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The Effect of Water Quality on Bat Activity over Streams in the Middle Chattahoochee River Watershed in Western Georgia

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Abstract

North American bat species are linked to freshwater ecosystems, and poor water quality could be having an impact on bat populations. Bat populations are sensitive to a variety of environmental factors such as disease, pollution events, and fluctuating population and diversity of prey species. We hypothesized that higher quality freshwater systems, having large diverse populations of macroinvertebrates and low amounts of *E. coli*, would be correlated with bat activity. Our results show that overall, bat activity is higher in healthier streams with diverse communities of macroinvertebrates. This study is important for demonstrating the link between water quality and the environment as well as to help further the study and protection of declining bat populations in North America.

Introduction

North America is experiencing an overall decline in bat populations (Rodhouse et al. 2019), partially due to the sensitivity to environmental change among members of this taxonomic group (Salvarina 2015, Phelps and Kingston 2018). The infection of many different bat species by *Pseudogymnoascus destructans* or White Nose Syndrome (WNS) is the dominant cause of decline in North American bats, resulting in a 90% reduction in population size of some species (Alves et al. 2014, Cheng et al. 2021). In addition, habitat destruction, biotic and abiotic factors, and pollution events contribute to declining bat populations (O'Shea et al. 2015). Understanding the ways in which bats interact with their environment and vice versa can help to further the protection of North American freshwater ecosystems.

Riparian habitats are of particular importance to bats. Healthy riparian habitats with a mixture of large open waterways and forest canopies support a diverse bat community, providing key habitat for both habitat specialists and generalists (Biscardi et al. 2017). These riparian habitats provide fresh drinking water, roosting trees, and many different insects, and can serve as corridors between foraging areas (Johnson et al. 2010).

Bat activity in general is linked to freshwater ecosystems, and bats are considered top predators in riparian streams. As a habitat generalist, *Nycticeius humeralis* is commonly found in freshwater ecosystems; however, previous studies have shown that they are less impacted by water quality, due to the large diversity in their diet. *Perimyotis subflavus* is a riparian habitat specialist and has been shown to be impacted by water quality, as their diet mainly consists of *Dipterans* (Kalcounis-Rueppell et al. 2007, Li and Kalcounis-Rueppell 2017, Feldhamer et al. 2008). As foragers, *P. subflavus*, *N. humeralis*, as well as *Eptesicus fuscus*, and several *Myotis* species rely on diverse aquatic and terrestrial insect communities to suit their nutritional needs. Poor water quality and degraded riparian habitats can impact foraging behavior and food availability for these species (Biscardi et al. 2007, Feldhamer et al. 2008, Johnson et al, 2010).

Stream health and pollution levels can be estimated using several different methods. Often stream health is assessed through aquatic insect biomonitoring (Bonada et al. 2006). Macroinvertebrates are typically unable to escape pollution events and are small enough to be collected in large numbers and easily identified. Some taxa of macro-invertebrates are sensitive to levels of pollutants in water as well as oxygen levels. Invertebrates in the order *Ephemeroptera*, *Plecoptera*, and *Trichoptera* (EPTs) are especially sensitive to unhealthy streams. Healthy freshwater ecosystems will often have both a large and diverse population of EPTs and other groups of macroinvertebrates. Species in the order *Diptera* can vary on their indication of stream health. Those in family *Athericidae* are very sensitive to pollutants; families *Tipulidae* and *Simuliidae* are somewhat tolerant of pollutants, while species in the *Chironomidae* family can be extremely tolerant of pollutants. Large quantities of macroinvertebrates, such as those in the order *Hirudinea* or class *Oligochaeta*, usually indicate poor stream health, as they are more tolerant of low oxygen levels and pollutants (Sallenave 2015, Kenney 2009).

Bacterial biomonitoring is another way to assess water quality in freshwater ecosystems. Levels of *Coliform* bacteria or *Escherichia coli* can indicate amounts of fecal matter or pollutants from sewage in aquatic systems. While a majority of *E. coli* found in a water supply is not disease causing, the higher the amount of *E. coli* in a body of water, the greater the probability for pathogenic strains to be present (Standridge 2008).

This study aims to investigate the link between bat activity in riparian areas, as measured by acoustic sampling of bat echolocation calls, and overall stream health. The authors hypothesize that streams that are considered healthier, having more diverse macroinvertebrate counts and low amounts of *E. coli*, will impact bat activity and bat species composition.

