

Effects of wastewater treatment plant effluent on bat foraging ecology in an urban stream system

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Abstract:

Wastewater treatment plant (WWTP) effluent in the Cape Fear River Basin headwaters in North Carolina, USA, has influenced stream water quality and aquatic components of the stream food web. To examine effects of WWTP effluent on terrestrial predators in this system we determined prey availability, bat community structure, and bat foraging and commuting behavior at sites above and below WWTPs. We predicted an effect of effluent in the riparian habitat specialist *Perimyotis subflavus* but not the habitat generalists *Eptesicus fuscus*, *Lasiurus borealis*, or *Nycticeius humeralis*. Nocturnal insect abundance was higher upstream of the WWTPs. There were more Diptera, Coleoptera, and Lepidoptera upstream of the WWTPs whereas there were more Odonata downstream of the WWTPs. There were more *E. fuscus* upstream of the WWTPs and more *P. subflavus* downstream of the WWTPs. Despite the difference in bat community structure up- and downstream of the WWTPs, bat commuting and foraging activity levels were the same; there was no difference in the total number of echolocation sequences we recorded per night up- and downstream of the WWTPs nor was there a difference in the proportion of those sequences that contained a feeding buzz. Our results suggest the effect of anthropogenic nutrients in the stream persists through higher food web trophic levels as we found impacts on nocturnal flying insects as well as two common species of insectivorous bats. *Perimyotis subflavus* and *E. fuscus* may serve as easily tractable terrestrial bioindicators of water quality as influenced by WWTP effluent in this, and other, urban watersheds.

Keywords: Riparian areas, Water quality, Insectivorous bats, Feeding, Insects, Echolocation

Article:

1. Introduction

Insectivorous bats are a major terrestrial food web component involved in the reciprocal flow of energy and nutrients in stream and riparian food webs (e.g., Power and Rainey, 2000; Power et al., 2004; Baxter et al., 2005; Ballinger and Lake, 2006). The close association between insectivorous bats and streams is evident worldwide (Rydell et al., 1994; Sanchez et al., 1996; Walsh and Harris, 1996; Carmel and Safriel, 1998; Holloway and Barclay, 2000; Warren et al., 2000; Fellers and Pierson, 2002; Law and Chidel, 2002; Russo and Jones, 2003; Evelyn et al., 2004; Lloyd et al., 2006) and across a broad range of bat species (Racey and Entwistle, 2003). The primary reason is that insectivorous bats are top predators on riparian and/or emergent stream insects (Walsh and Harris, 1996; Pierson, 1998; Racey, 1998; Racey et al., 1998; Racey and Entwistle, 2003; Baxter et al., 2005; Ballinger and Lake, 2006).

Anthropogenic inputs to streams effect the invertebrate community structure of streams (e.g., Lawrence and Gresens, 2004; Chambers et al., 2006; Guecker et al., 2006; Gulis et al., 2006) which, in turn, can affect the nocturnal insect food base for bats, and other predators, in riparian zones (e.g., Bank et al., 2006; Price et al., 2006). There have been few studies that have linked water quality to specific insect prey and habitat where bats are foraging (Agosta, 2002) despite the fact that top predators such as bats, at the interface of aquatic and terrestrial zones, are a good model to understand consequences of water quality on higher trophic levels (Power and Rainey, 2000; Power et al., 2004; Baxter et al., 2005; Ballinger and Lake, 2006).

Previous declines in populations of gray bats (*Myotis grisescens*) have been attributed to organochlorines found in the carcasses, milk, and feces of bats (Clark et al., 1978, 1988) and insects in the streams over which *M. grisescens* feeds (Clark et al., 1983; Clawson and Clark, 1989). However, for other bat species, links between water quality, insect prey, and bat diet are not as direct. For example, it has been shown that organochlorines and metals from contaminated sediment are present in chironomid (Diptera) larvae and adults (Reinhold et al., 1999) and that chironomid insects comprise a major portion of the diets of bats such as *Myotis* species (Belwood and Fenton, 1976; Flavin et al., 2001), hoary bats (*Lasiurus cinereus*: Rolseth et al., 1994), and Leisler's bat (*Nyctalus leisleri*: Shiel et al., 1998). However, an impact of contaminated chironomids on survival or reproduction by bats has not been demonstrated. Additionally, although population decline of pipistrelle bats (*Pipistrellus pipistrellus*) in an industrial area could be attributed to high levels of organochlorine residues in individual bats, the proximate cause of the decline could only be presumed to be poor foraging habitat and polluted water (Gerrell and Gerrell-Lundberg, 1993).

Eutrophic freshwater, and the associated increase in insect productivity, has been implicated as having an effect on bat foraging behavior and species distribution. The increase of Daubenton's myotis (*Myotis daubentonii*) on the mainland of western Europe has been attributed to increases in numbers of eutrophic streams across the mainland (Kokurewicz, 1995). Vaughan and colleagues (1996) looked for an effect of sewage effluent on the activity of vespertilionid bats because the overall decline of bat populations in the United Kingdom coincided with an overall decline in river water quality. Bats that do not depend heavily on aquatic insects (*Nyctalus* spp. and *Eptesicus serotinus*) showed no difference in feeding or commuting passes up- and downstream of the effluent input, whereas bats that are highly dependent on aquatic insects either concentrated most of their foraging activity downstream of the effluent (*Myotis* spp.) or had lower activity downstream from the effluent (*P. pipistrellus*) (Vaughan et al., 1996). These results suggest that *Myotis* spp. respond positively to increased availability of pollution tolerant insects that result from eutrophication and that *P. pipistrellus* are dependent on pollution sensitive insects (Vaughan et al., 1996). In contrast, Racey et al. (1998) found no difference in foraging activity between bats (*P. pipistrellus* and *M. daubentonii*) foraging over an oligotrophic river when compared to a eutrophic river. Thus, there is limited evidence that nutrient enriched water has effects on bats in riparian systems.

