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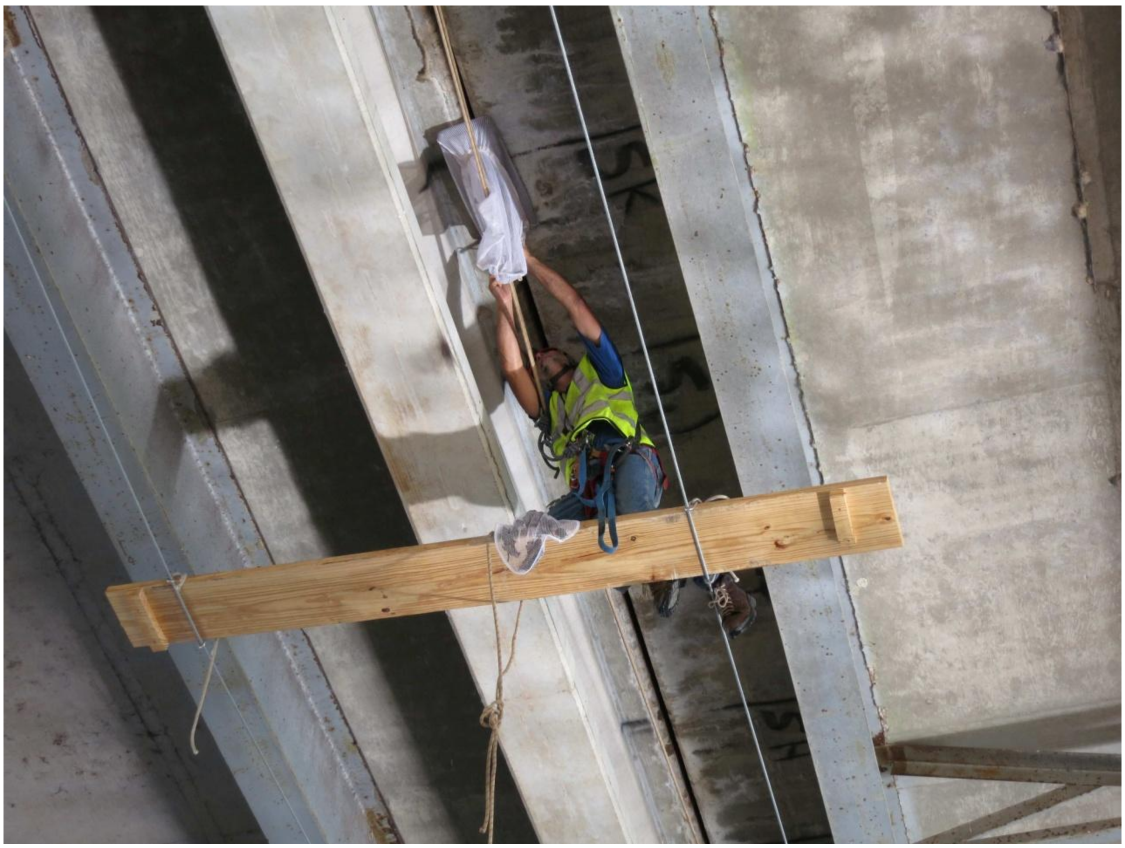
BAT SPECIES AND HABITAT MONITORING AT THREE BRIDGE SITES IN BELL AND CORYELL COUNTY, TEXAS



Biologist retrieving Brazilian free-tail bats (*Tadarida brasiliensis*), visible in mesh holding bag, from a bridge gap for mark-recapture population estimates.

Prepared for
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30 September 2013

Abstract

We studied three sites in central Texas in order to describe and monitor existing bat colonies at bridges that are scheduled to be replaced, and to facilitate the future installation and monitoring of bat houses at new bridges. Two bridges on Interstate Highway 35 over the Lampasas River and Salado Creek in Bell County (Lampasas and Salado bridges), have existing colonies of mainly Brazilian free-tailed bats (*Tadarida brasiliensis*), and a small portion of cave myotis (*Myotis velifer*) roosting in linear gaps between bridge segments. A third bridge, State Highway 9 over Turkey Run Creek in Coryell County (Turkey Run bridge), is new and is being constructed with bat houses. To characterize these populations, biologists measured the habitat dimensions, temperature and humidity, bat colony seasonality, abundance, diversity, and the upstream and downstream water quality in relation to guano for a year preceding the demolition of the existing two bridges in order to have a baseline for future comparison.

At the two occupied bridges, Lampasas and Salado, we measured 124 and 110 linear meters (m) of regularly utilized roosting area containing 2.18 m^3 and 0.43 m^3 of volume at Lampasas and Salado bridges, respectively. This quantity of gap habitat used is less than the quantity of habitat provided by the bat houses (132 linear meters and 2.54 m^3 at each bridge) that will replace the existing gap habitat once the bridges are removed and replaced. Technicians using boom lifts marked one meter sections of the accessible portions of the linear gaps, and used those marks to perform point counts with photography.

A photographer performed 82 sampling events over a period of a year (40 at Lampasas bridge, 42 at Salado bridge), and technicians counted individuals in the images. Point counts at the Lampasas bridge, based on 19 sampling events during the late summer through fall (15 June 15 – 15 November), averaged 4,025 ± 203. Sampling from bat traps at the Lampasas bridge indicates there may be between 1.4 and 2.6 times more bats inhabiting the gap than we are able to observe by counting the single layer of bats visible in the photographs. We estimate the maximum potential average late summer/fall bat count at 5,635 to 10,465 individuals, based on the 1.4 and 2.6 multipliers elucidated from the bat trap data. Based on 22 sampling events, the average number of bats counted at the Salado bridge during the late summer through fall (15 June – 15 November) is 2,577 ± 345. Sampling from bat traps at the Salado bridge yielded a single estimate (versus a range of estimates such as at Lampasas) of 2.2 times more bats inhabiting the gap than we are able to observe by counting the single layer of bats visible in the photographs. We estimate the maximum potential average late summer/fall bat count at 5,669 individuals (with as many as 11,725 on some occasions), based on the 2.2 multiplier elucidated from the bat trap data. Daily fluctuations were typically higher during the spring and fall, with changes in point counts of up to 1.4 times more bats between counts on day 1 and day 3. At the Lampasas bridge we documented the lowest number of bats in December and January, with less than 100 individuals that remained through the winter. Bats began arriving in late February, with a 'spring peak' of 1,500-2,200 (point count value, not including multiplier) from the middle of March to the middle of April. After that, numbers varied and were lower for about a month until late May, and from there to early November the point counts were highest (1,200-5,500), with the peak in late October. A pregnant bat was seen on 6 June 2012, and juveniles and a lactating bat were captured on 23 July 2012. The point counts at Salado bridge were similar, though slightly lower, than those at Lampasas, and no evidence of breeding was found there.

Biologists experimented with five captive Brazilian free-tailed bats and found no detrimental effect of placing Passive Integrated Transponder (PIT) tags subcutaneously on the back. After this experiment, 695 bats were tagged during 7 events and recaptured during 21

events to make population estimates using the POPAN formulation to estimate abundance in Program MARK. We estimated that the population at Lampasas bridge ranged between 7,540 and 11,198 individuals during the summer and spring of 2012, respectively, and that the population at Salado bridge ranged between 1,785 and 10,453 individuals during the summer and fall of 2012. These numbers are in the same range as our point counts after the multipliers are applied, with a maximum average at 10,465 for Lampasas bridge and 5,669 at Salado bridge.

To document species diversity, technicians performed acoustic surveys at all three sites from April 2012 to January 2013. Using a combination of autoclassifiers in Sonobat v3.1 and knowledge of species abundance and ranges, biologists identified seven species present at the two bridges with bat colonies, and three species at Turkey Run bridge. Bat activity as measured by bat passes, correlated well with point counts performed using photomonitoring. Peaks of activity, as defined by >50 bat passes in two hours, for the most abundant species, Brazilian free-tailed bats (*Tadarida brasiliensis*), were from June 2012 to November 2012. Early mistnetting attempts failed because the areas to sample were too large, and even if multiple net arrays were used, they would have likely been inundated by the most abundant species and failed to detect rare species such as the tricolored bat (*Perimyotis subflavus*) that only had a total of three passes at all sites over all seasons.

Temperature and humidity values were measured by 25 iButton® (Maxim Integrated$_{TM}$) dataloggers placed inside the gaps used by bats, inside mock bathouses placed in different sites, and outside the gaps (but under the bridge for control) yielded 167,253 datapoints. Temperature readings inside the gaps were higher than outside. This may be due to lack of airflow within the gap which would allow the ambient temperature of the gap to remain warmer, the presence of bats in the gap, or the thermal load resulting from the surrounding concrete. Temperature means were not different among four sample bat houses installed at Lampasas bridge, and all of the placement options were satisfactory in that they provided a buffer from the daily surface minimum. The range is within the temperatures the species is known to tolerate (25-38°C), though bats may exhibit some preference toward temperatures lower than 35-38°C. Two of the mock bathouses at the Lampasas bridge, designated as the 'southwest' and 'northwest,' had the least variance, meaning their minimum temperature is a little higher and maximum is a little lower. This is consistent with a larger thermal load on the main structure (versus frontage road bridge where the other two houses were), and points toward a minor preference to placing the houses on the main lanes instead of the frontage lanes.

Biologists documented the locations of 90 swallow nests at Lampasas bridge, and 96 at Salado bridge. These locations, together with bat locations, are memorialized for future comparison with water quality parameters related to guano deposition.

A final consideration in bat house placement is the effect of guano on water quality standards established by the Texas Commission on Environmental Quality (TCEQ). The standards are set with the goal of maintaining the quality of surface waters for public health, recreation, and aquatic life while allowing sustainable economic growth (TCEQ 2013b). We performed upstream and downstream sampling, and found water quality was impacted by the presence of bats, particularly downstream of the occupied bridges. Primary contact recreation standards for *Escherichia coli* bacteria were exceeded at all three bridges, particularly during storm events. The highest concentration if *E. coli,* 40,000 colonies/100 ml, was measured downstream of the Lampasas bridge. There was no significant variability in water quality due to the diurnal movements of the bat colonies and there was a lack of consistent seasonal trends among the occupied bridges. The most significant impacts to water quality occurred during storm events, likely due to the flushing of guano into the streams by storm pulses. The installation of suggested best management practices (e.g. berms and excluding bats from roosting over water) could mitigate impacts to water quality.

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1.0 Introduction and Overview

1.1 Background

More than half of America's bats are endangered or declining in numbers sufficient to warrant concern (Keeley and Tuttle 1999). Bats are especially susceptible to extinction due to low productivity (i.e. produce only one pup/year) and because most species form large colonies in vulnerable locations. Landscape change, agricultural intensification, development, and habitat fragmentation have also contributed to the loss of suitable bat habitat. As a consequence of losing natural roosts, bridges and culverts have become important roosting, migratory, and maternity locations. Twenty-four of the 45 U.S. species of bats have been documented to use highway structures as day and night roosts, and based on their known preferences at least 13 others are likely to use bridges and culverts (Keeley and Tuttle 1999).

The Texas Department of Transportation (TxDOT) has proposed the removal of two bridge structures on Interstate Highway (IH) 35 at the crossing of the Salado and Lampasas Rivers in Bell County and the construction of a new location bridge on State Highway (SH) 9 crossing Turkey Run in Coryell County. The Lampasas and Salado River bridges are both currently occupied by an unknown number of Brazilian free-tailed bats (*Tadarida brasiliensis*). In addition to Brazilian free-tailed bats, three additional species of bat known to use crevice roosts such as those found on bridges have ranges that include Bell and/or Coryell County, Texas (Davis and Schmidly 1997): cave myotis (*Myotis velifer*) (Keeley and Tuttle 1999), big brown bats (*Eptesicus fuscus*) (Lausen and Barclay 2002), and silver-haired bats (*Lasionycteris noctivagans*) (Mattson et al. 1996). A nationwide study of bats roosting in American bridges documented that Brazilian free-tailed bats were found most commonly roosting in bridges and structures, followed by big brown bats and cave myotis (Keeley and Tuttle 1999). Keeley and Tuttle (1999) did not document silver-haired bats roosting in the bridges they surveyed, but acknowledged the potential for that species to occur in bridge habitats.

Brazilian free-tailed bats are one of the most abundant bat species in the U.S and Mexico (Hristov et al. 2010), the either the most abundant (Schmidly 1994) or the second most abundant on the Edwards Plateau of central Texas (Davis and Schmidly 1997), and the species most commonly found to inhabit bridge structures (Keeley and Tuttle 1999). This is a migratory species[1] that overwinters in Mexico and the southwestern U.S., and is known to summer as far north as southern Oregon and Nebraska. In spite of this abundance, little information exists on seasonal variability in colony size and structure at bridge roosts. In addition, population trends are poorly understood because of a lack of repeatability of survey methods (Hristov et al. 2010). Researchers assert that the overall status of this species is truly unknown because of the lack of long-term monitoring at large colony sites (McCracken 2003). Estimating size of bat colonies has been logistically and technologically challenging, so few studies have been performed to make quantitative estimates of Brazilian free-tailed bat colony sizes (Hristov et al. 2010).

Cave myotis are either the most abundant (Davis and Schmidly 1997) or the second most abundant (Schmidly 1994) bat species on the Edwards Plateau, and the species third-most likely to inhabit bridge structures, following Brazilian free-tailed and big brown bats (Keeley and Tuttle 1999). This species ranges from the southwestern USA to Honduras (Barbour and Davis 1969). In caves, Myotis roost in large colonies, often numbering in the thousands. Like Brazilian free-tailed bats, cave myotis use of manmade structures like buildings and

[1] For our purposes, we assume all Brazilian free-tailed bats to be of the migratory subspecies, *T.b.mexicana.*

bridges is less well understood. In addition to sharing roosts with Brazilian free-tailed bats, cave myotis have been documented to sometimes roost with big-eared bats, big brown bats, Yuma myotis and ghost-faced bats (Davis and Schmidly 1997).

Big brown bat population studies are common in the literature (Davis et al. 2007, Ellison et al. 2007, Nuebaum et al. 2007 Grilliot et al. 2010, O'Shea et al. 2010, and others), likely because of their extensive range from northern Canada to southern Mexico (Bat Conservation International 2013a). While typically considered a forest dwelling species (Davis and Schmidly 1997), big brown bats were the species encountered at bridge surveys second most often, following Brazilian free-tailed bats (Keeley and Tuttle 1999).

Silver-haired bats are distributed throughout Texas but with relatively few county records (Schmidly 1994). This species typically roosts in tree cavities and under loose bark, particularly in coniferous or mixed old growth forests, but feed in along roadways or water courses (Bat Conservation International 2013b).

The goal of this study was to characterize the current bat usage at all three planned bridge locations prior to construction to enable comparison of pre-construction use of the bridge sites to use and occupancy of artificial structures designed to replace existing bat habitat post-construction. The objectives of this study were to:

1) Characterize pre- and post-construction bat diversity, abundance and occupancy patterns at the existing and planned bridge locations;
2) Measure and compare available habitat with habitat provided by artificial roost structures;
3) Develop design specifications for artificial roost structures;
4) Quantify impacts to water quality from bat roosts over water on water quality pre- and post-construction; and
5) Characterize importance of existing bridge structures to birds and compare nesting pre-and post-construction.

The work was designed to be repeated after the bridges are completed (post-construction) because the current (pre-construction) "opportunistic" bat habitat will be destroyed during construction, where it currently exists, and replaced by specifically designed artificial roosts. This report details findings during the pre-construction phase of the project.

This report is organized into seven chapters (including Chapter 1.0 Introduction) that detail each of six discrete tasks relating to the bat population and habitat use at the Lampasas and Salado bridges. In addition to this introductory chapter, the following chapters include:

- Chapter 2 Habitat Mapping, Microclimate, and Bat House Placement
    - Marked off one linear meter sections for monitoring
    - Calculated volume of habitable space
    - Microclimate recordings within and outside of habitat
    - Recommendations for bat house locations
    - Recommendations for exclusion
    - Recorded bird activity
- Chapter 3 Photomonitoring
    - Meter-by-meter photographic documentation of bat use
    - Estimated population size
    - Seasonal roosting activity estimates
- Chapter 4 Mark-recapture
    - Recorded sex ratios
    - Compared body-condition indices
    - Estimated population size
- Chapter 5 Acoustic Monitoring

    - Species diversity by site
- Chapter 6 Water Quality
    - Water quality monitoring upstream and downstream of bridge
        - *Escherichia coli*
        - ammonia, nitrate, nitrite, total Kjeldahl nitrogen [TKN] and phosphorus
    - Storm event sampling

1.2 Study Area

The study area includes three sites, two on IH 35 over the Lampasas River (Lampasas bridge), and Salado Creek (Salado bridge) in Bell County, and a third site on SH 9 over Turkey Run Creek (Turkey Run bridge) in Coryell County (Figure 1.1 to Figure 1.4). The riparian corridor near the Lampasas bridge consists of thick vegetation including sycamore (*Platanus occidentalis*), box elder (*Acer negundo*), chinaberry (*Melia azedarach*), hackberry (*Celtis lindheimeri*), poison ivy (*Toxicodendron radicans*), mountain dewberry (*rubus trivialis*), and mustang or sweet mountain grapes (*Vitis sp.)*. Water depth at the bridge does not exceed 0.6 m (2 ft), however up and downstream of the bridge water depth reaches up to 1.2 m (4 ft) (Figure 1.2). Riprap, silt and cobble line the banks just under the bridge, and most of the submerged substrate under the bridge is made up of silt and cobble. The riparian corridor near the Salado bridge has been heavily modified and landscaped to the east of the bridge (Figure 1.3). The first two sites have existing bridges with roosting colonies of Brazilian free-tailed bats, cave myotis, pigeons (rock doves, *Columba livia*) and nesting swallows (*Hirundo sp.*).

The Turkey Run bridge is located near Fort Hood military training installation. It is a newly constructed bridge, that was in fact still under construction at the time of this report, in a sparsely populated area. The area immediately surrounding Turkey Run has been heavily modified due to recent construction and consists mostly of bare, packed caliche. The areas up and downstream of the bridge are slightly less modified, but the area was subjected to overgrazing in the past (Figure 1.4).

The study area is located in the Cross Timbers and Prairies ecoregion of Texas (Griffith et al 2004). Alternating bands of wooded habitat scattered throughout a prairie region represents the ecoregion. All study sites are located within the Brazos River Basin, which drains an area of approximately 116,550 square km within Texas and New Mexico (Hendrickson 1999). Land use upstream of the Salado Creek and Lampasas River sites consists of rural residences, agricultural and livestock grazing. Land use upstream of the Turkey Run bridge site consists of urban residences, industrial, retail, and a golf course. A waste water treatment facility is located approximately 450 m upstream of the Turkey Run bridge.

The average annual rainfall within the study area is 80.5 cm, and the average high and low temperature is 26.1 and 12.3 °C, respectively (National Climate Data Center 2013). The Stillhouse Hollow Dam weather station is located approximately 4.43 km upstream of the Lampasas bridge site, and is the most intermediate weather station to all three sites with long-term, readily available data (Figure 1.1). The total precipitation recorded at the Stillhouse Hollow Dam weather station in 2011 was 52 cm, in 2012 it was 77.9 cm, and in January through April 2013 was 23.3 cm.

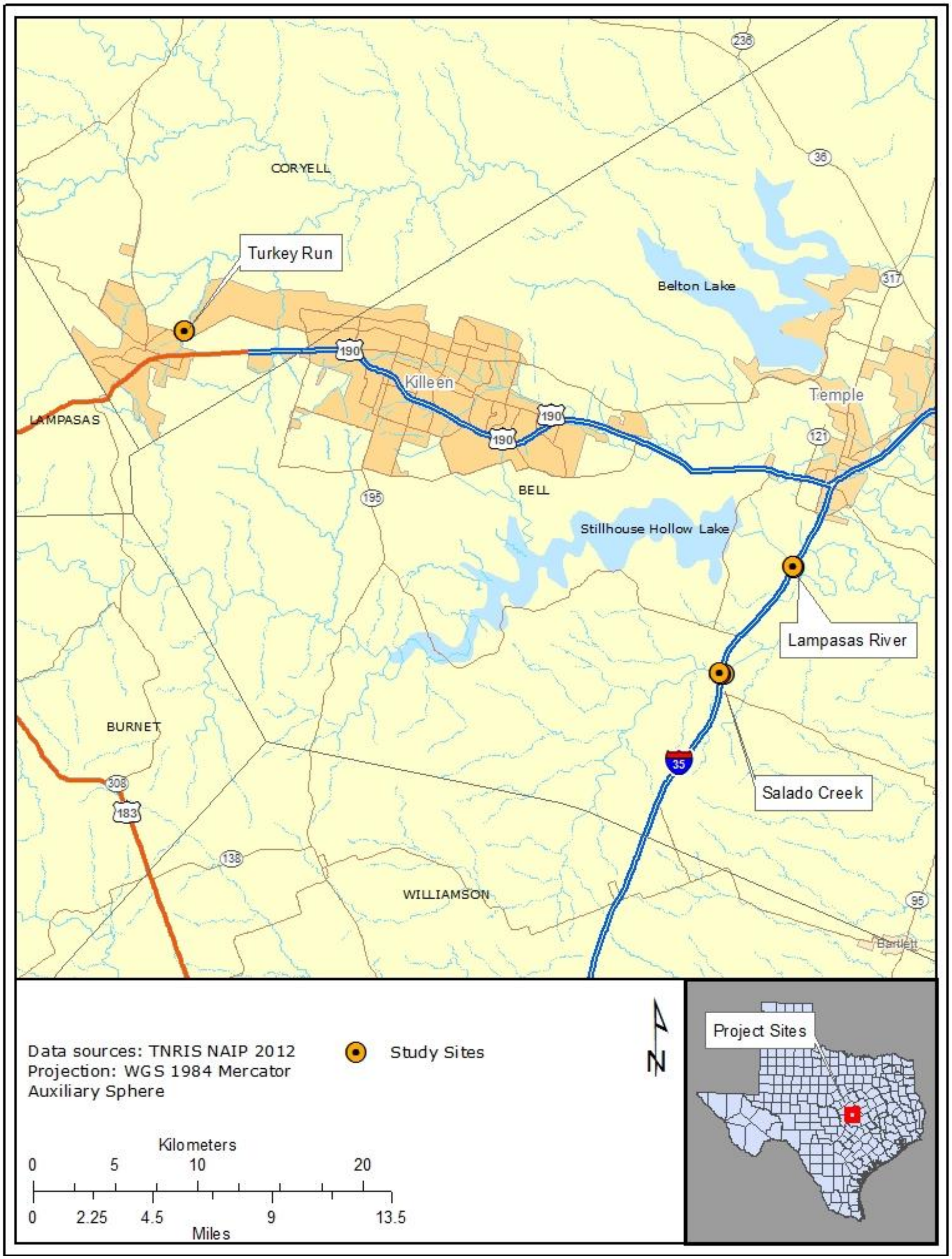

Figure 1.1. Location of the Lampasas bridge (Bell County), Salado bridge (Bell County), and Turkey Run bridge (Coryell County) sites, Texas.

Figure 1.2. The underside of the Lampasas Bridge in Bell County, Texas, showing the existing main lane bridge (left side) currently occupied by bats. The area to the right side of the photograph shows the new main lanes currently under construction.

Figure 1.3. The underside of Salado Creek bridge in Bell County, Texas, showing the existing main-lane bridge (left side), currently occupied by bats.

Figure 1.4. Turkey Run bridge in Coryell County, Texas. This site had no gap habitat and did not host a bat colony during the course of this study. The bridge is complete and the roadway is still under construction.

1.3 Methods

All researchers handling bats had current rabies pre-exposure vaccines. No obviously injured, daytime grounded bats, or very young pups were handled. All current recommended precautions regarding White Nose Syndrome were followed (Appendix A).

1.3.1 Sampling Frame

Our sampling schedule was based on five seasons relating to the approximate dates of major life history events for bats (Davis and Schmidly 1997; Harris 2005). Seasons were defined as winter (January), when only overwintering bats are present; spring (1 February-15 March), while breeding is occurring and bats are returning to their summer roosts; early summer (15 March - 15 June), during the gestational period; late summer (15 June - 30 August), during and after the birth of pups; and fall (1 October – 15 November), while migratory individuals are departing for their winter roosts. Some sampling fell outside of these named seasons, and those data are included in order to increase sample size and help establish trends. The number of times each bridge was sampled during the various seasons is detailed in Table 1.1.

In order to accomplish the overall goals of this study, it was essential to have landmarked field locations for reference across seasons and by multiple field crews at the Lampasas and Salado sites. Turkey Run had no gap habitat and no bat colony, therefore did not require landmarking. The inhabited bridge gaps were labeled each meter, and ropes were installed at various locations along the bridges to allow repeated access to the bats. Two and three letter segment designations in the figures in Chapter 2.0 are referenced in all other chapters.

Table 1.1. Bat monitoring techniques used in this study, showing number of replicates per bridge.

Activity/ Method	Date Range	Replicates	Lampasas	Salado	Turkey Run
Point Count (photography)	Winter (January)	7	5	2	0
Point Count (photography)	Spring (1 February - 15 March)	10	5	5	0
Point Count (photography)	Early Summer (15 March - 15 June)	16	8	8	0
Point Count (photography)	Late Summer (15 June - 30 August)	16	8	8	0
Point Count (photography)	September	6	3	3	0
Point Count (photography)	Fall (1 October - 15 November)	23	12	11	0
Point Count (photography)	December	6	3	3	0
Bat traps (photo verification)	Winter (January)	4	2	2	0
Bat traps (photo verification)	Spring (1 February - 15 March)	6	3	3	0
Bat traps (photo verification)	Early Summer (15 March - 15 June)	6	3	3	0
Bat traps (photo verification)	Late Summer (15 June - 30 August)	2	1	1	0
Bat traps (photo verification)	Fall (1 October - 15 November)	3	2	1	0
Acoustic Monitoring	Winter (January)	6	3	3	0
Acoustic Monitoring	Early Summer (15 March - 15 June)	14	6	3	5
Acoustic Monitoring	Late Summer (15 June - 30 August)	20	5	5	10
Acoustic Monitoring	Fall (1 October - 15 November)	14	5	5	4
Mist Netting	Fall (1 October - 15 November)	8	2	2	4
Mark Recapture	Spring (1 February - 15 March)	8	5	3	0
Mark Recapture	Early Summer (15 March - 15 June)	2	1	1	0
Mark Recapture	Late Summer (15 June - 30 August)	5	3	2	0
Mark Recapture	Fall (1 October - 15 November)	6	3	3	0

1.3.2 Population Estimation

In order to minimize the effect of inherent biases to population estimation methods, the

United States Geological Survey Working Group (2003) recommends the use of at least two sampling techniques. We used four methods to estimate population parameters, including photomonitoring, bat traps, mark-recapture, and acoustic monitoring. Mistnetting was attempted but abandoned because the area underneath the bridge and over the riparian corridor was too large to effectively cover. The various estimation methods were repeated over the five seasons described above, with multiple replicates per season (Table 1.1). More detail about the techniques employed and data analysis are provided in Chapter 2.0.

1.3.3 Habitat mapping

While bat houses are widely understood to be an effective tool for mitigating roost destruction, there are numerous cases where they go unoccupied. In this study we assume that the local environment has sufficient food and water to support the bats given the occupation of the current bridges. We focused on measuring the total volume of available habitat in relation to roosting locations, as well as the daily and seasonal structure of temperature and humidity fluctuations as measured using dataloggers.

1.3.4 Water Quality Monitoring

Bat guano contains a number of potentially harmful pollutants, including *Escherichia coli* (*E. coli*) bacteria, nutrients such as nitrogen and phosphorus and various other pathogens. There are limited data available on how bat colonies affect water quality; therefore, we collected water quality data to determine if impacts related to bat colony occupation could be detected during diurnal monitoring and storm events.

In addition to water quality impacts from bats, pigeons and swallows are also likely to contribute to nitrate and coliform counts. We performed visual counts of pigeons and swallows and mapped swallow nests in relation to the footprint of the river and areas that run off into the river.

2.0 Habitat Mapping, Microclimate, and Bat House Placement

2.1 Abstract

At two bridges occupied by Brazilian free-tailed bat (*Tadarida brasiliensis*) colonies on Interstate Highway 35 over Salado Creek and the Lampasas River in Bell County, Texas (Salado and Lampasas bridges), we marked and measured bat habitat in gaps between bridge sections. These marks were used to facilitate point counts of bats, and map locations of dataloggers, bat traps and bird nests. The measurements were used to compare available and occupied habitat of existing bridges scheduled for demolition with proposed bat houses to be installed at adjacent new bridges. At Lampasas bridge, 132 linear meters of available gap habitat had a total volume of 2.38 m^3. When overlaid with bat use as determined by point counts, we found bats use most of this volume, 2.18 m^3, and most of the linear area, 124 meters. At Salado bridge, 171 linear meters of available habitat had a total volume of 0.70 m^3, and a little more than half of the volume, 0.43 m^3, and 110 linear meters, was typically occupied by bats. Bat houses on new bridges at these sites will replace bridge gap habitat, and provide ample volume (2.54 m^3), and a comparable amount of linear gap space (132 m).

In order to help inform decisions regarding the placement of bat houses that will be installed under those bridges to provide alternate habitat to the bats, we investigated the microclimactic regimes to ensure that temperatures where the bat houses would be installed were comparable with temperatures inside the gap habitat where bats were roosting. We found that temperatures within the gap habitat were higher than temperatures outside of the gap habitat, and that temperatures within the mock bathouses were comparable to those within the gap.

Biologists documented the locations of 90 swallow nests at Lampasas bridge, and 96 at Salado bridge. We observed an average of 23 swallows and 11 pigeons at Lampasas bridge and 21 swallows and 17 pigeons at Salado bridge over 19 observation dates.

