SHIFLET, LINDSEY ANN. M.S. Trophic Patterns of an Insectivorous Bat Community Foraging Over Urban and Pristine Streams as Revealed by Stable Isotope and Fecal Analyses. (2007)
Directed by Dr. Matina C. Kalcounis-Rüppell. 39 pp.

The North Buffalo Creek wastewater treatment plant (WWTP) has a demonstrated impact on basal stream components and the foraging activity of common bat species in the watershed. I examined trophic patterns of common bat species upstream and downstream of the WWTP and in the relatively pristine Uwharrie National Forest. I used stable isotopes and fecal analysis to examine trophic positions and diets of bat species at these sites. Bat species in the Uwharrie National Forest had distinct δ^{15}N and δ^{13}C signals whereas these isotope signals converged among bat species along North Buffalo Creek. Fecal analysis showed that in the Uwharrie National Forest diets of bats differed among the species whereas diets were similar along North Buffalo Creek. Bi-plots of δ^{15}N and δ^{13}C for bats and insects support fecal analyses. In the Piedmont of North Carolina, the unique trophic roles of particular bat species are lost along North Buffalo Creek.

TROPHIC PATTERNS OF AN INSECTIVOROUS BAT COMMUNITY FORAGING

OVER URBAN AND PRISTINE STREAMS AS REVEALED BY STABLE

ISOTOPE AND FECAL ANALYSES

by

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A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2007

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ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Anthropogenic disturbances to streams include urban runoff from impervious surfaces or point source input such as wastewater treatment plants (Nedeau et al., 2003). Anthropogenic disturbance to streams has the potential to decrease macroinvertebrate assemblage diversity while increasing the abundance of pollution tolerant insects (Holt, 2000; Mvungi et al., 2003; Nedeau et al., 2003; Van Metre and Mahler, 2003). Disturbed streams commonly contain reduced numbers of pollution - intolerant Trichoptera, Ephemeroptera, and Plecoptera species and increased numbers of pollution - tolerant Diptera species (Avery, 1970). A decrease in diversity among basal stream food sources has the potential to affect the entire riparian food web including riparian predators, such as frogs, salamanders, spiders, birds, and bats, that feed on emerging aquatic insects (Power and Rainey, 2000; Baxter et al., 2005; Ballinger and Lake, 2006).

Insectivorous bats are nocturnal predators that forage over a variety of terrestrial habitats including agricultural fields, forests, urban areas, and rural areas. In these terrestrial habitats insectivorous bats often forage in close proximity to water (Kalcounis and Brigham, 1995; Walsh and Harris, 1996; Racey, 1998; Racey and Entwistle, 2003; Duchamp et al., 2004). Insectivorous bats use open water for drinking and feeding

(Racey, 1998; Korine and Pinshow, 2004; Kunz and Fenton, 2004) and feed on both emerging aquatic insects and flying terrestrial insects in the riparian zone. Thus, insectivorous bats are a link between aquatic and terrestrial food chains at the riparian zone interface (Ballinger and Lake, 2006). As top nocturnal predators in riparian systems, insectivorous bats may show responses to anthropogenic disturbance to riparian systems because they obtain basal resources from streams and riparian vegetation (Walsh and Harris, 1996; Racey, 1998; Golet et al., 2001; Baxter et al., 2005).

There is limited evidence that bats respond to nutrient enrichment in streams. In the United Kingdom, bats that do not depend heavily on aquatic insects (*Nyctalus* spp. and *Eptesicus serotinus*) showed no activity responses whereas bats that do depend on aquatic insects showed activity responses up- and downstream of sewage input such that, *Pipistrelle pipistrellus* was more active upstream, whereas *Myotis* spp. predominantly foraged downstream (Vaughan et al., 1996). In North Carolina, foraging activity of *Eptesicus fuscus* was more common upstream, whereas foraging activity of *Perimyotis subflavus* was more common downstream of wastewater treatment plants (WWTP) (Kalcounis-Rueppell et al., 2007). Indeed, the increase of Daubenton's myotis (*Myotis daubentonii*) in western Europe has been attributed to the eutrophication of streams (Kokurewicz, 1995). However, Racey et al. (1998) found no difference in bat species' foraging activity over two rivers in Scotland receiving different levels of nitrate input.

North Buffalo Creek is a headwater urban stream of the Cape Fear River Basin in the Piedmont region of North Carolina. The North Buffalo Creek Wastewater Treatment Plant (WWTP) is located east of the city limits and contributes to poor water quality

downstream of the plant. North Buffalo Creek was placed on the North Carolina's Clean Water Act 303 (d) list by the United States Environmental Protection Agency for high levels of fecal coliform bacteria (NC-DENR, 2006). The aquatic invertebrate community downstream of the WWTP appears impacted by WWTP effluent in the stream. Aquatic invertebrates and fish incorporated a wastewater-derived nitrogen signal up to 5.94 km downstream (Ulseth and Hershey, 2005; Northington and Hershey, 2006) and fish showed a trend toward lower species richness and abundance downstream of the WWTP (Northington and Hershey, 2006). Although terrestrial invertebrates did not contain an enriched signal (Northington and Hershey, 2006), there was a difference in the insect community with Coleoptera, Lepidoptera, and Diptera more abundant upstream, and Odonata more abundant downstream of the WWTP (Kalcounis-Rueppell et al., 2007). In addition, there were differences in the foraging activity and community structure of bats upstream and downstream of the WWTP (Kalcounis-Rueppell et al., 2007). Kalcounis-Rueppell et al. (2007) suggested that the differences in foraging activity up- and downstream of the WWTP were due to different diets and trophic patterns among bat species. For example, *P. subflavus* had higher levels of activity downstream of the WWTP and may feed on pollution - tolerant insects downstream of the WWTP, whereas *E. fuscus* had higher levels of activity upstream of the WWTP and may avoid those insects by foraging upstream.

A relatively undisturbed region of Piedmont forest exists in the nearby Uwharrie National Forest. The Uwharrie National Forest is 50,386 acres of primarily coniferous forest approximately 100 kilometers southwest of Greensboro, NC (Becker, 2005). The

Uwharrie River, part of the Yadkin-Pee Dee River Basin, flows through the forest. In contrast to North Buffalo Creek, invertebrates collected from streams in the Uwharrie National Forest were depleted in ^{15}N (Rushforth and Hershey, In Press), which suggests the Uwharrie National Forest is more pristine than North Buffalo Creek.

There are four common bat species in the Piedmont of North Carolina. The big brown bat (*Eptesicus fuscus*) has a diet composed mainly of Coleoptera (Brigham, 1990; Feldhammer et al., 1995; Agosta, 2002; Carter et al., 2003; Carter et al., 2004; Whitaker, 2004). The evening bat (*Nycticeius humeralis*) mainly forages on Coleoptera and Lepidoptera, however, Hemiptera, Homoptera, and Hymenoptera are also known to be prey items (Whitaker and Clem, 1992; Feldhamer et al., 1995). The eastern red bat (*Lasiurus borealis*) forages predominantly on Lepidoptera (Whitaker et al., 1997; Carter et al., 2003; Carter et al., 2004) although they have been found to eat ground dwelling insects and some Coleoptera (Shump and Shump, 1982). The eastern pipistrelle bat (*Perimyotis subflavus*) forages over waterways and along forest edges (Fujita and Kunz, 1984) and has a diet of small soft-bodied insects (Fujita and Kunz, 1984; Swift et al., 1985; Whitaker, 2004), such as Diptera, Trichoptera, Hemiptera, Homoptera, and Hymenoptera (Carter et al., 2003).