The Cape Fear River Basin (CFRB) headwaters originate in Greensboro, North Carolina, USA, where urban land use has had a negative impact on stream water quality. Point source discharges from Wastewater Treatment Plants (WWTPs) contribute to poor water quality found in streams

within the basin. Both North and South Buffalo Creek are CFRB headwaters and are recognized as impaired due to instream habitat degradation and impaired biological communities from point and non-point source effluent, nutrient enrichment, and the presence of fecal coliform bacteria (NCDENR, 2004). North and South Buffalo Creeks each have a WWTP, and reaches downstream of the WWTPs constitute some of the worst water quality problems in North Carolina (NCDENR, 2004). Sections of both creeks have been listed as impaired on the state of North Carolina's Federal Clean Water Act 303(d) list since 2000 (NCDENR, 2006). A study on the influence of anthropogenic nutrients on stream components of North Buffalo Creek found that nearly all aquatic food web components examined (seston, moss, algae, conditioned leaves, caddis- flies and damselflies) downstream from the WWTP had incorporated wastewater effluent-derived nitrogen up to 5.94 km away (Ulseth and Hershey, 2005). In addition, fish in the stream derive their nutrition from basal sources impacted by effluent-derived nitrogen and there was a trend toward lower fish species richness and abundance at impacted sites (Northington and Hershey, 2006).

Four species of bat are common in the Buffalo Creek watershed: big brown (*Eptesicus fuscus*), red (*Lasiurus borealis*), eastern pipistrelle (*Perimyotis subflavus* formerly *Pipistrellus* eastern pipistrelle (*Perimyotis subflavus* formerly *Pipistrellus subflavus* see *subflavus* see Menu, 1984; Hooper and Van den Bussche, 2003; Hooper et al., 2006), and evening (*Nycticeius humeralis*) (2003; Hooper et al., 2006), and evening (*Nycticeius humeralis*) bats. Less common, but present, are silver-haired (*Lasionycteris noctivaga*), hoary (*L. cinereus*), Brazilian free-tailed (*Lasiurus noctivagus*), hoary (*L. cinereus*), Brazilian free-tailed (*Tadarida brasiliensis*), and two *Myotis* bats (Webster et al., 1985; Brown, 1997; Lambiase et al., 2002). As with other bat species (e.g., *P. pipistrellus* and *P. pygmaeus*, Davidson- Watts et al., 2006) common bats in the Buffalo Creek watershed can be classified as habitat specialists or generalists. *E. fuscus* and *N. humeralis* are habitat generalists (Watkins, 1972; Kurta and Baker, 1990; Brigham, 1991) that have relatively strong jaws (Freeman, 1981) and primarily eat coleopterans (Watkins, 1972; Brigham, 1990; Brigham and Saunders, 1990; Whitaker et al., 1991; Whitaker and Clem, 1992; Carter et al., 1998; Menzel et al., 2000; Agosta, 2002; Carter et al., 2003; Whitaker, 2004). Although *L. borealis* is an obligate tree-roosting species (Kunz and Lumsden, 2003), it is a habitat generalist rarely associated with any particular habitat type (e.g., Ford et al., 2005; Menzel et al., 2005) and has a diet consisting primarily of lepidopterans (Whitaker, 1972; Whitaker et al., 1977; Shump and Shump, 1982; Whitaker et al., 1997; Carter et al., 2003). *Perimyotis subflavus* has a broad diet that includes aquatic and terrestrial insects (Whitaker, 1972; Whitaker et al., 1997; Carter et al., 1998; Carter et al., 2003; Whitaker, 2004) and can be considered a riparian habitat specialist as it mainly forages along or over water sources (Fujita and Kunz, 1984; Broders et al., 2003; Ford et al., 2005).

The presence of effluent-derived nitrogen enrichment in aquatic food webs (Ulseth and Hershey, 2005; Northington and Hershey, 2006) and habitat specialist vs. generalist bats species as top predators in this system make this watershed an ideal site to study how WWTP effluent input affects terrestrial wildlife. The objective of this study was to determine prey (insects) availability, bat community structure, and bat foraging and commuting behavior, at sites above and below WWTPs in this watershed. We predicted that an effect of WWTP effluent would be

seen in the riparian habitat specialist *P. subflavus* but not the habitat generalists *E. fuscus*, *L. borealis*, or *N. humeralis*.

2. Methods

The Cape Fear River Basin headwaters are North and South Buffalo Creeks (Fig. 1). Both Creeks receive point-source nitrogen effluent from WWTPs (one on each creek; Fig. 1). From June–August 2004 and 2005, we used a paired design to sample up- and downstream of each WWTP. At each site we measured insect abundance, insect diversity, bat species abundance, and bat foraging and commuting activity. Insect traps and bat detectors were set at sites within 2 h before sunset, sampled through the night, and were collected within 1 h after sunrise. We had 14 sample sites total; four upstream and four downstream on North Buffalo Creek and three upstream and three downstream on South Buffalo Creek (Fig. 1). We matched all sites up- and downstream of the WWTPs with respect to creek width and riparian vegetation to minimize site-specific habitat differences. On a given sampling night, an up- and downstream site on the same creek was selected at random for paired sampling.

2.1. Insect abundance and diversity

To sample insects up- and downstream of the WWTPs we used passive insect traps. We sampled emergent insects from the streams using a single emergence trap (Bioquip®). We sampled terrestrial insects associated with the riparian habitat using a single Malaise trap (Bioquip®). We set the Malaise trap adjacent to the emergence trap, within approximately 5 m of the waters edge. Insect samples were collected in the morning and were stored in 80% ethanol. We identified all insects to order, and a subset to family, using published keys and a reference library of insects in the region (Tietz, 1963; Borror et al., 1989; Arnett, 2000). We further subdivided Diptera into chironomid and non-chironomid forms because chironomids are particularly important as bat prey (e.g., Bel- wood and Fenton, 1976; Flavin et al., 2001). We pooled emerging aquatic and terrestrial insects for all analyses as both were available to foraging bats.