2.2 Background

The removal of the two existing bridge structures at Salado Creek and the Lampasas River will result in the destruction of roost sites that are potentially important for Brazilian free-tailed bats, one of which is being used as a maternity colony. As part of their commitment to the environment, TxDOT has opted to documented available habitat within the existing structures and to attempt to provide a similar amount of suitable habitat by providing artificial roost structures on the two bridges. Additionally, TxDOT will place artificial roost structures at a new bridge site over Turkey Run Creek as part of their Bats and Bridges program. In order to ensure that suitable habitat is provided, we quantified and mapped the gap habitat at Salado and Lampasas bridges and measured various other environmental parameters.

When choosing roost sites, bats require rather specific microclimate conditions (Bogan et al. 2003) that vary among species. Brazilian free-tailed bats (*Tadarida brasiliensis*) are extremely heat tolerant. Wilkins (1989) found the upper ambient temperature limit tolerated by Brazilian free-tailed bats is approximately 35°C, and according to Herreid (1967), Brazilian free-tailed bats in the lab avoided temperatures exceeding 35°C; however Wolf and Shaw (2002) cited studies indicating that caves where Brazilian free-tailed bats were commonly encountered ranged in temperature from 25-38°C. Earlier researchers reported them roosting in caves reaching 39°C, and have been recorded in barns with temperatures as high as 43°C (Licht and Leitner 1967).

Brazilian free-tailed bats are known to roost in gaps in the Interstate Highway (IH) 35 bridge over the Lampasas River (Lampasas bridge) and Salado Creek (Salado bridge). These two bridges are being demolished, and those, in addition to the SH 9 bridge over Turkey Run Creek (Turkey Run bridge), which currently harbors no bat colony, are having bat houses installed under the bridge structures. The research presented herein is among the first effort to study microclimate (temperature and humidity) in relation to use of gaps under bridges by Brazilian free-tailed bats, and relate that to an artificial roost mitigation effort.

In addition to investigating bathouse placement based on temperature and relative humidity, we sought to answer three very basic research questions. These questions were based on the following null hypotheses:

- there are no significant temperature or humidity differences within the gap and outside of the gap;
- there are no significant temperature or humidity differences between the cross gap at the Salado bridge and the main gap at the Salado bridge;
- there are no significant temperature or humidity differences between bridge sections typically containing bats and bridge sections not typically containing bats.

We also recorded the presence of pigeons, swallows or swallow nests to 1) document the number of birds nesting/roosting on the bridge; 2) because cave myotis (*Myotis velifer*) and Brazilian free-tailed bats have been known to temporarily inhabit swallow nests in culverts (Keeley and Tuttle 1999); and 3) because the bird guano could also affect water quality parameters (Chapter 6.0).

2.3 Methods

2.3.1 <u>Bridge Habitat mapping</u>

We mapped the occupied gaps at the Lampasas and Salado bridges in one meter (m) long sections, and marked each section with a unique letter code to identify that specific meter-long section of the bridge (Figure 2.1). We used a boom lift, ladders, and technical ropework to access bridge sections that were out of reach. The gap areas that were not accessible for labeling were those directly over the rivers. Because the labels were intended to facilitate bat counts within the gap habitat, there was no need to mark Turkey Run Bridge, which contained neither gap habitat nor bats.

Figure 2.1. Each linear meter of the gap at the IH 35 bridges over the Lampasas River and Salado Creek in Bell County, Texas, were assigned an alphabetical identifier, which was semi-permanently marked directly on the underside of the bridge using spray paint. Also visible in the image are stains from bat urine.

The width and depth of the gap at the end of each section was measured to the nearest centimeter (cm) (Figure 2.2). The top of the gap at the Lampasas bridge extended into a T shape (Figure 2.3), which we measured using a stiff bent wire of known length (Figure 2.4). The main gap measurements for width and depth over each meter long segment were multiplied together. For calculations at Lampasas, these were added to the volume of each segment of the T-shaped extension, whose volume was also calculated by multiplying the width and depth of each side of the T over the one meter segment.

Figure 2.2. The width and depth of each gap segment at the IH 35 bridges over the Lampasas River and Salado Creek in Bell County, Texas, were measured to the nearest centimeter in order to calculate the volume of the available habitat.

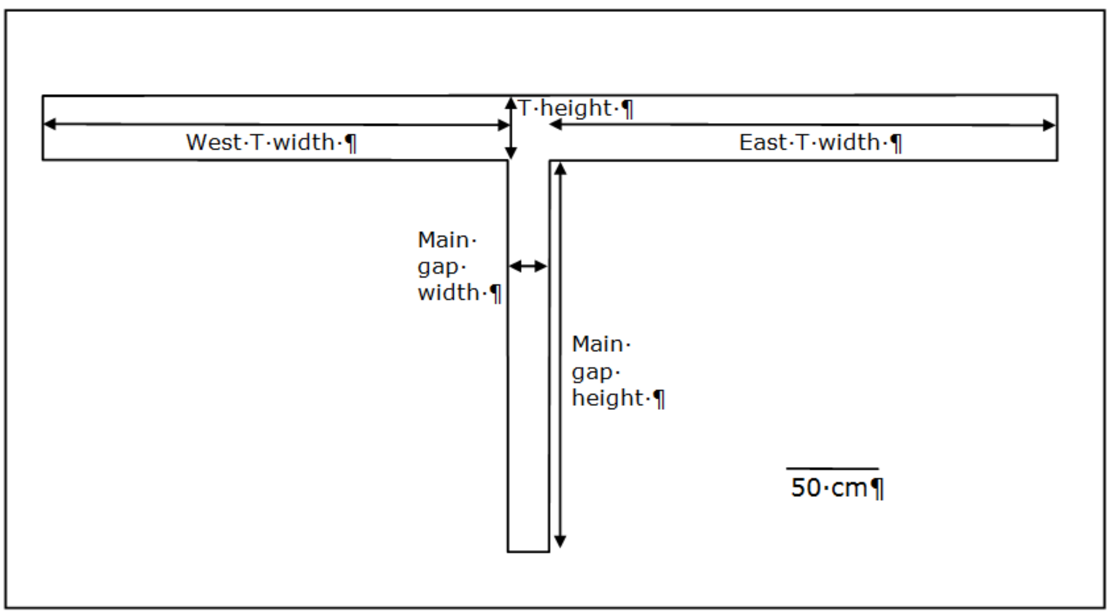

Figure 2.3. Cross sectional view of the T shaped gap in the IH 35 bridge over the Lampasas River in Bell County, Texas.

Figure 2.4. The width of the T at the top of the gap at Lampasas bridge, Bell County, Texas, varied along the length of the bridge. Stiff wire of varying known lengths was used to estimate the width at each meter.

The gap at the Salado bridge did not have the T-shaped extension; therefore, we measured the width and depth of the gap. In addition to the main gap at the Salado bridge, a single cross gap on the north end of the bridge was found to support roosting bats. Measurements for this cross gap were included in the gap volume calculations for the Salado bridge.

We added the volumes of each one meter segment together to calculate the total gap volume available for bat use. In areas where it was impossible to accurately measure each one meter segment due to the abundance of bats, inaccessible gap areas over the water or over an active roadway, or the presence of materials filling the gap, we used an average of all of the other measured segments for that bridge to fill the data point.

We refined the measure of total habitat volume to determine the volume of habitat that was actually used by bats, using point count data obtained from photomonitoring (Chapter 3.0) to justify our determinations. We calculated the volume of occupied gap habitat by using the sum of total available gap habitat volumes for all segments occupied by five or more bats for at least 20 percent of the time.

2.3.2 Microclimate

We installed 40 iButton DS1923 Hygrochron temperature/humidity loggers to record temperature and relative humidity within, alongside of, and away from the bat-inhabited gaps at Lampasas and Salado bridges, and on the underside of the Turkey Run bridge. Loggers were missioned and downloaded using an Embedded Data Systems Thermochron Server (Figure 2.5). Temperature and relative humidity were logged every 30 minutes at resolutions of 0.5°C and 0.6 percent, respectively (Figure 2.6) from September 2011 to

October 2012.

Figure 2.5. Thermochron Server from Embedded Data Systems used to download iButton data.

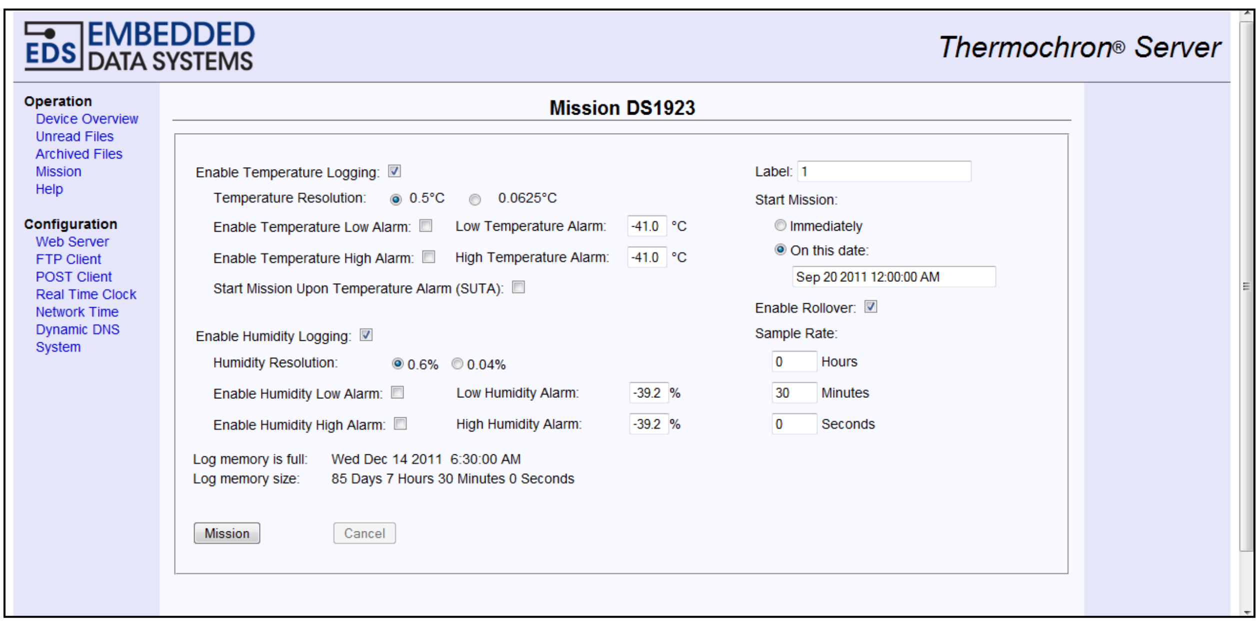

Figure 2.6. Mission settings for iButton data loggers.

Relative humidity (R.H.) readings are subject to saturation drift, or error derived from the sensors, and has been quantified by the manufacturer in their product specifications sheet Appendix B.1. When the sensor is reading a value of or greater than 70 percent for any amount of time, the subsequent values will begin to be recorded greater than the actual value and this will continue until the humidity drops below 70 percent. We attached loggers to the structures using a combination of rubber cement and Velcro tape.

Twenty loggers were deployed at the Lampasas Bridge, 15 at the Salado bridge and five at the Turkey Run bridge (Appendix B.2). Loggers were installed inside and outside the gap in areas known to regularly contain roosting bats and areas not known to regularly contain roosting bats (Table 2.1). Mock bathouses made of slatted plywood boxes were installed at four locations on the frontage road (Figure 2.7) to mimic the probable thermal regime of the bathouses that were planned for installation. We installed loggers in the mock houses to help record and predict the best location for the installation of the real bathouses (Figure 2.8). The 30 minute data was compiled in an access database to facilitate in quality control and assurance.

We used t-tests to determine whether there were significant differences between the means of temperature or humidity within the gap versus outside of the gap, within the cross gap at Salado versus the main gap, between bridge sections containing bats and bridge sections not containing bats.

In order to answer the question regarding the best place to install the new bat houses, we compared the temperature inside the gap versus outside the gap, and we compared the means, ranges, and variance of the temperature readings of the mock bat houses.

Table 2.1. Logger locations For Lampasas and Salado Bridges. Because depth in gap and distance out of gap were not recorded for the Salado Bridge "Yes" is used to symbolize presence of a logger and "No" is used to symbolize absence.

Bridge Name	Meter Name	Depth in gap(cm)	Distance out of gap(cm)
Lampasas	NAF	16	100
	NB	11	N/A
	ND	N/A	100
	NI	17	100
	NW	13	100
	SAN	19	N/A
	SBH	14	100/100
	SF	4	90
	ST	17	90
	SVA	13	N/A
Salado	NAB	Yes	No
	NEL	Yes	No
	NT	Yes	Yes
	NWJ	Yes	Yes
	SAZS	Yes	No
	SBOS	Yes	Yes
	SBYS	Yes	No

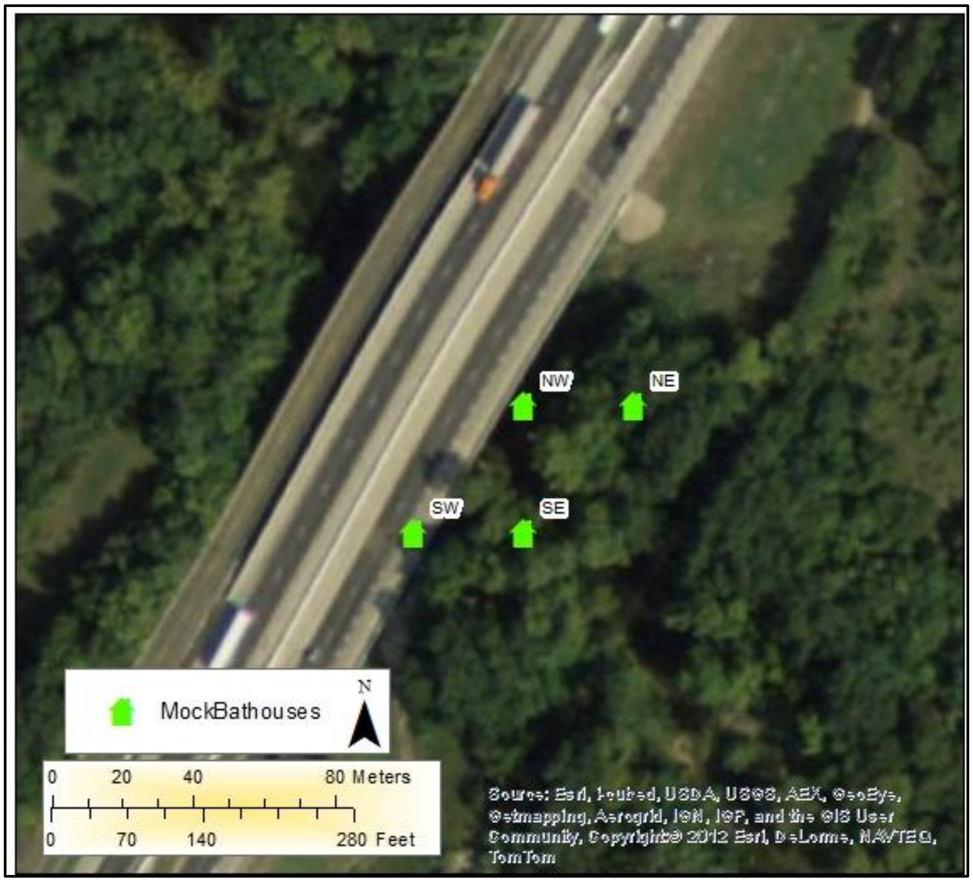

Figure 2.7. Map of mock bathouse locations in relation to the "old" Lampasas bridge, Bell County, Texas.

Figure 2.8. An installed bat house at the Lampasas bridge, Bell County, Texas, showing rope access for future monitoring.

2.3.3 Bat houses

We based bat house volume and gap length on engineering drawings provided by the Texas Department of Transportation (Appendix C, Figures C.1 and C.2).

2.3.4 Bird use of bridge habitat

We counted and mapped swallow nest sites in the field and confirmed nest locations and sizes during multiple site visits.

2.4 Results

2.4.1 Bridge habitat mapping

At the Lampasas bridge, 96 meter segments were marked along a 132 m span along which length, width and depth were measured (Appendix C, Table C.1), and a total of six ropes were installed to access areas that could not be reached with ladders (Appendix C, Figure C.3). The Lampasas Bridge gap included 21 unmeasurable segments where volume calculations were based on averages of all measured segments at this bridge. The total amount of gap habitat calculated for Lampasas was 2.38 m^3 over the available 132 m gap length, while the total occupied habitat was 2.18 m^3 over 124 m of gap length (Table 2.2).

At the Salado bridge, 129 meter segments were marked along a 144 m span of the main gap (Appendix C, Table C.2) and three ropes were installed to access areas that could not be reached with ladders (Appendix C, Figure C.4). An additional 28 meter segments were marked on a cross gap on the north side of Salado Creek that was occupied by bats. The Salado bridge gaps included 12 unmeasurable segments where volume calculations were based on averages of all measured segments at this bridge. The total amount of gap habitat calculated for Salado for both the main gap and cross gap was 0.70 m^3 over the total available 171 m gap length, while the total occupied habitat was 0.43 m^3 over 110 m of gap length (Table 2.2).

Table 2.2. Bat habitat summary values for the Lampasas and Salado bridges. Total length includes cross joints at Salado bridge.

Site	Total length (m)	Length used by bats (m)	Bat house length (m)	Total volume (m^3)	Volume used by bats (m^3)	Bat house volume (m^3)
Lampasas bridge	132	124	132	2.38	2.18	2.54
Salado bridge	171	110	132	0.70	0.43	2.54

2.4.2 Microclimate

Competent data were retrieved from 25 loggers; the rest of the data were not included in the analysis because of bad humidity readings or adhesive failure, largely a result of contact with bat urine.

A total of 167,253 temperature and humidity measurements were recorded and included in the analysis (Table 2.3). Each record consists of a time stamp, temperature and relative humidity.

Table 2.3. Total number of temperature and relative humidity values recorded and recovered by each logger. Differences in values are due to issues in data recovery or logger failure.

Bridge Name	Segment	In gap	Out of gap
Lampasas	NAF	11,076	6,981
	NB	2,259	---
	ND	---	11,078
	NI	746	11,079
	NW	6,981	6,981
	SAN	10,331	---
	SBH	4,096	4,096
	SF	---	740
	ST	---	8,192
	SVA	6,977	---
	BH NE	2,087	---
	BH NW	2,087	---
	BH SE	2,682	---
	BH SW	2,154	---
Salado	NAB	8,342	---
	NEL	8,209	11,678
	NT	4,845	7,551
	NWJ	752	8,492
	SAZS	7,555	---
	SBOS	748	4,242
	SBYS	4,216	---
Total		**86,143**	**81,110**

There were a total of eight bridge sections with competent paired temperature data (Table 2.4) and six with competent paired humidity data (Table 2.5) from within and just outside of the gap. The temperature and humidity in the gap was significantly different than the temperature and humidity outside of the gap for most of these sections, although not consistently higher or lower.

Table 2.4. Summary results of t-tests performed on temperature data within and outside of the gap for eight sections of the Lampasas bridge, Bell County, Texas. * denotes a significant difference between the means (p<0.05).

Bridge Section	Mean in gap	Mean out of gap	Variance in gap	Variance out of gap	Degrees of freedom	t Stat
NAF*	23.57	25.23	24.24	15.81	743	-5.07
NI*	29.27	25.76	8.56	12.12	745	14.54
NW*	25.51	23.81	29.49	23.63	6982	13.79
SBH*	9.38	11.92	15.94	18.69	982	-9.58
NEL	16.86	17.06	47.14	47.18	8206	-1.36
NT*	15.40	13.97	8.24	16.82	734	5.47
NWJ*	28.25	27.49	13.68	16.51	751	2.67
SBOS*	27.55	26.58	9.47	14.24	748	3.83

Table 2.5. Summary results of t-tests performed on humidity data within and outside of the gap for six sections of the Lampasas bridge, Bell County, Texas. * denotes a significant difference between the means (p<0.05).

Bridge Section	Mean in gap	Mean out of gap	Variance in gap	Variance out of gap	Degrees of freedom	t Stat
NAF*	66.44	44.33	216.35	137.03	742	22.69
NEL*	59.94	54.12	544.21	432.73	1710	5.44
NI*	48.49	43.60	177.63	120.92	744	5.46
NW*	54.96	43.17	239.36	125.09	744	11.93
NWJ	35.14	36.74	91.25	121.09	747	-2.12
SBOS*	35.86	37.96	107.16	116.83	748	-2.72

2.4.3 Bat houses

Each bat house contains 21.95 linear meters of gap for a total volume of 0.42 m^3 (Appendix C, Figures C.1 and C.2). Each bridge will have six bat houses installed (Figure 2.8), for a total length of 132 m and a total volume of 2.54 m^3. Table 2.2 summarizes this information, and demonstrates the bat houses are of adequate length and volume for replacing the bridge gap habitat.

In our analysis, we considered whether temperature within the gap was significantly different than ambient temperature within one meter outside the gap. We recorded significantly different temperatures (t (2762) =15.55, p<.01) within the gap (mean=29.0, s^2=10.7) than outside of the gap (mean=27.1, s^2=9.0) between May and July 2012 (Figure 2.9).

The temperature range commonly accepted by researchers as acceptable is indicated by the shaded area in Figure 2.10. All of the mock bat houses containing data loggers remained typically within this temperature range, although each dipped below the 25C threshold when ambient temperatures plummeted significantly in May and early June. Only the bathouse "NE" exceeded this temperature range, and then only when there was an unusually hot day and the ambient temperature rose to 40C (Figure 2.10).

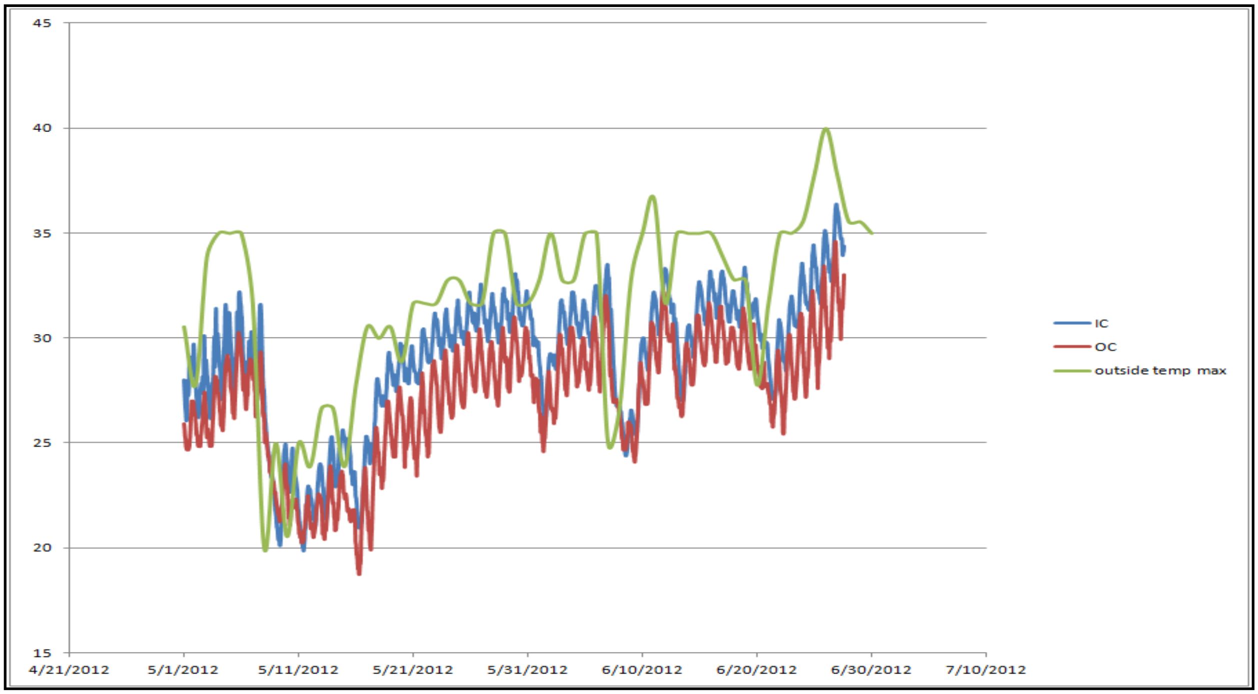

Figure 2.9. Average temperatures (in degrees Celsius) within (IC) and outside of (OC) the gap at Lampasas bridge, Bell County, Texas, compared with the maximum temperature recorded at a nearby weather station during the same 24 hour period (outside temp max).

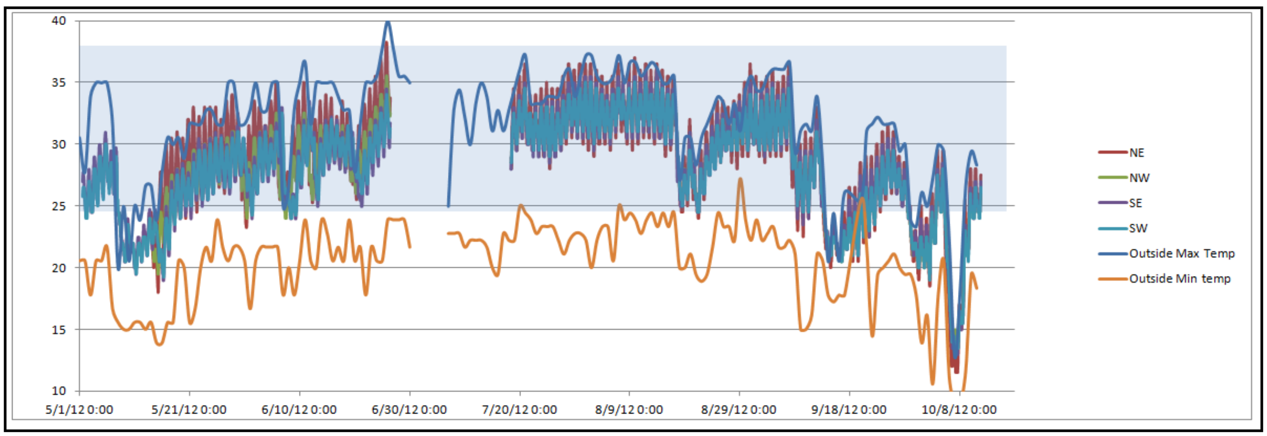

Figure 2.10. Daily temperatures (in degrees Celsius) within four mock bat houses at Lampasas bridge, Bell County, Texas, (NE, NW, SFE, SW - see Figure 2.7), compared with the daily minimum (daily surface min) and maximum (daily surface max) temperature recorded at a nearby weather station during the same 24 hour period. The blue shaded area represents the range of temperatures in which Herreid (1963) most often observed the largest cave populations of Brazilian free-tailed bats.

The bathouse labeled "NE" maintains higher overall temperatures than the other bathouses (Table 2.6); however ANOVA results indicate that between 14 May 2012 and 11 October 2012 there were no significant differences between the average temperatures of any of the four bat houses ($F(3,20556)=16.6$, $p<0.01$).

Table 2.6. Descriptive statistical results from comparing temperature data (in degrees Celsius) from dataloggers in each of the four "mock" bat houses at the Lampasas bridge, Bell County, Texas, with the average temperature within the gap and with data from two individual loggers located within the gap (loggers located in sections NAF and NX of the gap).

	NE* frontage	NW* main	SE* frontage	SW* main	Average IC**	NAF**	NX**
Mean Temp	28.8	28.5	28.5	28.2	29	30.6	29.8
Variance	22.4	18.0	19.2	18.4	10.7	10.8	12.4
Minimum Temp	11.5	13.5	13	13.5	19.9	21	20
Maximum Temp	38.3	35.5	36	35	36.4	38.5	37.5
Range	26.8	22	23	21.5	16.5	17.5	17.5

* Samples collected May 14, 2012 through October 11, 2012
**Samples collected May 14, 2012 through June 27, 2012

2.4.4 Bird use of bridge habitat

We documented approximately 90 swallow nesting sites at the Lampasas bridge (Appendix C.5) and approximately 96 at the Salado Bridge (Appendix C.6), with several of them supporting multiple nest cavities/openings. Pigeon activity at both bridges peaked in fall, and swallow activity at Lampasas peaked in early summer (Figure 2.11). We observed an average of 23 swallows and 11 pigeons at Lampasas bridge, and 21 swallows and 17 pigeons at Salado bridge over 19 observation dates (Appendix C, Table C.3). Occasionally, we found bats occupying nests built by swallows (Figure 2.12). We also documented pigeons roosting at both bridge sites.

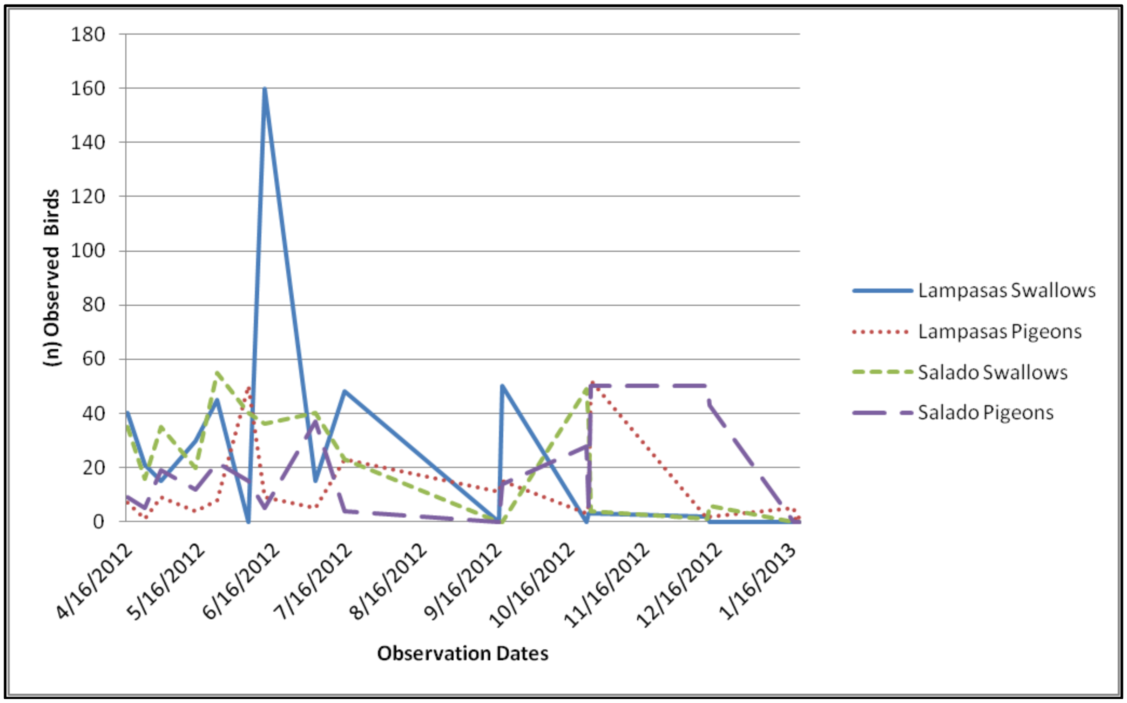

Figure 2.11. Observations of swallows and pigeons at the Lampasas and Salado bridges, Bell County, Texas.

