Menzel, S.M. Lohr, J.E. Ellenberger, and D.H. Van Lear. 1999. Bats roosting in deciduous leaf litter. Bat Research News 40:74–75.

Morris, A.D., D.A. Miller, and M.C. Kalcounis-Rueppell. 2010. Use of forest edges by bats in a managed pine forest landscape. The Journal of Wildlife Management 74:26–34.

Nam, D., D. Yates, P. Ardapple, D.C. Evers, J. Schmerfeld, and N. Basu. 2012. Elevated mercury exposure and neurochemical alterations in little brown bats (*Myotis lucifugus*) from a site with historical mercury contamination. Ecotoxicology 21:1094–1101.

Naylor, R.L., and P.R. Ehrlich. 1997. Natural pest control services and agriculture. Pp. 151–174 in Nature's Services: Societal Dependence on Natural Ecosystems (G.C. Daily, ed.). Island Press, Washington DC.

Newman, M.C. 2010. Fundamentals of Ecotoxicology. CRC Press, Boca Raton, FL.

Norberg, U.M., and J.M.V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London 316:335–427.

OWRB (Oklahoma Water Resources Board) 1983. Groundwater Investigation in the Picher Field. OWRB, Oklahoma City, OK.

Phelps, K.L., and K. McBee. 2009. Ecological characteristics of small mammal communities at a Superfund site. American Midland Naturalist l61:57–68.

Phelps K.L., and K. McBee. 2010. Population parameters of *Peromyscus leucopus* (white-footed deermice) inhabiting a heavy metal contaminated Superfund site. Southwestern Naturalist 55:363–374.

Phillips, J.C., and F.C. Lincoln. 1930. American waterfowl: their present situation and the outlook for their future. Houghton Mifflin, New York, NY.

Pikula, J., J. Zukal, V. Adam, H. Bandouchova, M. Beklova, P. Hajkova, J. Horakova, R. Kizek, and L. Valentikova. 2008. Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environmental Toxicology and Chemistry 29:501–506.

Price, P.W., B.J. Rathcke, and D.A. Gentry. 1974. Lead in terrestrial arthropods: evidence for biological concentration. Environmental Entomology 3:370–372.

Rattner, B.A. 2009. History of wildlife toxicology. Ecotoxicology 18:773–783.

Saugey, D.A., D.R. Heath, and G.A. Heidt. 1989. The bats of the ouachita mountains. Proceedings Arkansas Academy of Science 43:71–77.

Saugey, D.A., B.G. Crump, R.L. Vaughn, and G.A. Heidt. 1998. Notes on the natural history of *Lasiurus borealis* in Arkansas. Journal of the Arkansas Academy of Science 52:92–98.

Schmidt, A., and K. Tyrell. 2001. Bioaccumulation of environmental contaminants in bats collected from Missouri. Bat Research News 42:37–38.

Schmitt, C.J., J.J. Shyte, W.G. Brumbaugh, and D.W. Tillitt. 2005. Chemical effects of lead, zinc, and cadmium from mining on fish in the tri-states district of northeastern Oklahoma, USA. Environmental Toxicology and Chemistry 24:1483–1495.

Schmitt, C.J., W.G. Brumbaugh, G.L. Linder, and J.E. Hinck. 2006. A screening-level assessment of lead, cadmium, and zinc in fish and crayfish from northeastern Oklahoma, USA. Environmental Geochemistry and Health 28:445–471.

Shump, K.A., Jr., and A.U. Shump. 1982. Lasiurus borealis. Mammalian Species 183:1–6.

Sutton, R. 1987. Lead poisoning in flying-foxes (Chiroptera: Pteropodidae). Australian Mammalogy 2:125–126.

Teeling, E.C., O. Madsen, R.A. Van Den Bussche, W.W. de Jong, M.J. Stanhope, and M.S. Springer. 2002. Microbat paraphyly and the convergent evolution of a key innovation in old world rhinolophoid microbats. Proceedings of the National Academy of Sciences of the United States of America 99:1431–1436.

Thies, M., and D. Gregory. 1994. Residues of lead, cadmium, and arsenic in livers of Mexican free-tailed bats. Bulletin of Environmental Contamination and Toxicology 52:641–648.

USEPA (United States Environmental Protection Agency), Region 6. 1994. First Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

USEPA (United States Environmental Protection Agency), Region 6. 2000. Second Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

USEPA (United States Environmental Protection Agency), Region 6. 2005. Third Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

USEPA (United States Environmental Protection Agency), Region 6. 2010. Fourth Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

USFWS (United States Fish and Wildlife Service). 2000. Partial restoration plan and environmental assessment, addressing injuries to migratory birds and threatened and endangered species at the Tar Creek Superfund Site, Ottawa County, Oklahoma. Oklahoma Ecological Services Field Office, Tulsa, Oklahoma.

Verboom, B., and K. Spoelstra. 1999. Effects of food abundance and wind on the use of tree lines by an insectivorous bat, *Pipistrellus pipistrellus*. Canadian Journal of Zoology 77:1393–1401.

Voigt, C.C., K. Sorgel, and D.K. Dechmann. 2010. Refueling while flying: foraging bats combust food rapidly and directly to power flight. Ecology 91:2908–2917.

Weber, D.N., A. Russo, D.B. Seale, and R.E. Spieler. 1991. Waterborne lead affects feeding abilities and neurotransmitter levels of juvenile fathead minnows (*Pimephales promelas*). Aquatic Toxicology 21:71–80.

Whitaker, J.O., Jr. 1995. Food of the big brown bat *Eptesicus fuscus* from the maternity colonies in Indiana and Illinois. American Midland Naturalist 134:346–360.

Wickramasinghe, L.P., S. Harris, G. Jones, and N. Vaughan. 2003. Bat activity and species richness on organic and conventional farms: impact of agricultural intensification. Journal of Applied Ecology 40:984–993.

Wickramasinghe, L.P., S. Harris, G. Jones, and N.V. Jennings. 2004. Abundance and species richness of nocturnal insects on organic and conventional farms: effects of agricultural intensification on bat foraging. Conservation Biology 18:1283–1292.

Wolcott, K.A., and K. Vulinec. 2012. Bat activity at woodland/farmland interfaces in central Delaware. Northeastern Naturalist 19:87–98.

WHO (World Health Organization). 1992. Environmental health criteria 134, cadmium. International Programme on Chemical Safety (ICPS). Geneva, Switzerland: United Nations Environment, Programme, International Labor Organization, and World Health Organization.

WHO (World Health Organization). 1995. Environmental health criteria 165, inorganic lead. International Programme on Chemical Safety (ICPS). Geneva, Switzerland: United Nations Environment, Programme, International Labor Organization, and World Health Organization.

Zhang, L., G. Jones, S. Tossiter, G. Ades, B. Liang, and S. Zhang. 2005. Diet of flat-headed bats, *Tylonycteris pachypus* and *T. robustula*, in Guangxi, South China. Journal of Mammalogy 86:61–66.

Zook, B.C., R.M. Sauer, and F.M. Garner. 1970. Lead poisoning in Australian fruit bats (*Pteropus poliocephalus*). Journal of the American Veterinary Medical Association 157:691–694.

Zook, B.C., R.M. Sauer, and F.M. Garner. 1972. Lead poisoning in captive wild animals. Journal of Wildlife Diseases 8:264–272.

CHAPTER II

COMMUNITY COMPOSITION AND TISSUE METAL CONCENTRATIONS IN BATS (CHIROPTERA) FROM TAR CREEK SUPERFUND SITE

Abstract – Relatively few studies have focused on bats as bioindicators; however, multiple aspects of their ecology can contribute to their use as monitors of contamination. Little to nothing was known about the chiropteran community within the contaminated Tar Creek Superfund Site in northeastern Oklahoma. Mist-net surveys were conducted to test the hypothesis that contaminated communities (Tar Creek and Beaver Creek) would show lower diversity compared to reference communities (Plum Creek and Panther Creek). Tissue metal concentrations were determined via ICP-MS to test the hypothesis that bats from contaminated sites would have higher concentrations of lead (Pb), zinc (Zn), and cadmium (Cd) in liver, kidney, and hair tissues compared to bats from reference sites. Bat communities inhabiting contaminated sites had lower diversity compared to reference sites, although differences were not significant. Eastern Red Bat (*Lasiurus borealis*) was the most frequently caught species from contaminated and reference sites. More subadults were collected at reference sites and pregnant females were never collected at contaminated sites. Kruskal-Wallis nonparametric tests showed that males from Tar Creek (TC) had significantly higher concentrations of Zn in kidney compared to males from Beaver Creek (BC). Females from BC had significantly higher concentrations of Pb in hair compared to females from TC. Males from Panther Creek (PAN) had higher concentrations of Zn in hair compared to males from Plum Creek (PLM), and females from PAN had significantly higher concentrations of Pb in hair compared to females from PLM. All other comparisons were not significantly different among sites. Tissue concentrations in bats within contaminated sites were not as high as expected compared to previous studies of terrestrial and aquatic species, suggesting that volant mammals may experience lower levels of exposure to contaminants at Tar Creek Superfund Site.

Keywords – Lead, Zinc, Cadmium, *Lasiurus borealis*, Eastern Red Bat

INTRODUCTION

The Tri-State Mining District, a 500 mi^2 area composed of northeastern Oklahoma, southeastern Kansas, and southwestern Missouri, was active from 1891 to 1970 [1,2]. In 1983 the U.S. Environmental Protection Agency (EPA) added Tar Creek Superfund Site, the portion of the Tri-State Mining District located in Ottawa Co. OK, to the National Priorities List due to high levels of lead (Pb), zinc (Zn), and cadmium (Cd) in soils, and acid mine drainage [2]. Through the process of ore extraction, approximately 74 million tons of mine tailings, or chat, were created, with some piles reaching 200 feet in height [3]. These chat piles are still a constant source of contamination.

In 1994, blood Pb levels were above 10 μg/dL in 34% of children tested from within this contaminated area. Based on these findings, the EPA began remediating residential sites with soil contamination levels exceeding 500 ug/g Pb and 100 ug/g Cd within 0-12 inches in depth, and within 1000 ug/g Pb and 100 ug/g Cd within 12-18 inches in depth [4]. The Tar Creek Superfund Site area is not homogeneously contaminated, resulting in the EPA testing multiple sites and only remediating areas exceeding these levels. Of the locations tested, 61% of public sites and 65% of residential sites exceeded these levels and contaminated soil was excavated and clean topsoil was filled in [4]. Remediation is still in progress through the EPA with management through Quapaw Tribe of Oklahoma [5,6].

Several studies have investigated the impact of changes in land use and metal levels on local wildlife at Tar Creek Superfund Site [7-13]; however, none of these focused on bats. An acoustic study was conducted to gather data on species composition and abundance of bat communities in eastern Oklahoma along 6 transects near the Oklahoma-Arkansas border, with the northernmost transect located within this contaminated area (A. Korman, 2013, Master's thesis, Oklahoma State University, Stillwater, OK, USA). Although bat calls were detected within Tar Creek Superfund Site, they were collected in significantly lower numbers compared to the other transects, suggesting fewer bats are foraging in this area. Based on Korman's study I knew that bats were foraging in this area, but knew nothing else about this chiropteran community. Searches of Mammal Networked Information System (MaNIS; http://manisnet.org, Accessed 11 November 2012) showed no museum voucher specimen records of bats collected within the Tar Creek Superfund Site area.

Bats contribute to a healthy ecosystem by providing ecosystem services, which maintain natural checks and balances. These processes are required for healthy growth and development of the natural environment on which humans rely for survival. If these services are not provided production of certain items may cease; a loss to which a monetary figure can be placed. For example, benefits of insectivorous bats consuming agricultural pests can be observed as avoided costs, which save money for local and regional farmers [14]. A study conducted in southwest Texas, USA determined the avoided-cost value of about 1.5 million bats feeding over economically important agricultural fields in the Winter Gardens region [15]. These investigators found that bats could prevent 1 to 2 pesticide sprayings per year, an annual private savings of about $200,000 within this region. Likewise, any service provided by a healthy ecosystem, which increases the production of an item, can also be given a value. Insectivorous bats provide ecosystem services in the form of insect suppression and nutrient cycling via dispersal of guano [14,16-20].

Insectivorous bats can be used as bioindicators of environmental contamination due to several factors of their ecology [17,21]. First, they are known to accumulate toxicants in contaminated environments [22]. Second, for their size, bats are long lived and thus can give a longer view of the environment with which they are interacting. Third, insectivorous bats consume large amounts of arthropods each evening in order to maintain their high metabolism [23,24]. Bats may consume insects that are aquatic or that spend larval periods in the water or sediments. Both of these types of arthropods are known to be sources of contamination for species that prey on them [21,25,26].

The composition of insectivorous bat communities in an area can also be indicative of water quality. Kalcounis-Rueppel et al. [27] showed that insect community structure differed upstream compared to downstream from wastewater treatment plants, due to the high nutrient levels in the effluent. In turn, bats changed their foraging area to consume their preferred prey. By taking note of insects that benefit from increased nutrients, and the bats that consume them, pollution of a water system may potentially be recognized by observing which species of bats are foraging in an area [27].

