

Rapid increases in bat activity and diversity after wetland construction in an urban ecosystem

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Abstract:

Wetland construction can mitigate the biodiversity and water quality losses associated with reduced natural wetland coverage. While beneficial effects of wetland construction for bats have been observed in natural and rural settings, the effects of wetland construction on bats in an urban ecosystem are less understood. We used passive acoustic monitoring to measure bat activity levels and diversity at two constructed wetlands and two control sites on the University of North Carolina Greensboro campus, in Greensboro, North Carolina, USA. We monitored all 4 sites before and after wetland construction. Pre-wetland construction, there were few differences in bat activity and community structure at our sites. After wetland construction, we observed greater activity, attributable to all species we recorded, at wetland sites compared to control sites. Species diversity and species richness were also higher at wetland sites compared to control sites. When comparing the same sites before and after wetland construction, both bat activity and species richness increased after construction, but the effects were seen in Winter and not Spring. Our results demonstrate that bats use constructed wetlands in urban ecosystems similarly to other habitat settings. Increases in bat activity, diversity, and species richness occurred within one year of wetland construction.

Keywords: Chiroptera | Conservation | Management | Fresh water | Piedmont | Bioindicators | *Eptesicus fuscus* | Winter | Spring

Article:

Introduction

Wetlands are among the most threatened ecosystems globally (Bolpagni and Piotti 2016) and are declining in number and size (Adams 2010). Wetland loss and reduction can be attributed to anthropogenic activities (Gibbs 2000) such as destruction of wetlands for infrastructural development, agricultural (crop and animal) production, as well as impacts from climate change (Adams 2010). Estimates of global wetland loss range from 54 to 57% but may be as high as 87% (Davidson 2014). Wetland loss has slowed considerably in Europe and the United States since 1980, but there is a continued decline (Dahl 1990, 2006, 2011; Davidson 2014). Dahl (2011) estimates that North Carolina has lost approximately 49% of an estimated 11 million acres of wetlands that were present prior to 1787. Similarly, in North Carolina approximately 50% of palustrine wetlands have been altered so that they no longer support their original function (Cashin et al. 1992; Dahl 2011). In North Carolina, the majority of wetland loss is coastal and due to drainage for farming and managed forests (Cashin et al. 1992; Zedler and Kercher 2005). However, from 2004 to 2009, forested wetlands experienced the largest loss of any wetland type in North Carolina, due primarily to silviculture and urban and rural development (Dahl 2011). Recent efforts in wetland construction, restoration, and conservation aim to increase the positive effects wetlands can have on their local environments (Boyles et al. 2011). Wetlands improve local water quality by decreasing the nutrient load of nitrogen and phosphorus in surface and runoff water (Verhoeven et al. 2006; Hansen et al. 2018). Wetlands are areas of high plant productivity, serve as carbon sequesters, and abate flooding (Gopal et al. 2000; Zedler 2000; Zedler and Kercher 2005). Moreover, targeted wetland site development can significantly increase real estate values (Kaza and BenDor 2013).

Wildlife such as amphibians, reptiles, insects, and mammals rely on wetlands for refuge, food, and water (Fairchild et al. 2000). Bats use both natural and artificial wetland habitats (Vindigni et al. 2009; Stahlschmidt et al. 2012; Sirami et al. 2013). Bats can be used to assess the role of wetlands in improving biodiversity (Stahlschmidt et al. 2012) due to their (i) relatively stable taxonomy, (ii) ability to be sampled at several levels, (iii) wide geographic range, (iv) graded response to habitat degradation, that is correlated with responses to other taxa such as insects, (v) rich trophic diversity, and (vi) slower reproductive rates (Jones et al. 2009). Many species of bats forage and drink over open and calm water bodies (Vindigni et al. 2009; Salvarina 2016) due to higher insect abundance, decreased habitat complexity, and decreased ultrasonic interference (Vindigni et al. 2009; Salvarina 2016). Bats are sensitive to local resource availability and distribution while simultaneously reacting to landscape scale features (Mendes et al. 2017).

Bat activity levels at constructed wetlands have been found to be comparable or higher to that at natural wetlands (Menzel et al. 2005; Park and Cristinacce 2006; Vindigni et al. 2009; Stahlschmidt et al. 2012; Sirami et al. 2013; Kerbiriou et al. 2018). The increased response of bats to constructed wetlands can be demonstrated even within the first year of wetland placement (Menzel et al. 2005). Observations of foraging buzzes and high emergent aquatic insect abundance suggest that the heightened bat activity at constructed wetlands is due to the abundance of prey and reduced clutter (Park and Cristinacce 2006; Stahlschmidt et al. 2012). There are species specific differences in how bats benefit from wetlands. For example, less maneuverable bat species (Vindigni et al. 2009) and those that travel shorter distances while foraging (Lookingbill et al. 2010) use wetlands more than those that are maneuverable and travel long distances. While wetlands can have public health costs from increased abundance of disease

vectors (Dale and Knight 2008), bats could offset such risks in urban environments by consuming some of these vectors such as mosquitoes (Reiskind and Wund 2009). However, in urban environments there is little knowledge on how bats respond to new wetlands on the landscape.

The goal of this study was to investigate the effect of urban wetland construction on bat activity, species richness, and diversity by using bats as bioindicators (Kalcounis-Rueppell et al. 2007; Li and Kalcounis-Rueppell 2018). The University of North Carolina Greensboro (UNCG) recently constructed two wetlands on campus. We hypothesized that the new wetlands would increase diversity and activity of bats. We measured bat activity using acoustic monitoring and estimated species richness and diversity, before and after wetland construction and compare wetland sites to paired control sites on campus without wetlands. We predicted that bat species richness and diversity would increase at wetland sites compared to control sites.