2.2. Bat species presence and abundance

We use the terminology “calls” and “sequences” as in Burnett et al. (2004). At each site, we used a Pettersson D240x (Pettersson Elektronik AB, Uppsala, Sweden) bat detector attached to a Sony ICD-MX20 sound activated digital recorder to record bats flying over the site for species identification (sensu Kalcounis et al., 1999; Menzel et al., 2005). The detector was set to a time-expansion mode to preserve all characteristics of the recorded ultrasound (full spectrum recording). In this time-expansion mode, the detector sampled at 307 kHz with 8 bit resolution and continuously recorded a 1.7s loop of sound coming through the microphone. Upon detecting any sound in the range of 10–120 kHz the playback would be triggered and the previous 1.7s of recorded sound would be played back, time-expanded by a factor of 10, into the digital recorder. The digital recordings were downloaded as audio- files into a computer and resampled using SonoBat® software (DNDesign, Arcata, CA) at 44.1 kHz with 16 bit resolution to

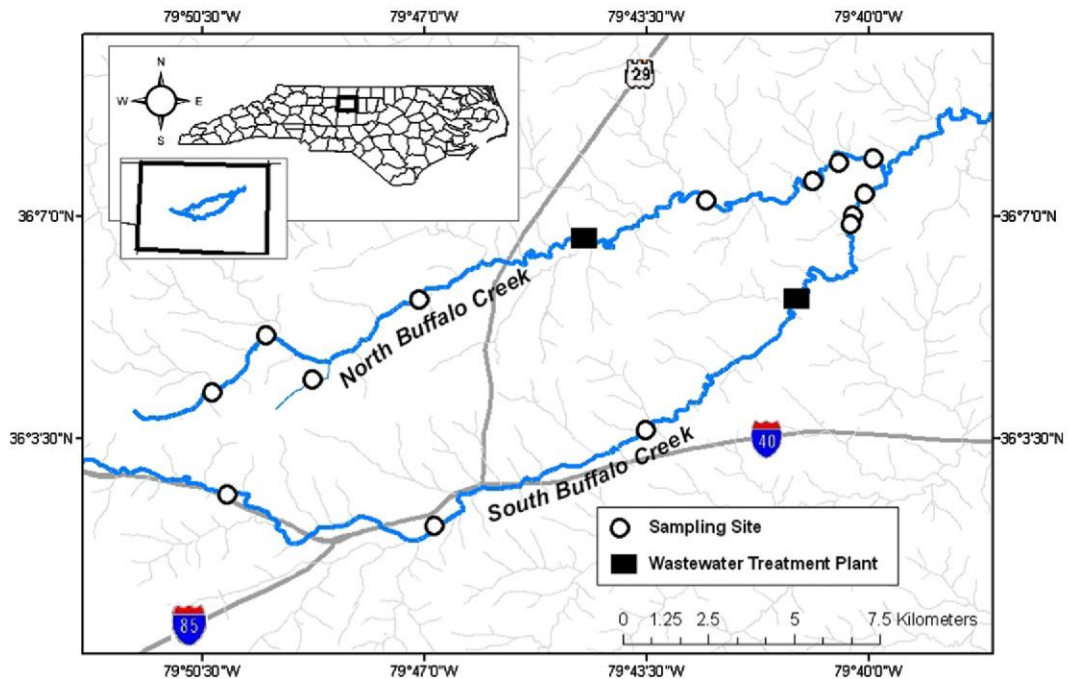


Fig. 1 – Study area showing the location of Buffalo Creek in Guilford County, North Carolina, USA. The location of Buffalo Creek within Guilford County is shown on the inset of Guilford County. The stream flow is eastward. The wastewater treatment plant (squares) and sampling site (open circles) locations along North and South Buffalo Creek are shown. Major highways are also shown.

retain the full signal quality of the original signal. We extracted time, amplitude, and frequency characteristics from sonograms rendered by SonoBat® which used 1024 point fast Fourier transforms, 192 point windows, and varied window overlap so as to always render the sonogram with resolution greater than the screen pixel resolution. Our recording system had a frequency response up to the 12 kHz necessary to capture ultrasound up to 120 kHz (with the time-expansion factor of ten). The maximum frequency resolution of the spectrographic analysis was 154 kHz. Therefore, this detector recorded high quality, full spectrum ultrasound to facilitate species identification but did not sample continuously through the night because of the 17s playback for every recording.

Because time-expanded calls were being used for species identification, we were only interested in sequences that consisted of commuting calls and we discarded all time-expanded sequences that contained a feeding buzz (Burnett et al., 2004). For every time-expanded sequence we randomly selected a single call for analysis in SonoBat®. Call variables measured were: high frequency, low frequency, frequency at maximum amplitude, duration, and slope. Using these variables we matched recorded calls to reference calls from a library developed from bats in the Piedmont and Coastal Plain regions of North Carolina (Kalcounis-Rueppell, unpublished) supplemented with reference calls from the Sonobat® library recorded from the southeastern United States. Many of the reference echolocation calls in our library were recorded, upon hand release, from bats captured in mist-nets near the sampling sites in this study. We matched unknown calls to reference calls manually (i.e., not automated) and a subset of calls was

analyzed independently by two people (first and second author) to validate the procedure. Using this procedure, we were able to distinguish among commuting calls for all species except we were unable to unequivocally distinguish between calls of *E. fuscus* and *L. noctivagans* and we were unable to unequivocally identify two *Myotis* species (due to a lack of *Myotis* reference calls from the study area). Therefore, we recognize an *E. fuscus/L. noctivagans* group (but refer to this as an *E. fuscus* group because *L. noctivagans* is not common in the area) and a *Myotis* group.