Despite their well-documented economic benefits to humans, populations of bats are declining due, in part, to anthropogenic factors. These include loss of appropriate habitat, roost site disturbance, and exposure to environmental contaminants [14,28]; additionally, the spread of white-nose syndrome is causing marked declines in bat populations, having a detrimental impact especially on populations of Little Brown Bats (*Myotis lucifugus*)

[14]. Given the recognized role of these animals in maintaining a healthy ecosystem and the impact that contamination has had on other resident taxa [7-13], I was interested in the impacts that contaminants at Tar Creek Superfund Site have on the Chiropteran community within this area. I hypothesized that the bat community within Tar Creek Superfund Site would have lower evenness and diversity compared to the community from reference sites, and that bats collected from within the contaminated area would have higher levels of Pb, Zn, and Cd in liver, kidney, and hair compared to bats from reference sites.

MATERIALS AND METHODS

Study sites

Bats were collected from 2 sites within the contaminated locality, Tar Creek (TC; N 36°57.495’, W 094°50.731’) and Beaver Creek (BC; N 36°56.2026’, W 094°45.3846’), and 2 sites within the reference locality, Oologah Wildlife Management Area, Plum Creek (PLM; N 36°35.5063, W 095°32.4197) and Panther Creek (PAN; N 36°37.747’, W 095°31.372’; Fig. 1). The site along TC was located between private land and land bought out by the EPA, while the site along BC was located on land owned by the Quapaw Tribe of Oklahoma. The contaminated area and the reference area are approximately 67 km apart from one another and are on different drainage systems, ensuring that the reference area is not contaminated via wind blowing metal contaminated chat dust or water-borne sediments, and that bat populations are unlikely to be shared between these two localities. Distance between the 2 contaminated sites is approximately 8 km, as is the distance between the 2 reference sites. This close proximity allowed for netting to occur simultaneously at the 2 contaminated sites, and simultaneously at the 2 reference sites.

Field methods

Bats were collected via mist nets placed over riparian areas for three consecutive nights each month during the time of year when bats are active (June-Sept 2012, and May-Sept 2013). Three to 4 nets were opened at sunset and left in place for 5 hours or until 1 am, and checked every 15 minutes. Total sampling effort was 278 net-nights, with 128 net-nights at reference and 150 net-nights at contaminated sites. Sampling was not possible during June 2013 due to flooding.

Captured bats were removed from the net manually, identified in the hand, and reproductive condition (scrotal/nonscrotal; pregnant/not pregnant; lactating/not lactating), and approximate age (as determined by shining a flash light through the extended fingers of the wing to determine degree of epiphyseal ossification) were recorded. Bats were then placed in cloth bags until they could be processed. Weight, sex, and standard external measurements (total length, tail length, forearm length, ear length, tragus length, hindfoot length) were measured with digital calipers while animals were restrained by hand or while individually held in cloth bags.

As approved by the Animal Care and Use Committee of Oklahoma State University under Animal Care and Use Protocol #AS129, an annual maximum of 5 males and 5 females per species from each site were euthanized for tissue collection. These bats were first anesthetized with isoflurane, then euthanized via cervical dislocation. Liver, and kidneys were removed and stored in sterile tubes and placed in liquid nitrogen for return to the laboratory where they were analyzed for Pb, Zn, and Cd. A 1 cm^2 patch of hair was also clipped from the venter [29] of each animal and stored in sterile tubes for analysis of metal content. Animals that were euthanized were prepared as voucher specimens and catalogued in the Oklahoma State University Collection of Vertebrates, adding the first collections data of Chiropteran species in Ottawa Co. [30]. Bats exceeding the total number allowed for euthanasia had only the hair sample collected and then were released after an 8.4 mm PIT tag had been inserted subcutaneously in the scapular region and site of insertion was sealed with tissue glue (Biomark, Inc., Boise, Idaho, USA) [29]. All field procedures were conducted following standards set forth by the American Society of Mammalogists [31].

To account for potential impact of temperature and precipitation on capture frequencies, weather data were collected from the nearest weather station, Nowata and Miami, for reference sites and contaminated sites, respectively, through Oklahoma Mesonet environmental monitoring stations [32]. The Miami weather station is an average of approximately 8.5 km away from the two contaminated sites, and the Nowata weather station is an average of approximately 16.5 km away from the two reference sites. Specific data collected included daily maximums and minimums for each sampling night, 10-year average maximums and minimums by month, and precipitation by month, because temperature and rainfall are known to effect netting success [33].

Insect and water samples were collected in 2013. A light trap [34] was used to collect insect samples for one sampling night for approximately 5 hours, at each of the four sites. The trap was located 15 – 20 m downstream from each netting location and consisted of a white cloth (218 x 218 cm) illuminated by a UV light draped over a rope approximately

1.5 m off the ground. Twice an hour insects present on the sheet were collected by using an aspirator and transferred to a collection jar. Insects were returned to lab, transferred to 70% ethanol, identified to order, and quantified. A homogenized subsample, excluding large beetles that bats could not have eaten, from each location was processed for metal analysis via ICP-MS. Water samples were collected in triplicate (130 ml each) from each of the 4 sites. Sample jars were acid washed prior to collection and water was analyzed via ICP-MS for Pb, Zn, and Cd.

Metal analysis

Materials used for tissue and insect digestion were acid washed. Tissue digestion methods are similar to those of Sanchez-Chardi et al. [35] except that only nitric acid was used for tissue digestion. Liver and kidneys from each euthanized animal were dried (60 °C) separately until a constant weight was maintained. The entire liver (mean DW 0.063 g), both kidneys (mean DW 0.033 g), and hair (mean DW 0.006 g) were each digested with 1 ml HNO_3 (Fisher Scientific; Trace Metal Grade) using an Ethos EZ closed vessel microwave digester (Milestone; Hair and Animal Tissue Protocols). Insect samples, separated by site (mass (g) PLM – 0.46, PAN – 0.06, BC – 0.34, TC – 0.08), were digested with 10 ml HNO_3. A 100 μl sample of each disgestate was diluted to a total volume of 5 ml with Ultrapure H_2O (Millipore) plus a 10 μl aliquot of internal standard (PerkinElmer Multi-element Calibration Standard 2% HNO_3). Analysis of water was conducted on a 5 ml sample of each triplicate sample plus 10 μl of internal standard made with ultra pure H_2O.

Concentrations of Pb, Zn, and Cd were measured by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Perkin-Elmer ELAN-6000. Quality control practices included use of duplicates for each sample, as well as blanks, and internal standards and followed standard operating procedures for the OSU Metabolic and Nutrition Phenotyping Core Facility. Metal concentrations were determined based on a range of 5 concentrations of calibration standards (0.025 ppb, 1 ppb, 1 ppm, 10 ppm, and 20 ppm). Tissue concentrations were determined by averaging measured values in duplicate samples for each tissue. Concentrations that were below ½ of the lowest calibration standard were considered as below detection limits (bdl) and were assigned values of ½ the lowest calibration standard [35]. Tissue and insect metal concentrations were calculated as μg/g on a dry weight basis, whereas metal concentrations in water samples were calculated as mg/L for Zn and μg/L for Cd and Pb.

Statistical analyses

Statistical analyses were performed using SAS 9.3. Species diversity of bat communities at each site was determined using Simpson's diversity index (*D*), which measures the probability that 2 individuals selected at random from a community will belong to the same species [36]. This index also takes into account the number of each species and is therefore indicative of evenness as well. Physical parameters, including total length, tail length, forearm length, hindfoot length, ear length, tragus length, and mass were compared among reference and contaminated sites (PROC NPAR1WAY WILCOXON). Precipitation data were compared between years using a Student's *t*-test. Metal concentrations were non-normally distributed and therefore were analyzed via nonparametric tests (PROC NPAR1WAY, PROC GLM). Correlations between tissue metal levels were examined using Pearson correlation coefficients (PROC CORR) [37].

RESULTS

Community structure

Due to heavy rains in late May/early June 2013 both PLM and PAN were flooded and I was not able to reach reference sampling sites in June; therefore, I decided to eliminate all June 2013 sampling. Forty-eight bats were collected, including 5 species (*Lasiurus borealis*—Eastern Red Bat, *Perimyotis subflavus*—Tricolored Bat, *L. cinereus*—Hoary Bat, *Myotis lucifugus*—Little Brown Bat, and *Nycticeus humeralis*—Evening Bat; Table 1). No PIT-tagged bats were recaptured. The annual permitted numbers of bats for tissue metal analyses were exceeded at only two sites: 7 female *L. borealis* were captured at TC in 2012, and 6 male *L. borealis* were captured at PLM in 2013. A single female captured at TC escaped before samples could be collected. A female *Nycticeus humeralis*, was collected at PAN and released without data collection. *Lasiurus borealis* comprised 89.6% of all bats collected. All other species were represented by only 1 or 2 individuals (Table 1).

Twenty-five bats, comprising 3 species, were collected from contaminated sites, and 23 bats, comprising 4 species, were collected from reference sites. Simpson Diversity values were calculated for combined contaminated sites (D = 0.16) and combined reference sites (D = 0.24). Diversity values for each site are presented in Table 1. Total bats per net-night from all sites combined was 0.17. Bats per net-night from individual sites are presented in Table 1. Field seasons 2012 and 2013 resulted in different capture numbers between contaminated and reference sites. In 2012, twice as many bats were collected from contaminated sites compared to reference sites (contaminated $n = 16$, reference $n = 8$), whereas 2013 showed the opposite result (contaminated $n = 9$, reference $n = 15$). Bats per net night separated by year were: 2012 contaminated – 0.24, reference – 0.14, and 2013 contaminated – 0.11, reference – 0.21.

Lasiurus borealis *age structure and reproduction*

Both the contaminated (BC, TC) and reference communities (PLM, PAN) were dominated by *L. borealis*; therefore, further analyses were conducted on only this species. Sex ratios (M:F) were equal at PAN, male biased at PLM and BC, and female biased at TC, where the number of females captured was twice that of males (Table 2). Seventeen percent of bats were identified as subadults based on epiphyseal closure [38]. Seventy-five percent of the subadults were collected from PLM, in June or early July; the other 25% were collected at TC in mid July. No pregnant or lactating females were captured in 2012 at any site. In 2013, 15% of females captured ($n = 2$) were pregnant; all were collected in May and only from reference sites. Both pregnant bats carried 4 embryos with crown rump lengths of 4 and 6 mm, respectively. Twenty-three percent of females captured ($n = 3$) in 2013 were lactating; all were collected in July and only from reference sites. All adult males captured were scrotal (27 June – 13 September). A single subadult male captured on 9 July 2013 from PLM was scrotal, with testis = 3 x 1 mm, of the same size as an adult male captured from the same location just one day prior.

Tail length was the only external morphological trait measured that was significantly different among sites ($z = -2.79$, $p = 0.005$; Fig. 2). Mean masses of males and females from TC were lower than for any other site (Table 2). Sixteen percent of bats captured had visible external parasites (Streblidae, Cimicoidea) with 86% of these animals being collected from reference sites.

Precipitation at all sites for 2012 was significantly lower than that recorded for 2013 ($p < 0.01$; Oklahoma Mesonet). Average rainfall level recorded in Nowata, OK for June through Sept 2012 was 35.5% lower compared to the 30-year average, whereas average rainfall level recorded at the same station for May through September 2013 was 44.2% above the 30-year average. Although not significantly different, mean daily maximum and minimum temperatures recorded for 2012 were consistently higher compared to 2013 at all locations (Fig. 3 a & b).

Metals analysis

Liver and kidney samples were collected from 40 *L. borealis*, 2 *P. subflavus*, 1 *L. cinereus*, and 1 *M. lucifugus*. Of these, 3 liver samples and 2 kidney samples (2 *L. borealis*, 1 *L. cinereus*) did not yield adequate sample after digestion and therefore were not analyzed. From all samples analyzed, 36% of Pb levels (46/127), 28% of Cd levels (35/127) and no Zn levels were bdl. Metal concentrations (X ± S.E.) in liver, kidney, and hair for *L. borealis* are given in Table 3. Due to low sample sizes for species other than *L. borealis*, statistical analyses of metal tissue concentrations were conducted only on *L. borealis*; however, concentrations (µg/g) for single individuals of other species were: *L. cinereus* (BC) liver (not enough sample collected after digestion) kidney Zn – 3.252, Cd – 0.019, Pb – 0.001, hair Zn – 4.00, Cd – BDL, Pb – 0.062; *P. subflavus* (BC) liver Zn – 2.566, Cd – 0.069, Pb – 0.001, kidney Zn – 2.094, Cd – 0.110, Pb – BDL, hair Zn –

3.387, Cd – BDL, Pb – 0.013; *P. subflavus* (PAN) liver Zn – 3.234, Cd – 0.010, Pb – 0.003, kidney Zn – 1.584, Cd – 0.002, Pb – BDL, hair Zn – 1.219, Cd – BDL, Pb – BDL; and *M. lucifugus* (PAN) liver Zn - 2.773, Cd – 0.022, Pb – 0.004, kidney Zn – 1.551, Cd – 0.022, Pb – BDL, hair Zn – 4.519, Cd – 0.006, Pb – 0.018.

Male bats from PAN had significantly higher hair Zn concentrations compared to males from PLM ($p = 0.04$). Males from TC had significantly higher kidney Zn concentrations compared to BC males ($p = 0.03$). Significantly higher concentrations of hair Pb were found in BC females compared to TC females ($p = 0.02$). Also, PAN females had significantly higher hair Pb concentrations compared to PLM females ($p = 0.04$). Combined sexes from BC had significantly higher concentrations of Cd in liver ($p = 0.05$), and Pb in hair ($p = 0.05$) compared to combined sexes from TC. The only significant ($p < 0.05$) positive correlation between hair and internal tissues for the same metal was between hair Pb and liver Pb. Additional significant positive correlations for metal by tissue combinations included liver Zn by kidney Zn, liver Cd by kidney Cd, kidney Cd by hair Zn, hair Zn by hair Pb, and hair Cd by hair Pb (Table 4).