Figure 2.12. A bat leaving a nest built by a swallow along a bridge beam.

2.5 Discussion

The specific habitat quantification of the existing gap habitat at each of the two bridges allowed us to determine that the bat houses would have a comparable amount of habitat. This will allow bat house habitation by Brazilian free-tailed bat colonies similar in size to those that currently inhabit the bridges. It is important that this habitat be preserved in order to prevent the bats from having to seek out new roosting sites. The use of swallow nests by bats was rare enough that we did not attempt to quantify the available habitat provided by the nests or anticipate changes to the availability of that habitat due to construction activities.

The influences of guano deposition and subsequent infiltration into the waterways needed to be considered when determining the placement of the bat houses and recommendations for best management practices below the bat houses. Mapping of bird roosts and bat use were logical steps in due diligence for preservation of water quality related to guano. Although more bats utilized the bridges than birds, bird use of the bridges may still contribute a significant amount of organic waste during storm events.

Our temperature readings inside the gaps were higher than outside. This may be due to lack of airflow within the gap which would allow the ambient temperature of the gap to remain warmer, the presence of bats in the gap, or the thermal load resulting from the surrounding concrete.

Temperature means were not different among four sample bat houses, and all of the placement options are good in that they are providing a buffer from the daily surface minimum, and providing a reasonable range of temperatures the species is known to tolerate (25-38°C, possibly with some preference away from 35-38°C). The southwest and northwest houses have the least variance, meaning their minimum temperature is a little higher and maximum is a little lower. This is consistent with a larger thermal load on the main structure (versus access road), and points toward a minor preference to placing the houses on the main lanes instead of the frontage lanes.

We recommend the exclusion of the bats from the current gap to be performed over the course of a week, and by excluding bats from only about 20 percent of the habitat at a time. Ideally the exclusion occurs outside of the times when many bats are present or when they are sensitive to disturbance (e.g. hibernation, mating, pupping). Ideal times are when nighttime lows do not fall below 50° F, but when populations are largely gone, which leaves a narrow gap in the spring and fall. According to our photomonitoring observations, spring point counts were at their lowest at the Lampasas bridge on 13 February 2012. During this time nighttime lows are typically below 50, and the overwintering bats, if excluded, will likely die. However, there are only between 100-300 individuals at that time. By 14 March 2012 the point counts were quite high (2,000-5,000), breeding has begun, and much more disturbance may occur as a result of the exclusion. During the fall, populations are quite high as late as 8 November 2012, and they have almost entirely left by 12 December 2012. Salado dates and counts are very similar. We recommend weekly monitoring leading up to the exclusion in order to time it correctly.

3.0 Population Estimation

3.1 Abstract

In order to estimate the population of Brazilian free-tailed bat (*Tadarida brasiliensis)* colonies at two bridges on Interstate Highway 35 over the Lampasas River and Salado Creek in Bell County, Texas (Lampasas and Salado bridges), we developed site specific point count techniques using photomonitoring to document bats in bridge gaps and employed a large—scale mark-recapture effort using passive integrated transponder (PIT) tags. We performed acoustic surveys at the two known bridge bat colonies and at a third site without a bat colony, the State Highway 9 bridge over Turkey Run (Turkey Run bridge), from April 2012 to January 2013.

We conducted photomonitoring events over one year to track seasonal population variability, and for three consecutive days during several events at each site to measure daily fluctuations in roosting activity. To estimate the number of bats roosting at each bridge, we photographed and then counted the number of bats visible in the photographs per one meter segment. We installed bat traps to assist with estimating the total number of bats present where bats were thought to occupy gap areas that were not visible, or where the bats were stacked such that a portion of them were not visible in photographs. These trap data provided a multiplier for the point counts. We estimated total colony size based on complete sampling efforts of bats in unmarked sections (e.g. those over the water) and multipliers determined with bat traps.

In order to verify that PIT tags would not damage individual bats, we captured five individuals from the Salado bridge, and after a 20-day observation period we injected them with PIT tags and held them for another 15-day observation period. The general condition and behavior of the captured bats did not change during the observation period, and the PIT tags did not migrate from their original injection site within the 15-day observation period. After the trial, we tagged a total of 695 individuals from two bridges, and recaptured bats during the spring and fall of 2012.

We performed 40 photographic sampling events at the Lampasas bridge; bats roosting over the water were photographed during 18 of those events. On average, 29 percent of the colony at Lampasas bridge roosted over the water. The average number of bats observed during point counts at the Lampasas bridge during late summer and fall 2012 (15 June - 15 November) was 4,025 ± 203. The average number of bats counted during all other [2012-2013] seasons was 906 ± 183. Sampling from bat traps at the Lampasas bridge indicates there may be between 1.4 and 2.6 times more bats inhabiting the gap than we are able to observe by counting the single layer of bats visible in the photographs. Using the multipliers to account for stacked bats in the gap, we estimate the maximum potential average late summer/fall bat count at 5,635 to 10,465 individuals. We performed 42 photographic sampling events at the Salado bridge, with 25 events including bats over the water and 14 events including bats in the cross gap. Eight of the sampling events included all bridge sections. On average, 28 percent of the colony at Salado bridge roosted over the water. The average number of bats counted at the Salado bridge between 15 June 2012 and 15 November 2012 was 2,577 ± 345. Based on 17 sampling events from all other times of year when bats were present during 2012, the average number of bats counted during all other dates is 533 ± 150.

An analysis of variance indicates that the months with greatest variance in three day point counts at Lampasas bridge were March and November, and at Salado they were October and August. The months with the least variance, and lowest bat numbers, at each bridge

were December and January. The average point counts at both bridges varied seasonally as bats reproduced and migrated, and there was a strong correlation between the number of bats any given month at either bridge (*r*=0.78). For the fall and late summer, when the most bats were present at either colony, there was not a significant difference in the variance between the bridges (ANOVA: $F(1,36)=3.07$, $p>0.05$).

During mark-recapture efforts at the Lampasas bridge there were significantly more males captured ($p<0.05$), and the females had a higher body condition index. This, along with observations of pups and pregnant and lactating females during the summer of 2012, demonstrates that the Lampasas bridge was occupied by a maternity colony in 2012. We recorded no significant difference between the number of male and female bats captured from the Salado Bridge, indicating this is a non-breeding mixed-sex colony ($p>0.05$). Eleven tagged bats were re-captured. Using the POPAN formulation to estimate abundance in Program MARK, we estimated that the population at Lampasas bridge ranged between 7,540 and 11,198 individuals during the summer and spring of 2012, respectively, and that the population at Salado bridge ranged between 1,785 and 10,453 individuals during the summer and fall of 2012. These numbers roughly correspond to observations made during point counts, with some differences that may be attributed to violation of assumptions related to emigration. We conclude that PIT tagging is a viable method for long term tracking of individuals and for the study of populations of Brazilian free-tailed bats.

We identified seven species present at the two bridges with bat colonies and three species at Turkey Run bridge using a combination of autoclassifiers in Sonobat v3.1 and knowledge of species abundance and ranges. Bat activity as measured by bat passes correlated well with point counts performed using photomonitoring. Peaks of activity, as defined by >50 bat passes in two hours, for the most abundant species, Brazilian free-tailed bats (*Tadarida brasiliensis*), were from June 2012 to November 2012. While the data collection and analysis was not an insignificant effort, it was the only way to measure diversity at these sites. Early mistnetting attempts failed because the areas to sample were too large, and even if multiple net arrays were used, they would have likely been inundated by the most abundant species and failed to detect rare species such as the tricolored bat (*Perimyotis subflavus*) that only had a total of three passes at all sites over all seasons. The acoustic data will provide an excellent baseline for comparison between pre-construction (this study) and post-construction (future study) bat diversity.

3.2 Background

In order to determine the success of artificial bat roosts being installed at the Interstate Highway (IH) 35 bridges over Lampasas River (Lampasas bridge) and Salado Creek (Salado bridge), it was necessary to estimate the population of bats roosting in the gap habitat prior to exclusion. The research presented herein includes photographic and mark-recapture techniques, and is among the first effort to study populations of this species through the use of passive integrated transponder (PIT) tags, which were installed in bats from the two bridges known to host colonies. Acoustic monitoring techniques were employed to examine species diversity and to establish background activity patterns of bats immediately local to the bridge site.

Researchers have used imaging techniques to estimate bat populations for many decades. We reviewed various methods in terms of their efficacy at our study sites, and determined that still photography of day roosts was the best method to perform point counts of the Brazilian free-tailed bat (*Tadarida brasiliensis*) populations at the Lampasas bridge and Salado bridge. The method is longstanding, with early researchers such as Constantine (1967) and Davis and others (1962) extrapolating density estimates over available roost space to estimate colony sizes of roosting Brazilian free-tailed bats in Carlsbad Caverns and in several caves in central Texas. Still photography provides a permanent record of bat occupancy and density, and can be used as a baseline for future comparison (O'Shea 2003; Humphrey 1971). This method employs less opportunity for observer bias, reduces the likelihood of inaccurate counts that may occur if bats are changing positions during a counting event, and enables straightforward replication and validation by other scientists.

Relatively recent technological advances in the use of infrared video imaging and analyses provide accurate estimates of bat colony sizes (e.g. - Hristov et al. 2010). Thermal imaging and analysis is useful when bats are emerging from a single point with a thermally stable background, such as a cave entrance. This technique is less well suited to estimating at our study sites because we observed the bats exiting along the entire span of the bridge, and exiting on both sides. The combination of this factor and the high cost associated with the equipment and analysis led us to dismiss this technique.

Another way to perform point counts is to record the exit flight. Video recordings (Altenbach and others 1979), still photography (Humphrey 1971, Altenbach and others 1979) and infrared imaging (Hristov et al. 2010) have been used to estimate the number of bats as they emerge from cave entrances. These estimates are problematic at our study sites for several reasons, including the tendency of Brazilian free-tailed bats to utilize non-uniform patterns that involve mid-flight directional changes, and even direction reversals so that some bats are entering the roost site during exodus (McCraken 2003). Still photography of roosting bats, particularly in a bridge gap where the habitat is consistent within the entire roost and bats typically cannot escape detection by moving to inaccessible areas, provides more accurate and precise estimates of colony size than photography of bats in flight or bats roosting in a cave.

In addition to measuring seasonal variation, we became interested in the day-to-day variation of our point counts at these sites. While much research focuses on seasonal variation of bat populations (Hristov et al. 2010), McCracken (2003) noted that day-to-day variations can be high, and Hristov et al. (2010) showed fluctuations in consecutive nightly emergence from Carlsbad Caverns as high as 291,000 individuals.

Brazilian free-tailed bats are a migratory species, and in some cases are known to segregate by gender during certain times of the year. A single site could provide roosts for an overwintering population, a spring migration stop-off, a summer colony, a maternity colony, a mating swarm, or a fall migration stop-off. Defining the use of these bridges in terms of sex, condition, and population levels is crucial for understanding their purpose and any potential impacts from the changes anticipated as a result of construction.

Marking individual animals can be a valuable and effective technique for estimating population size, documenting use of roosts, and tracking migration patterns. Different marking techniques have their own unique set of challenges. Leg bands may interfere with flight membranes; forearm bands may cause long term swelling, bleeding, and infections, and are subject to being chewed off. The injury rate to bats can be so extensive that Bat World Sanctuary (2010) issued a position statement discouraging the use of any type of bat banding. Instead, the position statement presented evidence for the success of tattoos on ears and wing membranes, freeze branding (Sherwin et al. 2002), necklaces (Gannon and Willig 1998) and radio transmitters (for short term study). The position statement discusses the use of subdermally implanted passive integrated transponder (PIT) tags as a method of marking individuals, but warns that the large size of the needle may present a significant route for infections. The paper encourages an examination of the effects of PIT tags on different, especially smaller, species of bat.

Passive integrated transponder tags are permanent radio frequency identification (RFID) tags that are inserted under the skin of the study organism. They are encoded with a unique letter-number combination that can be read via radio waves with a mounted or hand held RFID or PIT tag reader. The use of PIT tags enables the researcher to identify individual organisms when they are captured at a later time.

A brief literature review shows that subdermal PIT tags have been used successfully on at least seven species of bats. These include studies of big brown bats (*Eptesicus fuscus*) (Ellison et al. 2007, Nuebaum et al. 2007 Grilliot et al. 2010, O'Shea et al. 2010, and others), Beehstein's Bats (*Myotis beehstcinii*) (Reekardt and Kerth 2007), eastern pipistrelles (now tricolored bat) (*Permimyotis subflavus*) (Damm and Geluso 2008), Hemprich's long-eared bat (*Otonycteris hemprichii*) (Daniel et al. 2010), northern long-eared bats (*Myotis septentrionalis*) (Patriquin et al. 2010), little brown myotis (*Myotis lucifugus*) (O'Shea and Bogan 2003, p. 245), and Townsend's Big-Eared Bat (Froschauer 2011). The use of PIT tags in Brazilian free-tail bats has not been documented in the literature, in fact Davis et al. (2007) PIT tagged big brown bats and specifically did not tag Brazilian free-tailed bats in an experiment involving both species.

Acoustic monitoring of bat populations is an effective and widely utilized method for determining species richness, habitat use, activity patterns, and relative abundance (Fenton 1988; La Val 1988; O'Shea et al 2003). While acoustic methods are valuable in that they are non-invasive and effective at measuring certain parameters, there are also many limitations. The data should not be over-interpreted, with the largest limitation being that the method is not a direct measure of abundance like the point counts; rather, it is a measure of relative abundance. Five bat passes can indicate five bats or one bat flying by the detector five times. Bat detectors do not detect bats as they fly by silently, which they may often do when near a roost and flying by memory, they will not perform well with some types of background noise (rustling leaves, certain types of frogs and insects, man-made sources of sound at a similar frequency), and they only detect sounds within a certain distance and within a certain orientation of the microphone. However, the number of bat passes is a measure of bat activity and an index of the number of bats. Several researchers use an acoustic activity index (AI) as a less biased indicator of bat activity than bat passes (Miller 2001). The AI measurement requires manipulation of the data into single minute-long segments during which the researcher documents the number of species present during that minute. Because the output from Sonobat v3.1 automatically counts bat passes, and because the number of bat passes strongly correlates with AI (Miller 2001), we did not pursue this additional manipulation of the data.

Mistnetting is the traditional approach for measuring species richness and to evaluate activity patterns and relative abundance of bat species. In addition to these data, during mistnetting many other factors can be measured regarding life history and growth. We attempted mistnetting and used acoustic methods as a way to assess species composition and complement other quantitative abundance methods.

Our objectives were to create a permanent record for baseline occupancy, to evaluate the efficacy of using PIT tags in Brazilian free-tailed bats, to estimate the population sizes of the Lampasas and Salado colonies, and to establish a repeatable methodology in order to ultimately compare the diversity of bats at the existing three sites with the diversity after construction of new bat-house equipped bridges. In addition, we sought to investigate the demographics of the Brazilian free-tailed bat colonies roosting at the Lampasas and Salado bridges through evaluation of the following null hypotheses:

- there are no significant differences between the apparent roosting populations at

Lampasas or Salado;

- there are no significant seasonal variations of roosting populations at Lampasas or Salado;
- there are no significant daily variations of roosting populations at Lampasas or Salado;
- There are no significant differences between the numbers of male and female bats captured during any season;
- There are no significant differences between the body condition indices of male and female bats during any season

3.3 Methods

We used man-lifts and ropes to access, measure and mark the roost sites at Salado and Lampasas bridges. A photography specialist then imaged each uniquely marked one meter section of the occupied cervices at the Salado bridge and the Lampasas bridge from October 2011 to December 2012 (Table 1.1). In many cases, we also photographed the unmarked sections over the water in the channels of the Lampasas River and Salado Creek, and details on those sample dates are covered in section 3.4.1. In the cross gap at Salado photography was not possible because the size and shape of the cross gap prevented us from achieving the proper distance and angle with the camera. At those gaps we performed visual field counts. The third bridge discussed elsewhere in this report, SH 9 over Turkey Run Creek, was not included in the point-count or mark-recapture analysis because it did not have an existing bat population during this study.

3.3.1 Photographic Monitoring

The base of the camera/optics system is a Canon 7D protected and powered by a Viewfactor Contineo camera support cage. This (1.6x cropped Field of View) camera body provides sufficient reach with a Canon 24-70 2.8 USM L and 70-200 2.8 IS USM L to reach the highest point of most bridges. We used image stabilization on the longer lens to allow handheld operation on longer focal lengths.

The flash used in exposing the gap habitat is a 400 watt-second Bowens Gemini studio strobe attached to side of a large photography backpack. This gives a light sufficiently powerful and properly aimed to reach bats deep within the bridge gaps, which makes quick one handed adjustment possible by the camera operator. Unlike small portable flashes, this flash provides consistent output and throw throughout the entire day. The power system included a large lead-acid battery including an integrated pure sine wave inverter (Paul Buff Vagabond) allowing 120v output with sufficient amperage to operate both the camera and light. The battery and inverter fill the base of the backpack and have a powerstrip allowing a 120v output that attaches to the flash strobe lighting as well as 14v DC regulated output to the camera. This setup allows the entire power system to be charged all at once, overnight, with a sufficient amount of energy that gives an abundant supply for an entire day of use for the camera and strobe.

We reviewed photographs on the digital camera while in the field to ensure image quality. In the lab, we counted individual bats by enlarging the photographs on a computer screen and recording the number of bats in each marked one meter section and in the unmarked span over the water (Figure 3.1).

We discarded two sample events from both the Salado and Lampasas datasets. Data from August 2011 were not included in the analysis for either bridge because the data set was incomplete and the images were substandard. We also did not include data from the consecutive three days of photography at Lampasas in September 2012 because photographs were recorded at or immediately following sunset, when there was no assurance that bats had not already departed for the evening.

Figure 3.1. Image of one labeled meter of gap at Lampasas bridge, Bell County, Texas, from 11 August 2012, with section enlarged and lightened showing two bats.

To estimate the number of bats roosting at each bridge we simply counted the number of bats visible in the photographs per one meter segment, including the number of bats counted or estimated over the water and, at Salado, in the cross gap. Although both cave myotis and Brazilian free-tailed bats were readily distinguishable from the photos, only Brazilian free-tailed bats were counted. Discussions with other bat experts (Mylea Bayless and Jim Kennedy, Bat Conservation International, personal communication 2011) and field observations indicated that bats were stacked such that a portion of them were not visible in the photographs (Figure 3.2); therefore, we installed bat traps to measure the stacking. The traps encompassed the depth of the gap and a span of one-half of the one meter section (Figure 3.3). Bat traps consisted of a wooden framed structure with wire mesh that fit snugly inside of the gap, allowing bats to enter the gap but be easily removed in one motion by sliding the trap out. Field observations also suggested that the horizontal portion of the T shaped gap was typically not occupied, because when bats were disturbed, they retreated into the horizontal portion, becoming invisible when viewed directly from below.

To create an estimate of the number of bats in the vertical space of the gaps, and to verify and correct our point count estimates, we used the bat trap counts to calculate a multiplier for the point counts. This multiplier is used with the assumption that the vertical space of the gap is inhabited; in other words, we assumed that bats were stacked such that a portion of them were not visible in the photographs. As the determination of the multiplier involved reporting point counts of bats, determination of the multiplier is discussed in detail in Section 3.4.1.

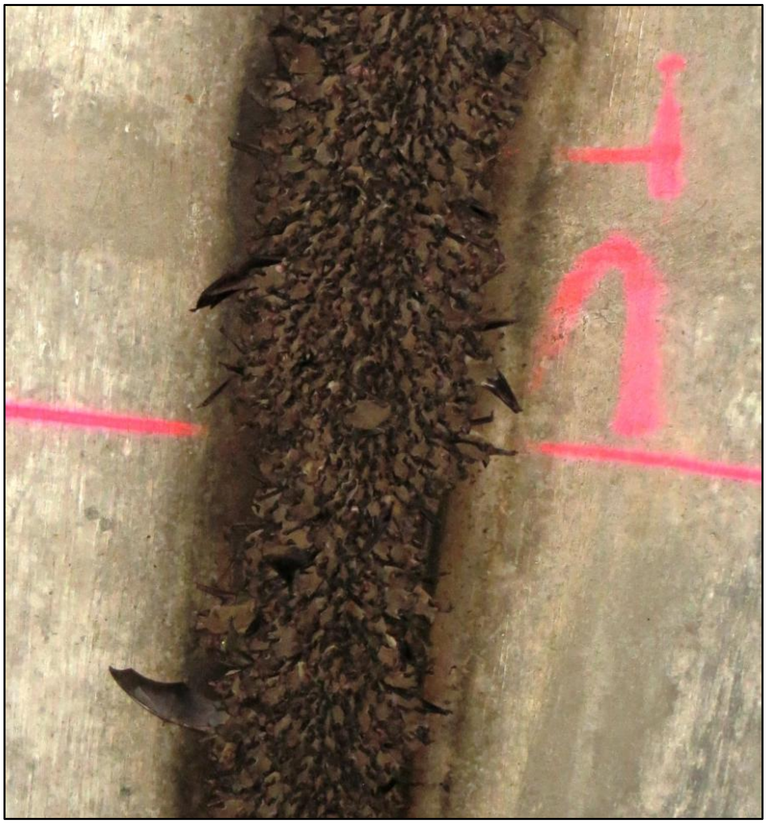

Figure 3.2. Bats stacked several deep into the gap and bulging out of the gap during periods of high occupation at the Lampasas bridge, Bell County, Texas.

Figure 3.3. Bat traps spanned half of the one meter section and allowed us to determine how deep the bats were stacking.

In order to estimate colony size during sampling events where bats over the water were not photographed or counted, we made estimates based on the 18 times when the counts were complete (i.e.-included the unmarked segments over the water). We calculated the average ratio of the colony counted from all of the events when bats were photographed over the water (i.e. average # bats over the water divided by average # bats in marked sections). We added the resultant proportion to each sampling event where bats over the water were not counted (i.e. # bats in marked sections plus [X proportion # bats in marked sections]).

At the Salado bridge only, in order to estimate colony size during sampling events where the cross gap and the section over the water were not counted, we compared two methods for estimating the variables. The incomplete count from the cross gap combined with the incomplete count for bats over the water left us with two unknown variables, compared to just one at Lampasas. Because there were some instances in which bats over the water were counted but bats in the cross gap were not, and vice versa, we could not always use the total number of bats inhabiting the rest of the bridge to determine the unknown variable. We performed a one tailed t-test in order to determine whether there would be significant differences in using the average proportion calculated from the grand total versus using the average proportion calculated from just the marked sections.

During several months, we collected data for three consecutive days in order to assess daily variation. We apply the term "consecutive" loosely, as data that were collected within 48 hours of one another. Specific dates of data collection are presented in the results section.

3.3.2 Mark-Recapture

Because no documentation was available to justify the use of PIT tags in Brazilian free-tailed bats, we performed a small trial on a test group of wild-caught bats to determine whether PIT tags were a valid marking method for this species.

We collected five bats from the IH 35 bridge over Salado Creek (Salado bridge) on 29 August 2011. These bats were maintained in captivity on a diet of mealworms and water until 3 October 2011 (Figure 3.4). We observed their individual eating and behavioral patterns for 20 days before implanting them with PIT tags (Biomark HPT9 ISO Compatible FDX-B High-Performance 9 mm PIT tag) on 19 September 2011. Injection sites were swabbed with rubbing alcohol prior to injection and coated with antibacterial gel after injection.

The five bats in the test group did not exhibit any obvious health or behavioral changes during the 14 day post-injection observation period. All of the injection sites appeared healthy, and the bats did not respond negatively to palpation at the injection site. When we released them under the Salado bridge on 3 October 2011, they did not appear to have any difficulty flying or re-integrating themselves with the bats roosting in the gap (Figure 3.5).

Figure 3.4. One of five Brazilian free-tailed bats maintained in captivity to test the efficacy of PIT tags in this species.

Figure 3.5. We observed released bats until they appeared to have re-integrated themselves and were no longer distinguishable (by an observer) from the rest of the colony.

Based on the lack of negative results from bats in the test group, PIT tags were injected subcutaneously into 347 bats at the IH 35 bridge over the Lampasas River (Lampasas bridge) and 349 bats at the Salado bridge in the fall of 2011 (Figure 3.6). The third bridge discussed elsewhere in this report, SH 9 over Turkey Run Creek, was not included in this analysis because it did not have an existing bat population during this study. In order to access the gap habitat for recapture, we bolted anchors into the concrete beams of the bridges, and hung ropes from those anchors. We then used standard single rope technique ascending and descending methods (Padgett and Smith 1987) to climb to the anchor and access the bats in that area. Unfortunately the bats quickly learned to avoid those areas, so we created a trolley system to access an entire span of gap habitat between bridge bents. This system consisted of tensioned cables rigged around the bents with a 2 in by 12 in lumber platform as the trolley, coupled with an independent safety line anchored into the bridge beams (Figure 3.7).

Figure 3.6. Field processing station for tagging bats at the Salado bridge, Bell County, Texas.

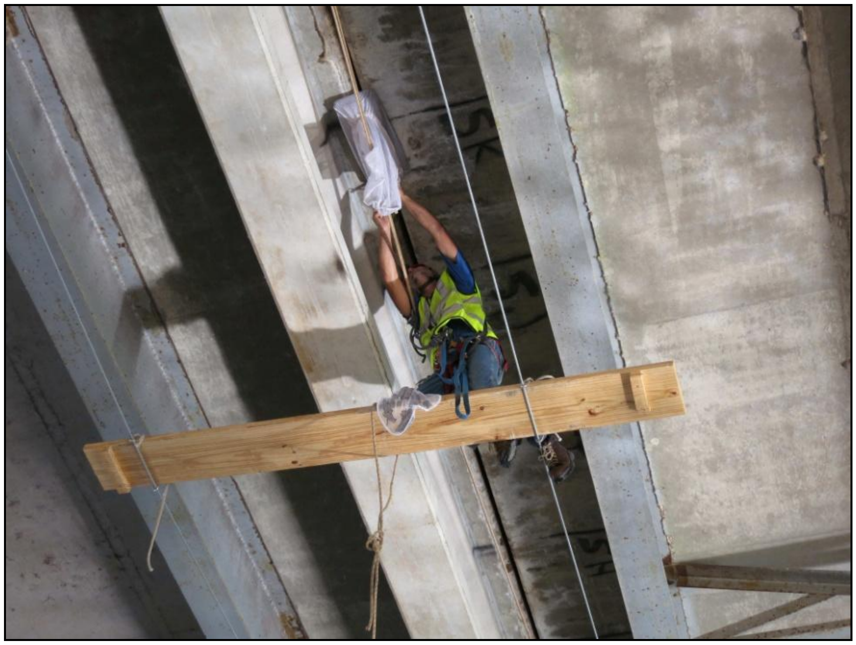

Figure 3.7. Trolley systems were installed under both the Lampasas and Salado bridges, Bell County, Texas, to allow us to access more of the gap than ropes alone.

Bats were captured for tagging by being gently prodded out of the gap into a net by an observer who was harnessed to a safety line. We did not mark very young (Figure 3.8) or obviously pregnant bats (Figure 3.9), or those exhibiting signs of stress (e.g. lethargy, panting) or previous injury, but released them near the point of capture.

Figure 3.8. Juvenile Brazilian free-tailed bat from Lampasas bridge, Bell County, Texas. Juveniles did not receive PIT tags.

Figure 3.9. A pregnant bat from the Lampasas bridge, Bell County, Texas. Pregnant bats did not receive PIT tags.

The metrics we recorded for tagged bats included weight, forearm length (Figure 3.10), gender, reproductive status when obvious, and age (juvenile or adult) based on wing joint ossification (Figure 3.11, Figure 3.12). We scanned tagged bats with a hand held Biomark 601 tag reader after injection to ensure that tags could be read (Figure 3.13). We recorded the same metrics for bats captured during the summer of 2012, but did not record metrics for bats captured during the spring of 2012. We performed a t-test to determine whether sex ratios were significantly different between the two bridges and whether body condition index (BCI) was different between sexes.

Figure 3.10. Calipers were used to measure forearm length of bats that would receive PIT tags.