The bat - insect link in the food chain presents a relatively straight forward model for understanding how anthropogenic disturbances to stream basal sources affect top riparian consumers. North Buffalo Creek is an ideal stream for this question because WWTP effluent enrichment was shown to be incorporated in basal sources (Ulseth and Hershey, 2005; Northington and Hershey, 2006) and we have a good understanding of

bat community structure and activity along this stream (Kalcounis-Rueppell et al., 2007). In addition, the community structure of bat species and the nocturnal flying insect community structure differed upstream and downstream of the WWTP (Kalcounis-Rueppell et al., 2007).

There are two methods to examine trophic behavior of bats, fecal analysis and natural abundance stable isotope analysis. Fecal analysis involves moistening and dissecting a fecal pellet collected from a bat and identifying insect parts in the pellet using a dissecting microscope (Whitaker, 1988). The percent volume of each insect order found in the pellet is then estimated (Whitaker, 1988). Natural abundance stable isotope analysis uses a small tissue sample (such as hair) from a consumer to identify ^{15}N and ^{13}C signals by gas isotope-ratio mass spectroscopy to identify the consumer's trophic position (DeNiro and Epstein, 1978; 1981; Peterson and Fry, 1987; Wayland and Hobson, 2001). As a consumer ingests a food source it preferentially metabolizes the lighter isotope and the heavier isotope is incorporated into tissues (DeNiro and Epstein, 1978; 1981; Minagawa and Wada, 1984). Isotope shifts, denoted as δ units, are expressed in parts per thousand (‰) or per mil. Isotope abundance is expressed relative to a standard;

$$\delta I^* = [(R_{sample}/R_{standard})-1] \times 1000,$$

where I* represents the isotope of interest and R designates the ratio of heavy to light isotopes. Standards for nitrogen and carbon are atmospheric nitrogen (N_2) and PeeDee Belemnite (PDB) respectively, (DeNiro and Epstein, 1978; 1981).

The purpose of my study was to examine trophic patterns of bat species upstream and downstream of the WWTP along North Buffalo Creek and along the Uwharrie River

and its tributaries in the Uwharrie National Forest. First, stable isotope (^{15}N and ^{13}C) values of bats were used to examine trophic positions and establish whether isotopic differences found in the basal stream sources were transferred to bats. I hypothesized that trophic positions of the four species of bats would differ among the three sites reflecting differences in basal source isotope signals (Ulseth and Hershey, 2005; Northington and Hershey, 2006). I predicted that bats sampled downstream of the WWTP would contain enriched wastewater - derived nitrogen and carbon signals from foraging on emergent aquatic insects that were enriched in isotopes whereas bats in the Uwharrie National Forest would be depleted in natural abundance stable isotopes of nitrogen and carbon. Bats upstream of the WWTP would be more enriched in ^{15}N and ^{13}C than bats caught in the Uwharrie National Forest, but less enriched than bats caught downstream of the WWTP. Second, I estimated diets of bats sampled at the three sites using fecal analysis. I predicted that bats in the Uwharrie National Forest would have more diverse diets compared to bats along North Buffalo Creek because the relatively pristine habitat has the potential to sustain a more diverse insect community. I predicted bat diet would differ upstream and downstream of the WWTP for both *E. fuscus* and *P. subflavus* but not for *N. humeralis* or *L. borealis* based on behavioral results from Kalcounis-Rueppell et al. (2007). Lastly, I compared diets derived from fecal analysis to trophic patterns determined from δ^{15}N and δ^{13}C values of bat species and insect orders.

CHAPTER II

METHODS

Sampling Sites

       There were 3 sampling sites for this study: upstream of the WWTP along North Buffalo Creek, downstream of the WWTP along North Buffalo Creek, and along the Uwharrie River and its tributaries in the Uwharrie National Forest (Figure 1). There were 3 sampling points upstream and 2 sampling points downstream of the WWTP along North Buffalo Creek (Figure 1). Sampling points were chosen to match riparian vegetation types and stream width upstream and downstream of the WWTP. There were 16 sampling points within the Uwharrie National Forest. Sampling points were along streams and road flyways near streams along the Uwharrie River and its tributaries (Figure 1). Sampling points along North Buffalo Creek were sampled during the summers of 2004-2006 and sampling points in the Uwharrie National Forest were sampled during the summers of 2004 and 2006.

Bat Sampling

       To sample bats, mist nets were set up at dusk, checked every ten minutes, and taken down at approximately 12:00 AM. Upon capture standard information [species, sex, age (adult/juvenile) and reproductive condition] was recorded. Hair samples were collected from each bat by carefully clipping hair from the back, between the scapulae.

Bats were placed into cloth bags for approximately 15 minutes to collect fecal samples. Bats were released at the site of capture after fecal sample collection. Hair and fecal samples were stored in sterile, dry, 1.5 ml microcentrifuge tubes. All samples were returned to the laboratory and stored in a -20º C freezer. Capture and handling of bats followed animal care guidelines of the American Society of Mammalogists, the UNCG Institutional Animal Care and Use Committee, and the Wildlife Resources Commission of the State of North Carolina.

Insect Sampling

To catch nocturnal flying insects, passive insect traps were set up at dusk, left throughout the night, and removed at dawn. Insect traps were set approximately 100 m away from mist nets where bats were captured. Insect traps were set at all sites along Buffalo Creek and at 4 of 16 sites in the Uwharrie National Forest (Figure 1). Sampling points where insects were trapped are indicated on Figure 1. Aquatic insects were sampled as they emerged from the water into an emergence trap (Bioquip®). Flying terrestrial insects were sampled using a Malaise trap (Bioquip®) set over the riparian vegetation approximately 1 meter from the water's edge. Captured insects were frozen at -20º C to facilitate identification to order using published keys for insects of the area (Borror et al., 1989). Coleoptera and Diptera were separated into aquatic or terrestrial forms because aquatic invertebrates had different isotopic signals than terrestrial invertebrates (Northington and Hershey, 2006). Identified insects were placed in

microcentrifuge tubes, separated by order and sampling point, and stored in a -20º C freezer.

Stable Isotope Processing

Insects and hair samples were processed for ^{15}N and ^{13}C by gas isotope-ratio mass spectroscopy. To prepare bat hair samples, hair was cleaned with non-deodorized soap to remove lipids. Cleaned hair and whole insects were dried in an oven at 60º C for 48 hours. Bat hair samples were pooled in the following way in order to meet stable isotope analysis mass requirements: individuals of a single species, caught at a given sampling point on a particular night. Insect samples were pooled to meet mass requirements by pooling insects of a single order or aquatic and terrestrial forms within a single order, caught at a given sampling point from a single night. Insects and hair samples were ground to a fine powder with a mortar and pestle. Powdered insect and hair samples were weighed (0.8 - 1.2 mg), placed into 4x6 mm pressed tin capsules, and sent to the Colorado Plateau Isotope Laboratory (CPSIL) of Northern Arizona University in Flagstaff, AZ, or the University of California at Davis Stable Isotope Lab at the Department of Plant Sciences in Davis, CA.

Bat Diet

Fecal analysis was used to determine the diet for all captured bats that produced a fecal pellet. One fecal pellet from each individual was placed in a Petri dish and soaked in 95% ethanol, teased apart with a dissecting probe and forceps, and examined with a dissecting microscope. Insect parts were identified to taxonomic order using a library of

insect parts collected from the region. The percent volume of each insect order was estimated as in Whitaker (1988; 2004) and diets are reported as the mean percent each insect order contributed to the diet of particular bat species.