Although not significantly different ($p > 0.05$), mean concentrations (µg/g) for all three metals in liver and Zn and Pb in kidney were higher in subadults ($n = 8$) compared to adults ($n = 30$; subadults liver Zn – 3.17, Cd – 0.03, Pb – 0.03, kidney Zn – 2.96, Pb – 0.011, adults liver Zn – 2.98, Cd – 0.02, Pb – 0.01, kidney Zn – 2.54, Pb – 0.003). In contrast subadults showed lower mean concentrations (µg/g) for all three metals ($p > 0.05$) in hair and for kidney Cd (hair Zn – 2.98, Cd – 0.001, Pb – 0.001, kidney Cd – 0.01) compared to adults (hair Zn – 3.573, Cd – 0.005, Pb – 0.033, kidney Cd – 0.174).

Three to 18 times more insects were collected at reference sites (PLM – 268, PAN – 1388) compared to contaminated sites (BC – 77, TC – 76). Insect orders listed by levels of abundance for each site are: PLM – Coleoptera > Diptera > Lepidoptera > Orthoptera, PAN – Diptera > Coleoptera > Trichoptera > Hemiptera, BC – Coleoptera > Lepidoptera > Diptera > Trichoptera, TC – Trichoptera > Lepidoptera > Diptera > Coleoptera. Contaminated sites had insect metal concentrations of Zn and Cd that were 42% and 34% higher compared to reference sites, respectively, while Pb levels were nearly the same (Table 3). Zn and Cd concentrations in insects were highest at TC, but Pb concentrations were low at all sites. Contaminated sites had water levels of Zn and Cd that were 99% and 94% higher compared to reference sites, respectfully. PAN had the highest Pb concentrations of any site, with concentrations at least 49% higher.

DISCUSSION

Total number of bats collected at combined contaminated sites and combined reference sites over both years were nearly identical, although during 2012 twice as many individuals were collected at contaminated sites as at reference sites and during 2013 nearly twice as many individuals were collected at reference sites. Had data only been collected during 2012, I would have concluded that contaminated sites had a bat community twice as large as reference sites. Creek levels within reference sites rise and fall with water levels in Lake Oologah, whereas TC and BC are fed through overflow from flooded mines. During 2012, when precipitation levels were 35.5% below the 30-year average, reference creeks were well below normal levels by July; however, contaminated water levels maintained near normal levels through September. Bats are drawn to clear, running water for drinking, foraging and as flyways. Therefore, low water levels at PLM and PAN most likely explains low capture success from reference sites during 2012. This study illustrates the importance of gathering data over multiple time periods and considering interactions between the focal species and abiotic factors, other than the presence of contaminants, that may influence population and community variables.

Two species, represented by single individuals, were captured from reference sites but not at contaminated sites, and one species, also represented by a single individual, was collected only at a contaminated site. TC had a slightly higher number of captures, but lower species diversity, compared to BC, which had fewer captures, but higher diversity. Similarly, PLM had higher captures, but lower diversity, and PAN had a lower number of captures with greater diversity. Presence of contaminants has been related to lower levels of species diversity [39]. Lowest values for D were at TC, which had the highest Zn and Cd concentrations for both insect and water samples, and PLM, which had the second highest concentration of Pb in insect samples. *Lasiurus borealis* was the most common species at all four sites, which is not surprising. This species ranges throughout much of North America and is often the most frequently captured species within the Ouachita Mountains of eastern Oklahoma and western Arkansas [40,41]. Based on capture results from this study, this trend is consistent for northeastern Oklahoma as well, although number of individuals per net-night for this study (0.17) was smaller than those reported in the Ouachita Mountains located further south [40,41,42].

Male biased sex ratios are common for *L. borealis* [40,41] in this part of the U. S. Although the total number of bats was smaller, TC had more than twice as many females (Table 2). Dumitrescu et al., [43] found that sex ratio was female biased in the F_1

generation when mother Wistar rats *(Rattus norvegicus)* were exposed to lead acetate (100 and 150 ppb) before mating and during pregnancy. Bouland et al., [44] observed a shift in sex ratios towards females in 3 different bird species (*Megaceryle alcyon, Sialia sialis, Tachycineta bicolor*) nesting along a mercury-contaminated river. These studies suggest that the female biased sex ratio observed at TC possibly is a subtle result of chronic exposure to low concentrations of metals.

Pregnant females were only collected in May and only from reference sites. All lactating females were collected in early July from reference sites, except for one *Perimyotis subflavus*, which was captured at BC on 27 May 2012. This suggests that she had already given birth to pups, which, for this species, is consistent with findings from Florida [45]. Although the sample size is small, no reproductive females were collected from TC, which had water Cd concentrations 6 times higher than the next highest water Cd concentration. Acute Cd exposure interferes with steroidogenesis, thereby decreasing estrogen levels in Sprague-Dawley rats (*R. norvegicus)* when dosed during diestrus [46]. Chronic Cd exposure possibly impeded reproduction in female bats; however, Cd doses used by these investigators (0, 3, or 5 mg Cd/kg body weight) were much higher than concentrations found in bats from either contaminated or reference areas (Table 3).

Lasiurus borealis subadults were captured as early as mid June and into July. *Lasiurus borealis* is estimated to become volant at 3 to 6 weeks of age, and may be weaned at 4 to 6 weeks of age [47] suggesting that the birth of the young captured on 16 June 2012 occurred in late May rather than mid-June, as reported from Indiana, Iowa, and lower Michigan [48]. This likely is due to Oklahoma's more southern location and warmer temperatures earlier in the year. Also, 75% of subadults were collected from

PLM, with the other 25% collected from TC, which is consistent with the higher levels of female reproduction found from reference sites.

Tail length was significantly longer in bats from contaminated sites compared to bats from reference sites ($p = 0.005$), possibly because TC had more than twice as many females as males, and females are consistently larger than males in *L. borealis* [49]. Secondly, 75% of smaller subadults were captured from reference sites. This difference is likely an artifact of population structure between sites. Although not significantly different TC males and females had the lowest mass compared to other sites. Dumitrescu et al. [43] found decreased body weight at maturation as determined by vaginal opening of F_1 generation females when mother Wistar rats *(R. norvegicus)* were exposed to lead acetate (100 and 150 ppb) before mating and during pregnancy. Possibly chronic exposure to metals prior to puberty impacted adult body mass in bats from TC.

A larger number of bats from reference sites were observed with external parasites. *Sigmodon hispidus* (Hispid Cotton Rat) captured from reference sites had higher parasite loads than animals captured from sites contaminated with heavy metals including Pb and Zn and organic compounds [50]. These authors suggested that parasites having free-living life stages may suffer direct effects of exposure to environmental contaminants or that contaminants may increase host immunity. Wilbur et al. [51] found a significantly lower frequency of sporulation of the intestinal parasite *Eimeria jemezi* in Northern Pocket Gophers (*Thomomys talpoides)* from a site with elevated radon levels compared to those from areas with near average soil concentrations of radon. They speculated that the elevated radon levels had an adverse effect on sporulation resulted in reduced parasite loads in gophers from the elevated radon site. Additionally, 87% of bats with external parasites in this study were subadults, so it may be that younger bats are not as adept at removing ectoparasites as older bats.

Although these data do not statistically support my hypothesis that the bat community within contaminated sites would have lower diversity and evenness compared to the reference community, they do show this trend. The combined contaminated sites had lower diversity ($D = 0.16$ compared to $D = 0.24$) than the combined reference sites.

Tissue metal concentrations were converted to ug/g WW for comparison to metal concentrations from *Myotis austroriparius* (Southeastern Myotis) collected by Clark et al. [52], and *Tadarida brasiliensis* (Mexican Free-tailed Bat) collected by Thies and Gregory [53]. Concentrations of Zn and Cd in liver and kidney found by Clark et al. [52] were approximately 20 – 30 times higher for Zn and over 100 times higher for Cd compared to those found in this study. Liver Cd and Pb concentrations collected by Thies and Gregory [53] were 50 – 100 times higher for Cd and about 1000 times higher for Pb compared to this study. These authors observed that there was no difference in reproduction in bats with Pb liver concentrations ranging from 0.74 – 49.44 μg/g WW. Walker et al. [54] found renal metal concentrations of *Plecotus auritus* (Brown Long-Eared Bats) in Britain that were over 1000 times higher for Pb and 55 times higher for Cd compared to concentrations from this study.

No significant difference in metal tissue levels was found between subadults and adults ($p = 0.69$). Similarly, Clark [55] found no relation between age, based on tooth wear, and Pb concentrations in whole body analyses from *M. lucifugus* and *Eptesicus fuscus* (Big Brown Bat). Thies and Gregory [53] also found no correlation between age and Pb liver concentrations of *T. brasiliensis* (Mexican Free-tailed Bat). Although not significant ($p = 0.14$), tissue concentrations for kidney Zn and kidney Pb were 14% and 73% higher, respectively in subadults compared to adults; however 55% lower Cd concentrations in kidneys of subadults compared to adults, although not significantly different ($p = 0.25$), is consistent with a study by Fritsch et al. [56], in which Cd tended to increase in concentration with age in rodents and shrews. Some possible reasons for younger animals having higher Cd levels include, subadults foraging in less optimal habitat due to best territories already claimed by adults, potential for metal transfer from mothers to young, or young of the year accumulating more metals due to higher growth rates.

Metal levels found in this study were low compared to other studies measuring metal levels in bats from other locations, as well as compared to studies of metal levels in other species from within Tar Creek Superfund Site [7,9,53,57,58]. The highest liver Pb level found in a bat from this study was 0.03 ug/g WW or 0.01 μg/g DW. Examples from other studies include: Thies and Gregory [53] – liver Pb levels of 3.93 ug/g WW, Zook et al. [58] – liver Pb levels of 500 ug/g WW, Hays and McBee [9] – turtle carapace Pb levels of 15 ug/g DW, and Beyer et al. [7] – liver Pb levels ranging between 7.5 – 94 ug/g WW. Beyer et al. [7] reported that out of multiple species of birds with elevated tissue metals concentrations, doves did not have elevated concentrations and suggested that they may not be feeding in heavily contaminated areas. Tar Creek Superfund Site is not a homogenously contaminated area; rather it is patchy with some highly contaminated areas, and others with background levels of metals. Bats as volant mammals may be able to forage selectively in less contaminated areas.

We hypothesized that bats collected within the contaminated Tar Creek Superfund Site would have higher tissue metal concentrations of Pb, Zn, and Cd in liver, kidney, and hair compared to bats collected from sites within a reference area; however this hypothesis was not entirely supported. Although some tissue metal concentrations were higher from bats collected within the contaminated area, a few tissue metal concentrations detected in bats collected from within the reference area were higher than levels found at contaminated sites (Table 3).

Kidney Zn concentrations were higher from TC males compared to BC males, which is consistent with insect and surface water concentrations. TC had the highest concentration of Cd of any site for both water and insects. Similarly PAN hair Zn concentrations in males were significantly higher compared to PLM concentrations in males, and PAN had the second highest concentration of Cd in water and insect samples. Pb concentrations from insects were highest at BC, and BC females had significantly higher hair Pb concentrations compared to females from TC ($p = 0.005$). Females from both reference and contaminated sites had higher concentrations of Pb in all three tissues compared to males, with the exception of PAN liver Pb, and hair Pb, and TC hair Pb. Clark [54] found that male *E. fuscus* had Pb concentrations 1.5 times higher than females, but noted that in mammals, Pb is generally at higher concentrations within female conspecifics.

The EPA collected surface water samples from multiple monitoring points throughout Tar Creek Superfund Site. Samples were analyzed for concentrations of Pb, Zn, and Cd in March 2009. Metal concentrations (µg/L) from monitoring sites located near my netting sites TC and BC, respectively, reported Zn – 5360, 549, Cd – 5.5, < 5, Pb – < 10,

< 10. Concentrations of Zn exceed both the acute (379 μg/L) and chronic (343 μg/L) levels for the Oklahoma Water Quality Standards. Concentrations of Pb and Cd reported by the EPA are similar to the concentrations found in this study from BC and TC, and Zn levels are much lower than those reported by the EPA [5].

I hypothesized that bats collected from contaminated sites would have significantly higher levels of Pb, Zn, and Cd in liver, kidney, and hair tissues compared to bats from reference sites. This hypothesis was supported in part, in that I found significant differences in some tissue metal levels within a sex when comparing BC to TC and PLM to PAN; however, differences were not significant across all four sites. This suggests that bats from both reference and contaminated sites may not take up the metals at high enough concentrations to cause impacts seen in terrestrial mammals examined within this same area.