Materials and Methods

Sites

During the week of March 15–20, 2017, UNCG constructed wetlands at two locations on the main campus in Peabody Park, which includes tributaries of North Buffalo Creek which is in the headwaters of the Cape Fear River Basin, to provide research and educational resources while simultaneously reclaiming wetland environments. One wetland (forested wetland) was constructed in a wooded area near a tributary to North Buffalo Creek (36° 4'23.97°N, 79° 48'32.89°W) (Supplementary Material A). A second wetland (open field wetland) was constructed an open lawn bordering a campus golf course and tennis court (36° 4'20.08°N, 79° 48'43.62 W), also near a tributary to North Buffalo Creek (Supplementary Material A & B).

UNCG is an urban campus. Written history of the sites of the constructed wetlands dates to 1897 when the land was purchased to establish a park in a wooded area for student exercise and recreation, to create a farm to supply the school with milk, pork, and produce, and to serve as a horticulture teaching laboratory (Bowles 1967; Trelease 2004). The wooded park was excavated to construct walking paths and bridges in 1902 and fell into disrepair in the 1930s. The forested area has been extensively renovated since the 1980s with paved and gravel walking paths that surround and follow a creek that runs northward into North Buffalo Creek. The forest is dominated by mature American beech (*Fagus grandifolia*), box elder (*Acer negundo*), sweetgum (*Liquidambar styraciflua*), shortleaf pine (*Pinus echinata*), longleaf pine (*P. palustris*), dogwood (*Cornus florida*), sugar maple (*Acer saccharum*), sycamore (*Platanus occidentalis*), white mulberry (*Morus alba*), willow oak (*Quercus phellos*) and winged sumac (*Rhus copallion*). Understory plants are typical of forested areas in the Piedmont of North Carolina (<https://peabodypark.uncg.edu/field-guide/>) and include a significant stand of roughed horsetail (*Equisetum hyemale*). In 1923, the farm was relocated off campus, and a golf course was constructed in its place. In 1941, a drainage creek that ran through the golf course was dammed, creating a recreational lake which was drained in 1954, followed by redevelopment of the golf course, walking paths, and other recreational fields and courts. Today, this area is primarily open lawn and walking paths that meander along the creek and through the recreation areas.

The wooded park and the site of the drained lake is where we constructed the wetlands. Design and excavation of the wetlands was directed and supervised by Thomas Biebighauser, Wetlands Restoration and Training, LLC, with the help of volunteers using a Hitachi 160 LC hydraulic excavator, shovels, and rakes. We refer to both wetlands as ‘constructed’ because there is no recorded history of these locations having been functional wetlands, even though they have held water in the past. To provide substrate in both constructed wetlands, branches and logs and small piles of stone were set in and around the wetlands, and native wetland plants were planted in the water and around the edge of each wetland in May 2017 and March 2018. In the forested wetland, the following were planted: seeds of purple top (*Tridens flavus*), little bluestem aldous (*Schizachyrium scoparium*), river oats (*Chasmanthium latifolium*), hop sedge (*Carex lupulina*), soft rush (*Juncus effuses*), creeping spike rush (*Eleocharis palustris*), broom sedge (*Andropogon virginicus*), indian grass cheyenne (*Sorghastrum nutans*) and starts of southern lady fern (*Athyrium filix-femina*), royal fern (*Osmunda regalis*), spice bush (*Lindera benzoin*), soft rush (*J. effuses*), square-stem spike rush (*Eleocharis quadrangulata*), little bluestem (*S. scoparium*), blazing star (*Liatris spicata*). In the open field the same species were planted as in the forested wetland with the addition of duck potato (*Sagittaria latifolia*), blue flag (*Iris virginica*), pickerelweed (*Pontedaria cordata*), swamp milkweed (*Asclepias incarnate*), and sweet grass (*Muhlenbergia capillaris*). The constructed wetlands retain water throughout the year and can be classified as vegetated, palustrine, persistent, emergent wetlands with mud substrates (Cowardin et al. 1979). Details of wetland construction and size are as follows.

The forested wetland is 6.4 × 12.19 m (0.0078 ha in area) and 0.46 m at maximum depth, with primarily silt/loam soil. Sand was removed during excavation to improve the ability of the wetland to retain water. The forested wetland lies on the west bank of a creek directly below a steep bank approximately 15 m high above which sits Gray Dormitory, which begins approximately 200 m from the wetland. Rainwater from the dormitory roof drains into a catchment that drains to the south side of the wetland through a concrete pipe. Prior to the wetland construction, the forested wetland was terrestrial with a thick leaf layer and soggy soil fed by rainwater drainage from the dormitory roof and runoff, and occasional flooding from the adjacent creek. A shallow ditch allowed drainage to overflow into the adjacent creek and was left unchanged during wetland construction except for stabilizing the soil by packing rocks approximately 1–2 ft deep on the most vulnerable edge of the ditch near the wetland. The wetland holds water continuously and can be considered emergent, vegetated, and persistent (Cowardin et al. 1979). The open field wetland is 30.5 × 24.4 m (0.0744 ha in area), and 0.46 m at maximum depth, with soil that is primarily sand and clay. The design includes an aquatic-safe liner and geotextile pads to prevent drainage. This wetland is 10 m from the bank of a tributary of North Buffalo Creek. Several large longleaf pine (*P. palustris*) and a sycamore (*P. occidentalis*) border the lawn 20–25 m south of the wetland.

Prior to wetland construction, each site was paired with a control site for monitoring the effect of constructing the wetland on biodiversity on the campus (see Supplementary Material A & B). The control site for the forested wetland is approximately 50 m southeast of the forested wetland on the opposite side of the creek. The forested wetland control site is very similar in terms of vegetation structure and understory but it is above the floodplain and does not hold water. The control site for the open field wetland is also on the opposite side of the creek approximately

200 m away. The open field wetland site is similar in terms of elevation and vegetation, and was likely part of the lake that was drained in 1954.

Bat Detection

Ultrasonic detectors (Wildlife Acoustics Inc., Maynard, MA; model SM_BAT4) and microphones (Wildlife Acoustics Inc., Maynard, MA; model SMM_U1_NOCAB) were outfitted to trees at all 4 sites, with one detector mounted to a tree at each site, prior to wetland construction, approximately 7 to 8 m above the ground. The detectors were semi-permanently mounted (via caballing around the tree) and powered by 5,000mAh C batteries. All detectors were installed on November 19th, 2016, and constantly recorded full spectrum recordings from sunset to sunrise each night. Recordings were downloaded weekly. The latest detector night used for this study was March 28th, 2018 (see Supplementary Material B for timeline and design details). Prior to the study we ensured that our 4 sites were far enough apart that a bat could not be recorded at two detectors simultaneously.

