A STUDY OF AMPHIBIAN POPULATIONS INSIDE AND OUTSIDE A COMBINED SEWAGE OVERFLOW WATERSHED IN MONTGOMERY COUNTY, TENNESSEE

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A STUDY OF AMPHIBIAN POPULATIONS INSIDE AND OUTSIDE A COMBINED SEWAGE OVERFLOW WATERSHED IN MONTGOMERY COUNTY, TENNESSEE

A Thesis

Presented to the Graduate School

In Partial Requirement for the Master of Science Degree

Austin Peay State University

Joshua Lucas Maloney

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DEDICATION

This is dedicated to my family and friends,
who have always been there for support and encouragement.

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I would like to first thank my major professor, Dr. A Floyd Scott, who introduced me to the field of Herpetology. With what seemed to be an endless amount of patience, he instilled organization, professionalism, and most importantly a sense of honor in my approach at becoming a field biologist. I would also like to thank Dr. Steven Hamilton and Dr. Mack Finley, who were instrumental in editing and improving this manuscript. And to Dr. Edward Chester for his superior ability to identify even the smallest local twig.

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ABSTRACT

Combined Sewer Overflows (CSOs) carry both sanitary sewage and storm-water runoff. When the carrying capacity of the system is exceeded some of the mixture of sewage and water may overflow from manholes and find its way into steams and other bodies of water in the surrounding drainage basin. This study looked at the amphibian fauna along a first-order stream in a CSO drainage basin of Clarksville, Tennessee and compared it with that of two other streams of similar size and character: 1) another urban stream in an adjacent drainage of Clarksville that had separate sewage and storm-water systems and 2) a Tennessee ecoregion reference stream in a rural setting 20 km to the southeast. Sampling involved time constrained searches that were conducted in spring, summer and fall at three sample sites along each stream from October 2004 through July 2006. Species richness of salamanders was lowest in the CSO drainage and highest in the reference stream, but no significant difference was detected between or among any of the sites. Frogs were absent in both of the urban streams, but were numerous in the reference stream, representing 6 species. Abundance of individuals (excluding the frogs) was lowest in the CSO drainage, somewhat greater in the adjacent urban stream, and highest in the rural reference stream. Results indicate that amphibian abundance in an urban setting is lower in streams with CSOs than in those where sewage and storm water are conveyed separately. Also suggested is a richer and more abundant amphibian fauna in rural versus urban drainages.

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CHAPTER I

INTRODUCTION

Most healthy freshwater ecosystems in temperate latitudes include a community of frogs and/or salamanders that interact with other biota as both predators and prey. Their abundance and diversity are usually highest in unpolluted aquatic habitats that are devoid of predatory fish. Amphibians are particularly important due to their ability to serve as indicator organisms that, by their presence or absence, help to determine the health of an aquatic ecosystem (Lannoo, 2005).

This study evaluated the possible pollution threat to freshwater amphibians posed by a combined sewer overflow system. This was achieved by comparing selected abiotic and biotic features of two urban streams, one inside a watershed with a combined sewer overflow system and the other in an adjacent watershed with separate storm-water and sewage conveyance. Both were also compared to a rural reference stream away from any municipal sewage/storm-water system. Species richness and relative abundance of amphibian (salamanders and frogs) populations in the two urban settings were compared with each other and with the reference stream.

Background and Literature Review

In order to better understand the premise of this study one must have a general comprehension of what a combined sewer system is, how it operates, and how it is regulated. Combined Sewer Systems (CSSs) were wastewater collection systems designed to carry sanitary sewage and storm-water (surface drainage from rainfall or snowmelt) in a single pipe to a treatment facility (U.S. Environmental Protection Agency, 1995). CSSs convey domestic, commercial, and industrial wastewater efficiently during dry weather, but in periods of heavy rainfall or snowmelt, total wastewater flows can exceed the capacity of the CSS. When this occurs, the CSS is designed to overflow directly to surface water bodies such as streams, lakes, and rivers (U.S. Environmental Protection Agency, 1995).

Regulation of water pollution in the United States began in 1972 with the passage of the Federal Water Pollution Control Act, which was further amended in 1977 when it became commonly known as the "Clean Water Act." This act was instrumental in the implementation of the National Pollution Discharge Elimination System (NPDES). The NPDES guidelines control water pollution by regulating it at the point source discharge. The United States Environmental Protection Agency (USEPA) is responsible for the regulation of point source discharge in the United States (33USC1251, 1972).

The EPA's Combined Sewer Overflow Policy was published on April 19, 1994, with the aim of controlling Combined Sewer Overflows (CSOs). This was to be accomplished through the NPDES permit program. The NPDES program provides guidance to both municipalities, and State and Federal permitting authorities on how to meet the Clean Water Act's pollution control goals. Obtaining a permit is required for discharge under this program (U.S. Environmental Protection Agency, 2001a). These permits ensure the development of a storm-water management plan that reduces pollutant discharge levels to the "Maximum Extent Practicable (MEP)" (U.S. Environmental Protection Agency, 2000). The management plans were required to: "identify major outfalls and pollutant loadings; detect and eliminate non-storm water discharges to the system; reduce pollutants in runoff from industrial, commercial, and residential areas;

and control storm water discharges from new development and redevelopment areas" (U.S. Environmental Protection Agency, 1996).

The provisions of the NPDES were expanded in 1990 to include regulation of point source discharge of harmful storm-water into the waters of the United States (EPA, 1996). Two phases of storm-water compliance were established requiring separate municipal storm and sewer water systems. Phase I applies to large and medium (populations greater than 100,000) municipal storm water systems (MS4), and phase II, for small (populations less than 100,000) MS4s. Phase I also required eleven categories of industrial activity to control damaging discharges (Appendix A). These industrial activities included construction that disturbed five or more acres of land (U.S. Environmental Protection Agency 1996). The requirements of Phase I and Phase II were identical except for timeline requirements, and went into effect in 1999 (U.S. Environmental Protection Agency, 2000).

In 1994 the USEPA issued the CSO Control Policy, a comprehensive policy which aimed to control the release of pollutants by CSOs (U.S. Environmental Protection Agency, 1994). The policy "required municipalities to meet specific health and environmental objectives, allowed control efforts to be tailored to site specific actions, allowed phased implementation to accommodate the municipalities fiscal abilities, and developed CSO strategies that reflect site specific wet weather effects of outfalls" (U.S. Environmental Protection Agency 1994). Additionally, municipalities were required to meet "nine minimum controls" (Appendix B) before 1 January 1997 (U.S. Environmental Protection Agency 1995).

Complications to the storm-water management program occurred in cities that operated CSSs (U.S. Environmental Protection Agency, 1994). These complications arose because the CSS collects sanitary sewage and storm water in the same system. During periods of heavy rainfall or snowmelt the system becomes overwhelmed. Old systems of this nature are designed to allow sanitary sewage to mix with storm water, which then exits the system prior to the publicly operated water treatment (POWT) facility (U.S. Environmental Protection Agency 2001b). This discharge of sewage and contaminated storm water is called an outfall, and may enter waters of the United States.

Combined sewer outfalls are capable of introducing an array of pollutants and pathogens into both lotic and lentic environments. Oxygen demanding chemicals are often included in these pollutants, which increase biochemical oxygen demand (BOD) while lowering the available oxygen in the water (SCR-PPP, 1998). This has been shown to cause fish kills during periods of high temperatures, affecting entire aquatic communities. Combined sewer outfalls have also been known to expel nitrogen to many watersheds throughout the United States (Puckett, 1994). High nitrogen concentration levels have been known to cause death and developmental anomalies in amphibians, and if high enough could have adverse effects throughout many watersheds in North America (Rouse et al., 1999). A study looking at chronic exposure to high concentrations of nitrogen in three species of amphibians (*Rana pretiosa*, *R. aurora*, and *R. boreas*) revealed larvae with reduced feeding activity, less vigorous swimming ability, disequilibrium, paralysis, and death (Blaustein et al., 1999).

Communities with CSSs are expected to develop long-term CSO control plans that will ultimately provide for full compliance with the Clean Water Act. The emphasis of these plans will be the attainment of water quality standards. CSO communities should begin developing these long-term control plans immediately with the ultimate goal of an expedient implementation. These plans should include "the characterization of the combined sewer system, monitoring the impacts of the CSO on local waterways, and discussing water quality and CSO control goals with the proper permitting and water quality authorities" (Data Source: http://cfpub1.epa.gov/npdes/cso/cpolicy.cfm).