The δ^{15}N and δ^{13}C values of bat and insects were used to estimate the possible diet items for each bat species to compare to fecal analysis. There are no published δ^{15}N and δ^{13}C fractionation values for insectivorous bat hair, therefore, I estimated fractionation values from other mammal and bird diet studies that used natural abundance stable isotopes (Table 1). Based on values from previous studies I used a δ^{15}N fractionation of 2 - 3‰ and δ^{13}C fractionation of 3 - 4‰ (Table 1). Possible diet items were chosen based on the insect orders and bat species mean and standard error values of δ^{15}N and δ^{13}C using diet fractionation values of δ^{15}N of 2 - 3‰ and δ^{13}C of 3 - 4‰.

Statistical Analysis

Trophic Positions Based on Stable Isotopes

Stable isotope values (δ^{15}N and δ^{13}C) were tested for normality using Shapiro-Wilks tests. Because some of the Uwharrie National Forest bats were captured over roads as opposed to streams, I examined differences in δ^{15}N and δ^{13}C for each species between stream and road captures using *t*-tests. There were no differences in δ^{15}N and δ^{13}C values between bats caught over roads versus streams (data not shown) therefore road and stream samples from the Uwharrie National Forest were pooled. Independent variables were bat species, site, and bat species * site interaction term. Dependent variables were δ^{15}N and δ^{13}C values. Normally distributed data were analyzed using two-

way analysis of variance (ANOVA) tests with bat species and site as main effects and site * bat species interaction term. Significantly different main effects were further examined with LSD Post Hoc tests. Statistical significance was accepted at $p < 0.05$ and all statistical tests were computed using SPSS version 15.1. Unless otherwise noted, means are presented ±1 standard error (SE).

Bat Diet from Fecal Analysis

Diets determined from fecal analyses were analyzed for differences among bat species using a multivariate analysis of variance (MANOVA). Because different insect orders were present at different sites, sites were analyzed separately. The independent variable was bat species and the dependent variables were the proportions of insect orders present in the diet. Significantly different main effects were further analyzed with Tukey HSD Post Hoc tests. Diet from fecal analysis was also analyzed for diversity using a one-way analysis of variance test (ANOVA). Independent variables were bat species, site, and the site * bat species interaction term. The dependent variable was diversity. Diversity ranged from zero to one, where zero represents maximum diversity and one represents no diversity. Diversity was calculated using the Simpson's index as Krebs (1989).

CHAPTER III

RESULTS

There were 93 bats from 4 species collected over the three years of this study: 22 *Eptesicus fuscus,* 44 *Lasiurus borealis,* 13 *Nycticeius humeralis,* and 14 *Perimyotis subflavus* (Table 2). Bats were caught upstream and downstream of the WWTP during the summers of 2004-2006. Bats were caught over roads and along streams in the Uwharrie National Forest during the summers of 2004 and 2006. *Perimyotis subflavus* was only caught in the Uwharrie National Forest (Table 2).

There were a total of 282 insects collected for the study. Captured insects belonged to Coleoptera, Diptera, Ephemeroptera, Hemiptera, Homoptera, Hymenoptera, Lepidoptera, Orthoptera, and Plecoptera (Table 3). Arachnidae are known prey items for bats and were also included with the insect samples. Not all insect orders were found at each site (Table 3). There were no aquatic Coleoptera collected downstream of the WWTP. Plecoptera and Ephemeroptera were found only in the Uwharrie National Forest. There were no Hemiptera collected in the Uwharrie National Forest (Table 3).

Trophic Positions from Stable Isotopes

Patterns of naturally occurring stable isotopes of $\delta^{15}N$ and $\delta^{13}C$ values for each bat species can be seen on Figure 2 and in Table 4. In the Uwharrie National Forest, *P. subflavus* had the highest $\delta^{15}N$ and the lowest $\delta^{13}C$ values (Figure 2a). *Lasiurus borealis*

was depleted in ^{15}N compared with *P. subflavus,* but had the same δ^{13}C as *P. subflavus* (Figure 2a). *Nycticeius humeralis* was depleted in ^{15}N relative to *L. borealis* and *E. fuscus* was depleted in ^{15}N relative to *N. humeralis* (Figure 2a). Upstream of the WWTP, the δ^{15}N values of *E. fuscus*, *N. humeralis*, and *L. borealis* were similar at approximately 7‰ (Figure 2b). Upstream of the WWTP, the δ^{13}C values of *E. fuscus*, and *N. humeralis* were similar, but *L. borealis* was depleted in ^{13}C compared with the other species (Figure 2b). Downstream of the WWTP, the δ^{15}N values of *E. fuscus*, *N. humeralis*, and *L. borealis* were similar at approximately 6‰ (Figure 2c). Downstream of the WWTP, *L. borealis* was depleted in ^{13}C compared with the other species (Figure 2c). The δ^{13}C values of *E. fuscus* and *N. humeralis* were similar (Figure 2c).

There was a significant site effect ($F_{2,9} = 4.36$, $p = 0.020$) and bat species effect ($F_{3,9} = 12.22$, $p < 0.001$) for δ^{15}N (Figure 3a). *P. subflavus* was enriched in ^{15}N relative to the other bat species (LSD post hoc test $p < 0.001$; Figure 3a). There was a significant site effect ($F_{9,2} = 5.48$, $p = 0.010$) and bat species effect ($F_{9,3} = 10.49$, $p < 0.001$) for δ^{13}C (Figure 3b). Bats in the Uwharrie National Forest were depleted in ^{13}C compared with bats from both North Buffalo Creek sites (LSD post hoc test $p < 0.001$; Figure 3b). The δ^{13}C values of *L. borealis* and *P. subflavus* did not differ (LSD post hoc test $p = 0.85$), and the δ^{13}C values of *E. fuscus* and *N. humeralis* did not differ (LSD post hoc test $p = 0.610$), however pair of species differed from each other (LSD post hoc test $p < 0.001$; Figure 3b).

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Bat Diet

Mean (±1SE) percent volume of each insect order in the diets of bats from fecal analyses is shown in Table 5. Diets were significantly different among bat species in the Uwharrie National Forest ($F_{3,48}$ = 2.07, p = 0.010; Figure 4a). The diets of *E. fuscus* and *N. humeralis* did not differ (Tukey post hoc test p = 0.919) and the diets of *L. borealis* and *P. subflavus* did not differ (Tukey post hoc test p = 0.919) but the percent volume of Coleoptera differed between the pair of species (Tukey post hoc test p < 0.019; Figure 4a). The diets of *E. fuscus* and *N. humeralis* consisted mainly of Coleoptera, followed by Lepidoptera and Diptera. The diets of *L. borealis* and *P. subflavus* contained primarily Lepidoptera followed by Coleoptera and Diptera (Table 5, Figure 4a). The diversity of insect orders in the diets of bat species in the Uwharrie National Forest was not significantly different ($F_{3,54}$ = 1.456, p = 0.137; Table 6).