We used the automatic identification (ID) function of Kaleidoscope 4.3 (Wildlife Acoustics Inc., Maynard, MA) to identify bat passes, using the Bats of North America 4.3 library with possible species set as *Eptesicus fuscus*, *Lasiurus borealis*, *L. cinereus*, *Lasionycteris noctivagans*, *Myotis lucifugus*, *M. septentrionalis*, *Nycticeius humeralis*, *Perimyotis subflavus*, *Tadarida brasiliensis* and No ID (Kalcounis-Rueppell et al. 2007; Grider et al. 2016; Schimpp et al. 2018). We defined a bat pass as a recording file generated by the bat detector that included at least 3 complete bat echolocation calls. We set Kaleidoscope identification accuracy to neutral. For species specific identifications, we were conservative and only included recording files with a match ratio larger than 0.60 (60% of all calls in the pass were identified to the same species) for statistical analysis. This criterion was appropriate for species specific identification because our previous manual vetting showed that at this criterion identifications were accurate in our study area (e.g., Schimpp et al. 2018).

For all bat passes (including all species and No ID), we measured bat activity on a nightly basis (number of passes per night) and combined them by units of one week to estimate Shannon's diversity index, Simpson's diversity index, and species richness because nights with no bat activity prevented the calculation of daily diversity indices. Further, we excluded bat passes identified as *Myotis spp.* from tests of species specific activity, diversity, and species richness because our previous work in our study area suggested it was unlikely for these species to be present (Kalcounis-Rueppell et al. 2007; Schimpp et al. 2018).

Statistical Analyses

We evaluated total bat activity, species specific activity, Shannon's diversity, Simpson's diversity, and species richness using Wilcoxon Rank Sum tests. We did not include habitat covariates at either site in our analyses and we pooled data at control sites and proposed wetland/wetland sites (see Supplementary Material B). We tested for differences between wetland and control sites, before and after wetland construction. For comparisons of change after wetland construction, we used nights between November 19th, 2016 and March 14th, 2017 for before construction, and November 19th, 2017 and March 14th, 2018 for after construction. To

avoid confounding effects of seasonality on bat activity, we further divided the data into Winter (November 19th to January 31st for both years) and Spring (February 1st to March 14th for both years, Supplementary Material B) and conducted tests separately. As late Spring and summer activity were only recorded after construction, if we used all detector nights to compare bat activity before and after, there would be an apparent increase regardless of treatment because bat activity is low in Winter (Geluso 2007).

We tested for significant differences in the medians between site types (wetlands and control) within time periods, and within site types between time periods using Wilcoxon Rank Sum tests. The Wilcoxon Rank Sum test implemented in Program R includes an estimate of differences between groups using location shifts, derived from a resampling technique that calculates the difference in the median of a sample of X and a sample of Y (Hollander et al. 2015). We used these median location shifts to indicate the magnitude of difference between groups (Gopal et al. 2000). All statistical analyses were performed in Program R (R Core Team 2018), using the packages ggplot2 for boxplot data visualization (Wickham and Chang 2008), and vegan for diversity indices (Oksanen et al. 2018).

Table 1. Wilcoxon median rank sum test results for comparisons of total bat activity, species specific bat activity, diversity indices, and species richness between the control and proposed wetland/wetland sites before the wetland construction (November 19th, 2016 – March 14th, 2017) and after the wetland construction (March 15th, 2017 – March 28th, 2018)

| | Pre-construction | | | | Post-construction | | | |
|------------------|------------------|-------------------------|---|--------------|-------------------|----------------|--|------------------|
| | Control median | Proposed wetland median | Median of differences between control vs proposed wetland | <i>p</i> | Control median | Wetland median | Median of differences between control vs wetland | <i>p</i> |
| Total Activity | 4 | 5 | -1.10×10^{-5} | 0.407 | 20 | 47 | -7.00 | <0.001 |
| EPTFUS Activity | 1 | 0.5 | 6.71×10^{-5} | 0.263 | 5 | 17.5 | -4.00 | <0.001 |
| LASBOR Activity | 0 | 0 | -1.02×10^{-5} | 0.001 | 0 | 0 | -6.57×10^{-6} | <0.001 |
| LASCIN Activity | 1 | 1 | -2.05×10^{-5} | 0.001 | 0 | 0 | -1.00×10^{-5} | 0.016 |
| LASNOC Activity | 0 | 0 | 4.72×10^{-5} | 0.562 | 0 | 1 | -4.09×10^{-5} | <0.001 |
| NYCHUM Activity | 0 | 0 | 1.10×10^{-5} | 0.478 | 0 | 2 | -1.00 | <0.001 |
| PERSUB Activity | 0 | 0 | -3.65×10^{-5} | 0.009 | 0 | 0 | -2.00×10^{-5} | <0.001 |
| TADBRA Activity | 0 | 0 | -1.02×10^{-5} | 0.382 | 0 | 0.5 | -3.76×10^{-5} | 0.002 |
| Shannon's Index | 0.97 | 1.13 | 0.12 | 0.463 | 0.94 | 1.09 | -0.16 | 0.019 |
| Simpson's Index | 0.47 | 0.62 | 0.07 | 0.364 | 0.44 | 0.52 | -0.06 | 0.049 |
| Species Richness | 6 | 5 | 5.81×10^{-5} | 0.946 | 6 | 7 | -3.50×10^{-5} | 0.050 |

For pre-construction control vs proposed wetland bat activity comparisons $n = 177$, diversity indices and species richness comparisons $n = 17$. For post-construction control vs wetland bat activity comparisons $n = 692$, diversity indices and species richness comparisons $n = 55$. Significant *p*-values are in bold. Activity units are number of passes per night

Results

There were 1980 detector nights of which 1770 were successful and 210 were failures due to equipment malfunction. We recorded a total of 250,178 acoustic files and identified 184,878 bat passes, including 7 bat species: *Eptesicus fuscus* (EPTFUS), *Lasiurus borealis* (LASBOR), *Lasiurus cinereus*, (LASCIN), *Lasionycteris noctivagans* (LASNOC), *Nycticeius humeralis* (NYCHUM), *Perimyotis subflavus* (PERSUB), and *Tadarida brasiliensis* (TADBRA). These 7

bat species generated 80,013 bat passes with a match ratio higher than 0.60 for species specific analysis. In the subset of dates used to directly compare before and after construction, we had 460 successful detector nights for the Winter and 309 for the Spring. We recorded 6008 bat passes in the Winter and 19,025 in the Spring. We only used nights on which all sites had recordings for statistical tests.