Methods

Three stream locations—Dixie Creek (33.06966 N/ 85.03667 W), Blue John Creek (32.99949 N/ 85.05141 W), and Flat Shoals Creek (32.84128 N/ 85.11643W)—in the Chattahoochee River watershed, Georgia, were selected as study sites. Each stream location was associated with continued water quality sampling by the Chattahoochee River Keepers and represented 3 levels of pollution (Table 1). Water quality levels were determined based on total *Coliform* and *Escherichia coli* quantified using Idexx Colilert ® kits to estimate Most Probable Number (MPN/ 100mL). These Idexx kits have been shown to be practical for field work and effective for measuring *Coliform* bacteria (Noble et al. 2003, Lee et al. 2014). The MPNs for both total *Coliform* and *E. coli* for sites were compared using a one-way ANOVA and Tukey's post hoc test in Jamovi (1.6.23).

Hester-Dendy (H/D) samplers were placed at each site to determine the composition of aquatic invertebrates. Evidence shows that H/D samplers are slightly more effective at measuring EPT richness than other methods of aquatic invertebrate sampling (Letovsky et al. 2012). The smooth Masonite boards of the H/D samplers also provide suitable habitat for some species of macroinvertebrates while being relatively easy to clean during processing and uniform in structure across all sample sites (Wilbanks et al. 2020). A cluster of three H/D samplers was left for 7 days—from 04/06/21 to 04/11/21— after which the samplers were collected. Samplers were stored in gallon-sized plastic bags with additional water from the stream in which collected. Samplers were rinsed with tap water into two fine mesh sieves and then transferred into collection jars. Larger plant material, sticks, rocks, and sand were also retained in the collection jars. Collection jars were stored in the refrigerator when not being examined. Invertebrates were identified visually under a microscope using the *West Virginia Department of Environmental Protection Field Guide to Aquatic Invertebrates*. Positively identified organisms were kept in a sample jar filled with ethanol.

Individuals of each taxonomic group were tallied for each stream sample to characterize community composition. EPT taxa richness or %EPTs was determined by dividing the total number of individuals in the orders *Ephemeroptera*, *Plecoptera*, and *Trichoptera* by the total number of individuals in the sample (Reif 2002). Total species richness was calculated using the Shannon-Wiener diversity index (H') (Nikleka et al. 2013).

Bat community composition and activity were determined using acoustic detection techniques. An Anabat Swift bat detector was placed at each site and left overnight. Acoustic sampling began 30 minutes prior to sunset and continued to 30 minutes after sunrise each evening. Each site was sampled 4 times; 2 times in April and 2 times in May (Table 1). Since only 2 detectors were available, detectors were rotated to ensure that each site sampled at least 2 evenings simultaneously with the other 2 sites. Detector microphones were oriented towards an open portion over the stream with slow-moving water and devoid of vegetational clutter to improve bat call quality (Hayes 2000).

Bat call data was downloaded using Anabat Insight. Only calls containing 5 or more pulses were considered for analysis. The software package BCID was used for initial call

Table 1. Study site locations and dates of acoustic sampling of bat echolocation calls at each site.

	UTM Coordinates		Acoustic Sampling Date					
Site	Northing	Westing	4/6/2021	4/7/2021	4/8/2021	5/13/2021	5/14/2021	5/15/2021
Dixie Creek	33.06966	-85.03667		X	X		X	X
Blue John Creek	32.99949	-85.05141	X		X	X		X
Flat Shoals Creek	32.84128	-85.11643	X	X		X	X	

