5-5-2017

Environmental Contaminant Exposure and Effects on Bats: Studies in Sichuan Province, China and Colorado, U.S.A.

Laura Heiker

UNIVERSITY OF NORTHERN COLORADO

Greeley, Colorado

The Graduate School

ENVIRONMENTAL CONTAMINANT EXPOSURE AND EFFECTS ON BATS: STUDIES IN SICHUAN PROVINCE, CHINA AND COLORADO, U.S.A.

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

Laura Heiker

College of Natural and Health Sciences
School of Biological Sciences
Biological Education

May 2017

This Dissertation by: Laura Heiker

Entitled: *Environmental Contaminant Exposure and Effects on Bats: Studies in Sichuan Province, China and Colorado, U.S.A.*

has been approved as meeting the requirements for the Degree of Doctor of Philosophy in College of Natural and Health Sciences in School of Biological Sciences, Program of Biological Education

Accepted by the Doctoral Committee

_____
Dr. Rick Adams, Ph.D., Research Advisor

_____
Dr. Lauryn Benedict, Ph.D., Committee Member

_____
Dr. Scott Franklin, Ph.D., Committee Member

_____
Dr. Thomas O'Shea, Ph.D., Committee Member

_____
Dr. David Diggs, Ph.D., Faculty Representative

Date of Dissertation Defense 24 Feb. 2017

Accepted by the Graduate School

_____
Linda L. Black, Ed.D.
Associate Provost and Dean
Graduate School and International Admission

ABSTRACT

Heiker, Laura. *Environmental Contaminant Exposure and Effects on Bats: Studies in Sichuan Province, China and Colorado, U.S.A.* Published Doctor of Philosophy dissertation, University of Northern Colorado, 2017.

As the world's only flying mammals, bats fill an important ecological role in most ecosystems, acting as agents of seed dispersal, pollination, fertilization, and insect control. The human-mediated release of environmental contaminants has been implicated in the decline of many bat populations over the past few decades. Given bats' ecological significance, I studied how bat presence and activity related to contaminated food and water sources in two global regions: 1) in and around the growing urban city of Chengdu, the capital of Sichuan Province, China, and 2) along the eastern slope of the Rocky Mountains in Colorado, U.S.A., which has been subject to 150 years of mining. In China (Chapter II), I tested mercury concentrations in fur and organochlorine concentrations in guano to assess their relationships to land use type and bat species, relative age, body condition, and phonic type. Because the Japanese pipistrelle (*Pipistrellus abramus*) had the highest fur mercury concentrations of bats sampled, in Chapter III I examined genetic identity and gene flow to confirm that all bats sampled were indeed *P. abramus* and to better understand local movements and potential implications of the contaminant concentrations. Finally, in Colorado (Chapter IV), I tested whether bat activity and feeding attempts differed locally above streams of high versus low metal contamination at high-elevation sites (>2,900 m). In China (Chapter II),

total mercury concentrations were significantly higher in adult *P. abramus* than in adult Chinese noctules (*Nyctalus plancyi*) ($P < 0.001$), and significantly higher in adult *N. plancyi* relative to juveniles ($P < 0.001$). There was no significant difference in concentrations by land use type (urban versus suburban), but 57% of adult pipistrelles had fur mercury concentrations above the threshold for reduced homeostatic control, with the maximum (33 ppm) from an adult female in an agricultural area. There was no relationship between fur mercury concentration and bat body condition for either species. Hexachlorobenzene, alpha-chlordane, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD were detected in guano but at levels well below those associated with harm. More bat phonic types were detected at a forested mountain site than agricultural or urban areas, though this could not be related to contaminant concentrations. In Chapter III, mitochondrial (*cyt* b) and nuclear studies confirmed that all individuals assumed to be *P. abramus* matched the species genetically and that there was weak population structure in Chengdu. This corroborated high gene flow in the area and a likely home range size of <10 km. Additionally, the *P. abramus* population had two mitochondrial clades, which may indicate ancient lineage separation due to glaciation and potential differences in susceptibility to physiological stresses. In Colorado (Chapter IV), there was no significant difference between the number of calls recorded at more contaminated sites and that at less contaminated sites. Though not statistically significant, the majority of feeding buzzes occurred above cleaner streams, suggesting that contamination could be an issue in habitats where fresh water is less available. Limited sample size and a short sampling period were constraints in all studies.

Key words: bats, mercury, organochlorines, China, Colorado, cytochrome b, aquatic-terrestrial subsidies, *Pipistrellus abramus*

ACKNOWLEDGMENTS

I first want to thank my friends and colleagues within the UNC Biological Sciences Department. In particular, Anna Schwabe and family, Randi Lupardus, Nadje Najar, Tom McCabe, and Lucas Dowers have provided endless personal and professional support throughout the years. Love you all.

Thanks to my committee members for their patience and flexibility. To my advisor, Rick Adams, I am grateful for the support and resources you provided for me to explore uncharted territory in China and the world of ecotoxicology. Lauryn Benedict, in addition to helping me on my project, you were an amazing teaching mentor. Scott Franklin, I appreciated your knowledge of statistics and ecology, and David Diggs, you introduced me to the world of GIS. (I now feel like my life would not be complete without attending the ESRI conference in San Diego!) Last, but definitely not least, Tom O'Shea, I am grateful for you sharing your knowledge of bat ecotoxicology. You have been a huge service to the field and a source of inspiration.

I am indebted to a large number of people in Chengdu without whom the foundation for this project would have been impossible. Sarah Bexell, your compassion for animals and years of pioneering conservation work in Chengdu are unparalleled. You gave me everything a visiting researcher could ask for in a foreign country. Professor Tang Ya provided me with a visa and invaluable in-country support. I am grateful to Hongyan Shi for sharing her knowledge of Chinese bats and providing access to the

Nyctalus plancyi colony at Xi Hua Normal. Feng Rui Xi, Deng Rui, and Du Li Li, thank you for your excellent field skills, patient translations, and friendship.

In addition to those in China, Kelsey Gonzales provided intrepid field support, a sense of adventure, and company overseas. Claire Ramos of Colorado State University, Pueblo made the bat fur mercury analysis possible.

I owe the third chapter of this dissertation to molecular ecology gurus Mitchell McGlaughlin, Anna Schwabe, Brandee Wills, and Nathan Redecker. You went above and beyond helping me in the lab.

I dedicate my fourth chapter to colleagues at USGS Fort Collins: Travis Schmidt, Johanna Kraus, and Holly Rogers, who were so gracious in sharing their knowledge and resources for the Mineral Belt project. Field support was also provided by Alex Wharry and Jenn Moore.

Monetary support was provided by the UNC Biological Sciences Department, the UNC Graduate School Association, Bat Conservation International, the Colorado Mountain Club, the Explorers Club, the Audubon Society of Greater Denver, and Luo Lan and Wang Ji Shan.

Thanks to all the bats that "donated" their tissues. It must have been scary, but I hope my work serves to help your future generations.

Finally, this dissertation would not have been possible without the love and support of my family and my beautiful dog, Maya. Thanks for seeing me through to the end.

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

Table

LIST OF FIGURES

Figure

CHAPTER I

INTRODUCTION

Background

Bats (Order Chiroptera) are the world's only flying mammals and have diversified into more than 1,300 living species (Fenton and Simmons 2014). They are an important component of most ecosystems worldwide and provide critical ecosystem services including seed dispersal, pollination, fertilization, and insect control (Altringham 2011; Boyles et al. 2011). To date, the International Union for Conservation of Nature (IUCN 2015) categorizes about 20% (255) of bat species as Critically Endangered, Endangered, Vulnerable, or Near Threatened, while 203 species lack sufficient data for evaluation. These numbers are likely conservative considering that species assessments do not keep pace with the identification of new species and that bats, being nocturnal and elusive, are quite difficult to study. In addition to habitat loss, habitat fragmentation, and human exploitation, pesticides and other environmental contaminants have been implicated in the decline of a number of bat populations (Cockrum 1970; Clark 1981; Gerell and Lundberg 1993; Clark 2001; Zhang et al. 2009). The mechanisms and impacts of these declines are not well understood and likely often go unnoticed until too late (see Clark and Shore 2001 for review). Although there is knowledge of how some substances directly kill bats, subtler, non-lethal effects that impair development, reproduction, and

long-term survival are understudied (Clark and Shore 2001). With the continued rise of pesticide production (Grube et al. 2011; Zhang et al. 2011) and industrial emissions (IPCC 2014), it is reasonable to assume that environmental contamination will continue to negatively affect bats and other wildlife. A finer understanding of how bats are affected is necessary to protect and manage these unique and ecologically important animals.

In general, bats have many traits that make them both vulnerable to pollutants and useful bioindicators of ecosystem health (G. Jones et al. 2009). First, bats are relatively long-lived for placental mammals of their size, with a usual lifespan of six or seven years and a maximum lifespan of over thirty years in the wild (Wilkinson and South 2002). This leaves ample time for contaminants to accumulate in tissues. Second, bats reproduce slowly, with most having only one young per year (Altringham 2011), and therefore require high adult survival for population growth and stability (O'Shea et al. 2011). Thus, population recovery can be difficult following reproductive impairment or mass mortality. Third, because insectivorous and carnivorous bats occupy relatively high positions in food webs, they are more susceptible to chemical bioaccumulation and bioconcentration than herbivores, nectarivores, and frugivores (Clark 1981). Lactating little brown bat (*Myotis lucifugus)* females, for example, may consume over 80% of their body weight in a single night (Kurta et al. 1989), so toxic chemicals ingested with prey items can quickly compound in their tissues. Fourth, many bat species undergo large shifts in body fat amounts during development, migration, and hibernation (Altringham 2011). Lipid-soluble compounds, like organochlorines, are sequestered in fat deposits and then released as those fats are broken down for energy use (Clark 1981). At such

times, harmful xenobiotics are released into the body, when individuals are already being taxed energetically (Geluso et al. 1976). Fifth, many bat species aggregate at agricultural sites to forage on large swarms of insects attracted to monoculture crops (Lee and McCracken 2005). These sites are often the target of night-time pesticide applications, and active bats may be sprayed directly or ingest newly sprayed prey items (Clark 1981). Finally, in arid environments, bats experience high rates of evaporative water loss that must be replenished nightly (Webb 1995; Webb et al. 1995). Drinking water is most critical for lactating females in arid environments where fresh water is limited (Adams and Hayes 2008), and pollution of natural fresh water sources or creation of mining drainage ponds expose bats to contaminant ingestion (Clark and Hothem 1991).

Project Objectives

Given bats' ecological significance and the ongoing possibility of harm from exposure to pollutants, I studied how bat presence, activity, and uptake of contaminants relate to food and water resources in two different biomes: 1) in and around the growing urban city of Chengdu, the capital of Sichuan Province, China, and 2) along the eastern slope of the Rocky Mountains in Colorado, U.S.A., which has been subject to 150 years of mining for precious metals.

Chapter II of this dissertation addresses the following hypotheses about bats in China: 1) Mercury concentrations in bat fur will differ between land use types (urban versus non-urban; 2) Organochlorine concentrations in bat guano will differ between land use types (urban versus non-urban); 3) Bats with higher contaminant concentrations in tissues will exhibit poorer body condition; 4) Tissue contaminant concentrations will

differ between species; 5) Tissue contaminant concentrations will differ between adults and juveniles within species; and 6) Bat species richness will be higher in areas where concentrations of mercury in bat tissues are lower.

Because fur mercury concentrations were found to be highest for the Japanese pipistrelle (*Pipistrellus abramus*), Chapter III examines genetic identity and gene flow of the species in the Chengdu area. The following hypotheses are addressed: 1) In-field identification of *P. abramus* will be confirmed genetically, and 2) *P. abramus* will exhibit high gene flow (and low structure) in and around Chengdu.

Chapter IV addresses contaminants from the standpoint of effects on foraging opportunities rather than from the standpoint of biological uptake. a related question about the degree to which contaminant effects carry across food web components in Colorado. I expected that 1) local bat activity would correlate negatively with level of stream heavy metal contamination, and 2) bat activity and the number of feeding buzzes across sites would correlate more strongly with aquatic emergent prey densities than with terrestrial insect densities or vegetation characteristics.

Although dissimilarities in climate, history, and community structure between subtropical Chengdu and the temperate Rocky Mountains made direct comparison of the two biomes infeasible, together I expected the studies would provide independent analyses of bat responses to contaminants under different environmental conditions.

CHAPTER II

BAT CONTAMINANT EXPOSURE IN AN URBAN LANDSCAPE, CHENGDU, CHINA

Background and Significance

Sichuan Province in south-central China is a biodiversity hotspot (Myers et al. 2000) thought to support over forty bat species from seven families (Appendix B). Recent studies estimate that some Chinese bat populations have declined by 60% in the last thirty years (Zhang et al. 2009). One suspected major cause is unregulated pesticide use and release of pollutants associated with ongoing industrial growth (Zhang et al. 2009), yet only a handful of studies have been published concerning bats and contaminants in central Asia and none in China (reviews by Clark and Shore 2001; Bayat et al. 2014; Zukal et al. 2015). My study is the first to address the presence of contaminants in Chinese bat tissues.

An important step in identifying contamination's role in bat declines is to characterize biological uptake (Sample et al. 1996). Contaminant concentrations in individual bats may provide clues to population and species effects by indicating which contaminants are of potential concern and whether individual effects may be severe enough to alter survival and reproduction (Sample et al. 1996). In concert with information about species richness and evenness, these concentrations can also help in understanding the potential role of pollutants in structuring community diversity and

dynamics, as some species are potentially more affected by contaminants than others (Tyler et al. 1989).

While there are likely thousands of pesticides and pollutants in China's environment, the present study concerns organochlorines (OCs) and mercury because they are both harmful to bats and other animals (discussed below) and useful for preliminary surveillance of bat exposure. Linkages between bat tissue concentrations and pathology have been defined more fully for these substances than for others, though numerous gaps still exist (Clark and Shore 2001; Bayat et al. 2014; Zukal et al. 2015). This foundation of information also makes it possible to sample bats non-lethally, which is desirable given the lack of prior exposure studies in the region and the sensitive conservation status of many bat species (Zhang et al. 2009).

Trace Metals

Background. Heavy metals are naturally occurring substances generally defined as being shiny, conducting electricity well, and having a specific density greater than 5 g/cm^3 (Järup 2003; Walker et al. 2006). Many metals occur in barely measurable quantities (parts per million = ppm, or parts per billion = ppb) in biological systems but still have large positive and negative impacts on organismal health. Metals like zinc, copper, iron, and magnesium are biologically necessary to animals, acting as cofactors for enzyme function (ATSDR 2005), whereas non-essential metals like mercury, cadmium, aluminum, and lead are more difficult to excrete and can be toxic even in small quantities (EFSA 2009). The metals of greatest concern to humans, and therefore potentially to other mammals, are cadmium, mercury, chromium, lead, and arsenic

(Tchounwou et al. 2012), although any substance in great enough concentration can become toxic. While arsenic is a metalloid, having chemical properties of both a metal and a non-metal (Walker et al. 2006), it has being grouped with heavy metals for the purpose of this study.

Metal toxicity operates at the cellular level by inducing oxidative stress, inhibiting DNA repair, and deregulating cell proliferation (Beyersmann and Hartwig 2008). This can lead to anemia, loss of coordination, lesions in vital organs, increased risk of cancer, infertility, behavioral changes, and death (IARC 1993; ATSDR 2005; EFSA 2009; Wibbelt et al. 2009). Most metal studies on mammals have been conducted on laboratory rats and mice. However, bat-specific effects have been found with lead (Zook et al. 1970; Sutton and Wilson 1983; Sutton and Hariono 1987), mercury (Nam et al. 2012), cadmium (Dixit and Lohiya 1974), iron (Farina et al. 2005), and copper (Hoenerhoff and Williams 2004) (reviewed in Zukal et al. 2015). Arsenic has also been noted at higher concentrations in bat tissues collected from polluted sites relative to those from reference sites, though the effects of this are unknown (O'Shea et al. 2001). Non-essential metals accumulate with age, such that adults tend to have higher tissue concentrations than juveniles (Walker et al. 2002).

Human practices affect metals' natural cycling in the environment, causing greater-than-normal accumulation in soils, waters, and air (Han et al. 2002). In general, some of the biggest contributors to environmental pollution are mining, waste burning and processing, fossil fuel burning, and production of steel, paints, rubbers, dyes, and fertilizers (ATSDR 2005; EFSA 2009; Pacyna et al. 2010; ATSDR 2012).

Relationship to bats. This chapter focuses on bats in urban areas, where heavy metals are emitted by industrial activities, commercial waste, and automobiles (Thornton 1990). A study on four species of *Pteropus* fruit bats in Australia found that those living in the city of Brisbane had higher lead concentrations in their organs than those from non-urban areas in the Northern Territory and Cape York (over 2,000 km away) (Hariono et al. 1993). Although this difference was not statistically significant, eleven of the 37 urban bats sampled had accumulated concentrations of lead in their livers or kidneys above the toxicity threshold established by Seawright (1989) for several domestic mammals (Hariono et al. 1993). At the time of the study, leaded gasoline was still in use in Australia (Australian Government 2015). Similarly, an eight-year study in Sweden found that common pipistrelles (*Pipistrellus pipistrellus*) captured near an industrialized area exhibited population declines and significantly lower body mass indices than those from a rural area about 25 km away (Gerell and Lundberg 1993). The drop in numbers and health was attributed to a decline in suitable aquatic foraging habitat due to drainage and pollution, and the urban bats carried greater concentrations of cadmium in their livers and kidneys and of DDT and polychlorinated biphenyls (PCBs) in their breast muscle (Gerell and Lundberg 1993). In Durban, South Africa, Naidoo et al. (2003) also linked higher foraging of *Neoromicia nana* at urban sites downstream of a wastewater treatment plant to greater uptake of cadmium, chromium, and nickel.

Testing for exposure and uptake. Upon entering an animal's body via ingestion, inhalation, or absorption, metals are carried through the bloodstream and eventually metabolized, incorporated into tissues, or excreted. The kidneys and liver, in particular, tend to accumulate metals over time, and, along with whole bat carcasses, are

the most common body parts tested for uptake (Zukal et al. 2015). Unincorporated metals are excreted in fur, urine, guano, nails, and milk, and each of these has been used as a form of non-lethal biomarker (e.g., Streit and Nagel 1993).

Fur is a particularly useful tissue for indicating metal exposure because it is relatively easy to collect and store and because concentrations of non-essential metals (e.g., lead, mercury, and cadmium) in fur have been correlated with significant accumulation in sensitive organs such as the brain, kidney, and liver (Nam et al. 2012; Zukal et al. 2015; Hernout et al. 2016a). It should be noted that concentrations of essential elements like copper and zinc tend to be less correlated between fur and vital organs because they are internally regulated as a part of normal body function (Hernout et al. 2016a). Because metals are transferred via the bloodstream to active hair follicles, which typically only produce fur once per year, fur provides a snapshot of metal uptake since the last molt (Beernaert et al. 2007; Hernout et al. 2016a). In contrast, blood, urine, feces, and stomach contents reflect more recent uptake—on the scale of hours to days—while bone reveals build-up over the years (Kales and Christiani 2005).

An important consideration in using fur to assess contaminant uptake is the timing of bat molting and shedding (Fraser et al. 2013). Most bats molt once per year during summer and fall (Fraser et al. 2013), and males and non-reproductive females typically molt sooner than reproductive females because the latter may lack sufficient energy to form new tissues until young are weaned (Dwyer 1963). Timing of growth in juveniles throughout the summer is highly variable and likely dependent on food availability and how early in the season young are born (Davis 1963; Fraser et al. 2013). Molting has been found to last from two weeks in *Lasiurus cinereus* (Cryan et al. 2004) to more than

four months in *Miniopterus schreibersii blepotis* (Dwyer 1963; Dwyer 1968; reviewed in Fraser et al. 2013). Fur shedding also may overlap temporally with fur growth, which can complicate interpretations of contaminant concentrations because older fur would reflect uptake from the prior year while newer fur would reflect that of the current year (Fraser et al. 2013).

Organochlorines

Background. Organochlorine compounds (OCs) are not naturally occurring and were first synthesized in the U.S. in the mid-1900s for vector control during warfare; shortly after, OCs became widely used as agricultural insecticides (Walker et al. 2006). When linked to mass mortality and reproductive failure in some wildlife species and regarded as an unacceptable health risk to humans, U.S. production and use of these substances was phased out during the 1970's and 1980's (U.S. EPA 1975; Keith 1991). However, some compounds are still used for malaria control, and first-world countries may sell these chemicals to developing countries where restriction and regulation of chemicals is less stringent (Smith 2001).

Organochlorines are highly lipophilic and persistent in the environment (U.S. EPA 1975). Residues of DDT and its by-products, for example, have been found in temperate soils more than 30 years after application (Dimond and Owen 1996), during which time the chemicals still readily entered the food chain (Welch 1994). Due to their size and non-polarity, OCs also bioaccumulate and biomagnify, as individuals from higher trophic levels ingest multiple individuals from lower trophic levels (Clark 1981). Harmful effects may not be immediately obvious because these substances are

sequestered in fats (Clark 1981). Yet when body fat is metabolized—for example, during migration or hibernation—high concentrations of chemicals can be released at once, resulting in mortality (Luckens 1973; Geluso et al. 1976).

Relationship to bats. DDT (1,1'-(2,2,2-trichloroethylidene)bis[4-chlorobenzene]) and its by-products (DDE and DDD) are the longest-studied contaminants in bats, with the first evidence of mortality observed in New Jersey in 1949 (Benton 1951; Clark and Shore 2001). Studies of bats in captivity given oral doses of DDT and DDE have demonstrated the compound's lethal effects (Luckens and Davis 1964; Clark and Stafford 1981). In the wild, DDT, DDE, and DDD have been associated with declines in some U.S. bat populations, including those of the Brazilian free-tailed bat (*Tadarida brasiliensis mexicana*) (Clark 1981; Clark 2001) and the gray bat (*Myotis grisescens*) (Clark et al. 1983; Clawson and Clark 1989). In the case of the free-tailed bat, populations had experienced unexplained declines and repeated mass die-offs from the 1950's to the early 1970's (Constantine et al. 1968; Cockrum 1970). Preliminary evidence that these declines were associated with DDT came from a study by Geluso et al. (1976), who showed that young free-tailed bats captured from Carlsbad Cavern, New Mexico with high concentrations of DDT died from neurotoxicity when they underwent conditions simulating migration in the lab. Concentrations of DDE also were high in birds and reptiles from the surrounding Rio Grande and Pecos River drainages (White and Krynitski 1983). To further support the connection, Clark (2001) tested the skin of museum specimens (*T. brasiliensis mexicana*) from 1930-1988 and found that concentrations of DDT compounds from the 1950's and 1960's were up to 4.8 times

higher than those in the 1970s, when DDT was being phased out. This pointed to DDT as the cause of the mass die-offs (Clark 2001).

DDT mainly acts on the brain, and signs of toxicity include altered metabolism, tremors, and convulsions (Luckens and Davis 1964; Clark and Kroll 1977; Clark and Prouty 1977; Clark 1981; Clark and Stafford 1981). Young bats are particularly susceptible because OCs can concentrate in mothers' milk (Ottoboni and Ferguson 1969; Clark 1981) and suckling juveniles appear to be 1.5 times more sensitive to brain DDT concentrations than adults (Clark et al. 1978; Clark 1981). Bat sensitivity to OCs appears to match that of wild-caught, like-sized mammals, such as shrews (reviewed in Clark 1981). However, amount of body fat plays a sizeable role in individual sensitivity (Luckens 1973; Clark 1981).

Testing for exposure and uptake. Through destructive sampling, concentrations of DDT and its by-products have been determined in the brains, livers, kidneys, muscle, body fat, and whole carcasses of bats (Bayat et al. 2014). Guano, fur, urine, milk, and nails have been used as non-lethal measures of exposure (Bayat et al. 2014). Correlations between organochlorine concentrations in guano and those in bat carcasses have not been strong enough to predict effects on bat health from guano alone (Clark et al. 1981, Clark et al. 1985). However, guano is one of the easiest tissues to collect and has been shown to reflect differences in DDT, DDE, and DDD contamination between sites (Clark and Prouty 1976, Clark et al. 1985). Thus, it can be a useful preliminary index of uptake within a colony over time and guide the need for examination of other tissues or demographic responses (Clark and Prouty 1976, Clark and Shore 2001).

Species Richness

The health of an ecological community can be measured, in part, by looking at the total number of species present, or species richness (Tilman 1996; Naeem and Li 1997). Because bat species differ in frequency and degree of contaminant exposure, in physiological tolerances, and in their ability to recover from disturbance (Hernout et al. 2015), some are expected to persist while others decline. As a consequence, if one habitat has fewer species than a similar, adjacent habitat, that community could be less resistant to future perturbation (i.e. the "insurance hypothesis") (Naeem and Li 1997; Yachi and Loreau 1999).

Environmental pollution is one factor implicated in the biotic homogenization of a variety of animal taxa within urban areas, whereby communities become dominated by a few species that can tolerate the disturbance (review by McKinney 2002). Reduced evenness, for example, has been demonstrated for bats (Avila-Flores and Fenton 2005; Coleman and Barclay 2012), birds (Blair 2001, Marzluff 2001), and many insects (McIntyre 2000) in urbanized settings relative to suburban and non-urban areas (review by McKinney 2002). The degree to which pollution contributes to this phenomenon is difficult to determine, so it must be examined from local, regional, and global scales (Grimm et al. 2008).

Questions

I had the following questions regarding bats in a heavily urbanized area in China:

Q1 Which contaminants are bats accumulating?

Q2 Do bats in an urban environment have greater contaminant concentrations in their tissues than bats from less urbanized areas?

Q3 What are the possible health ramifications of the contaminant levels measured in bat tissues?

Q4 Is there a relationship between contaminant concentrations in bat tissues and bat species richness at sampled sites?

Hypotheses

H1 Mercury concentrations in bat fur will differ between land use types (urban versus non-urban).

H2 Organochlorine concentrations in bat guano will differ between land use types (urban versus non-urban).

H3 Bats with higher contaminant concentrations in tissues will exhibit poorer body condition.

H4 Tissue contaminant concentrations will differ between species.

H5 Tissue contaminant concentrations will differ between adults and juveniles within species.

H6 Bat species richness will be higher in areas where concentrations of mercury in bat tissues are lower.

Methods

Study Location

Chengdu, the capital of Sichuan Province, is situated on the fertile Chengdu Plain (elev. 500 m, Fig. 1) and was established more than 2,500 years ago (Schneider et al. 2005). The city has experienced rapid urban expansion since the 1970's (Schneider et al. 2005) and at the time of this writing contains over fourteen million people. From 1988 to 2002, four concentric ring roads were built around the city to facilitate growth, and building density generally decreases from the city center to its periphery (Qiao et al. 2013). The city is surrounded by the Longquan Range and Penzhong Hills to the east, the

Qionglai Mountains to the west, and lowlands to the southeast. The area is part of the Sichuan Basin ecoregion, which contains endangered subtropical forests composed of evergreen broadleaf trees (World Wildlife Fund 2017).

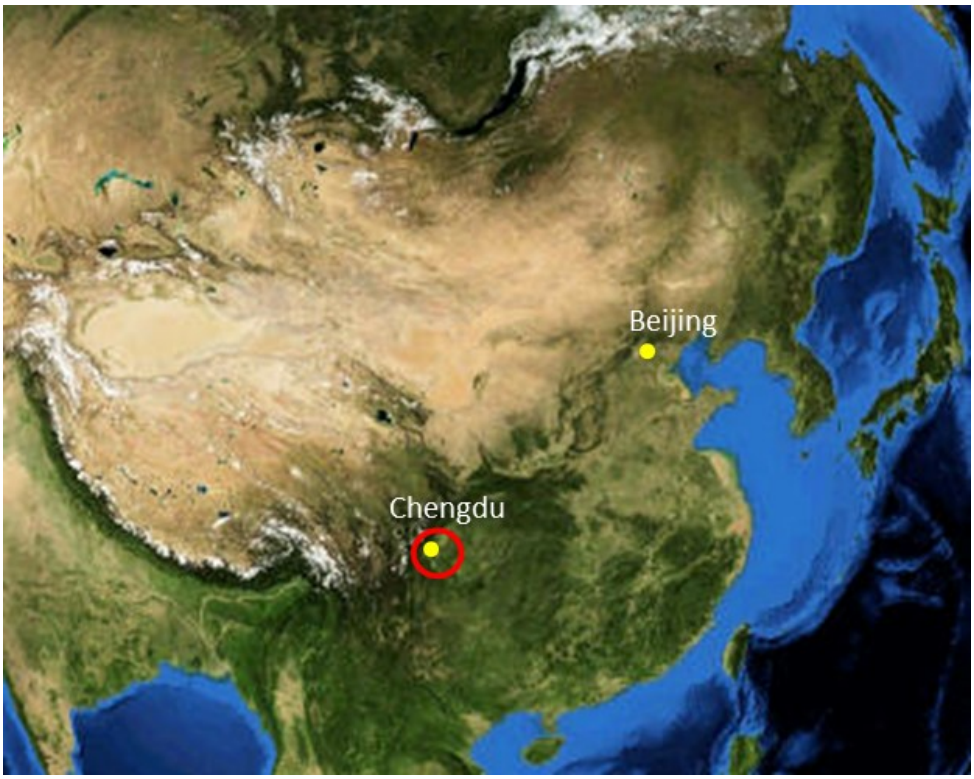


Fig. 1. Aerial image of China. Red circled region is Sichuan Province. (Map source: Google Earth 2016)

Chengdu lends itself to studies of trace metals because there is evidence of a gradient of metal pollution from higher within the city center to lower on the outskirts. Qiao et al. (2013) found that road dust from the innermost (first ring) road of the city had significantly higher concentrations of lead, copper, and zinc than the outermost (second and third) ring roads. A positive relationship between urbanization and soil metal pollution has been found in other Chinese cities for copper, lead, zinc, and cadmium, as

well (Wei and Yang 2010). Wei and Yang (2010) found that urban soils and urban road dust had higher concentrations of these metals than nearby agricultural soils, often in excess of China's safe soil standards for farming.

On a global scale, fine particulate matter in the air (<2.5 μg/m³) ($PM_{2.5}$), which typically contains heavy metals as a result of combustion and industrial activities (Craig et al. 2008), is relatively high in Chengdu, as is true for many Asian cities compared to other parts of the world (World Health Organization 2016, Fig. 2). In 2013, Chengdu's annual mean PM2.5, as measured at the U.S. Consulate (Lat. 30.624700, Long. 104.068388), was 97 (http://www.stateair.net/web/historical/1/2.html). (Values were averaged from "valid" hourly measurements only.) Using the conversion calculator provided at https://airnow.gov, this corresponds to an Air Quality Index (AQI) of 172, categorized as "unhealthy." According to the Environmental Protection Agency (https://airnow.gov), under these air conditions, members of the general population are at risk for respiratory and cardiovascular effects. Fine air particles also have been found to cause inflammation and oxidative stress in the brains of dogs (Calderón-Garcidueñas et al. 2003), mice (Veronesi et al. 2005), rats (Zanchi et al. 2008), and humans (Calderón-Garcidueñas et al. 2004), changes linked to Alzheimer's and Parkinson's diseases.

Chengdu also is a relevant location in which to study the effects of organochlorine pesticides (OCs) on wildlife. While OCs including DDT, dieldrin, and aldrin have been banned for production and use in the U.S. since the 1970's (further details below), a similar ban only occurred in China in 2005 (SCPOP 2007). Because organochlorines are highly persistent in the environment (see Background above), they may still be having effects today.

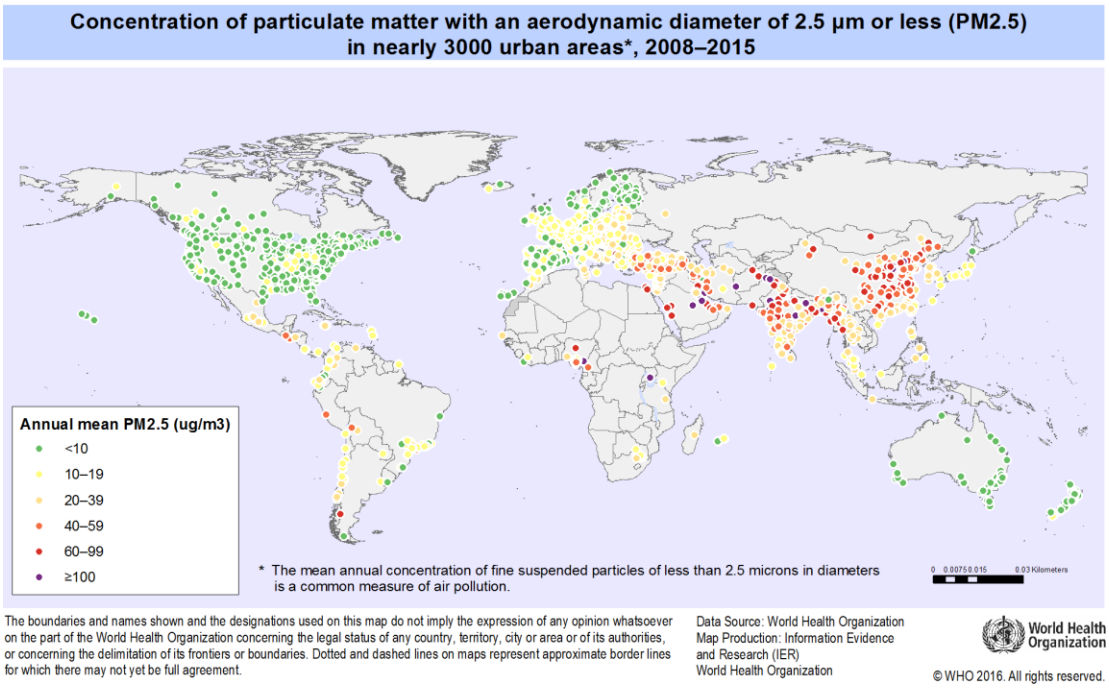


Fig. 2. Concentrations of particulate matter with an aerodynamic diameter of 2.5 um or less (PM2.5), 2008-2015. (Source: World Health Organization 2016)

Study Sites

Acoustic detectors were placed at ten sites of varying proximity to the urban center in 2012 and 2013, and mist-netting occurred at five of these in 2013 (Table 1, Figs. 3-7). Because this type of study was new to the area, site selection was opportunistic and dependent on personal contacts, access, and safety. Sampling locations were grouped into four categories: 1) highly urbanized (designated "urban"), 2) moderately urbanized (designated "suburban"), 3) agricultural, and 4) natural (a reserve). "Urban" and "suburban" definitions roughly followed those of McKinney (2002), whereby areas of high urbanization had more than 50% impervious surface cover and those of moderate urbanization had 20-50%. Surface coverage was considered within a 2.5-km diameter of

a site, an area analogous to that (2.5 x 2.5 km^2) used by Jaberg and Guisan (2001) to evaluate spatial trends in bat occupancy relative to land use types across a landscape.

Acoustic recordings without mist netting were conducted at three central urban sites (Jinjiang River, Jiulidi Park, and Southwest Jiaotong University), one peripheral urban site (Moon Bear Rescue Centre), and Longxi Hongkou Nature Reserve (Figs. 6-7). The first three urban sites were located in the northwest portion of the city, between the second and third ring roads (Fig. 6). The Moon Bear Rescue Centre was approximately 20 km north northeast of the city center, 6 km beyond the outermost ring road (Fig. 6). Although classified as urban according to the above definition of surface coverage, the Centre differed from the former three urban sites in that recordings took place at a 400 x 400 m^2 area of contiguous forest. Longxi Hongkou was 70 km northwest of the city, just above the Sichuan Basin and on the upper reaches of the Minjiang River (Fig. 7).

Starting from the city center and proceeding outward, netting sites were located as such: Sichuan University (urban) between the first and second ring roads, Chengdu Panda Base (suburban) between the third and fourth ring roads, Jiang'an campus (urban) just outside the fourth ring road, An Long organic farm (agricultural) about 50 km northwest of the city center, and Xi Hua Normal (urban) about 220 km east northeast of the city, in Nanchong (Figs. 3-5). Sichuan University is 2 km northeast of the U.S. Consulate where air quality measurements were regularly monitored.

Bat Tissue Collection

Triple-high mist nets (Avinet, Inc., Dryden, New York) were used to sample bats in June and July 2013 (Table 1), months when bat activity was expected to be high in the

region (Huang and Huang 1982). Nets were strung along bridges over bodies of calm, fresh water (Fig. 8) where bats were observed drinking and foraging. An exception to this was at Xi Hua Normal; captures there took place on the fourth floor of an older tenement building occupied by a *Nyctalus plancyi* maternity colony. Nets were opened from approximately half an hour before sunset (U.S. Naval Observatory 2013) to when activity reached a lull and no bats were detected for an hour. (Nets were typically closed by 22:00-23:00.) Sampling was approved by University of Northern Colorado IACUC Protocol 1205C-RA-B-15 (Appendix A).

All handling adhered to guidelines of the American Society of Mammalogists (Sikes et al. 2011). Technicians wore a leather glove on one hand and a disposable latex or nitrile glove on the other whilst removing and processing bats. When captured, bats were placed individually in cloth holding bags and left until they defecated or for up to 30 minutes. Holding bags were cleaned by hand in a mild bleach solution between netting events. Species were identified by external characteristics using a field key (Smith and Xie 2008) and sex assessed by observing genitalia. Relative age (juvenile versus adult) was determined by use of a headlamp to backlight and examine the degree of ossification at the metacarpal-phalangeal joints (Brunet-Rossinni and Wilkinson 2009). Bat mass was measured by placing a bagged bat on a small digital scale and then deducting the weight of the bag from the reading after the bat was released. Forearm length was measured with a handheld ruler to the nearest 0.25 mm. Amount of body fat, an indicator of body condition in bats, was estimated by calculating the ratio of body mass to forearm length (Pearce et al. 2008).

Table 1. Bat sampling locations in the area of Chengdu, China.

Site	Land Use Category	Latitude, Longitude	Elevation (m)	Call recording 2012[b]	Call recording 2013[b]	Mist netting 2013
An Long	Agricultural	30.851217, 103.796603	591	8/7 (partial)	7/23, 7/24, 7/25	7/25
Chengdu Panda Base	Suburban	30.738811, 104.144753	525	7/27, 7/28	6/24, 6/25 + 6/21, 6/23 (partials)	6/21, 6/23, 6/25
Jiang'an Campus	Urban	30.562304, 104.027022	481	--	--[c]	7/14
Moon Bear Rescue Centre[a]	Urban (forest)	30.823169, 104.157524	496	8/3, 8/4, 8/5	--	--
	Uurban (river)			8/3, 8/4, 8/5	--	--
Xi Hua Normal	Urban	30.816447, 106.067239	280	--	7/19 (partial)	7/18
Jinjiang River	Urban	30.707047, 104.054989	501	7/25, 7/26	--	--
Jiulidi Park	Urban	30.692229, 104.056397	498	7/26 (partial)	--	--
SW Jiaotong University	Urban	30.695399, 104.051387	507	7/27 (partial)	--	--
Sichuan University	Urban	30.634239, 104.087242	493	8/9 (partial)	7/15, 7/16 + 6/28, 7/2, 7/6 (partials)	6/28, 7/2, 7/6, 7/16
Longxi Hongkou[a]	Natural (river)	31.145983, 103.581483	1,828	7/30	--	--
	Natural (lake)			8/11	--	

[a]Forest = microphone faced a clearing within a natural treed (non-paved) area. River / Lake = microphone faced out towards an open river or lake at a forest edge.
[b]Recordings were done for whole nights (from a half hour before sunset to sunrise) for all except where noted as "partial." Partial nights occurred where recording devices could not be left overnight. For these, recordings began at half an hour before sundown and ended after a noticeable drop in activity, typically 2-3 hours later.
[c]No recordings due to adverse weather conditions.

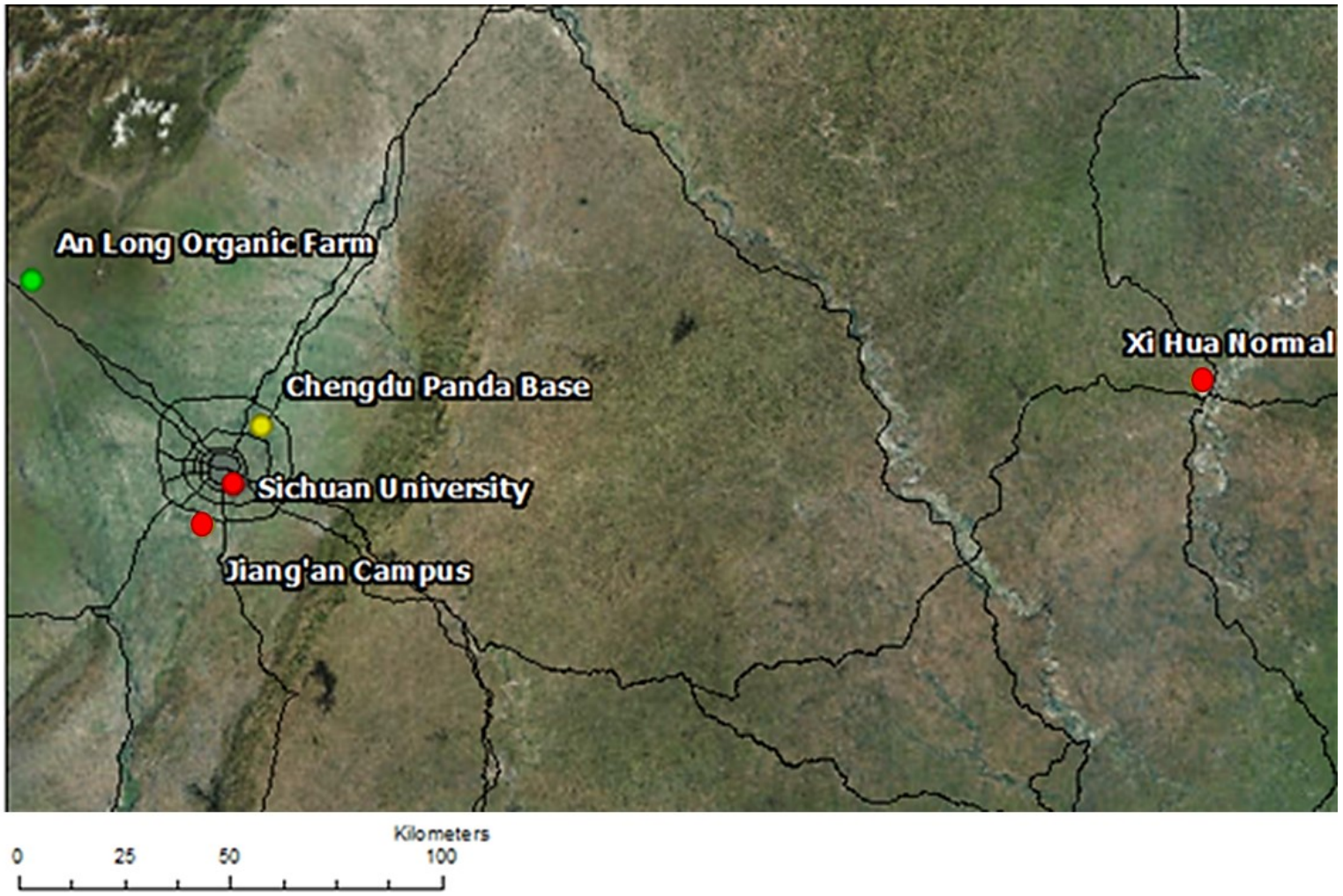


Fig. 3. Bat mist net locations in and around Chengdu, China. Colors indicate site type. Red = heavily urbanized. Yellow = moderately urbanized. Green = agricultural. (Map sources: Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.)