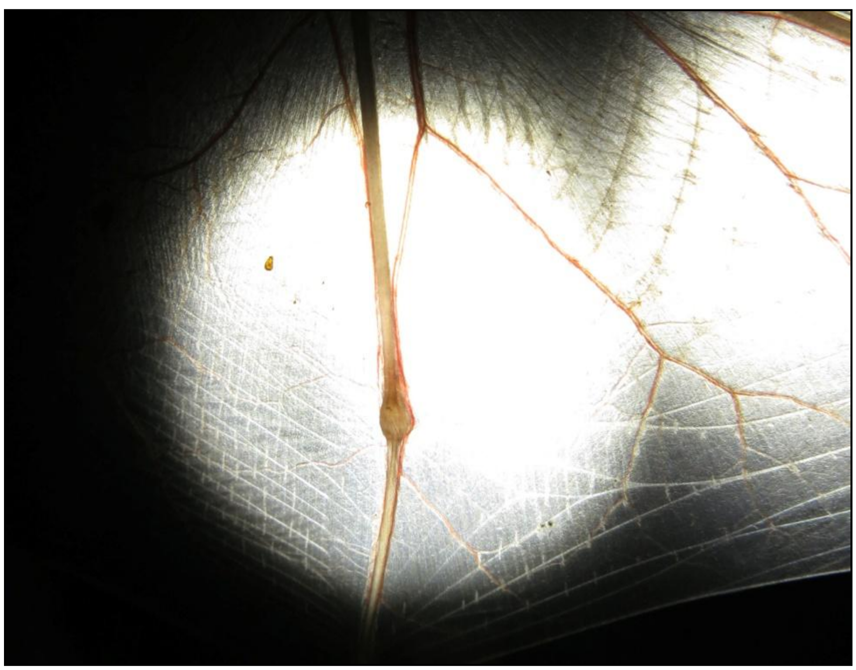

Figure 3.11. Fully ossified wing joint of adult Brazilian free-tailed bat.

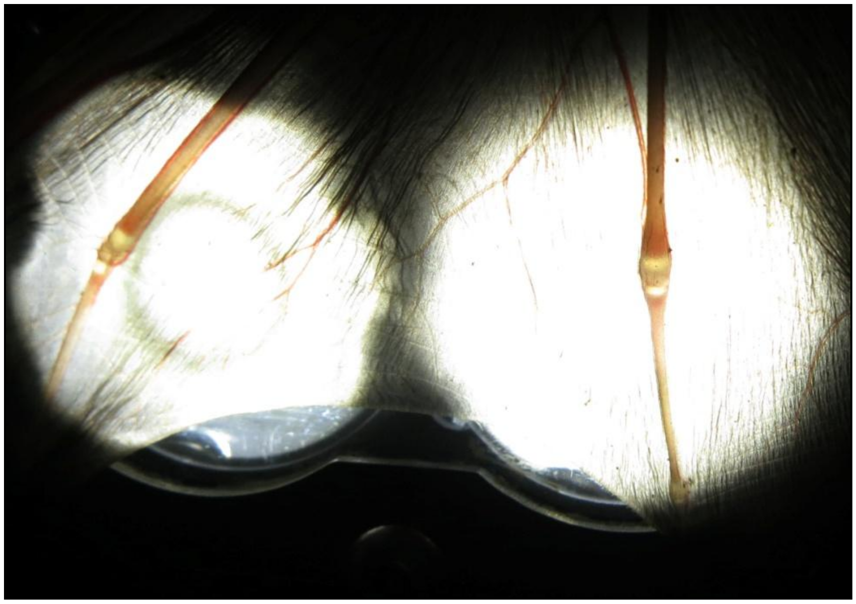

Figure 3.12. Wing joint of a juvenile Brazilian free-tailed bat, showing a lack of ossification.

Figure 3.13. Bats were scanned with a hand held tag reader after implantation.

We used the POPAN model in Program Mark to analyze our mark-recapture data (White and Burnham 1999). The POPAN model is an extension of the Cormack-Jolly-Seber live recapture model (CJS) (Cormack 1964, Jolly 1965, Seber 1965) that allows an estimation of abundance, which the basic CJS model does not. POPAN (Schwartz and Arnason 1996) uses a super-population approach, which consists of all animals that would ever be born into the population during the sampling period, and uses a parameter derived from the probability that an individual will enter into the population between sampling events (see Crosbie and Manly 1985 for details), rather than only estimating the number of animals present during a single sampling event.

We created separate encounter histories for each marked individual based on the tag code that individual received, and pooled the data by season for a total of three sampling occasions: fall (1 October – 15 November), spring (1 February – 15 March) and summer (early summer: 15 March – 15 June and late summer: 15 June – 30 August). Because of the migratory life history patterns of this species, we expect bats to arrive at the bridges during the spring, roost and breed during the spring and early summer, pup during the late summer, and migrate during the fall. We based our analysis on the following assumptions (Cooch and White 2013):

- tagged and untagged bats have the same probability of survival and capture between sampling occasions,
- tags are not lost and are detected when present,
- samples are collected instantaneously, and that
- the study area is constant

Life history observations that may have affected population estimates were recorded and are presented with the results below.

We defined a set of a priori candidate models to run in Program MARK based on data pooled into the three seasons. The parameters that MARK considers are the probabilities of capture (ρ), survival (ϕ), and the probability of entrance into the population (*PENT*) (Table 3.1). The AIC_c is an estimate of the information lost when a given model is used to generate data. The ΔAIC_c represents the difference between the selected model and the global model, and a low ΔAIC_c and high AIC_c weight is considered preferable.

Table 3.1. Parameter combinations used in modeling populations of Brazilian free-tailed bats at the Lampasas and Salado bridges.

Parameter Description	Parameter notation
Capture	
constant over time and colony	$\rho(.)$
constant over time, but differed by colony	$\rho(c)$
constant by colony, but differed over time	$\rho(t)$
differed by time and colony	$\rho(c*t)$
Survival	
constant over time and colony	$\phi(.)$
constant over time, but differed by colony	$\phi(c)$
constant by colony, but differed over time	$\phi(t)$
differed by time and colony	$\phi(c*t)$
Probability of Entrance into Population	
constant over time and colony	*PENT*(.)
constant over time, but differed by colony	*PENT*(c)
constant by colony, but differed over time	*PENT*(t)
differed by time and colony	*PENT*(c*t)

3.3.3 Acoustic Surveys and Mistnetting

We performed acoustic surveys at Lampasas, Salado, and Turkey Run bridges from April 2012 through January 2013, using a Petterson D240X heterodyne and full spectrum time expansion bat detector with a Samson Zoom H2 recorder to collect acoustic data based on auto-trigger. We chose time expansion (vs. heterodyne) as the method based on Limpens and McCracken (2004), discussions with local experts (Jim Kennedy, Bat Conservation International and Charles Pekins, Fort Hood, Personal Communication 2012)), and our own four nights of field experiments. We used the auto-trigger to enable the recording of individual files containing sounds within the auto-trigger threshold, rather than continuous recording of "noise" that may not have been within the frequency range of bat sounds. Appendix D details the setup instructions for the Zoom H2 and the D240X.

We performed two nights of experiments on exact placement of the detector to maximize bat detection and minimize interference from the ground (water reflections, insects, frogs), from trees, and from the bridge structures themselves. These experiments included observations of the direction that bats from the colony typically exited (e.g. upstream vs. downstream), field downloads from the detector to a laptop to evaluate file quality, then changing detector configuration, examining the files, and repeating until the quality was maximized. The recorder and external 12v battery were contained in a locking Pelican case with the D240X secured to the top of the case on a triangular shaped wood block (Figure 3.14). We designed the block to aim the microphone slightly upwards into the flight path. The entire setup was affixed to a 10 ft. galvanized steel pole which was placed in a concrete bucket stand that was put in the exact same place every night (Figure 3.15).

Figure 3.14. Zoom H2 recorded, external battery and Petterson D240X bat detector mounted within and on top of a Pelican case.

Figure 3.15. The bat detector was set up in the exact same spot during each evening of recording.

Although we attempted acoustic monitoring on more than 50 occasions, the data were heavily vetted for quality and consistency, and some data were not used. Examples of data collection efforts that did not make it into the analysis are occasions where the recording device was set up incorrectly (either having the autotrigger set up wrong or the date/timestamp set up wrong), occasions where the datacard came back blank, occasions where the batteries either died or overloaded and blew the fuses between the battery pack and the recorder, or occasions where it began raining prior to the end of the two hour sampling period. If any data inconsistencies were recognized or suspected, the data from that event was not included in the analysis. Careful, consistent and diligent data management are key to the success of acoustic monitoring.

We used Sonobat v3.1 with western autoclassifiers to analyze the data, because Texas-specific autoclassifiers were not available at the time of this analysis. Data files were run through the Dated Batch Attributer utility to assign bridge and date-specific file names and define metadata. The metadata attributed to each file contained the following information as in this example from the Salado bridge:

> USA, Texas, Bell County, South bank of the Salado River just east of the IH 35 Bridge; Coordinates (DD): 30.944084,-97.539092. Recorded: 20120611 using Petterson D240X and Zoom H2.

We did not scrub non-bat files prior to running files from each sampling event through SonoBatch, which is the process by which Sonobat v3.1 analyzes and reports the results of autoclassification. SonoBatch outputs results that includes the species classification (where possible), discriminate probability (a measure of closeness of the characteristics of the observed call to a known call), more than 50 call frequency characteristics, and relative abundance of species in files where more than one species may have been vocalizing concurrently. We accepted the classifiers assigned by the software by consensus or by vote when the species classification was one that was probable and that was appropriate to the geographic area (Davis and Schmidly 1997). When autoclassification did not yield appropriate species as determined by geographical range, we worked closely with the software developer, Joe Szewczak, to evaluate call similarity and determine which of the assigned classifiers were likely to be correct for our dataset, and to develop general assumptions about unlikely classifiers that were assigned (Table 3.2).

Table 3.2. We accepted classifiers with a discriminate probability of at least 0.9, but in certain cases made alternative classifications based on geography and call similarity.

Auto Classification*	Geographical Assessment	Combined determination
MYYU	out of range	Cave myotis (*Myotis velifer*)
EUMA	out of range	undetermined
MYTH	out of range	Brazilian free-tailed (*Tadarida brasiliensis*)
LANO	Probable	Silver-haired bat (*Lasionycteris noctivagans*)
MYCI	out of range	Cave myotis (*Myotis velifer*)
LACI	Probable	Hoary bat (*Lasiurus cinereus*)
EPFU	Probable	Big brown bat (*Eptesicus fuscus*)
ANPA	out of range	Big brown bat (*Eptesicus fuscus*)
PAHE	Possible (slightly out of range)	Tricolored bat *(Perimyotis subflavus*)
LABL	out of range	Eastern red bat (*Lasiurus borealis*)
COTO	TABR approach phase calls sound like COTO search phase calls	Brazilian free-tailed (*Tadarida brasiliensis*)
MYLU	out of range	Cave myotis (*Myotis velifer*)
TABR	Probable	Brazilian free-tailed (*Tadarida brasiliensis*)

* MYYU=Yuma myotis (*Myotis yumanensis*); EUMA=Spotted bat (*Euderma maculatum*); MYTH=Fringed myotis (*Myotis theysanodes*); LANO=Silver-haired bat (*Lasionycteris noctivagans*); MYCI=Western small0footed myotis (*Myotis ciliolabrum*); LACI=Hoary bat (*Lasiurus cinereus*); EPFU=Big brown bat (*Eptesicus fuscus*); ANPA=Pallid bat (*Antrozous pallidus*); PAHE=Western pipistrelle (*Parastrellus hesperus*); LABL=Western red bat (*Lasiurus blossevillii*); COTO=Townsend's big-eared bat (*Corynorhinus townsendii*); MYLU=Little brown bat (*Myotis lucifugus*); TABR=Brazilian free-tailed bat (*Tadarida brasiliensis*)

We used the number of bat passes, which the software tallies automatically, to quantify bat activity. Species richness and relative abundance were measured when the software was able to make a consensus on the species determination. We performed a few nights of dusk to dawn recording, but determined that due to site logistics and data quality we would only ultimately obtain and analyze data during the first two hours after sunset.

We are following the assumption that detectability does not change between visits, and therefore changes in these metrics over time indicate real changes in bat activity (Walsh et al. 2003). We are also following the assumption that the average spatial use of the habitat does not change before and after bridge construction, but recognize that if bats tend to fly lower or higher at our monitoring spot after bridge replacement, or happen to preferentially fly in another direction, e.g. upstream vs. downstream, after bridge replacement, this could artificially inflate or deflate our estimates and represent a change in behavior instead of a change in abundance or activity. Another acknowledgement that we make in our study design is that because we used only one detector per site, per night, we have no estimates of within-night (and within-habitat) variation, and no paired study designs, thus certain comparisons (among-habitat and among-night) are of limited value (see Hays 1997 for discussions of increased power using paired designs, and Gannon and Sherwin 2004 for discussion of data replication within habitat) and our ability to detect differences will be less because we don't have variance estimates for both the treatment and the replicate (see Jones et al. 2004 for discussion of this related to bat acoustic studies).

We deployed single-high mistnets on eight occasions during the fall of 2011. We set up mistnets over accessible portions of the waterway where we determined it likely for bats to fly. At the Lampasas and Turkey Run bridge, the net was set up near the bridge itself (Figure 3.16); however at the Salado bride the net was deployed upstream of the bridge near a still pool where we suspected bats would be more likely to drink (Figure 3.17).

Figure 3.16. Mistnetting at the Lampasas bridge, Bell County, Texas.

Figure 3.17. Mistnetting near the Salado bridge, Bell County, Texas.

3.4 Results

3.4.1 Photographic Monitoring

We analyzed data from 40 photographic sampling events at the Lampasas bridge, 18 of those times bats over the water were sampled. The proportion of the colony roosting over the water ranged from 14 to 42 percent, with a mean (± SD) of 29 ± 2 percent, with the exception of two sampling occasions during the winter sampling season (January 2013) when 94 percent of the observed bats were roosting over the water (Figure 3.18).

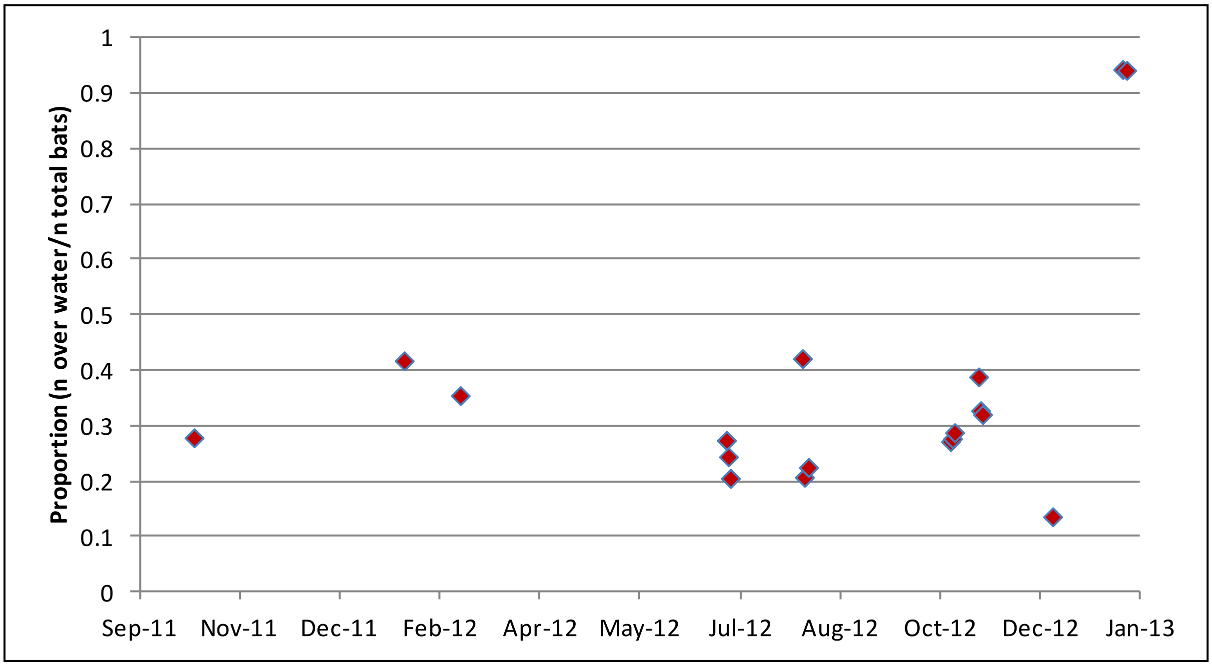

Figure 3.18. The proportion of bats roosting over the water at Lampasas bridge, Bell County, Texas, over 18 sampling occasions.

We used the results of point counts when bats were recorded over water to derive values for sampling events where bats were not recorded over the water by using the following equation (for all events except January 2013), where N represents the total number of bats we would have photographed if bats over the water were included and n represents the number of bats photographed: $N=n+0.29n$. Because the January 2013 discrepancy in the ratio of bats roosting over the water was so notable (Figure 3.18) and may represent actual differences in seasonal roosting patterns, derived values for the single day during that season when bats over the water were not recorded was based on the two events where bats were recorded. During both of these two events, 94 percent of the population was roosting over the water.

In order to create a generalized 'low' and 'high' population estimate for each site, we examined the dataset post-hoc and combined the seasons of late summer through fall as the time of year with the most bats, and the remainder as the time of year with the least. Given only a single full year of data collection, we did not have replicates by season in order to make a more detailed season-specific analysis. Based on 19 sampling events, the average number of bats observed during point counts at the Lampasas bridge during the late summer through fall (15 June – 15 November) is 4,025 ± 203. Based on 21 direct observations from the photographs, the average number of bats counted during all other dates is 906 ± 183.

During three late summer sampling events in 2011, the half-meter long traps in Lampasas bridge sections SX contained 47 bats on one occasion and SO contained 18 and 23 bats over two occasions (Table 3.3). Those bridge sections typically average 36 ± 3 bats and 29 ± 3 bats, based on 18 late summer/fall observations. The average distribution of bats across the span of the bridge is displayed in Figure 3.19.

The number of bats in the half-meter trap indicates that in section SX, there may be up to 94 bats (47 x 2) present in late summer/fall, and in section SO, there may be up to 46 (23 x 2). In photographs the maximum number observed in these two sections was 50 bats, with the averages being 36 and 29, respectively. These estimates suggest that there may be between 1.4 and 2.6 times (46/29 and 94/36) more bats inhabiting the gap than we are able to observe by counting the single layer of bats visible in the photographs, for a maximum potential average late summer/fall bat count of 5,635 to 10,465 individuals Figure 3.20). These extrapolated values were applied across all sampling occasions. Table 3.4 contains the estimated number of bats at the Salado Bridge at each sampling time.

Table 3.3. Bats recorded from half-meter long bat traps at Lampasas bridge, Bell County, Texas, (list the sampling dates), 2011. Sampling occasions when traps were checked but no bats were detected are not shown.

Date	Section	Bats in half-meter trap x 2 = 'stacked' bats per meter	Average number of bats photographed in section during season	Multiplier
8/31/2011	SX	94	36 ± 3	1.4
8/31/2011	SO	36	29 ± 3	2.6
10/5/2011	SO	46		1.4

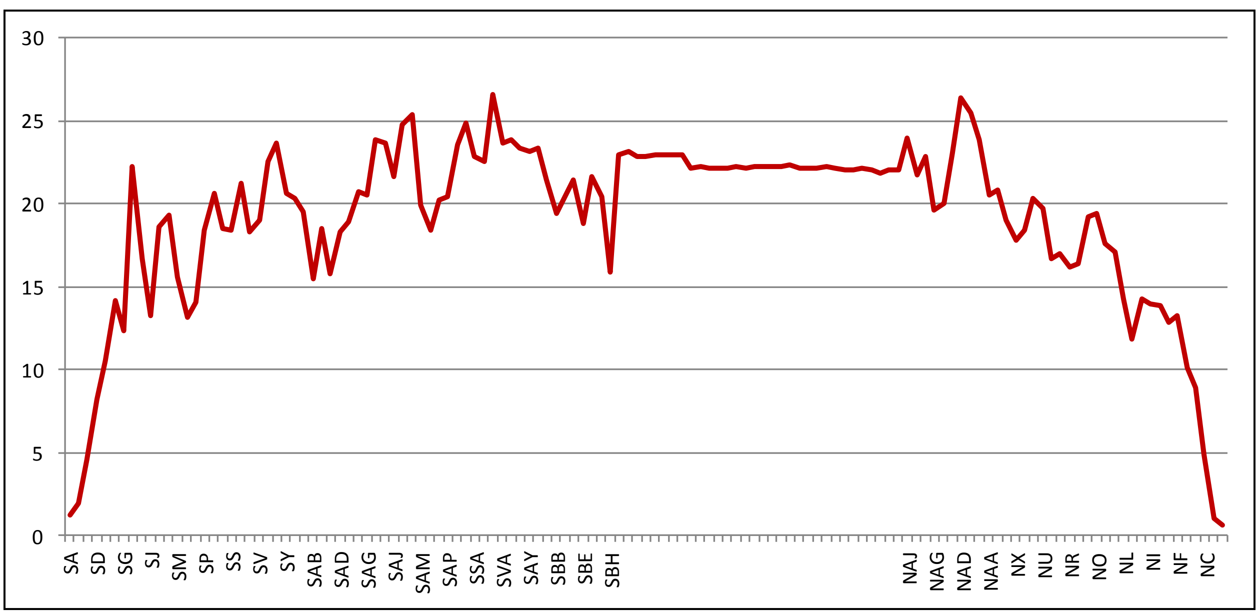

Figure 3.19. Average distribution of bats across the span of the Lampasas bridge, Bell County, Texas, from October 2011 through January 2013. The x-axis shows the marked

sections, while the unmarked sections in the middle of the graph are directly above the Lampasas River. The y-axis is the average number of bats per linear meter across all sampling events.

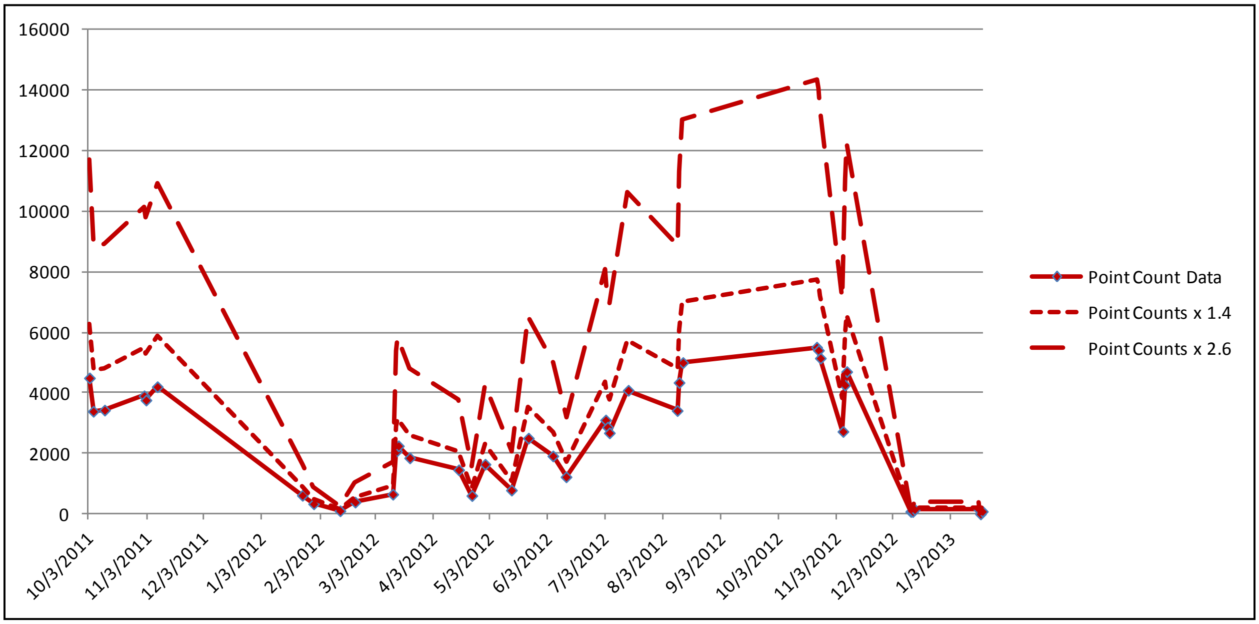

Figure 3.20. The number of bats observed during point count surveys and estimated based on multipliers of 1.4 and 2.6 at the Lampasas bridge, Bell County, Texas.

Table 3.4. Number of bats inhabiting bridge sections by date at the Lampasas bridge, Bell County, Texas including extrapolated estimates derived from bat trap data that account for stacking. River section raw count numbers in boldface type indicate actual rather than derived values.

Date	Marked sections	River section	Point counts	Point count (x1.4)	Point count (x2.6)
10/3/2011	3485	1011	4496	6294	11689
10/5/2011	2631	763	3394	4752	8824
10/11/2011	2477	**956**	3433	4806	8926
11/1/2011	3027	878	3905	5467	10153
11/2/2011	2918	846	3764	5269	9785
11/8/2011	3260	945	4205	5888	10934
1/24/2012	360	**258**	618	865	1607
1/30/2012	261	76	337	471	875
2/13/2012	82	24	106	148	275
2/21/2012	253	**139**	392	549	1019
3/12/2012	506	147	653	914	1697
3/14/2012	1612	467	2079	2911	5407
3/15/2012	1745	506	2251	3151	5853
3/21/2012	1437	417	1854	2595	4820
4/16/2012	1131	328	1459	2043	3793
4/23/2012	472	137	609	852	1583
4/30/2012	1275	370	1645	2303	4276
5/14/2012	612	177	789	1105	2053
5/23/2012	1943	563	2506	3509	6517
6/5/2012	1488	431	1919	2686	4989
6/12/2012	952	276	1228	1719	3193
7/3/2012	2256	**850**	3106	4348	8076
7/4/2012	2169	**700**	2869	4017	7459
7/5/2012	2131	**550**	2681	3753	6971
7/15/2012	3169	919	4088	5723	10629
8/10/2012	1978	**1440**	3418	4785	8887
8/11/2012	3444	**900**	4344	6082	11294
8/13/2012	3501	**1502**	5003	7004	13008
10/23/2012	4015	**1497**	5512	7717	14331
10/24/2012	3913	**1497**	5410	7574	14066
10/25/2012	3669	**1482**	5151	7211	13393
11/6/2012	1668	**1059**	2727	3818	7090
11/7/2012	2866	**1395**	4261	5965	11079
11/8/2012	3197	**1507**	4704	6586	12230
12/12/2012	63	18	81	114	211
12/13/2012	70	**11**	81	113	211
12/14/2012	117	34	151	211	392
1/17/2013	9	**150**	159	223	413
1/18/2013	2	33	35	49	91
1/19/2013	5	**81**	86	120	224

We performed 42 photographic sampling events at the Salado bridge, with 25 events including bats over the water and 14 events including bats in the cross gap. Eight of the sampling events included all bridge sections.

We used the eight complete sampling events to test for differences between two alternatives for estimating the uncounted sections during the incomplete sampling events. We determined that there was not a significant difference between the means of the portion of the colony roosting over irregularly sampled bridge sections when calculated using labeled sections vs. the grand total (Table 3.5). Because these differences were not significant for either the cross gap (p=0.2) or the segments over the water (p=0.1), we chose to use the means of the labeled sections to fill in the data gaps. We calculated the proportions of irregularly counted bridge sections by dividing those sections by the number of bats counted in the labeled sections of bridge.

Table 3.5. One-tailed t-test results comparing the proportion of bats in the cross gap to the bats counted in the labeled sections of bridge and to the grand total of bats counted in all other sections for the Salado bridge, Bell County, Texas.

	Mean of Labeled sections	Mean of grand total	Variance of labeled sections	Variance of grand total	t Stat	P
Cross Gap	0.054897	0.042892	0.002194	0.0013190	0.577182	0.286491
Over Water	0.227286	0.173955	0.010139	0.0039859	1.269191	0.112533

The proportion of the colony roosting over the water ranged from 0 to 80 percent, with a mean (± SD) of 28 ± 4 percent. During July and August 2012, between 56 and 81 percent of the population present was roosting over the water (Figure 3.18).

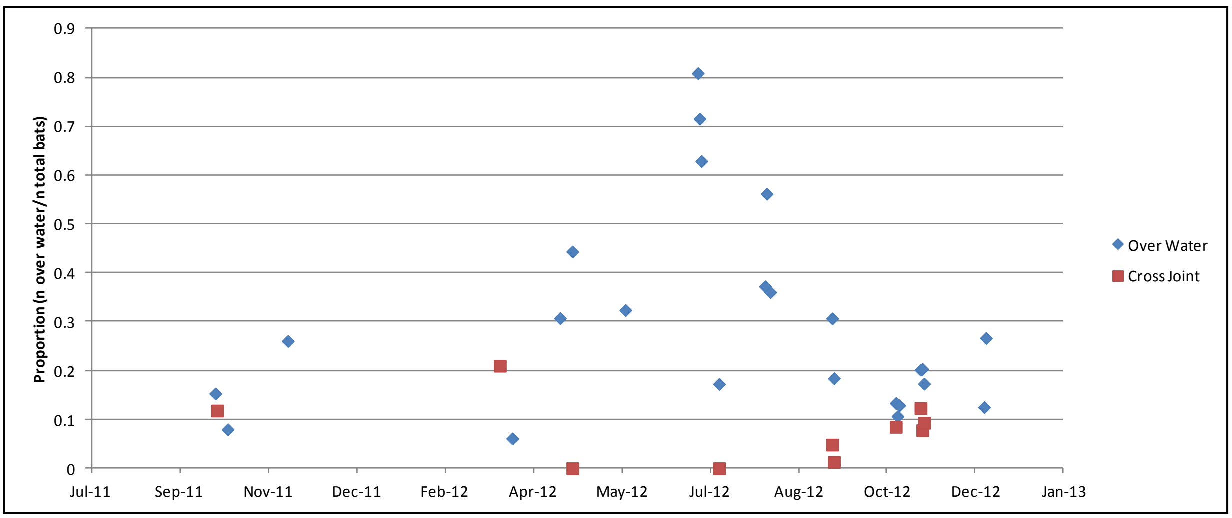

Figure 3.21. The proportion of bats roosting over the water and in the cross gap at the Salado bridge, Bell County, Texas, over 25 and 14 respective sampling occasions.