CSSs are remnants of this country's early infrastructure and so are typically found in older communities. CSSs serve roughly 772 communities containing about 40 million people, including Clarksville, Tennessee. Most communities with CSSs (and therefore with CSOs) are located in the Northeast and Great Lakes regions, and the Pacific Northwest (Data Source: http://cfpub.epa.gov/npdes/cso/demo). In March 1990, the Tennessee Department of Environment and Conservation (TDEC) issued an agreed order to Nashville, Tennessee requiring specific sewer line improvements, wastewater-treatment plant expansions, and elimination of all non-permitted CSO discharges by the year 2001 (Thackston et al. 1999). These orders were to have all metropolitan areas conform to the EPA's criteria for water quality (U.S. Environmental Protection Agency, 1976).

Kemp and Spotila (1997) stated that pollution released by CSOs adversely affects the health of the receiving body of water. This was further supported by Rochfort et al. (2000), claiming that urban sources of wet-weather pollution such as storm water and combined sewer overflow can indeed contribute significantly to the contaminants of receiving water.

Because of their life history, physiology, abundance, and ubiquity, stream salamanders make the most likely biological indicators in small streams where they may replace fish as the top predators (Southerland et al., 2004). It has been known for some time that stream salamanders are sensitive to environmental stressors such as contaminants, drought conditions, and floods (which may contain storm-water runoff) (Petranka, 1998; Corn et al., 2003). Observing only fish and macroinvertebrates when concerned about water quality is simply not enough though. Under the provisions of the Clean Water Act the EPA recommends biological indicators from more than one organism group be used to develop biological criteria as part of water quality standards (U.S. Environmental Protection Agency, 1990). Southerland et al. (2004) compiled data necessary to create a stream salamander index of biotic integrity (SS-IBI) used to assess stream conditions in small (first-order) streams in the Non-Coastal Plain regions of Maryland. Even so, not much information is available concerning the impacts of sewage on stream salamander populations, and thus water quality. Thus comparing organisms sampled from sites potentially polluted with sewage with organisms from non-impacted sites may provide useful information on the possible effects of sewage pollution on water quality. This study intended to contribute data not only to support the use of stream salamanders as indicators of biological integrity in small streams, but to show a possible correlation between sewage pollution and amphibian health. In this process we hoped to produce data necessary to help enable the city of Clarksville, Tennessee to comply with current EPA standards by modifying its current Combined Sewer System. We also aimed to promote awareness of CSO pollution to the public.

CHAPTER II

MATERIALS AND METHODS

Study Sites

The study area included portions of three 1st order streams in Montgomery County, Tennessee (Fig. 1). Two of these streams are Gallows Hollow and Lime Kiln Hollow branches, both urban tributaries of the Cumberland River in Clarksville, Tennessee (Fig. 2). The third, which served as a reference stream, is an unnamed tributary to Passenger Creek, located in the extreme eastern portion of the county (Fig. 1). All three streams fall under the provisions of Phase II of the Storm Water Management Program, under EPA guidelines. Both urban streams are near the northern limit of the EPA's Level IV ecoregion 71f (Western Highland Rim), whereas the rural reference stream is at the southern limit of the Western Pennyroyal Karst ecoregion 71e just to the north.

Gallows Hollow Branch (36.52141°N, 87.36044°W to 36.52257°N, 87.35617°W) originates from seeps just east of the R.J. Corman railroad line and runs roughly parallel to it for a distance of about 1.5 km before emptying into the Cumberland River (Fig.2). Numerous commercial operations surround it to the north and a five-lane city street (Crossland Avenue) and residential area lie to the south. The stream is less than a meter wide for most of its length and only a few centimeters deep, though pools of deeper and wider dimensions may be encountered. The substrate is composed primarily of chert gravel and limestone rock (Fig.3), except for the 250-meter stretch that flows through Valley Brook Park near the stream's terminus (lower reach), which is composed primarily of sand and large rip-rap rocks. There are two flow patterns present, slow-

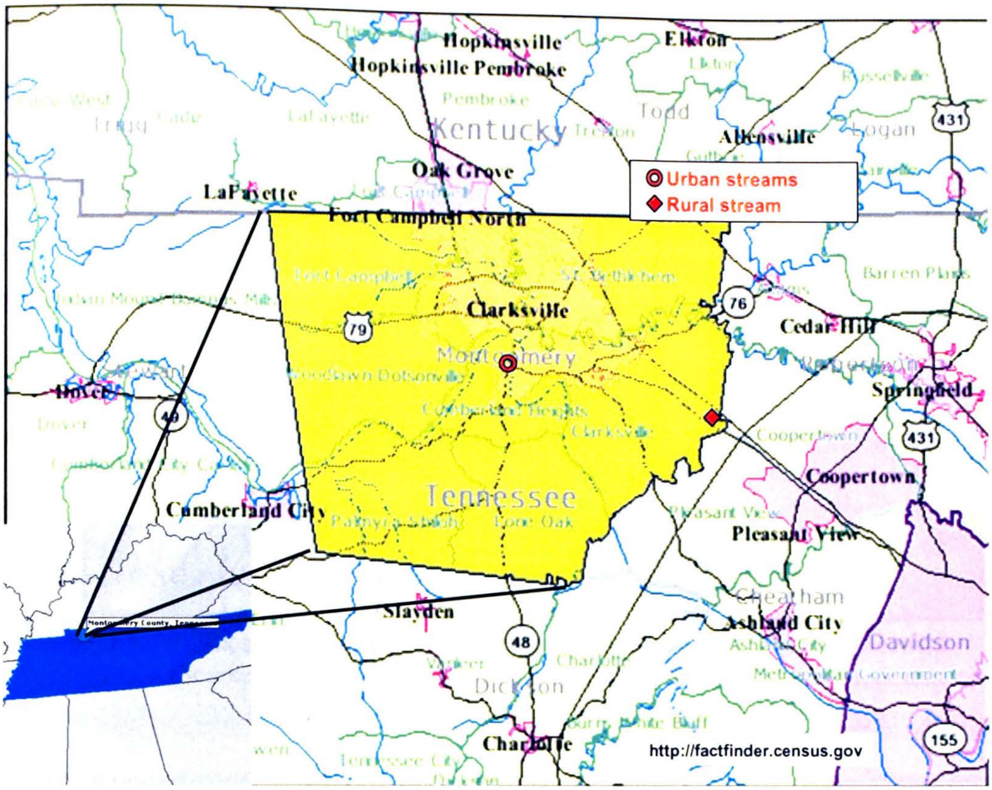

Figure 1. Location of the two urban drainages (Clarksville, Tennessee) and the rural reference drainage (I-24, Montgomery County, Tennessee).

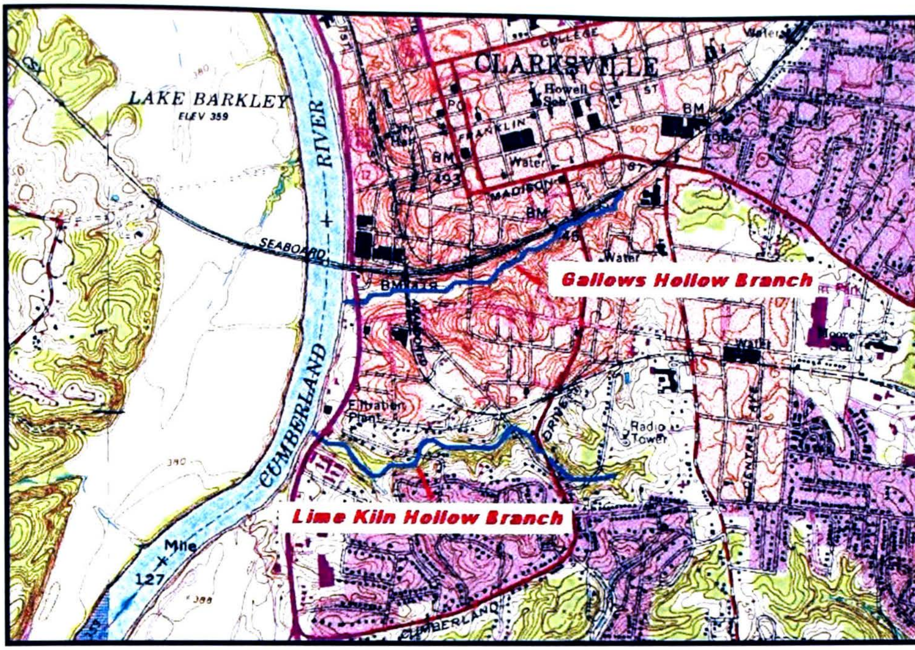

Figure 2. Location of Gallows Hollow and Lime Kiln Hollow branches in Clarksville, Tennessee. (Source of base map: Topozone topographic maps at http://www.topozone.com).