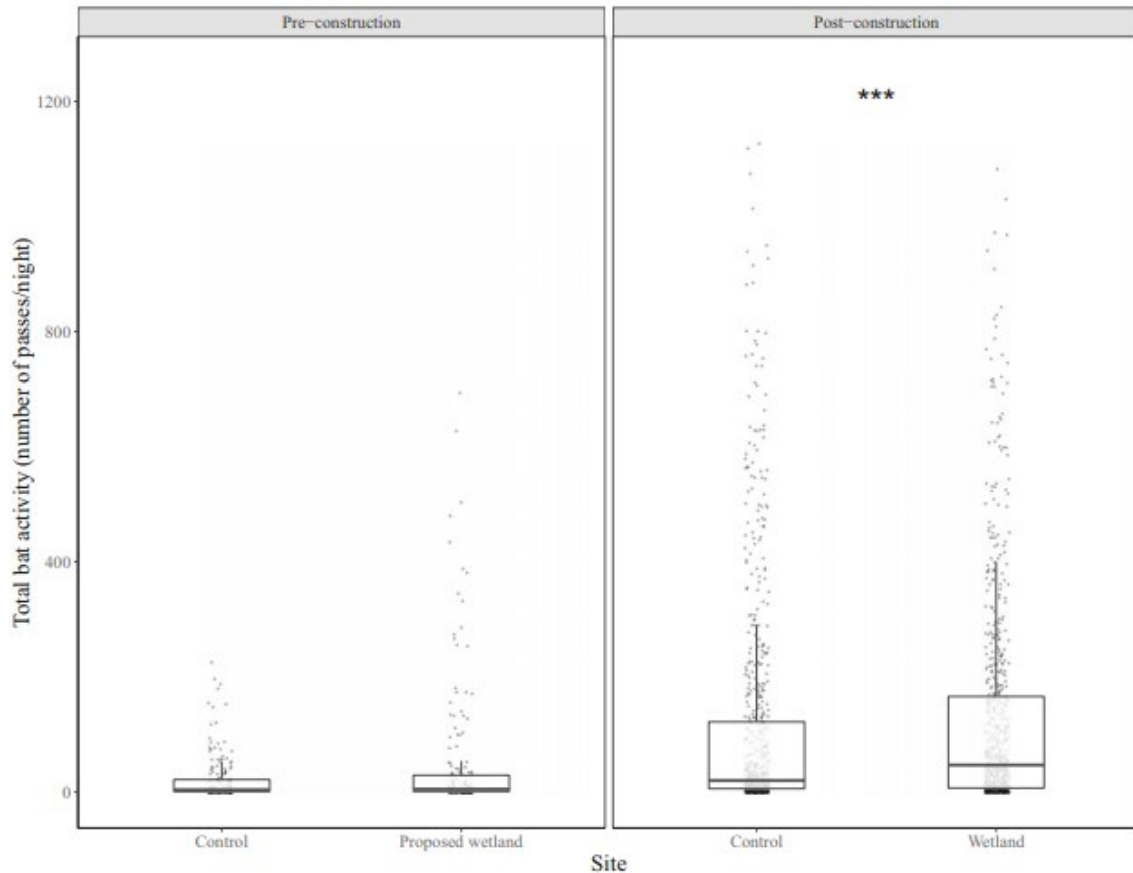


Fig. 1. Total bat activity (number of passes per night) in control and proposed wetland sites before the wetland construction (November 19th, 2016-March 14th, 2017) and in control and wetland sites after the construction (March 15th, 2017-March 28th, 2018) at the University of North Carolina Greensboro. Total bat activity was not different between sites before the construction ($p = 0.407$, $n = 177$, Wilcoxon rank sum test) and was higher at wetland when compared to control sites after the construction ($p < 0.001$, $n = 691$, Wilcoxon rank sum test)

There was no significant difference in total bat activity ($p = 0.407$) between control and proposed-wetland sites before construction (Table 1; Fig. 1). After wetland construction, total bat activity was significantly higher at the wetland sites than the control sites ($p < 0.001$; Table 1, Fig. 1). Considering each species, before construction, only activity of *L. borealis*, *L. cinereus*, and *P. subflavus* was significantly higher at the proposed-wetland sites than control sites (all $p < 0.050$, Fig. 2), whereas all 7 species exhibited significantly higher activity at wetland sites than control sites after wetland construction (all $p < 0.050$, Fig. 2). Before construction, there was no difference for Shannon's diversity index (H), Simpson's diversity index (SDI) or species richness between control and proposed-wetland sites (all $p > 0.050$, Table 1). After construction, all 3 indices were higher at wetland sites than control sites (all $p < 0.050$, Table 1).