2.3. Bat activity

In addition to the Pettersson D240x bat detector recording full spectrum echolocation sequences in time-expanded mode, we used a second Pettersson D240x bat detector attached to a second Sony ICD-MX20 digital recorder to record in real time (heterodyning transformation) mode. The purpose of this second detector was to record all bat activity through the night to facilitate our being able to count the total number of bat sequences recorded during the night. All real time call sequences were counted in SonoBat® and separated into sequences that consisted of only search phase calls (considered commuting behavior) and sequences that contained approach and feeding buzz calls (considered foraging behavior) (sensu Kalcounis and Brigham, 1995). Continuous recorded sequences were counted as separate sequences if there was silence of one second or greater between sequences. To ensure that activity counts were consistent between observers, all sequences were analyzed by at least two people. Although this recording mode produces continuous recordings through the night to facilitate activity and feeding indices, the heterodyned ultrasound precludes species identification.

Therefore, on a sampling night, at a site up- and downstream of the WWTP, there were two Pettersson D240x bat detectors, one emergence trap, and one Malaise trap. One detector served to record time-expanded full spectrum echolocation data for species identification whereas the other detector served to record all bat activity in real time. The detectors (and digital recorders) were fully automated, sound activated, and individually contained, within rain resistant Tupperware® containers that exposed only the detector microphone (sensu Kalcounis-Rueppell et al., 2006). The two Tupperware® containers were stacked on top of one another and were mounted on a wooden platform attached approximately 2 m high to a tree trunk in the riparian vegetation within 5 m of the water and Malaise trap. The microphone on each detector was perpendicular to the stream and angled at 45°.

2.4. Statistical analysis

We tested all data for normality using Shapiro-Wilk W tests. Where assumptions of normality were violated we used non-parametric statistics. We analyzed insect abundance, bat species abundance, and bat activity using Wilcoxon Matched Pairs tests. We analyzed years separately to compare results between years. We performed all analyses using Statistica version 7.1 (StatSoft Inc.). Statistical significance was accepted at $P < 0.05$. Unless otherwise noted results are presented as mean \pm 1 SE.

3. Results

3.1. Insect abundance and diversity

In 2004, we captured 4629 insects over 31 nights. In 2005, we captured 10,231 insects over 26 nights. During both years, our most representative orders were Diptera, Lepidoptera, Coleoptera, Homoptera, Hymenoptera, Hemiptera, Trichoptera, Odonata, and Orthoptera, however, the majority of the sample from both years was represented by Diptera (Fig. 2). In both years, insect capture data was not normally distributed due to zeros in the data matrix (not every insect order was represented in every sample; Shapiro-Wilk W Test-data not shown). Therefore, paired comparisons were made using a Wilcoxon Matched Pairs test. In 2004 there were significantly more insects upstream (90 ± 20 insects/night) of the WWTPs than downstream (59 ± 13 insects/night; $T = 127.0$, $n = 31$, $P = 0.030$; Fig. 3a). In 2005 there were significantly more insects upstream (248 ± 32 insects/night) than downstream (148 ± 22 insects/night) of the WWTPs ($T = 57.5$, $df = 26$, $P = 0.003$; Fig. 3b). There was a difference in the abundance of particular insect orders up- and downstream of the WWTPs (Table 1). In both years, there were significantly more nonchironomid and chironomid Diptera upstream than downstream of the WWTPs (Table 1). Additionally, in 2004, there were significantly more Coleoptera and Lepidoptera upstream than downstream of the WWTPs (Table 1). These differences

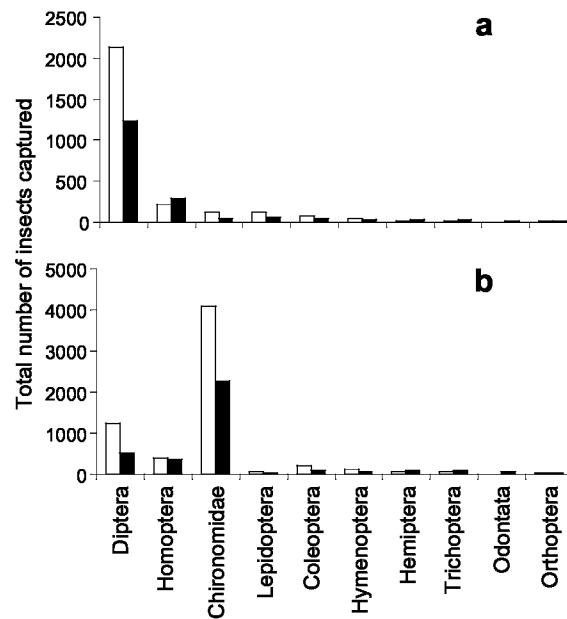


Fig. 2 – Total number of insects captured up- (open bars) and downstream (closed bars) of the WWTPs in 2004 (a) and 2005 (b) along Buffalo Creek in Guilford County, North Carolina, USA. Diptera represent non-chironomid Diptera only. Only the most commonly captured insect orders are shown.

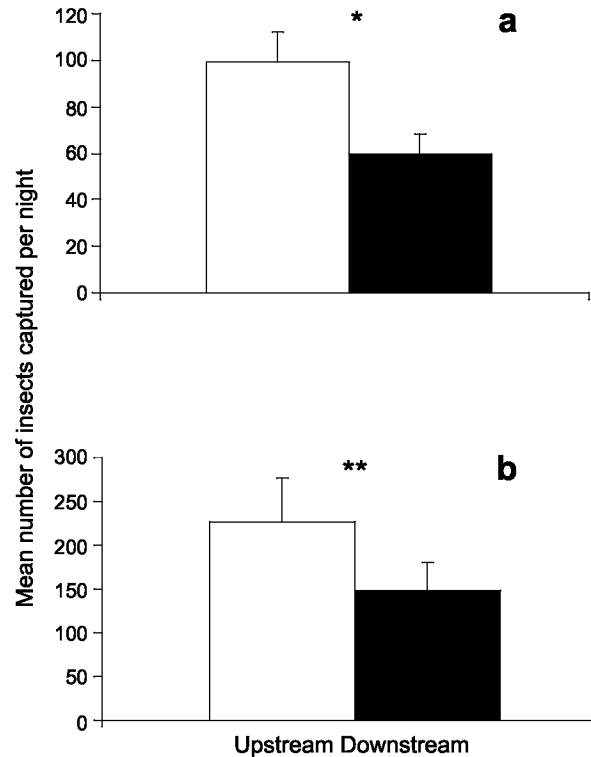


Fig. 3 – Mean (± 1 SE) insects captured per night up- and downstream of the WWTPs in 2004 (a) and 2005 (b) along Buffalo Creek in Guilford County, North Carolina, USA. Significant differences are denoted by asterisk ($P < 0.05$, ** $P < 0.005$).

in 2004 were supported by similar trends in 2005 (Table 1). In 2005 there were significantly more Odonata downstream of the WWTPs (Table 1).