Based on δ^{15}N and δ^{13}C bi-plots for bat species and insect orders there were potential diet items of *E. fuscus* and *N. humeralis* that differed from *L. borealis* and *P. subflavus* (Figure 2a). Using fractionation values of δ^{15}N of 2 - 3‰ and δ^{13}C of 3 - 4‰, the δ^{15}N and δ^{13}C values of terrestrial Coleoptera, Homoptera, Hymenoptera, and terrestrial Diptera were a fractionation shift away from *E. fuscus* and *N. humeralis* (Figure 2a). The enriched δ^{15}N signal from *P. subflavus* indicates the diet was composed of predominantly aquatic insect orders, such as Plecoptera, Ephemeroptera, and aquatic Diptera (Figure 2a). There was a trend for the diet of *L. borealis* to consist of both aquatic and terrestrial insects (Figure 2a).

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Despite relatively large proportions of Lepidoptera in the diet of *L. borealis,* there was no significant difference in diet among bat species upstream of the WWTP. Coleoptera was the largest percent of the diets for *E. fuscus* and *N. humeralis* (Figure 4b). The diversity of insect orders in the diet among bat species upstream of the WWTP was not significantly different ($F_{2,18}$ = 1.590, p = 0.231; Table 6). Based on the δ^{15}N and δ^{13}C bi-plot for bat species and insect orders, Lepidoptera, Coleoptera, aquatic Diptera and terrestrial Diptera should be diet items for all bat species (Figure 2b).

Despite relatively large proportions of Lepidoptera in the diet of *L. borealis,* there was no significant difference in diet among bat species downstream of the WWTP. Diets consisted of Coleoptera, Diptera, Hemiptera, Homoptera and Lepidoptera (Table 5, Figure 4c). The diversity of insect orders in the diet among bat species downstream of the WWTP was significantly different ($F_{2,18}$ = 12.604, p = 0.005), with *L. borealis* having low diet diversity relative to other species (Tukey post hoc test p = 0.007; Table 6). Based on the δ^{15}N and δ^{13}C bi-plot for bat species and insect orders, all insect orders except Homoptera should be diet item items for all bat species (Figure 2c).

CHAPTER IV

DISCUSSION

I found that the signals of natural abundance stable isotopes of carbon and nitrogen differed among the common bat species and among urban and pristine sampling sites in the Piedmont region of North Carolina. There were distinct δ^{15}N signals among bat species in the Uwharrie National Forest suggesting that each bat species occupies a unique foraging niche in this relatively pristine area. The δ^{13}C signals were similar between *E. fuscus* and *N. humeralis* and similar between *L. borealis* and *P. subflavus*, however the two pairs of species differed from one another. The δ^{13}C difference between the two pairs of species suggests that vegetation for basal food sources differs between the pairs. Along North Buffalo Creek, δ^{15}N values among the common bat species were similar suggesting that the bat species occupy similar foraging niches. Along North Buffalo Creek, the δ^{13}C values of *E. fuscus* and *N. humeralis* were similar but *L. borealis* was depleted in ^{13}C. The depleted ^{13}C signal of *L. borealis* suggests this bat species has different vegetation for basal food sources than *E. fuscus* and *N. humeralis*.

Overall, fecal analysis supports the isotopic results. Fecal analysis showed that in the Uwharrie National Forest diets differed among the common bat species. Specifically, diets of *E. fuscus* and *N. humeralis* were similar, and the diets of *L. borealis* and *P. subflavus* were similar, but the two pairs of species differed from one another. Along

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North Buffalo Creek, the diets among common bat species did not differ, however, there was a trend for the diet of *L. borealis* to be comprised mainly of Lepidoptera. This trend was not significant likely due to small sample sizes of *L. borealis*.

In general, examination of bi-plots of $\delta^{15}N$ and $\delta^{13}C$ for insects and bat species using fractionation values of $\delta^{15}N$ of 2 - 3‰ and $\delta^{13}C$ of 3 - 4‰ confirm that insect items found through fecal analysis could be potential diet items for the bat species. For example, Lepidoptera and Coleoptera are potential diet items for bat species along North Buffalo Creek and both insect orders were found in the fecal pellets of bat species. Bi-plots of $\delta^{15}N$ and $\delta^{13}C$ also confirm the fecal analysis diets along North Buffalo Creek, because potential diet items were similar among all bat species. However, congruence between isotope and diet results assumes that my fractionation estimates from previous studies (see Table 1) reflect $\delta^{15}N$ and $\delta^{13}C$ fractionation values for insectivorous bats in the study area. Ideally, I should have used known $\delta^{15}N$ and $\delta^{13}C$ fractionation values for insectivorous bats for determining potential diet items, but the fractionations are not known. Future studies should determine $\delta^{15}N$ and $\delta^{13}C$ fractionation values for insectivorous bats from hair. This would require hand feeding bats a representative insect diet and processing bat hair and insects for $\delta^{15}N$ and $\delta^{13}C$ signals. If fractionation values are different from the $\delta^{15}N$ of 2 - 3‰ and $\delta^{13}C$ of 3 - 4‰ that I used, there are implications for my results. Smaller fractionation values would eliminate many insect orders from the diet. For example, terrestrial insect orders would not be potential diet items of *L. borealis* in the Uwharrie National Forest. Along North Buffalo Creek, Lepidoptera and Homoptera would no longer be potential diet items.

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I found that in the relatively pristine Uwharrie National Forest, the common bat species had different foraging niches whereas along the urban North Buffalo Creek, foraging niches converged. Ecological niche differentiation in relatively pristine sites relative to anthropogenically disturbed sites has been documented for several species. For example, in Australia, basal resources differed among fragmented urban areas, which in turn affected the distribution of the predatory arthropod assemblage (Gibb and Hochuli, 2002). Moreover, the predatory arthropods with specific dietary niches were more severely impacted by changes in the basal resources than predatory arthropods with generalist dietary niches (Gibb and Hochuli, 2002). In Finland, carabid beetle distribution was correlated with habitat disturbance where specialist beetles with specific diet requirements were found in suburban and rural areas whereas generalist beetles were found in urban areas (Alaruikka et al., 2002). The abundance and diversity of both bird populations in California and salamander populations in North Carolina decreased as the amount of habitat degradation increased (Rottenborn, 1999; Wilson and Dorcas, 2003). Thus, there is evidence that anthropogenic disturbance that has primary impacts on basal food sources can impact higher trophic level organisms, as I found in my study.

Past studies have shown that ^{15}N signals of stream components were enriched downstream of the WWTP along North Buffalo Creek (Ulseth and Hershey, 2005; Northington and Hershey, 2006). Therefore, I expected that tissue from bat species collected downstream of the WWTP would be enriched in ^{15}N. However, I found that bats collected downstream of the WWTP were not enriched in ^{15}N. This result suggests that WWTP effluent enrichment was not transferred from the basal stream food sources

to the top terrestrial predators. The lack of ^{15}N enrichment downstream of the WWTP

may be explained through various mechanisms. First, bats along North Buffalo Creek

may not feed on emerging insects. However, this scenario is unlikely as bats have been

acoustically recorded and seen feeding directly over the water. Second, bats may feed on

emerging insects downstream of the WWTP, but because of their high vagility, bats can

forage at sites throughout the watershed, including sites upstream of the WWTP.