The proportion of the colony roosting in the cross gap ranged from zero to 21 percent, with a mean (± SD) of 7 ± 2 percent. Thus, we estimated the number of bats during sampling events where bats were not recorded in these irregularly counted sections by using the following post-hoc equation, where N represents the total number of bats we would have counted if bats over the water and in the cross gap were included and n represents the number of bats photographed in labeled sections: $N=n+0.28n+0.07n$.

In order to create a generalized 'low' and 'high' population estimate for each site, we examined the dataset post-hoc and combined the seasons of late summer through fall as the time of year with the majority of bats, and the remainder as the time of year with the least. Given only a single full year of data collection, we did not have replicates by season in order to make a more detailed season-specific analysis. Based on 22 sampling events, the average number of bats counted at the Salado bridge during the late summer through fall

(15 June – 15 November) is 2,577 ± 345. Based on 17 sampling events from when bats were present during the other times of year (16 November – 14 June) (Table 3.6), the average number of bats counted during all other dates is 533 ± 150; because this is an estimate of population size, this average does not consider three point counts from January 2013, when no bats were seen, which would artificially deflate the population estimate. Raw data are presented in Table 3.6. The average distribution of bats across the span of the Salado bridge is presented in Figure 3.23.

Capturing bats in the bat traps at the Salado Bridge was marginally successful. During two late summer/fall sampling events in 2012, a trap in the cross gap between sections NEJ and NEI contained 17 and 52 bats, a number on par with observations at the Lampasas bridge (Table 3.3). The number of bats in those sections fluctuates from as few as zero to as many as 140 (NEI) and 160 (NEJ) bats, even during counts taking place within 14 days of one another, but [when bats are present] average 46 ± 43 bats for NEI and 47 ± 51 bats for NEJ.

The number of bats in the half-meter trap indicates that in sections NEI and NEJ, there may be up to 104 bats (52 x 2) present in late summer/fall. During point counts the maximum number observed in these two sections was 140 and 160 bats, with the averages being 46 and 47, respectively. These estimates suggest there may be 2.2 times (104/46 and 104/47) more bats inhabiting the gap than we are able to observe by counting the single layer of bats visible in the photographs, for a maximum potential average late summer/fall bat count of 5,669 individuals (Figure 3.22). These extrapolated values were applied across all sampling occasions in Table 3.6.

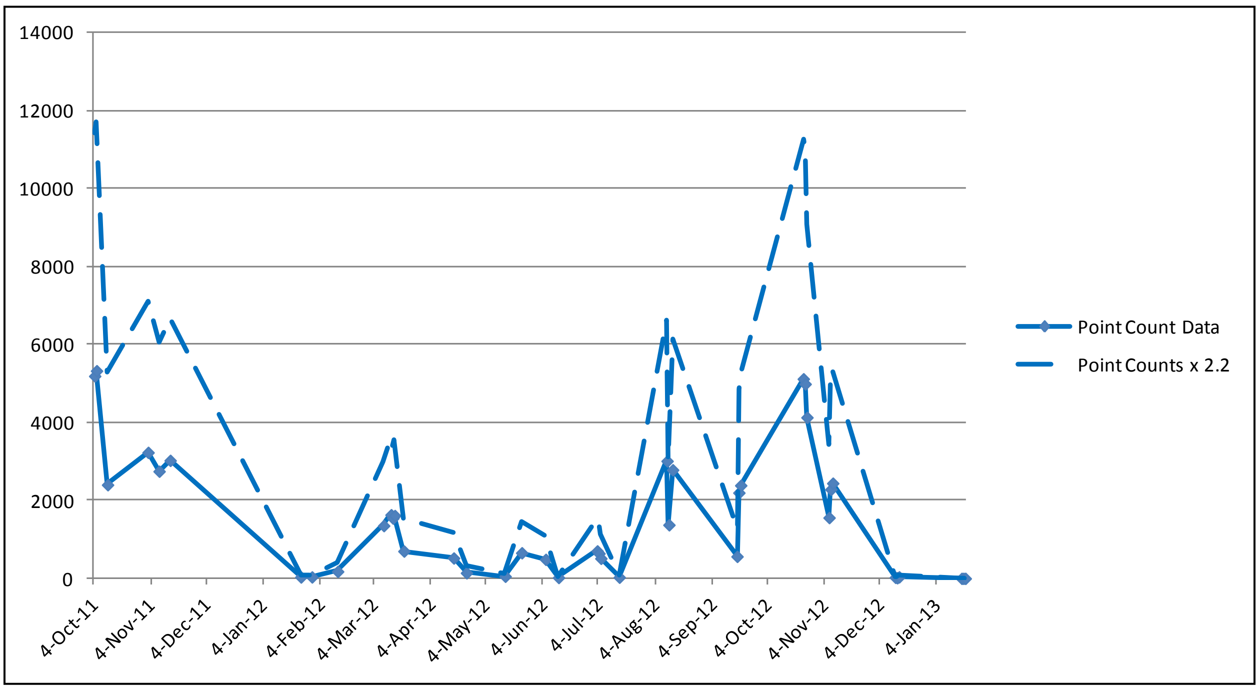

Figure 3.22. The number of bats observed during point count surveys and estimated based on a multiplier of 2.2 at the Salado bridge, Bell County, Texas.

Table 3.6. Bats inhabiting bridge sections by date at the Salado bridge, Bell County, Texas. River and cross gap section numbers in boldface type indicate actual rather than derived values.

Date	Marked sections	River section	Cross Gap	Point Count	Point Count (x2.2)
10/4/2011	4154	**750**	291	5195	11429
10/5/2011	3812	1067	**450**	5329	11725
10/11/2011	2078	**180**	145	2403	5288
11/2/2011	2395	671	168	3233	7113
11/8/2011	2037	570	143	2750	6050
11/14/2011	2129	**750**	149	3028	6662
1/24/2012	26	7	2	35	77
1/30/2012	27	8	2	36	80
2/13/2012	132	29	9	178	392
3/9/2012	1003	37	70	1354	2979
3/13/2012	1096	281	**230**	1633	3592
3/14/2012	1145	307	80	1546	3401
3/15/2012	1194	321	84	1612	3546
3/20/2012	617	**40**	43	700	1540
4/16/2012	345	**153**	24	522	1149
4/23/2012	79	**63**	**0**	142	312
5/14/2012	50	**0**	4	54	118
5/23/2012	424	**203**	40	657	1445
6/5/2012	361	101	25	487	1072
6/12/2012	17	5	**0**	22	48
7/3/2012	133	**562**	9	704	1549
7/4/2012	179	**450**	13	642	1411
7/5/2012	186	**315**	13	514	1131
7/15/2012	24	**5**	**0**	29	64
8/10/2012	1812	**1075**	127	3014	6630
8/11/2012	601	**731**	42	1374	3024
8/13/2012	1721	**945**	120	2786	6130
9/17/2012	373	**165**	**26**	564	1241
9/18/2012	1772	**400**	**28**	2200	4840
9/19/2012	1769	495	124	2388	5254
10/23/2012	4092	**630**	**400**	5122	11268
10/24/2012	4197	**500**	294	4991	10980
10/25/2012	3395	**500**	238	4133	9092
11/6/2012	1109	**280**	**171**	1560	3432
11/7/2012	1690	**431**	**165**	2286	5029
11/8/2012	1848	**387**	**208**	2443	5375
12/12/2012	21	**3**	1	25	56
12/13/2012	11	**4**	1	16	35
12/14/2012	28	8	2	38	83

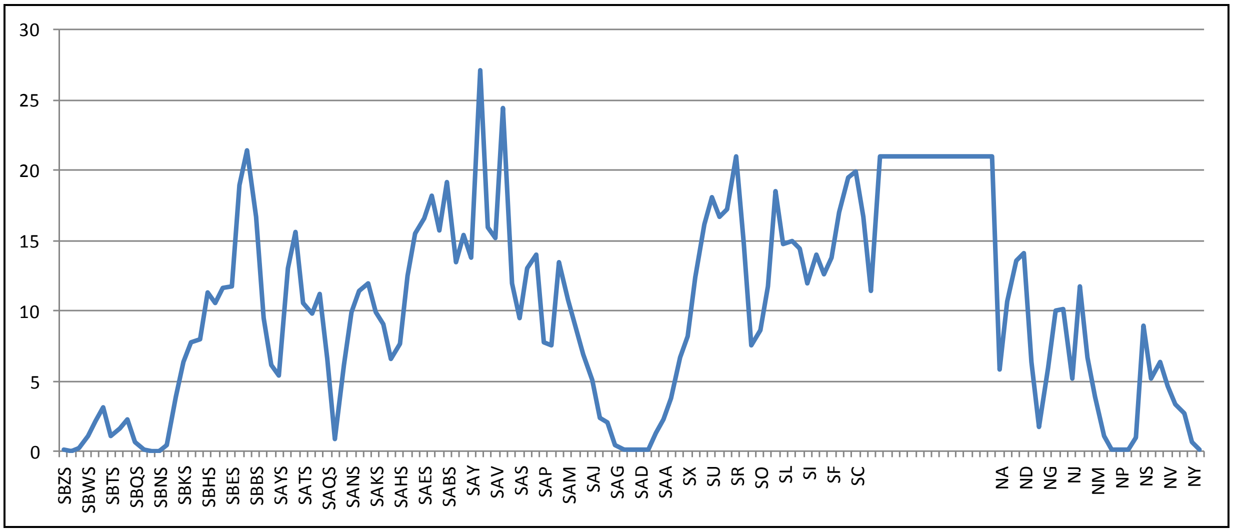

Figure 3.23. The average distribution of bats across the span of the Salado bridge, Bell County, Texas, between October 2011 and January 2013. The x-axis shows the marked sections, while the unmarked sections in the middle of the graph are directly above Salado Creek. The y-axis is the average number of bats per linear meter across all sampling events.

An analysis of variance (ANOVA) revealed that the months with greatest variance in three day point counts at Lampasas bridge were March and November, and at Salado they are October and August (Figure 3.24, Table 3.7). The months with the least variance, and lowest bat numbers, at each bridge were December and January.

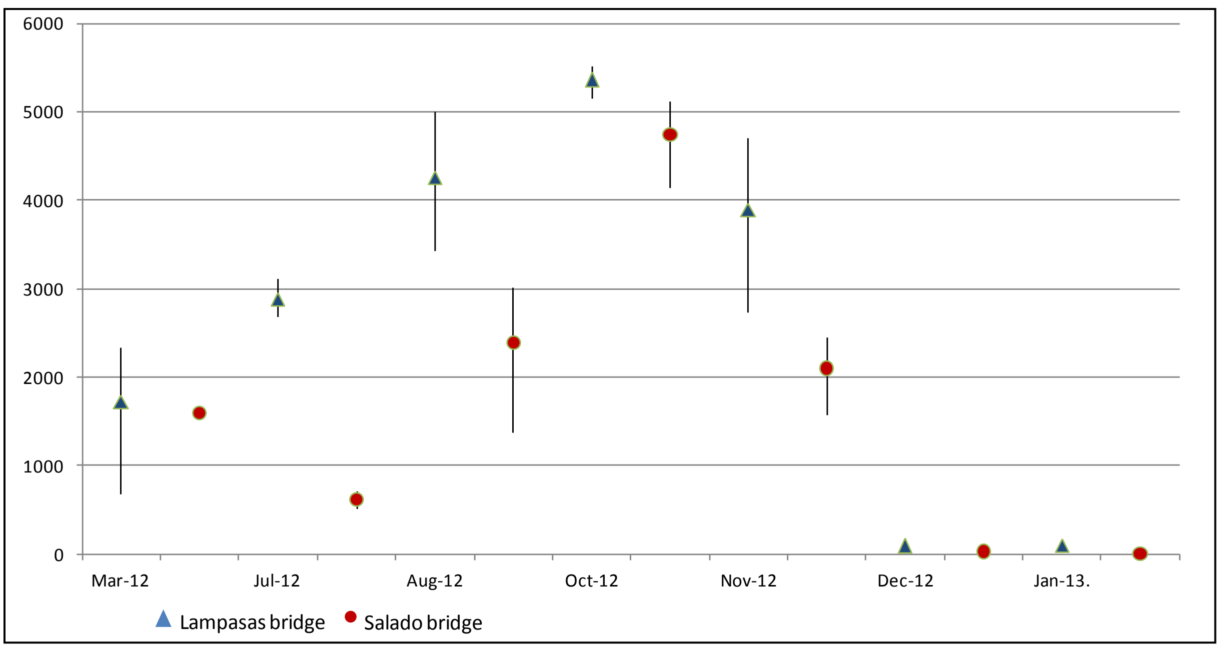

Figure 3.24. Range of values (max-min, represented by lines) and averages (points) for three day point count data at the Lampasas and Salado bridges, Bell County, Texas.

Table 3.7. Grand total from three day point count data at the Lampasas bridge, Bell County, Texas.

		Mar-12	Jul-12	Aug-12	Oct-12	Nov-12	Dec-12	Jan-13
Lampasas	Day 1	678	3106	3418	5512	2727	63	159
	Day 2	2160	2869	4344	5410	4261	81	35
	Day 3	2338	2681	5003	5151	4704	117	86
	Average	1725	2885	4255	5358	3897	87	93
	Variance	830,601	45,356	633,997	34,634	1,076,322	756	3884
Salado	Day 1	1633	704	3014	5122	1560	25	0
	Day 2	1546	642	1374	4991	2286	16	0
	Day 3	1612	514	2786	4133	2443	38	0
	Average	1597	620	2392	4748	2096	26	0
	Variance	2,068	9,402	788,837	288,739	221,902	122	0

The average point counts at both bridges varied seasonally as bats reproduced and migrated, and there was a strong correlation between the number of bats in any given month at either bridge (r=0.78) (Figure 3.25). For the fall and late summer, when the most bats were present at either colony, there was not a significant difference in the variance between the bridges (ANOVA: F(1,36)=3.07, p>0.05).

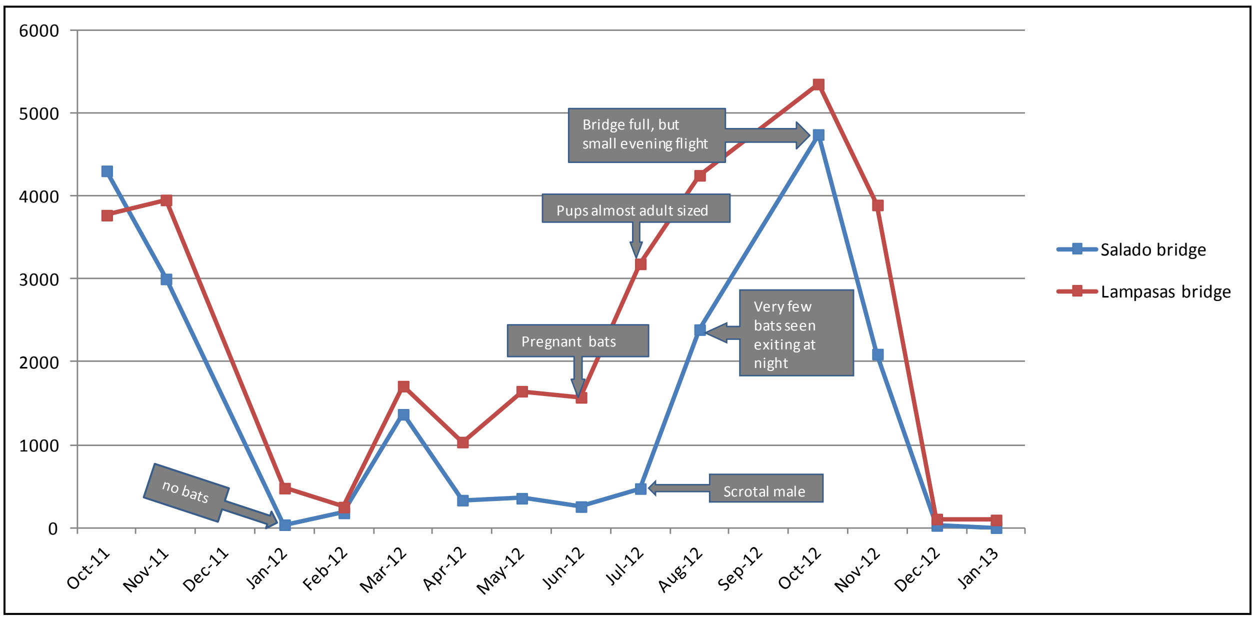

Figure 3.25. Point counts of bats at the Lampasas and Salado bridges, Bell County, Texas, tracked one another and varied with the season.

3.4.2 Mark-Recapture

Models considering each combination of parameters were run in program MARK. We assessed model fit using the information-theoretic approach, and we selected the most parsimonious model (i.e., that with the fewest parameters) with the most favorable combination of factors from the Akaike information criterion (AIC_c, ΔAIC_c, and AIC_c weight) (Table 4.2).

Table 3.8. Results from Program MARK for modeling capture (ρ), survival (ϕ) and

probability of entrance (*PENT*) into the population for Brazilian free-tailed bats at the Lampasas and Salado bridges. Only the five models with the strongest support are shown, with the selected model being displayed in boldface type.

Model	AIC_c	ΔAIC_c	AIC_c Weight	Parameters
φ(c*t)ρ(c*t)*PENT*(c*t)	184.5712	0	0.87528	11
φ(c)ρ(c)*PENT*(c*t)	188.5802	4.0090	0.11792	7
φ(.)ρ(.)*PENT*(c*t)	**194.2879**	**9.7167**	**0.00680**	**6**
φ(c*t)ρ(c)*PENT*(c*t)	230.3370	45.7658	0	10
φ(t)ρ(c)*PENT*(c*t)	289.3768	104.8056	0	9

We tagged a total of 695 bats, and recovered tags during 10 resampling occasions (raw data presented in Appendix C, Table C.1). Because life history patterns may contain valuable information relating to seasonality, population estimates, and gender and site biases, we also report these data (Table 3.9).

Table 3.9. Capture data and life history observations of Brazilian free-tailed bats from Lampasas and Salado bridges.

Bridge	Date	Bats Captured	Tags Installed	# recaptures	Life History Notes
Lampasas	10/18/2011	56	56	0	
	10/25/2011	271	271	0	
	11/14/2011	20	20	0	
	3/6/2012	23	0	1	
	3/9/2012	145	0	1	bats were cold and "locked together"
	3/12/2012	49	0	1	
	3/13/2012	98	0	2	
	3/14/2012	67	0	3	
	6/6/2012	2	0	1	pregnant bats observed
	7/15/2012	no mark-recapture activities			pups seemed to have almost reached adult size; section of bridge with previously only adult bats was completely empty
	7/23/2012	111	0	0	Several large juveniles and lactating females present; bats separated by gender with males on one half and juveniles/females on other half
	7/24/2012	85	0	1	
	7/25/2012	54	0	1	
Salado	10/13/2011	29	29	0	
	10/24/2011	309	309	0	
	11/13/2011	6	6	0	
	(Test Group)	5	5	0	
	1/17/2012	no mark-recapture activities			no bats present
	3/6/2012	132	0	2	
	3/12/2012	31	0	0	
	3/13/2012	190	0	4	
	3/14/2012	116	0	0	
	5/14/2012	no mark-recapture activities			very few bats observed
	6/6/2012	15	0	0	
	7/23/2012	32	0	0	one [potentially] juvenile; one [potentially] pregnant; one scrotal male
	7/24/2012	7	0	0	
	7/25/2012	0	0	0	no bats captured during third consecutive day of PIT effort; approximately 12 bats total present at bridge (six TABR, six MYVE)
	7/30/2012	no mark-recapture activities			no bats
	8/01/2012				no bats
	9/6/2012				fewer than 100 bats
	10/23/2012				only small exodus even though the bridge was full of bats
	10/25/2012				bats began exodus but returned to bridge when a cold front with wind blew through at 1815

TABR=Brazilian free-tailed bat; MYVE=cave myotis

For bats captured from Salado, the sex ratio (SR; M:F) was not biased toward either gender during the fall 2011 ($t(348)=0.267$, $p>0.05$) or during the summer 2012 ($t(61)=-1.276$, $p>0.05$). At Lampasas, SR was male biased both during the fall 2011 ($t(346)=-3.745$, $p<0.05$) and during the summer 2012 ($t(256)=-5.252$, $p<0.05$).

Body condition index (BCI; weight[g]/forearm length[mm]) was not significantly different between male and female bats at Salado during the fall 2011 ($t(342)=-0.701$, $p>.05$) or during the spring 2012 ($t(1.171$, $p>0.05$); however females at the Lampasas bridge had significantly higher BCI than males during the fall 2011 ($t(345)=3.232$, $p<0.05$) and summer 2012 ($t(250)=5.538$, $p<0.05$) (Table 3.10).

Table 3.10. Summary table of bat captures from three sampling events between fall 2011 and summer 2012. The estimated population sizes are N-hat estimates from Program MARK and represent the super-population during each seasonal sampling occasion.

<table>
<tr><th>Bridge</th><th>Date</th><th>Bats Captured</th><th>Sampling Season</th><th>Sex Ratio (M:F)</th><th>Average BCI (Male)</th><th>Average BCI (Female)</th><th>Est. Pop. Size</th></tr>
<tr><td rowspan="12">Lampasas</td><td>10/18/2011</td><td>56</td><td rowspan="3">Fall 2011</td><td rowspan="3">1.5</td><td rowspan="3">0.342</td><td rowspan="3">0.358</td><td rowspan="3">10,412</td></tr>
<tr><td>10/25/2011</td><td>271</td></tr>
<tr><td>11/14/2011</td><td>20</td></tr>
<tr><td>3/6/2012</td><td>22</td><td rowspan="5">Spring 2012</td><td rowspan="5" colspan="3">not recorded</td><td rowspan="5">11,198</td></tr>
<tr><td>3/9/2012</td><td>144</td></tr>
<tr><td>3/12/2012</td><td>49</td></tr>
<tr><td>3/13/2012</td><td>96</td></tr>
<tr><td>3/14/2012</td><td>64</td></tr>
<tr><td>6/6/2012</td><td>2</td><td rowspan="4">Summer 2012</td><td rowspan="4">1.9</td><td rowspan="4">0.271</td><td rowspan="4">0.291</td><td rowspan="4">7540</td></tr>
<tr><td>7/23/2012</td><td>111</td></tr>
<tr><td>7/24/2012</td><td>85</td></tr>
<tr><td>7/25/2012</td><td>54</td></tr>
<tr><td rowspan="10">Salado</td><td>10/13/2011</td><td>29</td><td rowspan="4">Fall 2011</td><td rowspan="4">0.95</td><td rowspan="4">0.354</td><td rowspan="4">0.357</td><td rowspan="4">10,453</td></tr>
<tr><td>10/24/2011</td><td>309</td></tr>
<tr><td>11/13/2011</td><td>6</td></tr>
<tr><td>(Test Group)</td><td>5</td></tr>
<tr><td>3/6/2012</td><td>130</td><td rowspan="3">Spring 2012</td><td rowspan="3" colspan="3">not recorded</td><td rowspan="3">10,242</td></tr>
<tr><td>3/12/2012</td><td>31</td></tr>
<tr><td>3/13/2012</td><td>186</td></tr>
<tr><td>6/6/2012</td><td>16</td><td rowspan="3">Summer 2012</td><td rowspan="3">1.38</td><td rowspan="3">0.291</td><td rowspan="3">0.301</td><td rowspan="3">1785</td></tr>
<tr><td>7/23/2012</td><td>32</td></tr>
<tr><td>7/24/2012</td><td>7</td></tr>
</table>

The estimated super-population size based on the POPAN formulation where survival and capture probability are constant is 24,625 for Lampasas Bridge and 18,110 for Salado Bridge. The super-population estimate represents the total number of bats ever present during the experiment. The full output from Program MARK is located in Appendix C.2.

3.4.3 Acoustic Surveys and Mistnetting

We analyzed data only from the first two hours after sunset in order to keep our data between nights and sites as comparable as possible; however we did record distinct peaks in bat activity during the hours just before dawn on sampling occasions when the detectors were deployed for the entire course of the night (Figure 3.26).

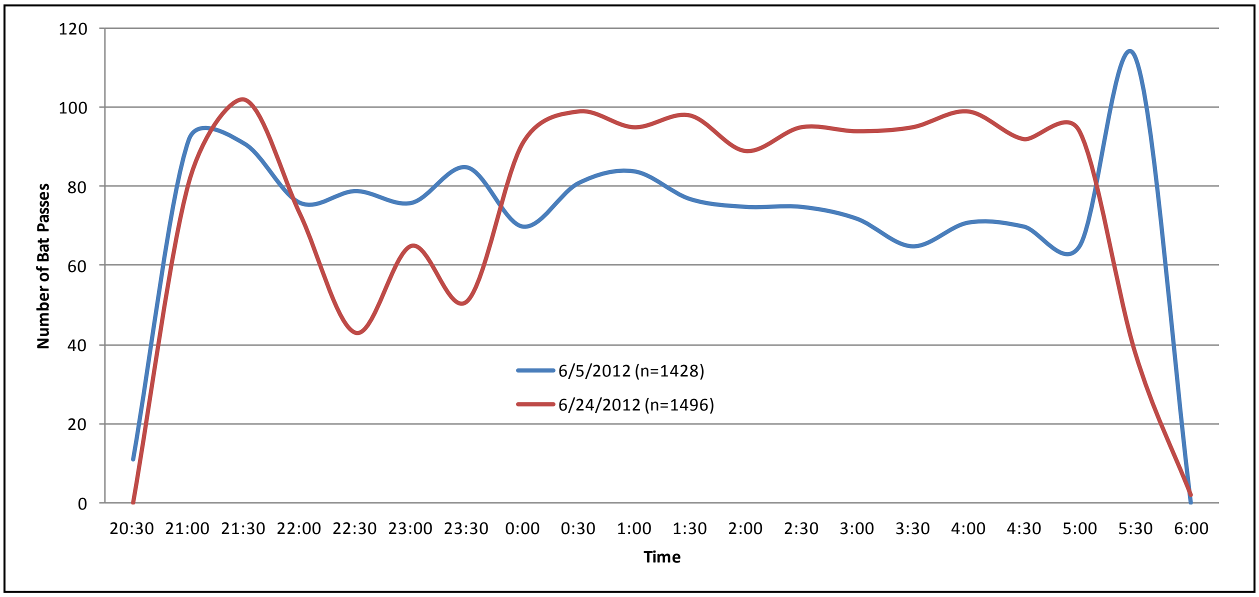

Figure 3.26. Bat passes recorded during two full nights at the Lampasas bridge, Bell County, Texas.

We analyzed 2,162 files recorded during the first two hours after sunset from 16 sampling events occurring between April 2012 and January 2013 at the Lampasas bridge (Appendix D). Of these files, 1,278 contained bat passes. The software was able to reach an autoclassification decision for 937 of these files, representing seven species after the corrections listed in Table 3.2 were applied. The vast majority (84 percent) of these calls belonged to Brazilian free-tailed bats, with the remaining 16 percent belonging to big brown bats, silver-haired bats, cave myotis, eastern red bats, hoary bats, and tricolored bats (Figure 3.27).

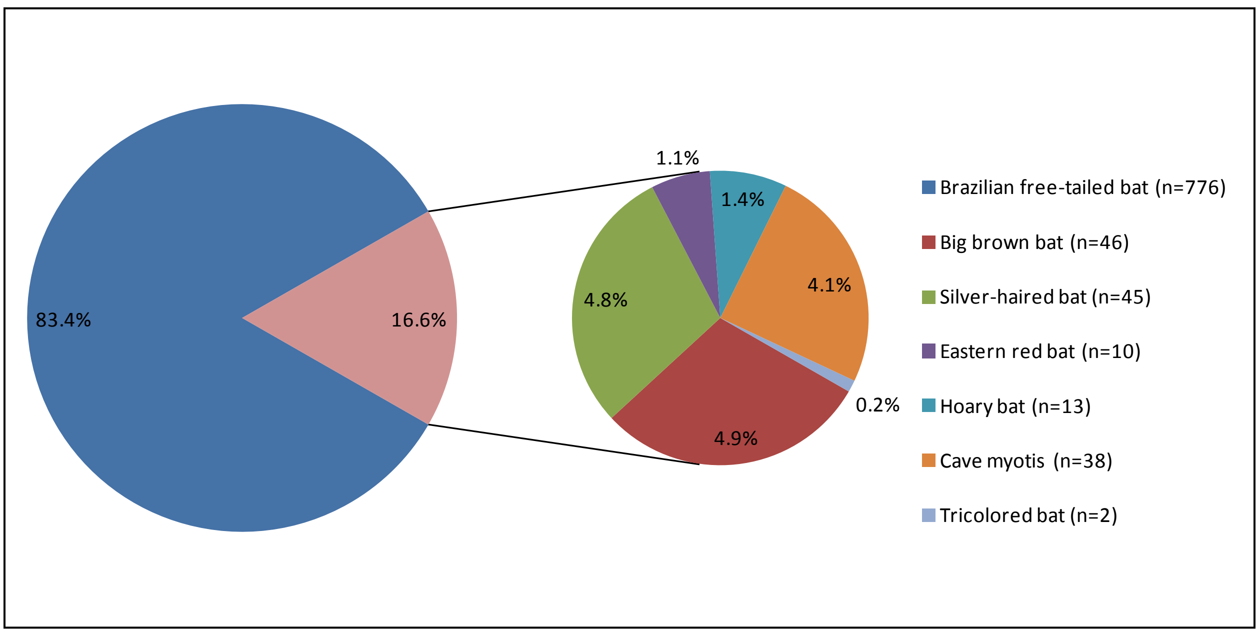

Figure 3.27. Relative abundance of seven species automatically classified by Sonobat v3.1 from 937 bat passes recorded from 16 sampling events occurring between April 2012 and

January 2013, near the Lampasas bridge, Bell County, Texas.