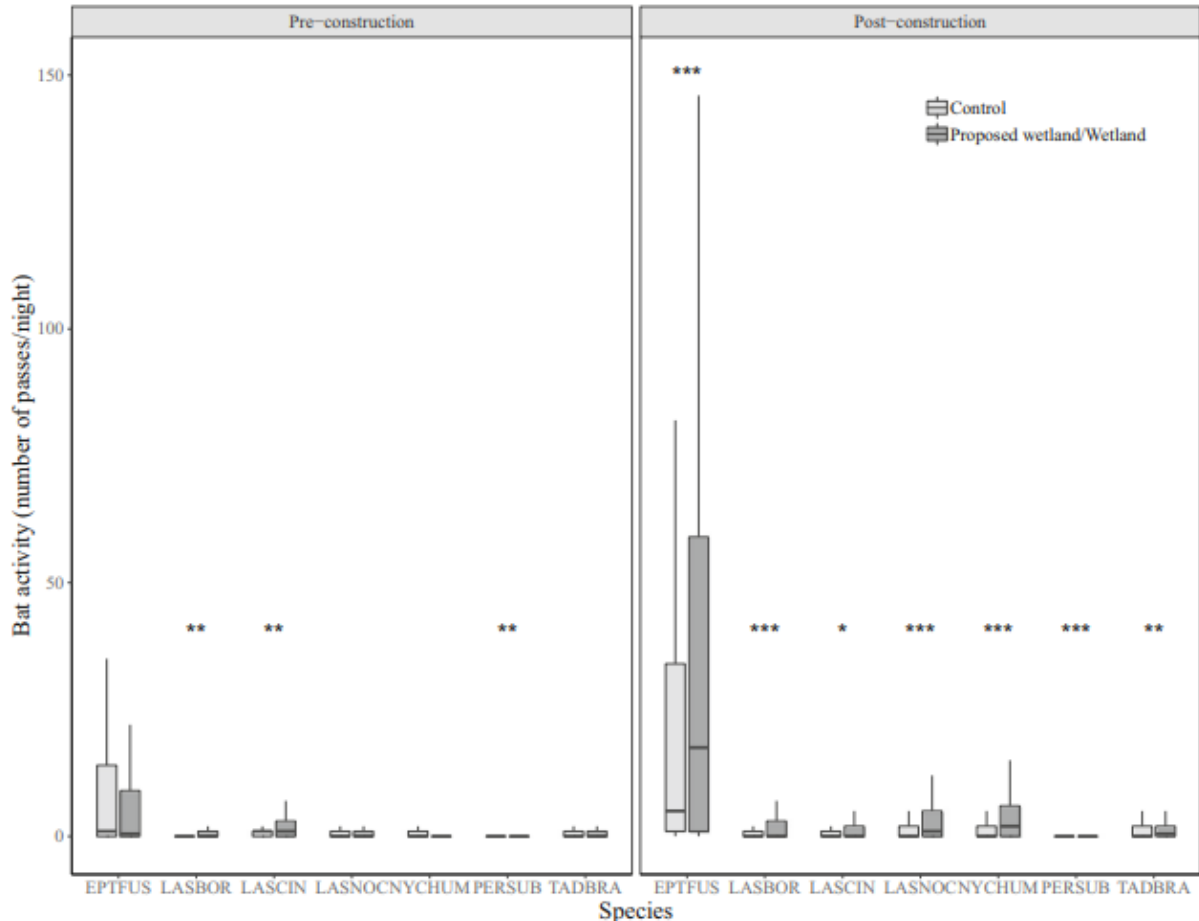


Fig. 2. Species specific bat activity (number of passes per night) in control and proposed wetland sites before the wetland construction (November 19th, 2016-March 14th, 2017) and in control and wetland sites after the construction (March 15th, 2017-March 28th, 2018) at the University of North Carolina Greensboro. Species abbreviations are: (*Eptesicus fuscus* (EPTFUS), *Lasiurus borealis* (LASBOR), *Lasiurus cinereus* (LASCIN), *Lasionycteris noctivagans* (LASNOC), *Nycticeius humeralis* (NYCHUM), *Perimyotis subflavus* (PERSUB), and *Tadarida brasiliensis* (TADBRA)). Before the wetland construction, LASBOR, LASCIN, and PERSUB activity was higher at the proposed wetland sites when compared to the control (all $p < 0.050$, $n = 177$, Wilcoxon rank sum test). After the construction, all 7 species had higher activity at the wetland sites when compared to the control (all $p < 0.050$, $n = 691$, Wilcoxon rank sum test)

For the subset of dates used for before-after construction comparisons, total bat activity increased significantly at both control ($p = 0.004$) and wetland ($p = 0.013$) sites after wetland construction in the Winter (Table 2, Fig. 3), but stayed the same in the Spring (control $p = 0.680$, wetlands $p = 0.225$, Table 3, Fig. 3). For the Winter dates used for direct before-after construction comparisons, *L. cinereus* activity significantly increased at the control sites ($p = 0.002$, Table 2, Fig. 4) and *N. humeralis* activity increased at the wetland sites ($p = 0.016$, Table 2, Fig. 4). For the Spring dates used for direct before-after construction comparisons, there was a significant increase of *P. subflavus* activity at the wetland sites ($p = 0.004$, Table 3, Fig. 4). For the dates used for direct before-after construction comparisons in both Winter and Spring, there was no significant change after construction in Shannon's diversity index (H) or Simpson's diversity index (SDI) at control sites or wetland sites (all $p > 0.050$, Tables 2 and 3). Species richness was higher in the Winter, but not Spring, at wetland sites compared to control sites after construction ($p = 0.038$, Table 2).

Table 2. Wilcoxon median rank sum test results for comparisons of total bat activity, species specific bat activity, diversity indices, and species richness between the Winter before the wetland construction (November 19th, 2016 – January 31st, 2017) and the Winter after the wetland construction (November 19th, 2017 – January 31st, 2018) at the control sites and the proposed wetland/wetland sites