Foraging home range sizes of bats are not well understood, however, limited evidence

suggest localized ranges on the order of 10 km (i.e., Menzel et al., 2001; Henry et al.,

2002; Owen et al., 2003). My sampling points were approximately 7 km downstream of

the WWTP, therefore bats captured downstream may have foraged both up- and

downstream of the WWTP on a given foraging bout. Third, bats may occasionally feed

on emerging insects downstream of the WWTP, but they may predominantly feed on the

terrestrial insects, which may be homogenous along the riparian zone of the North

Buffalo Creek watershed. An order - level analysis of the flying nocturnal insect

community structure in the North Buffalo Creek watershed supported a homogenous

terrestrial insect community in this watershed. The same types of insects are found

upstream and downstream of the WWTP despite relatively higher abundances of

Coleoptera, Lepidoptera, and Diptera upstream of the WWTP (Kalcounis-Rueppell et al.,

2007).

Kalcounis-Rueppell et al. (2007) found that the foraging activity of *L. borealis*

and *N. humeralis* were similar upstream and downstream of the WWTP. Therefore, I

expected the diets of *L. borealis* and *N. humeralis* to be similar up- and downstream of

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the WWTP. As expected, my results from the fecal analysis showed the diets of *L. borealis* and *N. humeralis* were similar upstream and downstream of the WWTP. The diet of *L. borealis* contained the same insect orders upstream and downstream of the WWTP along North Buffalo Creek with a trend toward large proportions of Lepidoptera. The diet of *N. humeralis* contained similar insect orders upstream and downstream of the WWTP with Coleoptera and Diptera as main diet components. These two bat species appear to forage on the same insect orders up- and downstream of the WWTP along North Buffalo Creek, supporting previous behavioral observations (Kalcounis- Rueppell et al., 2007).

Kalcounis-Rueppell et al. (2007) found that the foraging activity of *E. fuscus* differed upstream and downstream of the WWTP with higher levels of activity upstream of the WWTP. Therefore, I expected the diets of *E. fuscus* to be different up- and downstream of the WWTP. However, I found the diets of *E. fuscus* did not differ up- and downstream of the WWTP. Coleoptera and Diptera were main diet items for *E. fuscus* along North Buffalo Creek. The similar diets up- and downstream of the WWTP, despite higher levels of foraging activity upstream of the WWTP (Kalcounis- Rueppell et al., 2007), may be explained by several means. First, aquatic Coleoptera and aquatic Diptera can be pollution-intolerant or pollution-tolerant depending on the particular species and *E. fuscus* may be selectively feeding on particular species of Coleoptera and Diptera downstream of the WWTP. However, with my current analysis, I could not test this hypothesis because I did not identify Coleoptera and Diptera to lower taxonomic levels that would facilitate differentiation into pollution-tolerant versus intolerant forms. Thus,

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while foraging activity of *E. fuscus* was greater upstream of the WWTP, it may forage on the same insect orders downstream of the WWTP. Second, bats are capable of flying far distances to forage (Menzel et al., 2001; Henry et al., 2002; Owen et al., 2003) and *E. fuscus* could potentially forage on insects up- and downstream of the WWTP in a single night. Thus, *E. fuscus* sampled downstream of the WWTP may have foraged on insects upstream of the WWTP. Lastly, the similar diets of *E. fuscus* up- and downstream of the WWTP may reflect the homogeneity of the nocturnal terrestrial insect community along the riparian corridor of North Buffalo Creek.

Despite the clear result of convergence in trophic niches along North Buffalo Creek relative to the Uwharrie National Forest, the common bat species sampled along North Buffalo Creek were represented by small sample sizes. More importantly, I was not able to sample *P. subflavus* along North Buffalo Creek. *Perimyotis subflavus* is a major insect predator in the riparian zone and *P. subflavus* have been recorded foraging along North Buffalo Creek (Kalcounis-Rueppell et al., 2007). Interestingly, the foraging activity of *P. subflavus* was concentrated downstream of the WWTP (Kalcounis-Rueppell et al., 2007). Moreover, in the Uwharrie National Forest, *P. subflavus* had a δ^{15}N that was distinct, and higher than all other bat species sampled. Thus, it would be valuable to have diet and trophic position estimates of *P. subflavus* along North Buffalo Creek because this species would be the least likely to show a convergence in trophic position and the most likely to show a change in diet in the urban site. A future priority should be to carefully examine the foraging ecology of *P. subflavus* along North Buffalo Creek.

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Despite drawbacks of not sampling *P. subflavus* along North Buffalo Creek, I have made a major contribution to the study of the impact of anthropogenic disturbance to streams on common top terrestrial predators at the interface of the riparian-terrestrial zone. Although I did not capture *P. subflavus* along North Buffalo Creek, previous work demonstrates that they are present in the watershed (Kalcounis-Rueppell et al., 2007) and my results show that they are present in the Uwharrie National Forest. Thus, all *P. subflavus*, *E. fuscus*, *L. borealis*, and *N. humeralis* are present at all sites. My results show that although the same bat community occurs in the Piedmont region of North Carolina, the unique role of particular species within the bat community is lost in areas of anthropogenic disturbance resulting from urbanization. The individual and demographic significance of the convergence in trophic niches among bat species along North Buffalo Creek remains to be studied. It is possible that the loss of unique trophic niches may have negligible effects on populations of the common bat species along North Buffalo Creek if food resources are adequate for survival and reproduction.

CHAPTER V

CONCLUSION

There are four common bat species in the Piedmont region of North Carolina. All four species are found in the relatively pristine Uwharrie National Forest and along North Buffalo Creek, an urban stream where WWTP effluent enrichment was incorporated in basal food sources (Ulseth and Hershey, 2005; Northington and Hershey, 2006). Bats are major predators of insects associated with streams. Therefore, I was interested in examining diet and trophic position of bat species in both areas using stable isotopes and fecal analysis. I found that the bat species occupied unique foraging niches in the relatively pristine Uwharrie National Forest whereas foraging niches converged at sites along North Buffalo Creek. A lack of specialization in habitats with anthropogenic disturbance is evident in other predators such as spiders and salamanders (Welsh and Olliver, 1998; Gibb and Hochuli, 2002; Wilson and Dorcas, 2003). This result among bat species is even more striking because, as opposed to spiders and salamanders, bats are highly vagile and this mobility should have the potential to mitigate local habitat effects. Thus, in the Piedmont region of North Carolina, although the same four species of bat occur in the North Buffalo Creek watershed and the Uwharrie National forest, the unique trophic role of particular species within the bat community is lost in areas with anthropogenic disturbance that has resulted from urbanization.

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APPENDIX A: TABLES

Table 1. Published $\delta^{15}N$ and $\delta^{13}C$ fractionation values for different tissues from various mammals and birds. Values were used to estimate fractionation from hair of insectivorous bat as: $\delta^{15}N$ 2‰ - 3‰ and $\delta^{13}C$ 3‰ - 4‰.