3.2. Bat species presence and abundance

In 2004 we collected 19 nights of time-expanded recordings and analyzed a representative call from 892 echolocation sequences that did not contain feeding buzzes. However, due to equipment failure we only had complete paired data (automated detectors worked through the night at both up- and downstream sites) on 12 nights representing 386 echolocation sequences. For overall presence in the watershed we used the whole data set and for comparisons between sites up- and downstream of the WWTPs we only used the complete paired nights. Equipment failure was not a problem in 2005 and we collected 22 nights of complete time-expanded recordings and analyzed a representative call from 671 echolocation sequences that did not contain feeding buzzes.

In both years we recorded echolocation calls from *E. fuscus*, *N. humeralis*, *P. subflavus*, *L. cinereus*, *L. borealis*, *T. brasiliensis*, *N. humeralis*, *P. subflavus*, *L. cinereus*, *L. borealis*, *T. brasiliensis*, *N. humeralis*, *P. subflavus*, *L. cinereus*, *L. borealis*, *T. brasiliensis*, and 2 Myotis species (Fig. 4). Overall, the most representative species in both years was *E. fuscus*, followed by *P. subflavus*, species in both years was *E. fuscus*, followed by *P. subflavus*, and *N. humeralis* and *N. humeralis* (Fig. 4).

In both years the number of bats present per night at paired sites was not normally distributed (Shapiro-Wilk W Test-data not shown). Therefore, paired comparisons were made using a Wilcoxon Matched Pairs test. In 2004, there were more *E. fuscus* (n = 12, T = 3.50, Z = 2.78, P = 0.005) upstream of the WWTPs and more *P. subflavus* (n = 12, T = 0.00, Z = 2.67, P = 0.008) downstream of the WWTPs (Fig. 5a). There was no difference in *N. humeralis* (n = 12, T = 3.00, Z = 1.21, P = 0.22), *L. borealis* (n = 12, T = 4.00, Z = 0.37, P = 0.72), and *L. cinereus* (n = 12, T = 1.00, Z = 1.07, P = 0.29) up- and downstream of the WWTPs (Fig. 5a). The pattern was similar in 2005. In 2005, there were more *E. fuscus* (n = 22, T = 29.0, Z = 2.48, P = 0.005) upstream of the WWTPs and more *P. subflavus* (n = 22, T = 0.00, Z = 3.18, P = 0.001) downstream of the WWTPs (Fig. 5b). There was no difference in *N. humeralis* (n = 22, T = 21.00, Z = 1.98, P = 0.05) or *L. borealis* (n = 22, T = 9.50, Z = 0.76, P = 0.44) up- and downstream of the WWTPs (Fig. 5b). However, there were more *L. cinereus* (n = 22, T = 3.50, Z = 2.25, P = 0.02) upstream of the WWTPs (Fig. 5b).

3.3. Bat activity

In 2004, we collected 25 nights of real time echolocation data from paired sites up- and downstream of the WWTPs for a total of 7581 call sequences. In 2005, we collected 24 nights of real time echolocation data from paired sites up- and downstream of the WWTPs for a total of 12,589 call sequences. In both years, the number of bats present per night at paired sites was not normally distributed, therefore paired comparisons were made using a Wilcoxon Matched Pairs test. During both years, there was no difference in the mean number of total sequences recorded up- and downstream of the WWTPs (2004: n = 25, T = 148.00, Z = 0.39, P = 0.70; 2005: n = 24, T = 93.5, Z = 1.64, P = 0.12; Fig. 6). During both years, there was no difference in the proportion of the total number of echolocation sequences that contained feeding behavior

Table 1 – Mean ± 1 SE insects captured up- and downstream of the WWTPs

Order/group	2004					2005				
	Upstream	Downstream	T	n	P	Upstream	Downstream	T	n	P
Homoptera	6.81 ± 1.40	9.52 ± 2.66	172.50	31	0.487	14.85 ± 3.61	13.77 ± 1.66	127.00	26	0.511
Hemiptera	0.48 ± 0.19	0.74 ± 0.22	38.50	31	0.379	2.85 ± 1.21	3.58 ± 1.59	64.50	26	0.856
Coleoptera	2.65 ± 0.57	1.45 ± 0.29	60.00	31	0.031	8.08 ± 1.96	4.04 ± 0.79	107.60	26	0.082
Lepidoptera	4.00 ± 0.69	1.74 ± 0.33	44.50	31	0.004	2.73 ± 0.45	1.69 ± 0.31	69.00	26	0.062
Diptera	68.65 ± 17.31	39.81 ± 11.47	112.00	31	0.038	47.88 ± 10.80	20.04 ± 5.00	60.00	26	0.003
Chironomidae	4.03 ± 1.52	1.42 ± 0.59	57.50	31	0.044	157.69 ± 22.75	87.23 ± 17.50	52.00	26	0.003
Orthoptera	0.35 ± 0.16	0.65 ± 0.23	23.00	31	0.209	0.81 ± 0.28	1.73 ± 0.46	38.50	26	0.127
Hymenoptera	1.42 ± 0.33	1.10 ± 0.24	160.50	31	0.703	4.19 ± 1.13	2.69 ± 0.53	112.00	26	0.638
Odonata	0.06 ± 0.04	0.32 ± 0.16	6.00	31	0.176	0.08 ± 0.05	2.35 ± 0.69	0.00	26	0.002
Trichoptera	0.39 ± 0.15	0.97 ± 0.38	17.00	31	0.155	2.88 ± 0.62	3.58 ± 1.61	74.50	26	0.632

Wilcoxon matched pairs test statistics comparing the most representative insect orders for 2004 and 2005 are presented.