We obtained bat pass data over four seasons. Absolute numbers are not exactly comparable given the nuances of bat pass tallies (see methods section) and the unequal effort across seasons. However as a generalization, the number of bat passes recorded for silver-haired bats and cave myotis peaked during the early summer season, whereas big brown, eastern red and Brazilian free-tailed bat activity peaked during late summer (Table 3.11, Figure 3.28).

Table 3.11. Number of bat passes recorded for each species over three recording events during the early summer, late summer and winter and two during the fall near the Lampasas bridge.

Species	Early Summer n=3	Late Summer n=3	Fall n=2	Winter n=3
big brown bat	9	35	2	0
silver-haired bat	17	0	2	9
eastern red bat	4	17	2	0
hoary bat	3	5	0	8
cave myotis	19	2	9	3
tricolored bat	0	2	0	0
Brazilian free-tailed bat	213	423	67	73
TOTAL	**265**	**484**	**82**	**93**

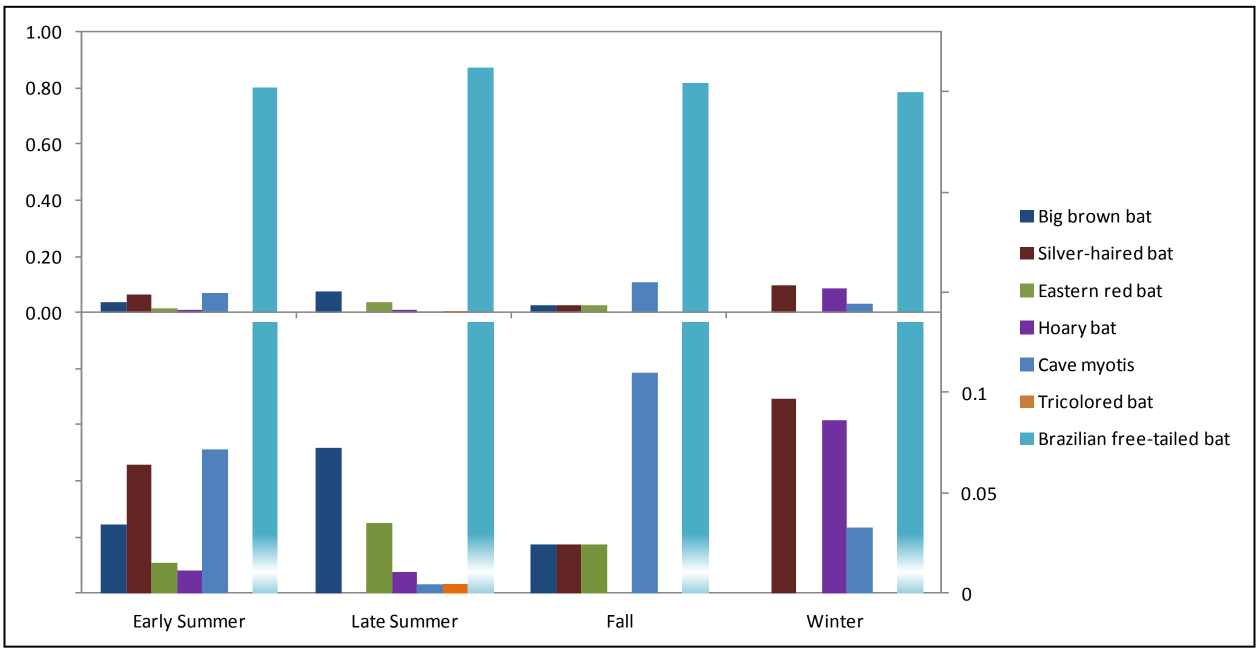

Figure 3.28. Proportion of bat passes by species across four seasons near the Lampasas bridge. The top half of the chart shows all values together, and the bottom half of the chart displays a close in view of the proportion of all values less than 12 percent.

We analyzed 2,928 files recorded during the first two hours after sunset from 15 sampling events occurring between May 2012 and January 2013 at the Salado bridge (Appendix D). Of these files, 1,061 contained bat passes. The software was able to reach an autoclassification decision for 850 of these files, representing seven species after the corrections listed in Table 3.2 were applied. The vast majority (87 percent) of these calls belonged to Brazilian free-tailed bats, with the remaining 16 percent belonging to big brown bats (six percent), silver-haired bats (three percent), cave myotis (two percent), eastern red bats (one percent), hoary bats (one percent) and tricolored bats (less than one percent) (Figure 3.29).

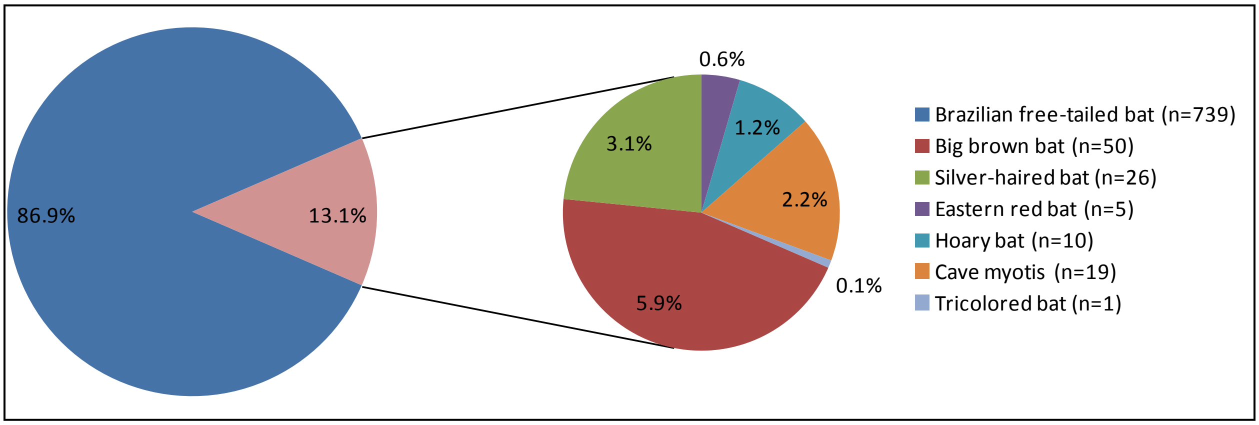

Figure 3.29. Relative abundance of seven species automatically classified by Sonobat v3.1 from 850 bat passes recorded near the Salado bridge.

We obtained bat pass data over the course of four seasons. Absolute numbers are not exactly comparable given the nuances of bat pass tallies and the unequal effort across seasons. However as a generalization, the number of bat passes recorded for big brown bats peaked during the early summer season, and silver-haired bats, cave myotis and Brazilian free-tailed bats peaked during the fall (Table 3.12, Figure 3.30).

Table 3.12. Number of bat passes recorded for each species over two recording events during the early summer, three each during late summer and winter and five during the fall near the Salado bridge.

Species	Early Summer n=2	Late Summer n=3	Fall n=5	Winter n=3
big brown bat	39	3	8	0
silver-haired bat	8	5	13	0
eastern red bat	4	0	1	0
hoary bat	1	3	6	0
cave myotis	6	1	12	0
tricolored bat	1	0	0	0
Brazilian free-tailed bat	149	187	376	27
TOTAL	**208**	**199**	**416**	**27**

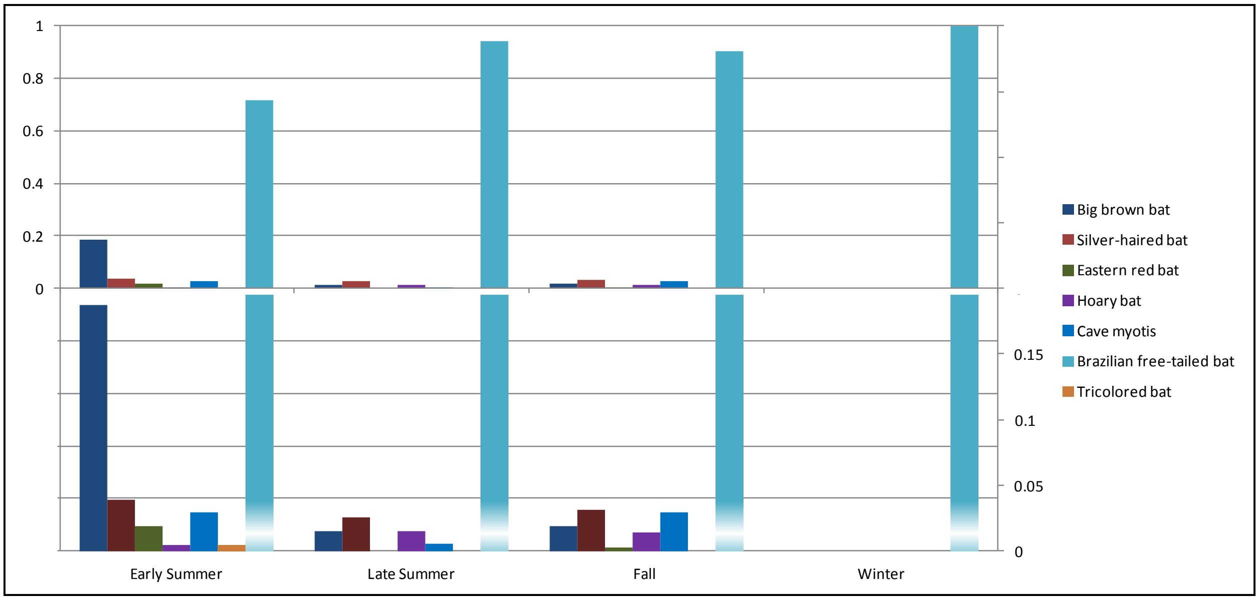

Figure 3.30. Proportion of bat passes by species across four seasons near the Salado bridge. The top half of the chart shows all values together, and the bottom half of the chart displays a close in view of the proportion of all values less than 20 percent.

There was a very strong correlation between photomonitoring point counts and acoustic activity patterns of Brazilian free-tailed bats at Lampasas bridge (r=0.84). There was almost no correlation at the Salado bridge (r=0.08).

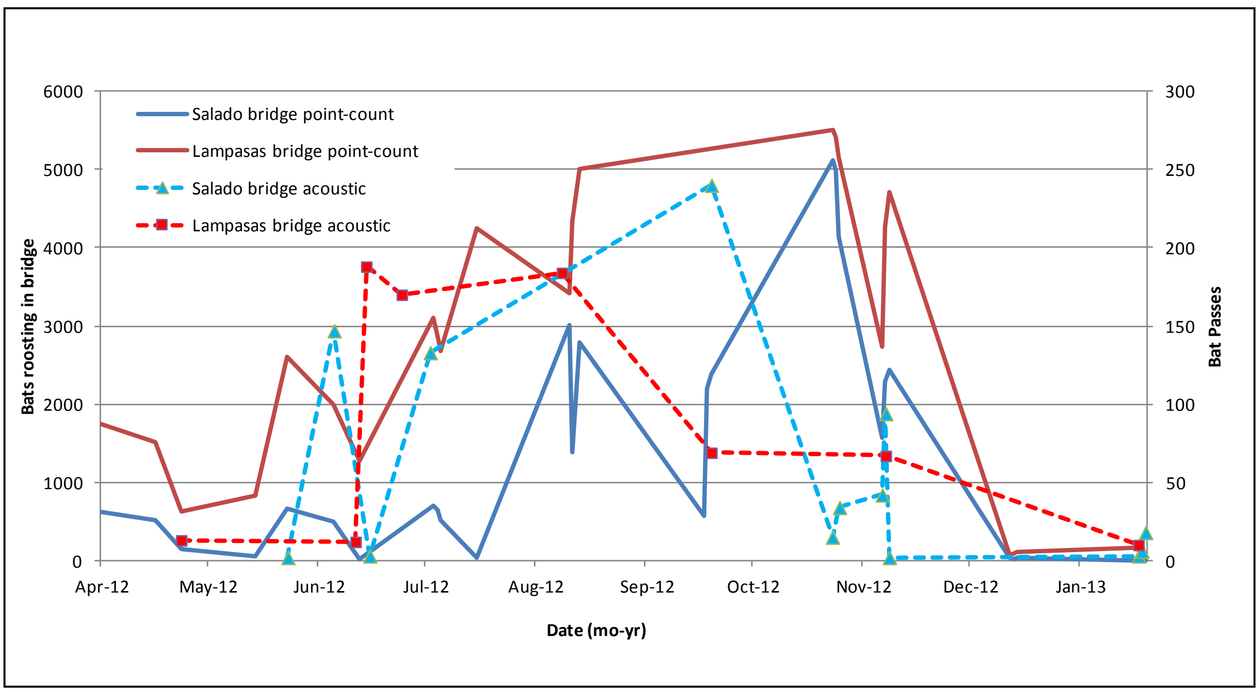

Figure 3.31. Comparison of point-count data and acoustic activity patterns of Brazilian free-tailed bats at Lampasas and Salado bridges.

We analyzed 911 files recorded during the first two hours after sunset from 16 sampling events occurring between April 2012 and November 2012 at the Turkey Run bridge. Of these files, 16 contained bat passes. The software was able to reach an autoclassification decision for five of these files, representing three species after the corrections listed in Table 3.2 were applied. Two of these calls belonged to Brazilian free-tailed bats, two belonged to cave myotis, and one belonged to an eastern red bat.

Table 3.13. Number of bat passes recorded for each species over two recording events during the early summer, three during late summer and one in late November (no established sampling season) near the Turkey Run bridge.

Species	Early Summer n=2	Late Summer n=3	Out of Season n=1
eastern red bat	1		
cave myotis		2	
Brazilian free-tailed bat	1		1
TOTAL	**2**	**2**	**1**

No bats were captured in the mistnets. Even if multiple net arrays were used, they would have likely been inundated by the most abundant species and failed to detect rare species such as the tricolored bat that only had a total of three passes at all sites over all seasons.

3.5 Discussion

Our goal with photographic monitoring was to use still photography combined with bat traps to estimate the actual number of bats roosting within the bridge gaps at the Lampasas and Salado bridges between October 2011 and January 2013. We aimed to estimate the actual number, rather than simply record the point count, because in this 'before and after' study design, the 'after' measurements will be in a bat house rather than the bridge gaps. In order for the pre and post-construction numbers to be comparable, we strived to estimate the actual number of bats, including those not detected through point counting of photographs. In order to estimate the number of bats not detected through photography, we combined our point counts with other population estimation methods, including mark-recapture.

We calculated correction factors for sections of the bridge that could not be photographed, and for "stacking" within the gaps. Our correction factors were based on a large dataset (see bold numbers in Table 3.4 and Table 3.6), and while there was some variance overall the values clustered and inspire confidence in our estimates. We attempted to use bat traps to verify the estimates we were able to make using direct observations of photographed bats, and to assess how much bats used the vertical space in the gaps that was not visible in the photographs. Overall the bat traps were only marginally successful in documenting bats, for several reasons. The environment degraded the condition of the traps quickly, and the warped traps were often found on the ground under the gap. While other researchers have used this design, we found on most occasions bats tended to roost on either side of the traps, avoiding the traps themselves. Since the bats quickly learned to avoid the traps we had only a small sample size from which to estimate the amount, which was quite variable (1.4 – 2.6 times). The habitation of the cross gap at the Salado bridge was so variable that these numbers should be interpreted with caution. The gap habitat at the Salado bridge was less deep than at the Lampasas bridge, and did not include the T-shaped extensions at the top. Future estimation techniques should focus on measuring these values more accurately.

Bats occurred at different densities in different sections of the bridges. Low numbers of bats were documented in the extreme north and south ends of the bridges where the gap is not high enough off the ground to provide an adequate drop zone. Additionally there are obvious dips in the average number of bats inhabiting one meter sections both at the Lampasas and Salado bridges (Figure 3.19, Figure 3.23). The reason(s) that some one meter sections are less densely populated that the surrounding bridge sections has not been thoroughly investigated. The apparently disproportionate distribution may be a result of slightly different shapes or compositions of the gaps in those areas, which may in turn exacerbate microclimatic variations. Some segments may have a lack of weather protection from leaks from above, or perhaps the bats avoid modifications we made in or near the gap to facilitate sampling, such as bolt placement or trap installation. There may be other unknown or unknowable variables at work that affect the distribution of the bats across the bridge habitat.

Large daily and seasonal fluctuations in cave-roosting Brazilian free-tailed bat point counts based on emergence data were documented in Hristov et al. (2010). We have observed changes in three day point count numbers of as many as 1,977 individuals (Lampasas bridge, November 2012, Table 3.7), representing a large increase, 1.4 times, in numbers between day one and day three. The high variances may be explained by a single population that sometimes roosts at other nearby structures during longer foraging trips, as a result of crowding after breeding, or during climactic extremes. Additionally, in the spring and fall, the individuals at these bridges may consist of other northern populations migrating between summer and winter roosts.

Photographic records of point count data for bridge roosting Brazilian free-tailed bats is a valid tool for creating a permanent record of roosting patterns on a daily, seasonal, annual and long term basis, including the documentation of roost-sharing species. We observed a few cave myotis periodically roosting at the bridges, but no effort was undertaken to discriminate between the species in the photographs unless it was very obvious that there were two different species. Davis and Schmidly (1997) also mentions Brazilian free-tailed bat roosts together with cave myotis, thus we were not surprised to find both species roosting at the bridges together.

Although Brazilian free-tailed bats are widespread and abundant, surprisingly little rigorous data collection has been performed on the life history patterns of this species. Monthly observations are useful for tracking breeding and migratory patterns; however due to potentially large-scale changes in the number of bats roosting at a bridge from one day to the next, care should be taken not to base conclusions on a single monthly visit. This is especially true for bridges hosting maternity colonies, like the Lampasas bridge. Recently born pups are small and well protected, and may grow fast enough to be undetected by photographic methods one month, but to cause an apparent doubling of the population the next.

Our efforts to use bat traps in order to measure stacking within the gaps were mostly unsuccessful. Besides point counts and estimates at emergence, other researchers have attempted to estimate the size of bridge roosting bat colonies by measuring the amount of available habitat in the gap, much as we did in Chapter 2.0, and then estimating how deeply within the gap bats were stacked during each visit. The Lampasas and Salado bridges had narrow gaps that did now allow us to estimate how deeply the bats may have been stacked without disturbing and removing bats during each count. Estimates that consider how deeply the bats are stacked will be more accurate than point counts of bats visible only in the top layer. Rather than disturbing the bats to measure how deeply they are stacked each time, we recommend the occasional extraction of all bats within a bridge section of known volume so that a metric may be developed to supplement point count data; however this step is only necessary when the desired outcome is an estimate of abundance. Life history patterns, relative abundance, and population trends can all be recorded and tracked by performing point counts without disturbance.

Photographic monitoring results are not directly comparable with our mark-recapture results because mark-recapture activities were not undertaken during each season that photography was performed. Bat populations recorded via photographic point counts peaked during summer and fall; however output from Program MARK indicated larger population sizes during fall and spring than during summer. It stands to reason that the point-count values may be more reliable than the theoretical values derived from the computation, and that alternative analyses within MARK may offer different seasonal estimates than those presented.

Keen (1988) discusses several general assumptions associated with mark-recapture analyses. We address each of these briefly as they relate to our study:

1. Survival rate of marked individuals is representative of the entire population.

   According to our preliminary study of five tagged bats, the short term survival rate of the tagged bats was 100 percent, suggesting that PIT tags do not negatively impact survival.

2. Individuals leave the population through death, not through emigration.

   Bels (1952), Stevenson and Tuttle (1981) and Humphrey and Cope (1976) indicate permanent switching of hibernacula is uncommon, and our data support that recaptured bats are loyal to their capture site; however, one bat tagged at Salado was recovered at Lampasas, indicating that roost switching does occur between these two sites. This switching may be related to microclimactic fluctuations, as the temperature within the gap at the Lampasas bridge appears to be more stable than that at the Salado bridge. This is supported by our 15 July 2012 observation of only 34 bats during an exceedingly high temperature point-count survey at Salado bridge, but notably higher than normal point-count result at Lampasas bridge on the same day. Ellison et al. (2007) reported similar results with big brown bats, which tended to move from building to building the most on hot days.

   Based on point-count surveys, we expected the population size estimates to be fairly similar for fall and summer, and notably lower in the spring. Instead we found a relatively constant estimate for fall 2011 and spring 2012, and a notably lower estimate for the summer of 2012. One possibility is that the timing of the PIT tagging combined with the low recapture rates swamped the mortality calculations in the population estimate formula. Since all PIT tags were placed during the fall of 2011, the majority of recaptures occurred during the spring sampling event, explaining the high population estimate. Fewer marked animals were recaptured during the summer, as tags are lost to mortality. More thoroughly vetted model selection and formulation in Program MARK may yield different estimates. Another option is that the bridges are actually used by two groups of bats. One group of bats may summer in the north, using the bridges as migratory stop-overs only in the fall and spring. A largely different group of bats may summer at these bridges, and migrate further south during the shoulder seasons. If this is the case, the marked bats were largely not present in the summer, which would explain the low summer mark-recapture estimates in relation to point-count estimates.

3. Recapture probability is equal.

   The Cormack (1964) model assumes marked individuals have equal probability of recapture over time, whereas the Jolly-Seber (1965) assumption states that there is an equal probability of catching any living individuals, marked or not, during a given sampling episode. The logistics of retrieving bats from the gaps under the bridges were a limiting factor in our study. During the first part of the study, we were only able to retrieve bats from specific sections where ropes had been installed to allow us to access the gap. Later, we installed a trolley system that allowed us to access a greater proportion of the gap; however we observed bats learning to evade us during recapture attempts on consecutive days. We assumed an equal probability of capturing any living individuals, marked or not, but note that the probability of capture was actually time dependent within the sampling episode. Pollock and Raveling (1982) indicted that survival estimates will be lower for populations containing trap-shy individuals.

4. Marks are not lost.

   The use of PIT tags under the skin provides a secure marking mechanism that prevents the loss of tags that may experienced with other marking methods, such as banding. Although not shown in our study, PIT tags may migrate under the skin of an animal.

5. Survival probability between episodes is equal for all marked individuals.

   Age-dependent survival rates may bias the survival estimates produced by the analysis; however Keen (1988, p. 162) indicates the bias is "inherent in the analysis and might be considered inconsequential when bats cannot be aged." Caughley (1966) gives age specific mortality rates in a U-shaped trend (higher death for very young and very old individuals). The Jolly-Seber analysis is "little influenced" by age dependent survival rates (Cormack 1972), and we did not consistently evaluate tooth wear or other factors as a method of aging individuals, though we evaluated reproductive maturity following volancy during the summer of 2012.

We reported life history observations because they may contain valuable information relating to seasonality, population estimates, and gender and site biases. For example, female bats at the Lampasas bridge had significantly higher BCI than male bats, which can be explained by the observation that Lampasas harbored a maternity colony on one half of the bridge, and pregnant females had higher BCI measurements than males.

Brazilian free-tailed bats are a migratory species that spends summers in caves and bridges throughout Texas and beyond, but overwinters in Mexico. Previous mark-recapture efforts with Brazilian free-tailed bats have resulted in recovery rates ranging from 0.3 to 2 percent (Cockrum 1969). We implanted bats with PIT tags just before fall migration, and recaptured them the following spring, which may also help explain the low tag recovery rates. Although low, successful tag recovery does indicate that the installation of PIT tags in Brazilian free-tailed bats does not negatively impact migration patterns or site fidelity, and is an effective method of marking this organism.

Acoustic activity patterns of the Brazilian free-tailed bat at Lampasas and Salado bridges correlated, though very weakly at Salado bridge, with the number of bats we observed roosting in the bridge gaps during point-counts, particularly with regard to the increased seasonal presence of bats during the summer and fall months. This overall result lends confidence to the data collected from other methods and supports the utility of acoustic methods.

The same seven bat species were recorded at both the Salado and the Lampasas bridges, with very similar relationships in their relative abundance. Activity at both bridges was dominated by Brazilian free-tailed bats, followed by big brown, silver-haired, and cave myotis. All else being equal, the geographic proximity of these two sites means we expect their species composition to be similar. Seasonal activity patterns in some species, namely big brown bats, differed between the two sites, but that could easily have been the result of low sample sizes and other sample biases discussed in the methods. The Turkey Run bridge, while the most rural of the three as it is not on a major interstate highway, had the least bat activity and diversity recorded. Bat abundance and diversity may be less for many reasons. First, Turkey Run Creek is much smaller and its flow is dominated by effluent from the city of Copperas Cove. The bridge had no existing bat colony; therefore, bat-bat interactions that may exist at the other sites are not present. Also, IH 35, while it is a major interstate supporting commerce from Canada to Mexico, may have an inflated number of bats because it follows a major topographic feature in central Texas, the Balcones Escarpment. This feature supports many springs, it is at the edge of two major ecotones (therefore has a higher diversity of habitat), and may be a significant navigational landmark for migratory species like bats.

Photomonitoring point-counts and acoustic activity patterns were well correlated at the Lampasas bridge, offering more support for the relative abundance patterns observed in the point-counts being more reliable than those indicated by the mark-recapture analysis.

If resources allow in the future, in order to more fully characterize the acoustic patterns at these sites, we recommend installing multiple receivers on each bridge each night to account for variable exit flight patterns (e.g. upstream vs. downstream) and to be able to calculate variance estimates within and among sites (e.g. Jones et al. 2004). Also, if more all night recordings are made, we may detect peaks of different species which may use the area at different times. Finally, we recommend more samples per season. The variability of bat passes per night can be extremely high as demonstrated by Hays (1997) who used a thorough 195 night dataset to show that in subsamples with seven or more nights, more than 60 percent of the datasets had means within 20 percent of the mean of the entire dataset. Based on that information, recommendations are that at least six to eight consecutive nights are needed to remove bias.

4.0 Water Quality

4.1 Abstract

We monitored water quality at Interstate Highway 35 over Lampasas River and Salado Creek in Bell County (Lampasas and Salado bridges) and at State Highway 9 over Turkey Run Creek in Coryell County (Turkey Run bridge), Texas. The Lampasas and Salado bridges are occupied by colonies of Brazilian free-tailed bats (*Tadarida brasiliensis*), and the Turkey Run bridge was built during the study and was not occupied by bats. Water quality was impacted by the presence of bats; however, the effects are variable both temporally and spatially. The diurnal patterns of the bats seem to have very little influence on water quality, if any at all. Our initial hypothesis that guano related constituents (such as *E. coli*, phosphorus and nitrogen species) would be elevated during occupation and decrease during nightly foraging was incorrect. We found no consistent seasonal trends among the occupied bridges, except for a stronger correlation between flow rates and *E. coli* at the Salado bridge in months where bats were present. Our hypothesis that the bat colonies would cause spatial variations in water quality was confirmed. At both occupied bridges, guano related constituents were generally elevated downstream of the bridges, particularly at the Salado bridge. The Salado bridge also consistently had the poorest water quality overall, with the exception of ammonia and phosphorus at the Turkey Run bridge that may be explained by an upstream wastewater treatment facility. Primary contact recreation standards for *E. coli* bacteria were exceeded at all three bridges, particularly during storm events. The highest in-stream concentration of *E. coli,* 40,000 colonies/100 ml, was measured downstream of the Lampasas bridge. The most significant impacts to water quality occurred during storm events, likely due to the flushing of guano into the streams by storm pulses. The installation of suggested best management practices (e.g. berms and excluding bats from roosting over water) could mitigate impacts to water quality.

4.2 Background

Mammal excrement, such as bat guano, contains a number of potentially harmful pollutants, including *Escherichia coli* (*E. coli*) bacteria, nutrients such as nitrogen and phosphorus and various other pathogens. These pollutants can lead to eutrophication of water bodies and illness in humans and other animals that come into contact with or consume the water. In fact, the Environmental Protection Agency has concluded "water bodies with substantial animal inputs can result in potential human health risks on par with those that result from human fecal inputs" (Dufour et al 2012, pg. 3). Previous studies have shown that bat colonies roosting over water bodies may impact water quality. Increases in nitrate, organic carbon, dissolved carbon dioxide, and *E. coli* have been detected at sites downstream of bat colonies (Wicks and Engeln 1997, Wooten et al. 1998, Gillen 2011). Other water quality parameters, such as total suspended solids, microbial pathogens, nitrogen compounds and phosphorus are likely impacted by bat colonies; however, there are limited data available on how bat colonies affect water quality. Of the studies identified above, only three were conducted in Texas, two at the Ann W. Richards Congress Avenue Bridge in Austin, Texas (Keeley and Tuttle 1999) and one at Buffalo Bayou in Houston, Texas (Guillen 2011).

At Buffalo Bayou, a significant increase in *E. coli* colonies was detected downstream of a Brazilian free-tailed bat (*Tadarida brasiliensis*) colony and DNA fingerprinting showed that bat guano was primarily responsible for the increase (Guillen 2011). Conversely, two studies conducted at the Ann W. Richards Congress Avenue Bridge in Austin, Texas found no significant impacts to water quality despite a population of approximately 1.5 million Brazilian free-tailed bats (Keeley and Tuttle 1999). The inconsistency of these studies shows the need for additional research to characterize how interactions of bat population, seasonal and diurnal movement, fecal loading, hydrology and geography influence water quality downstream of bat colonies. Further highlighting the need for more research is a survey of highway structures throughout the U.S. that identified approximately 4,250,000 bats of 24 species living in 211 highway structures (Keeley and Tuttle 2009).