Fig. 4. A heavily urbanized site in Chengdu. (Source: Google Earth 2016)

Fig. 5. A moderately urbanized site in Chengdu. (Source: Google Earth 2016)

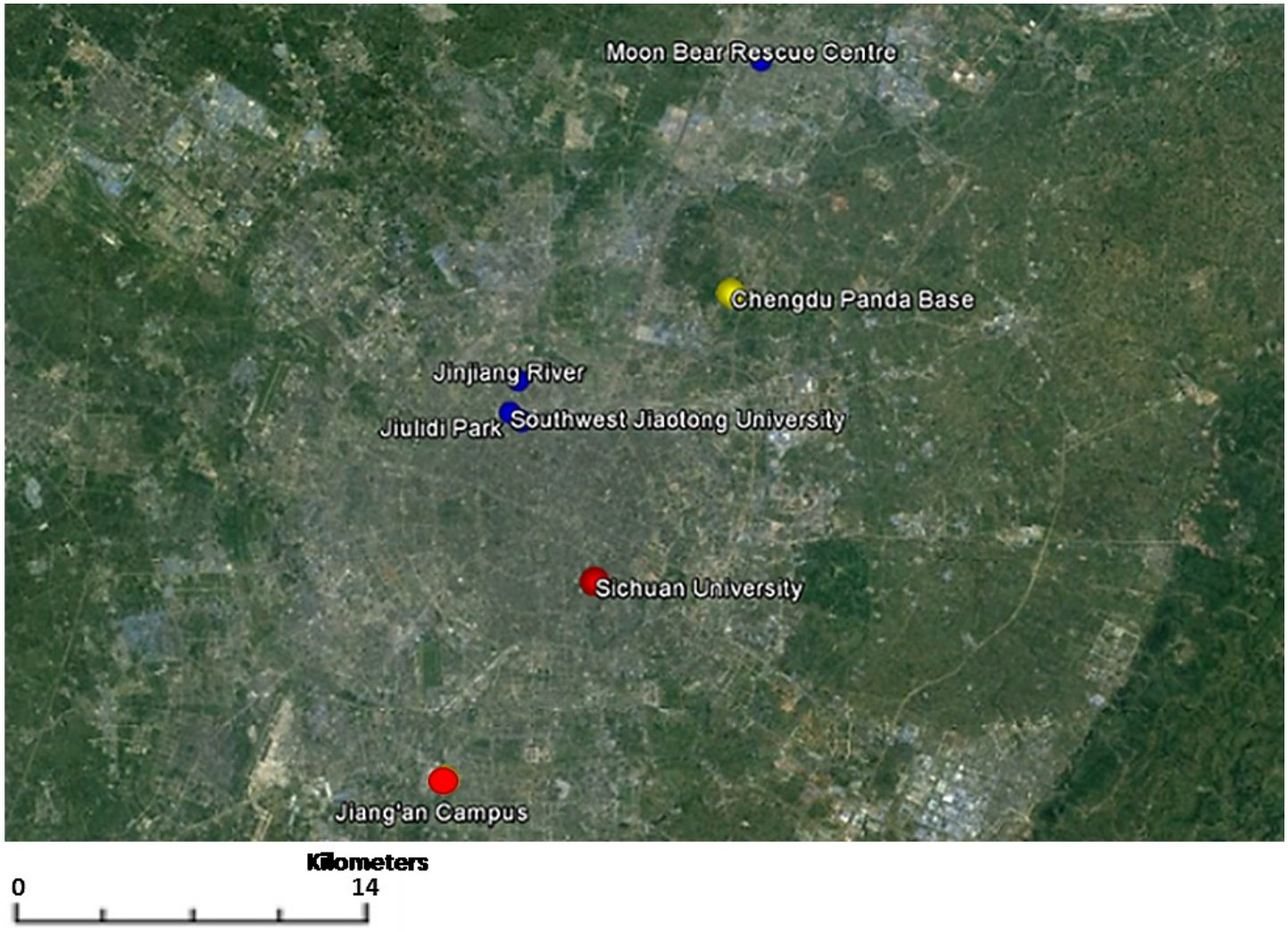


Fig. 6. Sites (blue dots) where bat calls were recorded but no mist netting took place in Chengdu, China. Compare to Fig. 3. (Source: Google Earth 2016)

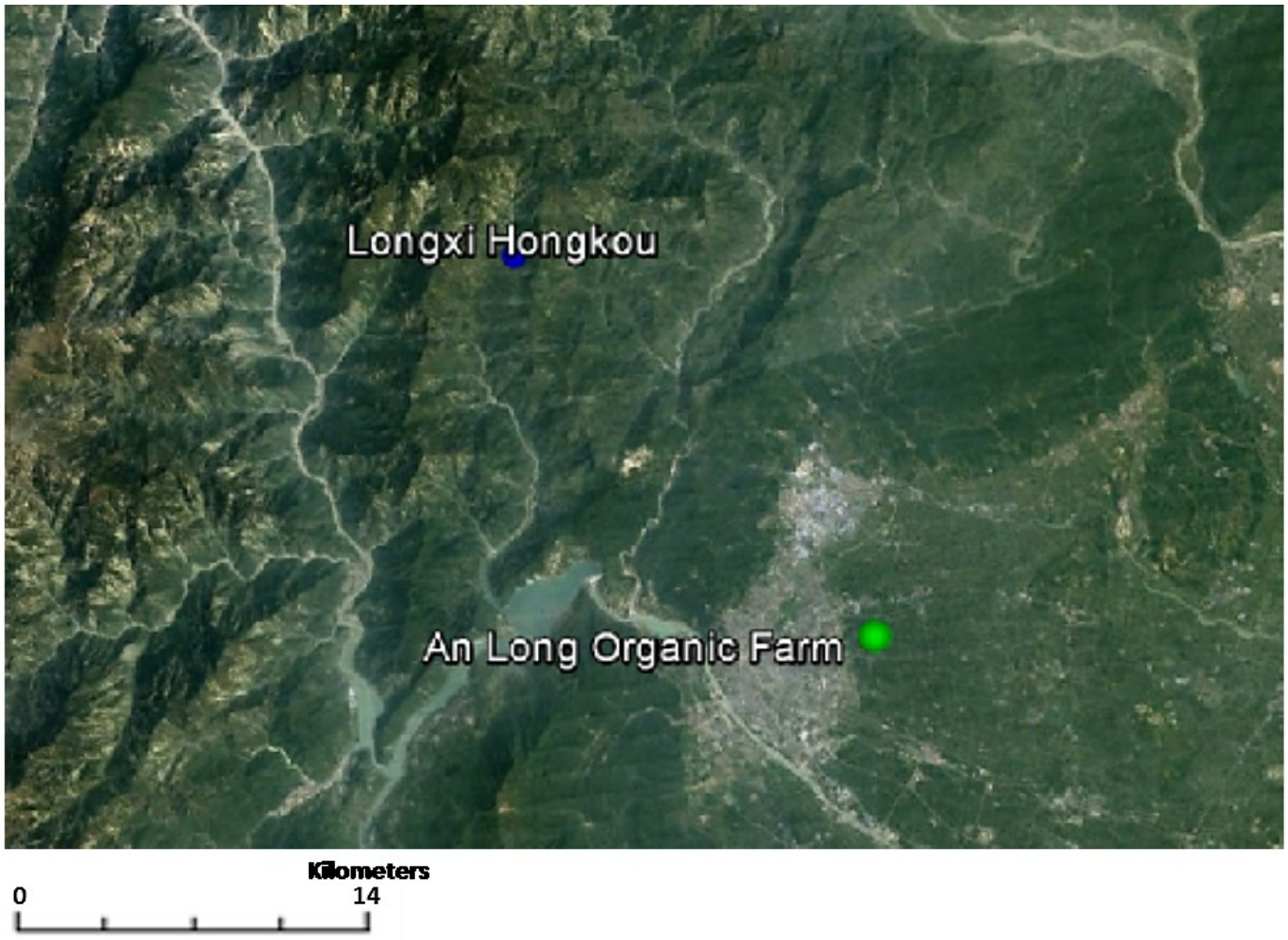


Fig. 7. Additional site (blue dot) where bat calls were recorded but no mist netting took place northwest of Chengdu, China. Compare to Fig. 3. (Source: Google Earth 2016)

Fig. 8. Triple-high mist nets erected over Swan Lake at the Chengdu Panda Base (2013). Photo by Kelsey Gonzales.

Guano samples were collected from cloth bags and directly off of bats using stainless steel forceps. They were then placed in individual, chemically clean glass jars (Eagle-Picher Environmental Services) and stored on ice until transfer to a -70°C freezer. Forceps were sterilized after each use by dipping in or spraying with 100% ethanol, passing the instrument through a flame, dipping in hexane, then wiping with lens paper. A small patch of fur (about 1 cm^2) was clipped from the dorsal interscapular region using stainless steel scissors that are similarly cleaned between each use. Fur was placed in chemically clean glass jars and stored at room temperature.

Tissue Analysis

Mercury in fur. Forty-five samples were analyzed for total mercury content with a direct mercury analyzer (MA-3000, Nippon Instruments Corporation, Japan) at Colorado State University, Pueblo. This instrument performs cold absorption spectroscopy, a process by which a sample is first combusted with high heat to vaporize mercury in the form of H^0 (Cizdziel and Gerstenberger 2004). The mercury is amalgamated onto a gold tube, while other products are flushed away. The tube is then heated to release the newly concentrated mercury, and this is subsequently measured by atomic absorption spectroscopy (Cizdziel and Gerstenberger 2004). Quality assurance and control included use of internal standards, initial and continued calibration verification, method blanks, and quality control samples (National Institute of Standards and Technology (NIST) standards "1947" (DORM-4 = dogfish muscle, Natural Resource Council Canada) and "1573a" (tomato leaves, Natural Resource Council Canada)).

Organochlorines in guano. Three guano samples from *Pipistrellus abramus* were analyzed for OC content by the Geochemical and Environmental Research Group at Texas A&M University (College Station) using gas chromatography. One sample came from a single adult female, and the other two were composites collected beneath pipistrelle roosts. The roosts, depicted in Fig. 10, were located beneath wooden slats that covered electrical outlets. For quality assurance and control, internal standards, initial and continued calibration verification, method blanks, and quality control samples (NIST standard "1947" (Lake Michigan fish tissue)) were used.

Fig. 9. Noctule roost site at Xi Hua Normal tenement. A *Nyctalus plancyi* maternity colony (indicated by arrow) resided in the rafters of the top floor. Photo by Laura Heiker.

Fig. 10. Pipistrelle roost site at Xi Hua Normal tenement. Photos by Laura Heiker.

Bat Call Collection and Analysis

Preliminary call collection occurred at eight sites in July and August 2012 (Table 1). A battery-operated SM2-BAT+ or EM3 sonar detector (Wildlife Acoustics, Maynard, Massachusetts) was used to record calls, depending on site conditions. When the threat of theft was minimal, SM2 units were used because they could be left overnight for longer sampling. These were mounted on trees at approximately 1.5 m above the ground, at the edge of a vegetative stand and facing towards open water; the microphone (SMX-US) faced out at 30 degrees above horizontal. EM3s were used for shorter periods, with the microphone pointing directly upward while being held or set down at breast height (approximately 1.5 m). Both detector types were set to record frequencies from 0-384 kHz. SM2s recorded calls in compressed WAC file format with the following settings: gain = +12.0 dB, dig HPH = fs/12, dig LPF = off, Trg Lvl = 18SNR, Trg Win = 2.0s, and a time expansion factor of 8. EM3s recorded calls in WAC or WAV format, also with a time expansion factor of 8.

In July and August 2013, the Chengdu Panda Base was revisited for recordings, and four sites—Sichuan University, Jiang'an campus, An Long organic farm, and Xi Hua Normal—were newly sampled (Table 1, Fig. 1). Further data collection at Longxi Hongkou Nature Reserve was not possible due to seasonal flooding and a massive landslide that took place in July 2013. The Moon Bear Rescue Centre was not revisited because it did not grant permission to mist net, which was the focus of the 2013 season. Similarly, Jinjiang River, Jiulidi Park, and Southwest Jiaotong University were not resampled because mist netting was impractical due to visibility to the general public.

Post-processing. WAC files were later decompressed into WAV format using Kaleidoscope software v. 4.0.0 (Wildlife Acoustics, Maynard, Massachusetts) (split to max duration = 5 s, time expansion ratio = 1). SM2 calls were additionally post-processed using SonoBat SM2 Batch Attributor with the SMX-UT button on, according to manufacturer recommendations to adjust for SMX-US microphone recordings. EM3 calls required no post-processing. So as to include as many bat files as possible, no files were scrubbed. SonoBat 3.1 (U.S. West, Arcata, California) was then used to view color sonograms and produce automated measurements of 76 different parameters for each call (via default settings).

Relative taxonomic richness. SonoBat 3.1 can be used for automated identification of sonar sequences in the U.S., and similar software has been developed for Europe. However, many fewer reference sequences exist for China. Exceptions are Jiang et al. 2008, Sun et al. 2008, Zhu et al. 2008, and Wei et al. 2011, and a collection of calls has been compiled in the Echobank database (Collen 2012). Unfortunately, this resource was unavailable at the time of this writing. As an alternative, to obtain an estimate of richness, I visually separated calls into phonic types. It is acknowledged that grouping into phonic types can limit usefulness of species diversity measurements because types do not necessarily distinguish between families or genera (Russo and Voigt 2016). However, in the absence of a validated call library, this was chosen as the best available approach. A discriminant function analysis or artificial neural network would have been preferable, but these rely on a set of known calls to train a program to then classify unknown calls (Parsons and Jones 2000).

Phonic types were grouped according to classifications from Collen (2012), who contributed substantially to the formation of Echobank, and Walters et al. (2012), who used European calls from Echobank to develop iBatsID, a publicly available tool for identifying European bat species. In addition, I matched the values of nine call parameter averages published for Chinese bat species by Collen (2012) with a list of possible species in China, derived from Wilson and Reeder (2005) (Appendix B). These call parameters—echolocation call type, bandwidth, call duration, characteristic frequency, dominant slope, maximum frequency, minimum frequency, peak frequency, and total slope—were viewed by Collen as the most useful for distinguishing species worldwide and have been used by many other authors for acoustic identification (e.g., Parsons and Jones 2000; Russo and Jones 2002; Hughes et al. 2011; and Walters et al. 2012). Although parameter values for the ~123 possible species in China (Appendix C) were too complex to be matched manually to my Chengdu calls, they served as a guide for potential identifications.

Data Analyses

Mann-Whitney U tests ($\alpha = 0.05$) were used to compare tissue contaminant concentrations between sites, species, age classes within species, and sexes within species. Spearman's rank correlations were used to assess the relationship between bat tissue concentrations and intraspecific body conditions. Statistical analyses were conducted in R package 3.3.1 (R Core Team 2013). Finally, tissue concentrations were compared to the literature to determine whether or not they crossed known threshold values for detectable physiological and behavioral effects in bats and other mammals.

Results

Bat Captures

For all sites combined, bats were successfully netted on a total of ten nights (Table 1), with 178 individuals captured: 43 *Pipistrellus abramus*, 134 *Nyctalus plancyi*, and one *Scotomanes ornatus* (Fig. 11, Appendix D). The high number of *N. plancyi* mostly resulted from a single night of netting at Xi Hua Normal, the site of a large maternity colony. To minimize handling time and safely release all bats, only a subset of these noctules was measured and sampled for tissues. In total, captures yielded 53 guano samples (all *P. abramus*) and 86 fur samples (42 *P. abramus*, 43 *N. plancyi*, and one *S. ornatus*) used for contaminant analyses. Sex, age, and site of these captures are listed in Table 2, and details for all individual captures are in Appendix D.

Mercury

Sample sizes were not large enough to statistically compare concentrations for all combinations of sex, age, species, and location (Tables 2-4). However, fur mercury concentrations of adult *P. abramus* across all sites (mean = 11.82 ppm, n = 11) were significantly different from those of adult *N. plancyi* (mean = 3.11 ppm, n = 17) ($P < 0.001$). Juvenile (mean = 0.89 ppm, n = 14) and adult (mean = 3.11 ppm, n = 17) *N. plancyi* concentrations also were significantly different from one another ($P < 0.001$), with those of the adults being higher. The mean concentration of Hg in pipistrelle fur from Sichuan University (all ages = 12.92 ppm (n = 4), adults only = 13.57 ppm (n = 3)) was higher than that from Chengdu Panda Base (all ages = 9.24 ppm (n = 7), adults only =

9.73 ppm, (n = 6)), but the difference was not significant (P = 0.315 for all ages, P = 0.262 for adults only) (Table 4).

a) 

b) 

c) 

Fig. 11. Bat species netted in Chengdu, China. a) Japanese pipistrelle (*Pipistrellus abramus*), b) Chinese noctule (*Nyctalus plancyi*), c) Harlequin bat (*Scotomanes ornatus*). (Photos by Kelsey Gonzales.)

Neither species exhibited a significant relationship between body condition index (BCI) and total fur mercury content (Figs. 12 and 13). For those bats tested for mercury, body condition was as follows: adult *N. plancyi* mean = 0.37 (range = 0.17-0.45, n = 17), juvenile *N. plancyi* mean = 0.33 (range = 0.25-0.39, n = 14), adult *P. abramus* mean =

Table 2. Summary of bats sampled for tissues in 2013. Weights are in grams. Individual capture data are in Appendix D.

Species	Site	AF	AM	JF	JM	Total n
		n (Avg. Weight)				
Chinese noctule (*Nyctalus plancyi*)	Sichuan University	1 (24.6)	-- --	1 (14.95)	1 (17.4)	3
	Xi Hua Normal	18[a] (20.48)	2 (24.5)	9 (17.74)	10 (17.6)	39
Japanese pipistrelle (*Pipistrellus abramus*)	An Long	2 (7.10)	--	1 (6.3)	--	3
	Jiang'an Campus	1 (8.10)	--	--	1 (5.10)	2
	Chengdu Panda Base	16 (7.19)	2 (6.03)	2 (6.00)	2 (5.10)	22
	Sichuan University	4 (6.05)	5 (5.30)	1 (3.10)	2 (4.08)	12
	Xi Hua Normal	3 (6.57)	--	--	--	3
Harlequin bat (*Scotomanes ornatus*)	Chengdu Panda Base	1 (38.30)	--	--	--	1

0.19 (range = 0.13-0.22, n = 11), and juvenile *P. abramus* = 0.13 (n = 1). For both species, juvenile BCI values fell at the lower range of those for adults.

Table 3. Total mercury concentrations in fur. Concentrations are in ppm, and means are geometric.

Site		AF	AM	JF	JM
Japanese pipistrelle (*Pipistrellus. abramus)*					
Sichuan University	Mean	18.61	11.59	-	11.15
	Range	-	6.28 - 21.38	-	-
	n	1	2	0	1
Chengdu Panda Base	Mean	9.03	14.14	6.79	-
	Range	4.60 - 16.07	-	-	-
	n	5	1	1	0
An Long	Mean	32.87	-	-	-
	Range	-	-	-	-
	n	1	0	0	0
Xi Hua Normal	Mean	9.04	-	-	-
	Range	-	-	-	-
	n	1	0	0	0
Chinese noctule (*Nyctalus plancyi*)					
Sichuan University	Mean	-	-	1.17	-
	Range	-	-	-	-
	n	0	0	1	0
Xi Hua Normal	Mean	3.15	2.60	0.91	0.85
	Range	0.63 - 6.90	-	0.38 - 2.03	0.46 - 4.42
	n	16	1	5	8

Table 4. Mercury concentrations in fur of adult bats (males and females combined) by land use type. Urban bats were captured at Sichuan University (SU) and Xi Hua Normal (XH), suburban at Chengdu Panda Base (PB), and agricultural at An Long farm (AL). Means are geometric.

Site type	Mean fur Hg (mg/kg)			
	n	*Pipistrellus abramus*	n	*Nyctalus plancyi*
Urban (SU)	3	13.57	0	--
Urban (XH)	2	7.16	7	3.00
Suburban (PB)	6	9.73	0	--
Agricultural (AL)	1	32.87	0	--

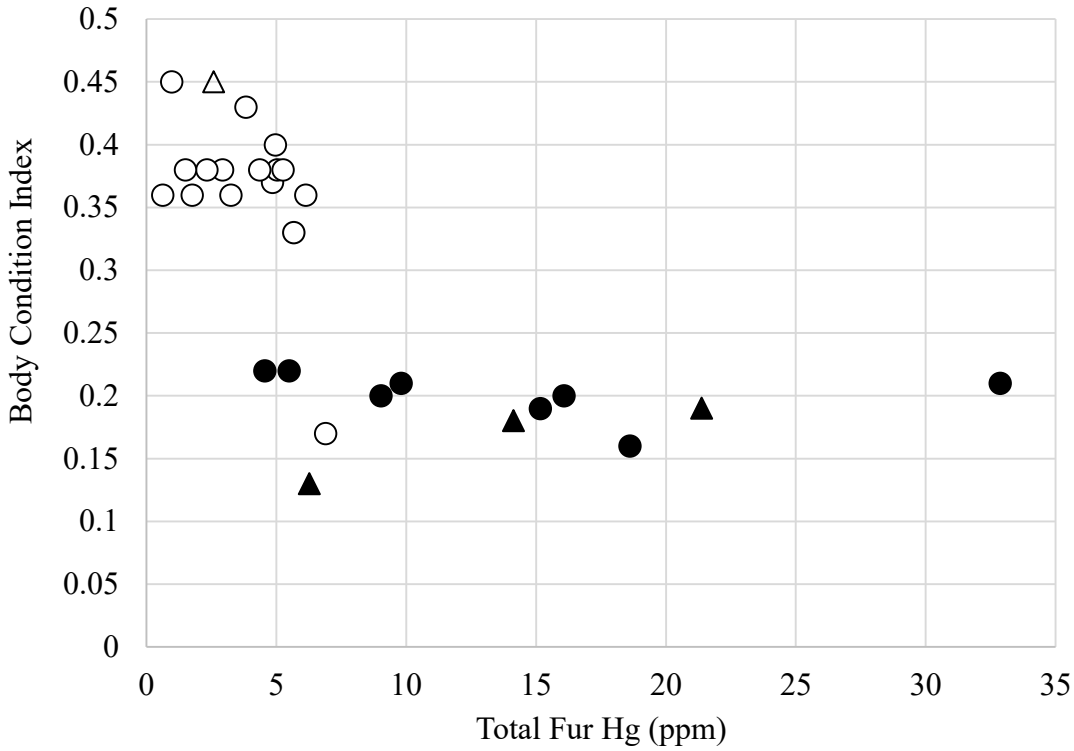


Fig. 12. Adult bat body condition index in relation to total mercury content of fur. Circles = females. Triangles = males. White = *Nyctalus plancyi* (r_s = -0.357, P = 0.160, n = 17). Black = *Pipistrellus abramus* (r_s = -0.321, P = 0.334, n = 11).

Organochlorines

Results are presented in Table 5 and Appendix E. Hexachlorobenze, alpha-chlordane, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD were detected in the two composite guano samples from Xi Hua Normal. The guano sample from the single adult female (Chengdu Panda Base) was too small to be properly analyzed, so only an estimated value for hexachlorobenzene could be produced, while other compounds were not detectable.

Alopecia

Three adult female *P. abramus* from the Chengdu Panda Base, two adult female *N. plancyi* from Xi Hua Normal, and one juvenile male *N. plancyi* from Sichuan

University exhibited hair loss on their backs or necks (Figs. 14 and 15, Appendix D). Two of these pipistrelles were pregnant, and the third was non-reproductive. One pregnant female had a fur Hg concentration of 4.56 ppm. Contaminant concentrations were not measured for the *N. plancyi*, nor was reproductive status noted in these adults. However, all females captured at the Xi Hua Normal colony were lactating or post-reproductive.

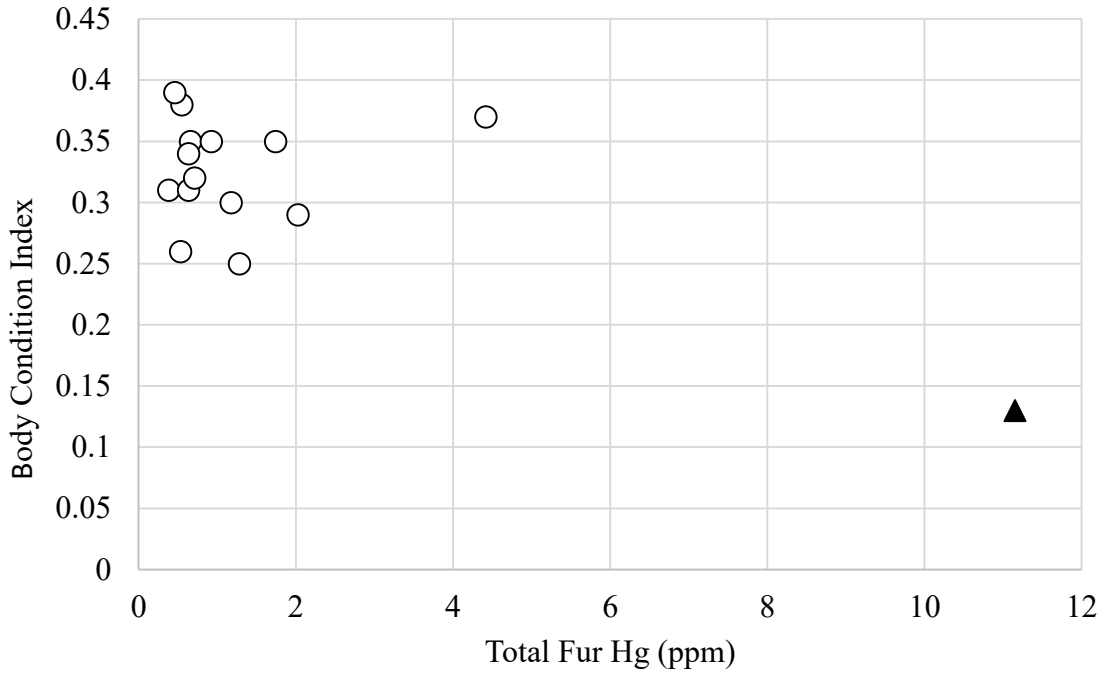


Fig. 13. Juvenile bat body condition index in relation to total mercury content of fur. Circles = females. Triangles = males. White = *N. plancyi* (r_s = -0.249, P = 0.353, n = 14). Black = *P. abramus* (n = 1).

Phonic Types

Phonic types and examples of calls from sites are depicted in Figs. 16-21. Under both criteria used, Longxi Hongkou had more phonic types than any other site (5 according to Walters et al. (2012), 4 according to Collen (2012) (Tables 6 and 7).

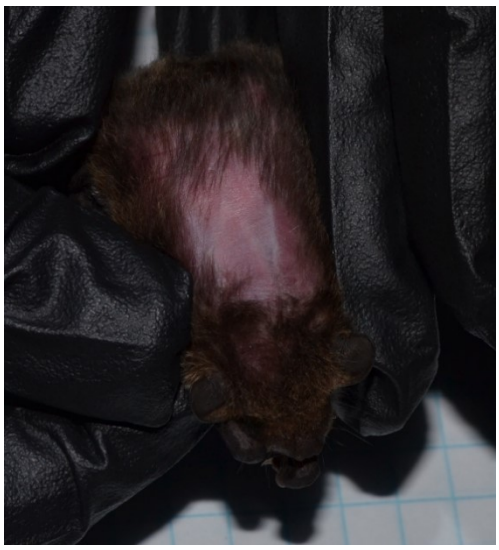
Referring to species possible in the Chengdu area (Appendix B), the first of these types (type 12 by Collen) is most likely a Rhinolophid, although a specific species could not be identified by characteristic frequency, one of the most useful parameters for this group (Walters et al. 2012). The characteristic frequency of this call was 67 kHz, which falls mid-way between average values for *R. pearsonii* (57 kHz) and *R. ferrumequinum* (82 kHz) (Collen 2012). Following Collen (type 3), the second call type is most likely *Vespertilio sinensis*. However, as demonstrated in Fig. 19c, many species of the family Vespertilionidae and Molossidae can produce calls of this shape depending on habitat.

The number of call types from more central urban areas (Jinjiang River, Jiulidi Park, Sichuan University, Southwest Jiaotong University, and the Bear Centre) did not differ substantially from those of peri-urban (Panda Base) or agricultural areas (An Long). All had one to three types, depending on whether the Walters et al. or the Collen classification was used (Tables 6 and 7). Types 2 and 3 according to Walters et al. (2012), and 7 and 8, according to Collen, were recorded at every site and characteristic of many species of Vespertilionidae and Molossidae. The second-most common type, 5 (Walters et al. 2012), was recorded at all sites except An Long (agricultural), Sichuan University (urban), and Southwest Jiaotong University (urban). Walters et al. (2012) principally associated this with bats of the *Myotis* genus (family Vespertilionidae).

Table 5. Organochlorine concentrations detected in guano from *Pipistrellus abramus*. Concentrations are in ppb. A complete listing of compounds tested for is in Appendix E.

Location	Sample identity	Wet weight (g)	Hexachloro-benzene	Alpha chlordane	*p,p'*-DDE	*o,p'*-DDD	*p,p'*-DDD
Chengdu Panda Base	Adult female	0.14	2.29[a]	ND[b]	ND	ND	ND
Xi Hua Normal	Roost composite	0.5	4.40	1.39	7.78	2.36	3.50
Xi Hua Normal	Roost composite	0.5	2.00	1.81	6.02	1.43	1.33

[a]Estimated because the sample was too small for proper analysis.
[b]ND = not detectable

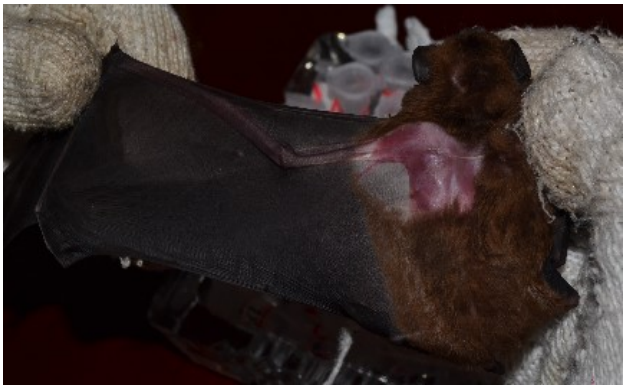
a) 

b) 

c) 

Fig. 14. *Pipistrellus abramus* individuals with fur loss. All three were captured at Chengdu Panda Base. a and b are pregnant adult females (23 June 2013). c is a non-reproductive adult female (25 June 2013). Photos by Kelsey Gonzales.

a) 

b) 

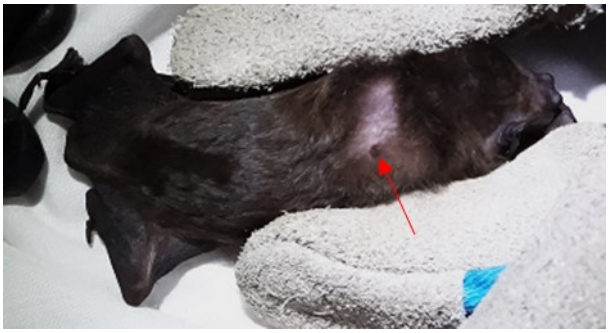
c) 

Fig. 15. *Nyctalus plancyi* individuals with fur loss. a and b are adult females, captured at Xi Hua Normal (18 July 2013). c is a juvenile male captured at Sichuan University (28 June 2013). Red arrow indicates an ectoparasite. Photos by Kelsey Gonzales.

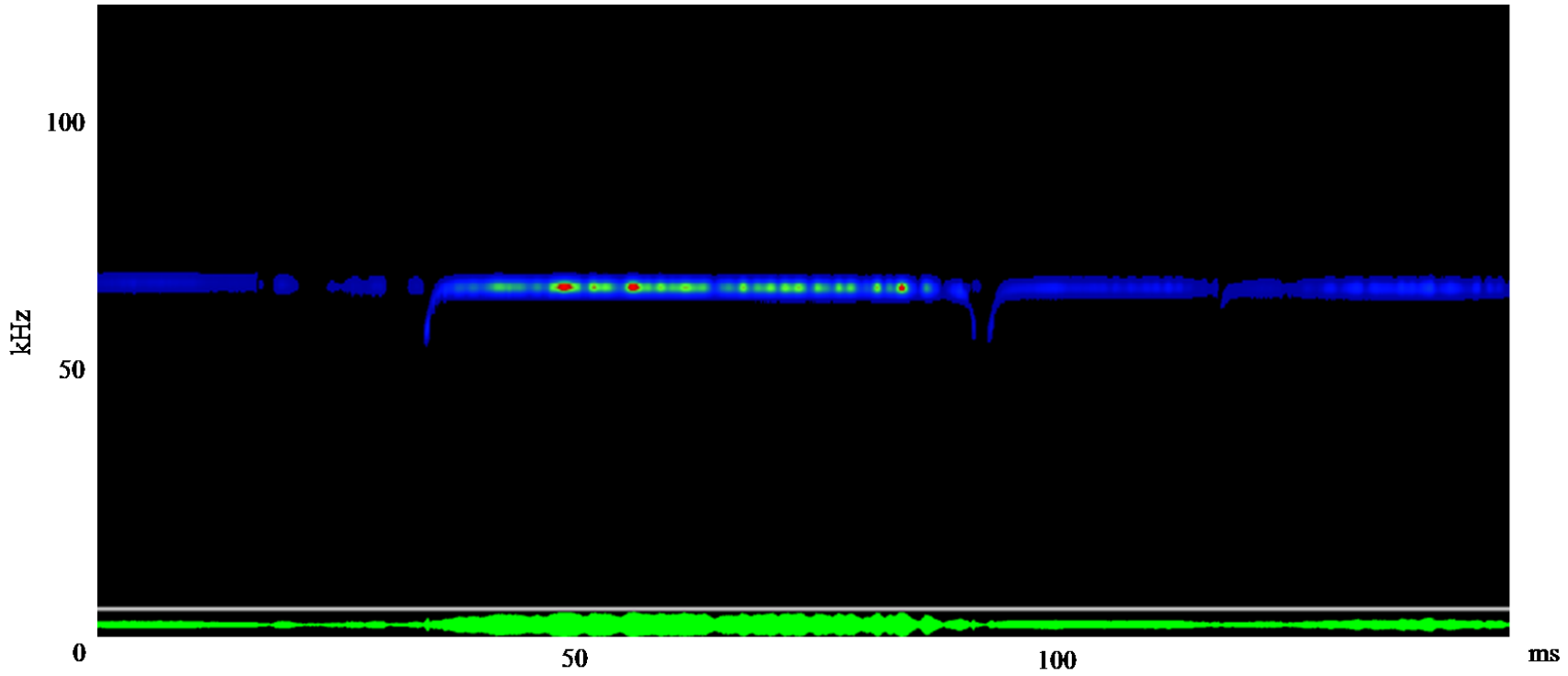


Fig. 16a. Call from Longxi Hongkou (11 Aug. 2013). This was visually matched to call types in Fig. 11b and 11c.

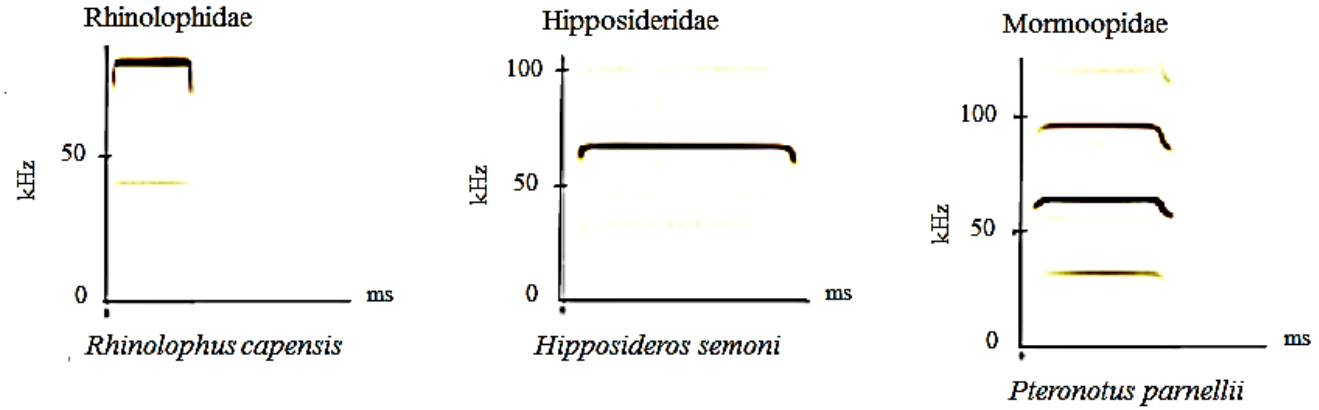


Fig. 16b. Call type 12 from Collen (2012), matched to the call from Fig. 11a. Described as "FM up-sweep, mid-length to long CF, FM down-sweep" (Collen 2012).

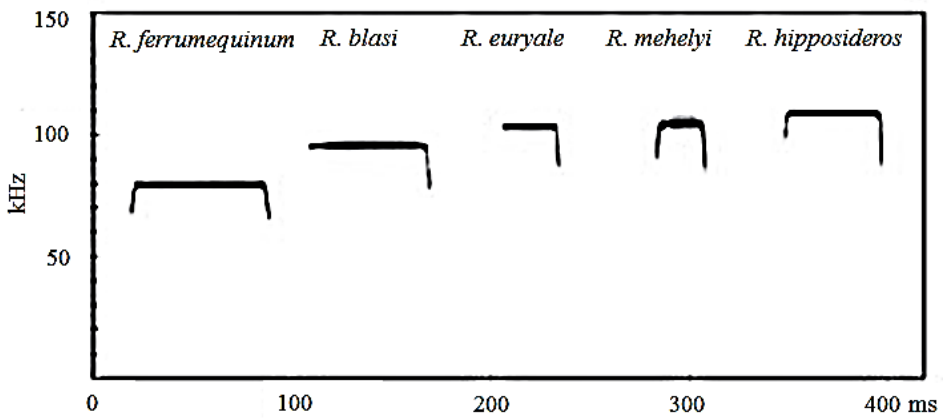


Fig. 16c. Call type 1 from Walters et al. (2012), matched to the call from Fig. 11a.

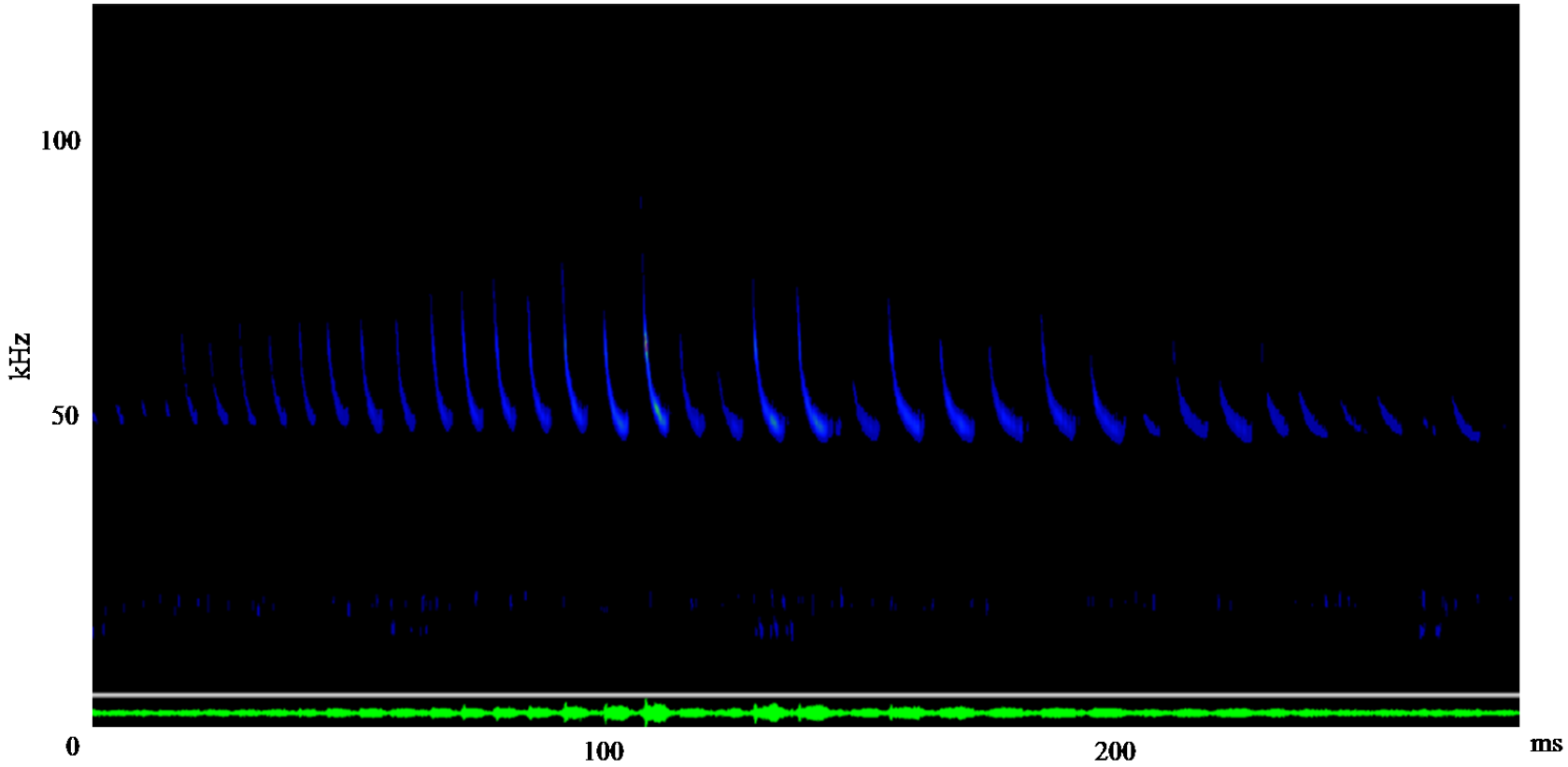


Fig. 17a. Call from An Long organic farm (7 Aug. 2012). This was visually matched to call types in Fig. 12b and 12c.

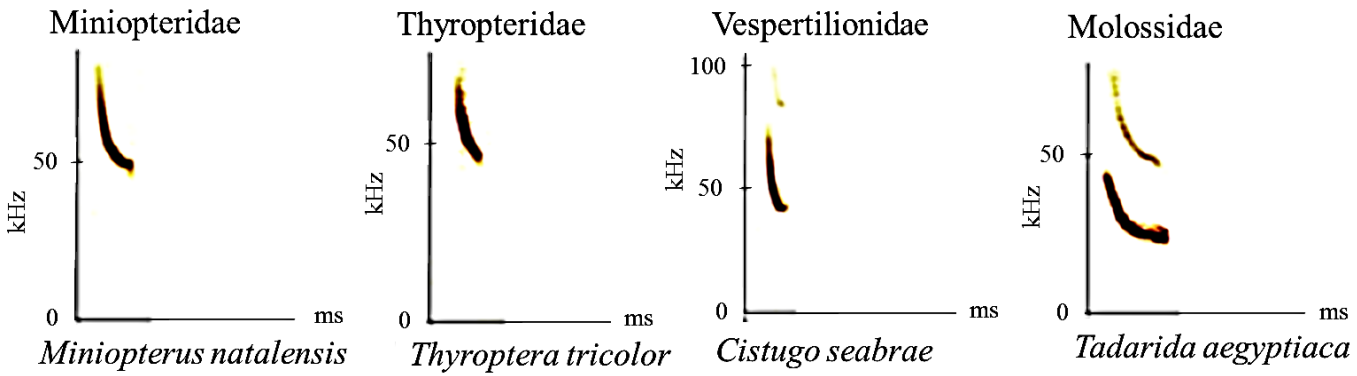


Fig. 17b. Call type 8 from Collen (2012), matched to the call from Fig. 12a. Described as "short, broadband, dominated by a single harmonic, mostly FM, but short narrowband ending" (Collen 2012).

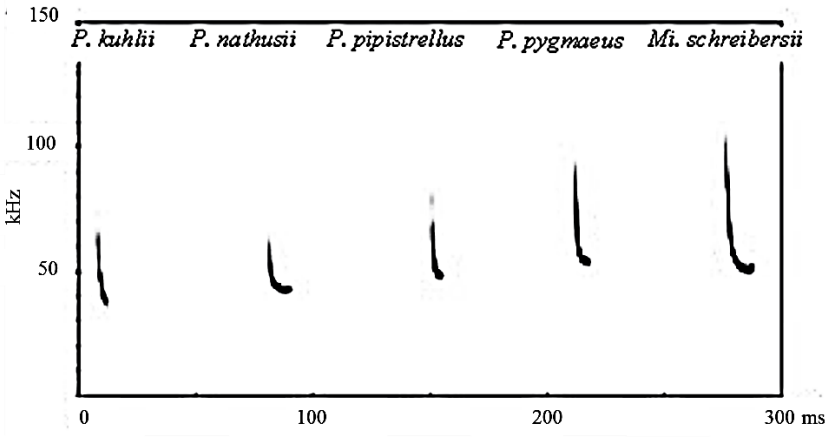


Fig. 17c. Call type 2 from Walters et al. (2012), matched to the call from Fig. 12a.