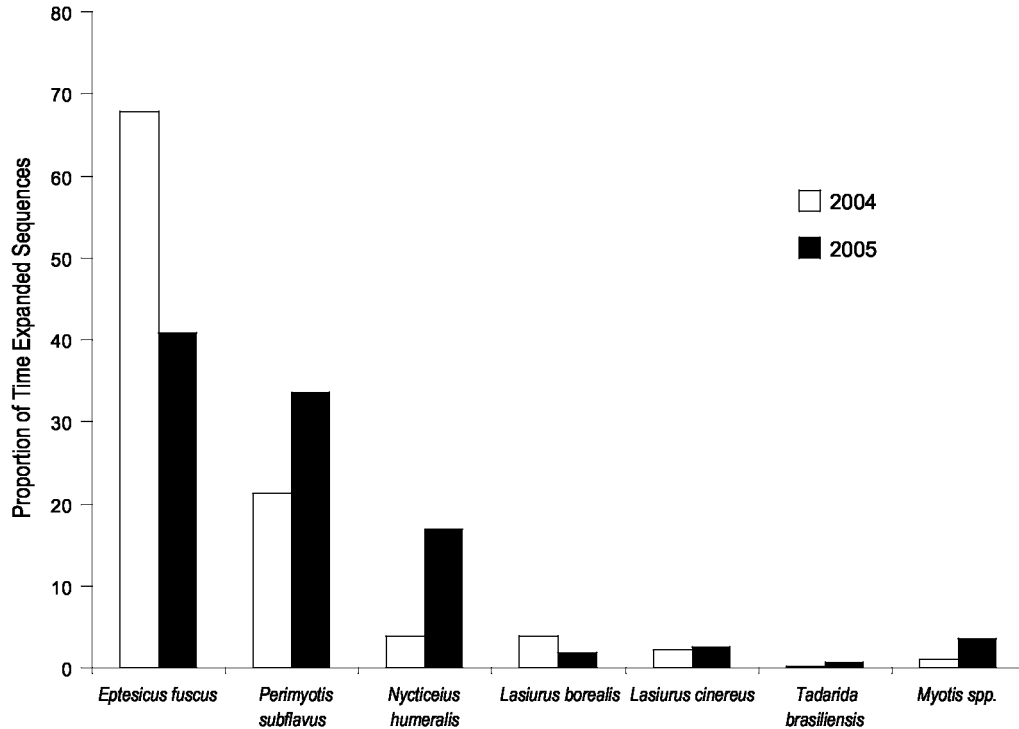


Fig. 4 – Bat species present along Buffalo Creek in Guilford County, North Carolina, USA in 2004 (a) and 2005 (b). Species were determined by analyzing one randomly selected call from a time-expanded sequence (see Section 2). The three most represented groups were *E. fuscus*, *N. humeralis*, and *P. subflavus*. Proportions are calculated from all recorded sequences: 2004 n = 892 and 2005 n = 671.

(ie, a feeding buzz) up- and downstream of the WWTPs (2004: n=25, T=101.00, Z=1.4, P=0.16; 2005: n=24, T=81.0, Z = 1.97, P = 0.05; Fig. 7).

4. Discussion

North and South Buffalo Creeks downstream of the WWTPs constitute some of the worst water quality problems in North Carolina, and the effect of anthropogenic nutrients on stream components can be seen in the aquatic food web components (Ulseth and Hershey, 2005; Northington and Hershey, 2006). Downstream from the WWTPs seston, moss, algae, conditioned leaves, caddisflies, damselflies, and fish have incorporated effluent-derived nitrogen up to 5.94 km away, and there is a trend toward lower fish species richness and abundance (Ulseth and Hershey, 2005; Northington and Hershey, 2006). To our knowledge, ours is the first study to examine how WWTP effluent affects bats and their prey in an impaired urban watershed where aquatic food web components are known to be influenced by WWTP effluent.

We found that the flying nocturnal insect community structure and abundance differed up- and downstream of the WWTPs. There were more insects captured upstream of the WWTPs. Diptera constituted the majority of the insect sample and were more abundant upstream of the WWTPs. Further, there were more Coleoptera and Lepidoptera

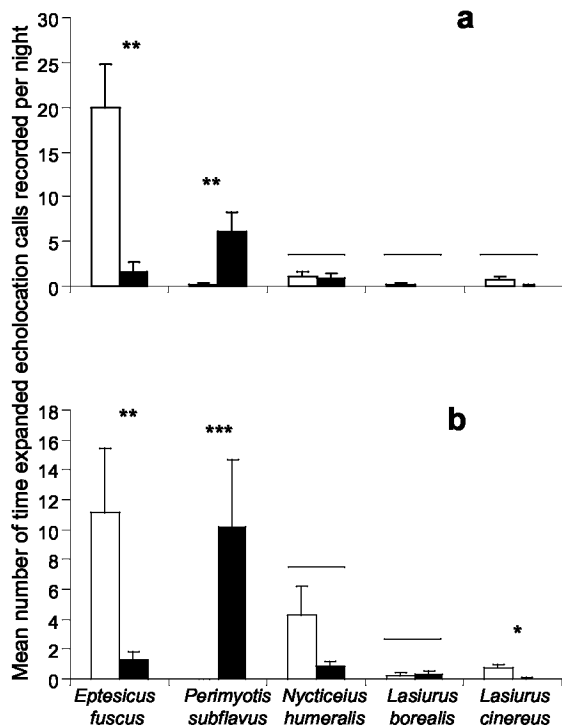


Fig. 5 – Mean (± 1 SE) number of time-expanded echolocation sequences recorded per night up- and downstream of the WWTPs in 2004 (a) and 2005 (b) along Buffalo Creek in Guilford County, North Carolina, USA. Upstream = open bars. Downstream = closed bars. Significant differences are denoted by asterisk ($P < 0.05$, $^{}P < 0.005$, $^{***}P < 0.001$). Line above pair denotes no difference. Sample sizes per year: 2004: $n = 12$ paired nights, 2005: $n = 22$ paired nights.**

