DEPARTMENT OF THE INTERIOR
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REGION 2

ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

OK - Assessment of Contamination in Caves Located at the Ozark Plateau National Wildlife Refuge, Oklahoma

Final Report
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by

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INTRODUCTION

Background

The Ozark Plateau National Wildlife Refuge (Refuge) is located in east central Oklahoma, and consists of ten parcels of land, totaling approximately 3,000 acres. The bulk of the Refuge lies south of the town of Stilwell, but a sizeable portion is also located along Spavinaw Creek, near the headwaters of Lake Eucha. The geography of the Refuge is comprised mainly of hilly oak-hickory uplands with pockets of wetlands in the hollows scattered around the region. The underlying strata is composed chiefly of the highly fractured, fine-grained limestone and cherty soils of the Boone Formation. Due to the fractured nature of the Boone Formation, numerous caves are featured and are located on or near Refuge property. These caves provide habitat for the Ozark big-eared bat *Corynorhinus townsendii ingens*, the gray bat *Myotis grisescens*, and the Indiana bat *Myotis sodalis*, all of which are Federally listed endangered species. The Ozark cavefish *Amblyopsis rosae*, which is a Federally listed threatened species, is also found in or near several Refuge caves. Nine Federal Species of Concern, including the Ozark cave crayfish *Cambarus tartarus* and the Ozark cave amphipod *Stygobromus ozarkensis*, are also found in the area.

The aquifer underlying the Boone Formation is classified as a karst aquifer (Osborn et al., 2000). Besides caves, sinkholes and disappearing springs are known to occur in this region; these features provide direct routes for surface runoff and precipitation to transport contaminants to the water table (Osborn and Hardy, 1999). Because of this phenomenon, any contaminants released aboveground have the potential to migrate directly into groundwater flowing through Refuge caves within the affected watershed.

Lake Eucha provides drinking water for the city of Tulsa, Oklahoma. In recent years, in response to complaints of foul taste and odor in the drinking water, the city funded studies to assess the problem by evaluating surface water (Oklahoma Water Resources Board, 2001) and ground water quality (Osborn et al., 2000). These studies concluded that Lake Eucha was receiving high concentrations of phosphorus and nitrate in surface and ground water, respectively. These high nutrient loads were causing large-scale algal blooms, which then produced chemicals that resulted in the smell and taste present in Tulsa's drinking water. In 1998, the USDA identified more than 700 poultry and 50 swine houses in the Eucha Priority Area, which covers 134,000 acres and includes the Spavinaw Creek watershed (USDA-NRCS, 1998). These houses, also called Concentrated Animal Feeding Operations (CAFOs), are well-known to produce considerable amounts of nutrients and heavy metals which can be carried offsite by surface runoff and by seepage into the water table. CAFOs also are probably a source of steroids found in downgradient streamwater and sediments (Miller et al., 1999). CAFOs, especially for poultry, are rapidly appearing throughout eastern Oklahoma. Currently, poultry CAFOs within the Eucha/Spavinaw watershed have the capacity to produce 84 million birds, and about 1,500 tons annually of waste phosphorus (Tulsa Metropolitan Authority, 2001).

The Nature Conservancy has also found evidence of organochlorines in groundwater flowing through a cave on a parcel of land owned by this organization, south of Grand Lake O' The Cherokees (Feirer, personal communication, 2001). This cave lies near several other caves which are on a tract of land proposed for acquisition by the Refuge.

In 1990, the U.S. Fish & Wildlife Service conducted a contaminants study at several bat caves throughout the refuge, to assess contaminant loads of bat guano and dead bats found in these caves (Martin 1992). The study concluded that endangered bats were being exposed to environmental contaminants, especially PAHs and trace elements. Whole-body concentrations of the following contaminants were found in gray bats: p,p'-DDE (0.31 to 1.9 ppm dry weight [DW]); oxychlordane (0.08 to 2.1 ppm DW); n-heptadecane (0.07 to 4.8 ppm DW); arsenic (0.3 to 3.0 ppm DW [2.3 to 66 ppm DW in guano]; and lead (up to 24 ppm DW [1.0 to 23 ppm DW in guano]). However, the scarcity of whole-body or organ analyses precluded definitive conclusions. The author of this study recommended further investigation, using surrogate species of bats and continued monitoring of trace elements in bat guano, to identify hot spots on and around Refuge lands, and to assess temporal trends of these contaminants.