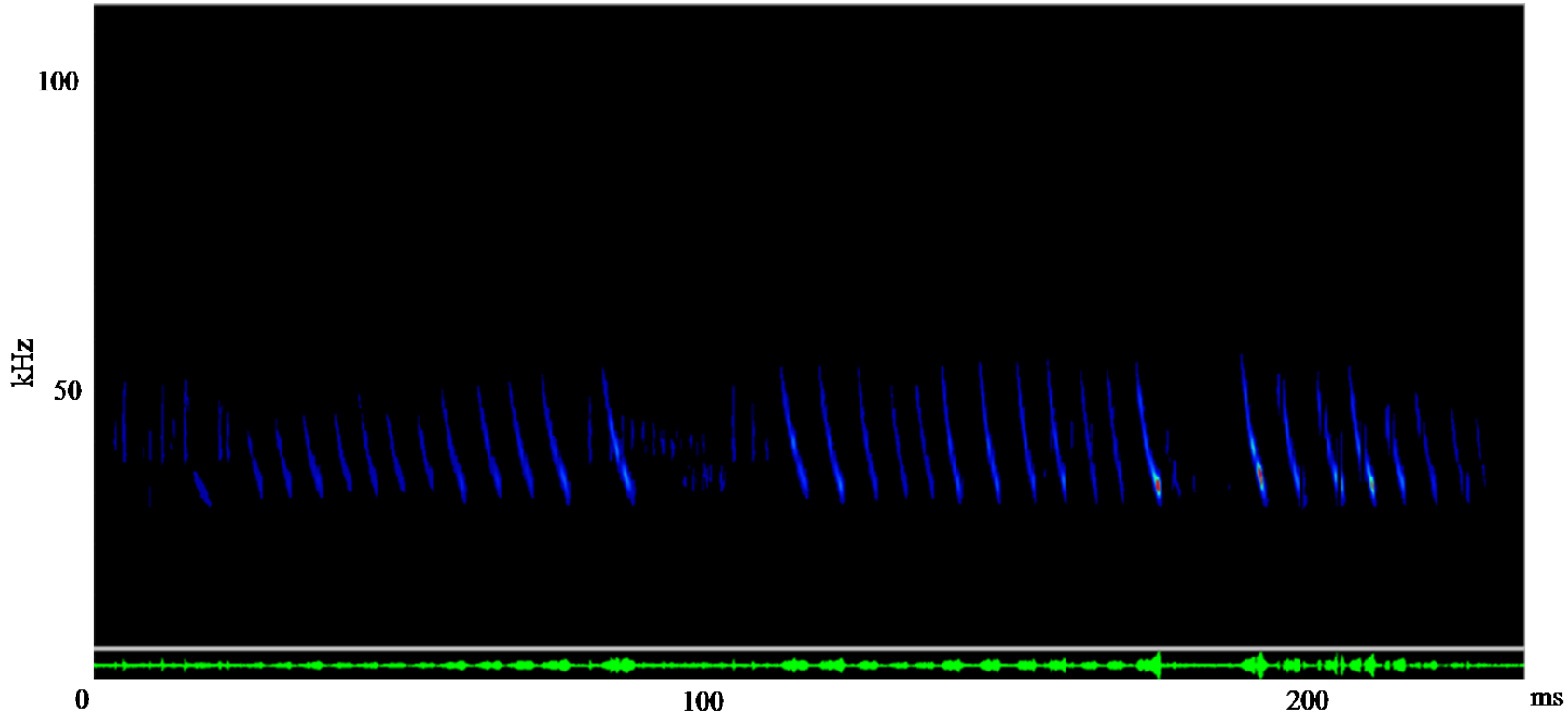


Fig. 18a. Call from Moon Bear Rescue Centre (3 Aug. 2012). This was visually matched to call types in Fig. 13b and 13c.

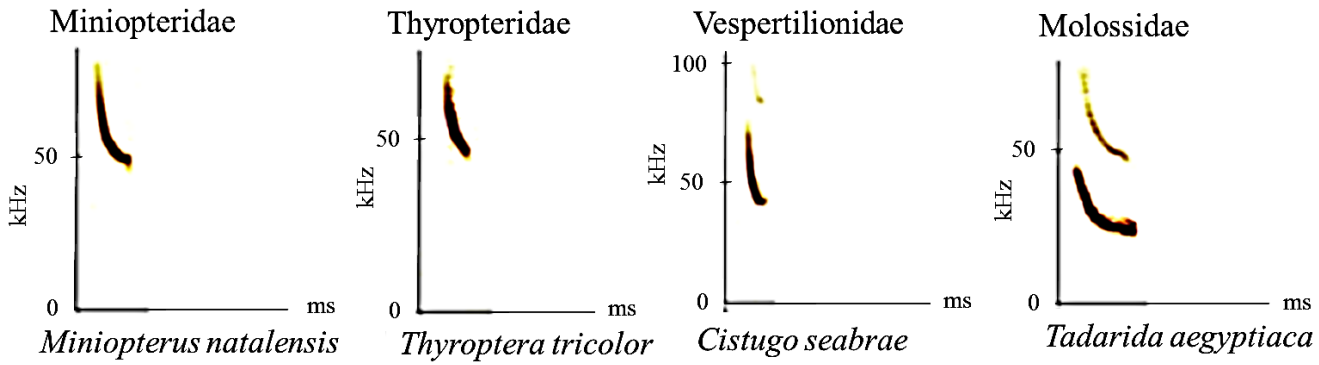


Fig. 18b. Call type 8 from Collen (2012), matched to the call from Fig. 13a. Described as "short, broadband, dominated by a single harmonic, mostly FM, but short narrowband ending" (Collen 2012).

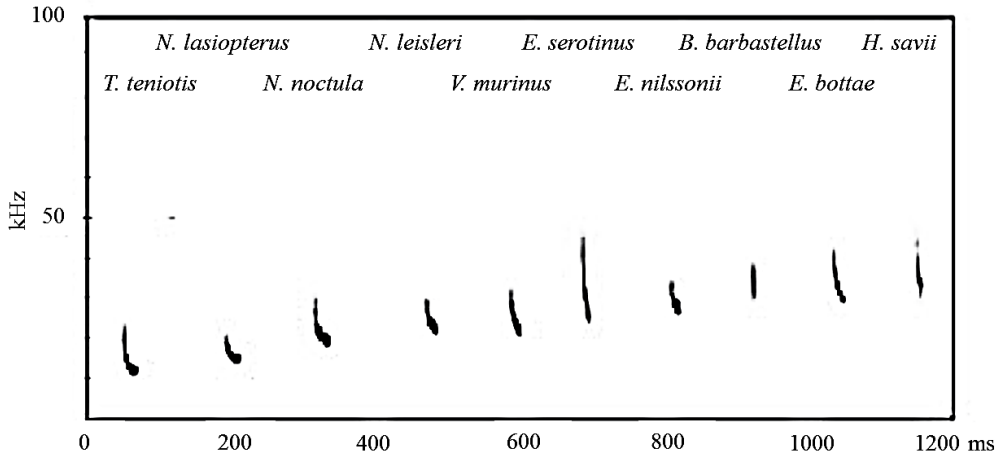


Fig. 18c. Call type 3 from Walters et al. (2012), matched to the call from Fig. 13a.

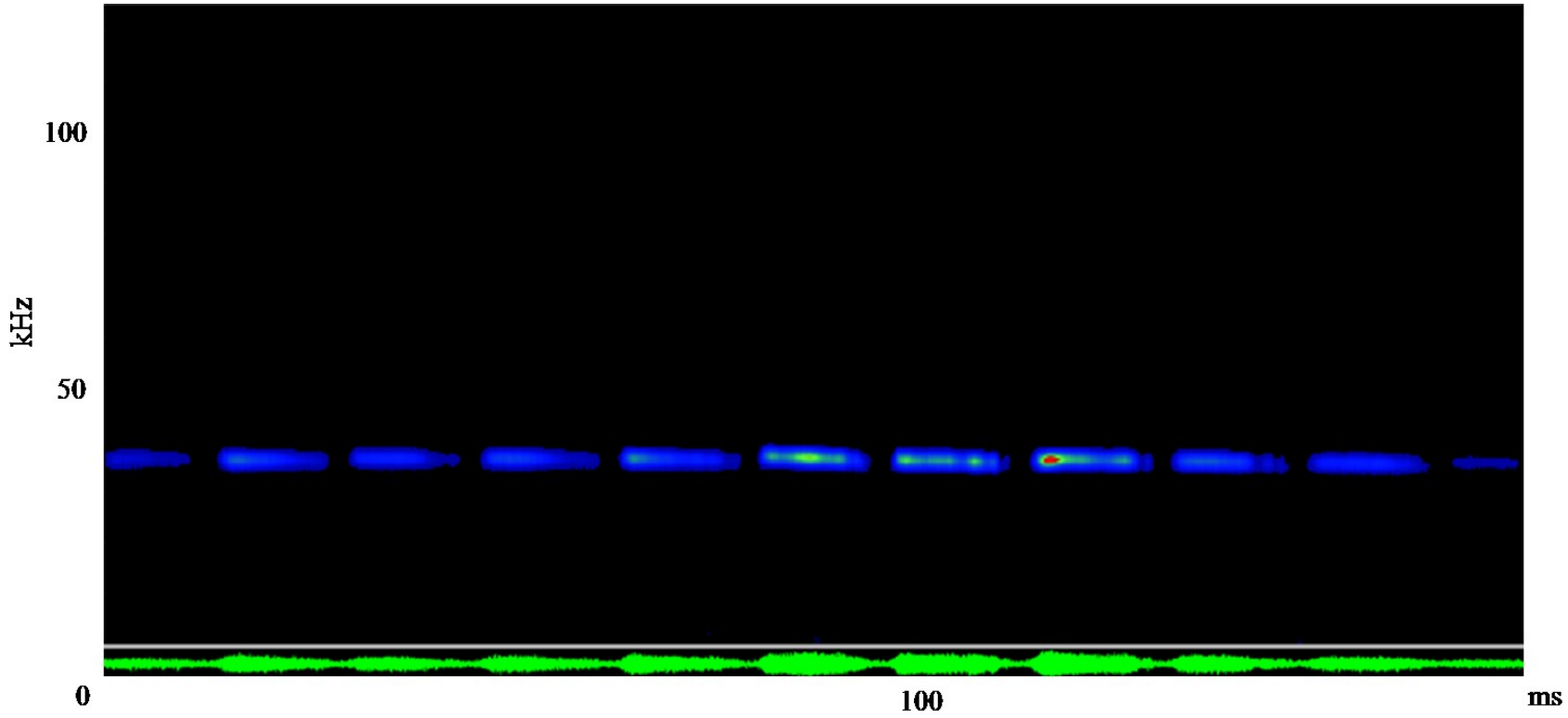


Fig. 19a. Call from Longxi Hongkou (11 Aug. 2012). This was visually matched to call types in Fig. 14b and 14c.

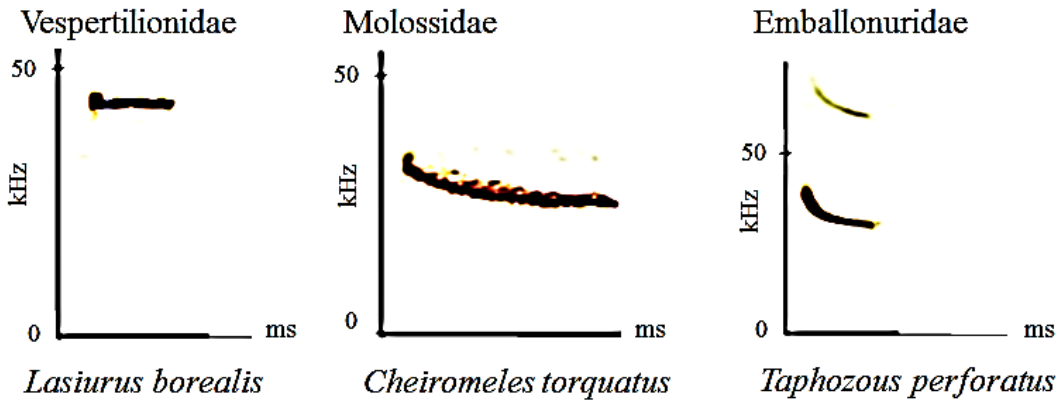


Fig. 19b. Call type 3 from Collen (2012), matched to the call from Fig. 14a. Described as "narrowband, dominated by a single harmonic" (Collen 2012).

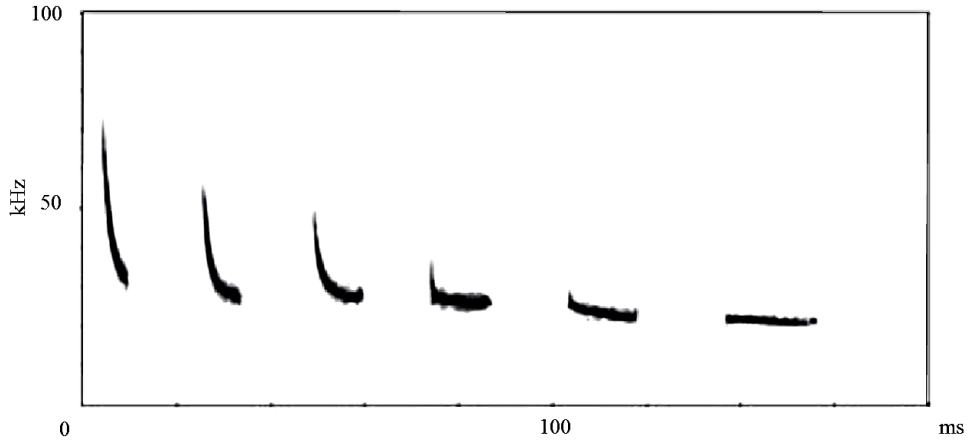


Fig. 19c. Call type 3 variant from Walters et al. (2012), matched to the call from Fig. 14a. (Example is all *Nyctalus leisleri.*)

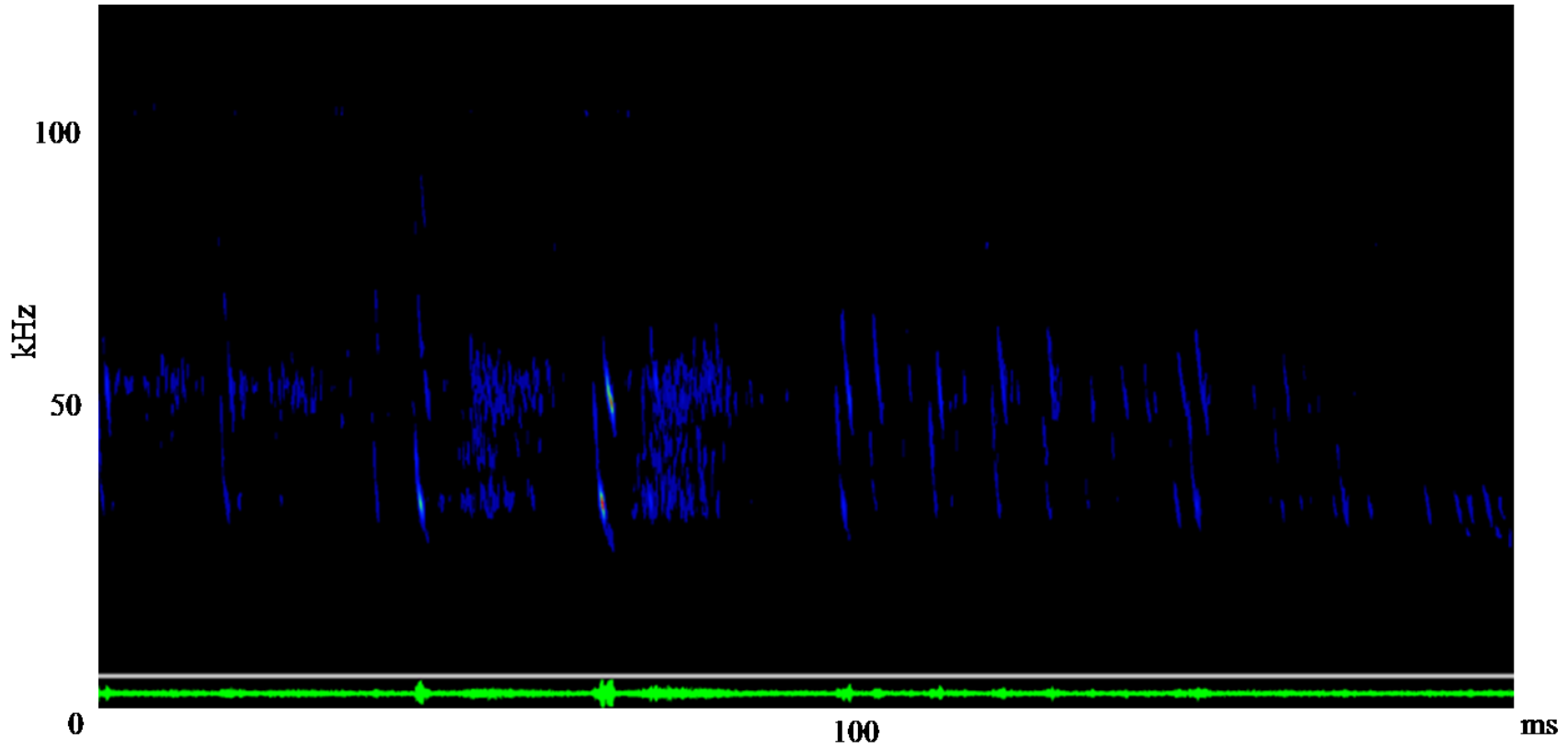


Fig. 20a. Call from Longxi Hongkou (30 July 2012). This was visually matched to call types in Fig. 15b and 15c.

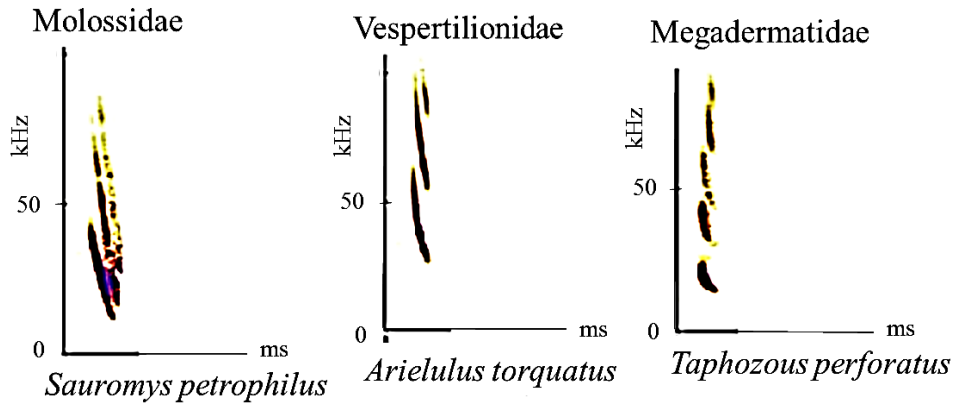


Fig. 20b. Call type 10 from Collen (2012), matched to the call from Fig. 15a. Described as "very short, broadband, multiharmonic, peak harmonic is the fundamental" (Collen 2012).

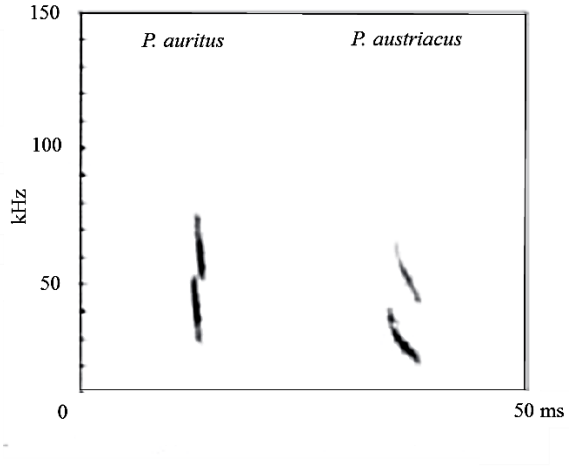


Fig. 20c. Call type 4 from Walters et al. (2012), matched to the call from Fig. 15a.

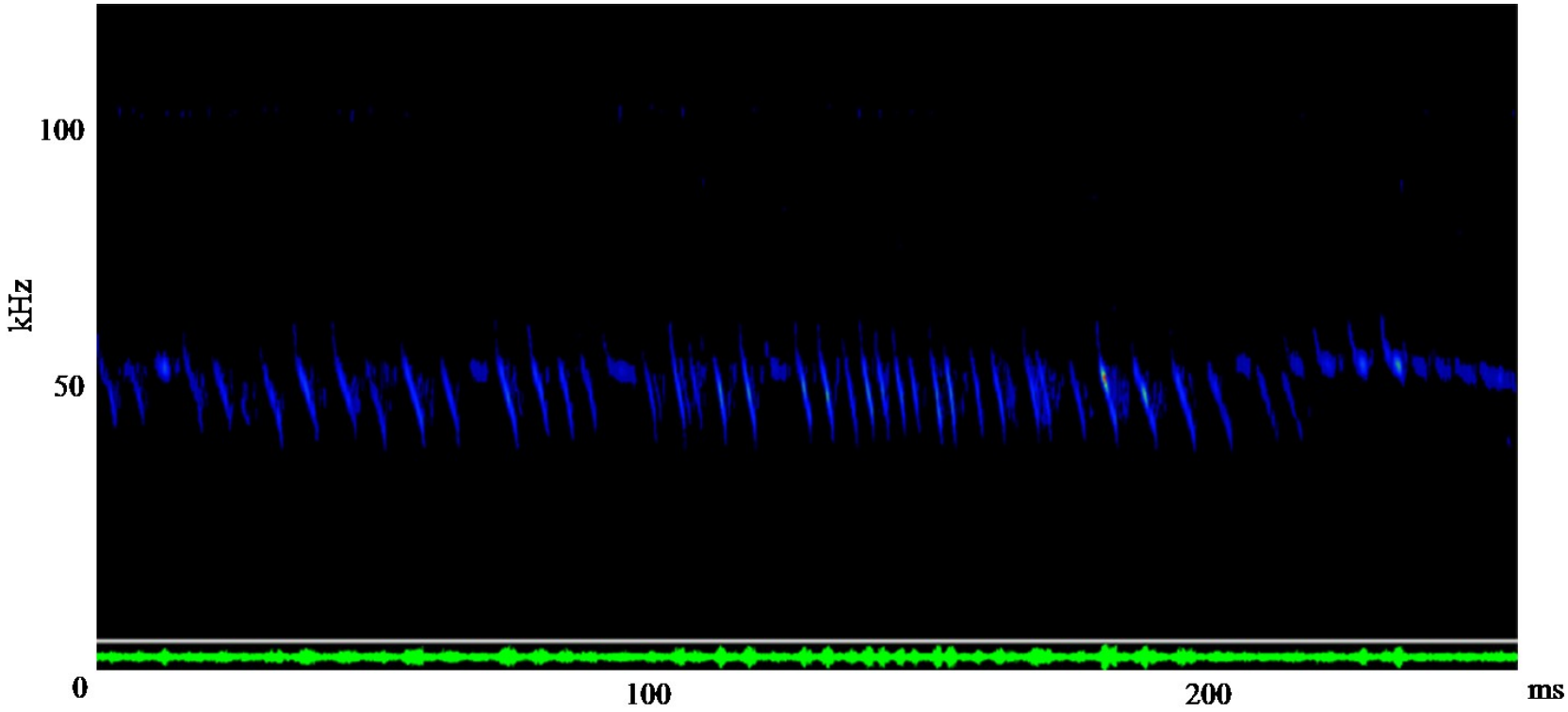


Fig. 21a. Call from Chengdu Panda Base (24 June 2013). This was visually matched to call types in Fig. 16b and 16c.

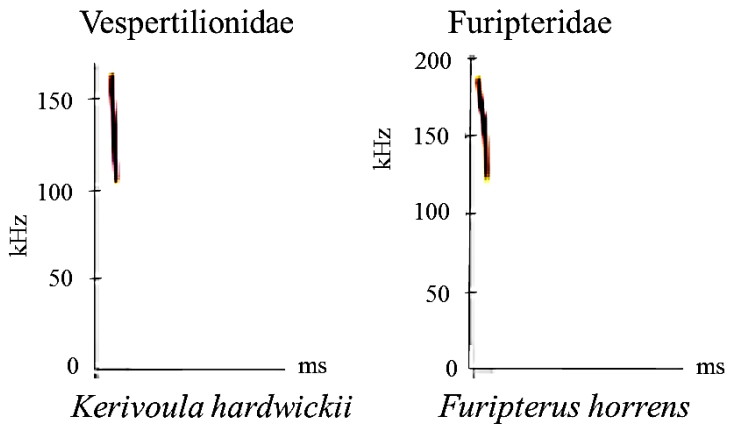


Fig. 21b. Call type 7 from Collen (2012), matched to the call from Fig. 16a. Described as "very short, broadband, dominated by a single harmonic" (Collen 2012).

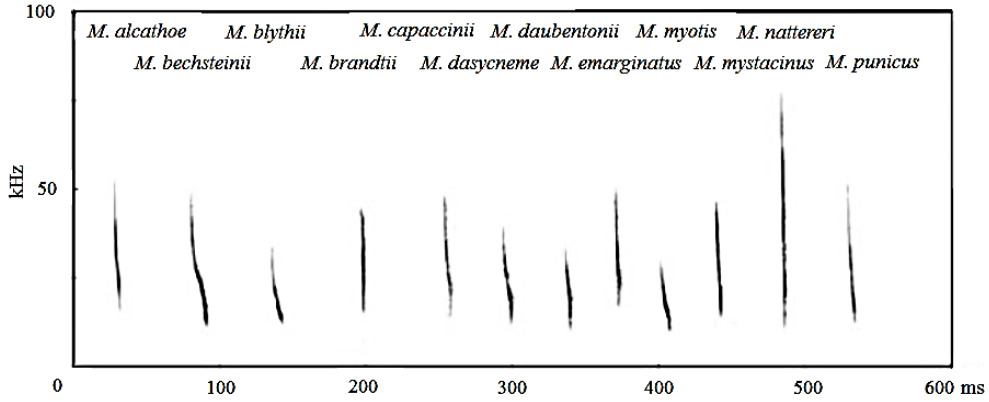


Fig. 21c. Call type 5 from Walters et al. (2012), matched to the call from Fig. 16a.

Table 6. Phonic types detected according to criteria from Walters et al. 2012.

Site	2012		2013	
	# of Call Types	Descriptions[a]	# of Call Types	Descriptions[a]
An Long	2	2, 3	3	2, 3
Bear Centre	3	2, 3, 5	-	-
Jinjiang River	3	2, 3, 5	-	-
Jiulidi Park	3	2, 3, 5	-	-
LXHK	5	1, 2, 3, 4, 5	-	-
Panda Base	2	2, 3	3	2, 3, 5
Sichuan Univ.	1	2	2	2, 3
SW Jiaotong	2	2, 3	-	-

[a]See Figs. 16-21.

Table 7. Phonic types detected according to criteria from Collen 2012.

Site	2012		2013	
	# of Call Types	Descriptions[a]	# of Call Types	Descriptions[a]
An Long	1	8	2	7, 8
Bear Centre	2	7, 8	-	-
Jinjiang River	2	7, 8	-	-
Jiulidi Park	2	7, 8	-	-
LXHK	4	3, 7, 8, 12	-	-
Panda Base	1	8	2	7, 8
Sichuan Univ.	1	8	1	8
SW Jiaotong	1	8	-	-

[a]See Figs. 16-21.

Discussion

Fur Mercury Concentrations

Mercury levels were detectable for all individuals sampled. The overall mean for *P. abramus* (10.74 ppm) was at the high end of values from reference areas in the U.S. and Canada for similarly sized bats (Table 8). For instance, the mean concentration from 149 *Myotis lucifugus* sampled in Novia Scotia, Canada was 9.33 mg/kg (Little et al. 2015), while means from *Perimyotis subflavus* and *Myotis austroriparius* were just over 6 ppm in South Carolina (Roach and Rumbold 2016). *Nyctalus plancyi*, on the other hand, exhibited concentrations (mean = 1.70 ppm) at the low end of reference values of similarly sized species from other parts of the world. For example, *Lasiurus intermedius* from uncontaminated sites in South Carolina had a mean value of 7.14 ppm, and *Eptesicus fuscus* from the same area had a mean of 15.4 ppm (Roach and Rumbold 2016). While the maximum concentration found in Chengdu (32.87 ppm) was substantially higher than these values, much higher concentrations have been reported from contaminated sites elsewhere (up to 708 ppm in *M. lucifugus* in Virginia (Karouna-Renier et al. 2014)).

Adult *N. plancyi* had significantly higher fur mercury concentrations than juveniles ($p < 0.001$), supporting the occurrence of metal accumulation with age. This adds to a large literature demonstrating this phenomenon in bats (including Pikula et al. 2010; Osborne et al. 2011; Karouna-Renier et al. 2014; and Yates et al. 2014).

Table 8. Concentrations of mercury in bat fur reported in the literature, ordered by species weight category[a]. This list is not exhaustive but provides a spectrum of concentrations from different geographic locations with various degrees of contamination. Weights and Hg concentrations include both male and female adults and juveniles, but no neonates, except where noted. Only references that provided values for individual species were included. NR = not reported.

Species	Weight Category	Sampling Location	Year	Contaminated?	n	Mean[b] Hg Concentration (mg/kg)	Hg range (mg/kg)	Source
Myotis leibii	1	New York	2008	Unknown	2	11.62	7.54 - 15.70	Scoch et al. 2010
		Ontario and Quebec, Canada	1998	Unknown	2	5.30	0 - 76.20	Hickey et al. 2001
Perimyotis subflavus	1	South Carolina	2015	No	14	6.35	1.12 - 12.20	Roach and Rumbold 2016
		Northeastern U.S.	2005-2009	Both	29	0.74[f]	NR - 2.75	Yates et al. 2014
Pipistrellus abramus	**1**	**Chengdu, China**	**2013**	**Unknown**	**14**	**10.74**	**4.56 - 32.87**	**Present study**
Myotis sodalis	1	New York	2008	Unknown	1 - 11[g]	10.04 - 16.48[g]	3.28 - 18.30[g]	Scoch et al. 2010
Rhinolophus cornutus cornutus	1	Japan	1890	No	6	2.63	NR	Miura et al. 1978
		Japan	1967, 1975	Yes	30	4.94[c]	NR	Miura et al. 1978
Myotis austroriparius	1	South Carolina	2015	No	10	6.60	0.33 - 15.00	Roach and Rumbold 2016
		Japan	1965	Yes	2	33	NR	Miura et al. 1978
Myotis lucifugus	1	Northeastern U.S.	2005-2009	Both	410	0.28[f]	NR - 3.76	Yates et al. 2014
		Virginia	2008	Yes	111[d]	118.4[d, f]	2.0 - 707.6[d]	Karouna-Renier et al. 2014

[a]Categories: 1 = 4-10 g, 2 = 10.1-20 g, 3 = 20.1-30 g. Standard weights were obtained from the PanTHERIA database (K. Jones et al. 2009), reported in Collen 2012. *M. leibii* – 5.22 g, *P. subflavus* (formerly *Pipistrellus subflavus*) – 5.74, *P. abramus* – 5.87, *M. sodalis* – 7.15, *R. cornutus* – 7.27, *M. austroriparius* – 7.35, *M. lucifugus* – 7.8, *N. humeralis* – 9.12, *L. seminolus* – 9.88, *M. grisescens* – 10.84, *L. borealis* – 12.33, *N. plancyi* – 15.15, *E. fuscus* – 17.49, *M. septentrionalis* – 18.28, *R. ferrumequinum* – 22.59, *L. intermedius* – 22.96, *L. cinereus* – 27.06.
[b]Geometric.
[c]Median. Mean not reported.
[d]Adults only.
[e]Juveniles only.
[f]Not stated whether mean is geometric or arithmetic.
[g]Results were from multiple sites reported individually.

Table 8, continued

Species	Weight Category	Sampling Location	Year	Contaminated?	n	Mean[b] Hg Concentration (mg/kg)	Hg range (mg/kg)	Source
		Virginia	2008	No	108[d]	3.3[d, f]	0.6 - 14.9[d]	Karouna-Renier et al. 2014
		Virginia	2008	Yes	29[e]	14.5[e, f]	1.2 - 152. 8[e]	Karouna-Renier et al. 2014
		Virginia	2008	No	35[e]	1.5[e, f]	0.3 - 13.5[e]	Karouna-Renier et al. 2014
		Virginia	2008	Yes	15	132	7.30 - 274	Nam et al. 2012
		Virginia	2008	No	11	3.09	1.39 - 5.50	Nam et al. 2012
		New York	2008	Unknown	2 - 19[g]	2.68 - 14.9[g]	0.94 - 3.65[g]	Scoch et al. 2010
		Nova Scotia, Canada	2001-2004, 2009-2012	Unknown	14 - 15[g]	3.76 - 27.22[g]	0.81 - 46.96[g]	Little et al. 2015
		Ontario and Quebec, Canada	1998	Unknown	39	1.5	1.30 - 2.50	Hickey et al. 2001
Nycticeius humeralis	1	South Carolina	2015	No	24	11.65	1.12 - 28.59	Roach and Rumbold 2016
Lasiurus seminolus	1	South Carolina	2015	No	116	5.04	1.53 - 34.83	Roach and Rumbold 2016
Myotis grisescens	2	Northeastern U.S.	2005-2009	Both	7	0.12[f]	NR - 0.46	Yates et al. 2014
Lasiurus borealis	2	South Carolina	2015	No	4	3.07	2.19 - 3.55	Roach and Rumbold 2016

[a]Categories: 1 = 4-10 g, 2 = 10.1-20 g, 3 = 20.1-30 g. Standard weights were obtained from the PanTHERIA database (K. Jones et al. 2009), reported in Collen 2012. *M. leibii* – 5.22 g, *P. subflavus* (formerly *Pipistrellus subflavus*) – 5.74, *P. abramus* – 5.87, *M. sodalis* – 7.15, *R. cornutus* – 7.27, *M. austroriparius* – 7.35, *M. lucifugus* – 7.8, *N. humeralis* – 9.12, *L. seminolus* – 9.88, *M. grisescens* – 10.84, *L. borealis* – 12.33, *N. plancyi* – 15.15, *E. fuscus* – 17.49, *M. septentrionalis* – 18.28, *R. ferrumequinum* – 22.59, *L. intermedius* – 22.96, *L. cinereus* – 27.06.
[b]Geometric.
[c]Median. Mean not reported.
[d]Adults only.
[e]Juveniles only.
[f]Not stated whether mean is geometric or arithmetic.
[g]Results were from multiple sites reported individually.

Table 8, continued

Species	Weight Category	Sampling Location	Year	Contaminated?	n	Mean[b] Hg Concentration (mg/kg)	Hg range (mg/kg)	Source
		Northeastern U.S.	2005-2009	Both	20	0.05[f]	NR - 0.22	Yates et al. 2014
		New York	2008	Unknown	1	5.30	NA	Scoch et al. 2010
Nyctalus plancyi	**2**				**30**	**1.70**	**0.38 - 6.90**	**Present study**
Eptesicus fuscus	2	South Carolina	2015	No	40	15.94	3.74 - 78.00	Roach and Rumbold 2016
		New York	2008	Unknown	1 - 7[g]	3.87 - 28.82[g]	1.98 - 42.97[g]	Scoch et al. 2010
		Virginia	2007	Yes	14	28.01	4.8 - 65.4	Wada et al. 2010
		Virginia	2007	No	15	10.94	~5 - ~13	Wada et al. 2010
		Northeastern U.S.	2005-2009	Both	127	0.10[f]	NR - 0.89	Yates et al. 2014
		Ontario and Quebec, Canada	1998	Unknown	3	1.5	0 - 15.40	Hickey et al. 2001
Myotis septentrionalis	2	Northeastern U.S.	2005-2009	Both	82	0.60[f]	NR - 3.70	Yates et al. 2014
		New York	2008	Unknown	1 - 9[g]	2.28 - 22.10[g]	2.28 - 41.53[g]	Scoch et al. 2010
		Ontario and Quebec, Canada	1998	Unknown	5	4.4	0 - 10.20	Hickey et al. 2001
Rhinolophus ferrumequinum nippon	3	Japan	1967, 1971	Yes	19	6.17[c]	NR	Miura et al. 1978
Lasiurus intermedius	3	South Carolina	2015	No	3	7.14	3.10 - 14.26	Roach and Rumbold 2016
Lasiurus cinereus	3	Northeastern U.S.	2005-2009	Both	6	0.02[f]	NR - 0.03	Yates et al. 2014
		New York	2008	Unknown	1	1.63	NA	Schoch et al. 2010

[a]Categories: 1 = 4-10 g, 2 = 10.1-20 g, 3 = 20.1-30 g. Standard weights were obtained from the PanTHERIA database (K. Jones et al. 2009), reported in Collen 2012. *M. leibii* – 5.22 g, *P. subflavus* (formerly *Pipistrellus subflavus*) – 5.74, *P. abramus* – 5.87, *M. sodalis* – 7.15, *R. cornutus* – 7.27, *M. austroriparius* – 7.35, *M. lucifugus* – 7.8, *N. humeralis* – 9.12, *L. seminolus* – 9.88, *M. grisescens* – 10.84, *L. borealis* – 12.33, *N. plancyi* – 15.15, *E. fuscus* – 17.49, *M. septentrionalis* – 18.28, *R. ferrumequinum* – 22.59, *L. intermedius* – 22.96, *L. cinereus* – 27.06.
[b]Geometric.
[c]Median. Mean not reported.
[d]Adults only.
[e]Juveniles only.
[f]Not stated whether mean is geometric or arithmetic.
[g]Results were from multiple sites reported individually.

The levels of Hg uptake found in the present study have not been viewed by many authors as cause for concern at the population level. Others, however, recognize that more subtle effects could exist, particularly with long-term exposure. In one of the few studies conducted directly on bats, Wada et al. (2010) saw no difference in adrenocortical response or glucocorticoid levels, biomarkers indicative of stress, in female *Eptesicus fuscus* with fur mercury levels as high as 28 ppm. Nam et al. (2012), however, found that *Myotis lucifugus* with brain concentrations greater than 1-5 ppm (dw) (corresponding to fur Hg concentrations >10 ppm) had altered monoamine oxidase (MAO) and acetylcholinesterase (ChE) activity, which play important roles in learning and memory, cognition, motor function, and thermoregulation. Bats from the Hg-contaminated site exhibited an increase in MAO and ChE activity with increasing Hg levels while bats from the reference site showed a more expected decrease in activity with increasing contamination. Thus, because bats from the reference site also seemed more capable of detoxifying mercury from the brain and liver, the authors speculated that the non-reference bats could have been suffering reduced homeostatic control.

The results of Wada et al. (2010) and Nam et al. (2012) are not necessarily contradictory. For one, the Wada et al. (2010) study was conducted on a substantially larger bat than that in the Nam et al. (2012) study, which should affect threshold interpretation. Wada et al. (2010) also tested wild-caught bats using repeated measure assays for cortisol and glucocorticoid response, which rely on the short-term induction of stress following capture. This is perhaps a very different stimulus than that induced by the chronic stress of metal exposure. Hormonal levels also may not necessarily reflect differences in behavioral response that rely on cognitive processing. Burton et al. (1977),

in point, found that white-footed mice (*Peromyscus maniculatus*) exhibited lower stress tolerance and motor function, measured by behavioral assays, above a fur Hg concentration of 10 ppm.

Based on the above literature, some bats from the present study are at risk for sublethal effects. Fifty-seven percent (8/14) of the *P. abramus* tested in Chengdu had fur Hg concentrations above 10 ppm. Even so, I expected fur mercury concentrations to be much higher given the levels of environmental pollution reported for Chengdu and China in general. These results can be explained by the netting locations, which occurred over running streams and maintained ponds and lakes. Methylmercury comprises the bulk of mercury found in bats and other mammals, with diet being the primary source of exposure (Driscoll et al. 2013; Yates et al. 2014). It is also the most toxic, bioaccumulative form of mercury, produced by bacteria that thrive under anoxic conditions in stagnant waters and undrained soils (Driscoll et al. 2013). Waters in an urban setting are more likely to be kept fresh for aesthetics and to minimize mosquito propagation, so although atmospheric levels of mercury may be high, environmental conditions upon deposition may not be suitable for conversion of mercury to the methylated form. Thus, even if urban bats are in the vicinity of an industrial point source, their risk of uptake may be more moderate if they reside in upland habitats with oxygenated waters and well-drained soils.

While there is evidence that the *P. abramus* in this study are undergoing sublethal effects (based on fur mercury values above the threshold associated with homeostatic changes in little brown bats (Nam et al. 2012) and reduced motor function in white-footed mice (Burton et al. 1977)), the degree to which these translate into population-

level effects is more difficult to evaluate. Reductions in motor control may have significant impacts on volant animals (De Francisco et al. 2003; Sutton and Hariono 1987), which rely on fine movements to quickly navigate their environment. Using a flight cage assay, Eguren (2014) found that *Lasiurus borealis* captured from Tar Creek Superfund Site (Oklahoma)—an area contaminated with lead, zinc, and cadmium—hit more obstacles than *L. borealis* from a reference site (although results were not statistically significant). With discoordination, bats could have more difficulty navigating their environment, capturing prey, and avoiding predators, but this would be unobservable in a bat held in the hand. A compromised homeostatic response could also be harmful to bats as they undergo torpor and hibernation but similarly would be difficult to detect. Ultimately, more long-term, widespread assessments are required. While pollutants alone may not be responsible for outright mortality, they likely create a greater strain on populations potentially already compromised by disease and disturbance (Kannan et al. 2010).

Species-specific effects. *Pipistrellus abramus* had significantly higher fur mercury concentrations than *Nyctalus plancyi* ($p < 0.001$), and there were likely a number of factors playing into this, including species-specific physiology and life history. Body mass was perhaps the biggest factor. *P. abramus* (weighing 6.31 ± 1.16 g in this study) is less than half the size of *N. plancyi* (19.10 ± 2.91 g). According to Kleiber's rule (1932), smaller animals have a higher mass-specific metabolism, meaning that they must consume more calories per gram of weight. This greater rate of consumption would result in faster accumulation of diet-sourced toxins. This conclusion is supported by the

fact that Miura et al. (1978) found a similar pattern of *P. abramus* having substantially higher fur mercury concentrations than larger bat species in rural Japan in 1978.

A second possible cause of many *P. abramus* individuals having higher fur mercury concentrations is that their diet may have contained insects with a greater tendency to accumulate toxins. However, current knowledge of their and *N. plancyi*'s foraging preferences are not sufficient to draw conclusions. *P. abramus* is an aerial hawking insectivore, and dietary analysis shows it to be an opportunistic generalist (Lee and Lee 2005). In northern Taiwan, Coleopterans make up the greatest proportion of their diet year-round (23% of volume), followed by Dipterans (15%), Hymenopterans (14%), Trichopterans (13%), Lepidopterans (11%), and Hemipterans (10%) (Lee and Lee 2005). To my knowledge, the diet of *N. plancyi* has not been evaluated, although it similarly is an aerial hawker (Tian et al. 2015).

Given that *P. abramus* are accumulating greater contaminant concentrations, the question remains whether they are experiencing relatively greater health effects. Like other pipistrelles (e.g., *P. kuhlii*, *P. pipistrellus*, and *Hypsugo savii* in Europe) (Russo and Ancillotto 2015), *P. abramus* seems to have adjusted well to city dwelling. Members of the species are able to utilize buildings as roost sites, forage on a variety of insect sizes and taxa, and tolerate light (Funakoshi and Uchida 1978). Although urbanization can force some species to travel farther from roost sites to forage than they would in more natural landscapes (Geggie and Fenton 1985), Chengdu seems to have enough vegetated parks and open water, including the Fu River, to afford urban bats sufficient water and insects locally. The drawback of living in this environment, however, is increased risk of

exposure to pollutants from industrial manufacturing, household products, and automobile exhaust.

Body Condition

There was no relationship between body condition and fur mercury load for either *P. abramus* or *N. plancyi*. I attribute this to the fact that fur mercury loads, as discussed above, were likely not high enough to elicit acute health effects in either species, and that bats in the Chengdu are likely exposed to numerous other contaminants not accounted for in this study.

Alopecia. An interesting side note was the occurrence of alopecia in 21% percent of *P. abramus* (n = 14) and 7% percent of *N. plancyi* (n = 30) examined. Fur loss in wild mammals has been associated with stress, nutritional deficiencies, parasites, and hormone imbalance, particularly in reproductive females when resources are limited (Noxon 1995; Bello-Gutierrez et al. 2010; Tang et al. 2012). Five of six alopecic bats in the present study were adult females, and four out of five of these were reproductive. Pederson et al. (2009) noted that frugivorous species on Montserrat may have experienced losses as a result of zinc-deficiencies caused by ingestion of volcanic ash. Bello-Gutierrez et al. (2010) found a greater incidence of hair loss in females of four species of frugivorous bats in a study in Mexico (although some males exhibited it as well). In that study, bats captured in urban areas had a significantly higher incidence of alopecia than those captured in peri-urban areas, and fur loss was most prevalent in the dry season, which coincided with the reproductive period and scarcer fruit availability (Bello-Gutierrez et al. 2010). Because no histopathological abnormalities were detected, the authors

attributed the difference to possible nutritional or hormonal issues associated with living in an urban environment (Bello-Gutierrez et al. 2010). Interestingly, those frugivorous bats experienced alopecia mostly on their chest and abdomen (Bello-Gutierrez et al. 2010), while bats in the present study only had bare patches on their backs and necks. These are areas that would not have been possible for a bat to groom on its own.

Bats in the Bello-Gutierrez et al. (2010) study also did not have any signs of tumors or inflammation, whereas two of six alopecic bats captured in Chengdu had visible growths in the centroid of the hair loss. The occurrence of these lesions points to fur loss possibly being caused by tumors (Rector et al. 2006), reactions to an insect or arthropod bite (Lollar and Schmidt-French 1998), or the result of dermal contact with an irritant (Lollar and Schmidt-French 1998). Mites and ticks were visible on many of the bats captured in China (Fig. 15c, Appendix D), although not just the ones with fur loss.

Guano Organochlorine Concentrations

Hexachlorobenzene, alpha chlordane, *p,p'*-DDE, *o,p'*-DDD, and *p,p'*-DDD were present in guano samples (Table 5) but well below levels associated with harm, in the ppb rather than in the ppm typically reported in other studies. Clark et al. (1995) found that organochlorine concentrations in the guano of Mexican free-tailed bats (*Tadarida brasiliensis*) from Mexican caves ranged from 0.13-0.99 ppm for *p,p'*-DDE and concluded that these levels were not of concern. DDE levels of 2.0–8.20 ppm were detected by Clark et al. (1988) in guano from a Missouri cave where gray bats (*Myotis grisescens*) were found dead and dying. In that study, because DDT, the parent compound of DDE and DDD, was not detected, the authors concluded that bats had not

been exposed to recent applications of the pesticide. A similar conclusion can be drawn regarding the bats from from Chengdu. Levels of *p,p*'-DDE also were well below those reported by Land (2001) (range: 24.5-240 ppb), who concluded that this compound was likely not a threat to populations sampled from Fort Hood, Texas.