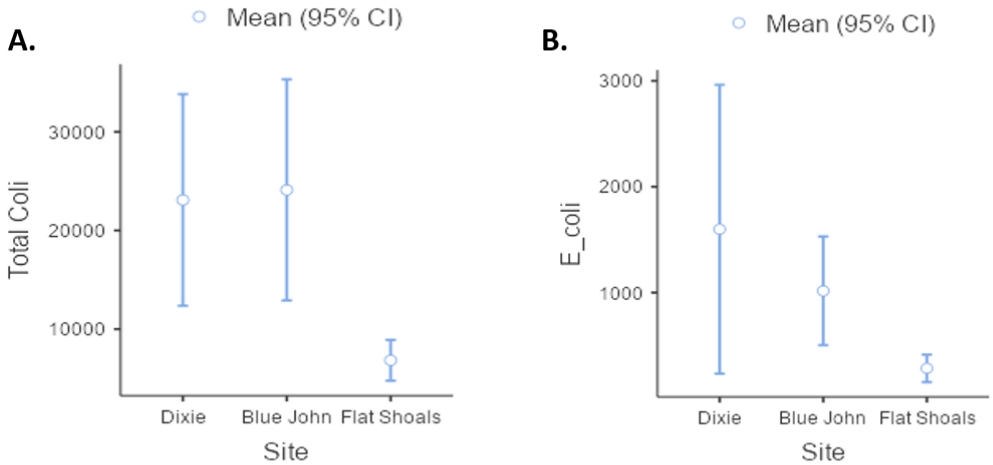

Fig. 1. Total *Coliform* concentration (A) and *E. coli* concentration (B) estimated as MPN/100ml for 3 stream locations in the Chattahoochee River watershed of western Georgia. Data downloaded from Chattahoochee River Keepers between June 2020 to April 2021.

identification, using the species list of potential species in Georgia. The default call filter was used to further reduce the number of echolocation calls for analysis. Calls were also visually identified to check automated identification accuracy. Relative abundance of bats was estimated by counting the number of echolocation call files recorded in a given 24-hour period. The numbers of call files for *E. fuscus*, *P. subflavus* and *N. humeralis* as well as for all bats combined were compared using a one-way ANOVA and a Tukey's post hoc test, in Jamovi (1.6.23), to determine if significant differences existed among study sites.

Results

Data collected by the Chattahoochee River Keepers from 06/18/2020 to 04/28/21 show that Dixie Creek had a total *Coliform* amount of 23092.6 MPN/ 100mL (SD = 32627 MPN/ 100mL) and a total *E. coli* amount of 1598.3 MPN/ 100mL (SD = 4149 MPN/ 100mL). Blue John Creek had a total *Coliform* amount of 24108.5 MPN/ 100mL (SD = 32635 MPN/ 100mL) and an *E. coli* amount of 1016.5 MPN/ 100mL (SD = 1493 MPN/ 100mL) (Figure 1A and 1B). Total *Coliform* amount for Flat Shoals Creek was 9685.7 MPN/ 100mL (SD = 6364 MPN/ 100mL), and total *E. coli* amount was 551.5 MPN/ 100mL (SD = 400 MPN/ 100mL). There was a significant difference in mean total *Coliform* MPN among the 3 study sites ($F=4.04$, $df= 2$, $p=<0.001$). There was no significant difference in mean total *Coliform* MPN between Dixie Creek and Blue John Creek ($p = 0.99$). Flat Shoals had significantly less mean total *Coliform* MPN than both Blue John Creek ($p = 0.011$) and Dixie Creek ($p = 0.012$) (Figure 1A). There was a significant difference in mean *E. coli* MPN among the 3 study sites ($F=5.63$, $df= 2$, $p=0.006$. There was no significant difference in mean *E. coli* MPN between the Dixie Creek site and the Blue John Creek site ($p=0.699$). Flat Shoals also had significantly less mean *E. coli* MPN than Blue John Creek ($p = 0.021$) but not Dixie Creek ($p = 0.141$) (Figure 1B).

Dixie Creek had the highest total number of macroinvertebrate individuals (497); however, 398 of those were *Chironomidae* (Table 2). This is possibly because this sampler became buried under the sediment of the stream during the time it was placed at Dixie Creek. Blue John Creek had the least number of individuals (12), and Flat Shoals Creek had a total of 152 individuals. Percent EPTs, a measure

Table 2. Total macroinvertebrate counts, percent EPTs, and H' for each of the three Hester-Dendy sampler locations from 04/06/21 to 04/11/21.

		Dixie Creek	Blue John Creek	Flat Shoals
Amphipoda	Gammaridae	31		
Bivalvia		3		
Coleoptera	Elmidae			4
Diptera	Chironomidae	398	3	8
	Simuliidae		3	47
Ephemeroptera	Heptageniidae			9
Megaloptera	Corydalidae			2
Nematoda		36		
Oligochaeta			4	1
Plecoptera				38
Prostigmata	Hydrachnidae			1
Trichoptera		29	2	42
Total Individuals		497	12	152
%EPT		5.8%	16.7%	58.6%
Shannon-Wiener Index (H')		0.74	1.38	1.61

Table 3. Mean number of bat call files (4 nights at each site) for total number of bats, big brown bats (EPFU), evening bats (NYHU) and tri-colored bats (PESU) for 3 stream sites in the Chattahoochee River watershed of western Georgia. For a given comparison, sites with different numbers of asterisks indicate significant differences.