It is imperative that contaminant levels, especially of heavy metals and organochlorines, be assessed in depth at key points in and around the Refuge. This data would provide further baseline data which can be incorporated in Refuge management plans, especially in addressing the proliferation of CAFOs and other industries within the watersheds of Refuge lands. This data can be used also as a reference for any future incidences that lead to contamination of trust resources and their habitats within the Refuge.

Scientific Objective

The scientific objective of this study is to expand the database of contaminants present in the aquatic habitats of the Ozark Plateau National Wildlife Refuge. This database includes information on concentrations of heavy metals and organochlorines in groundwater and sediment, and in bats and food sources collected from designated caves and riparian areas within the Refuge.

Management Action

The City of Tulsa is aggressively seeking alternatives in managing the Eucha / Spavinaw watershed to minimize nutrient loading of the city's water supply, including possible state regulation of nutrient-producing industries in the watershed. Because of this, there is considerable potential for the Refuge to benefit from an effort to further document contamination of groundwater and resources present in cave habitat. The Refuge management can then enter into cooperative efforts with the City of Tulsa, the Nature Conservancy, and other involved organizations and agencies to identify sources of nutrient loading of the Eucha / Spavinaw watershed, and to take steps to reduce such loading. These efforts would also reduce loading of the groundwater by other contaminants, further benefiting Federal trust resources which depend on cave habitat located on the Refuge. Knowledge of the extent and nature of contamination in caves on existing Refuge property may also help with the acquisition of up to 12,000 more acres to complete the integrity of the Refuge (USFWS 2002).

Project Sites

Three caves were assessed for groundwater contamination - one in the Spavinaw Creek basin, north of the town of Colcord, Delaware County, Oklahoma (code-named DL-39), and two caves southeast and southwest of the town of Stilwell, Adair County, Oklahoma (code-named AD-10 and CZ-18, respectively; Figure 1). All three caves contain permanent lotic bodies of groundwater. The cave DL-39 has considerable potential for contamination, due to the presence of chicken and hog houses upstream, along with a wastewater treatment plant recently built up gradient by the town of Colcord. The areas around the other two caves generally have not been subjected to intensive, concentrated agricultural pressure, as this region is mostly hilly, heavily forested, and sparsely populated, so these two caves served as reference and baseline sites.