Thus, *P. abramus* in Nanchong, to the east of Chengdu, are likely not at risk from exposure to organochlorines. The picture, however, is incomplete for *P. abramus* in central Chengdu and outlying agricultural areas, and that for *N. plancyi* could not be evaluated.

Contaminants and Land Use Type

Contrary to my hypothesis, there were no significant differences between fur mercury concentrations from the city center (Sichuan University) and those from the outskirts (Chengdu Panda Base) (Table 4). Sample sizes from the agricultural area (An Long farm) were not sufficient to compare to the other site types. However, there was an apparent trend with the highest mean mercury concentration for adult *P. abramus* recorded at the farm (32.87 ppm), the second-highest at the city center (Sichuan University, 13.57 ppm), and the lowest at the urban edge (Panda Base, 9.73 ppm) (Table 4). The concentration from the agricultural area is similar to values for *P. abramus* (33 ppm) detected by Miura et al. (1978) in Japan, where organomercurial fungicides were being applied. While these fungicides have been banned in Japan since 1968 (Miura et al. 1978), China has not implemented such restrictions and continues to be one of the largest miners of mercury in the world (Michael 2016). Although An Long has been farmed organically in more recent years, the land could have been subject to pesticide

applications in the past, as well as present-day drift from non-organic farms nearby. This does not negate the conclusion that bats in more central urban areas have a greater risk of mercury exposure than those in less densely populated urban areas. Rather, the picture of bat exposure across the landscape is complicated by a variety of current and historical factors.

There was not enough information to evaluate the relationship between land use type and organochlorine residues.

Contaminants and Phonic Diversity

The mountain site of Longxi Hongkou had the greatest number of phonic types (5 according to Walters et al., and 4 according to Collen) (Tables 6 and 7), with two calls, possibly a Rhinolophid and *Vespertilio sinensis*, not detected elsewhere. Adams and Bexell (2011) detected Rhinolophids at the Fu River in 2011, so it is possible that species of this family were merely missed at my urban and peri-urban locations. Unfortunately, tissue samples could not be collected from Longxi Hongkou to compare contaminant concentrations with other sites, so the potential role of pollutants in driving species richness differences could not be evaluated.

Regardless of pollution, urban areas have been known to harbor fewer bat species relative to natural areas, in large part due to habitat loss and fragmentation. Studies based on gene flow (Rossiter et al. 2012) and species richness and abundance in forest fragments (Struebig et al. 2008) support the idea that tree-roosting species are more susceptible to habitat loss and fragmentation than cave-roosters. In addition to the physical loss of roost sites, tree-roosting species experience disadvantages in terms of

lower mobility (Threlfall et al. 2011). While morphologically adapted to negotiate clutter (low wing loading, low aspect ratio), tree-roosting species are less equipped for long-distance flight (which requires high loading and a high aspect ratio) (Norberg and Rayner 1987; Kingston et al. 2003). In line with this, Avila-Flores and Fenton (2005), found bat species richness to be greater in natural areas relative to urban sites in Mexico, with vespertilionids (*Eptesicus fuscus* and *Myotis* species) and *Eumops perotis* (Molossidae) mostly inhabiting large parks and natural forest rather than built environments. *Nyctinomops macrotis* and *Tadarida brasiliensis* (both larger Molossids), occupied urban borders and central urban areas, respectively (Avila-Flores and Fenton 2005). Similarly, Threlfall et al. (2011) found that "open-adapted" bats were associated with areas of greater housing density, while "clutter-adapted" bats were associated with greater amounts of forest in Sydney, Australia.

Phonic diversity did not differ greatly between high density urban, low density urban, and agricultural sites. One distinction was the lack of phonic type 5 (Collen 2012), associated with some members of the *Myotis* genus, at An Long (agricultural), Sichuan University (urban), and Southwest Jiaotong University (urban). Relatively less sampling was conducted at An Long and Southwest Jiaotong, so it is possible that this phonic type could be detected with more recordings.

Limitations

Insufficient sample size was the single biggest obstacle to drawing larger conclusions from this study. Unfortunately, this is the norm rather than the exception for bat contaminant studies (e.g., Bayat et al. 2014; Zukal et al. 2015).

Another complication was that, particularly in an urban setting, environmental contamination is one of many factors that can affect bat species health. Habitat loss and modification, altered food and roost availability and quality, increased disturbance, and increased exposure to various pathogens combine to alter species success (Bradley and Altizer 2007; Bello-Gutiérrez et al. 2010). Moreover, comparing contaminant concentrations between species and across regions is not clear-cut because a variety of factors necessarily play into exposure, uptake, metabolism, and effects.

Thirdly, the phonic classifications I used were not robust enough to lead to strong conclusions regarding relative species diversity amongst site types. However, as stated previously, this was the best method available in the absence of a validated call reference library.

Despite these limitations, my call recordings and tissue contaminant concentrations serve as a unique reference for the region. In time, perhaps, the recordings can be further evaluated, with more refined call analysis technologies and greater public access to central Asian bat calls. Similarly, the fur mercury and guano organochlorine concentrations serve as a snapshot of bat contaminant exposure that should prove useful in the context of other regional samplings.

CHAPTER III

GENE FLOW AND GENETIC DIVERSITY OF THE JAPANESE PIPISTRELLE (*PIPISTRELLUS ABRAMUS*) IN CHENGDU, CHINA

Background and Significance

As members of the Order Chiroptera, bats share many evolutionary and ecological traits that make them susceptible to environmental contaminants (G. Jones et al. 2009). However, within this group of over 1,300 species (Fenton and Simmons 2014), inter- and intra-specific differences in contaminant exposure, uptake, and effect are expected due to variations in behavior, life history, environment, and genetic makeup (Streit et al. 1995; Frick et al. 2007; Hernout et al. 2015). In Chapter II, I presented a generalized picture of exposure to heavy metals and organochlorines in the Chengdu area by sampling multiple bat species. Because the Japanese pipistrelle (*Pipistrellus abramus*) was the most commonly encountered species and had the highest fur mercury concentrations, in this chapter I examined the species' genetic diversity and gene flow in Chengdu to better understand its local movements in relation to the urban environment. I also wanted to confirm that the individuals I sampled for contaminants were indeed *Pipistrellus abramus*, as opposed to a morphologically similar species.

Life History of *Pipistrellus abramus*

The Japanese pipistrelle (*Pipistrellus abramus*) is one of the most common and widespread bats in Asia, occurring in China, North and South Korea, Japan, Southeast Asia, and a small southeastern portion of Russia (IUCN 2015, Fig. 22). Its success is likely due, in part, to its ability to tolerate disturbance and roost in human-made structures (Bates and Tsytsulina 2008). As a generalist, *P. abramus* feeds opportunistically on a variety of insects throughout the year (Funakoshi and Uchida 1978; Lee and Lee 2005), and, like other pipistrelles, is known to forage around street lamps where some species of nocturnal insects aggregate (Blake et al. 1994; personal observation). *Pipistrellus abramus* is useful for regional comparisons of environmental contamination in urban landscapes because, aside from its ubiquity, it is relatively small and a year-round resident (Funakoshi and Uchida 1978). Weighing from about four to six grams (Bates and Harrison 1997), this bat consumes a large quantity of insects nightly to sustain its metabolic rate, thus acquiring pollutants quickly, if present (Clark 1981; Kurta et al. 1989). Females, in particular, have high roost fidelity throughout the year. In Japan, 25 separate colonies were found to roost and hibernate in their respective houses year-round, only occasionally emerging to forage during winter (Funakoshi and Uchida 1978). Females of the closely related *P. pygmaeus and P. pipistrellus* typically travel less than 2 km in one night to feed during the breeding season (Davidson-Watts and Jones 2006). Males, in contrast, tend to switch roosts regularly and often roost alone, although the distances they travel have not been well-studied (Funakoshi and Uchida 1978; Funakoshi et al. 2009). By mark-recapture, Funakoshi and Uchida (1978) found *P. abramus* to regularly move >5 km but not more than 10 km on a nightly basis, though

sex-specific differences were not mentioned. Thus, for adult females at least, it is reasonable to assume that tissue concentrations reflect exposure from a single, localized geographic area.

Fig. 22. Range of the Japanese pipistrelle (*Pipistrellus abramus*) (IUCN 2015).

Species Confirmation

My first aim was to confirm the identity of individuals sampled for tissue analysis because of the potential for morphological crypsis. When sampling bats, I minimized handling time and refrained from taking extensive morphometric measurements or noting dental characteristics. Yet species within the *Pipistrellus* genus can be difficult to distinguish from one another externally, and their taxonomy has often been the subject of revision (Hoofer and Van Den Bussche 2003). In Europe, for example, the common

pipistrelle (*Pipistrellus pipistrellus*) was recently split into two morphologically indistinguishable species based on differences in sonar calls, habitat use, and genetic uniqueness (Barlow and Jones 1997; Davidson-Watts and Jones 2006). Based on external characteristics alone, *P. abramus* individuals can appear very similar to *P. paterculus* and *P. javanicus*, with only minor differences in dentition and pelage color (Borissenko and Kruskop 2003). Because co-occurring species can accumulate contaminants differently (Chapter II), I thought it important to confirm species identity to justify regional comparisons.

Gene Flow and Population Diversity

My second objective was to assess phylogeographic history and genetic diversity of *Pipistrellus abramus* in and around Chengdu. Genetic variation in the Japanese pipistrelle has been studied in eastern China by Wei et al. (2010) and Dong et al. (2014). Wei et al. (2010) found evidence that historical and contemporary gene flow between continental China and offshore islands had been restricted by expanses of ocean currently ≥4 km wide. They also identified two mitochondrial clades, and Dong et al. (2014) further explored the role of natural selection in the clades' formation (discussed below). The present study concerns bats over a 240-km stretch from An Long organic farm in the west to Nanchong in the east. Nanchong is 45 km northwest of Yufeng, the western-most site sampled in previous studies. Widespread species, even highly mobile ones like bats, can differ in patterns of gene flow across their range (e.g., Rossiter et al. 2007). Genetic structure, in turn, can have conservation implications. Similar to the "insurance hypothesis" (Tilman 1996; Naeem and Li 1997) discussed for species richness in Chapter

II, a greater variety of genes provides more evolutionary options for a species to adapt to environmental changes. Genetic differences can also signify greater or lesser ability to cope with environmental stressors, including contaminants (Chatelain et al. 2016). Thus, I wanted to see whether patterns described for the populations in the eastern portion of the continent were upheld in a more western, localized portion of *P. abramus*' range.

Population structure—similarity in genetic signatures across individuals in an area, akin to spatial clustering—results from restricted gene flow, which can be due to a variety of factors such as geographic barriers, selective mating, colonization history, or a combination of these factors (Burland and Worthington Wilmer 2001). Across species, bats vary considerably in how much population structure they exhibit (reviewed in Burland and Worthington Wilmer 2001). Being relatively mobile and gregarious, they often have ample gene flow across their range, resulting in very little genetic structure (Burland and Worthington Wilmer 2001). This has been shown for both migratory (e.g., greater mouse-eared bat, *Myotis myotis* (Castella et al. 2000), common noctule, *Nyctalus noctula* (Petit and Mayer 2000), and Mexican free-tailed bat, *Tadarida brasiliensis* (McCracken et al. 1994)) and non-migratory species (common vampire bat, *Desmodus rotundus* (Honeycutt et al. 1981)) (reviewed in Burland and Worthington Wilmer 2001). Even relatively sedentary species can exhibit a continuum of gene flow across greater distances than any individual bat would travel (Burland et al. 1999). For example, both male and female brown long-eared bats (*Plecotus auritus*) stay within 2-3 km of their summer roost to forage, but little population structure is evident (Burland et al. 1999). This could either be due to mating at range edges or swarming behavior, where bats aggregate to mate at a single location prior to hibernation.

Hypotheses

H1 In-field identification of *P. abramus* individuals will be consistent with genetic species identification.

H2 *Pipistrellus abramus* populations will exhibit high levels of gene flow in and around Chengdu.

As a null hypothesis, all pipistrelles sampled were expected to have genotypes consistent with *Pipistrellus abramus* from other studies. I also predicted little population structure across Chengdu, in line with evidence from other parts of the species' range and the lack of any obvious geographic barrier to movement.

Methods

Sample Collection

The details of bat capture, including handling procedures and sampling locations, were the same as described under Methods in Chapter II. For each of 40 *P. abramus* individuals, a 3-mm biopsy of wing tissue was collected using a sterile punch. Tissues were stored in 95% ethanol in 1.5-mL plastic tubes at -25°C, then exported to the University of Northern Colorado.

Dioxyribonucleic Acid (DNA) Extraction

DNA was extracted from wing punches using a DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, U.S.A.), following the manufacturer's protocol. The only deviation from the protocol was incubating the wing punches in lysis buffer for up to two hours. (Following trial extraction, this method of chemical lysing to degrade wing tissue

was found to be comparable to the more labor-intensive use of liquid nitrogen and grinding with a mortar and pestle.)

Mitochondrial Analysis

In animals, mitochondrial DNA (mtDNA) is typically only passed down by the mother and so is useful for discerning population structure in species like the Japanese pipistrelle (Funakoshi et al. 2009) that have male-biased dispersal (Freeland et al. 2011; Fahey et al. 2014). Because mtDNA does not generally undergo recombination as does nuclear DNA, it tends to be well conserved through generations and is thus useful for detecting ancient patterns of gene flow (Allendorf and Luikart 2007). Cytochrome b (*cytb*) is a commonly used mitochondrial sequence in vertebrate studies.

Cytb regions were amplified for sequencing using polymerase chain reactions (PCR). For each wing biopsy sample, 1 µL of DNA was combined with 11.9 µL autoclaved MilliQ water, 4 µL Promega 5X Go Flexi buffer, 1 µL 25mM Promega stock $MgCl_2$, 1 µL 2.5 mM dNTP mix, 0.1 µL of Promega Go Flexi Taq polymerase, and 1 µL 10 mM of each forward and reverse primer (CY1 and CY2, obtained from Li et al. 2006 and Zhang et al. 2007, respectively), resulting in a total reaction volume of 20 µL. PCR was run using the following thermocycle profile: five minutes at 95ºC, followed by 30 cycles each of one minute at 95ºC, one minute at 52ºC, and 1 minute at 72ºC. After 30 cycles, samples were run an additional 30 minutes at 72ºC and held at 4ºC until collection. Amplified DNA quality was verified on an agarose gel with SYBR green fluorescent dye.

Following successful amplification, samples were cleaned using an Exo-Sap procedure. 5 µL of each PCR product was combined with 0.5 µL Exonuclease I and 1 µL FastAP Thermosensitive alkaline phosphate. The mixture was run on a thermocycler for 15 minutes at 37ºC, then 15 minutes at 87ºC, and held at 4ºC until collection. Samples were cycle-sequenced using 6.4 µL autoclaved MilliQ water, 0.33 µL BigDye v3.1 (Applied Biosystems Inc., Foster City, CA, USA), 2 µL 5X buffer, 0.8 µL cleaned PCR product, and 0.5 µL forward primer. Products were then run for one minute at 96ºC, followed by 30 cycles of 96ºC for 15 seconds, 50ºC for 20 seconds, and 60ºC for four minutes, and held at 4ºC until collection. Products were transferred to 96-well plates, dried down overnight, and sent to the Arizona State University molecular genetics lab for Sanger sequencing.

Once received, sequences were aligned in Geneious v.8.1.8 (http://www.geneious.com) (Kearse et al. 2012) using the Geneious algorithm. Based on nucleotide polymorphisms, or differences in base pair identity along the same regions of DNA, a phenetic tree was produced in Geneious Tree Builder and a posterior probability tree in Mr. Bayes v.3.2.6 (Huelsenbeck and Ronquist 2001). For the latter, default settings were adjusted to have a Subsampling Frequency of 1,000 generations, and the first 250,000 generations were discarded as burn-in. A haplotype network, which incorporates a step-wise mutation model, was constructed in TCS v.1.2 (Clement et al. 2000). Nucleotide diversity (π), a measure of heterozygosity that standardizes the number of nucleotide mismatches by the sequence length, was determined using DNA Sequence Polymorphism v.5.10.01 (http://www.ub.edu/dnasp/). Finally, sequences were

run in Genbank using BLAST v.2.3.0 (blast.ncbi.nlm.nih.gov/) (Morgulis et al. 2008) for comparison with accessions registered by other researchers worldwide.

Nuclear Analysis

Nuclear DNA (nDNA) provides a complementary perspective on gene flow because it is passed down by both parents and undergoes recombination. It tends to be highly polymorphic with a high mutation rate and therefore provides a more recent picture of gene flow than mtDNA (Allendorf and Luikart 2007). Tandem repeat sequences in the genetic code, called microsatellites, are used to assess molecular divergence. These can either come about from mutation or error on the part of RNA polymerase, which may inadvertently insert, delete, or change base pairs as it replicates a DNA strand.

Nine microsatellites (Table 9) were examined to compare heterogeneity amongst netting locations. Forward and reverse primer sequences were derived from Wei et al. (2009) and Racey et al. (2007), with some being modified in-house at the University of Northern Colorado to function with available solutions and dyes. The program Oligo Analyzer (www.IDTDNA.com) was utilized to assess primers for the correct melting temperature and probabilities of self-dimerization, hetero-dimerization, and hairpins. Fluorescent tagging of microsatellite regions was optimized by testing gradients of magnesium chloride, magnesium sulfate, and temperature. The following was combined for each sample: 2.4 µL of Promega 5X GoFlexi Buffer, 0.7 µL dNTP mixture (2.5 mM for each NTP, 10mM total NTP), 0.6 µL non-tagged 5 µM primer, 0.6 µL tagged 0.5 µM primer, 0.6 µL 5 µM fluorescent tag, 0.06 µL 100X Bovine Serum Albumin (BSA), 0.06

Table 9. Microsatellite primers and polymerase chain reaction conditions used for *Pipistrellus abramus*.

Primer name	Sequence (5'-3')	Repeat sequence	Allele size range (bp)	T_{ann} (ºC)	$MgCl_2$ / $MgSO_4$ (µL)	Tag	Dye
1-20[a]	F: TCTGGGTCCTGAAACTGG R: CCTGGCTCATAGGTGAATGT	$(AC)_{22}$	171-209	57.4	0.25 Mg_2SO_4	CAGT	FAM
1-26[a]	F: CAGAGTGCCACATTCTTCC R: GCATCCTCCATTCTCACAAC	$(GT)_{23}$	393-415	52.9	0.25 Mg_2SO_4[g]	M13	FAM
1-36[a]	F: CTCGCCTGTTCCCTCTTGC R: TTCATTCCTGCCACCACCC	$(GT)_{23}$	298-318	55.1	0.5 $MgCl_2$	T7	FAM
1-85[a]	F: [c]_CACTGGGTGACAGGGACAG R: ATTACTTACACGGGCGGCAG	$(GAA)_6$ . . . $(TG)_4$	237-244	59.6	0.5 $MgCl_2$	T7	FAM
1-451[a]	F: CCTTCAGAGTCTTCGCTT R: AAGACCAAGTTTTGCCTC	$(GC)_5AC(GC)_5(AC)_{20}$	254-284	59.6	2 $MgCl_2$	M13	PET
L45[a]	F: GGAATGCAGCAATGTTAGGTG R: AGGGCAGTGACAGGAAAG	$(AC)_{22}$	168-208	57.4	1 $MgCl_2$	CAGT	VIC
PA133[a]	F: GATCACCACATTGCCACTTT R: GTCAGTGCTGGGGTACA_[d]	$(AC)_{21}$	111-135	55.1	3 $MgCl_2$	CAGT	FAM
Ppi02[b]	F: GCTAGTTATTGCTCAGCGGAT CAGAGCCCCATTTTTATC R: GTCAATTTCCCTTCAACCAC	Dinucleotide[f]	123-141	59.6	4 $MgCl_2$	M13	PET
Ppi04[b]	F: [d]_[e]_CATCTAAGAGCTGTCCCC R: CACCCCATGACAAATGAAC	Dinucleotide[f]	174-208	55.1	1 $MgCl_2$	CAGT	VIC

[a]Wei et al. 2009
[b]Racey et al. 2007
[c]Removed guanine (G)
[d]Removed adenine (A)
[e]Removed thymine (T)
[f]Repeat sequences were unpublished but their motif type reported by S. B. Piertney (personal communication).
[g]Used 10 X Thermopol buffer

Promega GoFlexi Taq Polymerase (5 units/μL), and 1 μL DNA. (For one primer, "1-26," 1.2 μL of 10X ThermoPol buffer was used instead of GoFlexi.) Each solution was run separately with a low $MgSO_4$ concentration (0.25 μL 100 mM), a high $MgSO_4$ concentration (0.5 μL 100 mM), a low $MgCl_2$ concentration (1 μL 25 mM), and a high $MgCl_2$ concentration (2 μL 25 mM) and topped up with enough autoclaved MilliQ water to reach a total volume of 12 μL per sample. Samples were run on a thermocycler with a gradient of annealing temperatures to identify optimal conditions for amplification. Following successful amplification, samples were sent to the Arizona State University molecular genetics lab for fragment analysis. Multiplexing was used to reduce costs; this procedure involved combining the product fragments of different primers for the same individual into a single well (rather than separating fragments of each primer into different wells). Fragments that could possibly overlap for a specific sequence length were labeled with different dye colors to distinguish them in the final reading.

Fragments were aligned and peaks identified in Geneious. A peak indicated that a relatively large amount of PCR product had been generated at a specific location along a DNA fragment and provided the identity of an allele at a particular locus. When peak locations differed between individuals, these nucleotide polymorphisms indicated genetic variability in the population. For example, if one individual had peaks at base pair locations 122 and 126, while another individual had them at 122 and 132, this would indicate a difference in genetic structures, i.e. different haplotypes.

F_{ST}, F_{IS}, observed heterozygosity, expected heterozygosity, mean number of alleles per locus, mean effective number of alleles per locus (normalized based on frequency), and a principal component analysis for the five *Pipistrellus abramus*

subpopulations were computed in GenAlEx v.6.4 (Peakall and Smouse 2006). F_{ST} is a common way of measuring the degree of genetic divergence between two subpopulations or sampling locations. It utilizes deviations of observed heterozygosity from that expected by Hardy-Weinberg equilibrium to compare the degree of inbreeding in a single subpopulation to that of the total population (Freeland et al. 2011). Values range from zero (identical genetic sequences) to one (completely different sequences) (Wright 1951). In general, F_{ST} values from 0.05 to 0.25 indicate moderate differentiation, and those over 0.25 indicate strong differentiation, though norms vary by study subject (Freeland et al. 2011).

F_{ST} values were derived according to Nei's genetic distance model and also used to calculate the effective number of migrants between populations with the equation $N_e m = ((1/F_{ST}) -1)/4$ (Wright 1984). In this equation, N_e is the effective size of the population, or the estimated number of individuals actually contributing genes to the next generation (which is almost always less than the total number of individuals). *m* is the number of migrants moving from one population to another. Together, $N_e m$ is the number of migrants actually breeding in the new population to which they have moved. Although this equation has some complications, for example, the assumption that all populations exchange migrants at the same rate (Holsinger and Weir 2009), I chose to use it as a simple estimation, as has been done in other mammal studies (McManus et al. 2014).

F_{IS} is an inbreeding coefficient that estimates the amount of non-random mating in a population (Allendorf and Luikart 2007). It ranges from -1 to 1, with zero indicating the amount of heterozygosity expected under random mating. Positive values signify

inbreeding, meaning that more homozygotes are present than expected, and negative values signify outbreeding, meaning that more heterozygotes are present than expected.

The pairwise genetic distances (F_{ST}s) from above were coupled with pairwise geographic distances to determine isolation by distance (IBD) using IBD web service (Jensen et al. 2005). An adjusted measure of genetic distance ($F_{ST}/1\text{-}F_{ST}$) was used, as recommended by Rousset (1997). Geographic distances were obtained using Google Earth. In IBD web service, repetitions were set to 10,000, and one negative genetic distance was set to zero.

Nucleotide polymorphisms were run through STRUCTURE v.2.3.4 (Pritchard et al. 2000) and STRUCTURE Harvester Web v.0.6.94 (Earl and vonHoldt 2012) to identify the most likely number of genetic clusters via Bayesian analysis. In STRUCTURE, an admixture model was applied with a burn-in of 100,000, followed by 100,000 Markov chain Monte Carlo repetitions. Fifteen iterations were run for each cluster model from k = 1 to k = 8 (with k representing the number of populations). A ΔK graph, which signifies the most likely model based on the rate of change of likelihood (Evanno et al. 2005), was produced in Structure Harvester.

Results

Mitochondrial Deoxyribonucleic Acid (mtDNA)

The maximum sequence length retained across individuals was 1,176 base pairs. All sequences were identified as *Pipistrellus abramus* in Genbank, with most having ≥99% match by identity to other *P. abramus* records from China (Wei et al. 2010; Guo et al. 2013; Dong et al. 2014) and Japan (Nikaido et al. 2001; Sakai et al. 2003). (The two

exceptions, discussed below, still matched by 95%.) Variability was due to the presence of single nucleotide replacements, and no insertions or deletions. Because the largest genetic distance between any two sequences was 5%, the phenetic and posterior probability trees were relatively uninformative and therefore not pictured.

A total of six mitochondrial haplotypes were detected across sites. The Panda Base had the greatest number (5) but also the most individuals sampled (Table 10). Nucleotide diversity (π) ranged from 0 - 0.00890 (overall = 0.00541), with the Panda Base having the highest and Jiang'an, An Long, and Xi Hua the lowest (0). The haplotype network indicated the presence of two major mitochondrial clades (Fig. 23). Most individuals fell within the first clade, which was found at all geographic locations (Fig. 23). The second, differing from the first by ≥58 base pair regions, was comprised of two individuals: a juvenile male from Chengdu Panda Base and an adult female from Sichuan University. (These had the 95% matches by identity within Genbank.)

Nuclear Deoxyribonucleic Acid (nDNA)

Loci were variable for all nine microsatellites. H_o ranged from 0.444-0.870 and H_E from 0.414-0.669 (Table 10). Because sample sizes were uneven across sites and quite small at some locations, statistical significance of H_o relative to H_E was not assessed. An Long had the largest deviation from expected heterozygosity (0.285), while Xi Hua Normal had the smallest (0.031).

Pairwise F_{ST} for subpopulations ranged from 0.017-0.137, with the largest difference between An Long organic farm and Xi Hua Normal (Table 11). Using

Table 10. Mitochondrial (*cytb*) and nuclear (microsatellite) measures of genetic variability in *Pipistrellus abramus*. Locations: SU = Sichuan University, JA = Jiang'an Campus, PB = Chengdu Panda Base, AL = An Long organic farm, and XH = Xi Hua Normal. N = number of individuals, π = nucleotide diversity, H_O = observed heterozygosity, H_E = expected heterozygosity, N_a = mean # of alleles per locus, Ne = mean effective # of alleles per locus (normalized based on frequency), F_{IS} = inbreeding coefficient.

		Mitochondrial		Nuclear					
Location	N	Haplotypes observed	π	H_O	H_E	H_O - H_E	N_a	N_e	F_{IS}
SU	10	3	0.00034	0.583	0.669	-0.086	6.333	3.677	0.152
JA	2	2	0.00000	0.722	0.528	0.194	2.667	2.437	-0.333
PB	22	5	0.00890	0.530	0.614	-0.083	6.778	3.124	0.161
AL	3	2	0.00000	0.870	0.585	0.285	3.000	2.643	-0.500
XH	3	2	0.00000	0.444	0.414	0.031	2.444	1.956	0.000
Total	40	6	Overall = 0.00541				Mean = 4.244	Mean = 2.767	Mean = -0.104

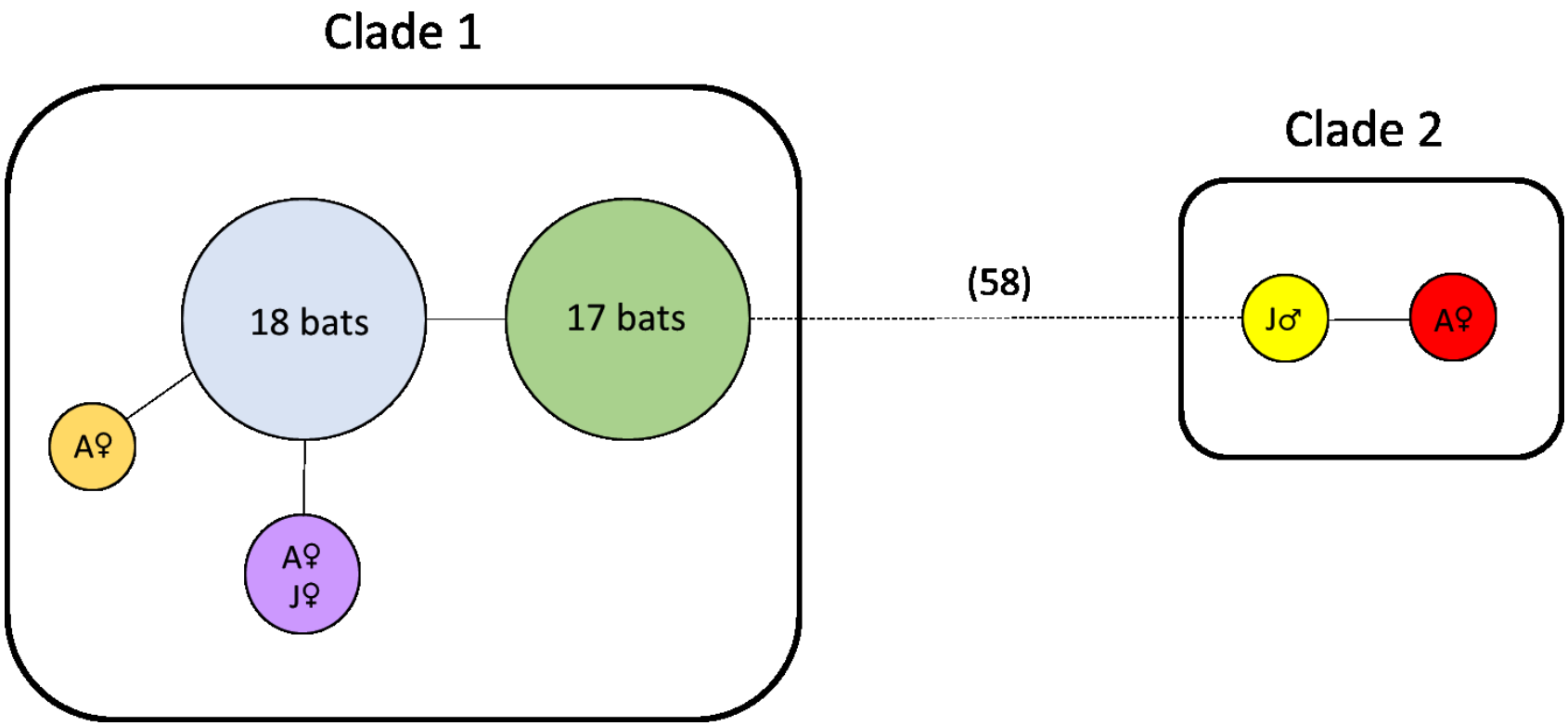


Fig. 23. Step-wise haplotype network for 40 *Pipistrellus abramus* individuals, using *cytb* regions. Solid lines represent single nucleotide polymorphisms, while the dotted line represents 58 polymorphisms. The 18 bats of the "blue" haplotype came from all five sampling locations, as did the 17 "green" bats. "Orange," "purple," and "yellow" individuals were from Chengdu Panda Base, and the "red" individual was from Sichuan University.

guidelines suggested by Freeland et al. (2001), differentiation was low ($F_{ST} < 0.05$) between Sichuan University and the Panda Base and moderate ($F_{ST} = 0.05$-0.25) for all other subpopulation combinations. Sichuan University and the Panda Base also had the largest effective number of migrants (14.301, Table 12); An Long and Xi Hua had the least (1.579). Xi Hua had the lowest effective number of alleles per locus (1.956), and Sichuan University had the highest (3.677). In line with these results, there was a positive, statistically significant relationship between pairwise genetic distance and geographic distance (km) (Fig. 24, $r^2 = 0.556$, $P = 0.0247$).

Table 11. Pairwise subpopulation F_{ST} values.

Location	Panda Base	Sichuan Univ.	Jian'ang	Xi Hua	An Long
Panda Base	0.000				
Sichuan Univ.	0.017	0.000			
Jiang'an	0.054	0.082	0.000		
Xi Hua	0.074	0.098	0.114	0.000	
An Long	0.063	0.058	0.100	0.137	0.000

Finally, F_{IS} showed Jiang'an and An Long to be outbred (-0.333, -0.500), Sichuan University and Chengdu Panda Base to be marginally inbred, (0.152, 0.161), and Xi Hua Normal to be neither (0.000).

Table 12. Number of effective migrants ($N_e m$) between subpopulations.

Location	Panda Base	Sichuan Univ.	Jiang'an	Xi Hua	An Long
Panda Base					
Sichuan Univ.	14.301				
Jiang'an	4.360	2.789			
Xi Hua	3.131	2.296	1.938		
An Long	3.712	4.076	2.241	1.579	

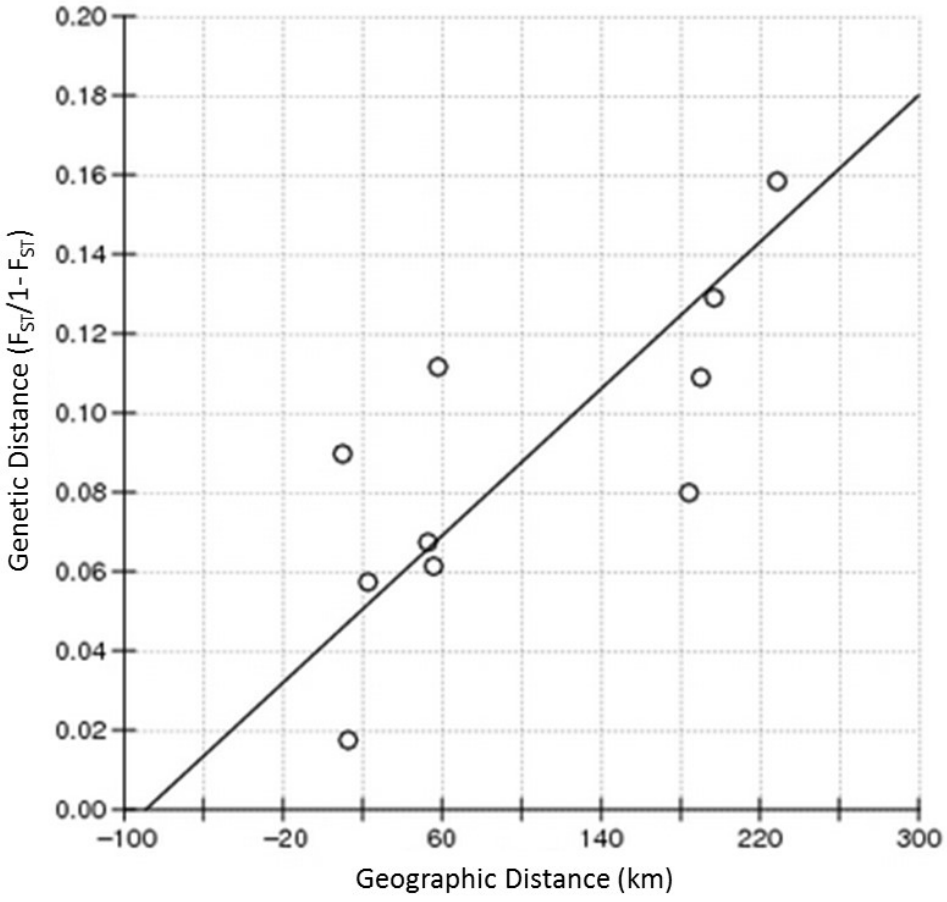


Fig. 24. Pairwise genetic distances (F_{ST}/1-F_{ST}) versus geographic distances (km). r = 0.7459, r^2 = 0.556, p = 0.0247. The line is a reduced major axis regression, plotted by IBD on the web (Jensen et al. 2005).

In the PCoA (Fig. 25), axis 1 explained 14.74% of variation, axis 2 explained 9.94%, and axis 3 explained 8.97%. The graph revealed substantial overlap of Sichuan University and Panda Base genotypes with those from all other locations, while bats from Xi Hua Normal formed a cluster distinct from An Long farm. A plot of axis 1 versus axis 3 and a plot of axis 2 versus axis 3 were also examined but did not yield any different results.

STRUCTURE Harvester identified k = 2 as the most likely number of clusters ($\Delta K = 15.677$). However, individual genotypes did not partition cleanly by subpopulation (Fig. 26), indicating a high degree of mixture.

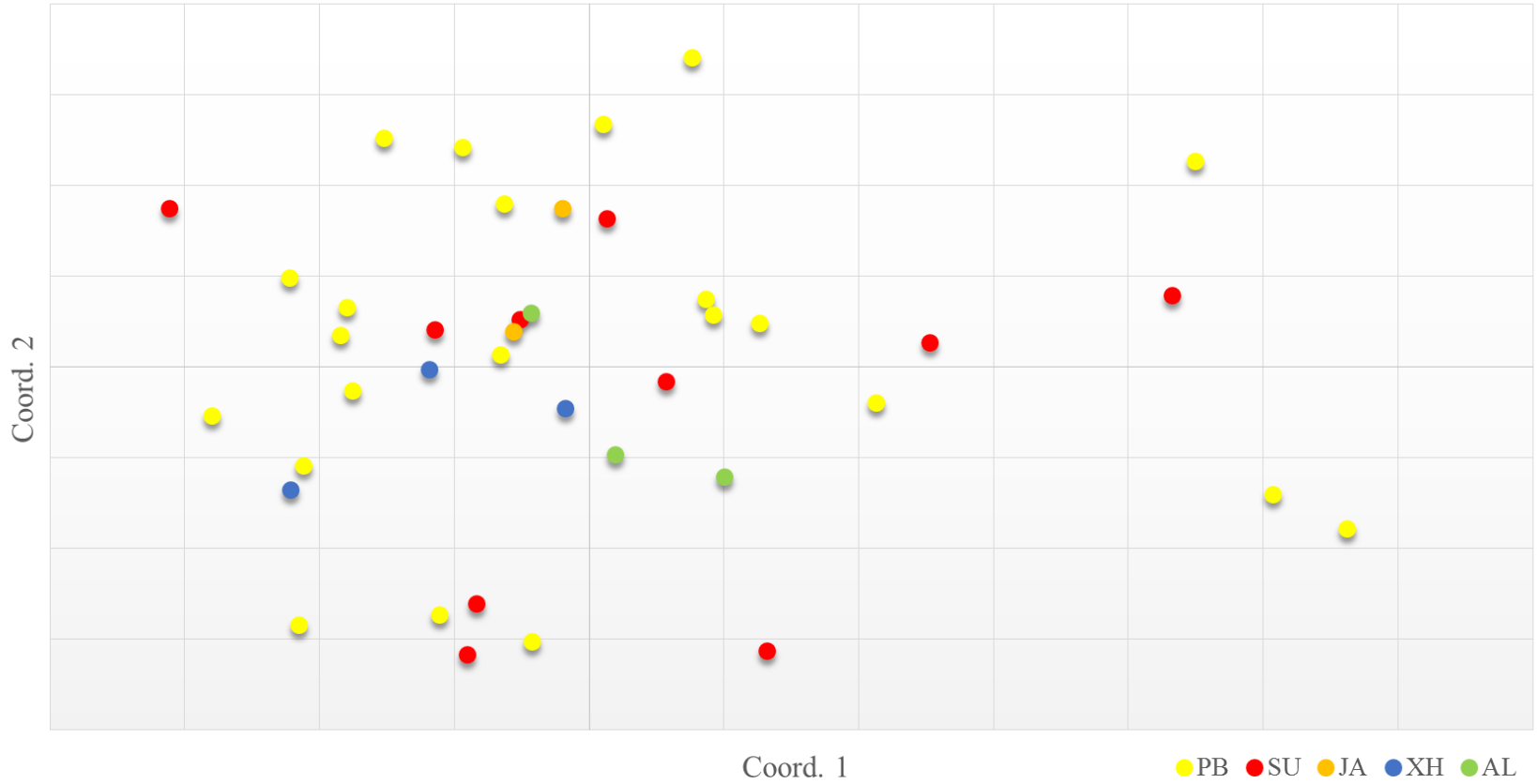


Fig. 25. Principal coordinate analysis of the five *Pipistrellus abramus* subpopulations from Chengdu, according to data from nine microsatellite loci. Axis 1 explained 14.74% of variation, and axis 2 explained 9.94%. (Axis 3, not pictured, explained 8.97%.)

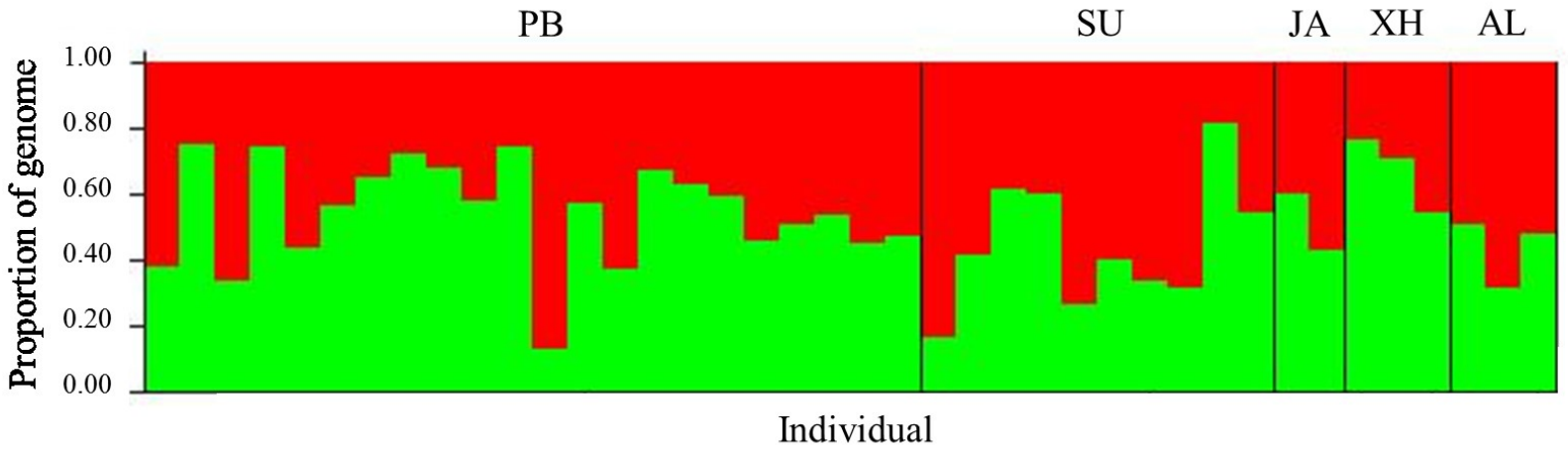


Fig. 26. STRUCTURE diagram of *Pipistrellus abramus* with two clusters, the most likely pattern selected by STRUCTURE Harvester. Each vertical bar along the x-axis represents an individual bat. The y-axis represents the proportion of an individual's genome attributed to each inferred cluster. Labels along the top indicate sampling location. PB = Chengdu Panda Base, SU = Sichuan University, JA = Jiang'an, XH = Xi Hua Normal, AL = An Long.

Discussion

Species Identification

The high *cytb* similarity of bats from this study with *Pipistrellus abramus* accessions in Genbank (≥95% match for all) confirms that in-field identifications were correct. This justifies use of these tissues to assess regional differences in pollutant uptake, as done in Chapter II. However, mitochondrial analysis also revealed two major clades (separated by 58 SNPs) that overlapped geographically, which could have interesting implications in terms of intra-specific differences in chemical susceptibility.

Mitochondrial Clades

One possible explanation for co-occurrence of mitochondrial clades, in this case, is a historical pattern of geographical separation followed by recolonization and rejoining. This has occurred for many species at temperate latitudes (e.g., the greater horseshoe bat, *Rhinolophus ferrumequinum* (Rossiter et al. 2007)) due to cycles of glacial expansion and shrinkage during the Pleistocene. Biota were restricted to refugia when glaciers were at their maximum, then expanded following glacial recession. Wei at al. (2010) identified sea level rise during the Pleistocene as the likely cause of mitochondrial differences in *Pipistrellus abramus* populations from eastern continental and off-shore China. They found the mainland and offshore clades to be separated by ≤35 step-wise mutations for *cytb*, with only slight geographical overlap. Andriollo et al. (2015) similarly concluded that glaciation likely caused present-day *Pipistrellus kuhlii* to have four main mitochondrial lineages in Europe and northern Africa, with a divergence as high as 6% in cytb and ND1 regions. In kind, ancient glaciation could have influenced

the movement of bat populations through what is today the Sichuan Basin. The Basin is directly adjacent to the Qinghai-Tibet plateau, which has been experiencing dramatic uplift since the Quaternary (Zhang et al. 2000). According to Zhang et al. (2000), this uplift caused substantial alterations to surrounding climates, which increased the intensity of glacial and inter-glacial events.