	Number of call files			One-way ANOVA
Species	Dixie Creek	Blue John Creek	Flat Shoals Creek	p-value
Total bat calls	6.5 (7.55)*	26.3 (20.4)*	325 (154)**	0.028
EPFU	0 (0)	1 (2)	3.75 (4.35)	0.195
NYHU	2 (2.31)*	8.5 (6.45)*	25.5 (18.1)**	0.04
PESU	0.5 (0.577)*	3.5 (2.89)*	236 (72.4)**	0.007

of *Ephemeroptera*, *Plecoptera*, and *Trichoptera* taxa richness, was measured by dividing the total number of individuals in those taxa by the total number of individuals in the sample (Reif 2002). Flat Shoals Creek had the highest %EPT at 58.6%. The %EPT of Blue John Creek was 16.7%, and for Dixie Creek it was 5.8% (Table 2). Using the Shannon-Wiener diversity index (H') (Nikleka et al. 2013), Flat Shoals had the greatest species richness, H' = 1.61. Species richness for Blue John Creek was H' = 1.36 and for Dixie Creek was H' = 0.74 (Table 2).

The Flat Shoals Creek site had a mean total number of bat call files of 325 (SD = 154) calls, Blue John Creek had a mean total number of bat call files of 26.3 (SD = 20.4) calls, and Dixie Creek had a mean total bat call files of 6.5 (SD = 7.55) calls (Table 3). There was a significant difference in the mean total number of bat calls among the 3 study sites (F=8.77, df=2, p=0.028). There was no significant difference between mean total number of bat calls between the Dixie Creek site and the Blue John Creek site (p=0.948). The mean total number of bat calls at the Flat Shoals Creek site was significantly greater than the mean total number of bat calls at both the Dixie Creek site (p=0.002) and the Blue John Creek site (p=0.003) (Table 3). There was a significant difference in mean number of bat calls identified as *P. subflavus* among the 3 study sites (F=20, df=2, p=0.007). There was no significant difference in mean number of P. subflavus calls between the Dixie Creek site and the Blue John Creek site (p=0.994). The mean number of *P. subflavus* was significantly greater at the Flat Shoals site than at the Dixie Creek (p=<0.001) and Blue John Creek sites (p=<0.001) (Table 3). There was a significant difference in the mean number of bat calls identified as *N. humeralis* among the 3 study sites (F=4.71, df=2, p=0.04). There was no significant difference in the number of *N. humeralis* calls between the Dixie Creek site and the Blue John Creek site (p=0.7). There was no significant difference in the number of *N. humeralis* calls between the Blue John Creek site and the Flat Shoals Creek site (p=0.134). The mean number of bat call files identified as *N. humeralis* was significantly greater at Flat Shoals Creek than at Dixie Creek (p=0.034, Table 3). There was no significant difference in the mean number of bat calls identified as *E. fuscus* among the 3 study sites (F=1.97, df=2, p=0.195, Table 3).

Discussion

The results of this study show a significantly greater bat activity at the healthier stream site than at the stream sites with lower stream health indicators. Flat Shoals Creek was determined to be the healthiest stream of all three sites, having the highest %EPTs and the highest aquatic invertebrate diversity level of all three study sites (Table 2). Flat Shoals Creek also had the lowest total *Coliform* and total *E. coli* amounts of all three stream sites (Figure 1A and 1B). Bat activity measured over Flat Shoals Creek site was significantly higher than at the other two sites as well (Table 3).