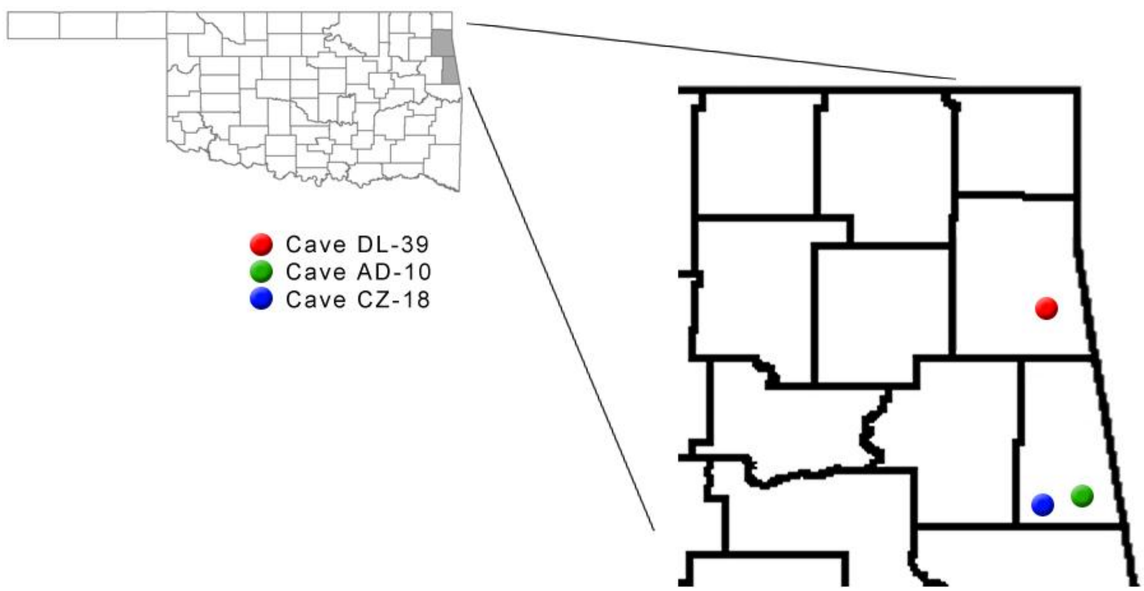

Figure 1: Location of Study Caves

METHODS

First Round of Collections

During the first round of data collection in 2002, three semi-permeable membrane devices (SPMDs) were completely submerged in pools of groundwater in each cave for a period of 28 days in January/February. The SPMDs were housed in a perforated stainless steel cylinder provided by the SPMD vendor, which allowed water to flow over the SPMDs. The SPMDs were removed at the end of this period and sent back to the vendor for preparation, then submitted to a contract chemical laboratory for analysis for organochlorines. A sediment sample was collected at the same time from depositional areas either in, or immediately downstream, of the location of each SPMD deployment point, and submitted for similar analysis. A grab sample of water was collected also at the end of the 28-day period from each of the three SPMD sampling locations, and submitted along with one field blank for metals analysis. In each of these caves three individuals of a surrogate species of bat, the eastern pipistrelle *Pipistrellus subflavus*, were collected by hand from the walls and ceilings in November 2002. The eastern pipistrelle is the most abundant species of bat in eastern Oklahoma, and its food preferences overlap those of the Federally listed species of bats that frequent these caves (Barbour and Davis 1969; Schwartz and Schwartz 1981; Harvey et al. 1999; Steve Hensley, personal communication, 2001). Attempts were made to locate carcasses of either gray bats or Ozark big-eared bats during sample collection, but none were found. Six female and three male eastern pipisrelles were collected, and were wrapped in aluminum foil and placed on wet ice in the field, then transferred to a freezer in the lab prior to being submitted for whole-body analysis for organochlorines and trace elements. Bat guano was collected from the floors of each study cave in November 2002 after the resident colonies of Federally listed bats had migrated to winter hibernacula. Guano deposits that were found in all three caves were probably composed chiefly of guano from gray bats, although in Caves CZ-18 and AD-10, there may also have been some contributions by big brown bats *Eptescius fuscus* and Northern long-eared bats *Myotis septentrionalis*, which can be present in these caves in small numbers. A stainless steel scoopula and chemically cleaned glass vials were used to composite the guano into one sample per study cave. These samples were kept refrigerated until being submitted to a contract chemical laboratory for analysis for trace elements and organochlorines. Moths, the primary food source of the Ozark big-eared bat and a major food source for other resident species of bats as well, were collected after dark in August 2002 near each of the study caves using a method described in the entomological literature: a white sheet was suspended over a section of clothesline stretched between two trees, and a black light was positioned to shine on the sheet. Latex gloves and chemically cleaned glass jars were used to hand-collect moths that landed on the sheet. Attempts were made to collect randomly and not favor any specific size or appearance of moth. All jars were placed on wet ice in the field, and then transferred to a freezer in the lab. An attempt was made in the lab to identify these moths to the family level. After this, all moths per location were combined into one sample per cave before submission to a contract chemical laboratory for whole-body analysis for organochlorines and trace elements.