Related to this concept, regional adaptation could have played a role in mitochondrial divergence. Because mitochondrial DNA is the template for production of proteins involved in energy and metabolism, it is important for physiological adaptation. In *Pipistrellus abramus* from eastern China, Dong et al. (2014) found evidence of selection in *cytb* regions that code for amino acids associated with trans- and intermembrane domains. These same domains aid in producing the proton gradient needed for ATP production and cellular respiration (Esposti et al. 1993). Dong et al. speculated that this selection could be due to differences in climate experienced by the mainland (temperate) clade versus the offshore (subtropical) clade. In the present study, however, the two mitochondrial clades occurred in the same location, despite being more genetically divergent from one other than those noted by Wei et al. (2010) and Dong et al. (2014). If climate is indeed a key driver, these differences could reflect historical influxes of bats from quite different areas, such as the deserts to the north or the tropics to the south.

Although contaminants are known to alter DNA integrity and cause mutations, for example through the creation of reactive oxygen species (Crespo-Lópeza et al. 2009), it was not possible to assess whether these could have contributed to mitochondrial

divergence in this case. However, it bears consideration whether one genotype over the other might impart a physiological advantage in coping with stressors.

Genetic Diversity

According to the *cytb* marker, the Panda Base had the greatest mitochondrial diversity, with the most haplotypes (5) and the highest nucleotide diversity (0.0089) (Table 10). This was also the location with the greatest number of individuals sampled, however. Nucleotide diversity across sites (0.00000 - 0.00890) was similar to that reported for many of the Wei et al. (2010) locations (0.00036 – 0.01878).

For nuclear data, Sichuan University and the Panda Base had the highest mean effective number of alleles per locus (3.677 and 3.124, respectively), followed by An Long (2.643), Jiang'an (2.437), and Xi Hua Normal (1.956) (Table 10). Again, Sichuan University and the Panda Base had the highest sampling effort, so results should be interpreted cautiously. Mean effective number of alleles per locus across sites (2.767) was slightly lower than allelic richness reported by Wei at al. (2010) (3.89), but this could also be due to a much lower sample size.

From these data, I conclude that overall genetic diversity in the Chengdu region is not substantially different from other parts of its range.

Gene Flow

Microsatellite data showed that recent gene flow amongst sub-populations was high, eliciting little population structure, as expected. STRUCTURE diagrams showed that genotypes did not separate out cleanly by location, even in the most highly supported

cluster (k=2) (Fig. 26). PCoA (Fig. 25) revealed that no location was completely distinct relative to all others. Additionally, N_em for all *P. abramus* locations in this study was >1 (Table 12). According to Wright (1931), the minimum number of effective migrants needed to maintain reproductive mixing between two populations is one per generation. So a sufficient degree of mixture to prevent genetic isolation had occurred across all sites.

Distance did, however, seem to play a role in limiting gene flow between the farthest sampling locations. At the most central sites, Sichuan University and Chengdu Panda Base had the highest amount of gene flow, as indicated by the lowest pairwise F_{ST} (0.017) (Table 11), the largest number of effective migrants (14.30) (Table 12), and broad overlap in the PCoA (Fig. 25). There was a significant degree of isolation by distance (Fig. 24, $r^2 = 0.556$, $p = 0.0247$), with Xi Hua Normal being most distinct from An Long organic farm. This was also supported by Xi Hua and An Long having the lowest number of effective migrants between them (1.58). Thus, microsatellite data indicated that while gene flow has occurred in recent generations, individuals probably do not span the entire 200 km from Xi Hua to An Long. Rather, successive overlap of breeding individuals at the edge of their ranges over time has likely allowed genes to spread over broader geographical areas. This is consistent with results for *Pipistrellus pipistrellus* (F_{ST} = -0.001-0.015) and *P. pygmaeus* (F_{ST} = -0.001-0.017) in Europe, where isolation by distance was also found to be significant (Bryja et al. 2009).

An Long and Jiang'an, had the largest deviations from expected heterozygosity (0.285 and 0.194, though significance was not tested) and F_{IS} values indicative of outbreeding (-0.333 and -0.500, respectively). In some cases, outbreeding can lead to a reduced ability to adapt to local conditions (Allendorf and Luikart 2007). However,

given that fewer than 10 bats were sampled from each of these locations, these heterozygosity and F_{IS} values may be more a result of small sample size.

Bats from Sichuan University and the Panda Base appeared to have some recent inbreeding (F_{IS} = 0.152 and 0.161), though the implications of this are unclear. The closely related *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* in central Europe have been reported to have F_{IS} values between 0.050 and 0.305, despite having high genetic diversity (Sztencel-Jabłonka and Bogdanowicz 2012). The authors of the study could not determine a reason for this, though they alluded to the possibility of bottlenecking, as has been determined for *Myotis bechsteinii* in Britain (F_{IS} = 0.145 - 0.289) (Durrant et al. 2009). While this could mean that *P. abramus* has undergone recent declines in central Chengdu, inbreeding can also occur as a function of non-random mating (Allendorf and Luikart 2007).

Contrasts Between Mitochondrial and Nuclear Data

As has been found in many other studies on bat species (e.g., Petit and Mayer 1999: *Nyctalus noctula*; Ruedi and Castella 2003: *Myotis myotis*; Weyandt et al. 2005: *Corynorhinus townsendii ingens*; Turmelle et al. 2011: *Eptesicus fuscus*), mitochondrial and nuclear DNA provided contrasting pictures of gene flow and diversity due to differing rates of mutation and mechanisms of inheritance (Allendorf and Luikart 2007). In each of the above bat studies, the presence of mitochondrial differentiation and lack of substantial nuclear diversity was attributed to male-biased dispersal. This is a strong possibility for *Pipistrellus abramus*, as well (Dong et al. 2014). As mentioned above, male Japanese pipistrelles tend to have lower roost fidelity than females, typically not

returning to their natal roost after the first year (Funakoshi and Uchida 1978; Funakoshi et al. 2009).

Limitations

Low sample size was the biggest limiting factor in this study, with less than 10 individuals sampled from three of the five sub-populations. Thus, results should be interpreted cautiously, particularly in regards to An Long farm (n = 3), Jiang'an (n = 2), and Xi Hua Normal (n = 3). Sex-based differences in diversity also could not be reliably assessed for this reason.

CHAPTER IV

ASSOCIATIONS BETWEEN STREAM HEAVY METAL CONTAMINATION AND BAT ACTIVITY IN THE EASTERN ROCKY MOUNTAINS, COLORADO, U.S.A.

Background and Significance

Predation on nocturnal insects is one of the many ecological roles performed by bats (Altringham 2011; Boyles et al. 2011). Insectivorous bats, in turn, rely on insect prey to meet their inherently high metabolic rates as well as the energetic demands of flight, endothermy, and reproduction (Kurta et al. 1989; Altringham 2011). Prey availability thus can alter bat behavior and spatial distribution (Lee and McCracken 2005; Fukui et al. 2006; Malison and Baxter 2010) and presumably bat fitness (Speakman and Racey 1989; Gerell and Lundberg 1993; Frick et al. 2007).

Aquatic emergent insects make up a significant portion of many bat species' diets (e.g., Belwood and Fenton 1976; Buchler 1976; Swift et al. 1985; Barclay 1991; Rydell et al. 1996; Clare et al. 2014). In the U.S., chironomids (Diptera), have been found to comprise at least 30% and as much as 76% of adult little brown bats' diets in the summer (Belwood and Fenton 1976; Buchler 1976; Freeman 1984; Swift et al. 1985; Barclay 1991; Anthony and Kunz 1997). Ephemeropterans (mayflies) and trichopterans

(caddisflies) also are commonly consumed and can supplement the diets of more generalist bat species, as well (Whitaker et al. 1997; Kurta and Whitaker 1998). In a study by Freeman (1984) in Moffat County, Colorado, for example, the stomach contents of *Myotis leibii*, *M. evotis*, *M. californicus*, *M. volans*, *M. thysanodes*, and *Lasionycterus noctivagans* were each composed of 13-21% trichopterans (caddisflies) from June through September. In the same study (Freeman 1984), trichopterans also comprised 30% of the stomach contents of *Lasiurus cinereus* and 45% of that of *Corynorhinus* (formerly *Plecotus*) *townsendii*, species that typically prefer moths (Black 1972; Whitaker et al. 1997).

Attempts to quantitatively describe such transfer, or flux, of energy and matter between trophic (feeding) levels have existed since at least the beginning of the last century (Paine 1980). Elton (1927) introduced the concept of trophic levels, i.e. energetic connections between functional groups (producers and consumers). Lindeman (1942) then focused on modeling the dynamics of these connections, and MacArthur (1955) posited that the nature of these connections influenced community stability. While there is a rich history of discussion in this field of ecology, a more recent focus has been on "aquatic-terrestrial subsidies" (*sensu* Polis et al. 1997), which specifically address spatial and temporal effects on nutrient and energy exchanges that flow from aquatic habitats to adjacent terrestrial ones. This area of study arose in response to earlier observations that terrestrial inputs, i.e. dropped vegetative matter, run-off, and detritus, could appreciably influence trophic dynamics within aquatic communities (Power et al 2004). It was generally believed that terrestrial habitats exerted stronger influences on aquatic habitats, as opposed to the other way around, in part due to the sheer area of terrestrial habitats

bounding inland streams (Power et al. 2004). However, Nakano and Murakami (2001) showed that the energetic contributions of one habitat to the other could change throughout the year, such that aquatically sourced insects supported terrestrial insectivores (songbirds) in winter months when terrestrial insects were scarce. In a field-based exclusion experiment, Fukui et al. (2006) similarly demonstrated that bats responded spatially to the presence or absence of aquatic insects emerging from Japanese riparian streams.

The potential for disturbance of these relationships by environmental pollutants is an important consideration for ecosystem health (Baxter et al. 2005; Schulz et al. 2015). Contaminants are unique in that they can affect terrestrial consumers both directly by being transferred through ingested food and water (Reinhold et al. 1999) and indirectly by influencing prey availability (Paetzhold et al. 2011). Toxin transference and accumulation across aquatic-terrestrial spheres, dubbed the "dark side of subsidies" (Walters et al. 2008), has been demonstrated for a number of substances including polychlorinated biphenyls (PCBs) (Walters et al. 2008), organic pesticides (Kelly et al. 2007), and non-essential metals (methyl mercury (Evers et al. 2005), cadmium (Currie et al. 1997; Naidoo et al. 2003), chromium (Naidoo et al. 2003), and nickel (Naidoo et al. 2003)). Other substances, including both non-essential and sufficiently high levels of essential metals, can limit larval growth but be partially or wholly excreted during pupation and metamorphosis in individual insects that make it to those stages (Timmermans and Walker 1989; Timmermans et al. 1992). Degree of toxicity to insects depends on the type of heavy metal, species, exposure concentration, and length of exposure (Timmermans et al. 1992). For contaminants that affect emergent insect

production, but are not exported via food webs, the effect on prey composition and quantity could be of greater importance to consumers than the toxins themselves (O'Shea et al. 2000; Kraus et al. 2014).

Stream Contaminants and Bats

There is a large literature examining aquatic insect vulnerability and response to different classes of contaminants (e.g., Hare 1992; review by Fleeger et al. 2003; Rico and Van den Brink 2015). There are comparatively fewer studies on bat foraging responses in this context. (Note that the present chapter focuses on differences in bat foraging patterns and *not* on biological uptake of contaminants, although both could be occurring in the same system. See Chapter II for information regarding toxin uptake and direct effects on bats.)

Many studies on bat foraging have concerned wastewater run-off, which often contains elevated nitrogen levels and can lead to eutrophication (Vaughn et al. 1996; Racey et al. 1998; Kalcounis-Rueppell 2007; Naidoo et al. 2013). For example, Vaughn et al. (1996) found that sewage effluent was associated with lowered *Pipistrellus pipistrellus* activity and foraging attempts on average, while *Myotis* species—including *Myotis daubentonii*, which prefers aquatic insects—foraged more frequently over contaminated reaches in England. The authors speculated that *Myotis* species were capitalizing on more pollution-tolerant insects, whereas *P. pipistrellus* were relying more heavily on pollution-sensitive insects (Vaughn et al. 1996). Kalcounis-Rueppell et al. (2007) similarly found species-specific differences in response to food availability and quality in North Carolina. In their study, insect abundance, particularly that of dipterans

(true flies), was lower downstream of a wastewater treatment plant. While overall bat activity and foraging did not differ between upstream and downstream of the point source, *Perimyotis subflavus* was more active downstream, *Eptesicus fuscus* was more active upstream, and *Nycticeius humeralis* activity did not differ (Kalcounis-Rueppell et al. 2007). Of these, *P. subflavus* is considered the most reliant on aquatic insects, while *E. fuscus* and *N. humeralis* are generalists, preferring coleopterans (beetles) and lepidopterans (moths) (Kalcounis-Rueppell 2007). Finally, Scott et al. (2010) used categories of insect tolerance to organic pollution (from the Biological Monitoring Working Party (1978) (Hawkes 1998)) to help define riparian areas in England as "disturbed" or "undisturbed" and relate bat activity to habitat quality. Additionally standardizing sites for flow quality, stream chemical composition, and vegetative structure (degree of fragmentation, bank vegetation complexity, and land use type), they found that "undisturbed" sites had 25% more bat passes and 72% more feeding buzzes than "disturbed" sites. (The number of feeding buzzes was significantly different, while the number of passes was not.) *Pipistrellus pygmaeus*, which favors wetlands, emitted significantly more calls and feeding buzzes at undisturbed sites, while neither the number of calls nor buzzes were significantly different between the two site types for *Pipistrellus pipistrellus* (Scott et al. 2010). In sum, these studies demonstrate that the ways in which aquatic contaminants affect patterns of bat foraging are largely species-specific.

In addition to species, sex, age, location, and time of year can affect degree of impact on individual bats and presumably on populations. Frick et al. (2007) found that a major spill of the pesticide metam sodium (an organosulfide) was associated with lowered survival of juvenile female Yuma myotis bats (*Myotis yumamensis*) at the

Sacramento River, California, most likely due to declines in aquatic insect availability (Frick et al. 2007). Clare et al. (2014), using molecular analysis of bat guano, found that adult female *Myotis lucifugus* at some locations in Canada shifted from eating primarily dipterans in May to mid-June (during pregnancy) to eating mostly lepidopterans from mid-June to September (during and after lactation). The authors also compared insect prey consumption patterns to indices describing insect tolerances to organic (Hilsenhoff 1988) and acid pollution (Fjellheim and Raddum 1990). They suggested that locations with more arthropods of a lower pollution tolerance were of better quality for bats than locations with more arthropods of higher pollution tolerance. The reasoning was that if a habitat was suitable for sensitive arthropod species only capable of surviving in cleaner environments, then bats would have lower exposure to contaminants and potentially more prey options there.

The above studies illustrate a trend towards implementing models that account for time, space, taxa-specific traits, and flux of both energy and contaminants. In one of the more comprehensive approaches to date, Baron et al. (1999) used the reduction in emergent insect biomass associated with stream contamination (heavy metals, PCBs, and radionuclides) to evaluate potential individual effects on *Myotis lucifugus* and *M. griscescens* for an ecological risk assessment. This information was paired with a wildlife exposure model (adapted from Sample and Suter 1994) that incorporated exposure routes, ingestion rate, contaminant concentrations in insects and surrounding media, and bat body weight. The resulting hazard quotients for each stream reach were compared to estimated levels of no adverse effect and lowest observed adverse effect (Baron et al. 1999). The authors produced a spatial distribution of risk, surmising that

bats were vulnerable to the effects of mercury in a single stream reach (Baron et al. 1999), the site of a settling basin for wastes discharged from an energy plant (Oak Ridge National Laboratory, Tennessee) (Cook et al. 1999). With this information, the authors were able to put forth a targeted management plan for the Clinch River/Poplar Creek system (Baron et al. 1999).

Heavy metals. Heavy metals are globally prevalent, with water contamination projected to increase in the near future (Tchounwou et al. 2012; Fernández-Luqueño et al. 2013). Acid mine drainage threatens the health of downstream ecosystems, and riparian areas are ecological hotspots at risk for degradation. Riparian habitats attract many species of bats because they provide open for foraging within complex vegetative structure alongside both aquatic and terrestrial carbon sources, as well as protection from wind and predators (Grindal et al. 1999; O'Shea et al. 2000; Scott et al. 2000; Rogers et al. 2006). *Myotis* bats, in particular, whose wing structure allows them to navigate vegetative clutter, tend to use these areas (Norberg and Rayner 1987; Grindal et al. 1999; O'Shea et al. 2000; Rogers et al. 2006). Thus, more closely examining the circumstances in space and time in which heavy metals exert significant influences on bats in riparian zones is important for directing conservation and remediation efforts (Vidon et al. 2010).

Study Objectives

The present study's aim was to better understand how heavy metal contamination of streams, as mediated by aquatic insect densities, affects bat habitat use in the Colorado Rockies. I predicted that both overall activity and the number of feeding buzzes, a measure of foraging intensity, would differ between streams with higher metal

contamination levels and streams with lower metal contamination levels, and that calls and buzzes would correlate positively with flux of aquatic emergent insects.

Hypotheses

H1 Bat activity will significantly differ between streams of higher metal contamination and streams of lower metal contamination.

H2 Number of feeding buzzes will significantly differ between streams of higher metal contamination and streams of lower metal contamination.

H3 Bat activity will correlate positively with aquatic emergent insect flux.

H4 Number of feeding buzzes will correlate positively with aquatic emergent insect flux.

Methods

Study Area

The study was conducted in the Colorado Mineral Belt (CMB), which extends from northeast of the city of Durango to west of the city of Boulder along the eastern slope of the Southern Rocky Mountains in Colorado. More than 150 years of mining have contributed to high concentrations of zinc, copper, and cadmium in streams above 2,330 m, producing highly acidic conditions to the detriment of aquatic life (Clements et al. 2000; Schmidt et al. 2012; Schmidt et al. 2013). The area has been a continued system of study for the effects of heavy metals on aquatic-terrestrial relationships (e.g., Custer et al. 1997; Clements et al. 2000; Schmidt et al. 2011; Schmidt et al. 2012; Schmidt et al. 2013; Kraus et al. 2014). Of note, Rocky Mountain streams with higher metal contamination have been noted to possess fewer emerging insects and lower

species richness than low contamination streams (Clements et al. 2000; Schmidt et al. 2013).

The region is mountainous and dominated by aspen (*Populus tremuloides*) forest and woodland, subalpine coniferous forest (*Abies lasiocarpa*, *Picea engelmanni*, *Pinus flexilis*, *Pinus ponderosa*), and alpine peaks (CNHP 2013). Rock crevices, abandoned mines, and man-made structures serve as potential bat roosts. Preliminary acoustic sampling by myself in late July 2014 showed bats to be present near streams at high elevations (>3,000 m), though at low densities relative to valley waters such as the Arkansas River. The climate is temperate, with an annual precipitation of about 50 cm in the form of winter snow and summer rain (Schmidt et al. 2013).

Metal Bioavailability Index

To identify likely areas of impaired insect emergence, I utilized the Chronic Criterion Accumulation Ratio (CCAR), a metal bioavailability index developed by Schmidt et al. (2010; 2011) specifically for the Colorado Mineral Belt. This model was developed using hydrologic, geologic, and water chemistry data from over 100 streams in the watershed and incorporates the additive effects of zinc, copper, and cadmium concentrations in freshwater to predict metal bioavailability to aquatic communities. CCAR values of 0.1 to 1.0 represent low metal availability, by which larval densities remain relatively constant. At values from 1.0 to 10.0, emergence of adults is close to zero, although low amounts persist. From a CCAR of 10.0 to 100.0, emergence of all but Chironomidae is virtually eliminated. Thus, in addition to tying a quantitative measure of

insect abundance to water quality, the model also potentially allows one to predict the site-specific composition of insect emergence.

Site Selection

A blocked design was used to control for the effects of elevation and season because they can significantly alter bat species presence and abundance on the landscape (Jaberg and Guisan 2001). Paired sites—each composed of one relatively less contaminated site and one relatively more contaminated site (Table 13, Figs. 27-30)—were selected for sampling via overlay of two data sets in ArcGIS 10.1. The first data set—supplied by Kirk Navo, former Colorado Parks and Wildlife administrator of the Bats/Inactive Mines project—was a map of abandoned mines occupied by bats in the study region from 1992 to 2009. Although most of these sites had since been closed for safety purposes, they indicated areas where bats were still likely to occur in nearby rocky crevices. The second data set comprised 154 sites at which CCAR was previously determined (Schmidt et al. 2013, Kraus et al. 2014). From these latter points, I chose suitable stream pairings with the largest available differences in CCAR.

The small number of suitable sites precluded randomization of site choices. Instead, pairings were chosen such that both points occurred no closer than 200 m and no greater than 2 km from one another. The 200-m separation was used to avoid sampling the same bat at the same time, given the detector's maximum (100-m) limit of detection (Wildlife Acoustics 2015). Streams were chosen within 2 km of one another based on the assumption that a bat would be just as likely to encounter one paired stream as the other. To control for additional variability, paired points were required to have similar

elevations, similar landscape conformations, the same approximate distance from nearby lakes and reservoirs, the same distance from towns and major roads, and similar habitat within a 100-m radius. A distance of ≥5 km between blocks was chosen based on the precedent of a bat landscape study by Grindal et al. (1999). Sites collectively ranged from near the town of Montezuma in Summit County south to the town of Saint Elmo in Chaffee County. Of those designated relatively clean, CCAR values ranged from 0.36 (at North Fork Lake Creek) to 14.71 (at Sts. John Creek). Relatively dirty sites ranged from 12.42 (at Chalk Creek Hancock) to 3605.66 (at South Fork Lake Creek).

Table 13. Paired streams of relatively lower and higher metal contamination level (CCAR, as measured in 2014).

Site pair	Latitude, Longitude	Elevation (m)	CCAR
North Fork Lake Creek	39.089713, -106.542508	3,235	0.36
South Fork Lake Creek	39.042563, -106.527112	3,205	3605.66
North Fork South Platte River	39.490073, -105.823400	3,134	3.32
Handcart Gulch	39.484590, -105.808043	3,033	902.86
West Tennessee Creek	39.346576, -106.332610	3,095	0.46
St. Kevin Gulch	39.291837, -106.366549	3,026	1737.28
Sts. John Creek	39.577840, -105.876453	3,221	14.71
Snake River	39.554606, -105.843803	3,363	375.79
Deer Creek	39.551272, -105.867896	3,327	0.73
Peru Creek	39.601834, -105.813468	3,312	3494.48
Chalk Creek East	38.714035, -106.312713	2,944	6.84
Chalk Creek Hancock	38.696738, -106.349442	3,079	12.42

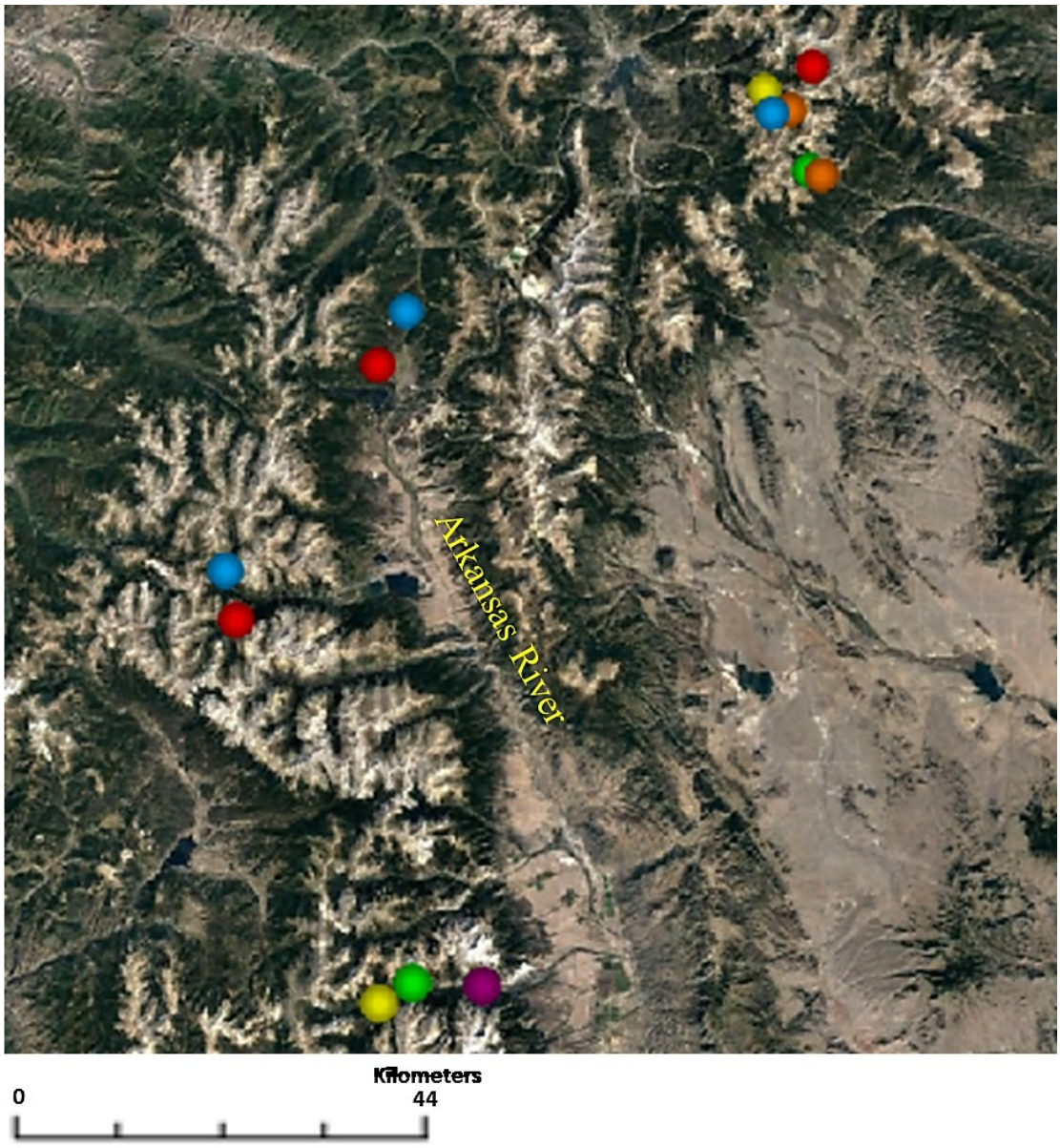


Fig. 27. Overview of sampling sites in the Colorado Mineral Belt, along the eastern slope of the Southern Rocky Mountains. Refer to figures 16-18 for color keys and site details. (Map source: Google Earth 2016)

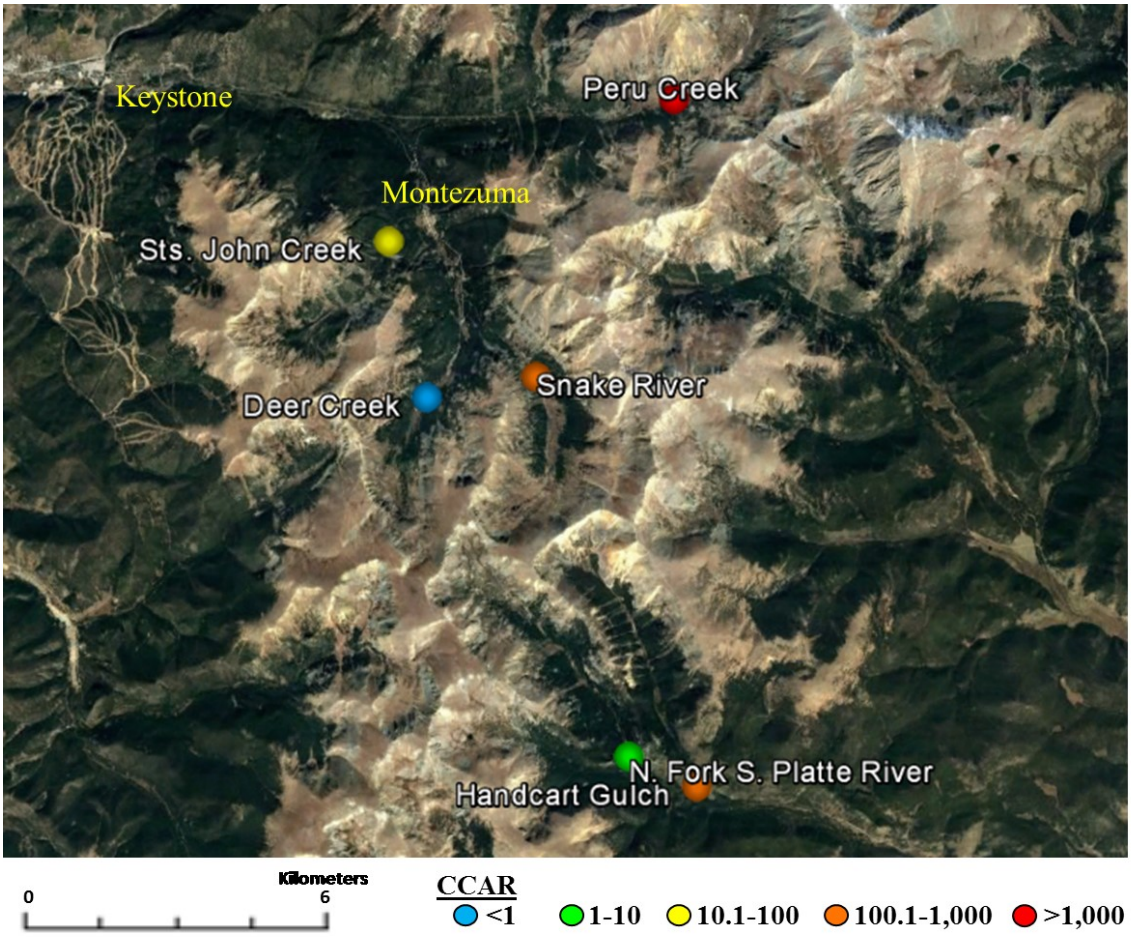


Fig. 28. Sampling sites in Summit and Park counties. (Map source: Google Earth 2016)

Sampling was conducted from 11 August to 3 October 2014 at a total of twelve sites. Ten of these (five site pairs) were sampled for two separate periods over the summer to compare potential seasonal changes in activity. While sampling at this time likely missed the peak of bat activity during the reproductive season (May through July), at least at lower elevations, this was deemed appropriate because 1) a high snow pack (>100% relative to the annual average from the past 30 years) (U.S. Department of Agriculture 2014) hindered earlier access to many sites, 2) bat calls were detected at most selected streams, and 3) aquatic insect productivity would still have been relevant to bats

at this time, prior to hibernation. Aquatic insects, terrestrial insects, bat activity, and water quality were sampled simultaneously at each site pair to account for temporal variation throughout the season (Hayes 1997). Vegetative characteristics were also noted once per site. The plot-level sampling design is depicted in Fig. 31.

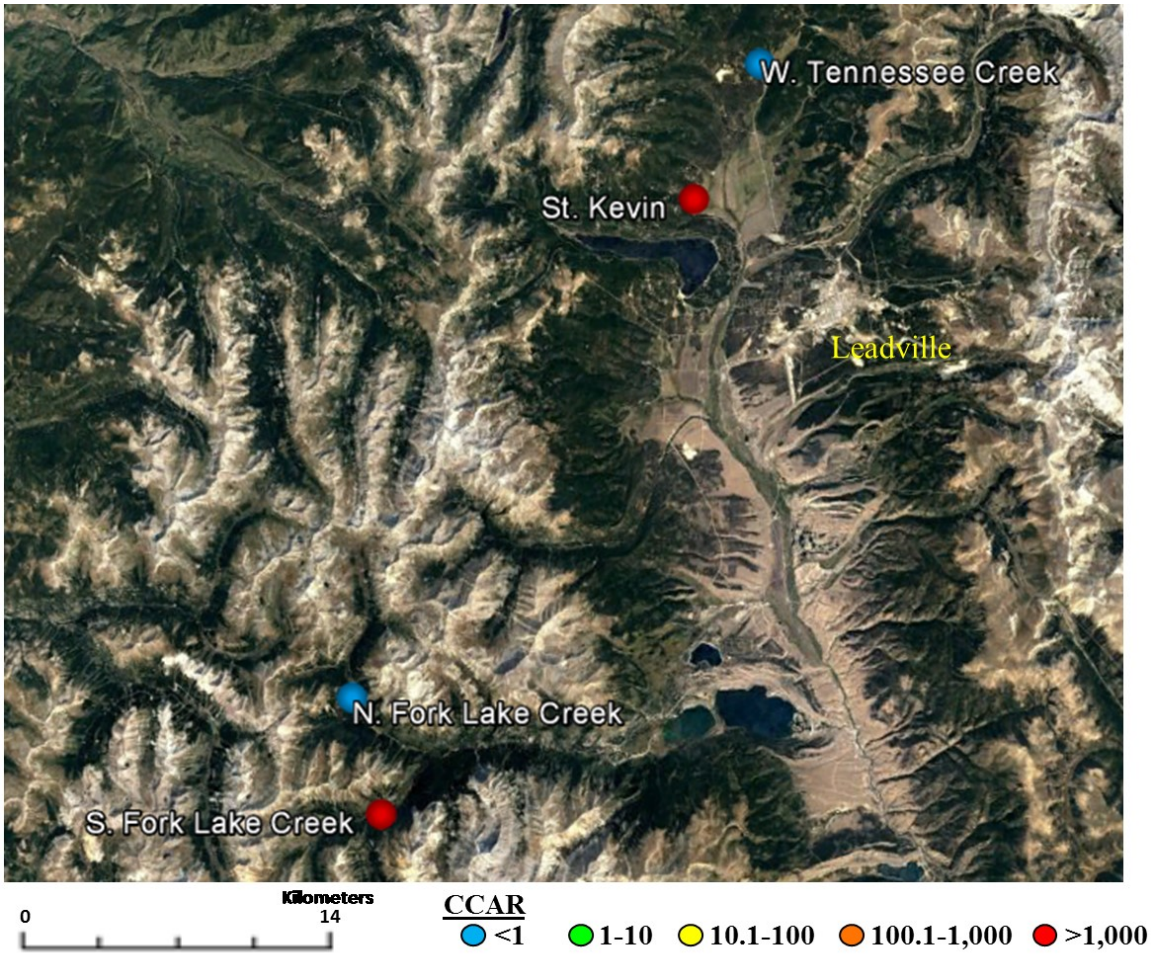


Fig. 29. Sampling sites in Lake and Chaffee counties. (Map source: Google Earth 2016)

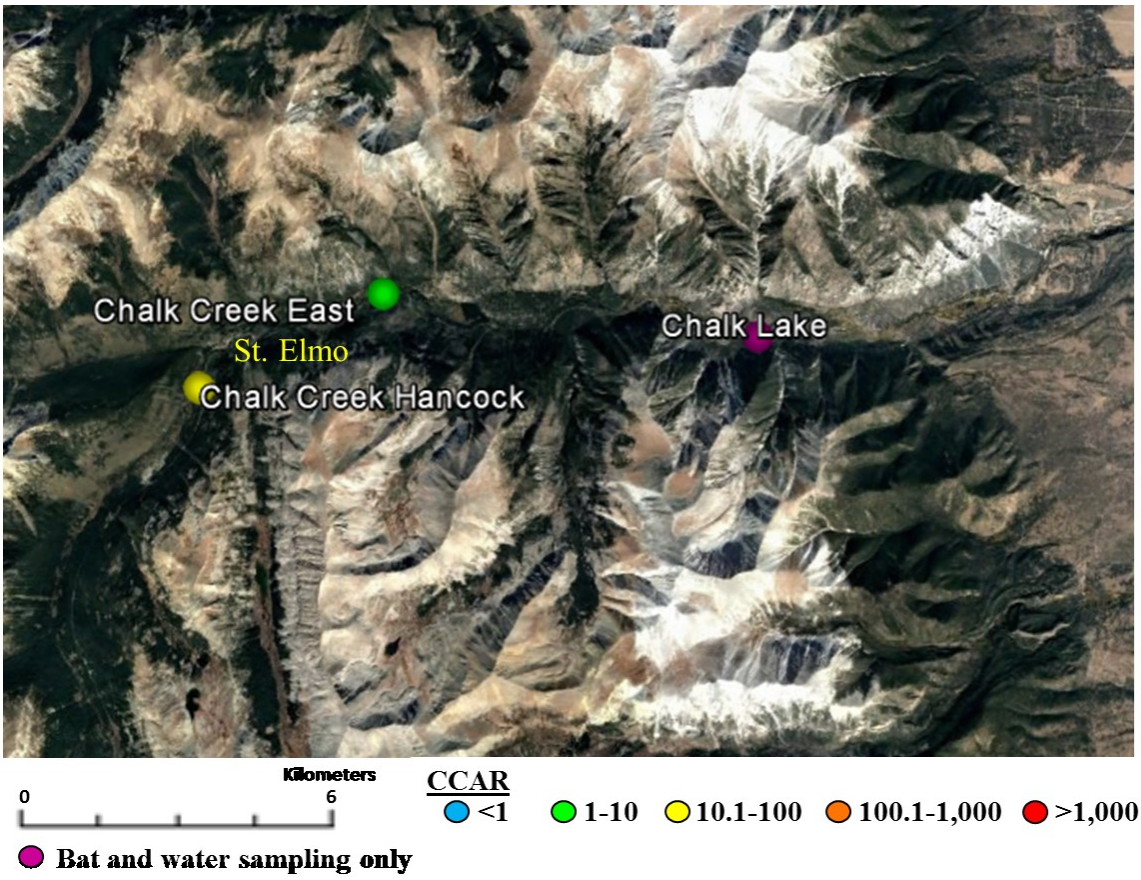


Fig. 30. Sampling sites in Chaffee County. (Map source: Google Earth 2016)

Water

Although water testing was done at most selected sites from 2003 to 2007 by Schmidt et al. (2013), testing was repeated to confirm that contamination levels had not changed. This sampling was concurrent with the period of biotic sampling. To have all necessary data for the CCAR model, pH, temperature, and conductivity were measured in-stream. Water samples were collected by dip method from calm portions of streams approximately 1 m from shore. They were sent to the USGS Denver (Colorado) lab to determine heavy metal concentrations and to the Colorado School of Mines' Advanced Water Technology Center (Golden, Colorado) for dissolved oxygen content. All water quality values were provided to the Aquatic Systems Branch of USGS, Fort

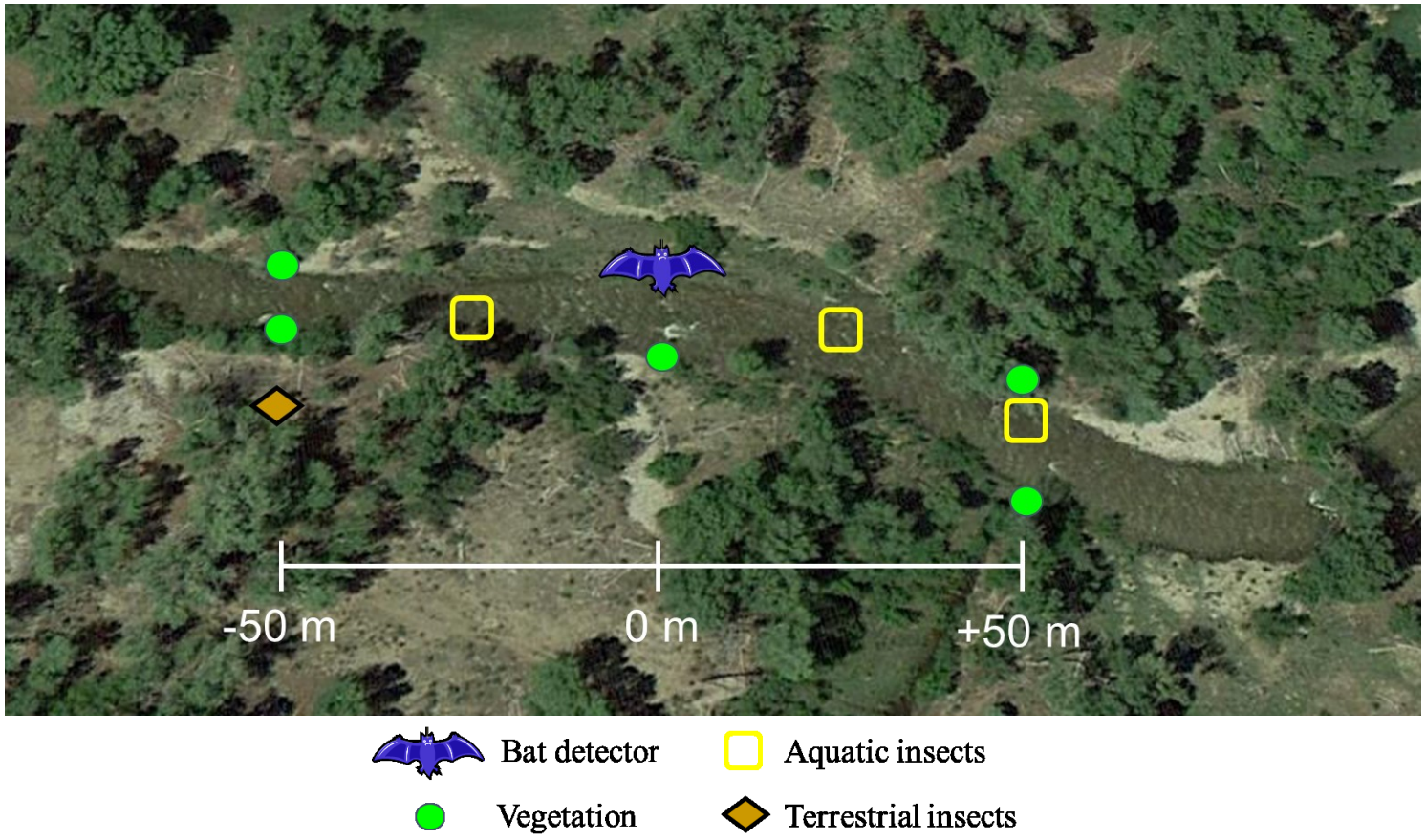


Fig. 31. Plot design for stream sampling in the Colorado Mineral Belt. The site pictured is along Chalk Creek, east of St. Elmo in Chaffee County.

Collins, who calculated the CCAR values. Channel widths at the bat detector, 25 m upstream, and 25 m downstream were also measured to obtain an estimate of surface area in the sampled reach.

An additional non-paired site, Chalk Lake (a 150 x 65-m body of water 6.8 km downstream of the Chalk Creek East site), was sampled for water quality because relatively higher bat activity for the area (i.e. three or four individuals seen at one time) was noted on consecutive nights.

Bat Activity

Recording. SM2-Bat+ detectors (Wildlife Acoustics, Maynard, Massachusetts) with a single SMX-US microphone per unit were used to sample bat activity. The maximum detectable distance of the SM2-Bat+, as stated by the manufacturer, was 100 m, but atmospheric conditions (humidity, temperature, air pressure, wind), habitat (clutter), and bat call characteristics (frequency, amplitude, direction) were expected to result in some call attenuation (Wildlife Acoustics 2015). Because vegetative clutter can interfere with both detector coverage and bat foraging (Hayes 2000; Wildlife Acoustics 2015), detectors were placed at the edge of a vegetative stand, facing towards open water. At all sites, units were placed within 2 m of the stream edge and at 1.5 m above the stream's surface, with the microphone oriented slightly above the horizontal (Ober and Hayes 2008a). In order to minimize the negative effects that swift-running water can have on bat foraging (Mackey and Barclay 1989) and call detection (Wildlife Acoustics 2015), detectors were placed along relatively calm portions of the stream. Finally, all

microphones were arbitrarily faced downstream to ensure sampling consistency (Ober and Hayes 2008a).

Calls were recorded for at least two consecutive nights per sampling period, with all but one site pair (North and South Fork Lake Creek) sampled for two periods throughout the season (Table 14). Thus, with periods combined, all sites were sampled for at least five nights. SM2s recorded calls in compressed WAC file format with the following settings: gain = +12.0 dB, dig HPH = fs/12, dig LPF = off, Trg Lvl = 18SNR, Trg Win = 2.0s, and a time expansion factor of 8.

Table 14. Nights on which bat calls were recorded concurrently at paired sites.

Site pair	Nights recorded (2014)
North Fork Lake Creek South Fork Lake Creek	9/7, 9/8, 9/9, 9/10, 9/11, 9/12
North Fork South Platte River Handcart Gulch	8/11, 8/12, 9/22, 9/23, 9/24
West Tennessee Creek St. Kevin Gulch	8/16, 8/17, 9/8, 9/9, 9/10, 9/11, 9/12
Sts. John Creek Snake River	8/31, 9/1, 9/2, 9/3, 9/4, 9/5, 9/14, 9/15, 9/16, 9/17, 9/18, 9/19
Deer Creek Peru Creek	8/31, 9/1, 9/2, 9/3, 9/4, 9/5, 9/14, 9/15
Chalk Creek East Chalk Creek Hancock	8/23, 8/24, 9/29, 9/30, 10/1, 10/2

Post-processing. WAC files were later decompressed into WAV format using Kaleidoscope software v. 4.0.0 (Wildlife Acoustics, Maynard, Massachusetts) (split to max duration = 5 s, time expansion ratio = 1). SM2 calls were additionally post-processed using SonoBat SM2 Batch Attributor with the SMX-UT button on, according to manufacturer recommendations to adjust for SMX-US microphone recordings. So as to include as many bat files as possible, no files were scrubbed. Call visualization and structure analysis were conducted using Sonobat 3.1 (U.S. West, Arcata, California).

Activity was measured as the number of bat passes per night (from sunset to sunrise). Fenton (1980) described a pass as a sequence of one or more echolocation pulses with at least a one-second pause before the next sequence. The sound files output by SonoBat generally met this definition, such that each file represented a distinct pass. However, for the few cases in which SonoBat parsed recordings into files less than one second apart, I only counted one of the files as a pass.