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REFERENCES

1. USEPA (United States Environmental Protection Agency), Region 6. 1994. First Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.
2. USFWS (United States Fish and Wildlife Service). 2000. Partial restoration plan and environmental assessment, addressing injuries to migratory birds and threatened and endangered species at the Tar Creek Superfund Site, Ottawa County, Oklahoma. Oklahoma Ecological Services Field Office, Tulsa, Oklahoma.
3. OWRB (Oklahoma Water Resources Board) 1983. Groundwater Investigation in the Picher Field. OWRB, Oklahoma City, Oklahoma.
4. USEPA (United States Environmental Protection Agency), Region 6. 2005. Third Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

5. USEPA (United States Environmental Protection Agency), Region 6. 2010. Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.
6. Quapaw Tribe of Oklahoma. 2013. Quapaw Tribe Announces First Tribally-Managed Superfund Site Cleanup in the Nation. News Release/Advisory: Environmental Protection Agency. Retrieved from: http://www.epa.gov/region6/6sf/oklahoma/tar_creek/. Accessed 2 May 2014.
7. Beyer, W.N., J. Dalgarn, S. Dudding, J.B. French, R. Mateo, J. Miesner, L. Sileo, and J. Spann. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology 48:108-117.
8. Conder, J.M., and R.P. Lanno. 1999. Heavy metal concentrations in mandibles of white-tailed deer living in the Picher Mining District. Bulletin of Environmental Contamination and Toxicology 63:80-86.
9. Hays, K.A., and K. McBee. 2007. Flow cytometric analysis of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Ecotoxicology 16:353-361.
10. Phelps, K.L., and K. McBee. 2009. Ecological characteristics of small mammal communities at a Superfund site. American Midland Naturalist l61:57-68.
11. Phelps, K.L., and K. McBee. 2010. Population parameters of *Peromyscus leucopus* (White-footed deermice) inhabiting a heavy metal contaminated superfund site. The Southwestern Naturalist 55:363-373.

12. Schmitt, C.J., J.J. Shyte, W.G. Brumbaugh, and D.W. Tillitt. 2005. Chemical effects of lead, zinc, and cadmium from mining on fish in the tri-states district of northeastern Oklahoma, USA. Environmental Toxicology and Chemistry 24:1483-1495.
13. Schmitt, C.J., W.G. Brumbaugh, G.L. Linder, and J.E. Hinck. 2006. A screening-level assessment of lead, cadmium, and zinc in fish and crayfish from northeastern Oklahoma, USA. Environmental Geochemistry and Health 28:445-471.
14. Kunz, T.H., E. Braun de Torrez, D. Bauer, T. Lobova, and T.H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences 1223:1-38.
15. Cleveland, C.J., M. Betke, P. Federico, J.D. Frank, T.G. Hallam, J. Horn, J.D. Lopéz Jr., G.F. McCracken, R.A. Medellín, A. Moreno-Valdez, C.G. Sansone, J.K. Westbrook, and T.H. Kunz. 2006. Economic value of the pest control service provided by Brazilian free-tailed bats in southcentral Texas. Frontiers in Ecology and the Environment 4:238-243.
16. Federico, P., T.G. Hallam, G.F. McCracken, S.T. Purucker, W.E. Grant, A.N. Correa-Sandoval, J.K. Westbrook, R.A. Medellín, C.J. Cleveland, C.G. Sansone, J.D. López Jr., M. Betke, A. Moreno-Valdez, and T.H. Kunz. 2008. Brazilian free-tailed bats as insect pest regulators in transgenic and conventional cotton crops. Ecological Applications 18:826-837.
17. Jones, G., D.S. Jacobs, T.H. Kunz, M.R. Willig, and P.A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8:93-115.

18. Kunz, T.H., J.O. Whitaker Jr., and M.D. Wadanoli. 1995. Dietary energetics of the insectivorous Mexican free-tailed bat (*Tadarida brasiliensis*) during pregnancy and lactation. Oecologia 101:407-415.

19. Lee, Y., and G.F. McCracken. 2005. Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. Journal of Mammalogy 86:67-76.

20. Whitaker, J.O., Jr. 1995. Food of the big brown bat *Eptesicus fuscus* from the maternity colonies in Indiana and Illinois. American Midland Naturalist 134:346-360.

21. McBee, K., and J.W. Bickham. 1990. Mammals as bioindicators of environmental toxicity. Current Mammalogy 2:37-88.

22. Clark, D.R., Jr. 1981. Bats and environmental contaminants: a review. United States Department of the Interior Fish and Wildlife Service. Special Scientific Report. Wildlife No. 235. 27 pp.

23. Maina, J.N. 2000. What it takes to fly: the structural and functional respiratory refinements in birds and bats. Journal of Experimental Biology 203:3045-3064.

24. Voigt, C.C., K. Sorgel, and D.K. Dechmann. 2010. Refueling while flying: foraging bats combust food rapidly and directly to power flight. Ecology 91:2908-2917.

25. Currie, R.S., W.L. Fairchild, and D.C.G. Muir. 1997. Remobilization and export of cadmium from lake sediments by emerging insects. Environmental Toxicology and Chemistry 16:2333-2338.

26. Price, P.W., B.J. Rathcke, and D.A. Gentry. 1974. Lead in terrestrial arthropods: evidence for biological concentration. Environmental Entomology 3:370-372.

27. Kalcounis-Rueppell, M.C., V.H. Payne, S.R. Huff, and A.L. Boyko. 2007. Effects of wastewater treatment plant effluent on bat foraging ecology in an urban stream system. Biological Conservation 138:120-130.

28. Clark, D.R., Jr., and R.L. Hothem. 1991. Mammal mortality at Arizona, California, and Nevada gold mines using cyanide extraction. California Fish and Game 77:61-69.

29. Wimsatt, J., T.J. O'Shea, L.E. Ellison, R.D. Pearce, and V.R. Price. 2005. Anesthesia and blood sampling of wild big brown bats (*Eptesicus fuscus*) with an assessment of impacts on survival. Journal of Wildlife Diseases 41:87-95.

30. Eguren, R.E., and K. McBee. 2013. New records of three species of bats from Nowata, Rogers, and Ottawa counties, Oklahoma. Proceedings of the Oklahoma Academy of Science 93:13-18.

31. Sites, R.S., W.L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235-253.

32. Oklahoma Mesonet. 2013. [on-line]. Available from: http://www.mesonet.org/index.php/weather/category/past_data_files. (Accessed March 15, 2013).

33. Kunz, T.H. 1974. Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). Ecology 55:693-711.

34. Paulson, G.S. 2005. Handbook to the construction and use of insect collection and rearing devices: A guide for teachers with suggested classroom applications. Springer. New York,NY.

35. Sanchez-Chardi, A., M.J. López-Fuster, and J. Nadal. 2007. Bioaccumulation of lead, mercury, and cadmium in the greater white-toothed shrew, *Crocidura russula,* from the Ebro Delta (NE Spain); sex- and age- dependent variation. Environmental Pollution 145:7-14.

36. Hair, J.D. 1980. Measurement of ecological diversity, p. 269-275. *In:* S. D. Schemnitz (ed.). Wildlife management techniques manual. The Wildlife Society, Washington, D.C.

37. Kalisinska, E., J. Gorecki, A. Okonska, B. Pilarczyk, A. Tomza-Marciniak, H. Budis, N. Lanocha, D.I. Kosik-Bogacka, K.M. Kavetska, M. Macherzynski, and J. Golas. 2014. Hepatic and nephric mercury and selenium concentrations in common mergansers, *Mergus merganser*, from Baltic region, Europe. Environmental Toxicology and Chemistry 33:421-430.

38. Brunet-Rossinni, A.K., and G.S. Wilkinson. 2009. Methods for age estimation and the study of senescence in bats. In T.H. Kunz and S. Parsons (eds.). Ecological and behavioral methods for the study of bats, 2nd edition, Johns Hopkins University Press, Baltimore, MD.

39. Kendall, R.J., T.A. Anderson, R.J. Baker, C.M. Bens, J.A. Carr, L.A. Chiodo, G.P. Cobb III, R.L. Dickerson, K.R. Dixon, L.T. Frame, M.J. Hooper, C.F. Martin, S.T. McMurry, R. Patino, E.E. Smith, and C.W. Theodorakis. 2001. Ecotoxicology, p. 1013-1045. *In*: C.D. Klaasen (ed.). Casarett and Doull’s toxicology: basic science of poisons. Sixth edition. McGraw-Hill Publishing, New York, NY.

40. Saugey D.A., D.R. Heath, and G.A. Heidt. 1989. The bats of the Ouachita Mountains. Proceedings of the Arkansas Academy of Science 43:71-77.

41. Perry R.W., S.A. Carter, and R.E. Thill. 2010. Temporal patterns in capture rate and sex ratio of forest bats in Arkansas. American Midland Naturalist 164:270-282.
42. Caire, W., R.M. Hardisty, and K. Lacy. 1988. Capture heights and times of *Lasiurus borealis* (Chiroptera: Vespertilionidae) in Southeastern Oklahoma. Proceedings of the Oklahoma Academy of Science 68:51-53.
43. Dumitrescu, E., T. Alexandra, and P. Snejana. 2008. Lead acetate impact of some markers of female reproductive system performances (litter size, sex ratio) and physical development (vaginal opening) in rats. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine 65:283-287.
44. Bouland, A.J., A.E. White, K.P. Lonabaugh, C.W. Varian-Ramos, and D.A. Cristol. 2012. Female-biased offspring sex ratios in birds at a mercury-contaminated river. Journal of Avian Biology 43:244-251.
45. Fujita, M.S., and T.H. Kunz. 1984. Pipstrellus subflavus. Mammalian Species 228:1-6.
46. Piasek, M., and J.W. Laskey. 1994. Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. Reproductive Toxicology 8:495-507.
47. Barbour, R.W., and W.H. Davis. 1969. Bats of America. University of Kentucky Press, Lexington, KY.
48. Shump, K.A., Jr., and A.U. Shump. 1982. Lasiurus borealis. Mammalian Species 183:1-6.
49. Williams, D.F., and J.S. Findley. 1979. Sexual size dimorphism in vespertilionid bats. American Midland Naturalist 102:113-126.

50. Faulkner, B.C., and R.L. Lochmiller. 2000. Ecotoxicity revealed in parasite communities of *Sigmodon hispidus* in terrestrial environments contaminated with petrochemicals. Environmental Pollution 110:135-145.

51. Wilber, P.G., K. McBee, D.J. Hafner, and D.W. Duszynski. 1994. A new coccidian (Apicomplexa: Eimeriidae) in the northern pocket gopher (*Thomomys talpoides*) and a comparison of oocyst survival in hosts from radon-rich and radon-poor soils. Journal of Wildlife Diseases 30:359-364.

52. Clark, D.R., Jr., A.S. Wenner, and J.F. Moore. 1986. Metal residues in bat colonies, Jackson County, Florida, 1981-1983. Florida Field Naturalist 14:38-51.

53. Thies, M., and D. Gregory. 1994. Residues of lead, cadmium and arsenic in livers of Mexican free-tailed bats. Bulletin of Environmental Contamination and Toxicology 52:641-648.

54. Walker, L.A., V.R. Simpson, L. Rockett, C.L. Weinburg, and R.F. Shore. 2007. Heavy metal contamination in bats in Britain. Environmental Pollution 148:483-490.

55. Clark, D.R., Jr. 1979. Lead concentrations: bats vs. terrestrial small mammals collected near a major highway. Environmental Science and Technology 13:338-341.

56. Fritsch, C., R.P. Cosson, M. Coeurdassier, F. Raoul, P. Giraudoux, N. Crini, A. de Vaufleury, and R. Scheifler. 2010. Responses of wild small mammals to a pollution gradient: host factor influence metal and metallothionein levels. Environmental Pollution 158:827-840.

57. Sutton, R.H., and P.D. Wilson. 1983. Lead poisoning in grey-headed fruit bats (*Pteropus poliocephalus*). Journal of Wildlife Diseases 19:294-296.

58. Zook, B.C., R.M. Sauer, and F.M. Garner. 1972. Lead poisoning in captive wild animals. Journal of Wildlife Diseases 8:264-272.

Table 1. Number of bats captured at each of 4 sites for 2012 and 2013 combined. Superscript r indicates a reference site and superscript c indicates a contaminated site. Simpson's diversity values (*D)* were calculated for each site. A net night equals one mist net set for one 5 hour period.

	Lasiurus borealis		*Lasiurus cinereus*		*Perimyotis subflavus*		*Myotis lucifugus*		*Nycticeus humeralis*				
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	Total	*D*	Bats/ net night
Plum Creek^{r}	6	10	0	0	0	0	0	0	1	0	17	0.12	0.27
Panther Creek^{r}	2	2	0	0	1	0	0	1	0	0	6	0.55	0.09
Beaver Creek^{c}	2	4	1	0	1	0	0	0	0	0	8	0.47	0.09
Tar Creek^{c}	12	5	0	0	0	0	0	0	0	0	17	0.00	0.23
Total	22	21	1	0	2	0	0	1	1	0	48		
Species Total	43		1		2		1		1				

Table 2. Sex ratios and average body mass (mean ± SE) separated by site and sex for *Lasiurus borealis* collected for 2012-2013 combined. Sample size is included in parentheses. Superscript r and c indicate a reference site and contaminated site, respectively.

Site	Sex ratio (male:female)	Average body mass (g)	
		Males	Females
Plum Creek^r	1.7:1	8.3 ± 0.4 (9)	11.5 ± 1.0 (6)
Panther Creek^r	1:1	9.0 ± 0.0 (2)	12.3 ± 0.3 (2)
Beaver Creek^c	2:1	9.6 ± 0.5 (4)	12.5 ± 2.0 (2)
Tar Creek^c	0.42:1	7.7 ± 0.6 (5)	10.6 ± 0.4 (12)

Table 3. Mean (± SE) metal levels in *Lasiurus borealis* (µg/g DW; n = 43), insects (µg/g DW; *n* = 1 sample night per site, means are average of two independent analyses), and water (Zn—mg/L, Pb and Cd—µg/L, *n* = 3 per site). Sample sizes for bats are in parentheses, and range is listed below mean.