| | Control | | | | Proposed wetland/Wetland | | | |
|------------------|-------------------------|--------------------------|--|--------------|--------------------------|--------------------------|---|--------------|
| | Pre-construction median | Post-construction median | Median of differences between pre- vs post- construction | <i>p</i> | Pre-construction median | Post-construction median | Median of differences between pre- vs post-construction | <i>p</i> |
| Total Activity | 2 | 5.5 | -2.00 | 0.004 | 2 | 4 | -1.00 | 0.013 |
| EPTFUS Activity | 0 | 1 | 1.27×10^{-5} | 0.476 | 0 | 1 | 6.78×10^{-5} | 0.305 |
| LASBOR Activity | 0 | 0 | 8.07×10^{-5} | 0.756 | 0 | 0 | 4.52×10^{-5} | 0.625 |
| LASCIN Activity | 0 | 1 | -5.60×10^{-5} | 0.002 | 1 | 0 | -1.15×10^{-5} | 0.098 |
| LASNOC Activity | 0 | 0 | -1.26×10^{-6} | 0.185 | 0 | 0 | 5.24×10^{-5} | 0.752 |
| NYCHUM Activity | 0 | 0 | 1.85×10^{-6} | 0.471 | 0 | 0 | -7.84×10^{-5} | 0.016 |
| PERSUB Activity | 0 | 0 | n/a* | n/a* | 0 | 0 | -3.49×10^{-6} | 0.477 |
| TADBRA Activity | 0 | 0 | -4.04×10^{-5} | 0.467 | 0 | 0 | -5.59×10^{-5} | 0.511 |
| Shannon's Index | 0.99 | 1.07 | -0.16 | 0.171 | 0.97 | 1.21 | -0.22 | 0.260 |
| Simpson's Index | 0.47 | 0.56 | -0.10 | 0.151 | 0.50 | 0.62 | -0.06 | 0.566 |
| Species Richness | 5 | 5 | -1.00 | 0.344 | 4.5 | 6 | -1.00 | 0.038 |

For control sites, pre- vs post-construction bat activity comparisons $n = 107$, diversity indices and species richness comparisons $n = 11$. For proposed wetland/wetland sites, pre- vs post-construction bat activity comparisons $n = 105$, diversity indices and species richness comparisons $n = 11$. Significant p -values are in bold. Activity units are number of passes per night.

*No high quality PERSUB recording was collected in both winter seasons. The Wilcoxon test was not applicable

Table 3. Wilcoxon median rank sum test results for comparisons of total bat activity, species specific bat activity, diversity indices, and species richness between the Spring before the wetland construction (February 1st, 2017 – March 14th, 2017) and the Winter after the construction (February 1st, 2018 – March 14th, 2018) at the control sites and the proposed wetland/wetland sites

| | Control | | | | Proposed wetland/Wetland | | | |
|------------------|-------------------------|--------------------------|--|----------|--------------------------|--------------------------|---|--------------|
| | Pre-construction median | Post-construction median | Median of differences between pre- vs post- construction | <i>p</i> | Pre-construction median | Post-construction median | Median of differences between pre- vs post-construction | <i>p</i> |
| Total Activity | 13.5 | 8.5 | -1.00 | 0.680 | 16 | 11 | -3.00 | 0.225 |
| EPTFUS Activity | 2 | 1 | -1.80×10^{-5} | 0.219 | 1 | 0 | -5.14×10^{-5} | 0.417 |
| LASBOR Activity | 0 | 0 | 4.76×10^{-5} | 0.789 | 0 | 0 | -6.38×10^{-5} | 0.541 |
| LASCIN Activity | 1 | 1 | 3.21×10^{-5} | 0.199 | 1 | 1 | -1.08×10^{-5} | 0.597 |
| LASNOC Activity | 0 | 0 | -6.20×10^{-5} | 0.726 | 0 | 0 | -3.03×10^{-5} | 0.551 |
| NYCHUM Activity | 0 | 0 | -7.10×10^{-6} | 0.240 | 0 | 0 | 4.92×10^{-7} | 0.619 |
| PERSUB Activity | 0 | 0 | 2.30×10^{-5} | 0.190 | 0 | 0 | -2.11×10^{-6} | 0.004 |
| TADBRA Activity | 1 | 1 | -4.03×10^{-5} | 0.545 | 1 | 1 | -1.34×10^{-5} | 0.714 |
| Shannon's Index | 0.90 | 0.91 | -0.13 | 0.277 | 1.24 | 1.39 | -0.12 | 0.609 |
| Simpson's Index | 0.50 | 0.53 | -0.11 | 0.337 | 0.67 | 0.67 | -0.04 | 0.609 |
| Species Richness | 5.5 | 5 | 1.00 | 0.284 | 6 | 6 | 2.2×10^{-5} | 0.582 |

For control sites pre- vs post-construction bat activity comparisons $n = 70$, diversity indices and species richness comparisons $n = 7$. For proposed wetland/wetland sites pre- vs post-construction bat activity comparisons $n = 72$, diversity indices and species richness comparisons $n = 7$. Significant p -values are in bold. Activity units are number of passes per night