The presence or absence of "feeding buzzes" (*sensu* Griffin et al. 1960) on any recorded night at a site was used to establish a dietary link to the riparian area (Kalko 1995). Bats increase the rate of their echolocation calls as they change from searching for a prey item to approaching and finally capturing. Griffin et al. (1960) described this as a change in pulse rate from about four or five pulses per second at 200-ms intervals to a pulse rate of one every five milliseconds. These changes were visible on a sonogram and could be heard when a call was slowed down by time-expansion software (SonoBat, in this case). Feeding buzzes were only counted when both a visible increase in pulse rate and an auditory "buzz" were detected.

Species Identification. To identify species, calls were first run through SonoBat's automated classification function, using default parameters. However, no clear identifications were elicited. Based on geographic location, habitat, and elevation, most bats were expected to be of the *Myotis* genus (Armstrong et al. 1994; Adams 2003), whose calls can be difficult to distinguish from one another due to similarities in call characteristics (Ober and Hayes 2008b; Scott et al. 2010). Frick (2013) also noted that the ability of the SM2-BAT+ to provide call recordings of high enough quality for species identification is limited at the edge of the microphone's range due to the signal to noise ratio, although these recordings are still sufficient to measure overall bat activity.

Thus, in the absence of strong species identifications, I obtained parameter measurements of each call using Sonobat (see Chapter II for more details) and classified calls as belonging to species with a high end frequency (~45 kHz) or a low end frequency (~25 kHz).

Insects

Insect traps were deployed at all sites to measure aquatic and terrestrial prey densities. For aquatic insects, methods followed those of Schmidt et al. (2013), using 1 m^2 plastic and mesh emergent traps with a removable plastic bottle attached at the apex. Three traps were set at each site: one 25 m upstream, one 25 m downstream, and one 50 m downstream of the bat detector (Fig. 31). Traps were placed over slack areas of water along the stream edge to maximize capture of emergent biomass (Iwata 2003). They were deployed for four nights, after which insects were removed using a plastic aspirator,

placed in acid-washed plastic containers, and stored on ice. As soon as possible, insects were transferred to a -20°C freezer.

Terrestrial insects were sampled to clarify their availability relative to aquatic insect prey. A CDC mini-light trap (attached to a six-volt battery and small solar panel) (BioQuip Products, Inc., Rancho Dominguez, CA) was deployed 50 m upstream from the bat detector and 10 m away from and perpendicular to the stream (Fig. 31). The trap was placed in habitat similar to that of the bat detector and at these distances from the stream in an attempt to lessen bias associated with potentially attracting bats or diminishing available prey. The side of the stream on which the trap was placed was determined by a coin flip, unless difficult terrain (for example, a steep, rocky slope) or lack of site access dictated placement on a particular side. Terrestrial insects were removed and stored as described above for aquatic insects.

Members of the Aquatic Systems Branch at USGS, Fort Collins identified insects to family and often genus with use of a compound microscope and insect key. Insects from separate taxonomic groups were then dried and weighed by site for biomass estimates. Emergence was calculated for aquatic insects as mg dry mass$\cdot m^{-2} \cdot d^{-1}$ (Kraus et al. 2014), then averaged over the three mesh traps per site.

Total productivity of each 50-m reach was extrapolated by multiplying average fluxes from the three m^2 traps by estimated channel surface area (as mentioned above). With this calculation, I held the assumption that all sections of the stream were equally productive. However, because traps were placed in what were assumed to be some of the most productive areas, these values should be considered maximum estimates.

Vegetation

Bat activity can be heavily influenced by local vegetative structure and composition (Brigham et al. 1997; Kusch et al. 2004; Ober and Hayes 2008a; Malison and Baxter 2010). To account for this, tree and shrub species richness and percent canopy cover were determined at all sites within 50 m upstream and 50 m downstream of the bat detector and 5 m upland from both stream edges (for a total of 250 m^2 on either bank and 500 m^2 per site) (Fig. 31). Percent canopy cover was measured using a spherical densiometer at nine pre-determined points within each plot, and the measurements were then averaged per site. (See Fig. 31 for the standard configuration used at all locations. Mid-stream measurements (not depicted) were also taken adjacent to the bat detector, 50 m upstream, and 50 m downstream.) A second approximation of the degree of vegetative clutter was made for each plot by measuring the area of tree and shrub cover (both dead and alive) at breast height (~1.5 m from the ground).

Statistical Analysis

Analyses were conducted in R statistical package v.3.3.1 (R Core Team 2013). Normality was tested with Shapiro-Wilk and Q-Q plots, and homogeneity of variance with Fligner-Killeen (which is suitable for non-parametric data). Because data were non-normal (see Results), a Wilcoxon signed-rank test was used to assess whether mean number of bat calls from relatively clean sites was different from that of bat calls from relatively dirty sites. The same test was performed without Chalk Creek East and Chalk Creek Hancock because the difference between their CCAR values (5.58) was much smaller than that of the other pairs (min. difference = 361.08, max. difference =

3,605.30), and it was worth examining whether the magnitude of difference had an effect. Wilcoxon signed-rank does not require the data from two populations ("clean") and ("dirty") to be normally distributed or to have equal variances, but it does assume that the difference between pairs is symmetrical about the median (Zar 2010). This was assessed with the symmetry test by Miao, Gel, and Gastwirth (2006) in R. (Paired-sample *t* tests, as opposed to Wilcoxon signed-rank tests, were not performed because normality of the differences between pairs could not be confirmed. While Shapiro-Wilk showed the distribution to be normal ($P = 0.071$), the Q-Q plot showed a positive skew.)

Factor relationships were tested using linear mixed models. CCAR and aquatic insect biomass were the main indespendent variables, while percent canopy cover, percent woody shrub cover, effect of blocking (as a random factor), and factor interactions were also included in different iterations. The average number of bat calls per night was the dependent variable in each case.

Spearman's rank correlations ($\alpha = 0.05$) also were used to test the relationships between bat activity and feeding attempts, bat activity and CCAR, aquatic insect biomass flux and CCAR, flux of aquatic insect numbers and CCAR, and bat activity and aquatic insect biomass flux across all sites. Because sampling was conducted over two separate periods at most sites throughout the season, the correlation between the average number of bat calls per night and emergent insect biomass flux also was tested separately for each period. Finally, average nightly number of bat calls was tested against stream calcium concentration *post-hoc* because pregnant and lactating females, in particular, rely on calcium to produce milk and support growth of developing young, as discussed above (Adams et al. 2003).

Results

Insects

Insect biomass flux per site, averaged over each set of three traps, ranged from 0.10 - 6.62 g/m^2/day, with an across-site average of 2.50 g/m^2/day (Table 15). Highest biomass emergence came from West Tennessee Creek (CCAR = 0.46) and lowest from South Fork Lake Creek (CCAR = 3,605.66). Insect flux by number ranged from 1.40 - 96.41 individuals/m^2/day, with Tennessee Creek again having the highest and South Fork Lake Creek the lowest. Overall average number was 28.30 individuals/m^2/day.

Accounting for the surface area of each 50-m stretch, average biomass flux per site ranged from 17.95 - 1,566.74 g/day, with an across-site average of 633.92 mg/day (Table 15). From this perspective, the most productive site was North Fork Lake Creek (1,566.74 mg/day, CCAR = 0.36) and the least productive was Peru Creek (17.95 mg/day, CCAR = 3494.48). Chalk Creek east had the greatest emergence by number of individuals (34,844.44 individuals/day, CCAR = 6.84), while Peru Creek had the least (311.44 mg/day, CCAR = 3,494.48). Reach biomass flux had a significant negative correlation with CCAR (r_s = -0.7972, P = 0.002, Fig. 32). Reach flux of individuals also correlated negatively with CCAR, though the relationship was not significant nor as strong (r_s = -0.559, P = 0.059, Fig. 33).

When not separated by sampling period, chironomids (Diptera) accounted for 44% of total insect biomass flux across sites (28.11 of 65.25 mg/night/m^2). Remaining flux was composed of 18% trichopterans (Lepidostomatidae, Limnephilidae, Hydroptilidae, and Rhyacophilidae) (11.53 mg/night/m^2), 16% other dipterans (Empididae, Culicida, Simuliidae, Ceratopogonidae, Blephariceridae, and Tipulidae)

Table 15. Aquatic emergent insect fluxes.

Site	CCAR	Channel area over 50-m length (m^2)	Average insect biomass flux over 3 traps ($g/m^2/day$)	Insect biomass flux over reach (g/day)	Average insect number flux over 3 traps ($\#/m^2/day$)	Insect number flux over reach (#/day)
NF Lake Cr	0.36	316.65	4.95	1566.74	62.07	19653.51
W. Tenn Cr	0.46	202.50	6.62	1340.52	96.41	19523.70
Deer Creek	0.73	289.56	2.85	826.28	5.83	1687.60
NF S Platte G	3.32	270.01	0.79	213.76	6.36	1716.79
Chalk Cr E	6.84	666.67	1.98	1322.07	52.27	34844.44
Chalk Cr Han	12.42	269.24	3.11	837.57	39.12	10533.27
Sts. John	14.71	112.27	1.80	201.89	20.14	2261.58
Snake River	375.79	251.97	0.84	211.62	15.59	3927.34
Handcart G	902.86	187.79	4.12	772.94	14.99	2815.24
St. Kevin G	1737.28	105.00	2.45	257.18	22.96	2410.80
Peru Creek	3494.48	129.17	0.14	17.95	2.41	311.44
SF Lake Cr	3605.66	391.16	0.10	38.47	1.40	547.62
Overall Average		266.00	2.50	633.92	28.30	8352.78

(10.06 mg/night/m^2), 13% ephemeropterans (Baetidae, Heptageniidae, Leptophlebiidae, and Ameletidae) (8.49 mg/night/m^2), and 11% plecopterans (Chloroperlidae and Perlodidae) (7.06 mg/night/m^2). Separated by site, chironomids always accounted for at least 50% of insect numbers and typically more than 90%.

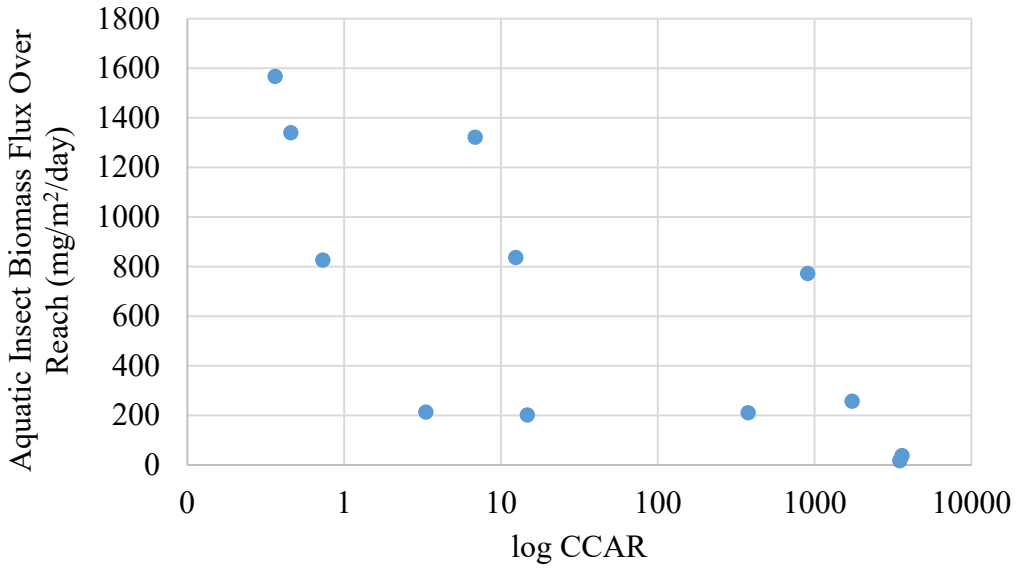


Fig. 32. Relationship between aquatic insect biomass flux (over each 50-m reach) and CCAR. r_s = -0.7972, P = 0.002.

Terrestrial trap deployment was hindered by inclement weather, theft, and vandalism. Cloudy weather, for example, prevented solar panels from charging uniformly across sites, so capture efforts could not always be accounted for. Because of this, terrestrial insect biomasses and numbers were not calculated. When traps were known to work, however, they were almost always void of captures, signifying that terrestrial prey availability was generally low.

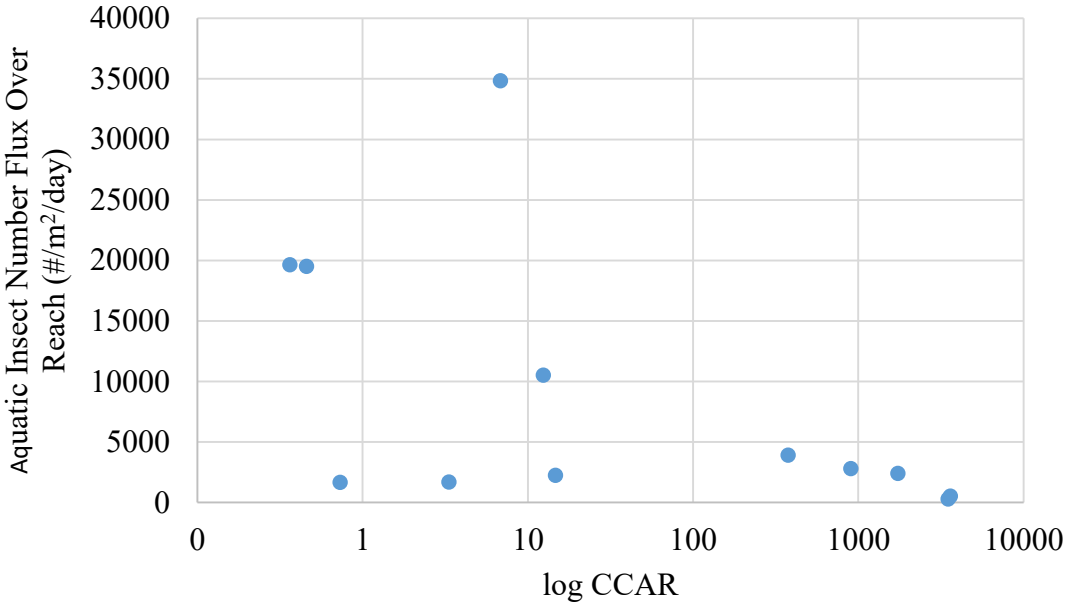


Fig. 33. Relationship between aquatic insect number flux (over each 50-m reach) and CCAR. r_s = -0.5590, P = 0.059.

Bat Activity

Bat calls were recorded on 60 of 88 detector-nights (Table 14), with a total of 1,875 calls and 13 feeding buzzes detected throughout the season. Mist netting at Chalk Lake was not successful due to consistently windy conditions. Both the Shapiro-Wilk test (P < .001) and a Q-Q plot indicated that the call data were not normal, but the assumption of homogeneity of variance was met (Fligner-Killeen P = 0.067). Attempts to transform the data (log, square root, and optimal lambda = 0.1, with zeros replaced with 0.5) improved normality but not to the point of significance. Average nightly number of feeding buzzes and average nightly number of calls were strongly positively correlated (r_s = 0.756, P = 0.004, Fig. 34). (A "strong" correlation was defined as r_s > 0.5, per Cohen (1992).)

The Wilcoxon signed-rank test showed no significant difference in the average number of bat calls from relatively clean sites and that from relatively dirty sites (P = 0.688). There also was no relationship between average nightly number of calls and CCAR across all sites and sampling dates (r_s = -0.042, P = 0.899, Fig. 35). When separated into two groups by sampling period (mid-season versus late), the relationship was significantly positive later in the season (8 Sep.-3 Oct.) (r_s = 0.677, P = 0.016, Fig. 36), but this was not the case for mid-season (11 Aug.-Sep. 13) (r_s = -0.247, P = 0.442, Fig. 36). Likewise, the correlation between average number of feeding buzzes per night and CCAR was not significant (r_s = -0.172, P = 0.594, Fig. 37). However, despite a low overall number of feeding buzzes, most buzzes were detected at a CCAR below 10. The highest number of calls and buzzes were detected at the North Fork of the South Platte; bat activity late in the season was 2.76 times greater than at the next most active site, Peru Creek. There was no relationship between average nightly bat activity and water calcium concentration (r_s = 0.98, P = 0.763).

For the linear model, spatial blocking was a significant factor in explaining call activity (α = 0.05, F = 4.6683, P = 0.0335), but level of contamination was not (α = 0.05, F = 1.0556, P = 0.3071). Due to limited sample size, there were not enough degrees of freedom to test for interactions nor to compare multiple linear models for best fit to the data.

Species. 99% of all passes recorded belonged to small clutter specialists of the *Myotis* genus (with a characteristic end frequency of 45 kHz). 1% of calls came from larger bats (with a characteristic end frequency of 25 kHz), potentially *Eptesicus fuscus*, *Lasiurus cinereus*, or *Lasionycterus noctivagans*. These latter calls were detected at a

number of sites throughout the sampling period, with no clear pattern of visitation: St. Kevin Gulch (Aug. 17 and Sep. 11), Sts. John Creek (Sep. 1 and 17), North Fork Lake Creek (Sep. 12), and Snake River (Sep. 19).

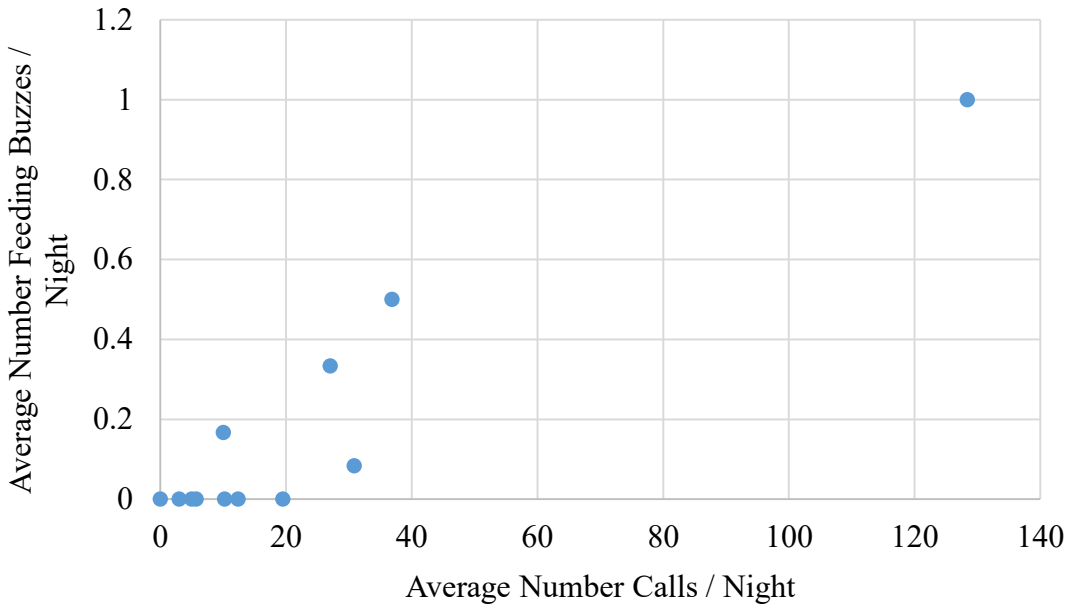


Fig. 34. Relationship between average number of calls per night and average number of feeding buzzes per night. $r_s = 0.756$, $P = 0.004$.

Vegetation

The number of woody species at sites ranged from zero to five (Table 18). Handcart Gulch had the greatest species richness, while North Fork Lake Creek had zero because no woody species were rooted in its plot area. Where percent canopy cover was substantially higher than bank area coverage, this indicated either 1) canopy hanging over the plot from nearby areas, or 2) thinner trees with relatively large canopies.

There was no relationship between average percent canopy cover and bat activity ($r_s = -0.133$, $P = 0.676$), nor between percent bank area coverage and bat activity ($r_s = 0.465$, $P = 0.128$).

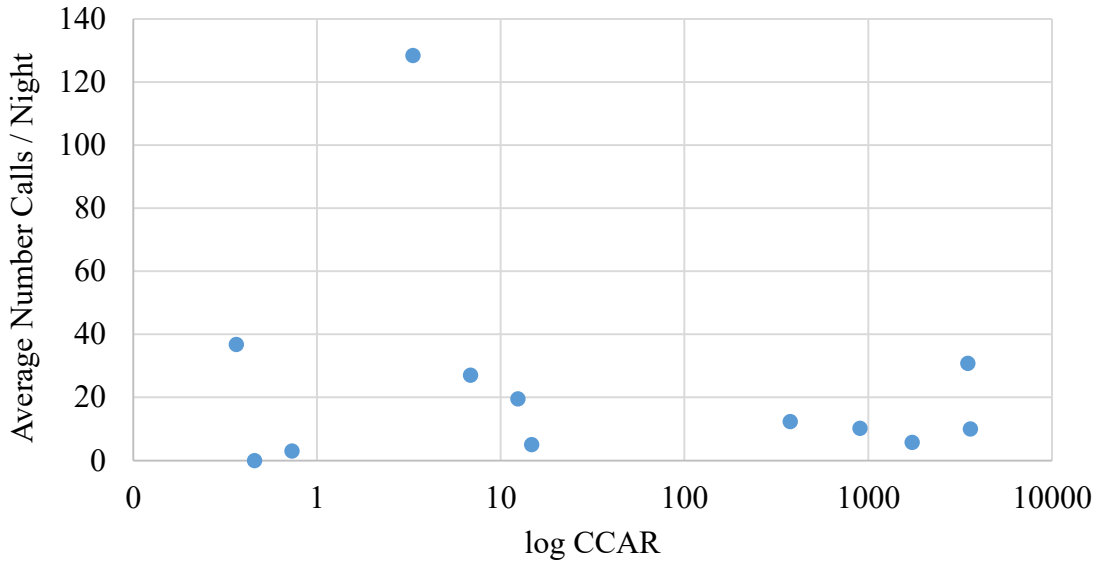


Fig. 35. Relationship between average number of calls per night and CCAR for all sampling dates. The highest number of calls was detected at the North Fork of the South Platte. r_s = -0.042, P = 0.899.

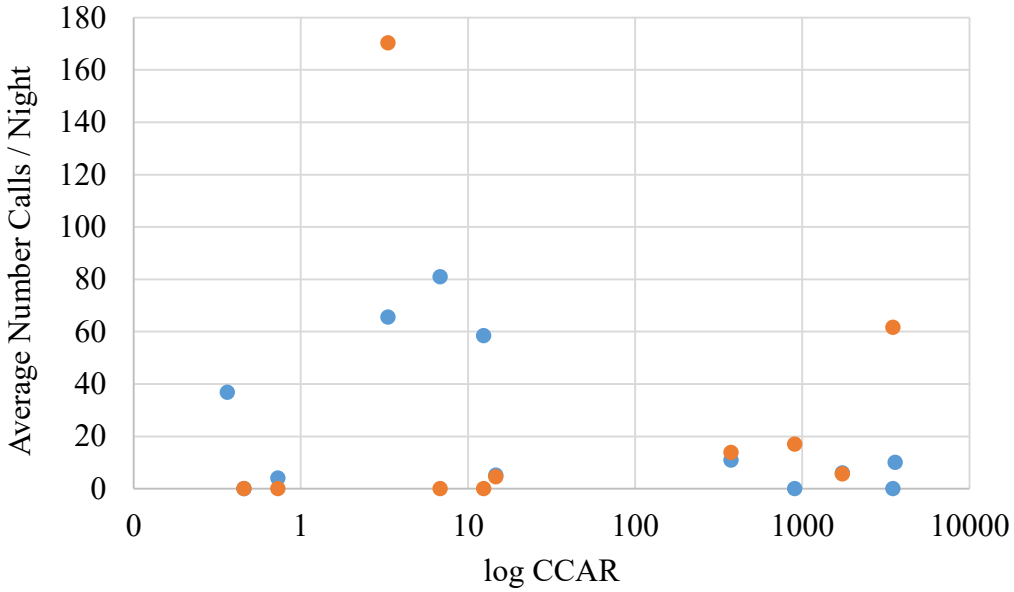


Fig. 36. Relationship between average number of bat calls per night and CCAR, separated into mid-season (blue dots, 11 Aug.-13 Sep.) and late season (orange dots, 8 Sep.-3 Oct.). The highest number of calls late in the season was detected at the North Fork of the South Platte. Mid-season r_s = -0.247, P = 0.442. Late season r_s = 0.677, P = 0.016.

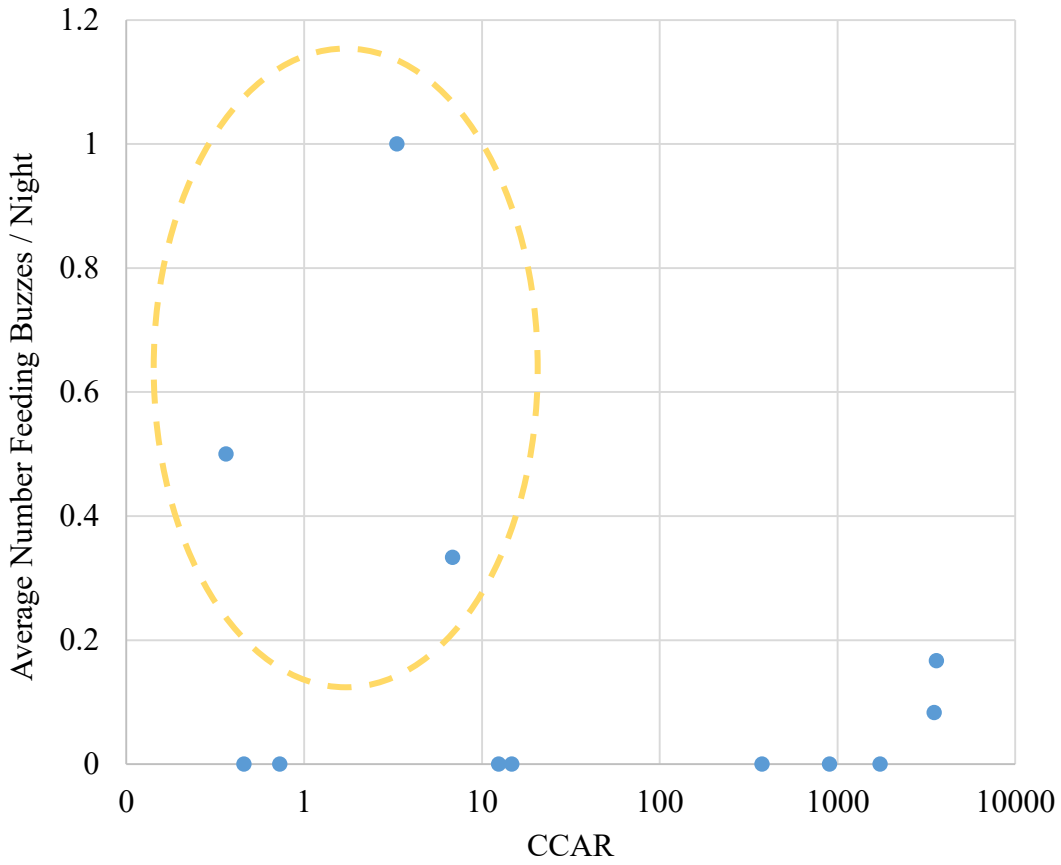


Fig. 37. Relationship between average number of feeding buzzes per night (normalized by sampling effort) and CCAR. While the correlation was not significant (r_s = -0.172, P = 0.594), most feeding buzzes were detected at a CCAR below 10.

Table 16. Site vegetative characteristics. Percent canopy cover was averaged across nine points per site (see Methods). Percent bank area coverage was the additive area of woody species measured at diameter breast height, as a percentage of 500 m^2 (the total bank area accounted for in each plot).

Site	Woody species	Average % canopy cover	% bank area coverage (at dbh)
North Fork Lake Creek	None	3	0
South Fork Lake Creek	*Dasiphora fruticosa* *Picea engelmannii* *Pinus flexilis* *Salix* spp.	19	1.185
North Fork South Platte River	*Abies lasiocarpa* *Picea engelmannii* *Salix* spp.	82	73.457
Handcart Gulch	*Abies lasiocarpa* *Picea engelmannii* *Pinus flexilis* *Populus tremuloides* *Salix* spp.	91	35.866
West Tennessee Creek	*Betula nana* *Pinus flexilis* *Salix* spp.	26	1.1765
St. Kevin Gulch	*Picea engelmannii* *Pinus flexilis* *Salix* spp.	51	3.6562
Sts. John Creek	*Picea engelmannii* *Salix* spp.	31	0
Snake River	*Betula nana* *Picea engelmannii* *Salix* spp.	29	0.001
Deer Creek	*Picea engelmannii* *Salix* spp.	87	0
Peru Creek	*Betula nana* *Pinus flexilis* *Salix* spp.	29	3.962
Chalk Creek East	*Alnus tenuifolia (?)* *Picea engelmannii* *Salix* spp.	51	5.199692438
Chalk Creek Hancock	*Picea engelmannii* *Salix* spp.	22	2.355611796

Discussion

Aquatic Insect Flux

Emergence of aquatic insects declined significantly with increasing stream metal contamination in terms of biomass (r_s = -0.7972, P = 0.002) but not numbers (r_s = -0.559, P = 0.059). Thus, though not a hypothesis of this study, the capacity of the CCAR model to predict emergent flux in terms of biomass was upheld.

Based on the availaibility of aquatic insects alone then, feeding opportunities would have been lower above more contaminated streams. The minimum dietary requirement for a 9-g *Myotis lucifugus* is about 2 g (wet weight) of prey per night (Anthony and Kunz 1997). The median biomass flux of emergent insects over a 50-m stretch in this study was 515 mg/night, which would meet 25% of one bat's nightly dietary requirement, assuming it could encounter and capture all available insects. The minimum emergence from the dirtiest streams was 18 mg/night (Table 17), which would meet only 1%, while maximum flux (from cleaner streams) was 1,567 mg/night, which would meet 78%. This is potentially a large difference for a species that, although highly mobile, would need to exert a substantial amount of energy in flight (Speakman and Racey 1989) searching for better resources.

Chironomid flies (Diptera) were the dominant taxon at all streams, which also was in line with prior studies of the area (Schmidt et al. 2013; Kraus et al. 2014). Because chironomids have a relatively high tolerance to metal pollution (Schmidt et al. 2013), this could have indicated that even streams considered to be relatively clean in this study were not pristine, as is often the case in human-dominated landscapes (Scott et al. 2010).

Bat Activity

The lack of difference in call activity and number of feeding buzzes between cleaner and dirtier sites, as well as the lack of correlation between average nightly number of calls and CCAR, was contrary to my hypotheses. These results indicated that bats were foraging evenly across sites. It seems likely that additional factors, such as the amount of open water available on the landscape and more profitable foraging activities at lower elevations (both discussed below) had a greater influence on bat presence than degree of stream contamination. The differing relationships between bat activity and CCAR in August-September (negative, insignificant linear correlation) versus September-October (positive, significant linear correlation) also indicated a seasonal component to bat habitat use, as did the significance of the blocking factor alone in the linear model.

At the elevations sampled (>2,900 m), bats detected were most likely males and non-reproductive females because reproductive females, being constrained by the energetic costs of pregnancy and lactation, tend to remain at lower elevations where temperatures are higher and insects more available (Barclay 1991; Grinevitch et al. 1995; Cryan et al. 2000). Willey (2010) found a significant drop in the number of reproductive females captured above 2,285 m in the Front Range of Colorado. In that study, males of an unknown species were netted as high as 3,371 m, at the Rocky Mountain Research Station in Boulder County, while no adult females were caught above 2,500 m (Willey 2010). This dominance of males and non-reproductive females at high elevations could have explained the lack of relationship between bat activity and calcium concentration, as lactating females are the ones who most covet this resource (Adams 2003).

Of possible interest was the fact that the majority of feeding buzzes came from sites with a CCAR <10. This conclusion was not strong because of low sample size and high variance in the data. However, a possible implication is that a CCAR ≈ 10 is an important threshold above with emergent insect flux is insufficient to draw bats to an aquatic resource in this system.

Bat species. The vast majority (99%) of bats detected in this study were small, clutter specialists (e.g., *Myotis* genus). Nine bat species occur along the Front Range of Colorado: Townsend's big-eared bat (*Corynorhinus townsendii*), silver-haired bat (*Lasionycterus noctivagans*), hoary bat (*Lasiurus cinereus*), western small-footed myotis (*Myotis ciliolabrum*), little brown myotis (*M. lucifugus*), long-eared myotis (*M. evotis*), fringed myotis (*M. thysanodes*), long-legged myotis (*M. volans*), and big brown bat (*Eptesicus fuscus*). Based on the calls recorded in this study and regional records (discussed below), the species most likely to be present were the little brown bat (*Myotis lucifugus*) and long-legged myotis (*Myotis volans*), though small-footed myotis (*Myotis ciliolabrum*) and western long-eared myotis (*Myotis evotis*) were also possible. *Myotis thysanodes* is excluded from this list because it tends to reside at "moderate" elevations and has not been recorded above 8,000 ft. (2,438 m) (Armstrong et al. 1994; Adams 2003).

Myotis lucifugus is an aerial insectivore that forages in clearings and directly over water to capture both flying emergent insects and those settled on the water's surface (Belwood and Fenton 1976; Anthony and Kunz 1977; Fenton and Bell 1979; Barclay 1991; Armstrong et al. 1994; Adams 1997). While considered to be more of an aquatic insect specialist than other species in the area, it also forages on moths (Armstrong et al.

1994). Though typically present in foothills and lower montane areas, the species has been recorded as high as 11,000 ft. (3,353 m) in Lake County (nine miles southwest of Leadville) (Armstrong et al. 1994). Hibernation occurs in caves and mines (Armstrong et al. 1994; Adams 2003).

Myotis volans can occupy a wide range of elevations in coniferous woodland and forest and has been recorded as high as 11,450 ft. (3,490 m) in Teller County, Colorado (Armstrong et al. 1994). While known to forage over water, the species tends to prefer moths (Freeman 1984, Armstrong et al. 1994). It hibernates in caves and mines (Armstrong et al. 1994; Adams 2003).

Myotis ciliolabrum occurs at low to moderate elevations, with the highest occurrence in Colorado recorded at 9,500 ft. (2,896 m) in the La Plata Mountains (La Plata County) (Armstrong et al. 1994; Adams 2003). Individuals can occupy a wide range of habitats, including rocky outcrops, grassland, woodland, and shrubland (Robbins et al. 1977; Armstrong et al. 1994) and primarily pursue flies and small beetles 1-6 m above the ground (Armstrong et al. 1994). Small-footed myotis reside in Colorado year-round, hibernating in caves, mines, and tunnels in the winter (Armstrong 1972; Armstrong et al. 1994).

The western long-eared myotis (*Myotis evotis*) resides in coniferous woodland and forest and, by means of slow, maneuvable flight, is able to glean insects from trees and other terrestrial surfaces in relatively dense habitats (Armstrong et al. 1994; Adams 2003). The highest record of this species in Colorado was recorded at 8,500 ft. (2,591 m) (Armstrong et al. 1994), though it has been noted as high as 9,500 ft. (2,895 m) in Utah (Mollhagen and Bogan 1997). Individuals are not known to hibernate in Colorado and

are therefore assumed to be migratory, though their destination is unknown (Armstrong et al. 1994). Like *M. lucifugus*, *M. evotis* may take advantage of aquatic insects but seems to forage more broadly over terrestrial paths, favoring moths (Barclay 1991).

Of the above species, *M. lucifugus* has the strongest preference for aquatic insects and therefore the potential to be most affected by reductions in aquatic insect biomass relative to other bat species. However, because *M. lucifugus* is not a strict dietary specialist and also commonly feeds on moths and other terrestrial insects (Armstrong et al. 1994), it could have readily switched prey. Aside from lack of fidelity to a specific prey type, the low bat activity near sampled streams could have been due to the presence of more profitable foraging areas within easy flying distance. The bats detected at Chalk Creek East, for example, were less than 7 km from Chalk Lake, a calmer and more open body of water. In habitats where freshwater is more limited, activity would predictably be more concentrated over individual water bodies, and contamination could have a larger effect on bats (Korine et al. 2016). The results of the present study, however, indicate that bats at high elevations (>2,900 m) within the Colorado Mineral Belt are not greatly affected by reductions in aquatic insect prey due to heavy metal contamination of streams, at least in late summer and early fall.

Limitations

There were a number of challenges inherent in studying bats in a high-elevation system. Because both bat and insect activity tend to be positively correlated with temperature and negatively correlated with precipitation (Erickson & West 2002; Parsons

et al. 2003), it was difficult to obtain a large number of bat calls. Adverse weather also limited success of terrestrial trap deployment and bat mist netting.

It would have been desirable to place more than one detector at a site for longer periods of time throughout the season so as to increase the number of calls and account for both horizontal and vertical bat activity (Hayes 2000). Bat species are known to stratify their foraging efforts vertically, with some individuals regularly foraging above the canopy (Kalcounis et al. 1999). Depending on the height of the surrounding canopy, calls could have been difficult to detect at the angle in which I faced my microphone (slightly above the horizontal). This study also would have been well-complimented by bat captures to confirm species presence, abundance, age structure, and sex ratio, and to assess dietary composition and tissue metal concentrations.

CHAPTER V

CONCLUSIONS

This dissertation scratches the surface of the relationship between environmental contaminants and bats and exemplifies some of the difficulties inherent in this area of study. Aside from the typical challenges of studying an elusive, nocturnal, highly mobile animal, identifying the effects of chemical contamination is complicated by the following: 1) Numerous contaminants are likely present and interacting in any given area, resulting in a variety of synergistic effects. 2) New pesticides are developed every year and put into use without good knowledge of their direct and indirect effects on wild animal populations (Sánchez-Bayo 2011). For example, it was only after neonicotinoids were released for general use that they were linked to the decline of native honey bee populations (Henry et al. 2012; Whitehorn et al. 2012). The effects of neonicotinoids on bats have not been evaluated but could potentially be sublethal or indirect (i.e. affect prey availability) (Gibbons et al. 2015). Similarly, many industrial effluents that are likely toxic, such as brominated flame retardants (Darnerud 2003), have not been evaluated for bats. 3) Contamination is often inextricable from other factors known to affect bats, such as urbanization (Russo and Ancillotto 2015). 4) A reliable method for aging bats beyond the first year has not been discovered (Brunet-Rossinni and Wilkinson 2009), which hinders examining accumulation over time. This knowledge is key to understanding

reproductive and long-term survival effects. While mark-recapture, PIT-tagging, and captive colony studies are some ways to get around this, they are relatively time- and energy-intensive. 5) Sublethal effects can be difficult to detect. For example, adverse behavioral effects do not always align with less obvious reproductive effects. Baron et al. (1999) found that rough-winged swallows in particular reaches of the Clinch River/Poplar Creek system in Tennessee were likely exposed to levels of mercury associated with reduced egg production and hatching but did not display obvious behavioral effects.

The fact that studying bats and contaminants is difficult, however, does not negate the value of such research. Bats are an evolutionarily unique taxon, important for helping structure ecological communities (e.g., by aiding in reforestation through seed transfer in tropical systems (Medellín and Gaona 1999) and performing ecosystem services (e.g., by preying on crop pests and saving agricultural industries billions of dollars (Boyles et al. 2011). Bats are also a sensitive group, with an estimated 20% of species threatened or endangered worldwide, and information about nearly as many inadequate for status assessment (IUCN 2015). Yet contaminants have definitive lethal and sublethal effects on bats and likely also increase sensitivity to disturbance and disease (e.g., white-nose syndrome (Kannan et al. 2010)). Despite some advances in determining health effects on bats in the last few decades, particularly at the level of the individual organism, key difficulties still lie in extrapolating individual effects to those of population, via survival and reproduction. So much work remains to be done.

Because of the complexity of assessing contaminant effects on animal populations, it is important to approach the topic from multiple angles. This was an

impetus behind the directions taken in this dissertation, which addressed bats and contaminants from the perspectives of biological uptake and species richness (Chapter II), genetic diversity (Chapter III), and prey availability (Chapter IV).

In the second chapter, I found that 1) the degree of mercury uptake was species-specific for *Nyctalus plancyi* and *Pipistrellus abramus* in Chengdu, China; 2) the smaller, more resident species (*P. abramus*) had the higher mercury levels, with 57% of sampled individuals having fur contaminant concentrations associated with neurochemical changes (Nam et al. 2012); 3) highest mercury concentrations in bat fur came from an agricultural area, followed by a central urban area, and finally by the edge of the city; and 4) bat species richness, as measured by a proxy of call categories detected, was higher in a forested mountain site than in agricultural or urban areas.

In the third chapter, I confirmed the following: 1) All individuals used for the above contaminant studies were indeed *Pipistrellus abramus*; 2) Population structure of *P. abramus* in Chengdu was consistent with that in eastern China. Specifically, there was a high degree of gene flow in and around the city, with a small amount of population structuring, possibly due to male-dominated dispersal, female philopatry, and a home range of 5-10 km in the species. 3) *Pipistrellus abramus* had two mitochondrial clades that overlapped geographically. These clades provided evidence of ancient lineage separation and recolonization, possibly due to Pleistocene glaciation, and, although not tested in the present study, may contribute to differences in the sub-populations' abilities to adapt to physiological stressors.

Finally, the fourth chapter showed that 1) bat activity over more metal-contaminated streams was no different than activity over less-contaminated streams at

high-elevation sites (>2,900 m) in the Colorado Rockies; and 2) there were more foraging attempts over cleaner streams (CCAR <10), though low sample size made this more of a possibility than a strong conclusion.

Together, in addition to existing literature, my data demonstrate that toxic effects are largely influenced by habitat, bat species, and form of contaminant. In China, two common species in the same habitat took up mercury differently, likely due to differences in body size, life history traits (e.g., migratory vs. non-migratory), and possibly genetic predisposition. In the southern Colorado Rockies, where contamination of streams by metals was greatest, bats did not seem to be greatly affected because 1) species likely had more suitable foraging habitat at lower elevations, and 2) the metals involved did not appear to cause acute toxicity to bats at natural exposure levels. Thus, in general, high-elevation mountain habitats may not strongly influence bat health at the individual or population level, despite acid mine drainage negatively affecting emergent insect production. This could, however, be otherwise in drier locations, where water and insects are more concentrated.

The conclusion that direct and indirect effects of contaminants on bats (and other animals) is influenced by traits of the pollutant, the organism itself, and the setting is not novel. However, the value of the data generated in this dissertation are three-fold. First, there has been a movement towards finding generalities in how bat individuals, populations, and species are affected by contaminants (e.g., Baron et al. 1999; Scott et al. 2010; Clare et al. 2014; Hernout et al. 2016b). This is desirable given the enormity of chemical compounds that currently exist and continue to be produced, and the global scale of risk to bat populations. So more information about differing habitats, bat

assemblages, and habitats is needed to make useful predictions. This present study is the first to address contaminant uptake by bats in China and the effects of heavy metal contamination on bats at high-elevation sites (>2,900 m). Second, the data from this study provided a snapshot of conditions to which future studies in similar regions may be compared. Even with the development of generalizations about bat susceptibilities to various contaminants, there will more specific effects based on the characteristics of an individual location and ecological community. Finally, the Colorado portion of this study integrated traditional principles of bat biology (e.g., life history and ecology) with those of biogeochemistry (e.g., metal flux and biotic availability) and aquatic toxicology (e.g., macroinvertebrate assemblages and development). Such collaborations are the minority in contaminant-related studies (Schulz et al. 2015) but will be necessary to draw realistic conclusions about ecological systems beyond the level of an individual species.

Looking forward, it is acknowledged that this dissertation's greatest weaknesses were small sample size and short-term sampling (<3 months of sample collection per project). While small sample sizes are not uncommon in bat contaminant studies (Zukal et al. 2015), this is an important consideration that should be surmounted in future studies to ensure statistical significance and defensibility of results. Longer-term studies have the potential to provide much-needed information about bats through all seasonal stages and, optimally, multiple life stages (e.g., growth and development, reproduction, migration, hibernation, and senescence). Hopefully, with more attention to this topic, future research can be improved in these regards.

LITERATURE CITED

Adams, R. A. 1997. Onset of volancy and foraging patterns of juvenile little brown bats, *Myotis lucifugus*. J. Mammal. 78:239-246.

Adams, R. A. 2003. Bats of the Rocky Mountain West: natural history, ecology, and conservation. Univ. Press of Colorado, Boulder, CO.

Adams, R. A., and S. M. Bexell. 2011. Year of the bat: estimates of urban and rural bat species presence and educational outreach in Sichuan Province, China. 41st Annual Meeting of the North American Society for Bat Research. Toronto, Canada.

Adams, R. A., S. C. Pedersen, K. M. Thibault, J. Jadin, and B. Petru. 2003. Calcium as a limiting resource to insectivorous bats: can water holes provide a supplemental mineral source? J. Zool. Lond. 260:189-194.

Adams, R. A., and M. A. Hayes. 2008. Water availability and successful lactation by bats as related to climate change in arid regions of western North America. J. Anim. Ecol. 77:1115-1121.

Agency for Toxic Substances and Disease Registry (ATSDR). 2005. Toxicological profile for zinc. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Agency for Toxic Substances and Disease Registry (ATSDR). 2012. Toxicological profile for cadmium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Allendorf, F. W., and G. H. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, MA.