	Plum^{r}	Panther^{r}	Beaver^{c}	Tar^{c}
Liver Zn	3.05 ± 0.31 (14)	3.22 ± 0.61 (3)	2.82 ± 0.43 (6)	2.98 ± 0.29 (15)
	0.296-4.530	2.11-4.22	1.98-6.31	1.98-6.31
Cd	0.02 ± 0.01 (13)	0.02 ± 0.01 (3)	0.02 ± 0.01 (6)	0.01 ± 0.00 (15)
	0.001-0.131	0.012-0.030	0.010-0.050	0.028-0.002
Pb	0.004 ± 0.000 (13)	0.006 ± 0.000 (3)	0.008 ± 0.000 (6)	0.008 ± 0.000 (15)
	0.000-0.014	0.003-0.008	0.002-0.016	0.002-0.026
Kidney Zn	2.482 ± 0.137 (13)	2.364 ± 0.088 (2)	2.339 ± 0.176 (6)	2.884 ± 0.157 (15)
	1.682-3.314	2.207-2.570	1.874-3.022	2.323-4.450
Cd	0.016 ± 0.003 (12)	0.049 ± 0.029 (4)	0.021 ± 0.008 (6)	0.015 ± 0.002 (15)
	0.002-0.038	0.013-0.136	0.009-0.061	0.001-0.029
Pb	0.005 ± 0.002 (13)	0.002 ± 0.001 (3)	0.005 ± 0.001 (6)	0.003 ± 0.001 (15)
	0.000-0.033	0.000-0.006	0.000-0.011	0.000-0.014

Table 3. Continued

	Plum[r]	Panther[r]	Beaver[c]	Tar[c]
Hair Zn	3.125 ± 0.227 (16)	4.438 ± 0.740 (4)	4.531 ± 0.823 (6)	3.146 ± 0.259 (16)
	0.942-4.662	2.692-5.694	1.440-7.552	1.835-4.961
Cd	0.009 ± 0.009 (16)	0.003 ± 0.002 (4)	0.004 ± 0.003 (6)	0.0002 ± 0.0001 (16)
	0.000- 0.143	0.000-0.007	0.000-0.017	0.000-0.003
Pb	0.012 ± 0.011 (16)	0.030 ± 0.019 (4)	0.120 ± 0.085 (6)	0.007 ± 0.006 (16)
	0.000-0.171	0.000-0.085	0.000-0.526	0.000-0.099
Insect Zn	3.002 ± 0.002	4.922 ± 0.005	4.543 ± 0.053	9.173 ± 0.003
Cd	0.009 ± 0.014	0.028 ± 0.065	0.009 ± 0.374	0.049 ± 0.196
Pb	0.012 ± 0.149	0.001 ± 0.362	0.024 ± 0.666	0.001 ± 0.211
Water Zn	0.007 ± 0.001	0.020 ± 0.013	0.139 ± 0.005	5.969 ± 0.611
Cd	0.038 ± 0.004	0.353 ± 0.148	0.115 ± 0.011	6.546 ± 0.952
Pb	0.219 ± 0.153	1.579 ± 1.356	BDL	0.934 ± 0.658

Table 4. Metal-tissue correlations for significant ($\alpha \leq 0.05$) Pearson Correlation Coefficients.

Tissues	Pearson Correlation Coefficient (*p*-value)
Liver Zn x Kidney Zn	0.5126 (0.001)
Liver Cd x Kidney Cd	0.7883 (<0.0001)
Liver Pb x Hair Pb	0.3165 (0.05)
Hair Zn x Hair Pb	0.5902 (<0.0001)
Hair Cd x Hair Pb	0.3020 (0.01)

Figure 1. Map of Oklahoma with the contaminated locality, Tar Creek Superfund Site, identified by a square and the reference locality, Oologah Wildlife Management Area, identified by a triangle. Contaminated and Reference localities are about 67 km apart. Individual collection sites, identified by circles, within each locality are about 8 km apart.

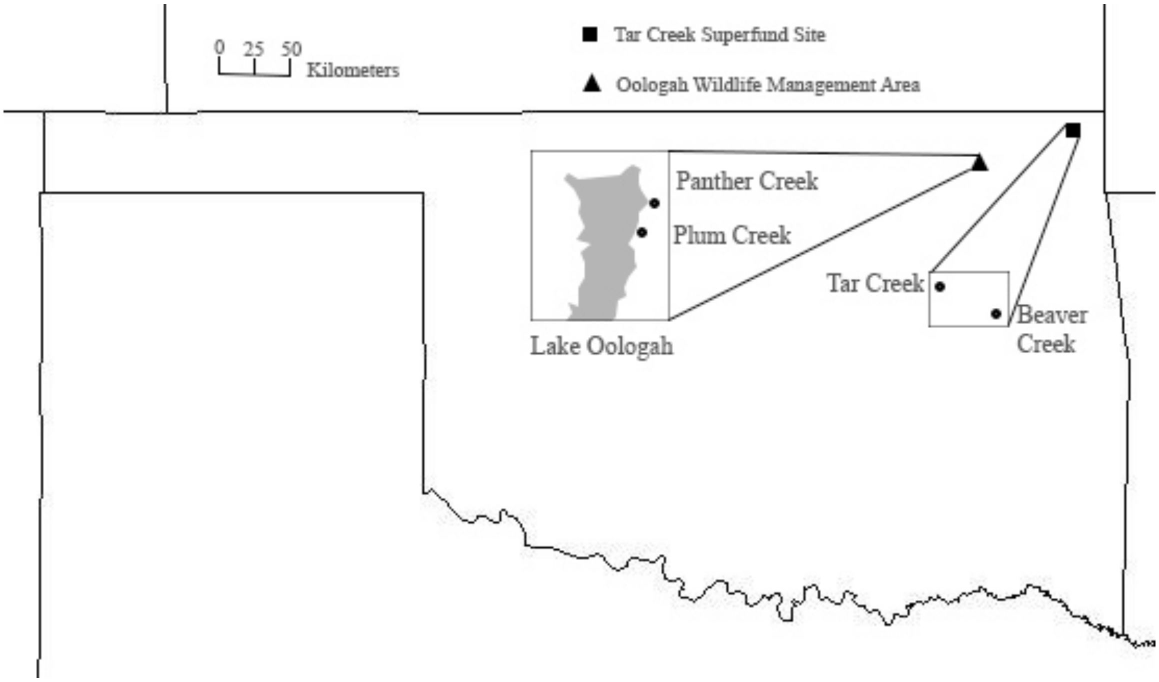


Figure 2**.** Tail Length (mm) by site showing standard error bars (Reference *n* = 19, Contaminated *n* = 21). *P* = 0.005.

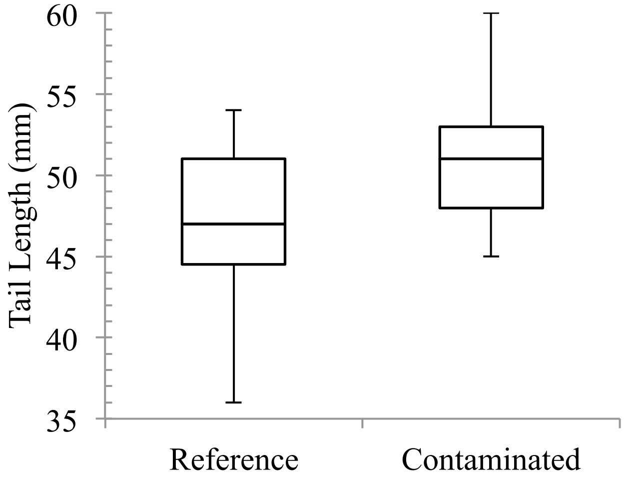
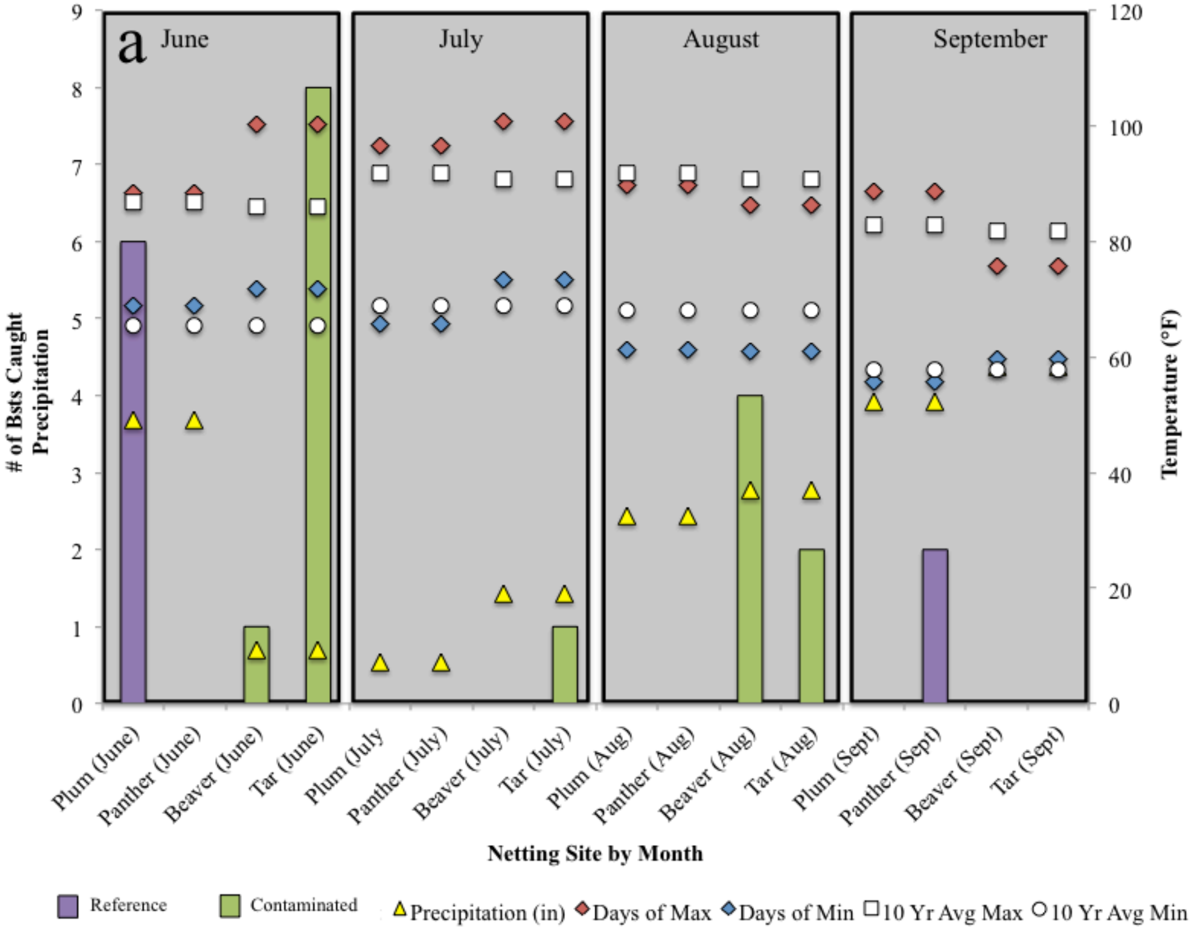
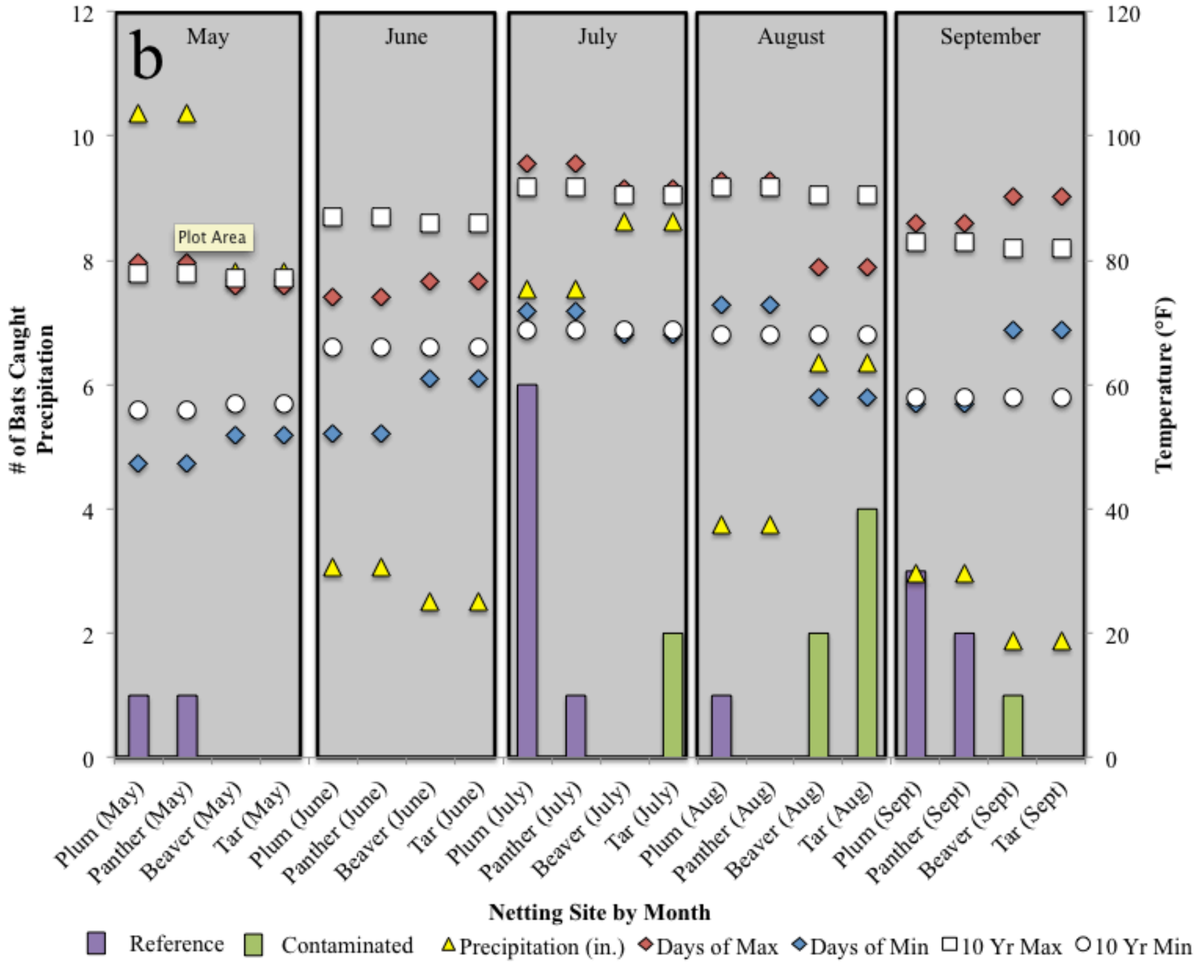