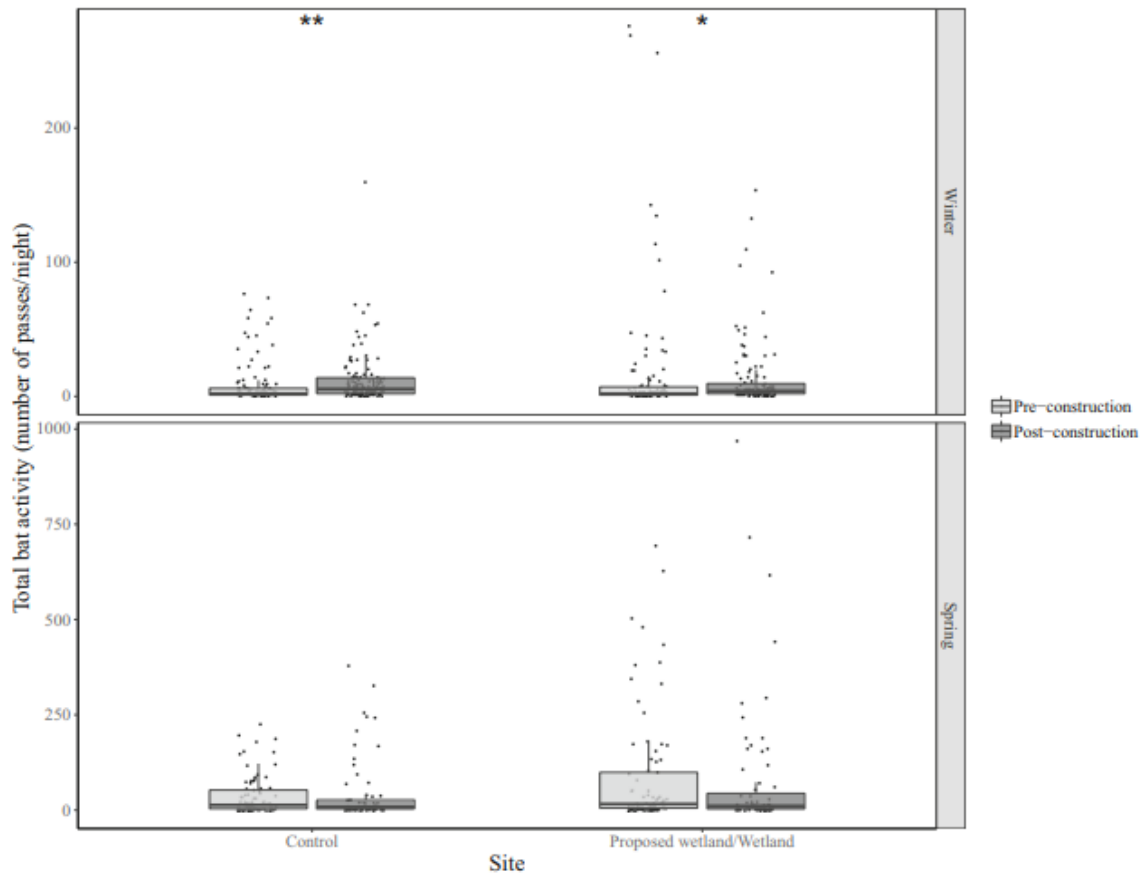


Fig. 3. Total bat activity (number of passes per night) in control and proposed wetland sites before the wetland construction in the Winter (November 19th, 2016-January 31st, 2017) and Spring (February 1st, 2017-March 14th, 2017), and in control and wetland sites after the construction in the Winter (November 19th, 2017-January 31st, 2018) and Spring (February 1st, 2018-March 14th, 2018) at the University of North Carolina Greensboro. After the wetland construction, total bat activity was higher at both control and wetland sites as compared to control and proposed wetland sites respectively in the Winter (control $p = 0.004$, $n = 107$, proposed wetland/wetland $p = 0.013$, $n = 105$, Wilcoxon rank sum test). No difference was found in the Spring (both $p > 0.050$, $n = 70$ for control, $n = 72$ for proposed wetland/wetland, Wilcoxon rank sum test)

Discussion

Our study indicates the constructed wetlands on the campus of University of North Carolina Greensboro are influencing bat activity, diversity, and species richness, in an urban environment. Our results suggest that constructed wetlands may fulfill the same habitat requirements of natural wetlands due to the bat responses we observed (Menzel et al. 2005; Park and Cristinacce 2006; Vindigni et al. 2009; Stahlschmidt et al. 2012; Sirami et al. 2013; Kerbiriou et al. 2018). Although habitat requirements (i.e., water quality, insect abundance, etc.) were not examined in our study, the increase in the median activity of bats supports the hypothesis that wetland construction has a positive effect on bat activity.