Altringham, J. D. 2011. Bats: from evolution to conservation, 2nd ed. Oxford University Press, New York.

Andriollo, T., Y. Naciri, and M. Ruedi. 2015. Two mitochondrial barcodes for one biological species: the case of European kuhl's pipistrelles (Chiroptera). PLoS One 10:e0134881.

Anthony, E. L. P., and T. H. Kunz. 1997. Feeding strategies of the little brown bat, *Myotis lucifugus*, in southern New Hampshire. Ecology 58:775-786.

Armstrong, D. M. 1972. Distribution of mammals in Colorado. Monograph, U. Kansas Museum of Nat. Hist. 3:1-415.

Armstrong, D. M., R. A. Adams, and J. Freeman. 1994. Distribution and ecology of bats of Colorado. Nat. Hist. Inventory of Colorado, Univ. of Colorado Museum 15:1-83.

Australian Government, Department of the Environment. 2015. Fuel quality standards. http://www.environment.gov.au/node/13512. Accessed September 13, 2015.

Avila-Flores, R., and M. B. Fenton. 2005. Use of spatial features by foraging insectivorous bats in a large urban landscape. J. Mammal. 86:1193-1204.

Barclay, R. M. R. 1991. Population structure of temperate zone insectivorous bats in relation to foraging behavior and energy demand. J. Anim. Ecol. 60:165-178.

Barlow, K. E., and G. Jones. 1997. Differences in songflight calls and social calls between two phonic types of the vespertilionid bat *Pipistrellus pipistrellus*. J. Zool. 241:315-324.

Baron, L. A., B. E. Sample, and G. W. Suter II. 1999. Ecological risk assessment in a large river-reservoir: 5. Aerial insectivorous wildlife. Environ. Toxicol. Chem. 18:621-627.

Bates, P. J. J., and D. L. Harrison. 1997. Bats of the Indian Subcontinent. Harrison Zoological Museum, Sevenoaks, Kent, U.K.

Bates, P., and K. Tsytsulina. 2008. *Pipistrellus abramus*. The IUCN Red List of Threatened Species. Version 2014.3.c. Accessed January 11, 2015.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biol. 50:201-220.

Bayat, S., F. Geiser, P. Kristiansen, and S. C. Wilson. 2014. Organic contaminants in bats: trends and new issues. Environ. Int. 63:40-52.

Beernaert, J., J. Scheirs, H. Leirs, R. Blust, and R. Verhagen. 2007. Non-destructive pollution exposure assessment by means of wood mice hair. Environ. Pollut. 145:443-451.

Bello-Gutiérrez, J., G. Suzán, M. G. Hidalgo-Mihart, and G. Salas. 2010. Alopecia in bats from Tabasco, México. J. Wildlife Dis. 46:1000-1004.

Belwood, J. J., and M. B. Fenton. 1976. Variation in the diet of *Myotis lucifugus* (Chiroptera: Vespertilionidae). Can. J. Zool. 54:1674-1678.

Benton, A. H. 1951. Effects on wildlife of DDT used for control of Dutch Elm disease. J. Wildlife Manage. 15:20-27.

Beyersmann, D., and A. Hartwig. 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch. Toxicol. 82:493-512.

Biological Monitoring Working Party. 1978. Final report: assessment and presentation of biological quality of rivers in Great Britain. Unpublished report. D.o.E. Water Data Unit.

Black, H. L. 1974. A north temperate bat community: structure and prey populations. J. Mammal. 55:138-157.

Blair, R. B. 2001. Birds and butterflies along urban gradients in two ecoregions of the U.S. Pp. 33-56 *in* J. L. Lockwood, and M. L. M. McKinney, eds. Biotic homogenization. Kluwer, Norwell, MA.

Blake, D., A. M. Hutson, P. A. Racey, J. Rydell, and J. R. Speakman. 1994. Use of lamplit roads by foraging bats in southern England. J. Zool. Lond. 234:453-462.

Borissenko, A. V., and S. V. Kruskop. 2003. Bats of Vietnam and adjacent territories: an identification manual. Hanoi and Moscow: Joint Russian Vietnamese Science and Technological Tropical Centre. Hanoi and the Zoological Museum of Moscow M. V. Lomonosov State University.

Boyles, J. G., P. M. Cryan, G. F. McCracken, and T. H. Kunz. 2011. Economic importance of bats in agriculture. Science 332:41-42.

Bradley, C. A., and S. Altizer. 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol. Evol. 22:95-102.

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: cytochrome-b sequences and mammals. J. Mammal. 82:960-973.

Brigham, R. M., J. E. Cebek, and M. B. C. Hickey. 1989. Intraspecific variation in the echolocation calls of two species of insectivorous bats. J. Mammal. 70:426-428.

Brigham, R. M., S. D. Grindal, M. C. Firman, and J. L. Morissette. 1997. The influence of structural clutter on activity patterns of insectivorous bats. Can. J. Zool. 75:131-136.

Brunet-Rossinni, A. K., and G. S. Wilkinson. 2009. Methods of age estimation and the study of senescence in bats. Pp. 315-325 *in* T. H. Kunz, and S. Parsons, eds. Ecological and behavioral methods for the study of bats. The Johns Hopkins University Press, Baltimore, MD.

Bryja, J., P. Kanuch, A. Fornuskova, T. Bartonicka, and Z. Rehak. 2009. Low population genetic structuring of two cryptic bat species suggests their migratory behavior in continental Europe. Biol J. Linn. Soc. 96:103-114.

Buchler, E. R. 1976. Prey selection by *Myotis lucifugus* (Chiroptera: Vespertilionidae). Am. Nat. 110:619-628.

Burland, T. M., E. M. Barratt, M. A. Beaumont, and P. A. Racey. 1999. Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. Proc. Roy. Soc. Lond. B 266:975-980.

Burland, T. M., and J. Worthington Wilmer. 2001. Seeing in the dark: molecular approaches to the study of bat populations. Biol. Rev. 76:389-409.

Burton, G. V., R. J. Alley, G. L. Rasmussen, P. Orton, V. Cox, P. Jones, and D. Graff. 1977. Mercury and behavior in wild mouse populations. Environ. Res. 14:30-34.

Calderón-Garcidueñas, L., W. R. Maronpot, R. Torres-Jardon, C. Henríquez-Roldán, R. Schoonhoven, H. Acuña-Ayala, A. Villareal-Calderón, J. Nakamura, R. Fernando, W. Reed, et al. 2003. DNA damage in nasal and brain tissues of canines exposed

to air pollutants is associated with evidence of chronic brain inflammation and neurodegeneration. Toxicol. Pathol. 31:524-538.

Calderón-Garcidueñas, L., W. Reed, R. R. Maronpot, C. Henríquez-Roldán, R. Delgado-Chavez, Calderón-Garcidueñas, A., I. Dragustinovis, M. Frano-Lira, M. Aragón-Flores, A. C. Solt, et al. 2004. Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. Toxicol. Pathol. 32:650-658.

Castella, V., M. Ruedi, L. Excoffier, C. Ibanez, R. Arlettaz, and J. Hausser. 2000. Is the Gibraltar Strait a barrier to gene flow for the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? Mol. Ecol. 9:1761-1772.

Chatelain, M., J. Gasparini, and A. Frantz. 2016. Trace metals, melanin-based pigmentation and their interaction influence immune parameters in feral pigeons (*Columba livia*). Ecotoxicology 25:521-529.

Cizdziel, J. V., and S. Gerstenberger. 2004. Determination of total mercury in human hair and animal fur by combustion atomic absorption spectrometry. Talanta 64:918-921.

Clare, E. L., B. R. Barber, B. W. Sweeney, P. D. N. Hebert, and M. B. Fenton. 2011. Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). Mol. Ecol. 20:1772-1780.

Clare, E. L., W. O. C. Symondson, H. Broders, F. Fabianek, E. E. Fraser, A. Mackenzie, A. Boughen, R. Hamilton, C. K. R. Willis, F. Martinez-Nuñez, et al.. 2014. The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. Mol. Ecol. 23:3618-3632.

Clark, Jr., D. R. 1981. Bats and environmental contaminants: a review. USFWS special scientific report—wildlife no. 235. U.S. Fish and Wildlife Service, Washington, D. C.

----------. 2001. DDT and the decline of free-tailed bats (*Tadarida brasiliensis*) at Carlsbad Cavern, New Mexico. Arch. Environ. Con. Tox. 40:537-543.

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

Clark, Jr., D. R., and J. C. Kroll. 1977. Effects of DDE on experimentally poisoned free-tailed bats (*Tadarida brasiliensis*): lethal brain concentrations. J. Toxicol. Env. Health 3:893-901.

Clark, Jr., D. R., and T. G. Lamont. 1976. Organochlorine residues in females and nursing young of the big brown bat *Eptesicus fuscus*. B. Environ. Contam. Toxicol. 15:1-8.

Clark, Jr., D. R., and R. M. Prouty. 1976. Organochlorine residues in three bat species from four localities in Maryland and West Virginia, 1973. Pestic. Monit. J. 10:44-53.

----------. 1977. Experimental feeding of DDE and PCB to female big brown bats (*Eptesicus fuscus*). J. Toxicol. Env. Health 2:917-928.

Clark, Jr., D. R., and R. F. Shore. 2001. Chiroptera. Pp. 159-214 *in* R. F. Shore, and B. A. Rattner, eds. Ecotoxicology of wild mammals. John Wiley & Sons Ltd., West Sussex, England.

Clark, Jr., D. R., and C. J. Stafford. 1981. Effects of DDE and PCB (Aroclor 1260) on experimentally poisoned female little brown bats (*Myotis lucifugus*): lethal brain concentrations. J. Toxicol. Env. Heal. A 7:925-934.

Clark, D. R., Jr., F. M. Bagley, and W. W. Johnson. 1988. Northern Alabama colonies of the endangered grey bat *Myotis grisescens*: organochlorine contamination and mortality. Biol. Conserv. 43:213-225.

Clark, Jr., D. R., R. L. Clawson, and C. J. Stafford. 1983. Gray bats killed by dieldrin at two additional Missouri caves: aquatic macroinvertebrates found dead. B. Environ. Contam. Tox. 30:214-218.

Clark, Jr., D. R., T. H. Kunz, and T. E. Kaiser. 1978. Insecticides applied to a nursery colony of little brown bats (*Myotis lucifugus*): lethal concentrations in brain tissues. J. Mammal. 59:84-91.

Clark, Jr., D. R., R. K. LaVal, and M. D. Tuttle. 1981. Estimating pesticide burdens of bats from guano analyses. Bull. Environ. Contam. Toxicol. 29:214-220.

Clark, Jr., D. R., A. Moreno-Valdez, and M. A. Mora. 1995. Organochlorine residues in bat guano from nine Mexican caves, 1991. Ecotoxicology 4:258-265.

Clawson, R. L., and D. R. Clark, Jr. 1989. Pesticide contamination of endangered gray bats and their food base in Boone County, Missouri, 1982. B. Environ. Contam. Tox. 42:431-437.

Clement, M., D. Posada, and K. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657-1660.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecol. Appl. 10:626-638.

Cockrum, E. L. 1970. Insecticides and guano bats. Ecology 51:761-762.

Cohen, J. 1992. A power primer. Psychol. Bull. 112:155-159.

Coleman, J. L., and R. M. R. Barclay. 2010. Urbanization and the abundance and diversity of prairie bats. Urban Ecosyst. 15:87-102.

Collen, A. 2012. The evolution of echolocation in bats: a comparative approach. Doctoral dissertation, University College London, London, England.

Colorado Natural Heritage Program. 2013. Ecological systems of Colorado map. Colorado Natural Heritage Program, Colorado State University, Fort Collins, CO. http://www.cnhp.colostate.edu/images/ecology/Ecol_website_map2.jpg. Accessed February 9, 2017.

Constantine, D. G., E. S. Tierkel, M. D. Kleckner, and D. M. Hawkins. 1968. Rabies in New Mexico cavern bats. Public Health Rep. 83:303-316.

Cook, R. B., G. W. Suter II, and E. R. Sain. 1999. Ecological risk assessment in a large river-reservoir: 1. Introduction and background. Environ. Toxicol. Chem. 18:581-588.

Craig, L., J. R. Brook, Q. Chiotti, B. Croes, S. Gower, A. Hedley, D. Krewski, A. Krupnick, M. Krzyzanowski, M. D. Moran, et al. 2008. Air pollution and public health: a guidance document for risk managers. J. Toxicol. Environ. Health A. 71:588-698.

Crespo-Lópeza, M. E., G. L. Macêdo, S. I. D. Pereira, G. P. F. Arrifano, D. L. W. Picanço-Diniz, J. L. M. do Nascimento, and A. M. Herculano. 2009. Mercury and human genotoxicity: critical considerations and possible molecular mechanisms. Pharmacol. Res. 60:212-220.

Cryan, P. M., M. A. Bogan, and J. S. Altenbach. 2000. Effect of elevation on distribution of female bats in the Black Hills, South Dakota. J. Mammal. 81:719-725.

Cryan, P. M., M. A. Bogan, R. O. Rye, G. P. Landis, and C. L. Kester. 2004. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. J. Mammal. 85:995-1001.

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

Custer, C. M., C. Yang, J. G. Crock, V. Shearn-Bochsler, K. S. Smith, and P. L. Hageman. 2009. Exposure of insects and insectivorous birds to metals and other elements from abandoned mine tailings in three Summit County drainages, Colorado. Environ. Monit. Assess. 153:161-177.

Darnerud, P. O. 2003. Toxic effects of brominated flame retardants in man and in wildlife. Environ. Int. 29:841-853.

Davidson-Watts, I., and G. Jones. 2006. Differences in foraging behavior between *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825). J. Zool. 268:55-62.

Davis, W. H. 1963. Aging bats in winter. Trans. Ky. Acad. Sci. 24:28-30.

De Francisco, N., J. D. Ruiz Troya, and E. I. Agüera. 2003. Lead and lead toxicity in domestic and free living birds. Avian Pathol. 32:3-13.

Dimond, J. B., and R. B. Owen. 1996. Long-term residue of DDT compounds in forest soils in Maine. Environ. Pollut. 92:227-230.

Dixit, V. P., and N. K. Lohiya. 1974. Histological changes in testis of the non-scrotal mammal, *Rhinopoma kinneari* (*Wroughton*) following the administration of cadmium chloride. Indian J. Exp. Biol. 12:200-202.

Dong, J., X. Mao, H. Sun, D. M. Irwin, S. Zhang, and P. Hua. 2014. Introgression of mitochondrial DNA promoted by natural selection in the Japanese pipistrelle bat (*Pipistrellus abramus*). Genetica 142:483-494.

Driscoll, C. T., R. P. Mason, H. M. Chan, D. J. Jacob, and N. Pirrone. 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47:4967-4983.

Durrant, C. J., T. J. C. Beebee, F. Greenaway, and D. A. Hill. 2009. Evidence of recent population bottlenecks and inbreeding in British populations of Bechstein's bat, *Myotis bechsteinii*. Conserv. Genet. 10:489-496.

Dwyer, P. D. 1963. Seasonal changes in pelage of *Miniopterus schreibersii blepotis* (Chiroptera) in north-eastern NSW. Aust. J. Zool. 11:290-300.

Dwyer, P. D. 1968. The biology, origin and adaptation of *Miniopterus australis* (Chiroptera) in New South Wales. Aust. J. Zool. 16:49-68.

Earl, D. A., and B. M. vonHoldt. 2012. Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Cons. Genet. Res. 4:359-361.

Eguren, R. E. 2014. Community composition, population demographics, and tissue metal concentrations in bats (Chiroptera) at Tar Creek Superfund site: with a focus on use of a behavioral flight cage assay. South Nazarene University, Bethany, OK.

Elton, C. 1927. Animal ecology. The MacMillan Company: New York.

Erickson, J. L., and S. D. West. 2002. The influence of regional climate and nightly weather conditions on activity patterns of insectivorous bats. Acta Chiropt. 4:17-24.

Esposti, M. D., S. De Vries, M. Crimi, A. Ghelli, T. Patarnello, and A. Meyer. 1993. Mitochondrial cytochrome b: evolution and structure of the protein. BBA Bioenerg. 1143:243-271.

European Food Safety Authority (EFSA). 2009. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on cadmium in food. EFSA J. 980: 1-139.

Evanno G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611-2620.

Evers, D. C., N. M. Burgess, L. Champoux, B. Hoskins, A. Major, W. M. Goodale, R. J. Taylor, R. Poppenga, and T. Daigle. 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14:193-221.

Fahey, A. L., R. E. Ricklefs, and J. A. Dewoody. 2014. DNA-based approaches for evaluating historical demography in terrestrial vertebrates. Biol. J. Linn. Soc. 112:367-386.

Farina, L.L., D. J. Heard, D. M. Leblanc, J. O. Hall, G. Stevens, J. F. X. Wellehan, and C. J. Detrisac. 2005. Iron storage disease in captive Egyptian fruit bats (*Rousettus aegyptiacus*): relationship of blood iron parameters to hepatic iron concentrations and hepatic histopathology. J. Zoo. Wildl. Med. 36:212–221.

Fenton, M. B. 1980. Adaptiveness and ecology of echolocation in terrestrial (aerial) systems. Pp. 427-446 *in* R. G. Busnel, and J. F. Fish, eds. Animal sonar systems. Plenum Press, New York.

Fenton, M. B., and G. P. Bell. 1979. Echolocation and feeding behavior in four species of *Myotis* (Chiroptera). Can. J. Zool. 57:1271-1277.

Fenton, B., and N. Simmons. 2014. Bats: a world of science and mystery. The University of Chicago Press, Chicago.

Fernández-Luqueño, F., F. López-Valdez, P. Gamero-Melo, S. Luna-Suárez, E. N. Aguilera-González, A. I. Martínez, M. D. S. García-Guillermo, G. Hernández-Martínez, R. Herrera-Mendoza, M. A. Álvarez-Garza, et al. 2013. Heavy metal pollution in drinking water – a global risk for human health: a review. Afr. J. Environ. Sci. Technol. 7:567-584.

Fjellheim, A., and G. Raddum. 1990. Acid precipitation: biological monitoring of streams and lakes. Sci. Total Environ. 96:985-993.

Fleeger, J. W., K. R. Carman, and R. M. Nisbet. 2013. Indirect effects of contaminants in aquatic ecosystems. Sci. Total Environ. 317:207-233.

Fraser, E. E., F. J. Longstaffe, and M. B. Fenton. 2013. Moulting matters: the importance of understanding moulting cycles in bats when using fur for endogenous marker analysis. Can. J. Zool. 91:533-544.

Freeland, J. R., H. Kirk, and S. D. Petersen. 2011. Molecular ecology, 2nd ed. Wiley-Blackwell, West Sussex, U.K.

Freeman, J. 1984. Ecomorphological analysis of an assemblage of bats: resource partitioning and competition. Doctoral dissertation. University of Colorado, Boulder, CO.

Frick, W. F. 2013. Acoustic monitoring of bats, considerations of options for long-term monitoring. Therya 4:69-78.

Frick, W. F., W. E. Rainey, and E. D. Pierson. 2007. Potential effects of environmental contamination on *Yuma myotis* demography and population growth. Ecol. Appl. 17:1213-1222.

Fukui, D., M. Murakami, S. Nakano, and T. Aoi. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. J. Anim. Ecol. 75:1252-1258.

Funakoshi, K., R. Katahira, and H. Ikeda. 2009. Night-roost usage and nocturnal behavior in the Japanese house-dwelling bat, *Pipistrellus abramus*. Mamm. Study 34:131-139.

Funakoshi, K., and T. A. Uchida. 1978. Studies on the physiological and ecological adaptation of temperate insectivorous bats: III. Annual activity of the Japanese house-dwelling bat, *Pipistrellus abramus*. J. Fac. Agr. Kyushu U. 23:95-115.

Geggie, J. F., and M. B. Fenton. 1985. A comparison of foraging by *Eptesicus fuscus* (Chiroptera: Vespertilionidae) in urban and rural environments. Can. J. Zool. 63:263-266.

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

Gerell, R., and K. Lundberg. 1993. Decline of a bat *Pipistrellus pipistrellus* population in an industrialized area in South Sweden. Biol. Conserv. 65:153-157.

Gibbons, D., C. Morrissey, and P. Mineau. 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ. Sci. Pollut. R. 22:103-118.

Griffin, D. R. 1958. Listening in the dark. Yale University Press, New Haven, CT.

Griffin, D. R., F. A. Webster, and C. R. Michael. 1960. The echolocation of flying insects by bats. Anim. Behav. 8:141-154.

Grimm, N. B., D. Foster, P. Groffman, J. M. Grove, C. S. Hopkinson, K. J. Nadelhoffer, D. E. Pataki, and D. P. C. Peters. 2008. The changing landscape: ecosystem responses to urbanization and pollution across climatic and societal gradients. Front. Ecol. Environ. 6:264-272.

Grindal, S. D., J. L. Morissette, and R. M. Brigham. 1999. Concentration of bat activity in riparian habitats over an elevational gradient. Can. J. Zool. 77:972-977.

Grinevich, L., S. L. Holroyd, and R. M. R. Barclay. 1995. Sex differences in the use of daily torpor and foraging time by big brown bats (*Eptesicus fuscus*) during the reproductive season. J. Zool. 235:301-309.

Grube, A., D. Donaldson, T. Kiely, and L. Wu. 2011. Pesticides industry sales and usage: 2006 and 2007 market estimates. U.S. Environmental Protection Agency.

Guo, W. P., X. D. Lin, W. Wang, J. H. Tian, M. L. Cong, H. L. Zhang, M. R. Wang, R. H. Zhou, J. B. Wang, M. H. Li, et al. 2013. Phylogeny and origins of hantaviruses harbored by bats, insectivores, and rodents. PloS Pathog. 9:e1003159.

Han, F. X., A. Banin, Y. Su, D. L. Monts, J. M. Plodinec, W. L. Kingery, and G. E. Triplett. 2002. Industrial age anthropogenic inputs of heavy metals into the pedosphere. Naturwissenschaften 89:497-504.

Hare, L. 1992. Aquatic insects and trace metals – bioavailability, bioaccumulation, and toxicity. Crit. Rev. Toxicol. 22:327-369.

Hariono, B., J. Ng, and R. H. Sutton. 1993. Lead concentrations in tissues of fruit bats (*Pteropus* sp.) in urban and nonurban locations. Wildlife Res. 20:315-320.

Hawkes, H. A. 1998. Origin and development of the Biological Monitoring Working Party score system. Water Res. 32:964-968.

Hayes, J. P. 1997. Temporal variation in activity of bats and the design of echlolocation-monitoring studies. J. Mammal. 78:514-524.

Hayes, J. P. 2000. Assumptions and practical considerations in the design and interpretation of echolocation-monitoring studies. Acta Chiropt. 2:225-236.

Hecker, K. R., and R. M. Brigham 1999. Does moonlight change vertical stratification of activity by forest-dwelling insectivorous bats? J. Mammal. 80:1196-1201.

Henry, M., M. Beguin, R. Requier, O. Rollin, J. F. Odoux, P. Aupinel, J. Aptel, S. Tchamitchian, and A. Decourtye. 2012. A common pesticide decreases foraging success and survival in honey bees. Science 336:348-350.

Hernout, B. V., C. J. McClean, K. E. Arnold, M. Walls, M. Baxter, and A. B. A. Boxall. 2016a. Fur: a non-invasive approach to monitor metal exposure in bats. Chemosphere 147:376-381.

Hernout, B. V., K. E. Arnold, C. J. McClean, M. Walls, M. Baxter, and A. B. A. Boxall. 2016b. A national level assessment of metal contamination in bats. Environ. Pollut. 214:847-858.

Hernout, B. V., S. Pietravalle, K. E. Arnold, C. J. McClean, J. Aegerter, and A. B. A. Boxall. 2015. Interspecies variation in the risks of metals to bats. Environ. Pollut. 206:209-216.

Hickey, M. B. C., M. B. Fenton, K. C. MacDonald, and C. Soulliere. 2001. Trace elements in the fur of bats (Chiroptera: Vespertilionidae) from Ontario and Quebec, Canada. B. Environ. Contam. Tox. 66:699-706.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. J. N. Am. Benthol. Soc. 7:65-68.

Hoenerhoff, M., and K. Williams. 2004. Copper-associated hepatopathy in a Mexican fruit bat (*Artibeus jamaicensis*) and establishment of a reference range for hepatic copper in bats. J. Vet. Diagn. Invest. 16:590–593.

Holsinger, K. E., and B. S. Weir. 2009. Genetics in geographically structured populations: defining, estimating, and interpreting F_{ST}. Nat. Rev. Genet. 10:639-650.

Honeycutt, R. L., I. F. Greenbaum, R. J. Baker, and V. M. Sarich. 1981. Molecular evolution of vampire bats. J. Mammal. 62:805-811.

Hoofer, S. R., and R. A. Van Den Bussche. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chiropt. 5:1-63.

Huang, W., and X. Huang. 1982. The seasonal activity of *Pipistrellus abramus* in relation to environmental factors. Acta Theriol. Sin. 2:143-155.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Hughes, A. C., C. Satasook, P. J. J. Bates, P. Soisook, T. Sritongchuay, G. Jones, and S. Bumrungsri. 2011. Using echolocation calls to identify Thai bat species: Vespertilionidae, Emballonuridae, Nycteridae, and Megadermatidae. Acta Chiropt. 13:447-455.

IARC (International Agency for Research on Cancer). 1993. Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Lyon, France.

IPCC. 2014. Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. [Core Writing Team, R.K. Pachauri and L.A. Meyer, eds.] IPCC, Geneva, Switzerland.

IUCN Red List of Threatened Species. Version 2015.1. www.iucnredlist.org. Accessed June 2, 2015.

Iwata, T. 2003. The roles of fluvial geomorphology in the trophic flow from stream to forest ecosystems. Doctoral dissertation. Kyoto University, Kyoto, Japan.

Jaberg, C., and A. Guisan. 2001. Modelling the distribution of bats in relation to landscape structure in a temperate mountain environment. J. App. Ecol. 38:1169-1181.

Järup, L. 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68: 167–182.

Jensen, J. L., A. J. Bohonak, and S. T. Kelley. 2005. Isolation by distance, web service. BMC Genetics 6: 13. v.3.23. http://ibdws.sdsu.edu/

Jiang, T., J. Feng, K. Sun, and J. Wang. 2008. Coexistence of two sympatric and morphologically similar bat species *Rhinolophus affinis* and *Rhinolophus pearsoni*. Prog. Nat. Sci. 18:523-532.

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

Jones, K., J. Bielby, M. Cardillo, and S. Fritz. 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology 90: E90-184.

Kalcounis, M. C., K. A. Hobson, R. M. Brigham, and K. R. Hecker. 1999. Bat activity in the boreal forest: importance of stand type and vertical strata. J. Mammal. 80:673-682.

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. Biol. Conserv. 138:120-130.

Kales, S. N., and D. C. Christiani. 2005. Hair and metal toxicity. Pp. 125-158 *in* D. J. Tobin, ed. Hair in toxicology: an important biomonitor. Royal Society of Chemistry, Cambridge, MA.

Kalko, E. K. V. 1995. Insect pursuit, prey capture, and echolocation in pipistrelle bats (Microchiroptera). Anim. Behav. 50:861-880.

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.

Karouna-Renier, N. K., C. White, C. R. Perkins, J. J. Schmerfeld, and D. Yates. 2014. Assessment of mitochondrial DNA damage in little brown bats (*Myotis lucifugus*) collected near a mercury-contaminated river. Ecotoxicology 23:1419-1429.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

Keith, J. O. 1991. Historical perspectives. Pp. 1-21 *in* T. J. Pearle, ed. Wildlife toxicology. Van Nostrand Reinhold, New York.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17:230-240.

Kelly, B. C., M. G. Ikonomou, J. D. Blair, A. E. Morin, and F. A. P. C. Gobas. 2007. Food web-specific biomagnification of persistent organic pollutants. Science 317:236-239.

Kingston, T., C. M. Francis, Z. Akbar, and T. H. Kunz. 2003. Species richness in an insectivorous bat assemblage from Malaysia. J. Trop. Ecol. 19:67-79.

Kleiber, M. 1932. Body size and metabolism. Hilgardia 6:315-353.

Korine, C., R. Adams, D. Russo, M. Fisher-Phelps, and D. Jacobs. 2016. Bats and water: anthropogenic alterations threaten global bat populations. Pp. 215-241 *in* C. C. Voigt, and T. Kingston, eds. Bats in the anthropocene: conservation of bats in a changing world. Springer International Publishing, New York.

Kraus, J.M., T.S. Schmidt, D.M. Walters, R.B. Wanty, R.E. Zuellig, and R.E. Wolf. 2014. Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. Ecol. Appl. 24:235-243.

Kurta, A., G. P. Bell, K. A. Nagy, and T. H. Kunz. 1989. Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). Physiol. Ecol. 62:804-818.

Kurta, A., and J. O. Whitaker. 1998. Diet of the endangered Indiana bat (*Myotis sodalis*) on the northern edge of its range. Am. Midl. Nat. 140:280-286.

Kusch, J., C. Weber, S. Idelberger, and T. Koob. 2004. Foraging habitat preferences of bats in relation to food supply and spatial vegetation structures in a western European low mountain range forest. Folia Zool. 53:113-128.

Land, T. A. 2000. Population size and contaminant exposure of bats using caves on Fort Hood Military Base. Master's thesis. Texas A&M University, College Station, TX.

Lee, Y.-F., and L.-L. Lee. 2005. Food habits of Japanese pipistrelles *Pipistrellus abramus* (Chiroptera: Vespertilionidae) in northern Taiwan. Zool. Stud. 44:95-101.

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

Li, G., G. Jones, S. J. Rossiter, S.-F. Chen, S. Parsons, and S. Zhang. 2006. Phylogenetics of small horseshoe bats from East Asia based on mitochondrial DNA sequence variation. J. Mammal. 87:1234-1240.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23:399-418.

Little, M. E., N. M. Burgess, H. G. Broders, and L. M. Campbell. 2015. Mercury in little brown bat (*Myotis lucifugus*) maternity colonies and its correlation with freshwater acidity in Novia Scotia, Canada. Environ. Sci. Technol. 49:2059-2065.

Lollar, A., and B. Schmidt-French. 1998. Captive care and medical reference for the rehabilitation of insectivorous bats. Bat World, Mineral Wells, TX.

Luckens, M. M. 1973. Seasonal changes in the sensitivity of bats to DDT. Pp. 63-75 *in* W. B. Deichmann, ed. Pesticides and the environment: a continuing controversy. Intercontinental Medical Book Corp., New York.

Luckens, M. M., and W. H. Davis. 1964. Bats: sensitivity to DDT. Science 146:948.

MacArthur, R. 1955. Fluctuations of animal populations and a measure of community stability. Ecology 36:533-536.

Mackey, R. L., and R. M. R. Barclay. 1989. The influence of physical clutter and noise on the activity of bats over water. Can. J. Zool 67:1167-1170.

Malison, R. L., and C. V. Baxter. 2010. The fire pulse: wildfire stimulates flux of aquatic prey to terrestrial habitats driving increases in riparian consumers. Can. J. Fish Aquat. Sci. 67:570-579.

Marzluff, J. M. 2001. Worldwide urbanization and its effects on birds. Pp. 19-47 *in* J. M. Marzluff, R. Bowman, and R. Donnelly, eds. Avian ecology and conservation in an urbanizing world. Springer, Boston, MA.

McCracken, G. F., M. K. McCracken, and A. T. Vawter. 1994. Genetic structure in migratory populations of the bat *Tadarida brasiliensis mexicana*. J. Mammal. 75:500-514.

McIntyre, N. E. 2000. Ecology of urban arthropods: a review and a call to action. Ann. Entomol. Soc. Am. 93:825-835.

McKinney, M. L. 2002. Urbanization, biodiversity, and conservation. Bioscience 52:883-890.

McManus, J. S., D. L. Dalton, A. Kotzé, B. Smuts, A. Dickman, J. P. Marshal, and M. Keith. 2014. Gene flow and population structure of a solitary top carnivore in a human-dominated landscape. Ecol. Evol. 5:335-244.

Medellín, R. A., and O. Gaona. 1999. Seed dispersal by bats in birds in forest and disturbed habitats of Chiapas, México. Biotropica 31:478-485.

Miao, W., Y. R. Gel, and J. L. Gastwirth. 2006. A new test of symmetry about an unknown median. Pp. 199-214 *in* A. Hsuing, C.-H. Zhang, and Z. Ying, eds. Random walk, sequential analysis and related topics—a festschrift in honor of Yuan-Shih Chow. World Scientific Publisher, Singapore.

Michael, G. W. 2016. Mercury. Pp. 108-109 *in* Mineral commodity summaries, U.S. Geological Survey. https://minerals.usgs.gov/minerals/pubs/commodity/mercury/mcs-2016-mercu.pdf.

Miura, T., T. Koyama, and I. Nakamura. 1978. Mercury content in museum and recent specimens of Chiroptera in Japan. B. Environ. Contam. Toxicol. 20:696-701.

Mollhagen, T. R., and M. A. Bogan. 1997. Bats of the Henry Mountains region of southeastern Utah. Occasional Papers, Museum of Texas Tech Univ.

Morgulis, A., G. Coulouris, Y. Raytselis, T. L. Madden, R. Agarwala, and A. A. Schäffer. 2008. Database indexing for production MegaBLAST searches. Bioinformatics 24:1757-1764.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853-858.

Naeem, S., and S. Li. 1997. Biodiversity enhances ecosystem reliability. Nature 390:507–509.

Naidoo, S., D. Vosloo, and M. C. Schoeman. 2013. Foraging at wastewater treatment works increases the potential for metal accumulation in an urban adapter, the banana bat (*Neoromicia nana*). Afr. Zool. 48:39-55.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. P. Natl. Acad. Sci. USA 98:166-170.

Nam, D.-H., 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.

Nikaido, M., K. Kawai, Y. Cao, M. Harada, S. Tomita, N. Okada, and M. Hasegawa. 2001. Maximum likelihood analysis of the complete mitochondrial genome of eutherians and a reevaluation of the phylogeny of bats and insectivores. J. Mol. Evol. 53:508-516.

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. Phil. Trans. R. Soc. Lond. B 316:335-427.

Noxon, J. O. 1995. Alopecia. Pp. 211-214 *in* S. J. Ettinger and E. C. Feldman, eds. Textbook of veterinary internal medicine, 4th ed. Saunders Co., Philadelphia, PA.

Ober, H. K., and J. P. Hayes. 2008a. Influence of vegetation on bat use of riparian areas at multiple spatial scales. J. Wildlife Manage. 72:396-404.

----------. 2008b. Prey selection by bats in the forests of western Oregon. J. Mammal. 89:1191-1200.

O'Shea, T. J., D. R. Clark, Jr., and T. P. Boyle. 2000. Impacts of mine-related contaminants on bats. Pp. 205-216 *in* K. C. Vories and D. Throgmorton, eds. Proceedings of bat conservation and mining: a technical interactive forum. U.S. Dept. of Interior, Office of Surface Mining, Alton, IL and Coal Research Center, S. Illinois Univ., Carbondale, IL.

O'Shea, T. J., L. E. Ellison, T. R. Stanley, and R. D. Stevens. 2011. Adult survival and population growth rate in Colorado big brown bats (*Eptesicus fuscus*). J. Mammal 92:433-443.

O'Shea, T. J., A. L. Everette, and L. E. Ellison. 2001. Cyclodiene insecticide, DDE, DDT, arsenic, and mercury contamination of big brown bats (*Eptesicus fuscus*) foraging at a Colorado Superfund site. Arch. Environ. Con. Tox. 40:112-120.

Osborne, C. E., D. C. Evers, M. Duron, N. Schoch, D. Yates, D. Buck, O. P. Lane, and J. Franklin. 2011. Mercury contamination within terrestrial ecosystems in New England and mid-Atlantic states: profiles of soil, invertebrates, songbirds, and bats. Biodiversity Research Institute, Gorham, ME.

Ottoboni, A., and J. I. Ferguson. 1969. Excretion of DDT compounds in rat milk. Toxicol. Appl. Pharm. 15:56-61.

Pacyna, E. G., J. M. Pacyna, K. Sundseth, J. Munthe, K. Kindbom, S. Wilson, F. Steenhuisen, and P. Maxson. 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmos. Environ. 44:2487-2499.

Paetzhold, A., M. Smith, P. H. Warren, and L. Maltby. 2011. Environmental impact propagated by cross-system subsidy: chronic stream pollution controls riparian spider populations. Ecology 92:1711-1716.

Paine, R. T. 1980. Food webs: linkage, interaction strength, and community infrastructure. J. Anim. Ecol. 49:667-685.

Parsons, K. N., G. Jones, and F. Greenaway. 2003. Swarming activity of temperate zone microchiropteran bats: effects of season, time of night and weather conditions. J. Zool. Lond.

Parsons, S., and G. Jones. 2000. Acoustic identification of twelve species of echolocating bats by discriminant function analysis and artificial neural networks. J. Exp. Biol. 203:2461-2656.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288-295.

Pearce, R. D., T. J. O'Shea, and B. A. Wunder. 2008. Evaluation of morphological indices and total body electrical conductivity to assess body composition in big brown bats. Acta Chiropt. 10:153-159.

Pederson, S. C., G. G. Kwiecinski, P. A. Larsen, M. N. Morton, R. A. Adams, H. H. Genoways, and V. J. Swier. 2009. Bats of Montserrat: population fluctuation and response to hurricanes and volcanoes, 1978-2005. Pp. 302-340 i*n* T. H. Fleming, and P. A. Racey, eds. Island bats: evolution, ecology, and conservation. The Univ. of Chicago Press, Chicago.

Petit, E., and F. Mayer. 1999. Male dispersal in the noctule bat (*Nyctalus noctula*): where are the limits? P. Roy. Soc. Lond. B Bio. 266:1717-1722.

Petit, E., and F. Mayer. 2000. A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). Mol. Ecol. 9:683-690.

Pikula, J., J. Zukal, V. Adam, H. Bandouchova, M. Beklova, P. Hajkova, J. Horakova, R. Kizek, and L. Valentikova. 2010. Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environ. Toxicol. Chem. 29:501-506.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. 28:289-316.

Power, M. E., W. E. Rainey, M. S. Parker, J. L. Sabo, A. Smyth, S. Khandwala, J. C. Finlay, F. C. McNeely, K. Marsee, and C. Anderson. 2004. River-to-watershed subsidies in an old-growth conifer forest. Pp. 217-240 *in* G. A. Polis, M. E. Power, and G. R. Huxel, eds. Food webs at the landscape level. The Univ. of Chicago Press, Chicago.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Qiao, X., A. H. Schmidt, Y. Tang, Y. Xu, and C. Zhang. 2013. Demonstrating urban pollution using toxic metals of road dust and roadside soil in Chengdu, southwestern China. Stoch. Env. Res. Risk A. 28:911-919.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Racey, P. R., S. M. Swift, J. Rydell, and L. Brodie. 1998. Bats and insects over two Scottish rivers with contrasting nitrate status. Anim. Conserv. 1:195-202.

Racey, P. A., E. M. Barratt, T. M. Burland, R. Deaville, D. Gotelli, G. Jones, and S. B. Piertney. 2007. Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. Biol. J. Linn. Soc. 90:539-550.

Rector, A., A. Mostmans, K. Van Doorslaer, C. A. McKnight, R. K. Maes, A. G. Wise, M. Kiupel, and M. Van Ranst. 2006. Genetic characterization of the first chiropteran papillomavirus, isolated from a basosquamous carcinoma in an Egyptian fruit bat: the *Rousettus aegyptiacus* papillomavirus type 1. Vet. Microbiol. 117:267-275.

Reinhold, J. O., A. J. Hendriks, L. K. Slager, and M. Ohm. 1999. Transfer of microcontaminants from sediment to chironomids, and the risk for the Pond Bat *Myotis dasycneme* (Chiroptera) preying on them. Aquat. Ecol. 33:363-376.

Rico, A., and P. J. Van den Brink. 2015. Evaluating aquatic invertebrate vulnerability to insecticides based on intrinsic sensitivity, biological traits, and toxic mode of action. Environ. Toxicol. Chem. 34:1907-1917.

Roach, N., and D. G. Rumbold. 2016. Survey of mercury in the fur of bats (Chiroptera) of coastal South Carolina. 7th Society of Environmental Toxicology and Chemistry World Congress. Orlando, FL.

Robbins, L. W., M. D. Engstrom, R. B. Wilhelm, and J. R. Choate. 1977. Ecogeographic status of *Myotis leibii* in Kansas. Mammalia 41:365-367.

Rogers, D. S., M. C. Belk, M. W. González, and B. L. Coleman. 2006. Patterns of habitat use by bats along a riparian corridor in northern Utah. Southwest. Nat. 51:52-58.

Rossiter, S. J., P. Benda, C. Dietz, S. Zhang, and G. Jones. 2007. Rangewide phylogeography in the greater horseshoe bat inferred from microsatellites: implications for population history, taxonomy and conservation. Mol. Ecol. 16:4699-4714.

Rossiter, S. J., G. Jones, R. D. Ransome, and E. M. Barratt. 2000. Genetic variation and population structure in the endangered greater horseshoe bat Rhinolophus ferrumequinum. Mol. Ecol. 9:1131-1135.

Rossiter, S. J., A. Zubaid, A. Mohd-Adnan, M. J. Struebig, T. H. Kunz, S. Gopal, E. J. Petit, and T. Kingston. 2012. Social organization and genetic structure: insights from codistributed bat populations. Molec. Ecol. 21:647-661.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219-1228.

Ruedi, M., and V. Castella. 2003. Genetic consequences of the ice ages on nurseries of the bat *Myotis myotis*: a mitochondrial and nuclear survey. Mol. Ecol. 12:1527-1540.

Russo, D., and L. Ancillotto. 2015. Sensitivity of bats to urbanization: a review. Mamm. Biol. 80:205-212.

Russo, D., and G. Jones. 2002. Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. J. Zool. Lond. 258:91-103.

Russo, D., and C. C. Voigt. 2016. The use of automated identification of bat echolocation calls in acoustic monitoring: a cautionary note for a sound analysis. Ecol. Indicators 66:598-602.

Rydell, J., A. Entwistle, and P. A. Racey. 1996. Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos 76:243-252.

Sakai, T., Y. Kikkawa, K. Tsuchiya, M. Harada, M. Kanoe, M. Yoshiyuki, and H. Yonekawa. 2003. Molecular phylogeny of Japanese Rhinolophidae based on variations in the complete sequence of the mitochondrial cytochrome b gene. Genes Genet. Syst. 78:179-189.

Sample, B. E., and G. W. Suter II. 1994. Estimating exposure of terrestrial wildlife to contaminants. ES/ER/TM-125, Oak Ridge National Laboratory, Oak Ridge, TN, USA.

Sample, B. E., D. M. Opresko, and G. W. Suter II. 1996. Toxicological benchmarks for wildlife: 1996 revision. No. ES/ER/TM-86/R3. ORNL Oak Ridge National Laboratory (US).

Sánchez-Bayo. F. 2011. Impacts of agricultural pesticides on terrestrial ecosystems. Pp. 63-87 *in* F. Sánchez-Bayo, P. J. van den Brink, and R. M. Mann, eds. Ecological impacts of toxic chemicals. Bentham Science Publishers Ltd., U.S.A.

Schmidt, T. S., W. H. Clements, R. B. Wanty, P. L. Verplanck, S. E. Church, C. A. San Juan, D. L. Fey, B. W. Rockwell, E. H. DeWitt, and T. L. Klein. 2012. Geologic processes influence the effects of mining on aquatic ecosystems. Ecol. Appl. 22:870-879.

Schmidt, T. S., W. H. Clements, R. E. Zuellig, K. A. Mitchell, S. E. Church, R. B. Wanty, C. A. San Juan, M. Adams, and P. J. Lamothe. 2011. Critical tissue residue approach linking accumulated metals in aquatic insects to population and community-level effects. Environ. Sci. Technol. 45:7004-7010.

Schmidt, T. S., J. M. Kraus, D. M. Walters, and R. B. Wanty. 2013. Emergence flux declines disproportionately to larval density along a stream metals gradient. Environ. Sci. Technol. 47: 8784-8792.

Schneider, A., K. C. Seto, and D. R. Webster. 2005. Urban growth in Chengdu, Western China: application of remote sensing to assess planning and policy outcomes. Environ. Plann. B 32:323-345.

Schulz, R., M. Bundschuh, R. Gergs, C. A. Brühl, D. Diehl, M. H. Entling, L. Fahse, O. Frör, H. F. Jungkunst, A. Lorke, et al. 2015. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. Sci. Total Environ. 538:246-261.

Scoch, N., D. Evers, O. Lane, D. Yates, M. Duron, R. S. Hames, and J. D. Lowe. 2010. Biogeography of mercury contamination in New York state: risk to species of greatest conservation need. Biodiversity Research Institute, Gorham, MN.

Scott, S. J., G. McLaren, G. Jones, and S. Harris. 2010. The impact of riparian habitat quality on the foraging and activity of pipistrelle bats (*Pipistrellus* spp.). J. Zool. 280:371-378.

Seawright, A. A. 1989. Lead. Pp. 211-216 *in* Chemical and plant poisons, animal health in Australia. Vol. 2, 2nd ed. Australian Government Publishing Service, Canberra.

Sikes, 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 animals in research. J. Mammal. 92:235-253.

Smith, C. 2001. Pesticide exports from U.S. ports, 1997-2000. Int. J. Occup. Env. Health 7:266-274.