Figure 3. Gallows Hollow Branch looking upstream (east) above Valley Brook Park.

shallow and fast-shallow. Water almost never reaches the base of either the right or left banks, and sediment deposition is high. There is some evidence of erosion on both banks, though this does not seem to be dramatically affecting the stream. Channelization does not seem to be present, and there is a good deal of natural meandering.

The Valleybrook Park reach is very open and is dominated primarily by stream-side herbaceous plants such as white snakeroot and rattlesnake weed (beefsteak plant), though the occasional maple, magnolia, sycamore, and weeping willow are present. The middle and upper reaches are bordered mostly by a second-growth hardwood forest composed of black locust, box elder, maple, redbud with a thick understory of privet and bush honeysuckle. At the terminus of the upper reach the stream banks become quite inundated by the invasive Japanese Kudzu plant (see Appendix C for complete list of plant species collected along Gallows Hollow Branch).

Lime Kiln Hollow Branch (36.51445°N, 87.36224°W to 36.51627°N, 87.35575°W) drains the basin adjacent to and just east of the Gallows Hollow Basin. It is located in a residential area where some lawns stretch down to the stream edge, though most houses present are situated just beyond short yet dense riparian vegetation (Fig.4). A considerably mature second growth forest of oaks, elms, poplars, and hickories are present, though privet and bush honeysuckle seem to dominate and surround the mowed residential lawns (see Appendix D for complete list of plant species collected at Lime Kiln Hollow Branch). Two streets cross perpendicular to the stream, Charlotte Street near the upstream (east) end of the study area and Barker Street approximately in the middle of the study area. The stream bed is composed of a mixture of sand, gravel, and cobble surrounded by fine sediment (Fig. 4). Two main flow patterns are represented

Figure 4. Lime Kiln Hollow Branch looking upstream (east) at point below Barker Street.

including slow-shallow and fast shallow. Sediment deposition is somewhat high and water rarely reaches the base of both the lower right bank and the lower left bank without channel substrate exposure. Channelization, however, appears to be absent here and the stream exhibits a normal flow regime. The occurrence of riffles is relatively frequent and natural meandering produces many bends. The north bank is very stable with little to no undercutting, while there is evidence of erosion along several reaches of the south bank. In the upper reach of the stream a sanitary sewer line can be seen running parallel to the stream. In addition, the stream is crossed by two 10" sewer lines, one just downstream from the Charlotte Street crossing and the other immediately above the Barker Street crossing. The sewer line in this watershed is a separate sewer system and the stream receives urban storm water runoff only.

The unnamed tributary to Passenger Creek (36.46863°N, 87.12951°W to 36.46853°N, 87.13051°W) is located in the Red River drainage basin just south of Interstate Highway 24 near the Montgomery–Robertson County line in the extreme eastern portion of Montgomery County (Fig. 5). This stream has a natural channel with substrate composed of fine sediment, sand, gravel, cobble, and exposed limestone bedrock (Fig. 6). Pools, runs, and riffles are present throughout the section studied, except during periods of low rainfall when the upper reach dries up leaving only a few isolated pools. Sediment deposition is moderately low here with some sand bars occurring throughout. Both banks are mostly stable, though a few undercut areas are present. A well developed hardwood forest borders the stream's south bank and includes such tree species as oak, maple, elm, walnut, sycamore, cedar, paw-paw, and ironwood, while a narrow zone of deciduous trees and shrubs such as privet, knotweed, coralberry,

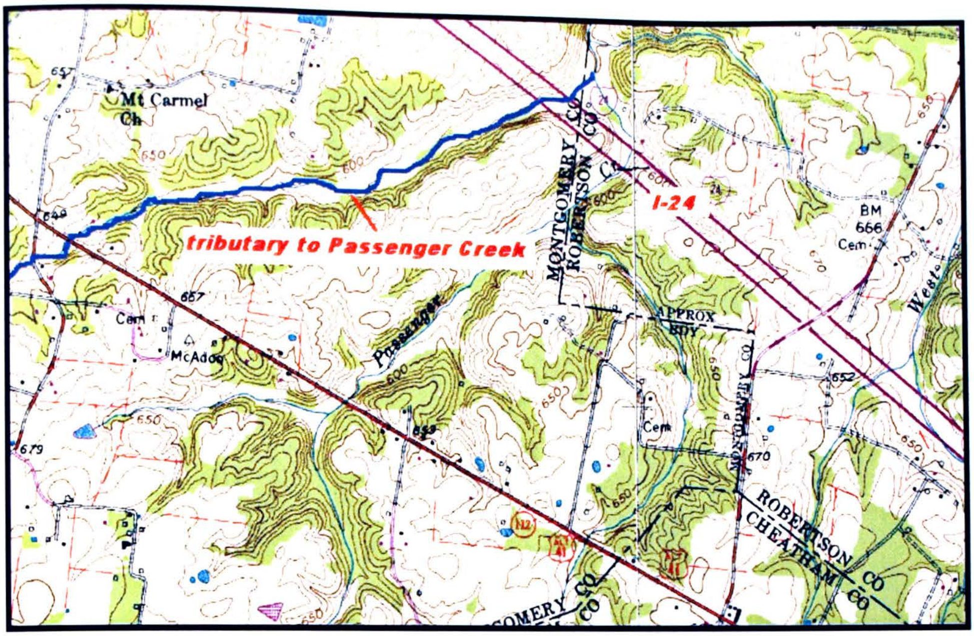

Figure 5. Location of the section of a tributary to Passenger Creek examined in this study in relation to topographic features, roads, and county boundaries. (Source of base map: Topozone topographic maps at http://www.topozone.com).

Figure 6. Tributary to Passenger Creek looking upstream just east of Interstate 24.

and hydrangea are found between it and open fields to the north (see Appendix E for complete list of plant species collected at the tributary to Passenger Creek).

Sampling Protocol and Procedure

Three comparable 1-km reaches were selected from each stream for study. During each sampling episode, each of the three stream reaches were searched for 15 minutes by two persons working upstream from three predetermined starting points. Starting points for each time-constrained search remained the same throughout the study and were located at 0 m, 333 m and 666 m from the downstream end of each 1-km reach. Figure 7 shows the location of stream reaches and starting points (designated lower, middle and upper) for time constrained searches along Gallows Hollow Branch and Lime Kiln Hollow Branch, and Figure 8 depicts the same information for the tributary to Passenger Creek. Table 1 gives the latitude/longitude coordinates of the starting points for each timed constrained search along all three stream reaches.

Time-constrained searches were conducted along each stream in spring (late May or early June), summer (late July or early August) , and fall (late September or early October). Gallows Hollow and Lime Kiln Hollow branches were surveyed on the same day and the tributary to Passenger Creek was sampled on the following day if at all possible. The order of days in which these streams were sampled was also alternated every sampling season to help reduce any time-related sampling bias (Table 2). During each time-constrained search, the two researchers proceeded upstream turning cover objects (principally rocks) in and along the edge of the water looking for amphibians. As animals were captured, they were secured in zip-lock bags labeled according to the stream and reach being sampled (e.g. Gallows Hollow, lower). When