Figure 3 a & b. Number and location of bat captures for 2012 (a) and 2013 (b) by month in relation to weather variables recorded from closest Mesonet weather station. Number of bats captured (left y-axis) is represented by purple (reference sites) and green (contaminated sites) bars. Temperature (°F) is located on the right y-axis. Diamonds represent the 3 day maximum (red) and minimum (blue) temperatures for the actual nights netted, whereas the 10 year average maximum (squares) and minimum (circles) temperatures are in white. Yellow triangles represent inches of precipitation recorded for the month from the closest Mesonet weather station.


a
June
July
August
September
# of Bsts Caught
Precipitation
Temperature (°F)
0
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
120
Plum (June)
Panther (June)
Beaver (June)
Tar (June)
Plum (July
Panther (July)
Beaver (July)
Tar (July)
Plum (Aug)
Panther (Aug)
Beaver (Aug)
Tar (Aug)
Plum (Sept)
Panther (Sept)
Beaver (Sept)
Tar (Sept)
Netting Site by Month
Reference
Contaminated
Precipitation (in)
Days of Max
Days of Min
10 Yr Avg Max
10 Yr Avg Min



*Nets were not set in June 2013 due to heavy rains during scheduled netting dates.

CHAPTER III

BEHAVIORAL ASSAY ON *LASIURUS BOREALIS* (CHIROPTERA) SHOWS DIFFERENCES BETWEEN BATS FROM CONTAMINATED AND REFERENCE SITES BASED ON FLIGHT MANEUVERABILITY

Abstract – I conducted a behavioral flight assay on Eastern Red Bats (*Lasiurus borealis*: Chiroptera) to measure impacts of metals on maneuverability and willingness to fly in bats from a contaminated, abandoned mining area, Tar Creek Superfund Site compared to bats from an uncontaminated reference site. Bats collected via mist nets were assessed for flight performance within a portable flight cage. Bats from the reference site showed strong relationships ($R^2 = 0.69$ and $R^2 = 0.97$) between flight cage variables measured that were not observed in contaminated bats. Tissue metal concentrations (Pb, Zn, and Cd) were determined in liver, kidney, and hair from bats analyzed via flight cage trial to compared metal levels to behavioral assay responses. Metal concentrations in bats from the contaminated sites were not significantly different from those found in bats from the reference sites; however, we were able to distinguish between reference bats and bats from the contaminated site using the flight cage assay. This study provides the first data on assessing impacts of metal levels with a non-invasive behavioral assay using a portable flight cage.

Keywords – Lead, zinc, cadmium, flight cage, maneuverability, obstacle course, Tar Creek Superfund Site

1. Introduction

Tar Creek Superfund Site is a 40 mi^2 area located in northeastern Oklahoma, encompassing the communities of Picher, Quapaw, Cardin, Commerce, and Miami (ATDSR, 2004). Lead (Pb) and zinc (Zn) mining occurred between the early 1900s and the 1970s, with an annual extraction of 130,410 tons of Pb and 749,254 tons of Zn at peak operation (USEPA, 2005). The Environmental Protection Agency (EPA) listed this area as a Superfund Site in 1983 due to high levels of cadmium (Cd), Pb, and Zn in soils and water (ATDSR, 2004; USEPA, 1994, 2005, 2010). Major sources of exposure for humans included soil, mine tailings, ingestion of homegrown produce and tap water, airborne dust, and use of biota by Tar Creek area tribal populations (ATSDR, 2004). Neuberger et al. (2009) recorded an excess of adult mortality within Ottawa Co., OK compared to all other counties in Oklahoma. Several studies have determined elevated Pb, Zn, and Cd levels (Beyer et al., 2004; Conder and Lanno, 1999; Hays and McBee, 2007; Schmitt et al., 2005, 2006), described histopathological lesions (Beyer et al., 2004), and documented effects on community structure and population demographics (Hays and McBee, 2010; Phelps and McBee, 2009, 2010) and genetic structure (Hays and McBee, 2007, 2010) in a variety of taxa present within Tar Creek Superfund Site; however, no studies have addressed potential impacts on behavior in wild vertebrates inhabiting the Site.

Behavioral assays have been used to determine effects of metals on wild species of vertebrates (Burger and Gochfield, 1995; Marentette et al., 2012). Often maneuverability is a measure used in these behavioral assays. Maneuverability in volant animals has been tested by use of an obstacle course within a flight cage (Brilot et al., 2009); however, no studies have addressed the impacts of metals on maneuverability in bats. In mammals, Pb poisoning can impact multiple systems within the body, including the nervous system, gastrointestinal tract, muscular, and blood systems (Eisler, 1988). Lead has no biological use in the body and requires a slow, energetically wasteful excretion process; therefore, any amount of body Pb only negatively impacts the organism. Reports of bats suffering from Pb toxicosis describe them as lethargic, and unwilling to fly (Sutton and Wilson, 1983; Zook, 1972).

Insectivorous bats require a high level of maneuverability in order to locate and reach prey, water sources, and roosts, as well as avoid predators. Due to the high-energy requirements associated with flight, bats have a high metabolism, requiring them to consume close to their body weight in insects each evening (Kunz et al., 1995). Interspecies variations in maneuverability allows for multiple species of bats to share a foraging area based on aspects of wing structure, which is closely related to life history. Bats with lower wing loading often will be found foraging in areas that have high clutter, such as within the forest and amongst branches of a tree. Bats with higher wing loading and aspect ratio forage in more open areas, such as above the canopy or over water. Bats with low aspect ratio and wing loadings have longer, thinner wings allowing them to fly at high speeds, but with limited maneuverability (Norberg and Rayner, 1987). By categorizing bats into three different groups: slow agile fliers, fast strong fliers with little maneuverability, and a group that lies in the middle, we gain an understanding of the foraging demands encountered by each group, and the types of habitats in which they will be found. Reduced maneuverability for any reason impacts foraging ability, which in turn, can have a real effect on survival and fitness. Even chronic low levels of exposure to a contaminant with neurological effects potentially can affect a bat's maneuverability. Bats within the contaminated areas have potential for exposure to metals, which may impact their maneuverability and willingness to fly (De Francisco et al., 2003; Sutton, 1987).

Insectivorous bats can be used as bioindicators of environmental contamination due to several factors of their ecology (McBee and Bickham, 1990; Jones et al., 2009). First, they are known to accumulate toxicants in contaminated environments (Clark, 1981). Second, for their size, bats are long lived and thus can give a longer view of the environment with which they are interacting. Third, insectivorous bats consume large amounts of arthropods each evening in order to maintain their high metabolism (Maina, 2000; Voigt et al., 2010). Bats may consume insects that are aquatic or that spend larval periods in the water or sediments. Both of these types of arthropods are known to be sources of contamination for species that prey on them (Currie et al., 1997; McBee and Bickham, 1990; Price et al., 1974).

Bats contribute to a healthy ecosystem by providing ecosystem services, which maintain natural checks and balances (Kunz et al., 2011). These processes are required for healthy growth and development of the natural environment on which humans rely for survival (Kremen, 2005). Cleveland et al. (2006) showed that bats feeding over agricultural fields in south Texas save individual farmers about $200,000 per year in costs for pesticide applications. In addition to insect suppression, insectivorous bats provide ecosystem services in the form of nutrient cycling via dispersal of guano (Federico et al., 2008; Jones et al., 2009; Kunz et al., 1995, 2011; Lee and McCracken, 2005; Whitaker, 1995).

Due to importance of maneuverability to foraging ability, mate location, and roost accessibility in bats, and their potential for exposure to Pb within the contaminated Tar Creek Superfund Site area, we developed a non-invasive behavioral assay based on thermographic videography to investigate maneuverability and willingness to fly in resident bats. I tested the hypotheses that bats collected within the contaminated site would have lower maneuverability and lower willingness to fly compared to bats from a reference population and that bats with lower maneuverability and willingness to fly would have higher levels of Pb, Zn, and Cd in liver, kidneys, and hair compared to bats collected from a reference population,

2. Methods

2.1 Study sites

During summer 2013, bats were collected from two contaminated sites, Tar Creek (N 36°57.495', W 94°50.731') and Beaver Creek (N 36°56.2026', W 94°45.3846'), and one site within the reference locality, Oologah Wildlife Management Area, Plum Creek (N 36°31.120', W 95°33.610'; Fig. 4). The contaminated area and the reference area are approximately 67 km apart from one another and on separate river drainages, ensuring that the reference area is not contaminated via wind-blown chat or sediments, and that no bat populations are shared between these two localities.

2.2 Field methods

Bats were collected via mist nets placed over riparian areas for 3 consecutive nights each month during the time of year when bats are active (May – Sept 2013). Three to 4 nets were opened at sunset and left in place for 5 hours or until 1am, and checked every 15 minutes. Total sampling effort was 120 net–nights, with 37 net–nights at reference and 83 net–nights at contaminated sites. Sampling was not possible during June 2013 due to flooding and continued rain.

Captured bats were removed from the net manually, identified in the hand, and reproductive condition (scrotal/nonscrotal; pregnant/not pregnant; lactating/not lactating), approximate age (as determined by shining a flash light through the extended fingers of the wing to determine degree of epiphyseal ossification), mass, and sex were recorded. Bats were placed in cloth bags to be transferred to the portable flight cage.

Bats were released, one at a time, into a portable flight cage made from a 10' x 15' pop-up canopy tent with an attachable mesh enclosed cage containing an obstacle course to test flight performance. The cage was divided into three equal sections, with obstacles present only in the middle section (Fig. 5). Three wooden poles (10' x 2" x 1"), were extended across the top of the mesh cage in the middle 5 foot section. Obstacles, consisting of lines made from size 9 nylon seine twine and weighted with small washers, were attached to magnetic strips glued to the underside of the poles via magnets attached to each line. The two outer poles had 6 obstacles and the inner pole had 5 obstacles, which were off set to prevent open flyways through the course. Width between obstacles was 450 mm, slightly longer than the average wingspan for *L. borealis* (293 ± 0.001 mm; Salcedo et al., 1995). The magnets connecting obstacles to the poles were weak enough that when hit by a bat, lines dropped to the floor of the cage. Flight trials lasted 10 min and were video recorded with a thermographic camera (Flir Scout TS-32 Pro). Thermal markers were attached to the inside the cage with Velcro® to delineate sections of the cage visibly on thermal video recordings. After recording for 10 min., number of obstacles dropped was counted by at least 2 people. After each trial, bats were recaptured with a hand net and returned to cloth bags until they could be processed for metal analysis.

Variables measured during each trial included: the number of movements between sections of the cage, the number of obstacles dropped, and time spent in flight, giving a measure of willingness to enter the obstacle course, maneuverability, and willingness to fly, respectively. Movement between sections of the cage and time spent in flight were scored from the video files recorded by the thermographic camera. Each time a bat moved from one third of the cage into another (e.g., from a section with no obstacles into the section with obstacles—Fig. 5), a score of 1 was recorded. The amount of time a bat spent flying, rather than roosting on or crawling on the mesh sides of the cage, during the 10 min trial was recorded in sec. One person scored all videos for movement and flight time. Twenty percent of the videos were scored 3 times each to verify that scores and flight times were assessed accurately.

After flight trials bats were anesthetized with isoflurane then euthanized via cervical dislocation. The entire liver and both kidneys were removed, stored in sterile tubes and placed in liquid nitrogen for return to the laboratory where they were analyzed for Pb, Zn, and Cd. A 1-cm^2 patch of hair was also clipped from the venter (Wimsatt et al., 2005) of each animal and stored in sterile tubes for analysis of metal content. All procedures were conducted following standards set forth by the Animal Care and Use Committee of the American Society of Mammalogists (Sites et al., 2011) according to Oklahoma State University (OSU) Animal Care and Use Protocol #AS129.