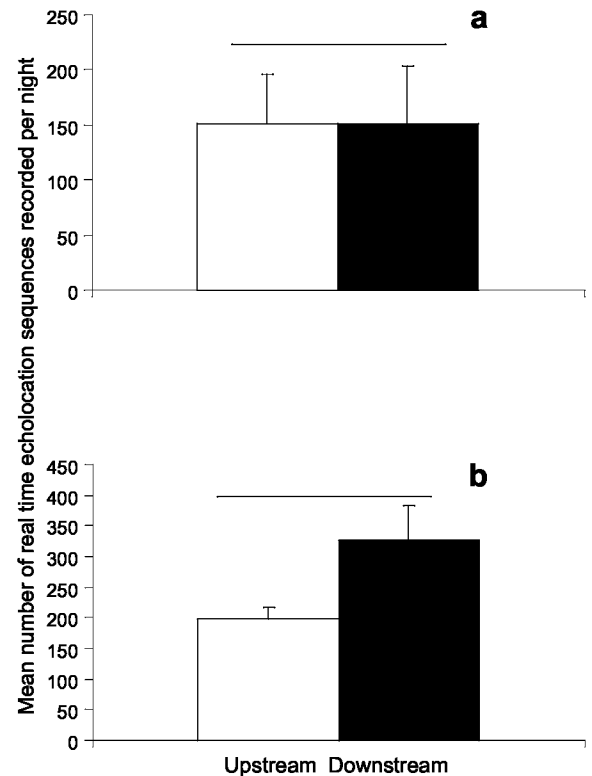


Fig. 6 – Mean (± 1 SE) number of real time echolocation sequences recorded per night up- and downstream of the WWTPs in 2004 (a) and 2005 (b) along Buffalo Creek in Guilford County, North Carolina, USA. Line above pair denotes no difference. Sample sizes per year: 2004: $n = 25$ paired nights, 2005: $n = 24$ paired nights.

upstream of the WWTPs, whereas there were more Odonata downstream of the WWTPs. We recorded seven bat species or species groups including big brown (*E. fuscus*), eastern pipistrelles (*P. subflavus*), evening (*N. humeralis*), red (*L. borepipistrelles* (*P. subflavus*), evening (*N. humeralis*), red (*L. borealis*), hoary (*L. cinereus*), free-tailed (*T. brasiliensis*), and two species of Myotis bats. The most commonly recorded species were *E. fuscus*, *P. subflavus*, and *N. humeralis*, and they differed in their distribution with respect to the WWTPs; there were more *E. fuscus* upstream of the WWTPs and more *P. subflavus* downstream of the WWTPs. Despite the difference in bat community structure up- and downstream of the WWTPs, bat commuting and foraging activity levels were the same; there was no difference in the total number of echolocation sequences we recorded per night up- and downstream of the WWTPs nor was there a difference in the proportion of those sequences that contained a feeding buzz. The patterns that we found with respect to insect abundance, bat species abundance, and bat activity were consistent between years. Our results suggest the effect of WWTP effluent in the stream persists through higher food web trophic levels as we found impacts on nocturnal flying insects as well as insectivorous bats. Impaired urban watersheds such as Buffalo Creek, that receive WWTP

effluent, are common worldwide. It is likely that the effects we found of WWTP effluent on bats and their prey base are pervasive.

Both aquatic and terrestrial insects present in this food web differed in abundance and diversity up- and downstream of the WWTPs. Our results are consistent with a study from the United Kingdom that examined the effect of anthropogenic inputs on bat food webs (Wickramasinghe et al., 2004). In a study of the effect of agrochemical use in conventional farming vs organic farming on the availability of bat prey, it was demonstrated that insect abundance, insect diversity, insect species richness, and moth diversity was higher over water sources on organic farms that did not use agrochemicals (Wickramasinghe et al., 2004). This result suggests that conventional agriculture has an impact on the flying nocturnal insect community that is the prey base for insectivorous bats.

Nutrient input into water systems can either enhance or decrease the prey base for insectivorous bats. If the prey base for insectivorous bats is particularly tolerant to nutrient input or to subsequent increases in primary productivity associated with the input, the prey base maybe enhanced. For example, the main component of the diet of the widespread *P. pipistrellus* and *P. pygmaeus* in the UK are Nematoceran Diptera (Barlow, 1997), and these insects are more abundant at sewage treatment plants (Park and Cristinacce, 2006) and eutrophic rivers (Racey et al., 1998).

The diet of bats reflects the availability of insects on a geographical, seasonal, or nightly scale (Fenton, 2001). Within the

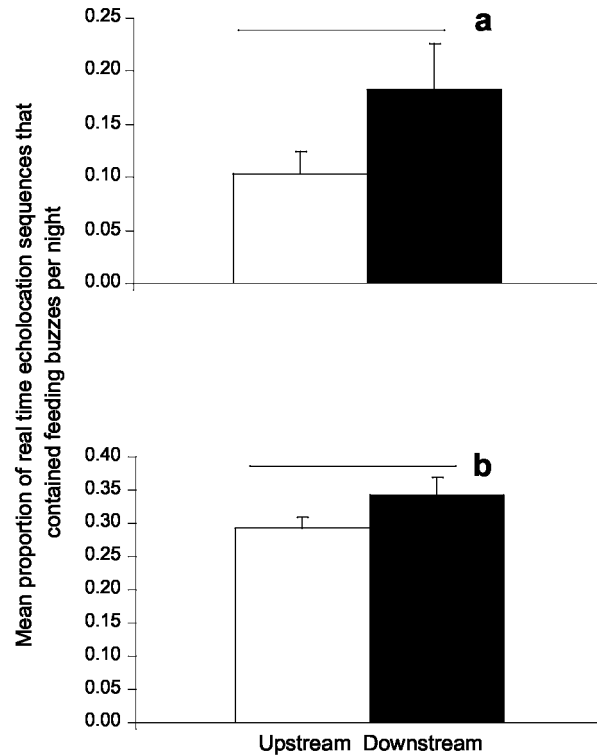


Fig. 7 – Mean (± 1 SE) proportion of total number of real time echolocation sequences that contained a feeding buzz recorded per night up- and downstream of the WWTPs in 2004 (a) and 2005 (b) along Buffalo Creek in Guilford County, North Carolina, USA. Line above pair denotes no difference. Sample sizes per year: 2004: $n = 25$ paired nights, 2005: $n = 24$ paired nights.