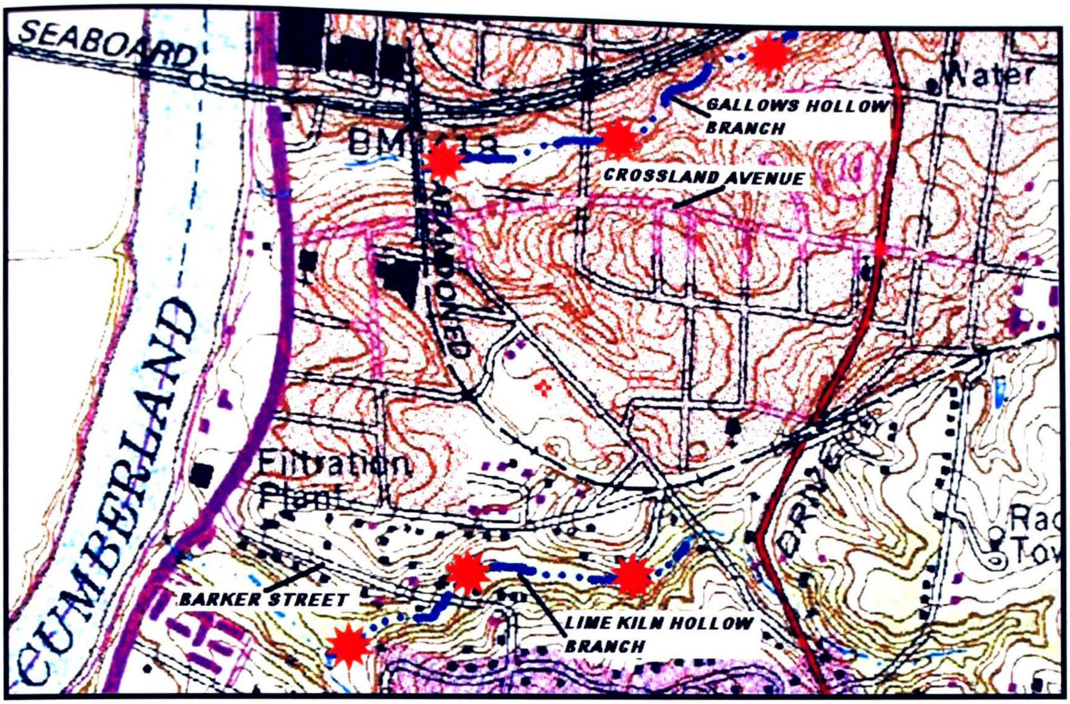

Figure 7. Locations of starting points for time-constrained searches (red stars) along the stretches (highlighted in blue) of Gallows Hollow and Lime Kiln Hollow branches selected for study as depicted on the USGS Clarksville, Tennessee topographic quadrangle, scale 1:25,000. (Source of base map: Topozone topographic maps at http://www.topozone.com).

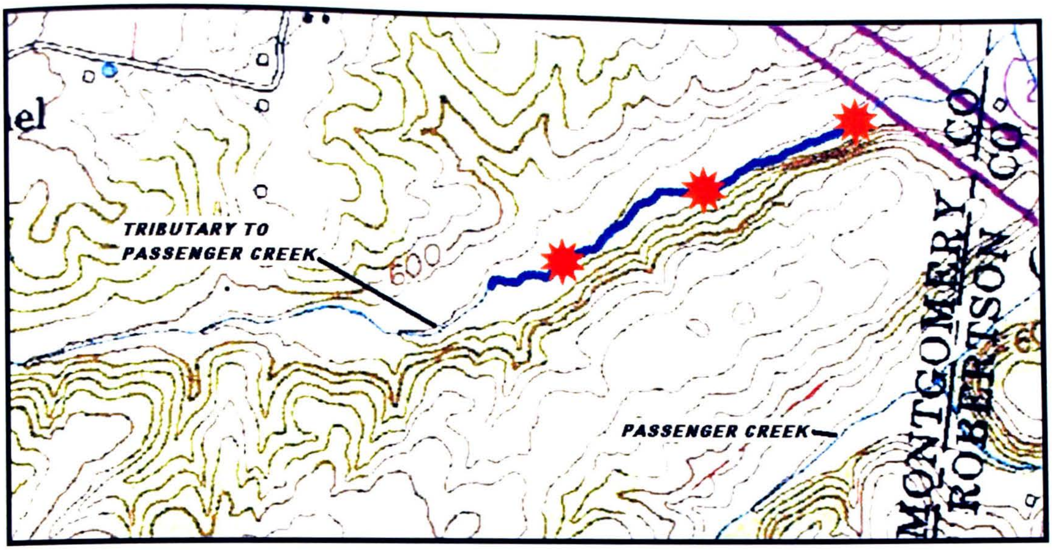

Figure 8. Locations of starting points for time-constrained searches (red stars) along the stretch (highlighted in blue) of a tributary to Passenger Creek selected for study as depicted on the USGS Clarksville, Tennessee topographic quadrangle, scale 1:25,000. (Source of base map: Topozone topographic maps at http://www.topozone.com).

Table 1. Latitude and longitude coordinates (decimal degrees) of beginning points for time-constrained searches along streams selected for study.

Stream name and reach	Latitude	Longitude
Gallows Hollow Branch		
Lower	36.521389	87.360833
Middle	36.521944	87.357222
Upper	36.523056	87.355278
Lime Kiln Hollow		
Lower	36.514444	87.362222
Middle	36.515556	87.360000
Upper	36.515556	87.356667
Tributary to Passenger Cr.		
Lower	36.468889	87.129444
Middle	36.467500	87.132778
Upper	36.466667	87.135278

Table 2. Seasons and dates over a two-year period during which sampling for amphibians was conducted at study streams. The asterisk (*) indicates bodies of water located in a CSO drainage.

	Stream		
Season	Gallows Hollow Branch*	Lime Kiln Hollow Branch	Trib. to Passenger Cr.
Fall 2004	4 Oct	4 Oct	5 Oct
Spring 2005	10 May	10 May	11 May
Summer 2005	29 Jul	29 Jul	1 Aug
Fall 2005	10 Oct	11 Oct	14 Oct
Spring 2006	24 May	24 May	23 May
Summer 2006	25 Jul	25 Jul	26 Jul

time expired and the search was over for a given reach, the animals (except voucher specimens) were released where they were captured after the following data were recorded for each individual amphibian captured: species name, sex (if determinable from external features), life stage (e.g., larva, metamorph, juvenile, or adult), reproductive-state (also if reflected externally), and microhabitat (e.g., under rock just above water at edge of riffle).

The following abiotic data were recorded during each sampling session: date, time start and finish, names of person(s) taking samples, general weather conditions, previous day's general weather, air temperature, water temperature, and pH of water. In the lab, all data were entered into a Microsoft Excel Worksheet for management and analysis. The occurrence of each amphibian species was recorded at each sampling site, and voucher specimens were brought back to the lab, fixed in 10% formalin then transferred to 40% isopropanol before being accessioned into the Austin Peay State University Museum of Zoology.

Statistical Analyses

Beyond running the usual descriptive statistics (means, modes, medians, standard deviations, and standard errors), a variety of inferential tests were employed when analyzing the data. A single factor Analysis of Variance (ANOVA) was the test chosen to compare the grand means of air temperature, water temperature and pH at the three streams. This test was also used to compare the means of snout-vent lengths of *E. cirrigera*, present in all three streams. The Chi Square Test for Goodness of Fit was used to determine if there was a significant difference among the ratios of the numbers of individual amphibians observed at the three streams versus those expected. The alpha level for significance for all tests was set at 0.05. Both the single factor ANOVA and Chi Square Test for Goodness of Fit were retrieved by utilizing the data analysis package in the Microsoft Excel program.

CHAPTER III

RESULTS

Abiotic Data

Abiotic data collected at the three streams during the study are summarized in Table 3. Mean temperatures and pH at the three streams fluctuated from sample to sample (Figs. 9, 10, and 11), although water temperature at Gallows Hollow Branch was noticeably higher during the last two sample sessions. The grand mean water temperature at Gallows Hollow Branch was the highest, and the tributary to Passenger Creek exhibited the lowest grand mean pH. No significant variation, however, was detected among the overall means for any of the abiotic variables measured at the study streams (single factor ANOVA- air temperature: $F = 0.17$, $P = 0.98$; water temperature: $F = 0.18$, $P = 0.85$); pH: $F = 2.08$, $P = 0.16$).

Amphibian Diversity

Salamanders were found in all three drainages, but varied in the numbers of species and individuals. Two species were found in Gallows Hollow Branch, and four species in both Lime Kiln Hollow Branch and the tributary to Passenger Creek (Table 4). Of the four species present, only the Two-lined Salamander (*Eurycea cirrigera*) was found in all three streams. The combined number of individuals encountered in each stream during the study is shown in Figure 12. Gallows Hollow Branch (within the CSO drainage) had the fewest animals (salamanders only), Lime Kiln Hollow Branch (in the neighboring drainage next to the CSO) had many more animals (salamanders only), and the tributary to Passenger Creek (outside the city of Clarksville in rural Montgomery County) had the most animals (both frogs and salamanders). An analysis of the

Table 3. Means for abiotic variables measured during each amphibian sampling session at each stream, during a study of the water quality inside and outside the Gallows Hollow drainage basin, October 2004 through July 2006. A dash (-) in a cell indicates missing data.