Activity levels of *P. subflavus* have been shown to be linked with the water quality of freshwater ecosystems. *P. subflavus* has a diet associated with soft-bodied aquatic insects, with a preference for *Chironomidae* (Feldhamer et al 2008). Several studies have indicated that *P. subflavus* activity tends to increase as water quality declines and *Chironomidae* populations increase (Kalcounis-Rueppell et al. 2007, Li and Kalcounis-Rueppell 2017, Feldhamer et al. 2008). However, the results of this study indicated that *P. subflavus* activity was greater at the healthy stream site. Biscardi et al. (2007) also found that even though *Chironomidae* is the preferred food source for many species of bats, these species still prefer the healthier riparian systems with diverse aquatic insect communities. *P. subflavus* prefers *Chironomidae* as a large portion of its diet; however, they also show a preference for *Trichopterans* when they are available in large numbers. Like many other species native to freshwater riparian systems, *P. subflavus* is a dietary generalist and requires a somewhat diverse diet to meet its nutritional needs (Biscardi et al. 2007, Feldhamer et al. 2008, Johnson et al, 2010, Kalcounis-Rueppell et al. 2007, Li and Kalcounis-Rueppell 2017). While the amount of *Chironomidae* was greatest at Dixie Creek, other stream quality measures indicated this site to have lower stream health than Flat Shoals Creek (Table 2, Figure 1A and 1B). The lack of macroinvertebrate diversity at this site seems to be correlated with lower levels of bat activity (Tables 2 and 3).

Many other habitat variables may influence bat use and community composition at a site. Stream width, depth, and flow can impact a species' use of that habitat as well as the composition of riparian vegetation, which can also impact aquatic macroinvertebrate communities (Salvarina 2015). It should be noted that the physical characteristics of the three sites may have impacted the results of this study. While all sites had similar canopy cover and vegetation surrounding the streams, the width, depth, and flow of all the streams were different, which may have impacted macroinvertebrate composition during sampling. The H/D sampler placed at Dixie Creek became buried in sediment at some point during the week when it was placed. This may have artificially inflated the number of *Chironomidae* individuals in the sample, as *Chironomidae* are known to prefer silt sediment habitats (Sallenave 2015). It should also be noted that bat trapping may have been a more accurate method of determining species composition and bat activity at each of the sites; however, due to concerns of potential cross-species transmission of COVID-19, acoustic sampling was deemed the safest method for sampling the bats.

The cause of the relatively low total amount of macroinvertebrate individuals at Blue John Creek is unknown. The stream had a depth, width, and flow similar to that of Dixie Creek; however, the H/D sampler for Blue John Creek did not become buried in sediment. Further testing is needed to determine more accurately the community composition of macroinvertebrates at these two sites, and to determine the impact of the physical characteristics of the riparian habitat on the bat community composition.

Works Cited

Alves, Davi M.C.C., Levi C. Terribile, Daniel Brito. 2014. "The Potential Impact of White-Nose Syndrome on the Conservation Status of North American Bats." *PLoS ONE*. 9(9): e107395.

Biscardi, S., D. Russo, V. Casciani, D. Cesarini, M. Mei, L. Boitani. 2007. "Foraging Requirements of the Endangered Long-Fingered Bat: the Influence of Micro-Habitat Structure, Water Quality and Prey Type." *Journal of Zoology*. ISSN 0952-8369.

Bonada, Nuria, Narcis Prat, Vincent H. Resh, and Bernhard Statzner. 2006. "Developments in Aquatic Insect Biomonitoring: A Comparative Analysis of Recent Approaches." *Annual Review of* Entomology, 51: 495-523.

Cheng, T. L., J.D. Reichard, J.T.H. Coleman, T.J. Weller, W.E. Throgmartin, B.E. Reichert, A.B. Bennett, H.G. Broders, J. Campbell, K. Etchison, D.J. Feller, R. Geboy, T. Hemberger, C. Herzog, A.C. Hicks, S. Houghton, J. Humber, J.A. Kath, R.A. King, S.C. Loeb, A. Masse, K.M. Morris, H. Niederriter, G. Nordquist, R.W. Perry, R.J. Reynolds, D.B. Sasse, M.R. Scafini, R.C. Stark, C.W. Stihler, S.C. Thomas, G.G. Turner, S. Webb, B.J. Westrich, W.F. Frick. 2021. "The Scope and Severity of White-Nose Syndrome on Hibernating Bats in North America." *Conservation Biology*. 2021; 1-12.

Feldhamer, George a., Timothy C. Carter, and John O. Whitaker Jr. 2008. "Prey Consumed by Eight Species of Insectivorous Bats from Southern Illinois." *American Midland Naturalist*, 162: 43-51.

"Field Guide to Aquatic Invertebrates" West Virginia Department of Environmental Protection. 2021.

Hayes, J.P. 2000. "Assumptions and Practical Considerations in the Design and Interpretation of Echolocation-Monitoring Studies." *Acta Chiropterologica*, 2: 225-236.