$\delta^{15}N$ Fractionation	$\delta^{13}C$ Fractionation	Tissue	Organisms	Citation
4.4 ± 0.2‰	2 ± 0.2‰	Blood	Bats (Amaranth diet)	Mirón et al., 2006
3.3 ± 0.2‰	0.1 ± 0.1‰		Bats (Soya diet)	
2.7 ± 0.7‰	-	Blood	Mammals and Avians	Robbins et al., 2005
3-3.2‰	-	Blood	Bats (Nectarivores)	Voigt and Matt, 2004
-	2.7 ± 0.45‰	Blood, Wing, Hair	Bats (Nectarivores)	Voigt et al., 2003
3.4‰	2.6‰	Blood, Hair	Pygmy Raccoon	McFadden et al., 2006
3.3 ± 0.4‰	0.2 ± 0.01‰	Blood	Chicken, Quail, Gulls	Hobson and Clark, 1992
3.0‰	2.0‰	Blood, Muscle	Marten	Ben-David et al., 1997
3.2‰	2.7‰	Serum, Blood, Liver, Muscle, Fur	Red Fox	Roth and Hobson, 2000
4.0 - 4.7‰	-	Wing Membrane	Bats (Nectarivores)	Voigt and Matt, 2004
-	1‰	Hair, Wing	Vampire Bats	Voigt and Kelm, 2006
-	2‰	Hair	Gray Wolf	Darimont and Reimchen, 2002
-	3.2‰	Hair, Feces	Alpaca, Cattle, Goat, Horse, Llama, Rabbit	Sponheimer et al., 2003a,b
2.8 - 6.4‰	-			
3.7 - 5.6‰	2.5‰ - 3.8‰	Feather	Avian	Mizutani et al., 1992
2.7 ± 0.5‰	2.1 ± 0.08‰	Feathers	Chicken, Quail, Gulls	Hobson and Clark, 1992
3 - 5‰	0-1‰	Skin	Fur Seal	Kurle and Worthy, 2001
2.4‰	3.3‰	Bone	Grassland Vole	Harding and Stevens, 2001
2.3‰	4.4‰		Marsh Vole	

Table 2. Hair and fecal samples collected from bats at each site. Number represents individual bats from which hair was collected and individual fecal pellets examined. Each row represents a different sampling nights. Discrepancies between number of individual bats and number of individual fecal pellets reflect that not every bat collected produced a fecal pellet.

	Perimyotis subflavus		*Eptesicus fuscus*		*Lasiurus borealis*		*Nycticeius humeralis*	
	Individual Number of Bats	Individual Number of Pellets	Individual Number of Bats	Individual Number of Pellets	Individual Number of Bats	Individual Number of Pellets	Individual Number of Bats	Individual Number of Pellets
Uwharrie National Forest	1	1	3	3	3	3	1	1
	3	3	1	1	1	1	2	2
	2	2	-	-	1	1	1	1
	2	2	-	-	3	3	-	-
	2	2	-	-	2	2	-	-
	1	1	-	-	3	3	-	-
	2	2	-	-	2	2	-	-
	1	1	-	-	2	2	-	-
	-	-	-	-	3	3	-	-
	-	-	-	-	2	2	-	-
	-	-	-	-	7	7	-	-
	-	-	-	-	3	3	-	-
	-	-	-	-	1	0	-	-
	-	-	-	-	1	1	-	-
	-	-	-	-	4	4	-	-
	-	-	-	-	1	1	-	-
Upstream of the WWTP	-	-	3	3	1	1	2	1
	-	-	5	5	1	1	2	1
	-	-	3	3	-	-	1	1
	-	-	1	1	-	-	-	-
	-	-	1	1	-	-	-	-
Downstream of the WWTP	-	-	1	1	2	2	1	1
	-	-	1	1	1	-	1	1
	-	-	1	1	-	-	1	1
	-	-	1	1	-	-	1	1

Table 3. Insect samples collected at each site from different sampling nights. Each row represents a different sample analyzed for stable isotopes. Numbers indicate the number of individual insects pooled in a sample for stable isotope analysis.

	Arachnidae	Aquatic Coleoptera	Terrestrial Coleoptera	Aquatic Diptera	Terrestrial Diptera	Ephemeroptera	Hemiptera	Homoptera	Hymenoptera	Lepidoptera	Orthoptera	Plecoptera
						Number of Individual Insects						
Uwharrie National Forest	3	1	1	47	1	9	-	15	5	2	1	2
	-	-	-	-	2	-	-	25	-	3	1	2
	-	-	-	-	-	-	-	-	-	15	-	-
Upstream of the WWTP	4	1	14	2	1	-	7	2	4	1	2	-
	-	2		4	5	-	-	1	1	1	2	-
Downstream of the WWTP	1	-	1	15	15	-	4	9	15	10	1	-
	1	-	2	2	-	-	3	4	1	2	1	-
	-	-	-	-	-	-	-	-	-	4	-	-

Table 4. Mean ± 1SE δ^{15}N and δ^{13}C values for bat species and insect orders caught at each sampling site. The number represents the number of samples averaged in the mean is "n" (refer to Tables 2 and 3). Aquatic (Aq) and terrestrial (T).

Uwharrie National Forest				Upstream of the WWTP				Downstream of the WWTP			
		δ^{15}N	δ^{13}C			δ^{15}N	δ^{13}C			δ^{15}N	δ^{13}C
Organism	n	Mean ± SE	Mean ± SE	Organism	n	Mean ± SE	Mean ± SE	Organism	n	Mean ± SE	Mean ± SE
E. fuscus	2	4.6 ± 0.21	-21.70 ± 0.86	*E. fuscus*	6	7.2 ± 0.17	-20.65 ± 0.04	*E. fuscus*	4	6.19 ± 0.29	-21.00 ± 0.12
L. borealis	16	6.6 ± 0.21	-23.00 ± 0.14	*L. borealis*	2	7.41 ± 0.15	-23.38 ± 0.44	*L. borealis*	2	6.77 ± 0.45	-22.21 ± 0.08
N. humeralis	3	5.6 ± 0.94	-22.30 ± 0.22	*N. humeralis*	3	7.10 ± 0.37	-20.95 ± 0.52	*N. humeralis*	4	5.99 ± 0.26	-20.4 ± 0.17
P. subflavus	8	9.04 ± 0.80	23.04 ± 0.22	*P. subflavus*	-	-	-	*P. subflavus*	-	-	-
Arachnidae	1	8.84	-26.8	Arachnidae	1	4.39	-27.97	Arachnidae	2	7.56 ± 0.34	-26.87 ± 0.20
Aq. Coleoptera	1	7.51	31.7	Aq. Coleoptera	2	7.81 ± 0.48	-25.53 ± 0.68	Aq. Coleoptera	-	-	-
T. Coleoptera	1	2.09	-27.47	T. Coleoptera	2	5.87 ± 0.21	-25.95 ± 0.21	T. Coleoptera	3	4.30 ± 1.35	-26.62 ± 1.41
Aq. Diptera	1	5.30	-24.37	Aq. Diptera	2	5.58 ± 0.22	-26.33 ± 0.61	Aq. Diptera	2	6.29 ± 5.91	-27.91 ± 2.06
T. Diptera	2	1.48 ± 1.44	-26.45 ± 0.86	T. Diptera	2	4.39 ± 0.39	-25.28 ± 0.33	T. Diptera	1	5.42	-26.54
Ephemeroptera	1	6.58	-26.77	Ephemeroptera	-	-	-	Ephemeroptera	-	-	-
Hemiptera	-	-	-	Hemiptera	1	3.23	-18.79	Hemiptera	2	3.45 ± 2.01	-21.43 ± 7.02
Homoptera	2	1.14 ± 0.77	-25.92 ± 4.98	Homoptera	2	1.79 ± 1.43	-27.76 ± 0.68	Homoptera	2	0.64 ± 0.15	-26.26 ± 0.37
Hymenoptera	1	2.15	-25.54	Hymenoptera	1	5.54 ± 0.47	-26.89 ± 0.66	Hymenoptera	1	6.21 ± 0.71	-24.73 ± 1.79
Lepidoptera	3	0.63 ± 0.02	-32.42 ± 1.83	Lepidoptera	2	5.19 ± 1.04	-28.28 ± 0.55	Lepidoptera	3	3.98 ± 1.21	-27.48 ±0.66
Orthoptera	2	0.82 ± 0.17	-14.26 ± 1.33	Orthoptera	1	1.93	-17.02	Orthoptera	2	4.74 ± 1.02	-25.86 ± 0.59
Plecoptera	2	5.86 ± 3.09	-27.17 ± 066	Plecoptera	-	-	-	Plecoptera	-	-	-

Table 5. Mean ± 1SE percent volume of each insect order in the diets of bats as determined through fecal analysis from (a) the Uwharrie National Forest, (b) upstream of the WWTP and (c) downstream of the WWTP

a.