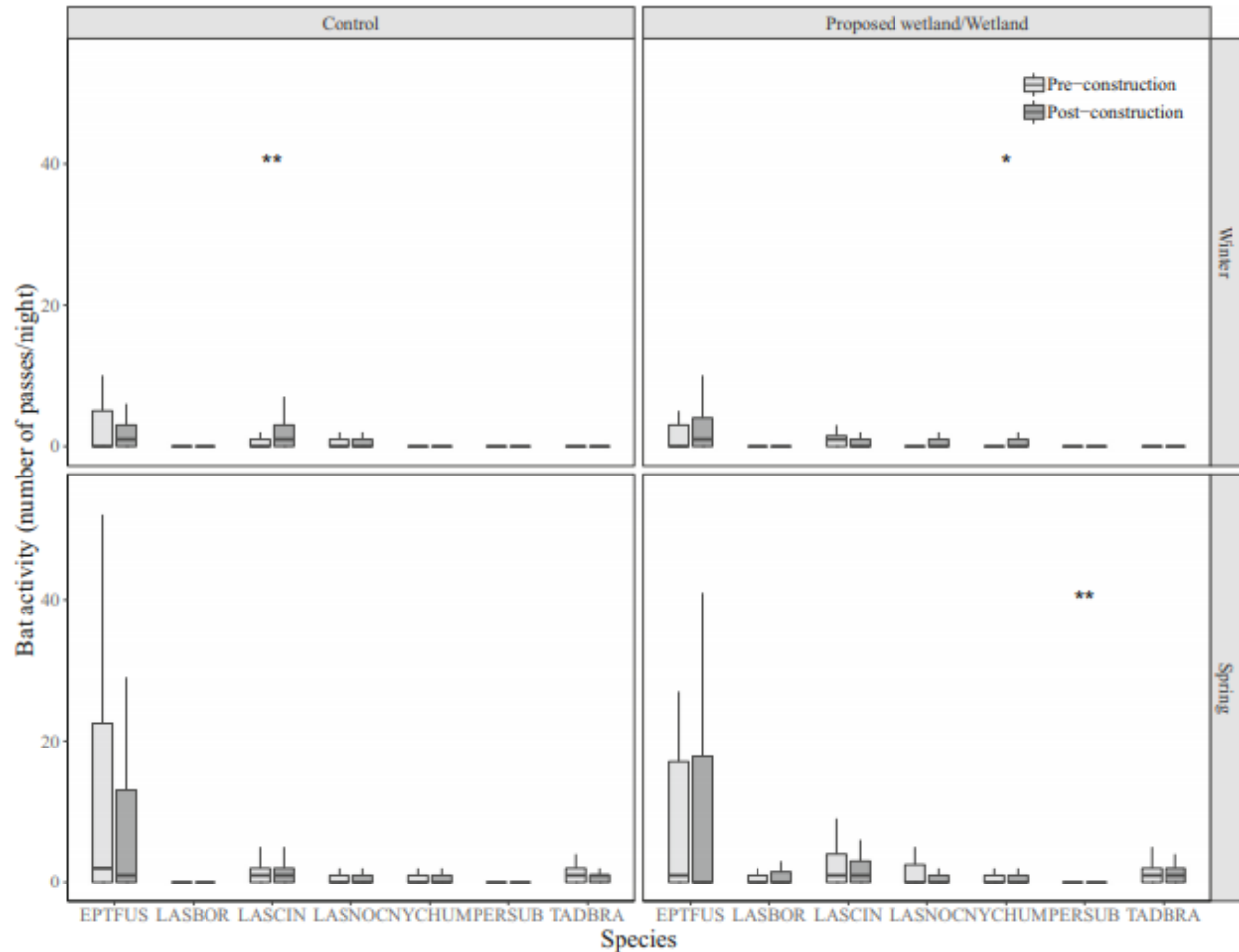


Fig. 4. Species specific bat activity (number of passes per night) in control and proposed wetland sites before the wetland construction in the Winter (November 19th, 2016-January 31st, 2017) and Spring (February 1st, 2017-March 14th, 2017), and in control and wetland sites after the construction in the Winter (November 19th, 2017-January 31st, 2018) and Spring (February 1st, 2018-March 14th, 2018) at the University of North Carolina Greensboro. Species abbreviations are: *Eptesicus fuscus* (EPTFUS), *Lasiurus borealis* (LASBOR), *Lasiurus cinereus* (LASCIN), *Lasionycteris noctivagans* (LASNOC), *Nycticeius humeralis* (NYCHUM), *Perimyotis subflavus* (PERSUB), and *Tadarida brasiliensis* (TADBRA). In the Winter, LASCIN activity significantly increased at the control sites ($p = 0.002$, $n = 107$, Wilcoxon rank sum test) and NYCHUM activity increased at the wetland sites after the wetland construction ($p = 0.016$, $n = 105$, Wilcoxon rank sum test). In the Spring, only PERSUB activity increased at the wetland sites as compared to the proposed wetland sites ($p = 0.004$, $n = 72$, Wilcoxon rank sum test)

Our results also demonstrate that a rapid increase in bat activity coincides with the construction of an urban wetland. Our study included only 1 year of post construction monitoring. Similar to a study by Menzel et al. (2005) that showed bat activity responded rapidly to wetland construction in a natural environment, our study showed a rapid response to wetland construction in an urban site, emphasizing the importance of wetlands as habitats for bats regardless of the surrounding habitat. Bats are an excellent model organism to evaluate the effects of wetland construction or restoration as they (i) quickly respond to new wetlands, (ii) can be related to water quality and insect communities (Kalcounis-Rueppell et al. 2007; Li and Kalcounis-Rueppell 2018), (iii) can be sampled throughout the year using acoustics with less effort than traditional trapping methods,

and (iv) serve as indicators of seasonal differences in abundance or community structure (e.g. Whitsitt and Tappe 2009).

Interestingly, when we compared sites pre- and post-construction while accounting for season, the effect we saw was in the Winter, and not the Spring. Although many bats in the piedmont region, where Greensboro is located, migrate during the Winter, some bats are resident (Grider et al. 2016, K. Parker, H. Li, and M.C. Kalcounis-Rueppell unpublished data). Our results suggest that constructed wetlands may be important for overwintering bats in urban areas. Alternatively, resident bats may have discovered the wetlands, on the landscape, in the Winter more quickly than the migratory/non-resident bats in the Spring. If discovery time is part of the pattern we report, we would expect that differences between the Winter and Spring seasons will be reduced over time. Yet another alternative is that constructed wetlands in the Winter are used for drinking, whereas in the Spring they are used for feeding. As we primarily recorded our data in early Spring, it may be that any effects of the wetlands based on food (i.e., insect emergence) were not seen because we were too early in our Spring sampling.

We were very conservative in our bat species identification from recording and there is bias in confidence in species identification. For that reason, it is difficult to speculate on particular species' responses to wetland construction. Rather, our results show us that all species respond, and the mechanism of response likely differs by species. For example, both *E. fuscus* and *P. subflavus* demonstrated an increase in activity following wetland construction. Appropriate freshwater sources are an essential component of many bat species habitats (Salvarina 2016) and previous literature has shown correlations, albeit with different patterns and mechanisms, between *E. fuscus* and *P. subflavus* activity and water sources, and water quality (Kalcounis-Rueppell et al. 2007; Li and Kalcounis-Rueppell 2018). The presence of constructed wetlands may allow for further study on the impact of water quality on bat abundance and activity and we are not aware of studies of wetlands construction that have focused on the relationship between water quality and bat abundance and activity.

Descriptive studies on the effect of wetland restoration on bats in the Southeast lack before and after sampling data (Menzel et al. 2005) making our study unique and useful as a model for assaying bat biodiversity in future studies. Using our acoustic monitoring approach and the results of our study, we envision several additional areas of future research. First, continuous monitoring of the wetlands for several years will help us understand the temporal process of response of bat communities to wetland construction. Our results are consistent with the Menzel et al. (2005) study, that increases in bat activity after wetland restoration can occur within a short timescale because our analyses included less than one year of post-construction monitoring. Furthermore, Kerbiriou et al. (2018) observed that older, artificial wetlands supported greater bat activity than more recent wetlands. We are not aware of studies that have focused on the *process* of bat community change over long periods of time. Second, we expect that much of the increased activity of bats at wetlands is a response to increased insect abundance and consequent opportunities for foraging. While the increase in bat activity at constructed wetlands is consistent with the hypothesis that bats use constructed wetlands to forage (Park and Cristinacce 2006; Stahlschmidt et al. 2012), we could directly test that hypothesis by comparing the proportions of foraging buzzes produced at wetland and control sites or before and after construction. Third, the study could be expanded to additional woodland and open wetlands with

a variety of habitat types to determine the impact of the habitat type surrounding wetland on bat activity and community structure. Fourth, monitoring data at the wetland sites could also be used to observe the impact of wetland construction on direct competition and other social behaviors in bats.

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