Johnson, Joshua B., W. Mark Ford, John w. Edwards, Michael A. Menzel. 2010. "Bat Community Structure within Riparian Areas of Northwestern Georgia, USA." *Folia Zoology*. 59 (3): 192-202.

Kalcounis-Rueppell, M.C., V.H. Payne, S.R. Huff, A.L. Boyko. 2007. "Effects of Wastewater Treatment Plant Effluent on Bat Foraging Ecology in an Urban Stream System." *Biological Conservation*, 138 (2007): 120-130.

Kenney, Melissa A., Ariana E. Sutton-Grier, Robert F. Smith, and Susan E. Gresens. 2009. "Benthic Macroinvertebrates as Indicators of Water Quality: The Intersection of Science and Policy." *Terrestrial Arthropod Reviews*, vol. 2, no. 2, July 2009, pp. 99-128.

Lee, Lee H., Meiyin Wu, Alexandra Peri, Tin-Chun Chu. 2014. "Method Evaluations for *Escherichia coli* and Coliforms Detection in Northern New Jersey Water Bodies." *GSTF Journal of BioSciences*. Vol.3 No.1, August 2014.

Letovsky, Erin, Ian E. Myers, Alexandra Canepa, Declan J. McCabe. "Differences between Kick Sampling Techniques and Shot-Term Hester-Dendy Sampling for Stream Macroinvertebrates." *BIOS*, 83(2): 47-55.

Li, Han and Matina Kalcounis-Rueppell. 2017. "Separating the Effects of Water Quality and Urbanization on

Temperate Insectivorous Bats at the Landscape Scale." *Ecology and Evolution*. 2018; 8: 667-678.

Nikleka, Enkeleda, Spase Shumka, Rozeta Hasalliu. 2013. "Application of Shannon-Wiener Index and other Parameters as a Measure of Pollution in a Drinking Reservoir." *International Journal of Ecosystems and Ecology Sciences*. Vol.3 (3): 453-458 (2013).

Noble, Rachel T., Stephen B. Weisberg, Molly K. Leecaster, Charles D. McGee, Kerry Ritter, Kathy O. Walker, Patricia M. Vainik. 2003. "Comparison of Beach Bacterial Water Quality Indicator Measurement Methods." *Environmental Monitoring and Assessment*. 81: 301-312, 2003.

O'Shea, Thomas J., Paul M. Cryan, David T.S. Hayman, Raina K. Plowright, Daniel G. Streicker. 2015. "Multiple Mortality Events in Bats: a Global Review." *Mammal Review*. ISSN 0305-1838

Phelps, Kendra L., Tigga Kingston. 2018. "Environmental and Biological Context Modulates the Physiological Stress Response of Bats to Human Disturbance." *Oecologia*. (2018) 188: 41-52.

Reif, Andrew G. 2002. "Assessment of Stream Quality Using Biological Indices at Selected Sites in the Red Clay and White Clay Creek Basins, Chester County, Pennsylvania, 1981-97." *U.S. Geological Survey*. USGS Fact Sheet FS-118-02.

Rodhouse, Thomas J., Rogelio M. Rodriguez, Katharine M. Banner, Patricia C. Ormsbee, Jenny Barnett, Kathryn M. Irvine. 2019. "Evidence of Region-Wide Bat Population Decline from Long-Term Monitoring and Bayesian Occupancy Models with Empirically Informed Priors." *Ecology and Evolution*. 2019; 9: 11078-11088.

Sallenave, Rossana. 2015. "Stream Biomonitoring Using Benthic Macroinvertebrates." New Mexico State University. Circular 677, pp. 1-12.

Salvarina, Ioanna. 2015. "Bats and Aquatic Habitats: a Review of Habitat Use and Anthropogenic Impacts." *Mammal Review*. ISSN 0305-1838

Standridge, Jon. 2008. "'*E. coli*' as a Public Health Indicator of Drinking Water Quality." *American Water Works Association Journal*, vol. 100, no. 2, Feb. 2008, pp. 65–75

Wilbanks, Kelsey A., Damon L. Mullis, J. Checo Colon-Gaud. "Comparison of a Wood Sampler for Macroinvertebrate Bioassessment of Non-Wadeable Streams in the Southeastern Coastal Plain." *Journal of Freshwater Ecology*, vol. 35, no. 1, Jan. 2020, pp. 429–448.