Uwharrie National Forest

Insect Order	E. fuscus Mean ± SE	L. borealis Mean ± SE	N. humeralis Mean ± SE	P. subflavus Mean ± SE
Coleoptera	65.00 ± 12.08	34.18 ± 3.29	56.25 ± 6.57	30.82 ± 4.19
Diptera	5.75 ± 4.25	19.97 ± 2.30	15.00 ± 6.46	32.36 ± 4.58
Hemiptera	1.75 ± 1.75	4.82 ± 1.46	18.75 ± 6.57	5.45 ± 3.60
Homoptera	10.00 ± 10.00	1.54 ± 0.76	0.00 ± 0.00	2.73 ± 2.73
Lepidoptera	17.5 ± 13.00	38.59 ± 4.00	10.00 ± 5.40	28.18 ± 7.84
Other	0.00 ± 0.00	1.03 ± 0.74	0.00 ± 0.00	0.00 ± 0.00

b.

Upstream of the WWTP

Insect Order	E. fuscus Mean ± SE	L. borealis Mean ± SE	N. humeralis Mean ± SE
Coleoptera	45.50 ± 6.43	15.00 ± 10.00	45.00 ± 9.30
Diptera	15.36 ± 2.65	5.00 ± 5.00	31.25 ± 8.16
Hemiptera	11.43 ± 3.25	0.00 ± 0.00	6.25 ± 8.30
Homoptera	3.21 ± 1.79	0.00 ± 0.00	0.00 ± 0.00
Lepidoptera	24.50 ± 9.00	80.00 ± 15.00	2.50 ± 4.00
Other	0.00 ± 0.00	0.00 ± 0.00	15.00 ± 8.00

c.

Downstream of the WWTP

Insect Order	E. fuscus Mean ± SE	L. borealis Mean ± SE	N. humeralis Mean ± SE
Coleoptera	51.25 ± 3.15	10.00 ± 10.00	41.25 ± 7.18
Diptera	33.75 ± 10.87	0.00 ± 0.00	26.25 ± 3.75
Hemiptera	6.25 ± 3.75	0.00 ± 0.00	3.75 ± 3.75
Homoptera	2.50 ± 2.50	0.00 ± 0.00	0.00 ± 0.00
Lepidoptera	5.00 ± 5.00	90.00 ± 10.00	28.75 ± 11.25
Other	1.25 ± 1.25	0.00 ± 0.00	0.00 ± 0.00

Table 6. Mean ± 1SE diversity values for diets determined through fecal analysis. The number of bats averaged in the mean is represented at "n".

Fecal Analysis

Site	Bat species	n	Diversity Mean ± SE
Uwharrie National Forest		47	0.46 ± 0.02
	E. fuscus	4	0.59 ± 0.14
	L. borealis	39	0.45 ± 0.02
	N. humeralis	4	0.43 ± 0.05
	P. subflavus	11	0.40 ± 0.046
Upstream of the WWTP		21	0.49 ± 0.04
	E. fuscus	14	0.49 ± 0.51
	L. borealis	2	0.71 ± 0.21
	N. humeralis	5	0.40 ± 0.05
Downstream of the WWTP		10	0.49 ± 0.07
	E. fuscus	4	0.43 ± 0.04
	L. borealis	2	0.84 ± 0.16
	N. humeralis	4	0.38 ± 0.03