The Texas Commission on Environmental Quality (TCEQ) sets and implements surface water quality standards to establish criteria for the quality of surface water bodies throughout the state, with the goal of maintaining the quality of surface waters for public health, recreation, and aquatic life while allowing sustainable economic growth (TCEQ 2013b). Standards are based on intended use, which can be categorized into three types; aquatic life, recreation, or public water supply. Water bodies are evaluated based several criteria including dissolved oxygen, temperature, pH, dissolved minerals, toxic substances and bacteria. A high aquatic life use designation means that total dissolved oxygen should never be less than 3.0 mg/L and that the species assemblage should be an association of regionally expected species that is highly diverse with sensitive species present. A primary contact recreation designation requires that the geometric mean of *E. coli* bacteria not exceed 126 colonies/100 ml, and that no single sample exceed 399 colonies/100 ml. For secondary contact recreation the geometric mean of *E. coli* bacteria may not exceed 630 or 1,030 colonies/100 ml depending on the subclassification. Noncontact recreation waters may not exceed a geometric mean of 2,060 *E. coli* bacteria colonies/100 ml [30 Texas Administrative Code §1.307.7(b)(a)(A)].

Our study involved water quality monitoring bridge three sites at Interstate Highway (IH) 35 over Lampasas River and Salado Creek in Bell County (Lampasas and Salado bridges) and at State Highway (SH) 9 over Turkey Run Creek in Coryell County (Turkey Run bridge), Texas. The Lampasas and Salado bridges are occupied by colonies of Brazilian free-tailed bats (*Tadarida brasiliensis*), and the Turkey Run bridge was built during the study and was not occupied by bats.

To provide a basic unit for assigning site-specific standards and for applying water quality management programs, the TCEQ assigns identification numbers to stream segments, particularly major streams. These segments are intended to have relatively homogeneous chemical, physical, and hydrological characteristics. The Lampasas bridge site (Segment 1215) is within a segment designated by the TCEQ as a high aquatic life use segment with primary contact recreation use. In 2010, it was listed by the TCEQ as impaired for bacteria, but was delisted in 2012. The Salado Creek bridge site (Segment 1243) is within a segment designated by the TCEQ as a high aquatic life use segment with primary contact recreation use, and was listed in 2010 as fully meeting the requirements of those designations [30 Texas Administrative Code §1.307.10(3)]. Turkey Run Creek is a small tributary to the Leon River and is not a classified water body. In Texas, unclassified perennial waters, like Turkey Run Creek, are designated as high aquatic life use and primary contact recreation water bodies.

We monitored the effects of large roosting bat colonies on water quality at two bridges with existing colonies located at IH 35 over the Lampasas River and Salado Creek in Bell County (Lampasas and Salado bridges) and at a new bridge without a bat colony at SH 9 over Turkey Run Creek in Coryell County (Turkey Run bridge). Our intent was to test the following hypotheses:

1. Water quality will vary diurnally based on the diurnal movements of the bat colonies.
2. The presence of bat colonies may cause spatial variability in water quality with the greatest impacts occurring downstream of the roosting area.

3. During storm events, water quality may be degraded downstream of the roosting areas due to the flushing of guano into the streams.

To test these hypotheses, we set up sampling stations at each site so that there was one upstream control station and at least two sampling stations downstream of the area where bat influences are likely. Samples were collected seasonally, diurnally and during storms. At least one storm event was observed beneath each bridge to determine runoff patterns and potential points where guano may be washed into the streams by storm runoff.

4.2.1 Water Quality Study Area

The project area is located in the Cross Timbers and Prairies ecoregion in Bell and Coryell County, Texas (Griffith et al 2004). Alternating bands of wooded habitat scattered throughout a prairie region represents the ecoregion. All study sites are located within the Brazos River Basin, which drains an area of approximately 116,550 km^2 within Texas and New Mexico (Hendrickson 1999). Land use upstream of the Salado Creek and Lampasas River sites consists of rural residences, agricultural and livestock grazing. Land use upstream of the Turkey Run bridge site consists of urban residences, industrial, retail, and a golf course. A waste water treatment facility is located approximately 450 m upstream of the Turkey Run bridge.

The average annual rainfall within the study area is 80.5 cm, and the average high and low temperature is 26.1 and 12.3 °C, respectively (National Climate Data Center 2013). The Stillhouse Hollow Dam weather station is located approximately 4.43 km upstream of the Lampasas bridge site, and is the most intermediate weather station to all three sites with long-term, readily available data (Figure 4.1). The total precipitation recorded at the Stillhouse Hollow Dam weather station in 2011 was 52 cm, in 2012 it was 77.9 cm, and in January through April 2013 was 23.3 cm. Rainfall and temperature records recorded at the Stillhouse Hollow Dam weather station during the study are presented in Appendix F.1.

Continuous streamflow data are not available for Salado Creek and Turkey Run Creek during the time period of our study. United States Geological Survey (USGS) flow monitoring did not begin in Salado Creek until 14 March 2013, and no USGS flow monitoring has occurred at Turkey Run Creek. The USGS maintains a stream gauging station at the Lampasas bridge sampling site, and data are available for the entire study period (Appendix F.1). Discharge ranged from approximately 0.03 to 25,349 m^3/s and is highly dependent on storm runoff and releases from Stillhouse Hollow Dam, which is located 4.43 km upstream (Figure 4.1).

The wetted width of the Lampasas River is approximately 12 m, and the bankfull width is approximately 20 m. The topographic floodplain is steep at the study site. Three sampling stations are located at the Lampasas bridge; LR-1, LR-2 and LR-3 (Figure 4.2). LR-1 is located 50 m upstream of the existing bridge centerline, LR-2 is located at the centerline, LR-3 is located 50 m downstream of the existing bridge centerline and underneath the new northbound frontage road bridge. The depth during normal flow conditions is variable across the site, ranging from approximately 20 cm at LR-2 (station located in a riffle) up to 1 m at LR-1 and LR-3. The substrate consists of silt sand, cobble, and organic debris.

The wetted width of Salado Creek is approximately 28 m and the bankfull width is approximately 45 m. The topographic floodplain is broad and may have been modified by development near the stream. Four sampling stations are located at the Salado bridge site; SC-1, SC-2, SC-3 and SC-4 (Figure 4.3). SC-1 is located 40 m upstream of the Salado bridge centerline, SC-2 is located at the centerline, SC-3 is located 50 m downstream of the centerline, and SC-4 is located 275 m downstream of the centerline (25 m downstream of a small dam). The depth during normal flow conditions is variable across the site, ranging from approximately 20 cm at SC-4 up to 2 m at the upstream stations. A dam is located between stations SC-3 and SC-4 and is responsible for lower flow velocity and greater depth of SC-1, SC-2 and SC-3. The substrate consists of cobble, silt sand, bedrock and debris. Bedrock is only exposed downstream of the dam.

The wetted width of Turkey Run Creek is approximately 1.5 m and the bankfull width is approximately 8 m. The topographic floodplain is relatively flat and broad at the study site. Three sampling stations are located at the Turkey Run bridge site; TR-1, TR-2 and TR-3 (Figure 4.4). TR-1 is located 50 m upstream of the SH 9 bridge centerline, TR-2 is located at the centerline, TR-3 is located 50 m downstream of the existing bridge centerline. The depth during normal flow conditions is variable across the study area, ranging from approximately 10 cm at TR-3 (station located in a riffle) up to 0.5 m at TR-1 and TR-2. The substrate consists of silt, sand and cobble. Flood pulses often rework the channel morphology.

At the Lampasas and Salado bridges, storm water outfalls discharge highway runoff under the bridges. On the north side of the Lampasas bridge and the north and south side of the Salado bridge, runoff flows through concrete ditches to the streams (Figure 4.5). On the south side of the Lampasas bridge runoff flows through ditches eroded by storm flows and into a recently constructed retention structure. Additional photos of all sites are included in Appendix F.2. Roadway construction was still underway at Turkey Run bridge during this study, and no outfalls were constructed under the bridge.

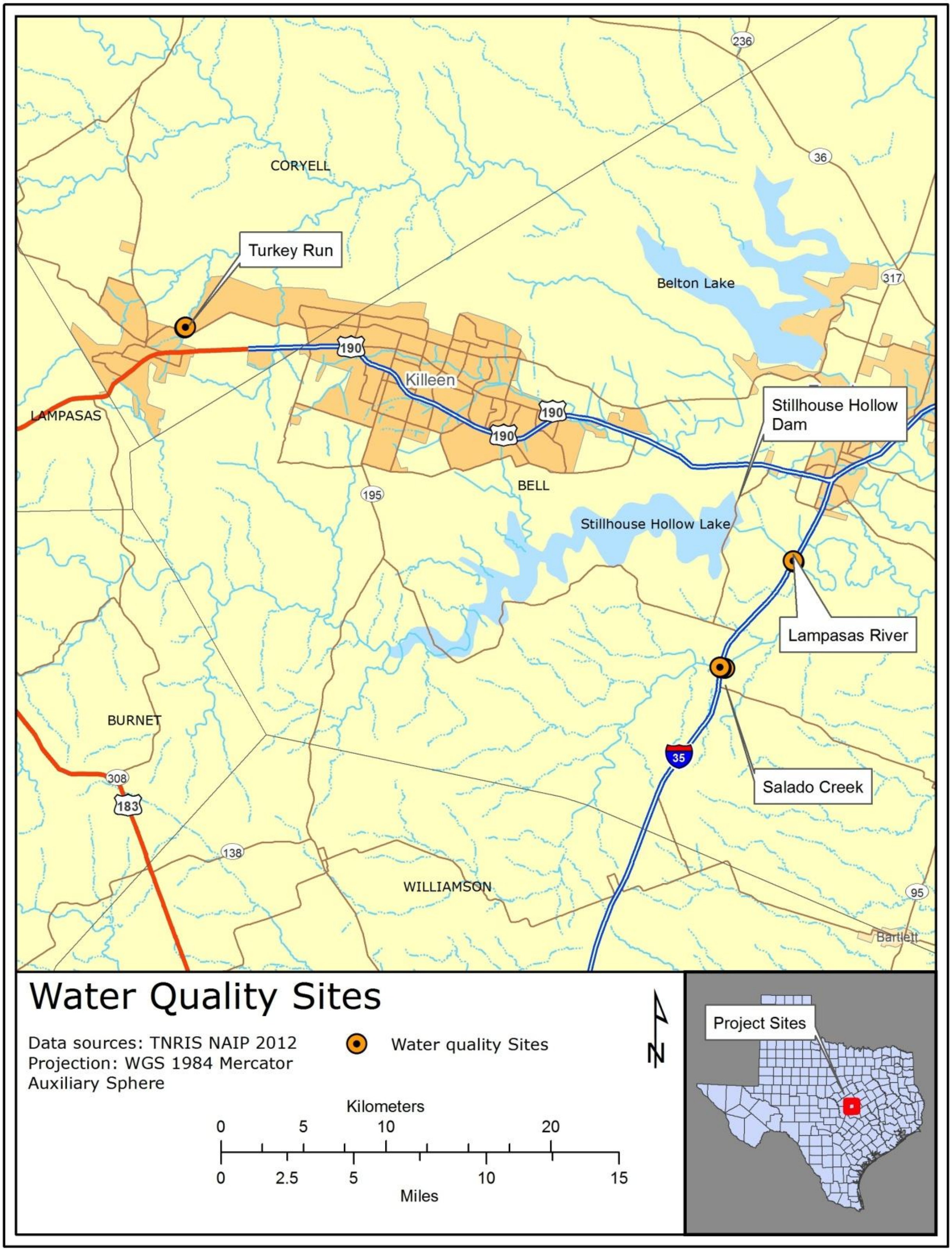

Figure 4.1. Location of the Lampasas, Salado, and Turkey Run study areas and the Stillhouse Hollow Dam and Reservoir in Bell and Coryell counties, Texas.

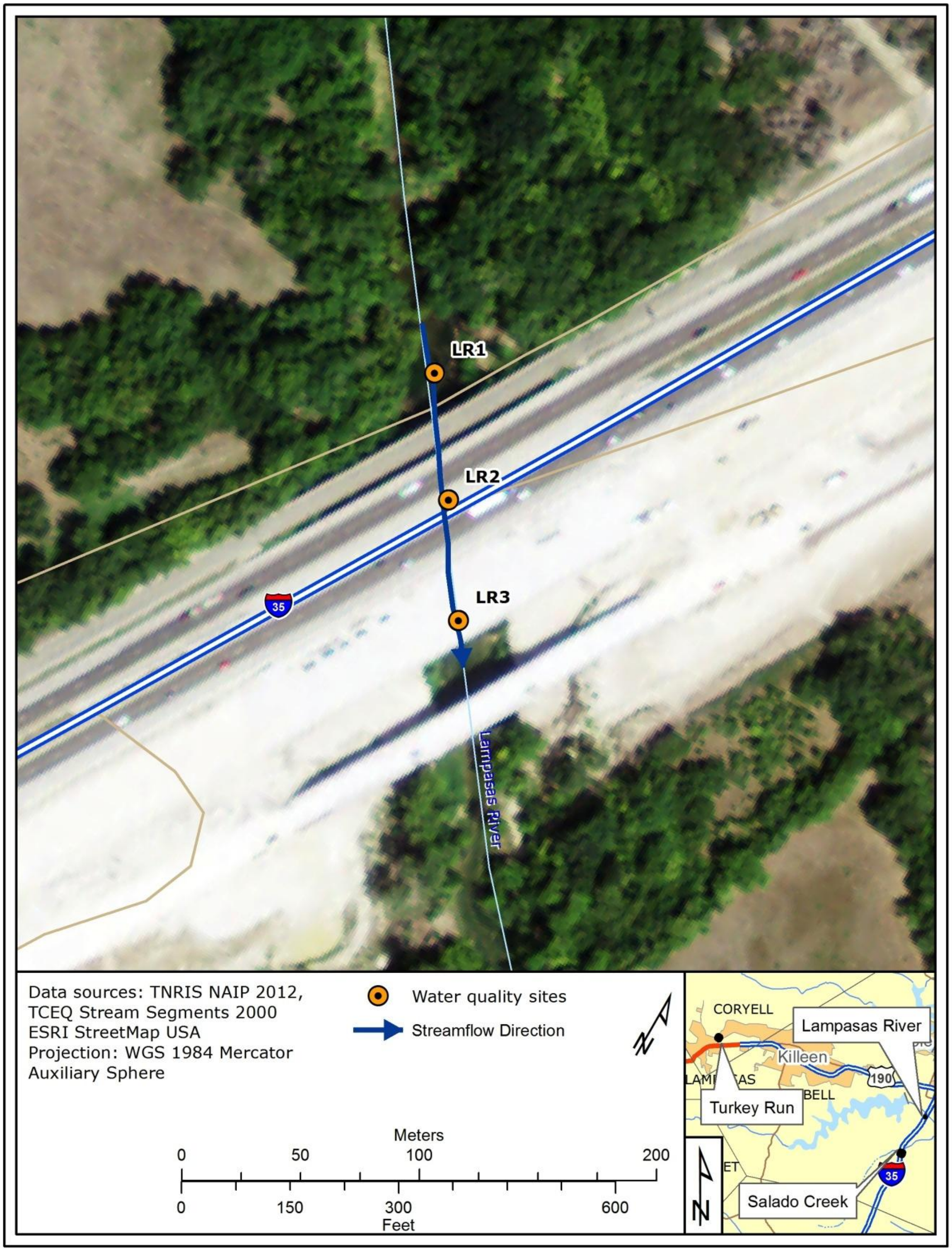

Figure 4.2. Water quality sampling locations at the Lampasas bridge, Bell County, Texas

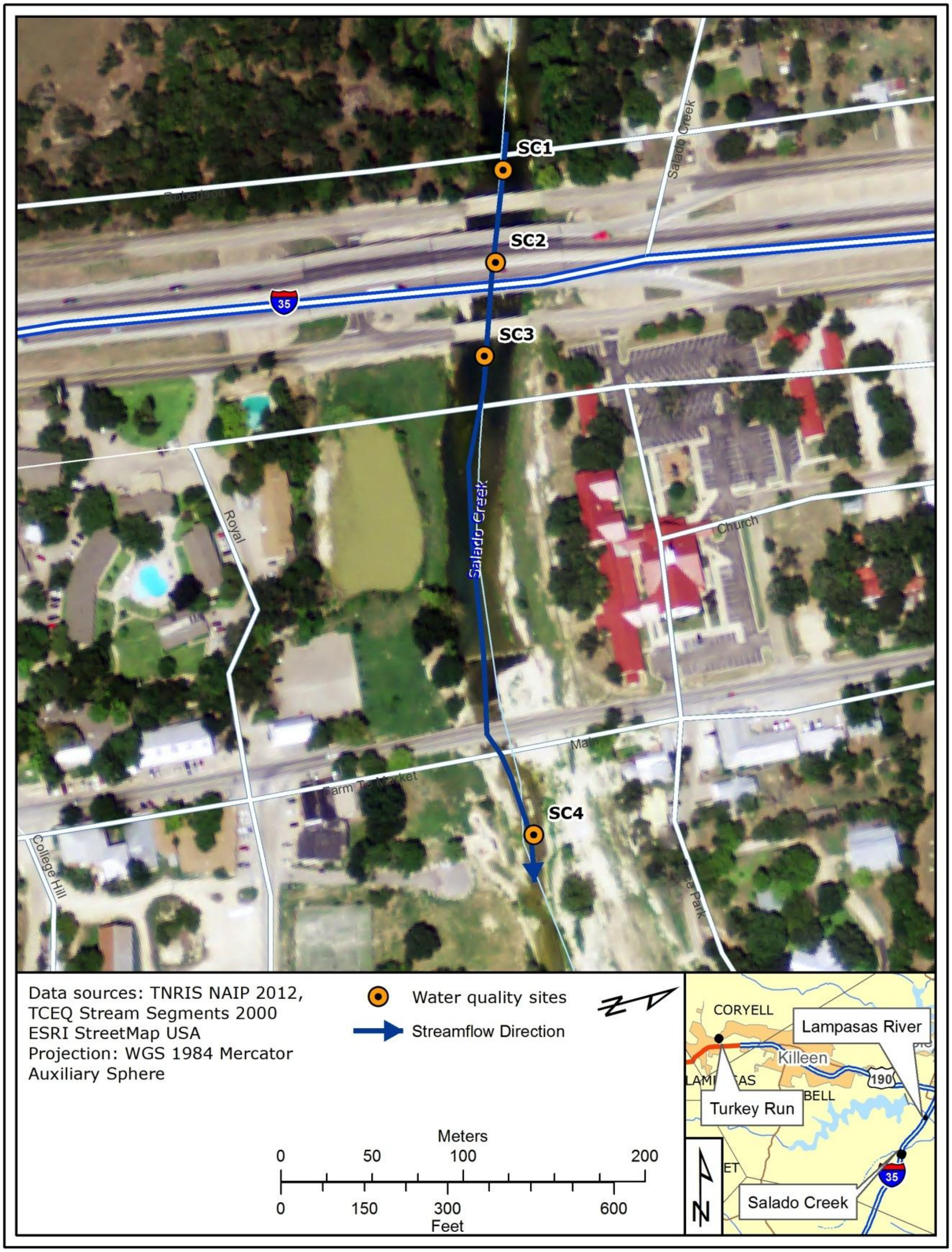

Figure 4.3. Water quality sampling locations at the Salado Creek bridge, Bell County, Texas.

Figure 4.4. Water quality sampling locations at the Turkey Run bridge, Coryell County, Texas.

Figure 4.5. Concrete drainage gutter that conveys storm water from the outfall underneath the north side of the Lampasas bridge, Bell County, Texas. Bat guano can be seen in the gutter.

4.3 Methods

Four sampling stations were established at the Lampasas bridge (Figure 4.2), Salado bridge (Figure 4.3) and three at Turkey Run bridge (Figure 4.3). At each site, station one was located approximately 50 m upstream of the bridge centerlines, station two was located at the bridge centerlines and station three was located approximately 50 m downstream of the bridge centerlines. At the Salado bridge, a fourth station was located approximately 275 m downstream of the bridge centerline to assess water quality downstream of an impoundment. Roadway runoff and guano accumulation patterns were mapped at the Salado and Lampasas bridges. Roadway construction was still underway at Turkey Run bridge during this study, and no outfalls were constructed under the bridge, therefore no runoff map was created for the Turkey Run bridge.

This study was designed to examine potential bat colony impacts to water quality at three temporal scales: seasonal, diurnal and storm. To assess seasonal changes in water quality, samples were taken at all stations bimonthly beginning in October 2011 and ending in October 2012. *E. coli* and nutrient samples were collected beginning in October 2011. Nutrient sampling (for ammonia, nitrate, nitrite, total Kjeldahl nitrogen [TKN] and phosphorus) continued through June 2012, but was discontinued after it was determined that efforts were better spent focusing on *E. coli* sampling.

To assess water quality during storm events, samples were collected when storm events of sufficient intensity to generate significant runoff occurred. Because *E. coli* was a primary constituent of interest, storm collection was limited to Monday 2 am through 12 pm Thursday as the hold time for *E. coli* samples is six hours and the laboratory only accepts these samples until 4 pm on Thursdays.

Two diurnal sampling events occurred simultaneously at all three study sites on 4-5 October

2011 and 25-26 June 2012. Six samples were collected at each station during each event. Sample one was collected before the colony emergence, sample two was collected during colony emergence, sample three was collected when emergence was nearly complete, sample four was collected prior to the return the colony, sample five was collected during the peak of the colonies return and sample six was collected after much of the colony had returned. The sampling times were determined by biologists who were familiar with the timing and duration of emergence through previous fieldwork at the sites.

Because the goal of the study is to determine if bat colonies that roost in the bridges are impacting water quality, we consider station one at each bridge a control point that is representative of water quality before any influence by the bat colonies. We consider station two at each bridge site to represent the location where bat colony influences begin, as the colonies tend to roost in cracks located at the bridge centerlines. At station three, we expect the bat colony influences to be strong because all inputs of bat guano (direct or washed in by runoff) will occur just upstream. Station four at the Salado bridge site is located near a spring that harbors the federal candidate Salado Salamander (*Eurycea chisholmensis*) and is meant to assess the influence of the upstream bat colony on water quality near the spring.

4.3.1 Sample Collection

All field technicians were trained and overseen by a State of Texas Licensed Professional Geoscientist. All field technicians were required to demonstrate proper sampling technique before collecting samples in the field. All stream samples were collected from the center of flow, requiring the field technicians to enter the streams to collect samples.

To ensure that the field technicians did not introduce particles or dissolved constituents into the samples, downstream samples were collected first and upstream samples were collected last. Powder free gloves were worn when taking all samples and changed between sampling stations. Nutrient samples (nitrate, nitrite, ammonia, TKN, phosphorus) were collected in EPA approved 1000 mL QEC Level 6 Precleaned HDPE bottles and all *E. coli* samples were collected in US EPA approved 120 mL IDEXX Vessels with Sodium Thiosulfate. All samples were immediately placed on ice after collection and kept on ice until delivered to the laboratory for *E. coli* analyses or until chemical analyses were performed. Detailed sample collection procedures are given in Appendix F.3.

4.3.2 Analytical Methods

The Lower Colorado River Authority Environmental Laboratory Services, a National Environmental Laboratory Accreditation Conference accredited laboratory, performed all *E. coli* analyses. All nutrient samples were analyzed in the Zara Environmental LLC laboratory using a Hach DR2800 Spectrophotometer and a Hach DRB200 Digital Reactor Block. Nitrite was analyzed according to Hach Method 8507, phosphorus according to Hach Method 8190, TKN, nitrate and total nitrogen according to Hach Method 10242 and ammonia according to Hach Method 10205. All analytical methods are outlined in Appendix F.4. Water quality measurements were taken with a Horeba U52 water quality sonde and include conductivity, pH, temperature, dissolved oxygen, turbidity and ORP. Because the Horiba U52 sonde was rented, we were unable to utilize it for short notice storm sampling. The Horiba U52 was calibrated by Pine Environmental prior to each sampling event (Appendix F.5) and was checked for accuracy by Zara personnel using standard solutions. All flow measurements were taken with a Flowtracker ADV using the Mid Section Discharge Equation, the same equation used by the USGS, and was operated according to the Flowtracker Technical Manual (SonTek/YSI 2007).

4.4 Results

Table 4.1 provides a summary of all sampling events. Due to prolonged regional drought and the sampling criteria, only two storm events were sampled on 10 July 2012 and 8 January 2013. Samples were not collected from the Turkey Run bridge site during the 8 January 2013 storm event as no bat colony occupied that bridge during the study.

During the 10 July 2012 storm, samples were collected only from the in-stream sampling stations. An increase in *E. coli* was observed at the downstream sampling stations when compared to the upstream sampling stations, and sonde measurements taken from storm drains discharging under the bridges indicated a high level of dissolved constituents in the water, suggesting that the roadway runoff could be the source of elevated *E. coli* levels measured at the downstream stations. To test this hypothesis, additional road runoff samples were collected during the 8 January 2013 storm event from inside each pipe before it could interact with guano deposits (LR-R1, LR-R3, SC-R1 and SC-R3) and at points just before the runoff entered the streams after it had interacted with guano deposits (LR-R2, LR-R4, SC-R2 and SC-R4). Drawings relating the location of the bat-occupied bridge gaps to runoff patterns beneath the Lampasas and Salado bridges are shown in Figure 4.6 and Figure 6.7. Sampling locations are shown in Figure 4.2 and Figure 4.3.

Table 4.1. Summary of all water quality sampling events.

Date	Event Type	*E. coli* Collected	Nutrients Collected
10/3/11-10/4/11	Diurnal	Yes	Yes
12/7/11	Bi-monthly	Yes	Yes
3/7/12	Bi-monthly	Yes	Yes
4/12/12	Bi-monthly	Yes	Yes
6/14/12	Bi-monthly	Yes	Yes
6/25/12-6/26/12	Diurnal	Yes	Yes
7/10/12	Storm	Yes	Yes
8/27/12	Bi-monthly	Yes	No
10/23/12	Bi-monthly	Yes	No
1/8/13	Storm	Yes	No

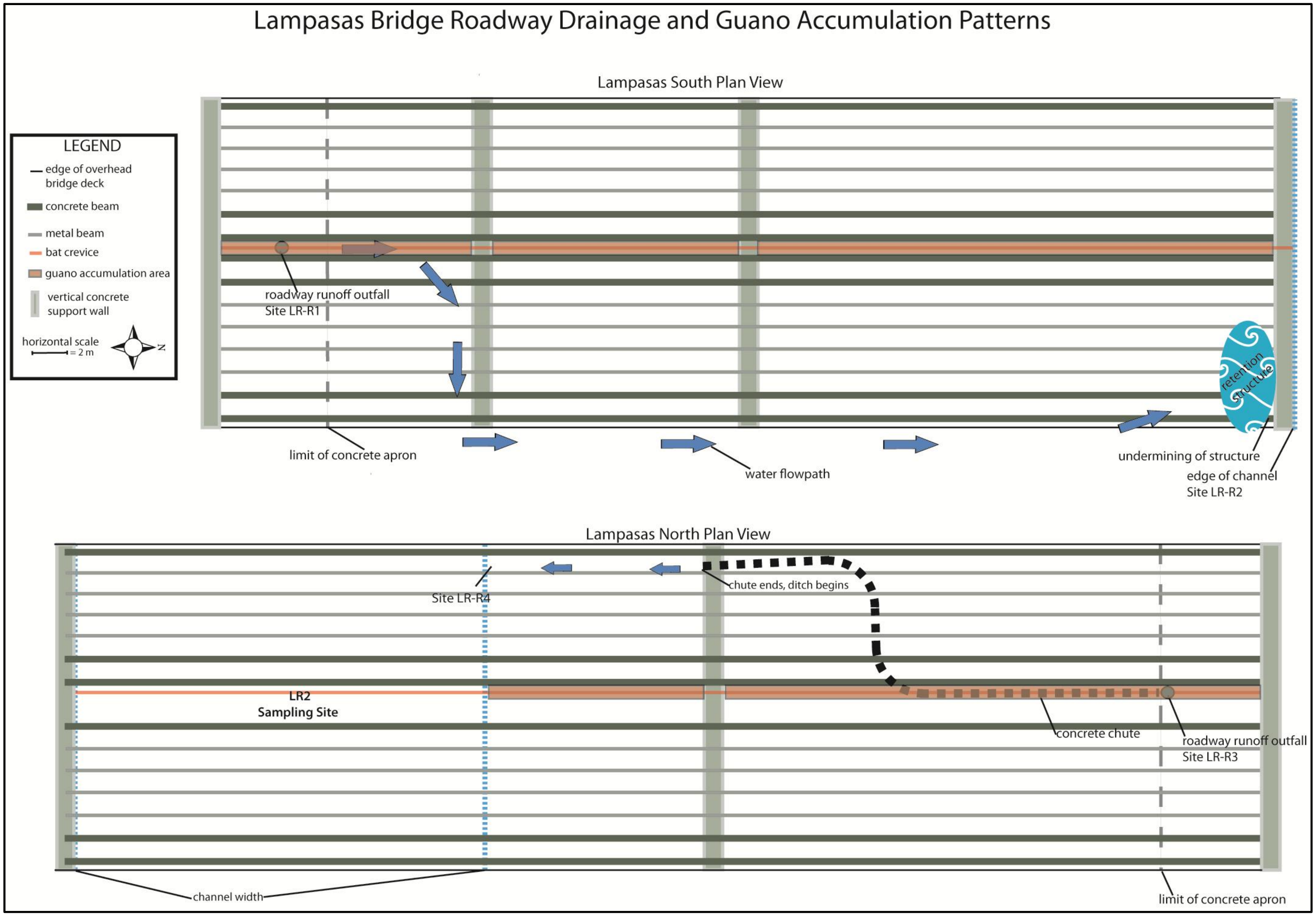

Figure 4.6. Drainage and bat roosting patterns underneath the Lampasas bridge, Bell County, Texas.