2.3 Metal analysis

The entire liver, both kidneys, and hair samples were analyzed following modified methods of Sanchez-Chardi et al. (2007) which are outlined in Ch. 2 of this thesis.

Samples were digested with 1 ml HNO_3 (Fisher Scientific; Trace Metal Grade) using an Ethos EZ closed vessel microwave digester (Milestone; Hair and Animal Tissue Protocol). A 100 μl sample of each digestate was diluted to a total volume of 5 ml with Ultrapure H_2O (Millipore) plus a 10 μl aliquot of internal standard (PerkinElmer Multi-element Calibration Standard 2% HNO_3). Concentrations of Pb, Zn, and Cd were measured by an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Perkin-Elmer ELAN-6000. Quality control practices included use of duplicates for each sample, as well as blanks and internal standards and followed standard operating procedures for the OSU Metabolic and Nutrition Phenotyping Core Facility.

2.4 Statistical analyses

Statistical analyses were performed using SAS 9.3. Data were not normally distributed; therefore, nonparametric analyses were used. Due to low sample sizes, flight cage variables were compared between reference and pooled contaminated sites (PROC NPAR1WAY; PROC CORR). Tissues metal concentrations were compared between bats from reference and contaminated sites and correlations between metal concentrations and the three flight variables were determined using PROC NPAR1WAY and PROC CORR.

3. Results

Lasiurus borealis was the most frequently caught species and was therefore the only species used for flight trials. Six bats (4M:2F) from the reference site, and 8 bats (4M:4F) from combined contaminated sites were video recorded in the flight cage. Females and males did not differ ($\alpha = 0.05$) for any of the flight variables; therefore sexes were pooled for remaining analyses. Bats from contaminated sites hit more obstacles (mean ± S.E.; 4.1 ± 0.72) than did bats from the reference site (3.5 ± 1.31). Bats from contaminated sites were less likely to move through the obstacle course into the other sections of the cage (mean number of movements = 52 ± 17) compared to bats from the reference site (mean = 61 ± 38), but spent more time in flight (51 ± 15 s); however, none of the 3 variables were significantly different between reference and contaminated groups. Bats from the reference site showed strong relationships between amount of time spent flying and number of obstacles dropped ($R^2 = 0.6945$) and between amount of time spent flying and movement between sections of the cage ($R^2 = 0.9758$; Fig. 6a). Bats from contaminated sites did not show strong relationships between flight variables (obstacles dropped and flight time—$R^2 = 0.0004$; movement and flight time—$R^2 = 0.2422$; Fig. 6b).

Means and ranges for metal concentrations separated by tissue and site are in Table 1. Bats at reference and contaminated sites did not differ statistically for any metal ($\alpha = 0.05$); however, bats from contaminated sites had higher levels of Pb in liver and higher levels of Zn in kidney and hair. Pearson correlation coefficients were calculated for the 3 flight variables and each of 7 metal/tissues combinations for all bats (Table 2). Liver, kidney, and hair Zn were all negatively correlated with amount of time spent in flight. Kidney and hair Zn were negatively correlated to movement scores. Liver Pb and Cd were positively correlated with number of obstacles dropped, whereas kidney Pb was negatively correlated with number of obstacles dropped. Although correlation coefficients indicated strong relationships between liver Pb and number of obstacles dropped and between liver Zn and amount of time spent in flight, none of the *p* values values were below 0.05.

4. Discussion

Lasiurus borealis is considered an aerial hawker, meaning it hunts and catches insects in flight (Norberg and Rayner, 1987). This type of foraging behavior requires a high level of maneuverability. For bats that are actively flying we would expect to see a strong relationship between amount of time spent in flight, number of obstacles dropped, and number of movements between sections of the cage as was seen in bats from the reference site (Fig. 6a). I did not find these relationships in bats from the contaminated sites (Fig. 6b). Interestingly, bats from contaminated sites showed no obvious relationships between amount of time spent flying and number of obstacles dropped or number of movements between sections of the cage. My hypothesis that bats collected within the contaminated area would have lower willingness to fly compared to bats from an uncontaminated reference site was not supported; however, they did drop more obstacles and were less willing to maneuver through the portion of the cage that contained the obstacles (i.e., number of movements between sections of the cage).

Pb is a neurotoxin that can cause uncoordinated movement, muscle tremors, and a lack of appetite in bats (Sutton, 1987). Shrews (*Blarina brevicauda)* exposed to 25 mg/kg lead acetate daily in drinking water were more hyperactive than animals that received sodium acetate in water (Punzo and Farmer, 2003). A similar hyperactive response may explain why bats from contaminated sites flew 60 s more on average compared to bats from the reference site.

I hypothesized that bats collected within contaminated sites would have higher tissue metal concentrations of Pb, Zn, and Cd in liver, kidney, and hair compared to bats collected from PLM. My data did not support this hypothesis; rather, I found that metal tissue concentrations were not significantly different ($p > 0.05$) between the reference site and contaminated sites. Although metal levels were not significantly different, negative correlation between liver, kidney and hair Zn and time spent in flight suggests that bats spent less time flying with increased Zn levels. Concentrations of Pb and Cd in liver were positively correlated with number of obstacles dropped, suggesting that animals with higher Pb and Cd concentrations in liver hit more obstacles; however, because kidney Pb was negatively correlated with number of obstacles dropped the relationship between metal uptake and maneuverability is not straightforward.

Bats with higher Zn concentrations made fewer movements between sections of the cage and spent less time flying. Despite a tendency to make fewer movements between sections of the cage, bats with higher Cd levels in liver and kidney tended to drop more obstacles. Finally, higher concentrations of Pb in liver suggest a trend towards hyperactivity and poor maneuverability; with more movements, time in flight and obstacles dropped. Although tissue metal levels in *L. borealis* were not significantly different between sites, bats from contaminated sites and bats from the reference site showed differences in flight behavior. Despite lower tissue metal concentrations than those associated with Pb toxicity and loss of ability to fly in other bats (Sutton and Wilson, 1983; Zook et al., 1972), bats from contaminated sites did show decreased ability to maneuver through an obstacle course. This study provided the first behavioral flight assay to assess impacts of metals on bats.

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References

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Report to Congress, Tar Creek Superfund Site, Ottawa County, Oklahoma. Centers for Disease Control and Prevention, Atlanta, Georgia. 35 pp.

Beyer, W.N., J. Dalgarn, S. Dudding, J.B. French, R. Mateo, J. Miesner, L. Sileo, and J. Spann. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology 48:108-117.

Brilot, B.O., L. Asher, and M. Bateson. 2009. Water bathing alters the speed-accuracy trade-off of escape flights in European starlings. Animal Behaviour 78:801-807.

Burger, J., and M. Gochfeld. 1995. Effects of varying temporal exposure to lead on behavioral development in herring gull (*Larus argentatus*) chicks. Pharmacology Biochemistry and Behavior 52:601-608.

Clark, D.R., Jr. 1981. Bats and environmental contaminants: a review. United States Department of the Interior Fish and Wildlife Service. Special Scientific Report. Wildlife No. 235. 27 pp.

Cleveland, C.J., M. Betke, P. Federico, J.D. Frank, T.G. Hallam, J. Horn, J.D. Lopéz, Jr., G.F. McCracken, R.A. Medellín, A. Moreno-Valdez, C.G. Sansone, J.K. Westbrook, and T.H. Kunz. 2006. Economic value of the pest control service provided by Brazilian free-tailed bats in south central Texas. Frontiers in Ecology and the Environment 4:238–243.

Conder, J.M., and R.P. Lanno. 1999. Heavy metal concentrations in mandibles of white-tailed deer living in the Picher Mining District. Environmental Contamination and Toxicology 63:80-86.

Currie, R.S., W.L. Fairchild, and D.C.G. Muir. 1997. Remobilization and export of cadmium from lake sediments by emerging insects. Environmental Toxicology and Chemistry 16:2333-2338.

De Francisco, N., J.D. Ruiz Troya, and E.I. Agüera. 2003. Lead and lead toxicity in domestic and free living birds. Avian Pathology 32:2-13.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Services Biological Report 85:1-134.

Federico, P., T.G. Hallam, G.F. McCracken, S.T. Purucker, W.E. Grant, A.N. Correa-Sandoval, J.K. Westbrook, R.A. Medellín, C.J. Cleveland, C.G. Sansone, J.D. López, Jr., M. Betke, A. Moreno-Valdez, and T.H. Kunz. 2008. Brazilian free-tailed bats as insect pest regulators in transgenic and conventional cotton crops. Ecological Applications 18:826–837.

Hays, K.A., and K. McBee. 2007. Flow cytometric analysis of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Ecotoxicology 16:353-361.

Hays, K.A., and K.McBee. 2010. Population demographics of red-eared slider turtles (*Trachemys scripta*) from Tar Creek Superfund Site. Journal of Herpetology 44:441-446.

Jones, G., D.S. Jacobs, T.H. Kunz, M.R. Willig, and P.A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8:93-115.

Kremen, C. 2005. Managing ecosystem services: what do we need to know about their ecology? Ecology Letters 8:468-479.

Kunz, T.H., E. Braun de Torrez, D. Bauer, T. Lobova, and T.H. Fleming. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences 1223:1–38.

Kunz, T.H., J.O. Whitaker, Jr., and M.D. Wadanoli. 1995. Dietary energetics of the insectivorous Mexican free-tailed bat (*Tadarida brasiliensis)* during pregnancy and lactation. Oecologia 101:407-415.

Lee, Y., and G.F. McCracken. 2005. Dietary variation of Brazilian free-tailed bats links to migratory populations of pest insects. Journal of Mammalogy 86:67–76.

Maina, J.N. 2000. What it takes to fly: the structural and functional respiratory refinements in birds and bats. Journal of Experimental Biology 203:3045-3064.

Marentette, J.R., S. Tong, G. Wang, N.M. Sopinka, M.D. Taves, M.A. Koops, and S. Balshine. 2012. Behavior as biomarker? Laboratory versus field movement in round goby (*Neogobius melanostomus*) from highly contaminated habitats. Ecotoxicology 21:1003-1012.

McBee, K., and J.W. Bickham. 1990. Mammals as bioindicators of environmental toxicity. Current Mammalogy 2:37-88.

Neuberger J.S., S.C. Hu, K.D. Drake, and R. Jim. 2009. Potential health impacts of heavy-metal exposure at the Tar Creek Superfund Site, Ottawa County, Oklahoma. Environmental Geochemical Health 31:47-59.

Norberg, U.M., and J.M.V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 316:335-427.

Phelps, K.L., and K. McBee. 2009. Ecological characteristics of small mammal communities at a Superfund site. American Midland Naturalist l61:57–68.

Phelps K.L., and K. McBee. 2010. Population parameters of *Peromyscus leucopus* (white-footed deermice) inhabiting a heavy metal contaminated Superfund site. Southwestern Naturalist 55:363–374.

Price, P.W., B.J. Rathcke, and D.A. Gentry. 1974. Lead in terrestrial arthropods: evidence for biological concentration. Environmental Entomology 3:370-372.

Punzo, F., and C. Farmer. 2003. Effects of lead on exploratory behavior and running speed in the shrew, *Blarina brevicauda* (Insectivora). Journal of Environmental Biology 24:411-414.

Salcedo, H.D., M.B. Fenton, M.B.C. Hickey, and R.W. Blake. 1995. Energetic consequences of flight speeds of foraging red and hoary bats (*Lasiurus borealis* and *Lasiurus cinereus*; Chiroptera: Vespertilionidae). The Journal of Experimental Biology 198:2245-2251.

Sanchez-Chardi A., M.J. López-Fuster, J. Nadal. 2007. Bioaccumulation of lead, mercury, and cadmium in the greater white-toothed shrew, *Crocidura russula*, from the Ebro Delta (NE Spain); sex- and age-dependent variation. Environmental Pollution 145:7–14.

Schmitt, C.J., W.G. Brumbaugh, G.L. Linder, and J.E. Hinck. 2006. A screening-level assessment of lead, cadmium, and zinc in fish and crayfish from northeastern Oklahoma, USA. Environmental Geochemistry and Health 28:445-471.

Schmitt, C.J., J.J. Shyte, W.G. Brumbaugh, and D.W. Tillitt. 2005. Chemical effects of lead, zinc, and cadmium from mining on fish in the tri-states district of northeastern Oklahoma, USA. Environmental Toxicology and Chemistry 24:1483-1495.

Sites, R.S., W.L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235-253.

Sutton, R. 1987. Lead poisoning in flying-foxes (Chiroptera: Pteropodidae). Australian Mammalogy 10:125-126.

Sutton, R.H. and R.D. Wilson. 1983. Lead poisoning in grey-headed fruit bats (*Pteropus poliocephalus*). Journal of Wildlife Diseases 19:294-296.

USEPA (United States Environmental Protection Agency), Region 6. 1994. First Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

USEPA (United States Environmental Protection Agency), Region 6. 2005. Third Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

USEPA (United States Environmental Protection Agency), Region 6. 2010. Fourth Five-year Review, Tar Creek Superfund Site, Ottawa County, Oklahoma. United States Environmental Protection Agency, Washington, D.C.