<u>Second Round of Collections</u>

In 2003, collections were made at each of the three caves of the following: moths, eastern pipistrelles, and bat guano. The same methods of collection and preservation were used as in the previous year, and a similar number of samples were collected. Moths were collected in August, and bats and guano were collected in November. Seven male and two female eastern pipistrelles were collected. All samples were submitted for analysis for metals and organochlorine (whole-body in bats and moths). As in the previous year, no carcasses of either gray bats or Ozark big-eared bats were found.

RESULTS AND DISCUSSION

SPMDs

Deployed only during the first round of data collection, the SPMDs did not show any organochlorine concentrations above detection limits (Appendix A), except for some components of chlordane (alpha and gamma, and trans-nonachlor), and p,p'-DDE and p,p'-DDT. These contaminants were present in trace quantities (0.002 – 0.015 ppm DW) in all three caves. These concentrations probably indicate a minor issue of concern, especially when compared to low concentrations of oxychlordane (an epoxide metabolite of chlordane) found in the bats collected in this study (see below). Use of chlordane as a pesticide was banned in the 1980s, so the low levels of chlordane components present in the SPMDs may indicate past applications of pesticides upstream of the caves, possibly to control underground termites (Eisler 1990) or other residential pests. DDT was also banned several decades ago, so the trace amounts of DDT metabolites detected in SPMDs probably are also a result of past use. No analysis for elements was performed on the SPMDs.

Sediment

Likewise collected only during the first round of data collection, no sediment samples indicated any organochlorine concentrations above detection limits (Appendix A). The preponderance of sand in each sediment sample (73-83% by grain size) probably precluded the adsorption of contaminants to grains in the sediment being sampled. Attempts were made during the study to find permanently submerged cavern sediments with a more organic composition, but were unsuccessful. No analysis for elements was performed on the sediment samples.

Water

Also collected only during the first round of data collection, in general concentrations of elements in water samples were below detection limits (Appendix B). The only elemental concentration that exceeded 1 ppm (DW) was magnesium (0.847 – 1.41 ppm DW), which probably arose from the weathering of surface and subterranean rock formations in the vicinity of the caves. No organochlorine analyses were performed on the water samples.

Bats

Organochlorine concentrations in bats in both rounds of collections generally were low (< 1.0 ppm DW) or below detection limits (Appendices A, C), with the exception of three organochlorine compounds, which appeared in all bat samples in both collections:

oxychlordane, p,p'-DDE, and total PCBs. As mentioned above, oxychlordane is a metabolite of the pesticide chlordane; it is also more toxic than its parent compound, and is of significant concern in environments, especially aquatic, known to be contaminated (Eisler 1990, EPA 2000). However, at the concentrations detected in this study (0.0096 – 0.393 ppm DW), there did not appear to be a significant issue with the presence of oxychlordane beyond the need for continued monitoring. These concentrations were also comparable to, or lower than, those in the previous study of contaminants in gray bats assessed in the same region (0.08 – 2.1 ppm DW; Martin 1992). The same appears to be true of p,p'-DDE in this study; concentrations in bats ranged from 0.0115 to 0.936 ppm DW, but probably were not significantly elevated. These concentrations were comparable to those found in Yuma myotis bats *Myotis yumanensis* collected from a reference cave in a study in Arizona (0.35 – 0.72 ppm DW; King et al. 2001). The concentrations of p,p'-DDE in this study generally were also lower than those in the previous study of contaminants in gray bats conducted in the same region (0.31 – 1.9 ppm DW; Martin 1992). Considering the persistence of DDT's child compounds in the environment, attempts to locate ongoing sources of DDT application in the vicinity may be fruitless; such applications might have taken place decades ago. However, the presence of DDT and its metabolites in any organism is cause for concern, so continued monitoring may be warranted. Concentrations of total PCBs in bats in this study ranged from less than the detection limit (0.00421 ppm DW) to 1.58 ppm DW. According to a study of pregnant bats and their ability to produce live young, the presence of PCBs in the mothers was not significantly correlated with still birth vs. live birth (Kamrin and Ringer 1996); however, maternal bats with levels over 2 ppm of PCBs appeared more likely to produce dead young. Considering the highest concentration of PCBs found in bats in this study, continued monitoring also appears to be warranted for this compound and the others mentioned above, to ensure that whole-body concentrations of these compounds in bats continue to trend downward. Because PCB congeners in general are highly water-insoluble, they have a high potential for bioaccumulating in aquatic ecosystems. Since some species of bats, especially gray bats, are known to forage over ponds and lakes for emergent aquatic insects (Barbour and Davis 1969; Schwartz and Schwartz 1981; Harvey et al. 1999), future monitoring should include an assessment of organochlorine contaminants in lentic aquatic ecosystems in the vicinity of Refuge caves that offer shelter for bats.