Smith, A. T., and Y. Xie. 2008. Mammals of China. Princeton University Press, Princeton, NJ.

Speakman, J. R., and P. A. Racey. 1989. Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements, and mass. J. Anim. Ecol. 58:797-813.

Stockholm Convention on Persistent Organic Pollutants (SCPOP). 2007. China: National report pursuant to Article 15, Parts A, B, and C. http://chm.pops.int/Countries/NationalReports/FirstRoundofPartyReports/tabid/254/Default.aspx. Accessed January 14, 2015.

Streit, B., and A. Nagel. 1993. Element assessment in tissue samples from European bats (Microchiroptera). Fresen. Environ. Bull. 2:162-167.

Streit, B., S. Winter, and A. Nagel. 1995. Bioaccumulation of selected organochlorines in bats and tits: influence of chemistry and biology. Environ. Sci. Pollut. R. Int. 2:194-199.

Struebig, M. J., T. Kingston, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2008. Conservation value of forest fragments to Palaeotropical bats. Biol Conserv. 141:2112-2126.

Sun, K., J. Feng, L. Jin, Y. Liu, and Y. Jiang. 2008. Identification of sympatric bat species by the echolocation calls. Front. Biol. China 3:227-231.

Sutton, R. H., and B. Hariono. 1987. Lead poisoning in flying-foxes (Chiroptera: Pteropodidae). Aust. Mammal. 10:125-126.

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

Swift, S. M., P. A. Racey, and M. I. Avery. 1985. Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. II. Diet. J. Anim. Ecol. 54:217-225.

Sztencel-Jabłonka, A., and W. Bogdanowicz. 2012. Population genetics study of common (*Pipistrellus pipistrellus*) and soprano (*Pipistrellus pygmaeus*) pipistrelle bats from central Europe suggests interspecific hybridization. Can. J. Zool. 90:1251-1260.

Tang, Z.-H., G.-L. Zhang, L.-X. Sheng, T.-Y. Hong, G.-J. Zhu, J. Yang, Y.-Y. Gong, Y. Zeng, H.-H. Hu, and L.-B. Zhang. 2012. Alopecia in Rickett's big-footed bat *Myotis ricketti* (Chiroptera: Vespertilionidae) in relation to age and sex. Zool. Stud. 51:494-499.

Tchounwou, P. B., C. G. Yedkou, A. K. Patlolla, and D. J. Sutton. 2012. Heavy metal toxicity and the environment. EXS 101:133-164.

Thornton, I. 1990. Soil contamination in urban areas. Palaeogeogr. Palaeocl. 82:121-140.

Threlfall, C., B. Law, T. Penman, and P. B. Banks. 2011. Ecological processes in urban landscapes: mechanisms influencing the distribution and activity of insectivorous bats. Ecography 34:814-826.

Tian, L.-X., Y.-X. Pan, W. Metzner, J.-S. Zhang, and B.-F. Zhang. 2005. Bats respond to very weak magnetic fields. PLoS One 10:e0123205.

Tilman, D. 1996. Biodiversity: population versus ecosystem stability. Ecology 77:350-363.

Timmermans, K. R., W. Peeters, and M. Tonkes. 1992. Cadmium, zinc, lead, and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. Hydrobiologia 241:119-134.

Timmermans, K. R., and P. A. Walker. 1989. The fate of trace metals during the metamorphosis of chironomids (Diptera, Chironomidae). Environ. Pollut. 62:73-85.

Turmelle, A. S., T. H. Kunz, and M. D. Sorenson. 2011. A tale of two genomes: contrasting patterns of phylogeographic structure in a widely distributed bat. Mol. Ecol. 20:357-375.

Tyler, G., A.-M. Balsberg Påhlsson, G. Bengtsson, E. Bååth, and L. Tranvik. 1989. Heavy-metal ecology of terrestrial plants, microorganisms, and invertebrates. Water Air Soil Pollut. 47:189-215.

U.S. Department of Agriculture, Natural Resources Conservation Service. 2014. Colorado basinwide snowpack summary for June 1, 2014. https://www.wcc.nrcs.usda.gov/ftpref/support/water/SummaryReports/CO/BSnow_6_2014.pdf?

U.S. Environmental Protection Agency. 1975. DDT: aenviron review of scientific and economic aspects of the decision to ban its use as a pesticide. EPA-540/1-75-022. Washington, D.C.

U.S. Naval Observatory, Astronomical Applications Department. 2013. Sun or moon rise/set table for one year. http://aa.usno.navy.mil/data/docs/RS_OneYear.php. Accessed September 14, 2015.

Vaughn, N., G. Jones, and S. Harris. 1996. Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. Biol. Conserv. 78:337-343.

Veronesi, B., O. Makwana, M. Pooler, and L. C. Chen. 2005. Effects of subchronic exposures to concentrated ambient particles. VII. Degeneration of dopaminergic neurons in Apo E-/- mice. Inhal. Toxicol. 17:235-241.

Vidon, P., C. Allan, D. Burns, T. P. Duval, N. Gurwick, S. Inamdar, R. Lowrance, J. Okay, D. Scott, and S. Sebestyen. 2010. Hot spots and hot moments in riparian zones: potential for improved water quality management. J. Am. Water Resour. As. 46:278-298.

Wada, H., D. E. Yates, D. C. Evers, R. J. Taylor, and W. A. Hopkins. 2010. Tissue mercury concentrations and adrenocortical responses of female big brown bats (*Eptesicus fuscus*) near a contaminated river. Ecotoxicology 19:1277-1284.

Walker, C. H., S. P. Hopkin, R. M. Sibly, and D. B. Peakall. 2006. Principles of ecotoxicology, 3rd ed. CRC Press, Boca Raton, FL.

Walker, L. A., L. J. Bailey, and R. F. Shore. 2002. The importance of the gut and its contents in prey as a source of cadmium to predators. Environ. Toxicol. Chem. 21:76-80.

Walters, D. M., K. M. Fritz, and R. R. Otter. 2008. The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. Ecol. Appl. 18:1835-1841.

Walters, L. W., R. Freeman, A. Collen, C. Dietz, M. B. Fenton, G. Jones, M. K. Obrist, S. J. Puechmaille, T. Sattler, B. M. Siemers, et al. 2012. A continental-scale tool for acoustic identification of European bats. J. Appl. Ecol. 49:1064-1074.

Webb, P. I. 1995. The comparative ecophysiology of water balance in microchiropteran bats. Pp. 203-218 *in* P. A. Racey, and S. M. Swift. Ecology, evolution, and behaviour of bats, eds. Oxford Univ. Press, Oxford.

Webb, P. I., J. R. Speakman, and P. A. Racey. 1995. Evaporative water loss in two sympatric species of vespertilionid bat, *Plecotus auritus* and *Myotis daubentonii*—relation to foraging mode and implications for roost site selection. J. Zool. 225:269-278.

Wei, B., and L. Yang. 2010. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchem. J. 94:99-107.

Wei, L., J. R. Flanders, S. J. Rossiter, C. M. Miller-Butterworth, L. B. Zhang, and S. Y. Zhang. 2010. Phylogeography of the Japanese pipistrelle bat, *Pipistrellus abramus*, in China: the impact of ancient and recent events on population genetic structure. Biol. J. Linn. Soc. 99:582-594.

Wei, L., Y. Gan, Z. Li, Z. Lin, T. Hong, and L. Zhang. 2011. Comparisons of echolocation calls and wing morphology among six sympatric bat species. Acta Theriol. Sin. 31:155-163.

Wei, L., P. Hua, W. Shao, C. M. Miller-Butterworth, and S. Zhang. 2009. Isolation and characterization of microsatellite loci in the Japanese pipistrelle (*Pipistrellus abramus*). Conserv. Genet. 10:677-679.

Welch, L. T. 1994. Contaminant burdens and reproductive rates of bald eagles breeding in Maine. M.S. Thesis, University of Maine, Orono.

Weller, T. J., and C. J. Zabel. 2002. Variation in bat detections due to detector orientation in a forest. Wildl. Soc. Bull. 30:922-930.

Weyandt, S. E., R. A. Van den Bussche, M. J. Hamilton, and D. M. Leslie. 2005. Unravelling the effects of sex and dispersal: Ozark big-eared bat (*Corynorhinus townsendii ingens*) conservation genetics. J. Mammal. 86:1136-1143.

Whitaker, J. O., Jr., C. Maser, and L. E. Kelle. 1997. Food habits of bats of western Oregon. Northwest Sci. 51:46-55.

White, D. H., and A. J. Krynitsky. 1983. Wildlife in some areas of New Mexico and Texas accumulate elevated DDE residues. Arch. Environ. Con. Tox. 15:149-157.

Whitehorn, P. R., S. O'Connor, F. L. Wackers, and D. Goulson. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336:351-352.

Wibbelt, G., S. Speck, and H. Field. 2009. Methods for assessing diseases in bats. Pp. 775-794 *in* T. H. Kunz, and S. Parsons, eds. Ecological and behavioral methods for the study of bats. The Johns Hopkins Univ. Press, Baltimore, MD.

Wildlife Acoustics. 2015. Frequently asked questions. http://www.wildlifeacoustics.com/support/product-faqs. Accessed June 16, 2015.

Wilkinson, G. S., and J. M. South. 2002. Life history, ecology and longevity in bats. Aging Cell 1:124-131.

Willey, M. 2010. Effects of elevation and associated temperature and insect biomass on abundance, diversity, and distribution of male and female bats in the Front Range of Colorado. Master's thesis. Univ. of Northern Colorado, Greeley, CO.

Wilson, D. E., and D. M. Reeder. 2005. Mammal species of the world. A taxonomic and geographic reference, 3rd ed. The Johns Hopkins Univ. Press, Baltimore, MD.

World Health Organization. 2016. Concentration of particulate matter with an aerodynamic diameter of 2.5 μm or less (PM2.5) in nearly 3000 urban areas, 2008-2015. http://gamapserver.who.int/mapLibrary/Files/Maps/Global_pm25_cities.png?ua=1

World Wildlife Fund. 2017. Eastern Asia: southern China. http://www.worldwildlife.org/ecoregions/pa0437. Accessed February 28, 2017.

Wright, S. 1931. Evolution of Mendelian populations. Genetics 16:97-159.

----------. 1951. The genetical structure of populations. Annals of Eugenics 15:323-354.

----------. 1984. Evolution and the genetics of populations. Experimental results and evolutionary deductions. Univ. of Chicago Press, Chicago.

Yachi, S., and M. Loreau. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. P. Natl. Acad. Sci. 96:1463-1468.

Yates, D. E., E. M. Adams, S. E. Angelo, D. C. Evers, J. Schmerfeld, M. S. Moore, T. H. Kunz, T. Divoll, S. T. Edmonds, C. Perkins, et al. 2014. Mercury in bats from the northeastern United States. Ecotoxicology 23:45-55.

Zanchi, A. C., C. D. Venturini, M. Saiki, P. H. N. Saldiva, H. M. T. Barros, and C. R. Rhoden. 2008. Chronic nasal instillation of residual-oil fly ash (ROFA) induces

brain lipid peroxidation and behavioral changes in rats. Inhal. Toxicol. 20:795-800.

Zar, J. H. 2010. Biostatistical analysis, 5th ed. Prentice Hall, Inc., Upper Saddle River, NJ.

Zhang, D., L. Fengquan, and B. Jianmin. 2000. Eco-environmental effects of the Qinghai-Tibet Plateau uplift during the Quaternary in China. Environ. Geol. 39:1352-1358.

Zhang, J. S., N. J. Han, G. Jones, L. K. Lin, J. P. Zhang, G. J. Zhu, D. W. Huang, and S. Y. Zhang. 2007. A new species of *Barbastella* (Chiroptera: Vespertilionidae) from north China. J. Mammal. 88:1393-1403.

Zhang, L., G. Zhu, G. Jones, and S. Zhang. 2009. Conservation of bats in China: problems and recommendations. Oryx 43:179-182.

Zhang, W., F. Jiang, and J. Ou. 2011. Global pesticide consumption and pollution: with China as a focus. P. Int. Acad. Ecol. Environ. Sci. 1:125-144.

Zhu, G., N. Han, T. Hong, M. Tan, D. Yu, and L. Zhang. 2008. Echolocation call, roost and ND 1 sequence analysis of new record of *Nyctalus plancyi* (Chiroptera: Vespertilionidae) on Hainan Island. Zoological Research 29:447-451.

Zook, B. C., R. M. Sauer, and F. M. Garner. 1970. Lead poisoning in Australian fruit bats (*Pteropus poliocephalus*). J. Am. Vet. Med. Assoc. 157:691.

Zukal, J., J. Pikula, and H. Bandouchova. 2015. Bats as bioindicators of heavy metal pollution: history and prospect. Mamm. Biol. 80:220-227.

APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

UNIVERSITY of
NORTHERN COLORADO

IACUC Memorandum

To: Dr. Rick Adams

From: Laura Martin, Director of Compliance and Operations

CC: IACUC Files

Date: 5/15/2012

Re: IACUC Protocol 1205C-RA-B-15 Approval

The UNC IACUC has completed a final review of your protocol "Roost Site Locations, Landscape Use and Home Range Calibration Using Mist-net Capture and Radio Telemetry". The protocol review was based on the requirements of Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training; the Public Health Policy on Humane Care and Use of Laboratory Animals; and the USDA Animal Welfare Act and Regulations. Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol as submitted to the Committee. This protocol has been assigned the following number 1205C-RA-B-15

The next annual review will be due before May 9, 2012.

Sincerely,

Laura Martin, Director of Compliance and Operations

Memorandum

To: Laura Martin: Director of Compliance and Operations

From: Rick Adams, Biological Sciences

Date: 5/31/12

Re: Addendum for addition to Approved Protocol 1205C-RA-B-15: *Roost Site Locations, Landscape Use and Home Range Calibration Using Mist-net Capture and Radio Telemetry*

We are seeking to extend the approved IACUC Protocol to comparable sites located in Sichuan Province, China. We will be working with Professor Tan Ya, Professor of Environmental Ecology, College of Architecture and Environment at Sichuan University.

List of Bat Species expected in Sichuan, China

Pteropodidae

Leschenault's Fruit Bat, *Rousettus leschenaulti*

Rhinolophidae

Javan Horseshoe Bat, *Rhinolophus affinis*

Lesser Japanese Horseshoe Bat, *Rhinolophus cornutus*

Greater Horseshoe Bat, *Rhinolophus ferrumequinum*

Short-winged Horseshoe Bat, *Rhinolophus lepidus*

Wooly Horseshoe Bat, *Rhinolophus luctus*

Great-eared Horseshoe Bat, *Rhinolophus macrotis*

Pearson's Horseshoe Bat, *Rhinolophus pearsoni*

Lesser Horseshoe Bat, *Rhinolophus pusillus*

Guizhou Horseshoe Bat, *Rhinolophus rex*

Temminck's Horseshoe Bat, *Rhinolophus rouxi*

Hipposideridae Tailless Leaf-nosed Bat, *Coelops frithi*

Himalayan Leaf-nosed Bat, *Hipposideros armiger*

Bicolored Leaf-nosed Bat, *Hipposideros pomona*

Pratt's Leaf-nosed Bat, *Hipposideros pratti*

Vespertilionidae

Lesser Painted Bat, *Kerivoula hardwickei*

Asiatic Barbastelle, *Barbastella leucomelas*

Big Brown Bat, *Eptesicus serotinus*

Greater Evening Bat, *Ia io*

Southwestern Mouse-eared Bat, *Myotis altarium*

Chinese Mouse-eared Bat, *Myotis chinensis*

Water Mouse-eared Bat,*Myotis daubentoni*

Hodgson's Mouse-eared Bat, *Myotis formosus*

Fujian Mouse-eared Bat,*Myotis frater*

Hairy-footed Mouse-eared Bat, *Myotis macrodactylus (Myotis fimbriatus)*

Whiskered Mouse-eared Bat, *Myotis mystacinus*

Beijing Mouse-eared Bat, *Myotis pequinius*

Fine-haired Noctule, *Nyctalus velutinus*

Indian Pipistrelle, *Pipistrellus coromandra*

Javan Pipistrelle, *Pipistrellus javanicus*

Common Pipistrelle, *Pipistrellus pipistrellus*

Dusty Pipistrelle, *Pipistrellus pulveratus*

Savi's Pipistrelle, *Pipistrellus savii*

Gray Long-eared Bat, *Plecotus auritus*

Harlequin Bat, *Scotomanes ornatus*

Flat-headed Bat, *Tylonycteris pachypus*

Eastern Bat, *Vespertilio superans*

Lesser Tube-nosed Bat, *Murina aurata*

White-bellied Tube-nosed Bat, *Murina leucogaster*

Schreiber's Long-fingered Bat, *Miniopterus schreibersi*

Molossidae

Wrinkle-lipped Bat, *Tadarida teniotis*

UNIVERSITY of NORTHERN COLORADO

IACUC Memorandum

To: Dr. Rick Adams
From: Laura Martin, Director of Compliance and Operations
CC: IACUC Files
Date: 7/9/13
Re: IACUC Protocol 1205C-RA-B-15 Annual Renewal Approval

The UNC IACUC has reviewed your annual renewal request for animal use protocol 1205C-RA-B-15.

The committee's review was based on the requirements of the Government Principles, the Public Health Policy, the USDA Animal Welfare Act and Regulations, and the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of live vertebrate animals at the University of Northern Colorado.

Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol for an additional year.

UNIVERSITY of NORTHERN COLORADO

IACUC Memorandum

To: Dr. Rick Adams
From: Laura Martin, Director of Compliance and Operations
CC: IACUC Files
Date: 5/5/2017
Re: IACUC Protocol 1205C-RA-B-15 Amendment Approval

The UNC IACUC has reviewed your request for an amendment to animal use protocol 1205C-RA-B-15.

The committee's review was based on the requirements of the Government Principles, the Public Health Policy, the USDA Animal Welfare Act and Regulations, and the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of live vertebrate animals at the University of Northern Colorado.

Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified modification as submitted to the committee on May 31, 2012 (addition of species and site).

APPENDIX B

BAT SPECIES DISTRIBUTED IN SICHUAN PROVINCE AND THEIR AVERAGE CALL PARAMETERS

Bat species were derived from Wilson and Reeder (2005) and Smith and Xie (2008), while search-phase call parameters came from Collen (2012). Grey highlighting indicates that a species is unlikely to occur in Chengdu.

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
Pteropodidae	*Rousettus*	*leschenaultii*	2	-	-	-	-	-	-	-	-	-
Rhinolophidae	*Rhinolophus*	*affinis*	12	15.78	27.88	80.65	0.01	80.65	64.87	80.55	0.01	13.7
	Rhinolophus	*cornutus*	No data									
	Rhinolophus	*ferrumequinum*	12	12.55	48.09	82.34	0	82.34	69.81	82.18	0	22.59
	Rhinolophus	*lepidus / shortridgeii*	12	18.37	35.34	103.3	0.01	103.3	84.75	103.2	0.01	5.46
	Rhinolophus	*luctus*	12	1.49	64.97	31.07	0	31.11	29.65	31.07	0	34.07
	Rhinolophus	*macrotis*	12	6.44	28.47	47	0.01	47	40.58	46.95	0	6.18
	Rhinolophus	*pearsonii*	12	5.33	42.3	56.72	0	56.72	51.42	56.68	0	11.55
	Rhinolophus	*pusillus*	12	12.18	36.08	109.93	0	109.93	97.72	109.85	0	5.15
	Rhinolophus	*rex*	12	3.99	43.52	26.01	0	26.04	22.06	26.01	0	32.62
	Rhinolophus	*sinicus*	12	13.46	32.33	84	0.39	86.84	71.74	84	0.4	10.12
Hipposideridae	*Aselliscus*	*stoliczkanus*	13	13.34	3.55	127.61	0.19	127.61	114.25	127.19	0.17	6.09
	Hipposideros	*armiger*	13	8.24	8.87	67.16	0.06	67.16	58.89	67.07	0.08	49.99
	Hipposideros	*lylei*	No data									

[a]See Collen (2012) for descriptions of Echolocation Call Type.

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
	Hipposideros	*pratti*	13	7.35	8.01	60.83	0.06	60.83	53.45	60.77	0.04	84.38
Megadermatidae	*Megaderma*	*lyra*	9	23.29	1.39	80.5	8.4	80.5	57.32	68.25	10.94	39.27
Molossidae	*Tadarida*	*insignis*	5	14.22	3.19	10.17	3.52	24.15	10.17	16.32	4.54	28.53
Vespertilionidae	*Barbastella*	*leucomelas*	10	11.33	2.52	28.55	3.48	39.82	28.55	34.49	3.55	15.05
	Eptesicus	*pachyotis*[b]	8	24.09	5.86	36.34	2.59	61.89	35.55	39.92	4.95	8.1
	Eptesicus	*serotinus*[b]	7	25.6	6.39	26.42	3.2	52.15	26.42	31.17	5.24	23.09
	Harpiocephalus	*harpia*	7	52.03	2.55	36.6	27.38	90.12	34.57	48.42	26.67	13.65
	Hypsugo	*alaschanicus*	7	21.66	4.41	40.33	4	61.56	39.41	42.52	6.48	5.6
	Hypsugo	*pulveratus*	7	21.71	4.4	40.45	4	61.76	39.53	42.65	6.5	5.53
	Ia	*io*	8	18.86	3.8	25	6.9	37.2	22.87	25	8.33	49.3
	Myotis	*altarium*	7	29.77	4.56	33.55	7.53	75.41	32.95	43.68	10.42	11
	Myotis	*chinensis*	7	33.68	4.56	25.3	7.02	59.1	25.3	34.47	8.55	41.99
	Myotis	*fimbriatus*	7	58.95	2.8	15.45	20.77	74.56	15.45	26.34	23.03	12.34
	Myotis	*formosus*	7	54.36	2.35	38.92	28.42	93.39	38.92	54.26	25.92	7.07
	Myotis	*frater*	7	60.6	3.5	51.01	15.56	110.8	50.2	68.17	18.76	7.54
	Myotis	*laniger*	7	47.75	3.56	34.78	12.85	85.08	33.75	46.25	15.61	7.66
	Myotis	*muricola*	No data									
	Myotis	*pequinius*	7	71.39	5.72	17	16.55	84	17	33	19.13	17.41

[a]See Collen (2012) for descriptions of Echolocation Call Type.

[b]Apparent gap in range map. Possibly present but not yet documented.

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
	Murina	*aurata*	7	66.67	1.6	67.83	36.91	137.59	63.75	85.88	36.37	4.27
	Nyctalus	*plancyi*	10	29.27	1.85	23.37	16.26	52.75	23.37	34.96	17.63	15.15
	Pipistrellus	*abramus*	8	8.83	8.33	43.33	0.56	52.19	43.33	44.6	1.36	5.87
	Pipistrellus	*pipistrellus*	8	23.69	5.22	45.44	1.22	69.14	45.44	47.61	5.17	5.3
	Pipistrellus	*tenuis*	8	23	7	48	1.58	68	45	48	3.84	3.48
	Plecotus	*austriacus*	8	18.98	2.94	22.74	4.87	41.75	22.74	29.66	7.18	6.75
	Scotomanes	*ornatus*	8	22.17	4.69	27.49	5.8	46.5	26.52	30.3	7.73	22.24
	Scotophilus	*heathii*	8	29.24	3.96	30.42	7.04	56.6	29.11	34.81	9.45	36.13
	Tylonycteris	*pachypus*	8	43.73	3.76	43.86	2.95	88.35	43.86	48.95	11.21	4.1
	Vespertilio	*sinensis*	3	27.17	1.95	25.71	12.58	47.21	19.7	25.71	13.61	24.3
Miniopteridae	*Miniopterus*	*magnater*	8	36.87	3.7	46.62	7.19	80.35	45.02	49.85	11.13	14.14
	Miniopterus	*schreibersii*	8	27.11	5.47	50.44	1.9	77.5	50.44	53.41	6	11.46

[a]See Collen (2012) for descriptions of Echolocation Call Type.

APPENDIX C

ADDITIONAL BAT SPECIES IN CHINA AND THEIR AVERAGE CALL PARAMETERS

These species are not projected to be in Sichuan Province but should still be considered as possibly present given the limited number of records from the country. Bat species were derived from Wilson and Reeder (2005) and Smith and Xie (2008), while search-phase call parameters came from Collen (2012).

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
Pteropodidae	*Cynopterus*	*brachyotis*	1	-	-	-	-	-	-	-	-	-
	Cynopterus	*sphinx*	1	-	-	-	-	-	-	-	-	-
	Eonycteris	*spelaea*	1	-	-	-	-	-	-	-	-	-
	Macroglossus	*sobrinus*	No data									
	Pteropus	*dasymallus*	No data									
	Pteropus	*giganteus*	1	-	-	-	-	-	-	-	-	-
	Pteropus	*lylei*	No data									
	Pteropus	*vampyrus*	No data									
	Rousettus	*amplexicaudatus*	No data									
	Sphaerias	*blanfordi*	1	-	-	-	-	-	-	-	-	-
Rhinolophidae	*Rhinolophus*	*formosae*	No data									
	Rhinolophus	*monoceros*	12	16.27	40.39	106.45	0.01	107.29	90.99	106.45	0.01	7.49
	Rhinolophus	*osgoodi*	2	11.24	28.67	72.1	0.04	76.16	61.87	71.66	0.06	12.39
	Rhinolophus	*paradoxolophus*	12	5.07	48.76	44	0.83	46.23	38.71	44	0.84	8.17
	Rhinolophus	*rouxii*[b]	No data									
	Rhinolophus	*siamensis*	No data									
	Rhinolophus	*subbadius*	No data									
	Rhinolophus	*thomasi*	No data									

[a]See Collen (2012) for descriptions of Echolocation Call Type.
[b]Questionable. Based on one record.

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
	Rhinolophus	*trifoliatus*	No data									
	Rhinolophus	*yunanensis*	12	4.74	49.34	49.77	0	49.77	45.03	49.7	0	19.33
Hipposideridae	*Coelops*	*frithii*	13	17.4	5.51	141	0.59	137.12	122.98	141	0.23	7.52
	Hipposideros	*fulvus*	No data									
	Hipposideros	*grandis*	No data									
	Hipposideros	*larvatus*	13	11.4	5.87	93.79	0.11	93.79	82.43	93.57	0.1	19.95
	Hipposideros	*lylei*	No data									
	Hipposideros	*turpis*	No data									
Emballonuridae	*Taphozous*	*melanopogon*	4	8.05	4.06	29.11	0.84	32.08	24.19	29.11	1.21	25.99
Molossidae	*Chaerephon*	*plicatus*	10	12.69	7.66	19.11	1.92	29.4	16.63	20.78	2.45	21.83
	Tadarida	*latouchei*	3	8.3	9.22	16.14	0.66	22.43	15.06	18.36	0.92	27.66
	Tadarida	*teniotis*	No data									
Vespertilionidae	*Arielulus*	*circumdatus*	10	34.51	3.75	31.69	9.52	65.64	30.6	37.37	12.16	10.4
	Arielulus	*torquatus*	No data									
	Eptesicus	*bottae*	3	14.99	7.76	30.47	0.92	45.56	30.47	33.56	2	15.66
	Eptesicus	*gobiensis*	8	22.6	6.28	32.92	2.28	56.18	32.17	36.31	4.47	10.95
	Eptesicus	*nilssonii*	8	9.53	11.72	26.5	0.6	35.95	26.5	28.55	1.07	10.72
	Falsistrellus	*affinis*	7	34.87	3.54	40.57	12.91	71.93	38.17	44.12	15.42	10.77
	Falsistrellus	*mordax*	No data									
	Hesperoptenus	*tickelli*	8	19.21	7.05	28.19	1.58	45.72	27.39	30.69	3.54	16.3
	Kerivoula	*hardwickii*	7	95.09	1.99	92.97	39.78	188.17	92.97	148.17	39.87	4.55
	Kerivoula	*picta*	7	66.93	0.58	116	51.04	160.28	101.09	116	48.86	4.5
	Murina	*cyclotis*	7	56.34	2	77	31.22	125.66	64.14	77	30.85	9.35
	Murina	*fusca*	7	64	1.72	60.89	35.77	128.37	57.17	77.4	35.04	6.14

[a]See Collen (2012) for descriptions of Echolocation Call Type.

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
	Murina	*hilgendorfi*	7	75.22	1.37	40.96	48.02	116.47	40.96	58.22	46.26	8.08
	Murina	*huttoni*	7	62.31	1.92	49.35	33.49	116.64	46.62	65.83	32.65	7.56
	Murina	*puta*	No data									
	Murina	*ussuriensis*	No data									
	Myotis	*adversus*	No data									
	Myotis	*annectans*	No data									
	Myotis	*blythii*	8	53.72	3.54	29.94	18.49	83.39	29.94	44	18.3	23.82
	Myotis	*bombinus*	7	66.9	4.12	24	16.06	87.12	22.94	38.71	18.78	9.74
	Myotis	*brandtii*	No data									
	Myotis	*dasycneme*	7	32.9	4.45	28.15	5.38	60.86	28.15	39.43	6.46	15.16
	Myotis	*daubentonii*	7	42.78	3.26	33.09	11.57	75.79	33.09	46.98	12.72	7.63
	Myotis	*davidii*	7	47.08	4.05	30.3	11.63	78.87	29.55	42.31	14.05	13.24
	Myotis	*horsfieldii*	7	41.71	4.12	39.92	7.91	85.56	39.33	51.21	11.09	6.05
	Myotis	*ikonnikovi*	7	59.03	2.18	40.02	32.42	98.73	40.02	48.34	28.24	5.86
	Myotis	*longipes*	No data									
	Myotis	*montivagus*	7	46.64	3.14	38.4	14.54	88.13	37.49	50.45	16.99	8.3
	Myotis	*nipalensis*	7	47.75	3.56	34.78	12.85	85.08	33.75	46.25	15.61	7.66
	Myotis	*pilosus/ricketti*	7	39.36	3.65	30.62	10.01	70.1	30.62	40.52	10.83	26.19
	Nyctalus	*aviator*	7	27.72	1.89	22.76	13.56	50.81	22.76	40.29	16.42	31.87
	Pipistrellus	*ceylonicus*	8	16.82	5.64	41.22	1.28	58.17	41.18	43.6	3.27	8.05
	Pipistrellus	*coromandra*	8	20.37	6.64	46.39	1.5	67.11	44.97	48.33	3.61	4.59
	Pipistrellus	*javanicus*	8	18.95	6.65	45.97	1.36	65	44.7	47.51	3.38	4.92

[a]See Collen (2012) for descriptions of Echolocation Call Type.

Family	Genus	Species	Call parameters									
			Echolocation Call Type[a]	Bandwidth (kHz)	Call Duration (ms)	Characteristic Frequency (kHz)	Dominant Slope (kHz/ms)	Maximum Frequency (kHz)	Minimum Frequency (kHz)	Peak Frequency (kHz)	Total Slope (kHz/ms)	Body mass (g)
	Pipistrellus	*kuhlii*	No data									
	Pipistrellus	*paterculus*	8	17.67	5.39	44.04	1.45	62.02	44.04	46.43	3.55	4.91
	Plecotus	*auritus*	10	25.51	2.51	15.57	7.38	51.18	25.57	36.39	10.77	8.19
	Plecotus	*tairanus*	No data									
	Scotophilus	*heathii*	8	29.24	3.96	30.42	7.04	56.6	29.11	34.81	9.45	36.13
	Scotophilus	*kuhlii*	No data									
	Tylonycteris	*robustula*	8	61	3.78	45	10.98	103	45	55	16.03	7.98
	Vespertilio	*murinus*	3	8.53	14.07	22.46	0.46	31.11	22.46	24.35	0.9	15.42
Miniopteridae	*Miniopterus*	*magnater*	8	36.87	3.7	46.62	7.19	80.35	45.02	49.85	11.13	14.14
	Miniopterus	*medius*	8	37.43	3.58	48.86	7.3	83.25	47.18	52.04	11.32	11.4
	Miniopterus	*pusillus*	No data									

[a]See Collen (2012) for descriptions of Echolocation Call Type.

APPENDIX D

INDIVIDUAL CAPTURE DATA FOR BATS MIST NETTED IN THE AREA OF CHENGDU, CHINA

Date	Location	Species[a]	Capture Time	Sex	Age	Repro. Status[b]	Wt. (g)	Forearm (mm)	Fur Hg (mg/kg)	Guano OC[c]	Genetic Analysis	Notes
21 June 2013	Chengdu Panda Base	*P. abramus*	20:52	F	J	--	6.9	32.69	--		√	
		P. abramus	21:01	F	A	Lac	6.6	33.85	--		√	
		P. abramus	21:10	M	A	Scrot	5.7	33.64	--		√	
23 June 2013	Chengdu Panda Base	*P. abramus*	20:17	F	A	Lac	8.7	34.10	--		√	
		P. abramus	20:31	F	A	Preg	7.7	34.28	4.559		√	Missing patches of fur; small lesion on back of head (Fig. 10b)
		P. abramus	20:38	F	A	Preg	7.5	32.50	--		√	
		P. abramus	--	F	A	Preg	6.9	34.00	--		√	Missing patches of fur on back (Fig. 10a)
		P. abramus	21:10	F	A	Lac	7.2	33.00	--		√	
25 June 2013	Chengdu Panda Base	*P. abramus*	20:15	F	A	Lac	6.3	34.00	15.163		√	
		P. abramus	20:28	F	A	Lac	7.0	34.50	16.073		√	
		P. abramus	20:28	F	A	Lac	6.8	33.75	--		√	
		P. abramus	20:35	M	J	--	4.2	N/A	--		√	
		P. abramus	20:38	M	J	--	6.0	N/A	--		√	
		P. abramus	20:45	M	A	Scrot	6.6	33.50	--		√	
		P. abramus	20:45	F	A	Preg	7.3	34.00	9.804		√	
		P. abramus	20:48	M	A	Scrot	5.8	32.00	14.14		√	
		S. ornatus	20:55	F	A	Lac	38.3	57.00	--		--	
		P. abramus	21:01	F	A	Lac	7.5	33.50	--		√	
		P. abramus	21:10	F	A	Lac	7.2	33.00	--	√	√	
		P. abramus	21:16	F	A	Non-repro	7.6	34.00	--		√	Growth on chin; dark growth under skin on back (Fig. 10c)

[a]*N. = Nyctalus*; *P. = Pipistrellus*; *S. = Scotomanes*

[b]Lac = lactating; Non-repro = non-reproductive; Preg = pregnant; Scrot = scrotal

[c]Does not include composite samples collected beneath roosts because the bats' identities were unknown.

Date	Location	Species[a]	Capture Time	Sex	Age	Repro. Status[b]	Wt. (g)	Forearm (mm)	Fur Hg (mg/kg)	Guano OC[c]	Genetic Analysis	Notes
		P. abramus	21:16	F	A	Lac	7.4	33.00	5.498		√	
		P. abramus	21:49	F	J	--	5.1	N/A	6.790		√	
		P. abramus	23:45	F	A	Lac	6.2	33.50	--		√	
		P. abramus	00:25	F	A	Lac	7.2	34.00	--		√	
28 June 2013	Sichuan University	*P. abramus*	21:00	F	A	Lac	7.4	33.75	--		√	
		N. plancyi	21:40	F	A	Lac	24.6	55.00	--		--	Lots of mites
		N. plancyi	21:40	F	J	--	13.8	51.50	--		--	Missing spot of fur on back; lots of mites (Fig. 11c)
2 July 2013	Sichuan University	*P. abramus*	20:48	M	A	Scrot	5.6	32.00	--		√	
		P. abramus	20:48	M	A	Scrot	4.3	32.50	6.278		√	
		N. plancyi	21:45	F	J	--	16.1	53.00	1.174		--	
6 July 2013	Sichuan University	*P. abramus*	20:26	M	J	--	5.1	34.50	--		√	
		P. abramus	20:29	F	J	--	3.1	31.50	--		√	
		P. abramus	20:35	M	A	Scrot	5.3	34.00	--		√	
		P. abramus	21:45	M	A	Scrot	6.6	34.50	21.382		--	
		N. plancyi	21:56	M	J	--	17.1	49.00	6.897		--	
14 July 2013	Jiang'an Campus	*P. abramus*	20:30	F	A	Preg	8.1	34.75	--		√	
		P. abramus	21:30	M	J	--	5.1	N/A	--		√	
16 July 2013	Sichuan University	*P. abramus*	20:08	M	A	Scrot	4.7	33.75	--		√	
		P. abramus	20:20	F	A	Lac	5.8	32.50	--		--	
		P. abramus	20:41	F	A	Post-lac	5.2	32.00	18.614		√	
		P. abramus	20:45	M	J	--	4.5	34.00	11.150		√	

[a]*N. = Nyctalus*; *P. = Pipistrellus*; *S. = Scotomanes*

[b]Lac = lactating; Non-repro = non-reproductive; Preg = pregnant; Scrot = scrotal

[c]Does not include composite samples collected beneath roosts because the bats' identities were unknown.

Date	Location	Species[a]	Capture Time	Sex	Age	Repro. Status[b]	Wt. (g)	Forearm (mm)	Fur Hg (mg/kg)	Guano OC[c]	Genetic Analysis	Notes
		P. abramus	20:50	F	A	Lac	5.8	33.50	--		√	
18 July 2013	Xi Hua Normal University	*P. abramus*	19:20-20:20	F	A	Lac / Post-lac	6.9	34.03	--		√	Missing fur
		P. abramus	19:20-20:20	F	A	Non-repro	6.3	32.63	--		√	
		P. abramus	19:20-20:20	F	A	Post-lac	6.5	32.67	9.036		√	
		N. plancyi	19:20-20:20	F	A	Post-lac	20.9	55.18	2.929		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	20.2	54.58	4.857		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	22.6	54.12	--		--	
		N. plancyi	19:20-20:20	M	J	--	20.0	52.88	--		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.6	54.07	6.142		--	
		N. plancyi	19:20-20:20	F	J	--	23.6	51.23	--		--	
		N. plancyi	19:20-20:20	F	J	--	19.6	52.40	--		--	
		N. plancyi	19:20-20:20	M	J	--	19.4	52.20	4.415		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.6	53.80	1.759		--	
		N. plancyi	19:20-20:20	F	J	--	23.6	51.23	--		--	
		N. plancyi	19:20-20:20	F	J	--	19.6	52.40	--		--	

[a]*N. = Nyctalus*; *P. = Pipistrellus*; *S. = Scotomanes*

[b]Lac = lactating; Non-repro = non-reproductive; Preg = pregnant; Scrot = scrotal

[c]Does not include composite samples collected beneath roosts because the bats' identities were unknown.

Date	Location	Species[a]	Capture Time	Sex	Age	Repro. Status[b]	Wt. (g)	Forearm (mm)	Fur Hg (mg/kg)	Guano OC[c]	Genetic Analysis	Notes
		N. plancyi	19:20-20:20	F	J	--	23.6	51.23	--		--	
		N. plancyi	19:20-20:20	F	J	--	19.6	52.40	--		--	
		N. plancyi	19:20-20:20	M	J	--	19.4	52.20	4.415		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.6	53.80	1.759		--	
		N. plancyi	19:20-20:20	F	A	Lac	20.3	53.54	2.325		--	
		N. plancyi	19:20-20:20	M	J	--	19.7	51.48	0.547		--	
		N. plancyi	19:20-20:20	F	J	--	15.2	53.11	2.025		--	
		N. plancyi	19:20-20:20	F	J	--	18.3	51.57	0.657		--	
		N. plancyi	19:20-20:20	F	A	Lac	21.2	53.32	4.966		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.4	50.70	5.040		--	
		N. plancyi	19:20-20:20	F	J	--	16.0	51.64	0.383		--	
		N. plancyi	19:20-20:20	F	A	Non-repro	23.5	55.12	3.839		--	
		N. plancyi	19:20-20:20	M	J	--	16.6	51.10	--		--	
		N. plancyi	19:20-20:20	M	J	--	21.0	53.47	0.460		--	
		N. plancyi	19:20-20:20	M	J	--	19.2	53.11	--		--	

[a]*N. = Nyctalus*; *P. = Pipistrellus*; *S. = Scotomanes*

[b]Lac = lactating; Non-repro = non-reproductive; Preg = pregnant; Scrot = scrotal

[c]Does not include composite samples collected beneath roosts because the bats' identities were unknown.

Date	Location	Species[a]	Capture Time	Sex	Age	Repro. Status[b]	Wt. (g)	Forearm (mm)	Fur Hg (mg/kg)	Guano OC[c]	Genetic Analysis	Notes
		N. plancyi	19:20-20:20	M	A	Scrot	24.5	54.03	2.596		--	
		N. plancyi	19:20-20:20	F	J	--	18.4	53.20	1.743		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.2	53.22	0.633		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	17.5	53.60	5.679		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.5	53.51	--		--	
		N. plancyi	19:20-20:20	M	J	--	13.3	50.19	0.532		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	19.2	53.21	3.252		--	
		N. plancyi	19:20-20:20	M	J	--	12.2	49.73	1.283		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	20.2	52.60	4.360		--	
		N. plancyi	19:20-20:20	M	A	Scrot	22.4	52.60	--		--	
		N. plancyi	19:20-20:20	F	A	Post-lac	N/A	53.98	--		--	
		N. plancyi	19:20-20:20	F	J	--	15.9	51.96	0.633		--	
		N. plancyi	19:20-20:20	M	J	--	17.4	49.88	0.924		--	
		N. plancyi	19:20-20:20	F	J	--	15.9	53.15	--		--	
		N. plancyi	19:20-20:20	F	A	Lac	21.2	55.53	5.264		--	

[a]*N. = Nyctalus*; *P. = Pipistrellus*; *S. = Scotomanes*

[b]Lac = lactating; Non-repro = non-reproductive; Preg = pregnant; Scrot = scrotal

[c]Does not include composite samples collected beneath roosts because the bats' identities were unknown.

Date	Location	Species[a]	Capture Time	Sex	Age	Repro. Status[b]	Wt. (g)	Forearm (mm)	Fur Hg (mg/kg)	Guano OC[c]	Genetic Analysis	Notes
		N. plancyi	19:20-20:20	F	J	--	16.8	51.87	0.713		--	
		N. plancyi	19:20-20:20	M	Juv.	--	17.2	49.92	0.633		--	
		N. plancyi	19:20-20:20	F	A	Lac	20.4	53.12	1.506		--	
		N. plancyi	19:20-20:21	F	A	??[d]	23.7	52.94	0.978		--	
25 July 2013	An Long organic farm	*P. abramus*	20:00	F	A	Post-lac	6.9	33.00	--		√	
		P. abramus	20:05	F	A	Post-lac	7.3	34.25	32.870		√	
		P. abramus	20:05	F	J	--	6.3	33.75	--		√	

[a]*N. = Nyctalus*; *P. = Pipistrellus*; *S. = Scotomanes*

[b]Lac = lactating; Non-repro = non-reproductive; Preg = pregnant; Scrot = scrotal

[c]Does not include composite samples collected beneath roosts because the bats' identities were unknown.

[d]Bat was released before reproductive status was noted.

APPENDIX E

CONCENTRATIONS OF ORGANOCHLORINES IN GUANO OF *PIPISTRELLUS ABRAMUS* FROM THE CHENGDU AREA

Samples were analyzed via gas chromatography—electron capture detection and are reported in ng/g (ppb).

Location	Panda Base	Xi Hua Normal	Xi Hua Normal
Sample Descriptor	Adult female	Roost composite	Roost composite
Wet Weight (g)	0.14	0.50	0.50
Collection Date	6/25/2013	7/18/2013	7/18/2013
Receive Date	3/26/2014	3/26/2014	3/26/2014
Extraction Date	3/28/2014	3/28/2014	3/28/2014
Analysis Date	4/23/2014	4/23/2014	4/23/2014
Surrogate Compounds	**%Recovery**	**%Recovery**	**%Recovery**
DBOFB	50.9	52.4	52.8
PCB103*	98.2	95.2	103.5
PCB198	67.5	66.3	70.9
	Concentration	**Concentration**	**Concentration**
Total PCBs	ND	ND	ND
Chlorinated Benzenes			
Tetrachlorobenzene 1,2,4,5	ND	ND	ND
Tetrachlorobenzene 1,2,3,4	ND	ND	ND
Pentachlorobenzene	ND	ND	ND
Hexachlorobenzene	2.29	4.40	2.00
Hexachlorocyclohexanes			
Alpha HCH	ND	ND	ND
Beta HCH	ND	ND	ND
Gamma HCH	ND	ND	ND
Delta HCH	ND	ND	ND
Chlordane-related Compounds			
Heptachlor	ND	ND	ND
Heptachlor Epoxide	ND	ND	ND
Oxychlordane	ND	ND	ND
Alpha Chlordane	ND	1.39	1.81
Gamma Chlordane	ND	ND	ND
Cis-Nonachlor	ND	ND	ND
Trans-Nonachlor	ND	ND	ND
Other Cyclodiene Pesticides			
Aldrin	ND	ND	ND
Dieldrin	ND	ND	ND
Endrin	ND	ND	ND
Pesticides			
Other Chlorinated Pesticides			
Pentachloroanisole	ND	ND	ND
Chlorpyrifos	ND	ND	ND
Mirex	ND	ND	ND
Endosulfan II	ND	ND	ND
DDTs and Related Compounds			
2,4' DDE	ND	ND	ND
4,4' DDE	ND	ND	6.02
2,4' DDD	ND	2.36	1.43
4,4' DDD	ND	3.50	1.33
2,4' DDT	ND	ND	ND
4,4' DDT	ND	ND	ND