Voigt, C.C., K. Sorgel, and D.K. Dechmann. 2010. Refueling while flying: foraging bats combust food rapidly and directly to power flight. Ecology 91: 2908–2917.

Whitaker, J.O., Jr. 1995. Food of the big brown bat *Eptesicus fuscus* from maternity colonies in Indiana and Illinois. American Midland Naturalist 134: 346–360.

Wimsatt, J., T.J. O'Shea, L.E. Ellison, R.D. Pearce, and V.R. Price. 2005. Anesthesia and blood sampling of wild big brown bats (*Eptesicus fuscus*) with an assessment of impacts on survival. Journal of Wildlife Diseases 41:87-95.

Zook, B.C., R.M. Sauer, and F.M. Garner. 1972. Lead poisoning in captive wild animals. Journal of Wildlife Diseases 8:264-272.

Table 5. Mean tissue concentrations (± S.E.) with ranges in parentheses reported by tissue and site (in μg/g DW; *n* = 14). Concentrations below 0.000025 μg/g DW were below detection limits (BDL) and assigned a number ½ that of the detection limit for statistical analyses.

	Reference (*n* = 6)	Contaminated (*n* = 8)
Liver Zn	3.184 ± 0.411	2.733 ± 0.205
	(2.094-4.389)	(2.052-3.738)
Cd	0.009 ± 0.004	0.015 ± 0.005
	(0.002-0.028)	(0.002-0.050)
Pb	0.003 ± 0.001	0.007 ± 0.002
	(0.000-0.007)	(0.002-0.026)
Kidney Zn	2.351 ± 0.161	2.452 ± 0.143
	(1.799-2.970)	(1.874-2.934)
Cd	0.016 ± 0.005	0.020 ± 0.006
	(0.002-0.038)	(0.006-0.061)
Pb	0.007 ± 0.005	0.004 ±0.001
	(0.000-0.033)	(0.000-0.014)
Hair Zn	3.642 ± 0.275	4.228 ± 0.249
	(2.691-4.662)	(3.274-5.302)
Cd	BDL	BDL
Pb	BDL	BDL

Table 6. Pearson correlation coefficients for 3 flight variables by 7 metal/tissue combinations, *p*-values are reported in parentheses. Metal concentrations for all hair Cd and Pb samples were below detection limit (BDL) and therefore not included in the analysis.

	Liver Zn	Liver Cd	Liver Pb	Kidney Zn	Kidney Cd	Kidney Pb	Hair Zn	Hair Cd	Hair Pb
Obstacles	-0.107	0.396	0.456	-0.124	0.239	-0.329	-0.049	BDL	BDL
	(0.72)	(0.16)	(0.10)	(0.67)	(0.41)	(0.28)	(0.87)		
Movement	-0.251	-0.046	0.126	-0.334	-0.049	-0.107	-0.323	BDL	BDL
	(0.39)	(0.88)	(0.67)	(0.24)	(0.86)	(0.72)	(0.26)		
Flight (s)	-0.451	0.003	0.294	-0.318	-0.065	-0.145	-0.336	BDL	BDL
	(0.11)	(0.99)	(0.31)	(0.27)	(0.83)	(0.62)	(0.24)		

Obstacles: the number of obstacles dropped – a measure of maneuverability.

Movement: movement between sections of the cage – a measure of willingness to enter the obstacle course.

Flight (s): time spent in flight – a measure of willingness to fly.

Figure 4. Map of Oklahoma with Tar Creek Superfund Sites (Contaminated-Tar Creek and Beaver Creek), and Oologah Wildlife Management Area (Reference-Plum Creek) labeled.

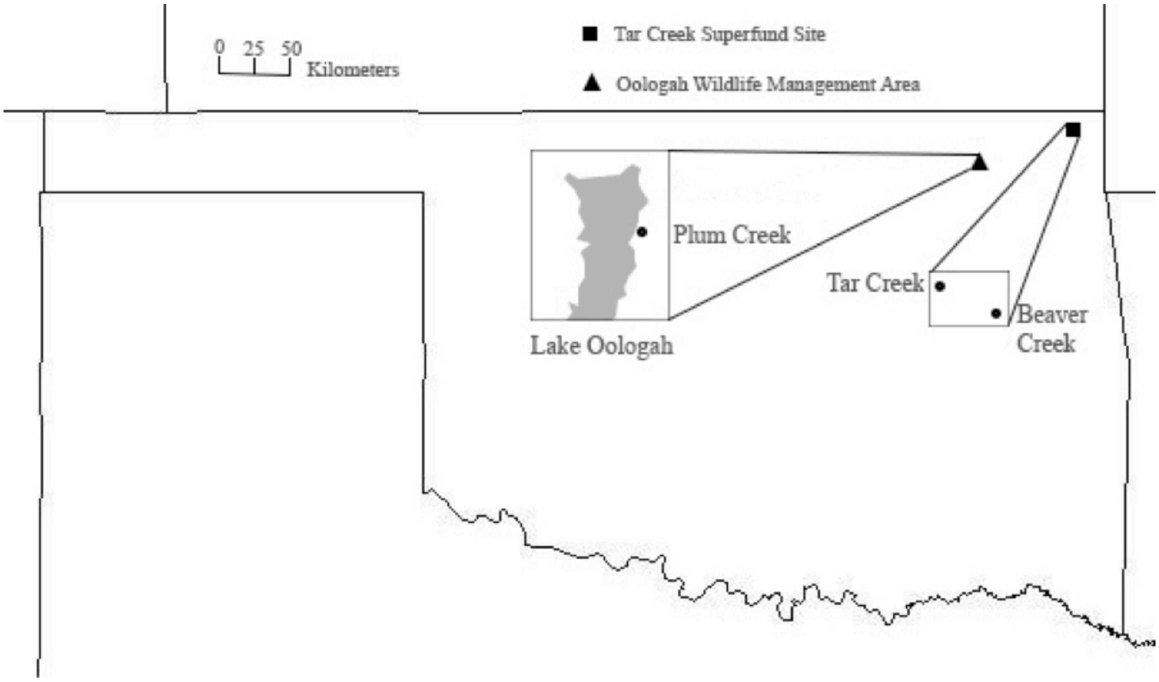


Figure 5. Flight cage diagram as viewed from above. Black dots represent hanging obstacles, which were spaced 450 mm apart, and red dots represent thermal markers that delineate the cage into 3 sections when viewed through thermal camera. Movement is measured as passing from one section into another.

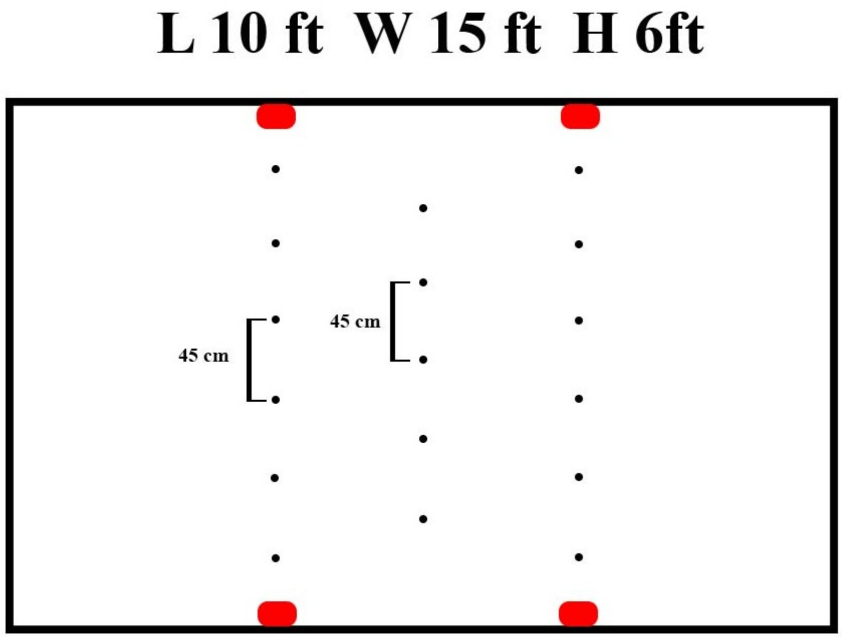
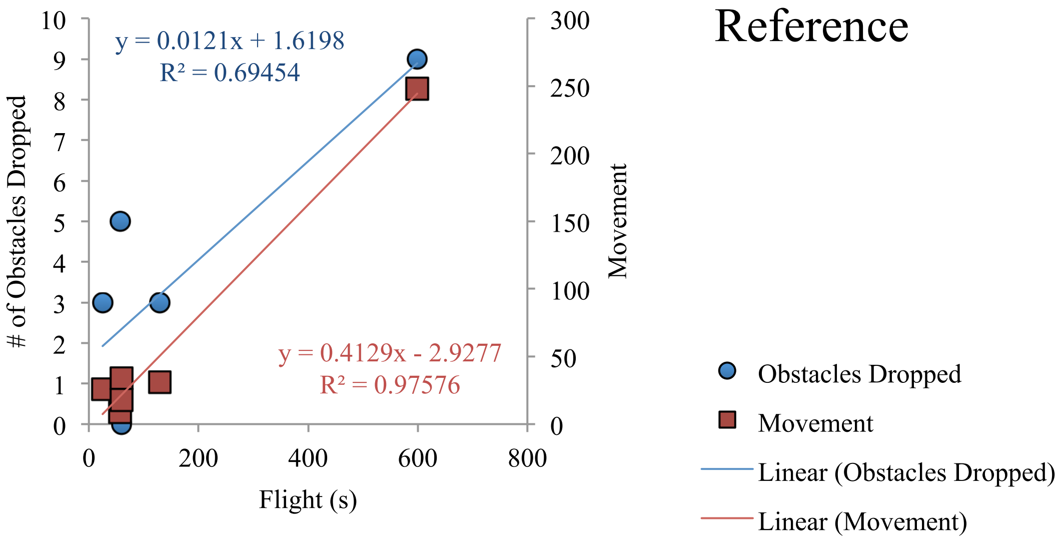
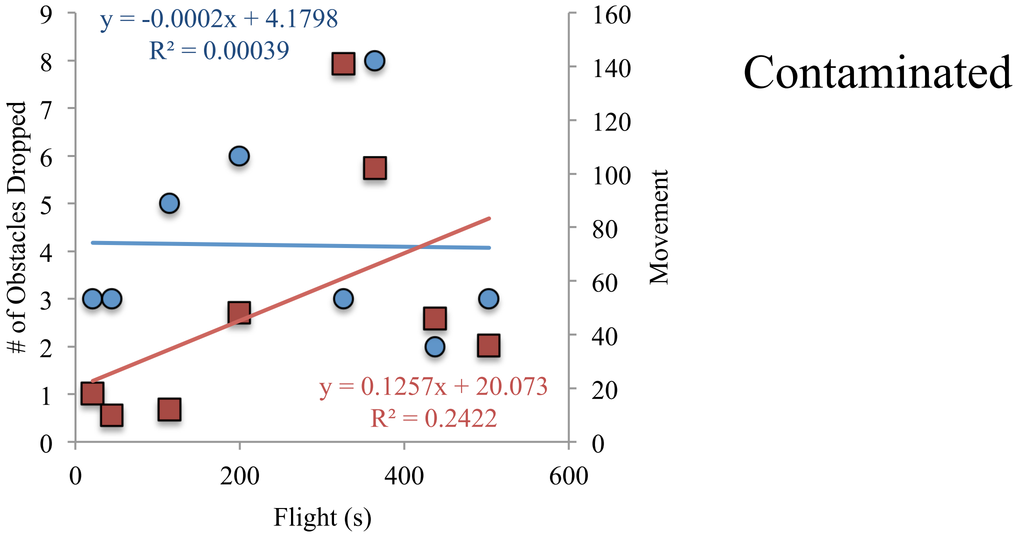


Figure 6 a & b. Flight cage scores for bats from a) Reference (*n* = 6) and b) Contaminated (*n* = 8). Blue dots represent number of obstacles dropped by flight time. Red squares represent movement scores by flight time (total *n* = 14).

a



b



APPENDIX A

Specimens examined.—Specimens are housed in the Oklahoma State University Collection of Vertebrates.

Lasiurus borealis.—**United States: Oklahoma;** *Nowata County*; Panther Creek (N36°37.747, W95°31.372), OSU13297, 13319, 13320, 13321; *Ottawa County;* Tar Creek (N36°57.495, W94°50.731), 13298, 13299, 13300, 13301, 13302, 13303, 13304, 13305, 13306, 13322, 13323, 13324, 13325, 13326, 13327; Beaver Creek (N36°56.2026, W94°45.3846), 13307, 13308, 13309, 13328, 13329, 13330; *Rogers County;* Plum Creek (N36°35.5063, W95°31.4197), 13310, 13311, 13312, 13313, 13314, 13315, 13331, 13332, 13333, 13334, 13335, 13336, 13337, 13338, 13339

Lasiurus cinereus.—**United States: Oklahoma;** *Ottawa County*; Beaver Creek (N36°56.2026, W94°45.3846), 13316

Myotis lucifugus.—**United States: Oklahoma;** *Nowata County*; Panther Creek (N36°37.747, W095°31.372), 13317

Perimyotis subflavus.—**United States: Oklahoma;** *Nowata County*; Panther Creek (N36°37.747, W95°31.372), 13340; *Ottawa County*; Beaver Creek (N36°56.2026, W94°45.3846), 13318

VITA

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Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in July 2014.

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