Sample Session	Gallows Hollow Branch			Lime Kiln Hollow Branch			Trib. to Passenger Creek		
	Air temp (C)	Water temp (C)	pH	Air temp (C)	Water temp (C)	pH	Air temp (C)	Water temp (C)	pH
Oct 2004	15.0	13.7	6.93	20.3	14.0	7.10	11.0	13.0	6.74
May 2005	20.7	15.0	7.76	25.0	15.9	7.77	24.7	15.0	7.18
Jul 2005	23.7	20.0	7.90	26.0	20.0	7.80	26.8	22.5	7.75
Oct 2005	16.7	14.3	7.70	15.3	15.7	-	22.0	17.0	7.20
May 2006	25.5	20.7	7.90	20.3	15.7	8.00	21.7	14.7	7.70
Jul 2006	28.3	24.7	7.70	25.7	20.3	7.80	26.7	20.5	7.20
Grand Mean **(± SD)**	21.7 (±5.16)	18.1 (±4.11)	7.65 (±0.36)	22.1 (±4.23)	16.9 (±2.59)	7.69 (±0.34)	22.2 (±5.89)	17.1 (±3.68)	7.30 (±0.38)

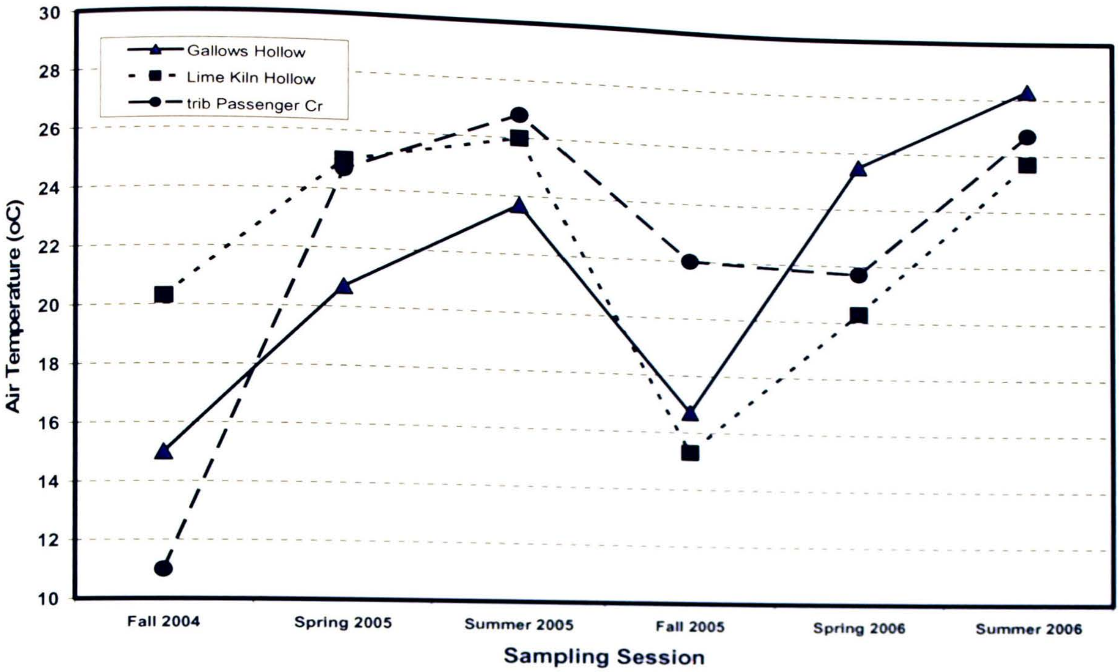

Figure 9. Means of air temperatures recorded at the three study streams during each sampling session.

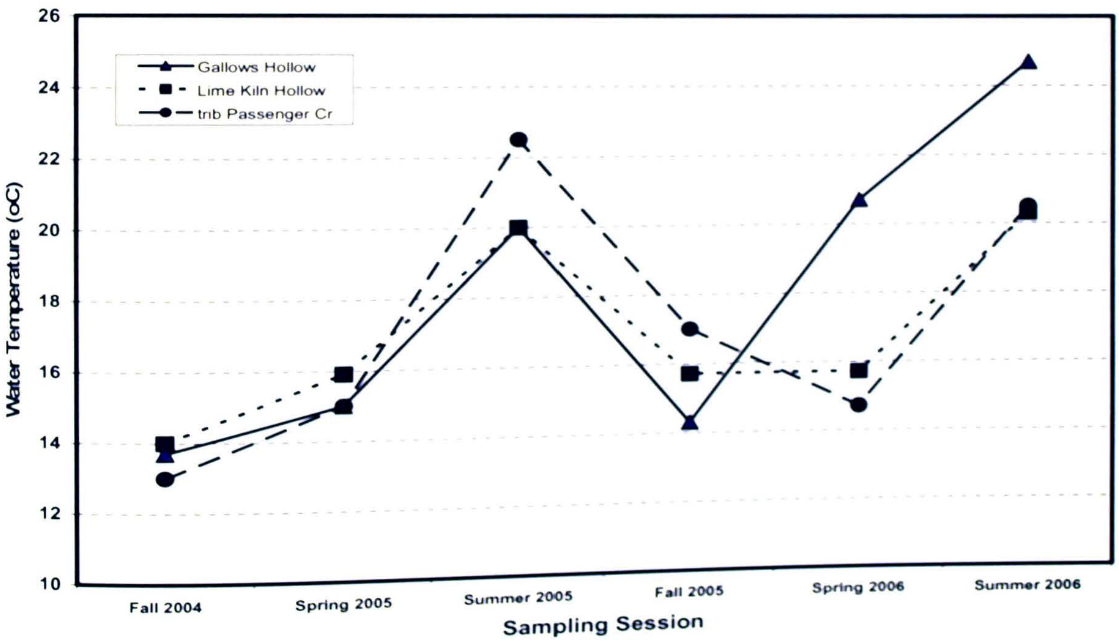

Figure 10. Means of water temperatures recorded at the three study streams during each sampling session.

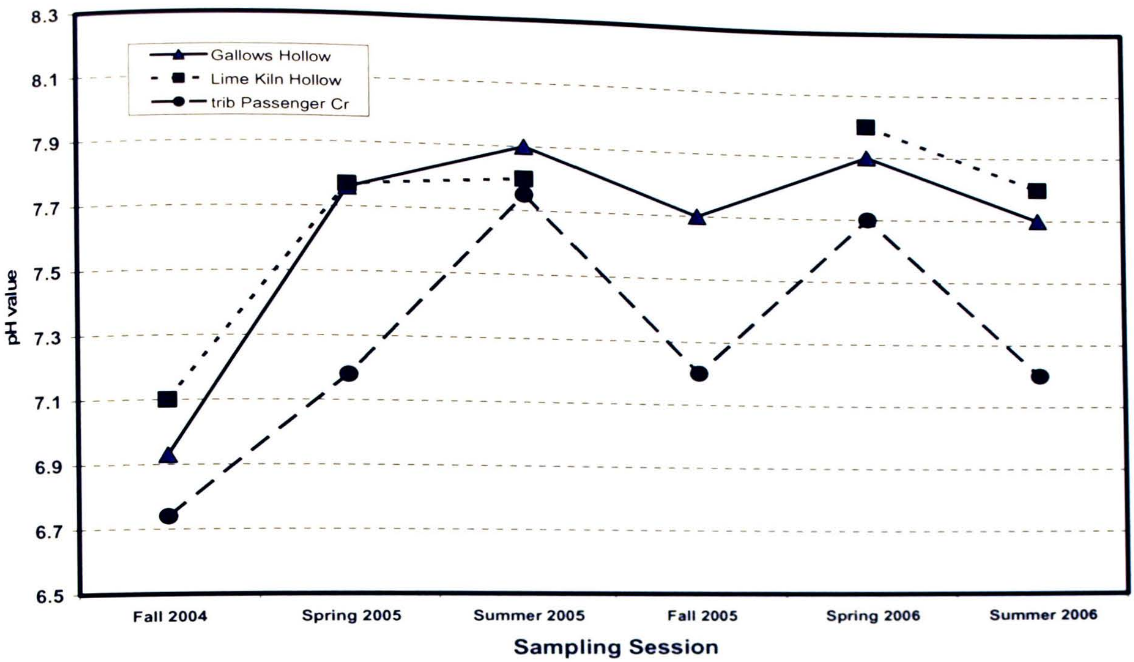

Figure 11. Means of pH values recorded at the three study streams during each sampling session.