Concentrations of elements in bats in this study (Appendices B, D) generally were comparable or lower than those reported in Martin (1992) and the reference site bats in King (2001). The range of concentrations of a selected set of elements in bats in this study was so great that ANOVA tests did not return any significant differences in the concentrations of each element in bats between caves. Nickel concentrations in bats in the first round of collection were generally higher (0.560 – 1.68 ppm DW) than those reported in the other two studies. However, the levels of nickel probably were not high enough to warrant significant concern, especially since the levels of nickel in bats collected during the second round generally were much lower (< 0.0517 – 0.472 ppm DW). One bat in the first collection, taken from Cave AD-10, exhibited markedly higher levels of barium, chromium, iron, strontium, and zinc than any other bat in the study, but because whole-body concentrations of these elements as a whole were comparable to those at reference sites in other studies, this data point probably is an outlier. However, monitoring of land use of residential and commercial areas in the vicinity of these caves, both for organic and inorganic contaminants, would be worthwhile.

Bat guano

Organochlorine concentrations in bat guano in this study (Appendices A, C) were below detection limits in all samples except in the case of p,p'-DDE. Two of three guano samples in the first round, and one of three guano samples in the second round, contained trace amounts (0.0205, 0.254, and 0.0160 ppm DW, respectively). The two higher readings came from guano collected at the cave DL-39, and, as in the case of p,p'-DDE traces in the whole-body bat samples, may indicate a residual issue with past uses of DDT.

In general, there was a marked difference in concentrations of some elements in guano between caves (Appendices B, D), with concentrations in guano from Cave DL-39 up to several magnitudes lower than in guano from the other two caves. This trend was true in both collection years; however, concentrations of other elements were comparable between caves, or, in a few instances, slightly higher in Cave DL-39 than in the other caves. Concentrations of elements in guano generally were also higher than in whole-body samples of bats; this trend was also reported in Martin (1992). However, because eastern pipistrelles were sacrificed for analysis rather than a Federally listed species of bat, and because the guano collected in this study probably came chiefly from gray bats, no direct links between guano and whole-body concentrations can be made. Although the dietary preferences of these three species (eastern pipistrelles, gray bats, Ozark big-eared bats) are known to overlap, little is known about the efficiency with which each species can eliminate contaminants from their bodies. No carcasses of Federally listed species (i.e., gray bats) were found in any of the caves that could be submitted for analysis to support such direct links and comparisons. Until this can be done, no definitive inferences can be formulated.

Moths

Several taxonomic families of moths were identified amongst the composite samples: *Arctiidae, Noctuidae, Notodontidae, Saturniidae, Sphingidae,* and *Yponomeutidae.* No composite samples of moths had concentrations of any organochlorines above detection limits in either round of collections (Appendices A, C). Concentrations of elements in moths generally were comparable to, or lower than, whole-body concentrations in bats, although in several instances, whole-body concentrations of several elements were higher in moths than in some bats. Data varied though between caves and collections, so no general trend could be discerned. Since gray bats, and especially Ozark big-eared bats, feed largely on moths, the presence of comparable levels of elements in both organisms may point to efficient transfer of these elements from ingested prey to fecal matter, as mentioned above. However, no definitive conclusions can be made in the absence of whole-body analysis of either of these bat species to complete the link.