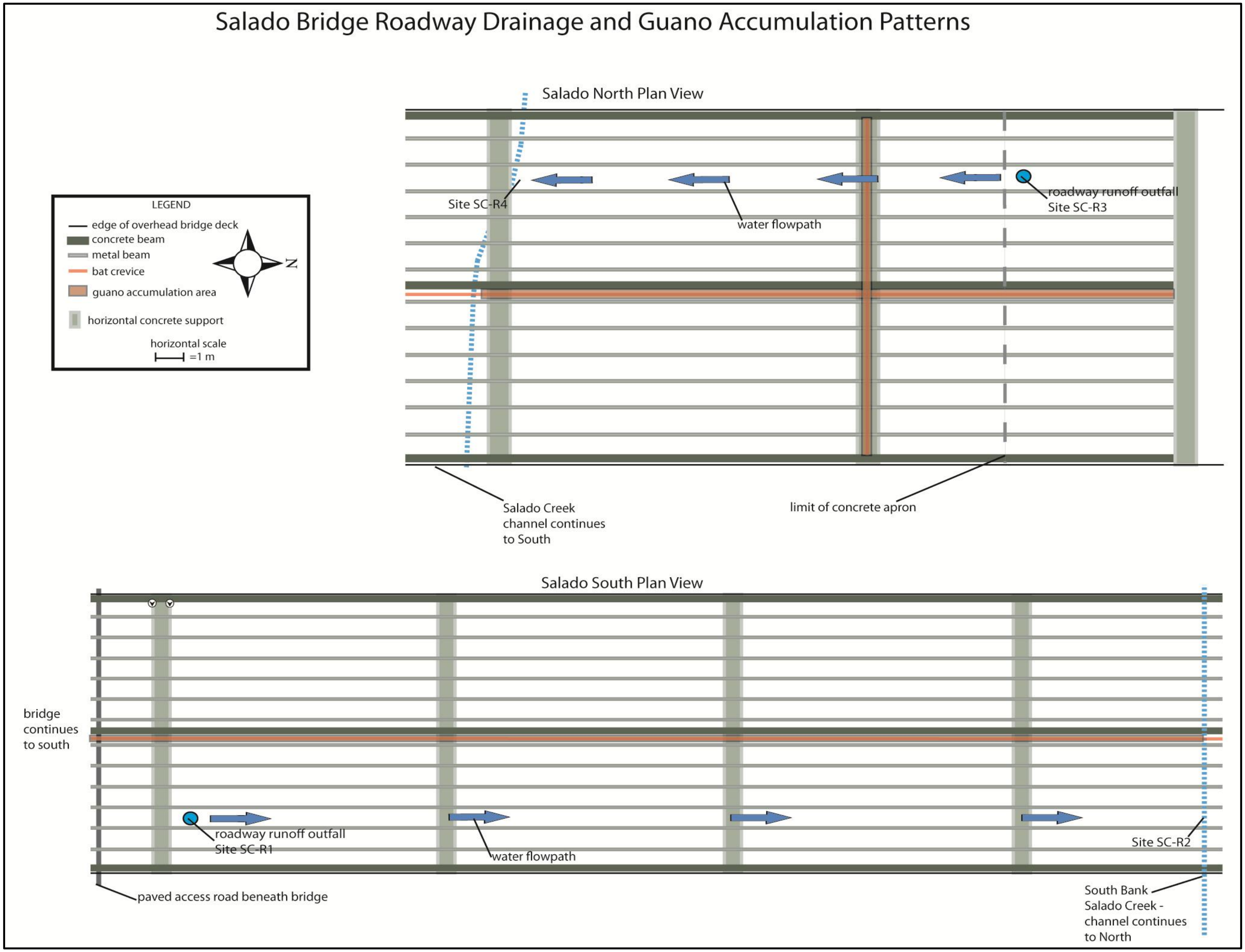

Figure 4.7. Drainage and bat roosting patterns under the Salado bridge, Bell County, Texas.

4.4.1 Bi Monthly Grab Samples

The maximum, minimum, and mean concentration of all measured constituents in grab samples from the Lampasas bridge are given in Table 4.2. The mean concentration of *E. coli* in grab samples taken from the site is 294 colonies/100 ml. These *E. coli* concentrations are not well correlated with the discharge at Lampasas River (Appendix F.6). All analytical results and measurements taken during bi-monthly sampling at the Lampasas bridge are presented in Appendix F.6.

The maximum, minimum and mean concentration of all measured constituents in grab samples from the Salado bridge is given in (Table 4.2). The highest total nitrogen, nitrate, TKN and *E. coli* bacteria concentrations in bi-monthly grab samples were measured in waters collected at the Salado bridge (Table 4.2). Out of all monitored sites, *E. coli* concentrations in grab samples were most elevated at Salado bridge with a mean concentration of 571 colonies/100 ml. *E. coli* concentrations in grab samples were well correlated with the discharge at Salado bridge, particularly when the bat colony was present (Figure 4.8, Figure 4.9, and Table 4.2). *E. coli* concentrations at SC-1 were less than those measured at the downstream sites and the correlation between flow and *E. coli* at SC-1 was weaker than at the downstream sites (Figure 4.8 and Figure 4.9). All analytical results and measurements take during bi-monthly sampling at Salado bridge are presented in Appendix F.6.

The highest concentrations of phosphorus and ammonia in grab samples were measured in water collected at the Turkey Run bridge. The maximum, minimum and mean concentration of all measured constituents in grab samples from the Turkey Run bridge is given in Table 4.2. The mean concentration of *E. coli* in grab samples taken from the site is 172 colonies/100 ml. These E. coli concentrations were not well correlated with discharge at the Turkey Run bridge (Appendix F.6). All analytical results and measurements take during bi-monthly sampling at the Turkey Run bridge are presented in Appendix F.6.

Table 4.2. Summary of bi-monthly water quality grab sample results at Lampasas Salado and Turkey Run bridges, Bell and Coryell counties, Texas, from December 2011 to October 2012 (n= 6 events). All data is given in Appendix F.6.

Site	Parameter	MEAN	MAX	MIN
Lampasas bridge	E.coli	141	740	40
	Total Nitrogen	0.80	1.50	0.02
	Nitrate/Nitrite	0.99	1.78	0.24
	TKN	0.21	1.06	BDL
	Phosphorus	0.01	0.03	0.001
	Ammonia	0.01	0.02	0.003
Salado bridge	E.coli	255	2,420	13
	Total Nitrogen	2.29	3.09	0.99
	Nitrate/Nitrite	2.23	3.02	0.02
	TKN	0.25	1.19	BDL
	Phosphorus	0.01	0.03	BDL
	Ammonia	0.01	0.02	0.004
Turkey Run bridge	E.coli	127	520	29
	Total Nitrogen	2.33	2.96	0.60
	Nitrate/Nitrite	1.76	1.83	1.70
	TKN	0.80	1.13	0.46
	Phosphorus	3.81	6.17	1.38
	Ammonia	0.08	0.15	0.02

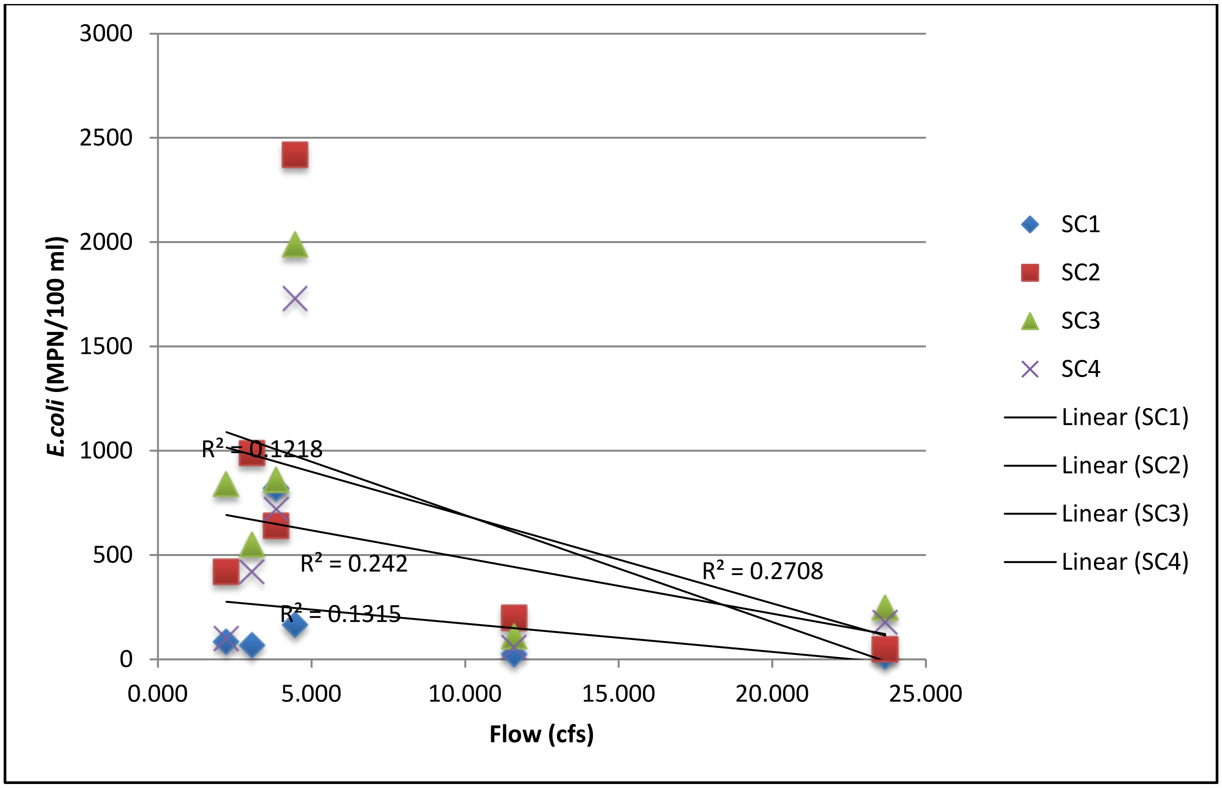

Figure 4.8. Correlation between discharge and *E. coli* concentration measured in all grab samples at the Salado bridge, Bell County, Texas (n = 6 events).

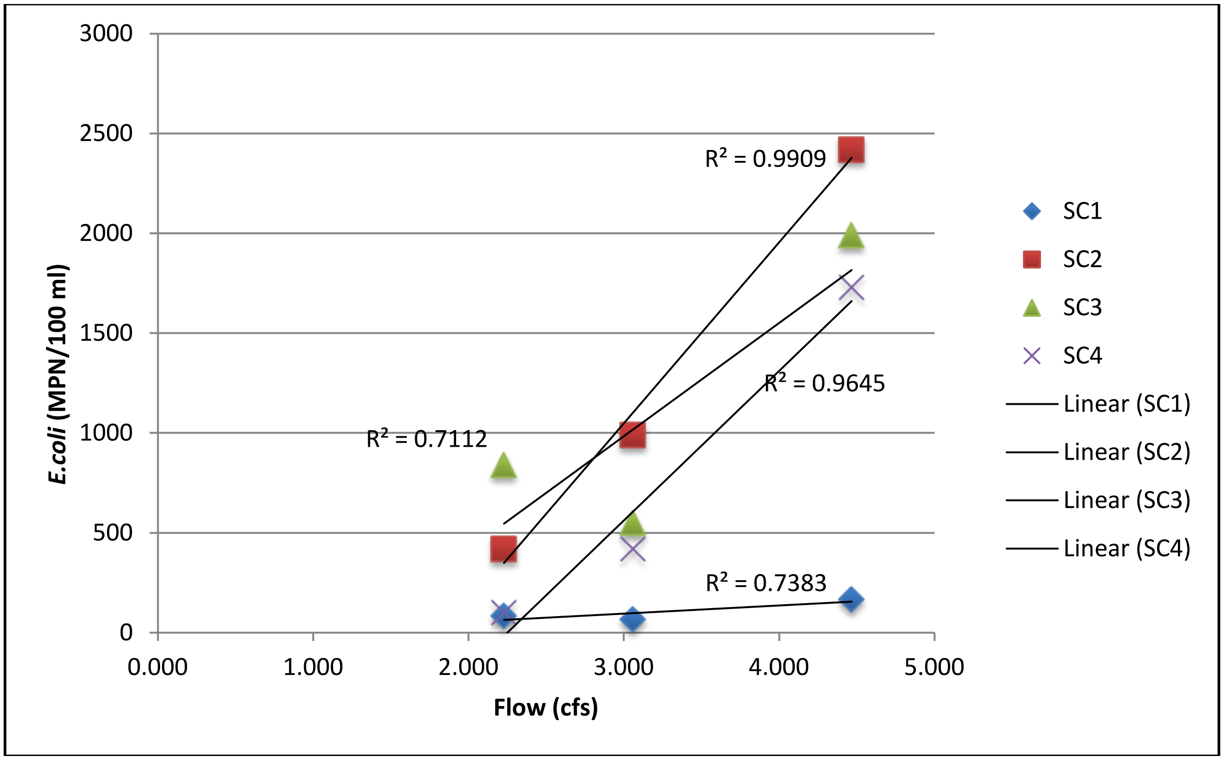

Figure 4.9. Correlation between discharge and *E. coli* concentration measured in grab samples at the Salado bridge, Bell County, Texas, when the bat colony was present (n = 3 events).

4.4.2 Diurnal Sampling Events

The maximum, minimum, and mean concentration of all measured constituents in diurnal samples from Lampasas River are given in Table 4.3 and Table 4.4. No distinct diurnal pattern was observed in nutrient concentrations at the Lampasas River (Appendix F.6). The mean concentration of *E. coli* at the site during diurnal sampling was 187 colonies/100 ml. There was no clear pattern suggesting bat influence during diurnal sampling at the Lampasas bridge (Appendix F.6). All analytical results and measurements take during diurnal sampling at the Lampasas bridge are presented in Appendix F.6 and Appendix F.7.

The maximum, minimum, and mean concentration of all measured constituents in diurnal samples from the Salado bridge is given Table 4.3 and Table 4.4. No distinct diurnal pattern was observed in nutrient concentrations at the Salado bridge (Appendix F.6). During diurnal sampling, *E. coli* concentrations were most elevated at the Salado bridge, with a mean concentration of 1,285 colonies/100 ml. Although there is no clear pattern suggesting bat influence during diurnal sampling, it does appear that *E. coli* concentrations may have been elevated during bat emergence and return, particularly during the 28-29 June 2012 monitoring event (Appendix F.5). All analytical results and measurements take during diurnal sampling at Salado bridge are presented in Appendix F.6 and Appendix F.7.

The maximum, minimum, and mean concentration of all measured constituents in diurnal samples from the Turkey Run bridge is given in Table 4.3 and Table 4.4. No distinct diurnal pattern was observed in nutrient concentrations at the Turkey Run bridge (Appendix F.5). The mean concentration of *E. coli* at the site is 159 colonies/100 ml. There was no clear pattern suggesting bat influence at the Turkey Run bridge (Appendix F.6). All analytical results and field measurements taken during diurnal sampling at Turkey Run bridge are presented in Appendix F.6 and Appendix F.7.

Table 4.3. Summary of results from the 3-4 October 2011 diurnal water quality sampling event at the Lampasas Salado and Turkey Run bridges, Bell and Coryell counties, Texas. BDL = below detection limit. All analytical results are provided in Appendix F.6.

Site	Parameter	MEAN	MAX	MIN
Lampasas bridge	*E. coli*	205	820	56
	Total Nitrogen	0.79	1.08	0.60
	Nitrate/Nitrite	0.42	0.54	0.04
	TKN	0.31	0.55	0.04
	Phosphorus	0.02	0.05	BDL
	Ammonia	0.04	0.05	0.03
Salado bridge	*E. coli*	594	2,420	70
	Total Nitrogen	3.57	4.72	2.60
	Nitrate/Nitrite	3.47	4.16	3.14
	TKN	0.12	0.55	BDL
	Phosphorus	0.02	0.05	0.01
	Ammonia	0.05	0.10	0.02
Turkey Run bridge	*E. coli*	195	460	120
	Total Nitrogen	2.73	3.33	2.13
	Nitrate/Nitrite	1.88	2.30	BDL
	TKN	0.85	2.93	0.34
	Phosphorus	5.33	5.74	4.48
	Ammonia	0.09	0.16	0.05

Table 4.4. Summary of results from the 25-26 June 2012 diurnal water quality sampling event at the Lampasas Salado and Turkey Run bridges, Bell and Coryell counties, Texas. BDL = below detection limit. All analytical results are provided in Appendix F.6.

Site	Parameter	MEAN	MAX	MIN
Lampasas bridge	*E.coli*	146	2,000	38
	Total Nitrogen	0.78	1.03	0.60
	Nitrate/Nitrite	0.26	0.82	BDL
	TKN	0.63	1.04	0.17
	Phosphorus	0.04	0.05	0.03
	Ammonia	0.02	0.03	0.01
Salado bridge	*E.coli*	764	4,600	170
	Total Nitrogen	3.58	4.13	3.09
	Nitrate/Nitrite	3.11	3.48	BDL
	TKN	0.47	3.34	BDL
	Phosphorus	0.03	0.05	BDL
	Ammonia	0.01	0.02	BDL
Turkey Run bridge	*E.coli*	114	820	50
	Total Nitrogen	1.11	4.82	BDL
	Nitrate/Nitrite	0.49	0.91	BDL
	TKN	0.69	3.91	BDL
	Phosphorus	4.44	4.98	3.68
	Ammonia	0.06	0.11	0.01

4.4.3 Storm Events

During the first storm event, samples were only taken from the in-stream sampling stations. During the second storm event, samples were measured in the in-stream sampling stations and in water discharging from roadway outfall pipes before it exited the pipe (and interacted with guano deposits), and just before it flowed into the streams (after it interacted with guano deposits) on the north and south side of the Salado bridge and Lampasas bridge (Figure 4.6 and Figure 6.7). No storm samples were taken from the Turkey Run bridge during the 8 January 2013 storm event.

During the 11 May 2012 storm event, nutrient concentrations at the Lampasas bridge were generally elevated at the downstream sampling stations (Appendix F.6). No nutrient samples were collected at the Lampasas bridge during the 8 January 2013 storm event. *E. coli* concentrations were measured at in-stream sampling stations during both storm events, and were generally elevated at the downstream sites (Appendix F.6). During the 8 January 2013 storm event, the concentration of *E. coli* in samples taken from station LR-R1 was 6,800 colonies /100 ml, and in the sample taken from LR-R2 the concentration was 5,500 colonies /100 ml. During the same storm event, the concentration of *E. coli* in samples taken from station LR-R3 was 1,500 colonies /100 ml, and in the sample taken at SC-R4 the concentration was 12,000 colonies /100 ml. A summary of the storm sampling results are given in Table 4.5 and Table 4.6 and graphs of all data are shown in Appendix F.6.

During the 11 May 2012 storm event, nutrient concentrations at the Salado bridge were generally elevated at the downstream sampling stations. No nutrient samples were collected at the Salado bridge during the 8 2013 storm event. *E. coli* concentrations were measured at in-stream sampling stations during both storm events, and were generally elevated at the downstream sites (Appendix F.6). During the 8 January 2013 storm event, the concentration of *E.coli* in samples taken from station SC-R1 was 8,200 colonies /100 ml and in the sample taken from SC-R2 was 10,000 colonies /100 ml. During the same storm event, the concentration of *E.coli* in samples taken from station SC-R3 was 46,000 colonies /100 ml and in the sample taken at SC-R4 was 483,920 colonies /100 ml. A summary of the storm sampling results is given in Table 4.5 and Table 4.6, and graphs of all data are shown in Appendix F.6.

During the 11 May 2012 storm event, nutrient concentrations at the Turkey Run bridge were not greatly elevated at downstream sites like at the Salado bridge and Lampasas bridge sampling stations. *E.coli* concentrations measured at in-stream sampling stations during the 11 May 2012 storm event were slightly elevated at the downstream sites compared to TR1, but not elevated above the concentrations measured during seasonal and diurnal sampling. Samples were not collected from the Turkey Run bridge during the 8 January 2013 storm event. A summary of the storm sampling results is given in Table 4.5 and Table 4.6 and graphs of all data are shown in Appendix F.6.

Table 4.5. Summary of results from 11 May 2012 storm sampling event at the Lampasas Salado and Turkey Run bridges, Bell and Coryell counties, Texas. All analytical results are provided in Appendix F.6.

Site	Parameter	MEAN	MAX	MIN
Lampasas bridge	*E. coli*	14,900	40,000	1,500
	Total Nitrogen	0.86	1.19	0.66
	Nitrate/Nitrite	0.34	0.62	0.16
	TKN	0.78	0.91	0.57
	Phosphorus	0.04	0.10	0.01
	Ammonia	0.05	0.08	0.03
Salado bridge	*E. coli*	4,742	12,098	170
	Total Nitrogen	3.41	3.57	3.09
	Nitrate/Nitrite	2.99	3.18	2.67
	TKN	0.43	0.47	0.39
	Phosphorus	0.02	0.08	BDL
	Ammonia	0.07	0.15	0.02
Turkey Run bridge	*E. coli*	137	180	110
	Total Nitrogen	1.80	1.95	1.69
	Nitrate/Nitrite	0.29	0.30	0.28
	TKN	1.50	1.67	1.39
	Phosphorus	5.12	5.16	5.08
	Ammonia	0.04	0.04	0.03

Table 4.6. Summary of results from the 8 January 2013 storm sampling event at the Lampasas Salado and Turkey Run bridges, Bell and Coryell counties, Texas. All analytical results are provided in Appendix F.6.

Site	Station ID	*E. coli*
Lampasas bridge	LR-R1	8,200
	LR-R2	10,000
	LR-R3	46,000
	LR-R4	> 483,920
	LR-1	1,100
	LR-2	290
	LR-3	1,700
Salado bridge	SC-R1	6,800
	SC-R2	5,500
	SC-R3	1,500
	SC-R4	12,000
	SC-1	320.00
	SC-2	750
	SC-3	700
	SC-4	490

4.5 Discussion

The hypotheses tested were:

1. Water quality will vary diurnally based on the diurnal movements of the bat colonies.
2. The presence of bat colonies will cause spatial variability in water quality with the greatest impacts occurring downstream of the roosting area.
3. During storm events, water quality will be degraded downstream of the roosting areas due to the flushing of guano into streams.

Water quality does appear to be affected by the presence of the bat colonies, however these effects are variable both temporally and spatially. The diurnal patterns of the bats seem to have very little influence on water quality, if at all. Our initial hypothesis that guano related constituents (such as *E. coli*, phosphorus and nitrogen species) would be elevated during occupation and decrease during nightly foraging was incorrect. Our hypothesis that the bat colonies would cause spatial variations in water quality was confirmed. At both occupied bridges, guano related constituents were generally elevated downstream of the bridges, particularly at the Salado bridge. Finally, our hypothesis that water quality would be degraded downstream of the bat bridges during storm events was generally confirmed; however, without DNA fingerprinting or determining which specific *E. coli* strain may be causing water quality degradation, it is impossible to directly relate these data to bats roosting at any of the sites. Determining the exact biological source of the *E. coli* was not within the scope of this study and it is likely that there are other contributing sources, such as nesting birds and other anthropogenic sources. Below is a discussion of the water quality patterns observed at each site and a short discussion of the potential causes of the observed patterns.

4.5.1 Lampasas Bridge Site

The nutrient and *E.coli* concentrations were typically most elevated during higher flow events like the storm events that were sampled on 11 May 2012 and 8 January 2013 and the grab sample taken on 14 June 2012. The lack of good correlation between *E. coli* concentration and discharge at the Lampasas bridge, even when bats are present (Appendix F.6), may be due to the highly altered flow regime at the site due to managed discharges from Stillhouse Hollow Dam, located approximately 4.42 km upstream. The highest concentration of *E. coli* at the Lampasas bridge (40,000 colonies /100 ml) was measured at station LR-3 during the 11 May 2012 storm event. At this site, roadway runoff is directed via pipes to flow immediately underneath the bat roosts, and this runoff interacting with guano deposits was responsible for the increase in *E.coli* concentrations downstream of the bridge. On both sides of the creek, samples taken from inside of the roadway outfall pipes at LR-R1 and LR-R3 (before water could interact with guano deposits) had a significantly lower *E. coli* concentration than at LR-R2 and LR-R4, after it had interacted with guano deposits. This suggests that roadway runoff flowing below the roosts is likely the source of elevated in-stream *E. coli* concentrations during storm events.

4.5.2 Salado Bridge Site

Of the three sites studied, the Salado bridge seems to be the most impacted by the bat colonies, as the site consistently has the poorest water quality at and below the bridge Table 4.2 through Table 4.6 and Appendix F.6). Grab samples collected from all of the sampling stations at Salado bridge exceeded the *E. coli* primary contact recreation standard for the geometric mean of samples taken at each station (126 colonies/100 ml), and several samples exceeded the single sample limit (399 colonies/100 ml). The elevated *E.coli* concentrations at SC-1, the upstream site, suggest that there is an upstream *E.coli* source, likely from ranching and livestock operations. Although an upstream source is present, *E.coli* concentrations at SC-2, SC-3 and SC-4 were greatly elevated compared to SC-1, suggesting that the bat colony is degrading water quality at the Salado bridge. Like the bimonthly grab samples, nitrogen, nitrate, TKN and *E.coli* bacteria concentrations in the diurnal samples were generally elevated in waters collected at the Salado bridge, even when the colony had vacated the bridge for the night. The nutrients and E.coli concentrations were typically most elevated during higher flow events like the storm events that were sampled on 11 May 2012 (Table 4.5) and 8 January 2013 (Table 4.6) and the grab sample taken on 14 June 2012. Runoff that has interacted with guano accumulations on the banks is likely responsible for the increase in *E. coli* concentrations in the stream.

It is possible that the dam located between stations SC-3 and SC-4 is increasing water residence time and decreasing water velocity near the bridge. This would allow guano to accumulate in the impoundment underneath the bridge and downstream to the dam instead of being flushed downstream and diluted like at the Lampasas bridge. *E. coli* concentrations were typically least elevated at SC-1, and often increased significantly downstream of the bridge, suggesting that a source of *E. coli* is present at the bridge.

The highest concentration of *E. coli* in Salado Creek (12,098 colonies /100 ml) was measured at station SC-3 during the 11 May 2012 storm event. Roadway runoff interacting with guano deposits was assumed to be responsible for the increase in *E. coli* concentrations downstream of the bridges. During the 8 January 2013 sampling event, a sample taken from inside of the north roadway outfall pipe (SC-R3, before it could interact with guano deposits) had a significantly lower *E. coli* concentration than at points just before the runoff entered the streams (SC-R4, after it had interacted with guano deposits); however the opposite was true with roadway runoff on the south side of the creek (Figure 6.7, Appendix F.6). Although the *E. coli* concentration at SC-R4 was not elevated as expected, the concentration of *E. coli* in all of the roadway runoff samples prior to interacting with guano were elevated well above the in-stream concentrations. This suggests that the roadway runoff prior to interacting with guano contributes to elevated in-stream *E. coli* concentrations, and that the interaction of the runoff with guano deposits increases the already elevated *E.coli* concentration of the roadway runoff. These results also suggest that the runoff on the south side of the creek may not be interacting with guano deposits as extensively as runoff on the north side.

4.5.3 Turkey Run Creek Site

No bat colony inhabited the bridge at the Turkey Run Creek during the study, which allows us to assess normal variations at a site that is not impacted by bat roosting. A wastewater treatment facility is located approximately 1,000 feet upstream from the site but only seems to significantly impact the concentration of ammonia and phosphorus at the site as elevated concentration of both constituents were consistently measured at the site. Texas water quality standards are likely exceeded at the site as grab samples exceeded the *E. coli* primary contact recreation standard for the geometric mean of samples taken at each station (126 colonies /100 ml) and TR-2 and TR-3 exceeded the single sample limit (399 colonies /100 ml) one time each.

There is not a good correlation between *E. coli* concentration and discharge (Appendix F.5), possibly due to the lack of a roosting bat colony at the site. The highest concentration of *E. coli* at the Turkey Run bridge (820 colonies/100 ml) was measured at station TR-3 during the 25 June 2012 diurnal monitoring event. Although this value exceeds the primary contact recreation standard, it is much lower than the peak concentrations measured at the other sites with roosting bat colonies present. Because there is no roosting colony at the Turkey Run bridge, there is no accumulation of guano for runoff to interact with prior to entering the stream.

4.5.4 Minimizing Water Quality Impacts

Bat colonies are known to have a variety of positive impacts on the ecosystem and on agricultural operations, but as urbanization continues in the central Texas region, suitable roosts are likely to be disturbed or destroyed. Ongoing monitoring shows that the IH 35 bridges crossing Salado Creek and Lampasas River provide suitable roosting habitat for large bat colonies. It is therefore important to maintain suitable roosting habitat as improvements are made to IH 35. Currently, the location of suitable bat roosting habitat is directly above storm water outfalls and drainages. This can cause negative impacts to water quality because the guano that is deposited beneath the roosting location is periodically flushed into the streams (Figure 4.10), causing spikes in *E. coli* bacteria and several nutrient concentrations (Table 4.5 and Table 4.6).

Figure 4.10. Storm water outfall under the south side of the main lanes of the Lampasas bridge, Bell County, Texas. Note the newer guano deposits (black) that have accumulated since the last rainfall event and the older, thicker guano deposits (brown) that are not washed away by storm water.

The realignment of IH 35 presents an opportunity to improve water quality at these sites while still providing roosting habitat within the bridges. Excluding bats from all areas where guano would directly interact with stormwater runoff (e.g. over outfalls and drainage channels) may improve water quality by reducing the amount of guano that is deposited directly into runoff; however, the greatest improvements to water quality might be achieved by ensuring that stormwater runoff has little to no contact with bat guano. Suggested options for reducing potential guano impacts on water quality include:

- providing berms around areas where guano will be deposited so that storm water is diverted away and does not wash the guano into water bodies,
- providing vegetative or other non-vegetative filter materials to filter guano from stormwater before it enters a larger waterbody, and
- removing guano deposits on a regular basis (e.g. quarterly or monthly).

These actions may help reduce the number of instances that water quality standards are exceeded, particularly the primary contact recreation standard for *E.coli*.

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