APPENDIX B: FIGURES

Figure 1. Maps showing sampling sites in: (a) Guilford, Richmond, Montgomery, Randolph, and Stanly counties in North Carolina. Sampling sites were (b) upstream and downstream of WWTP along North Buffalo Creek, and (c) along the Uwharrie River and tributaries. Sampling sites (open circles), WWTP (black square). North Buffalo Creek (b) flows from left to right. Circles with an x are points where insects were collected in the Uwharrie National Forest.
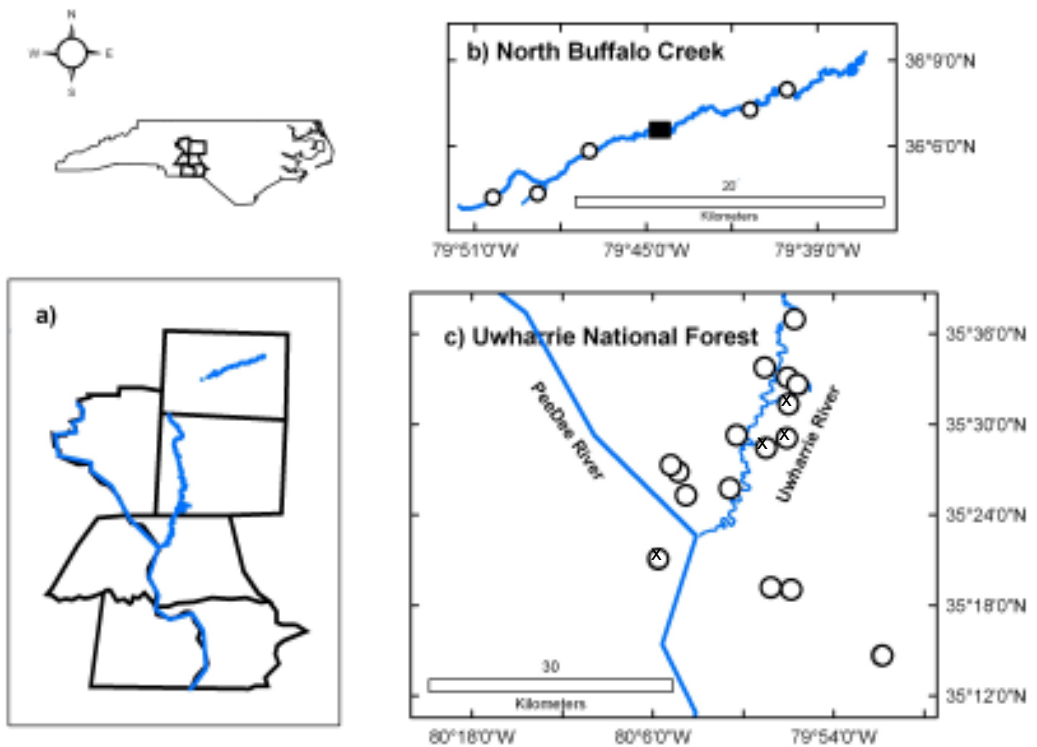
Figure 2. Bi-plots of mean ±1SE δ^{15}N and δ^{13}C values from bats and insects collected from (a) the Uwharrie National Forest, (b) upstream of the WWTP, and (c) downstream of the WWTP. Bats are shown as squares, and insects are shown as diamonds. Coleoptera and Diptera are differentiated as being aquatic (Aq) or terrestrial (T).
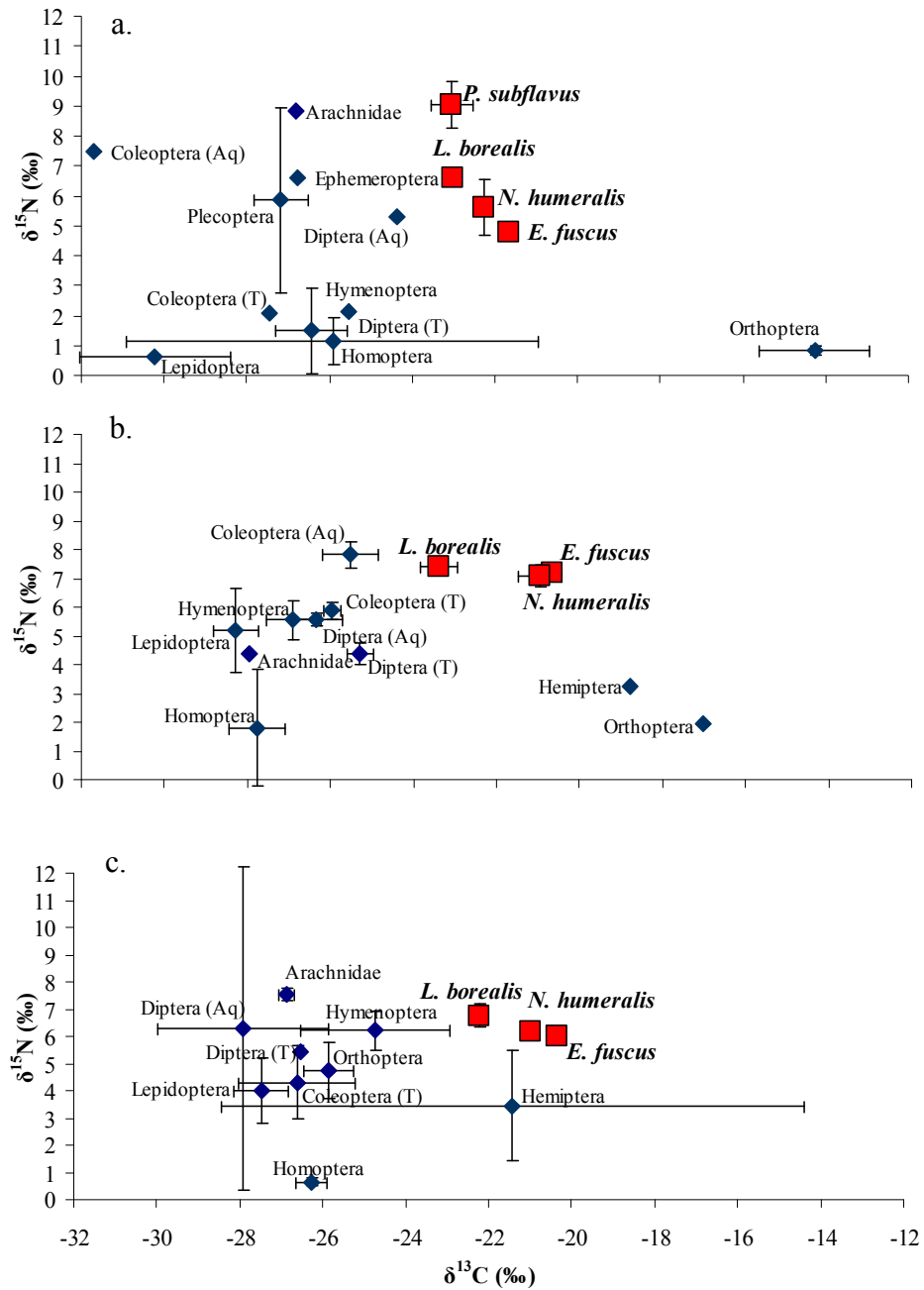
Figure 3. Mean ±1SE values for (a) δ^{15}N and (b) δ^{13}C for bat species in the Uwharrie National Forest, upstream of the WWTP, and downstream of the WWTP. Bats are shown as *E. fuscus* (diamonds), *L. borealis* (squares), *N. humeralis* (triangles), and *P. subflavus* (circles). *P. subflavus* and *L. borealis* have the same δ^{13}C value at the Uwharrie National Forest.
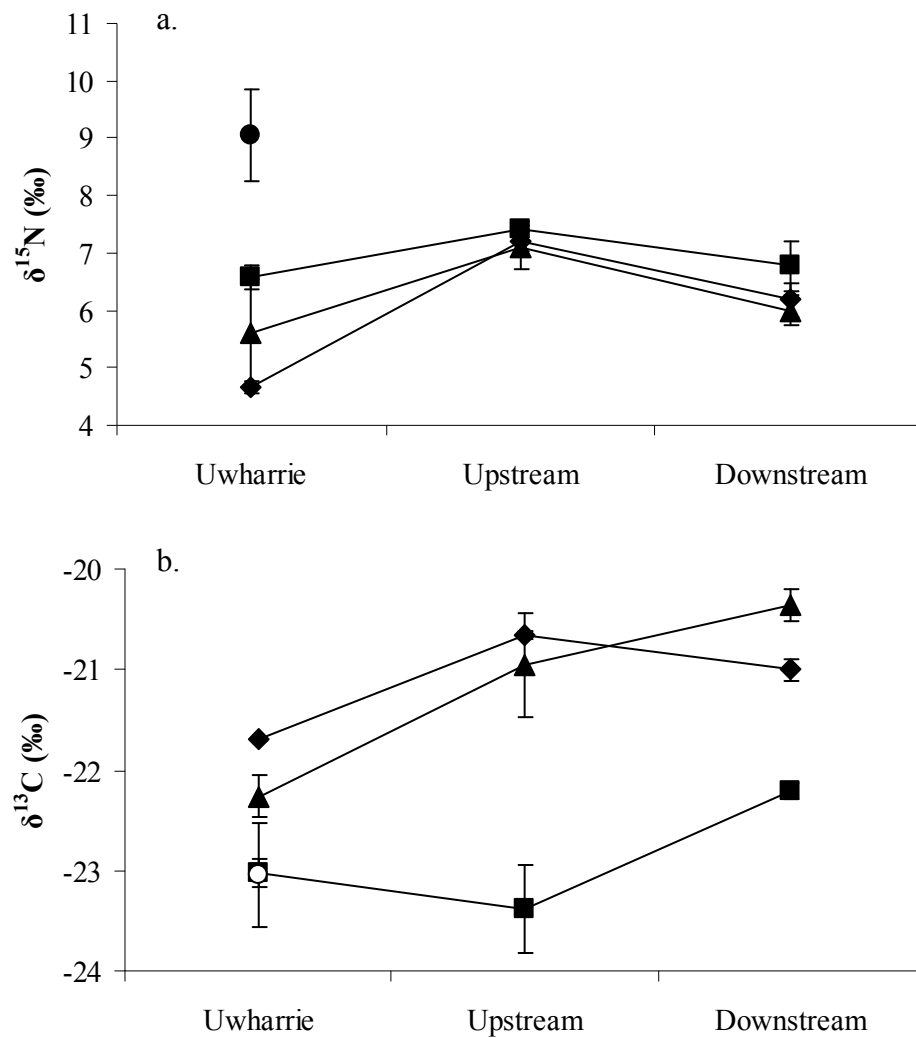
Figure 4. Mean percent volume of each insect order in the diets of bats as determined through fecal analysis for each bat species from (a) the Uwharrie National Forest, (b) upstream of the WWTP, and (c) downstream of the WWTP, years are combined. The "n" below each bar is the number of individuals examined from that species.