CONCLUSIONS AND RECOMMENDATIONS

Based on the data in this study, there appears to be slight organic and inorganic contamination of the cave habitats which were assessed, including groundwater flowing through the caves. The data in this study did not indicate that at present there is significant contamination of Cave DL-39, the cave that potentially could be affected by nearby CAFO and sewage treatment plant effluents. Most of the contamination detected in this study may be caused by natural sources, except in the case of components of chlordane, PCBs, and DDT, which are of man-made origin. Homeowner applications of pesticides around nearby residences, and runoff from nearby towns and highways, are possibly the source of these organochlorines. One eastern pipistrelle collected in this study contained high levels of several elements, and because this species of bat tends to remain in a specific area throughout their lifetimes, there may be an outstanding source of contamination in the vicinity of Cave AD-10. The elements found at elevated concentrations in this bat (barium, chromium, iron, strontium, and zinc) are known to be present in industrial effluvia, including oil and gas well wastes, sludge disposal, and in the byproducts of waste burning. In the absence of elevated levels of these elements in the other bats collected in this study, no conclusion can be made except that this source may be small, and frequented only by a few bats. However, it may be prudent to recommend a search of the area contained within the range of eastern pipistrelles for potential sources of contamination. If any such sources can be found, a follow-up study can be conducted which includes assessment of contamination of any aquatic ecosystems potentially affected by these industrial sources. Because bats are known to forage largely over water, and because many contaminants persist in sediments and other aquatic media, inclusion of this type of ecosystem in a follow-up study probably would be productive. Such a study would benefit the Ozark big-eared bat, which also probably has a limited range (Schwartz and Schwartz 1981; Harvey et al. 1999). However, the long-distance migrations of gray bats, between winter and summer caves up to several hundred miles apart (Schwartz and Schwartz 1981; Steve Hensley, personal communication, 2005) may limit the effectiveness of a local study. A cooperative study between agencies in which jurisdictions these winter and summer caves are located may provide a more comprehensive picture of potential contamination of this species of bat. Follow-up monitoring studies should also have provisions for a more exhaustive search for carcasses of Federally listed species of bats in study caves, to complete a direct link between ingestion of prey, uptake of contaminants in the gut, and elimination of these contaminants in fecal matter. Considering the present goal of adding about 12,000 more acres of Ozark Plateau habitat to the Refuge, it may be prudent to continue monitoring of these caves and their immediate surroundings, and to assess contaminant conditions at other caves proposed for acquisition. This may provide a clearer, more comprehensive picture of contaminant loading and “hot spots” in and around the areas to be incorporated into the Refuge. Knowledge of such potential contaminant sources can allow the Refuge personnel to work with the involved parties to reduce or eliminate these sources.

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APPENDIX A: Raw Data, Elements, Round One Collection (2002)

Sample Number [1]	Sample Matrix	Sample Weight (grams)	Percent Moisture
CC04	Bat Guano	12	65.4
DF04	Bat Guano	5	59.2
JS04	Bat Guano	9	69.3
CC06	Water	473	
DF06	Water	473	
JS06	Water	473	
CC01	Eastern Pipistrelle	8	46.8
CC02	Eastern Pipistrelle	7	44.9
CC03	Eastern Pipistrelle	11	44.9
CC07	Moth Composite	6	48.2
DF01	Eastern Pipistrelle	6	46.6
DF02	Eastern Pipistrelle	5	43.0
DF03	Eastern Pipistrelle	7	45.2
DF07	Moth Composite	1	48.2
JS01	Eastern Pipistrelle	11	43.6
JS02	Eastern Pipistrelle	1	49.4
JS03	Eastern Pipistrelle	6	44.5
JS07	Moth Composite	6	54.1

[1] CCxx = Cave CZ-18; DFxx = Cave AD-10; JSxx = Cave DL-39