Table 4. Counts of individual amphibians by species recorded at each stream during each sampling session. Fractions in the salamander portion of the table indicate the numbers of larvae (l) over post metamorphs (pm) in each sample.

	Streams																					
	Gallows Hollow Branch							Lime Kiln Hollow Branch							Trib. to Passenger Creek							
Taxon	Oct 2004	May 2005	Jul 2005	Oct 2005	May 2006	Jul 2006	Subtotals	Oct 2004	May 2005	Jul 2005	Oct 2005	May 2006	Jul 2006	Subtotals	Oct 2004	May 2005	Jul 2005	Oct 2005	May 2006	Jul 2006	Subtotals	Totals
Salamanders																						
Desmognathus conanti	0	0	0	0	0	0	**0**	0	2/0	0	0/2	0/1	0	**2/3**	2/10	0/30	0/30	0/13	0/35	0/31	**2/149**	**4/152**
Eurycea cirrigera	0	0/1	0/2	0/22	0	0/4	**0/29**	2/9	0/10	0/22	0/38	9/20	0/17	**11/107**	2/0	0/6	0	0/5	0	0	**2/11**	**13/147**
Eurycea longicauda	0	0	0	0	0	0	**0**	0	0	0	0	0	0	**0**	0	0	0/2	0	0	0	**0/2**	**0/2**
Eurycea lucifuga	0	3/0	1/0	0	0	0	**4/0**	0	0	0	0	0	0	**0**	0	0	0	0	0/3	0	**0/3**	**4/3**
Eurycea sp.	0	0	0	0	0	0	**0**	0	4/0	3/0	7/0	6/0	4/0	**24/0**	0	0	0	0	0	0	**0**	**24/0**
Plethodon dorsalis	0	0	0	0	0	0	**0**	0	0/1	0	0	0	0	**0/1**	0	0	0	0	0	0	**0**	**0/1**
Totals (l/pm)	0	3/1	1/2	0/22	0	0/4	**4/29**	2/9	6/11	3/22	7/40	15/21	4/17	**37/111**	4/10	0/36	0/32	0/18	0/38	0/31	**4/165**	**45/305**
Totals (all individuals)	0	4	3	22	0	4	**33**	11	17	25	47	36	21	**148**	14	36	32	18	38	31	**169**	**350**
Frogs																						
Bufo fowleri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	**2**	**2**
Pseudacris crucifer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	**1**	**1**
Pseudacris feriarum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	**1**	**1**
Rana clamitans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	4	5	4	4	**40**	**40**
Rana palustris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	1	0	**8**	**8**
Rana sphenocephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	5	**8**	**8**
Totals	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	3	6	7	5	11	**60**	**60**
Grand Totals	0	4	3	22	0	4	**33**	11	17	25	47	36	21	**148**	42	39	38	25	43	42	**229**	**410**

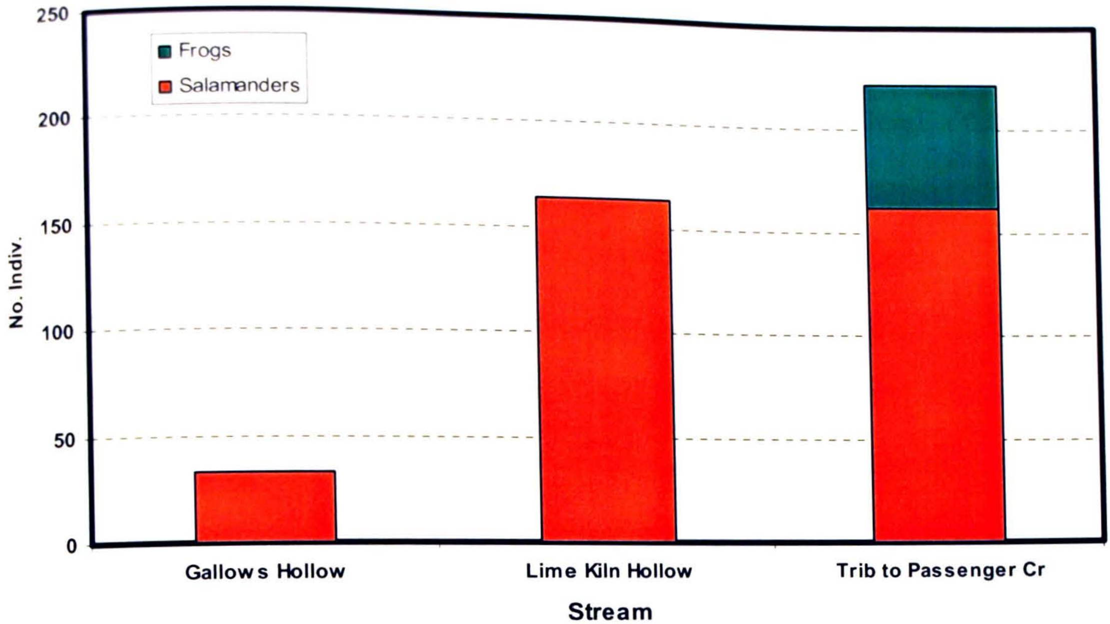

Figure 12. Numbers of salamanders (larvae and adults combined) captured over the course of the study at each stream.

distribution of abundance revealed a significant deviation from a ratio of 1:1:1 (Chi Square Test for Goodness of Fit- $X^2 = 91.86$, df = 2, $P < 0.001$, alpha level of 0.05), which is the expected ratio if each stream were equally suited for salamander populations. Further analysis reveals that the ratio of observed versus expected numbers of salamanders between both urban drainages (Gallows Hollow Branch and Lime Kiln Hollow Branch) also deviate significantly ($X^2 = 73.07$, df = 1, $P < 0.001$), whereas the ratio for Lime Kiln Hollow Branch versus the tributary to Passenger Creek (both outside the CSO influence) does not ($X^2 = 1.39$, df = 1, $P > 0.10$). Means of the snout-vent lengths of the post-metamorphic *Eurycea cirrigera* from each of the three streams were not significantly different ($F(2,138) = 2.50$, $P = 0.086$), though there were many more individuals present in the Lime Kiln Hollow Branch than either of the others.

Frogs were found only in the tributary to Passenger Creek, the stream in rural eastern Montgomery County (Table 4). Sixty individuals representing six species were captured overall. The most abundant species was the Green Frog (*Rana clamitans*) which accounted for 66.66% (40/60) of the total, while the Southeastern Chorus Frog (*Pseudacris feriarum*) and Spring Peeper (*Pseudacris crucifer*) were the least common species with only one specimen represented for each. Other species present in small numbers were the Pickerel Frog (*R. palustris*), Southern Leopard Frog (*R. sphenocephala*), and the Fowler's Toad (*Bufo fowleri*).

CHAPTER IV

DISCUSSION

Abiotic data such as water temperature, air temperature and pH often play an important role in the life cycle of amphibians (Dunson and Travis, 1991). They are important in body temperature regulation and hence reproductive success/fitness. This study, however, documented no significant differences in the abiotic variables measured and concluded, therefore, that these factors were not affecting the numbers of individuals residing at the three study sites.

Amphibian populations differed significantly between the three streams based on numbers of individuals. Gallows Hollow Branch (urban drainage within the CSO basin), produced the least number of individuals with 33, representing two species (both salamanders). Lime Kiln Hollow Branch (adjacent urban drainage outside the CSO basin) had 148 individuals and four species (all salamanders). Not surprisingly, the tributary to Passenger Creek (rural reference stream) proved to be the most productive with 229 individuals and 12 species (6 salamanders and 6 frogs). The remainder of this paper will attempt to explore the possibilities as to why this phenomenon may have occurred.