insectivorous bat ensemble of any riparian community, some species are considered specialized predators while others are considered generalist predators (Herd and Fenton, 1983; Arlettaz et al., 1997). Therefore, the response of bats to a change in the abundance or community structure of their prey will differ depending on the bat species considered. The three commonly recorded bat species in this study had markedly different responses to WWTP effluent. *P. subflavus* was more common below the WWTPs, *E. fuscus* was more common above the WWTPs, and *N. humeralis* did not differ in activity above the WWTPs, and *N. humeralis* did not differ in activity up- and downstream of the WWTPs. We also found a difference in the response of *L. cinereus* to WWTP effluent; however, this species was not common in this watershed.

In the UK, *P. pipistrellus*, *P. pygmaeus*, and *Myotis daubentonii* feed on emergent insects in riparian zones and there is evidence that water quality and subsequent nocturnal flying insect community structure and abundance is having an effect on those bat species. The range expansion of *M. daubentonii* through mainland Europe has been suggested to be the result of the concomitant increase in eutrophic freshwater (Kokurewicz, 1995). In a study of the effect of agrochemical use in conventional farming vs organic farming on bat activity and species composition, it was shown that overall activity was higher over water sources on organic farms relative to water sources over conventional farms and that some species of bats were either only

recorded on organic farms (*Rhinolophus* spp.) or recorded significantly more often on organic farms (*Myotis* spp.) (Wickramasinghe et al., 2003). This difference between farm practices in bat activity correlates with the nocturnal insect availability (Wickramasinghe et al., 2004). Although specific details about water sources and inputs are not given in Wickramasinghe et al. (2003, 2004), these results suggest that conventional agrochemical inputs to water sources may affect bats and their insect prey.

Interestingly, the effect of wastewater effluent on bat foraging behavior varies based on the type of wastewater effluent treatment; pipistrelles in the UK forage more readily over percolating filter sewage beds as opposed to activated sewage sludge (Park and Cristinacce, 2006). The reason for the difference between sewage treatment types is that percolating sewage filter beds support a bio-film that facilitates the presence of a wide diversity of macroinvertebrates whereas activated sludge treatment involves constantly aerating the sewage which prevents the establishment of macroinvertebrates (Park and Cristinacce, 2006).

Similar to the responses of riparian specialist bats in the UK, and as we predicted, *P. subflavus* demonstrated a significant response to water quality in our study. We recorded higher levels of *P. subflavus* activity downstream of the WWTPs suggesting that not only is this species tolerant to anthropogenic input to the watershed but it may benefit. Of the three common bat species recorded in our study, *P. subflavus* has the broadest diet (Carter et al., 1998; Carter et al., 2003; Whitaker, 2004). In the southeastern United States, *P. subflavus* has a highly diverse and evenly apportioned diet (Carter et al., 2003) and consumes fewer coleopterans than are available (Carter et al., 1998). It is likely that components of the diets of *P. subflavus* may be tolerant of the wastewater derived nitrogen enrichment downstream of the WWTPs. Although we did not see significantly higher amounts of any of the major orders of insects that comprise the *P. subflavus* diet downstream of the WWTPs there was a trend for increased numbers of trichopterans and hymenopterans downstream of the WWTPs and both of these orders are components of *P. subflavus* diet in this region (Carter et al., 1998).

In contrast, we found significantly more *E. fuscus* upstream of the WWTPs suggesting a lack of a tolerance to anthropogenic change in this species. This is surprising because *E. fuscus* is considered to be a habitat generalist that is very tolerant and flexible in its roosting habitat choices (Brigham, 1991; Everette et al., 2001; Agosta, 2002; Gehrt, 2004; Sullivan et al., 2006) and is found frequently in building roosts despite roosting in tree cavities in undisturbed areas (Kalcounis and Brigham, 1998; Willis et al., 2003). Our results suggest that this flexibility and tolerance of urban habitat may be limited to roost sites and that foraging habitat selection may be less tolerant to anthropogenic change that affects water quality. Our insect community results demonstrate that major coleopteran prey items for *E. fuscus* are more abundant upstream of WWTPs and it may be that *E. fuscus* is responding to this change in the insect community structure. Limited foraging habitat flexibility in *E. fuscus* is supported by data on the foraging home ranges of *E. fuscus* in the southeastern United States where bats traveled great distances from urban roost sites to forage in less developed areas (Menzel et al., 2001), and by data from western Canada that suggests that foraging home range size of *E. fuscus* is related to the quality and availability of insect prey (Wilkinson and Barclay, 1997).

An assumption we are making is that the differences in bat community structure and bat activity in our study relates to bat diet, especially with respect to *E. fuscus* and *P. subflavus*. Although we demonstrate differences in the community composition and availability of nocturnal flying insect prey up- and downstream of the WWTPs, we do not show that it is indeed bat diet that is the underlying factor responsible for the differences we see between bat species. Future investigations in the Buffalo Creek watershed should (1) examine diets of bats to determine if dietary preferences underlie the patterns reported herein, and (2) examine effects of WWTP effluent on insects and bats in relation to distance from the WWTP. Regardless, our results suggest that *P. subflavus* and *E. fuscus* may serve as easily tractable terrestrial bioindicators of water quality as influenced by WWTP effluent in this, and other, urban watersheds.

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