Land use and cover changes accompanying urbanization impacts natural ecosystems at multiple spatial scales (Faulker, 2004). The observed higher number of amphibians (salamanders) found in the urban reference stream (Lime Kiln Hollow Branch) as compared to the CSO drainage (Gallows Hollow Branch), may have resulted from one or a combination of several factors. First, there is a reach of Gallows Hollow Branch where it flows through Valley Brook Park that has been highly modified by human activity. In this area most of the stream's natural substrate has been replaced with limestone rip-rap and the shoreline vegetation and surrounding forest removed. This type of change in stream geomorphology can dramatically degrade the habitat for stream-side amphibians. For example, Gamradt and Kats (1997) documented the loss of habitat suitable for oviposition by stream-breeding California newts due to erosion from previous fire. Second, during dryer periods of the year in Valley Brook Park, water disappears completely when it reaches the point where rip-rap has replaced the natural substrate. This creates drought conditions that could limit reproductive success of many amphibians that require water to breed. Lastly, a manhole located on the slope just above the park at the lower reach has contributed sewage overflow to the stream in the past as a result of heavy rains. There is reason to believe that this sewage has contributed to the degradation of the habitat at the park end of the drainage. Pollution contained in sewage enriches the stream and introduces bacteria and toxic substances into the water potentially adversely affecting organisms living and drinking from the stream (EPA).

Concerning the absence of frogs in both of the urban streams, one or more limiting factors present in the urban habitat that are not present in the rural environment might be responsible. One example of a limiting factor that may be involved is an increased amount of noise pollution associated with a higher population density of humans in and around the city. Sun and Narins (2005) found that man-made acoustic inference, such as traffic, affected anuran chorus behavior. This is important in that chorus behavior in anurans is commonly used in lek reproductive strategy to attract mates, which in turn directly correlates to reproductive success. Highway noise pollution, therefore, may adversely affect the reproductive success of some species of anurans through interference of chorus behavior.

Both urban streams (Gallows Hollow Branch and Limekiln Hollow Branch) are bordered and crossed by frequently utilized streets. Jones et al. (2000) stated that "at the landscape scale, certain definable geometric interactions involving peak flows (floods) and debris flows (rapid movements of soil, sediment, and large wood down steep stream channels) are influenced by the arrangement of the road network relative to the stream network." They go on to discuss how this may influence the rates and patterns of survival and recovery of disturbed patches in stream networks, affecting ecosystem resilience. When an ecosystem's resilience is altered, all animals in that ecosystem are affected. Baker et al. (2004) stated that urban development creates more impervious surfaces causing streams to be "flashy". Flashiness is a decrease in the lag time of peak flow levels of stream water during a storm event. This flood event is capable of washing away amphibian communities, as well as their food and habitat. Because base flow levels return more rapidly, the water does not have efficient time to seap back down to the water column (http://www.epa.gov/owow/nps/urbanize/report.html). This typically results in a lower base level flow, another factor possibly affecting amphibian larval success.

Discrediting the hypothesis that noise pollution from city streets is partially responsible for the absence of frogs in the two urban streams is the fact that Interstate highway 24 (I-24) crosses the rural reference stream (tributary to Passenger Creek) a short distance from where sampling occurred. Despite the rural setting, I-24 is a frequently traveled highway which produces a fairly loud and constant noise pollution. So, the rural reference stream and its inhabitants (frogs included) are not devoid of such man-made acoustic interference either. More research will be needed before a connection can be made between the noise pollution and reduced amphibian abundance in urban versus rural streams.

Another possible factor contributing to the absence of frogs in the urban watersheds might be an increased amount of run-off pollution resulting from precipitation over a more impervious area surrounding the stream (i.e. streets, parking lots, mowed lawns, etc.). During periods of heavy precipitation, both the urban drainages are inundated with urban runoff containing such pollutants as oil, gasoline, lawn fertilizer, road salt, and sulfur from acid rain. The impact of this unfiltered storm water and its pollutants may have significant affects on the stream's herpetofauna. For instance, studies have found reduced salamander species richness and abundance at streams with higher impervious surface area in the surrounding basin (Boward et al., 1999).

It could be argued, however, that this too may not be a primary cause as to why there was an absence of frogs in the urban drainages. Although there is riparian vegetation present along the banks of the tributary to Passenger Creek in my study area to the north, it is enveloped by agricultural fields, another type of impervious surface. Compared to natural grasslands, agricultural fields are far more impervious in nature and have the tendency to facilitate run-off of fertilizers and pesticides containing such pollutants as ammonia, phosphorus and nitrogen resulting in a loss of biodiversity (Carpenter et al., 1998). Atrazine, the most widely used pesticide in the United States, can be present at several parts per million in agricultural runoff (Storrs and Kiesecker, 2004). An example of how this may have directly affected this study can be seen by Storrs and Kiesecker (2004), who found a significantly lower survival rate for amphibian tadpoles exposed to atrazine at early and late developmental stages. The tributary to Passenger Creek was deemed the reference stream for this study primarily due to its comparable size and geomorphology, but due to the agriculturally modified surrounding environment, this stream may not contain an amphibian fauna typical of a pristine first order rural reference stream. Despite the impervious nature of these fields, however, they are sure to absorb more water than urban pavement.

It should also not be overlooked that in addition to the typical urban run-off pollution Gallows Hollow Branch drainage receives sanitary sewage overflow, and during events of heavy precipitation may receive untreated sewage discharged from manholes. This can be harmful to the receiving body of water as wastewater has been known to contain such harmful elements as unionized ammonia and pathogenic micro-organisms (probably thriving on the organic matter present), which contribute to a decrease in dissolved oxygen (Rauch et al., 1998), a necessity for stream-dwelling amphibian larva (Wassersug and Seibert, 1975). However, because frogs were absent in both urban drainages, sewage contamination cannot be the sole cause for this phenomena. More research will be necessary before CSO's can be deemed as the direct cause for the low amphibian abundance in the Gallows Hollow Branch.

Urban riparian corridors have a much different composition of flora when compared to that of rural areas. As population increases, anthropogenic manipulation of natural flora is sure to follow. One possible outcome of this may be a higher number of invasive plant species present in and around an urban watershed. For example, Faulkner (2004) stated "hydrologic changes caused by habitat fragmentation generally reduce species richness and abundance of plants, macroinvertebrates, *amphibians*, and birds with greater numbers of invasives and exotics." This may suggest that the urban development in and around the Gallows Hollow Branch basin is a possible determining factor for the high number of invasive plant species.

Different flora compositions house different organisms. They also have the ability to act as barriers, keeping pollutants out and useful minerals and resources in. This barrier system can, and quite often does, protect the entire aquatic ecosystem. For example, vegetative buffer strips adjacent to water courses help to reduce contaminants such as nitrogen from entering surface waters (Rouse et al., 1999). Recall that nitrogen is a major pollutant associated with combined sewage overflow. If the composition of the vegetative buffer zone is dramatically altered by such an event as urbanization, it is quite possible that the filtering properties of the buffer will also change. This could contribute to a higher influx of pollution concentrations such as nitrogen, a problem this stream may not be equipped to handle. How this may have impacted this study is difficult to determine due of the large number of possible contributing factors.

Extensions of this study may include a more comprehensive strategy that would incorporate measuring dissolved oxygen and nitrate levels. This is important because dissolved oxygen levels have been shown to directly affect the larval behavior of amphibians (Wassersug and Seibert, 1975). Also, Solla et al. (2001) claim that a higher biochemical oxygen demand (less dissolved oxygen) may contribute to lower reproductive success and ultimately reduced population viability of amphibian populations. Nitrogen pollution, on the other hand, can also enter bodies of water through anthropogenic sources of pollution such as agriculture runoff. Rouse et al. (1999) stated that nitrate concentrations in some watersheds in North America are high enough to cause death and developmental anomalies in amphibians and impact other animals in aquatic ecosystems.

It may also be beneficial to extend the study area for each watershed/drainage. As our knowledge of habitat fragmentation impacts on aquatic ecosystems grows, it becomes more evident that we must look not only at riparian buffer zones, but examine with as much care as is necessary the entire watershed to better understand the influence a growing population and habitat destruction may have on aquatic ecosystems, including amphibian fauna (Willson and Dorcas, 2003).

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APPENDICES

APPENDIX A

Eleven Categories for Storm Water Phase One Compliance (USEPA 2000)

1. Category One (i): Facilities with effluent limitations
2. Category Two (ii): Manufacturing
3. Category Three (iii): Mineral Metal Oil and Gas
4. Category Four (iv): Hazardous Waste, Treatment, or Disposal Facilities
5. Category Five (v): Landfills
6. Category Six (vi): Recycling Facilities
7. Category Seven (vii): Steam Electric Plants
8. Category Eight (viii): Transportation Facilities
9. Category Nine (ix): Treatment Works
10. Category Ten (x): Construction Activity
11. Category Eleven (xi): Light Industrial Activity

APPENDIX B

Nine Minimum Controls for Combined Sewer Overflows (USEPA 1995)

1. Proper operation and regular maintenance programs for the sewer system and the CSOs
2. Maximum use of the collection system for storage
3. Review and modification of pretreatment requirements to assure CSO impacts are minimized
4. Maximization of flow to the publicly owned treatment works for treatment
5. Prohibition of CSOs during dry weather
6. Control of solid and floatable materials in CSOs
7. Pollution prevention
8. Public notification to ensure that the public receives adequate notification of CSO occurrences and CSO impacts
9. Monitoring to effectively characterize CSO impacts and the efficacy of CSO controls

APPENDIX C

Riparian vegetation along the three sample stream reaches (lower, middle, upper) of Gallows Hollow Branch. An asterisk(*) denotes an invasive plant species.

Species	Sample Reach		
	Lower	Middle	Upper
Beefsteak Plant (*Perilla frutescens*)	X	X	X
Black Locust (*Robinia pseudoacacia*)		X	
Boxelder (*Acer negundo*)		X	X
Bush Honeysuckle (*Lonicera morrowii*)		X	X
Butterweed (*Senecio glabellus*)	X		
Canadian Honewort (*Cryptotaenia canadensis*)	X		
Carya sp.		X	
Curly Dock (*Rumex crispus*)		X	X
Dandelion (*Taraxacum officinale*)	X		
Eastern Daisy Fleabane (*Erigeron annuus*)	X		
Eastern Redbud (Cercis canadensis)		X	X
European Privet (*Ligustrum vulgare*)*		X	X
Great Ragweed (*Ambrosia trifida*)	X	X	
Ivyleaf Speedwell (*Veronica hederifolia*)	X		
Japanese Hedge Parsley (*Torilis japonica*)	X		
Japanese Knotweed (*Polygonum cuspidatum*)		X	
Kudzu (*Pueraria montana*)			X
Narrowleaf Plantain (*Plantago lanceolata*)		X	X
Partridge Pea (*Chamaecrista fasciculata*)		X	X
Polygonum sp.	X		
Red Maple (*Acer rubrum*)		X	X
Rose of Sharon (Hibiscus syriacus)		X	X
Senecio sp.	X		
Smartweed (*Polygonum cespitosum*)		X	X
Southern Hackberry *(Celtis laevigata)*		X	
Southern Magnolia (*Mangolia grandiflora*)	X		
Sugar Maple (*Acer saccharum*)	X	X	
Sycamore (*Platanus occidentalis*)	X		
Tall Fescue (*Festuca arundinacea*)	X		
Virginia Wild Rye (*Elymus virginicus*)	X		
Weeping Willow *(Salix babolonica)*	X		
White Snakeroot (*Eupatorium rugosum*)	X	X	X
Winter Creeper (*Euonymus fortunei*)*	X	X	X

APPENDIX D

Riparian vegetation along the three stream reaches (lower, middle, upper) of Limekiln Hollow Branch. An asterisk(*) denotes an invasive plant species.

Species	Stream Reach		
	Lower	Middle	Upper
American Elm (*Ulmus americana*)	X		
Aster sp.	X		
Bearded Beggar Ticks (*Bidens aristosa*)	X		
Beefsteak Plant (*Perilla frutescens*)		X	
Black Cherry (*Prunus serotina*)	X		
Black Oak (*Quercus velutina*)			X
Bush Honeysuckle (*Lonicera morrowii*)	X	X	X
Carya sp.	X		
Christmas fern (*Polystichum acrostichoides*)		X	X
Coralberry (*Symphoricarpos orbiculatus*)	X	X	
Crataegus sp.	X	X	X
English Ivy (*Hedera helix*)			X
European Privet (*Ligustrum vulgare*)*			X
Green Ash (*Fraxinus pennsylvanica*)			X
Ground Ivy (*Glechoma hederacea*)*		X	
Honey Locust (*Gleditsia triacanthos*)			X
Microstegium sp.		X	X
Northern Red Oak (*Quercus rubra*)	X		
Osage Orange (*Maclura pomifera*)	X	X	
PawPaw (*Asimina triloba*)	X		
Possum Haw (*Ilex decidua*)	X	X	
Red Elm (*Ulmus ruber*)		X	
Richweed (*Collinsonia canadensis*)	X		
Roundleaf Greenbrier (*Smilax rotundifolia*)	X	X	
Rubus sp.	X		
Silver Maple (*Acer saccharinum*)		X	
Smallspike False Nettle (*Boehmeria cylindrica*)	X		
Smartweed (*Polygonum cespitosum*)	X	X	
Spicebush (*Lindera benzoin*)	X		
Sugar Maple (*Acer saccharum*)	X	X	X
Sycamore (*Platanus occidentalis*)	X	X	X
Tulip Poplar (*Liriodendron tulipifera*)		X	X
White Mulberry (*Morus alba*)	X	X	X
White Snakeroot (*Eupatorium rugosum*)	X	X	
Wild Grape (*Vitis aestivalis*)		X	
Winter Creeper (*Euonymus fortunei*)*	X		
Wood Oats (*Casmanthium latifolium*)	X		

Appendix E

Riparian vegetation along the three stream reaches (lower, middle, upper) of the Tributary to Passenger Creek. An asterisk(*) denotes an invasive plant species.

Species	Stream Reach		
	Lower	Middle	Upper
Allegheny Sperge (*Pachysandra procumbens*)	X		
American Elm (*Ulmus americana*)	X		X
American Plum (*Prunus americana*)			X
Bearded Beggar Ticks (*Bidens aristosa*)			X
Beefsteak Plant (*Perilla frutescens*)	X		X
Bitternut Hickory (*Carya cordiformis*)	X	X	
Black Cherry (*Prunus serotina*)		X	
Black Walnut (*Juglans* nigra)		X	
Canadian Clearweed (*Pilea pumila*)			X
Chinkapin Oak (*Quercus muehlenbergii*)			X
Christmas fern (*Polystichum acrostichoides*)	X		
Common Blue Violet (*Viola sororia*)		X	
Coralberry (*Symphoricarpos orbiculatus*)			X
Cumberland Mock Orange (*Philadelphus hirsutus*)	X		
Curlytop Knotweed (*Polygonum lapathifolium*)			X
Dutchman's Pipe (*Aristolochia macrophylla*)	X	X	X
Eastern Wahoo (*Euonymus atropurpurous*)*			X
European Privet (*Ligustrum vulgare*)*	X		X
Hairy White Aster (*Aster pilosus*)			X
Ironwood (*Carpinus caroliniana*)	X		
Limestone Wild Petunia (*Ruellia strepens*)		X	X
Multiflora Rose (*Rosa* multiflora)*		X	
Nepalese Browntop (*Microstegium vimineum*)*			X
Northern Red Oak (*Quercus rubra*)		X	X
PawPaw (*Asimina triloba*)	X		
Northern Red Oak (*Quercus rubra*)			
Red Elm (*Ulmus ruber*)	X	X	
Red Cedar (*Juniperus virginiana*)			X
Red Maple (*Acer rubrum*)	X		
Roundleaf Greenbrier (*Smilax rotundifolia*)	X		X
Saw Greenbrier (*Smilax bona-nox*)			X
Smartweed (*Polygonum cespitosum*)	X	X	X
Southern Hackberry (*Celtis laevigata*)			X
Sugar Maple (*Acer saccharum*)	X	X	X
Swamp Chestnut Oak (*Quercus michauxii*)			X
Sycamore (*Platanus occidentalis*)	X		X
Wallrue (*Asplenium ruta-muraria*)	X		
White Crownbeard (*Verbesina virginica*)	X		
White Oak (*Quercus alba*)	X	X	
White Snakeroot (*Eupatorium rugosum*)	X	X	X
Wild Hydrangea (*Hydrangea arborescens*)	X		
Wood Oats (*Casmanthium latifolium*)	X		X

APPENDIX F

Clarksville Gas and Water Department Project
Field Data Sheet for Streams

Page 1 of ___

Stream Name ________________ Date__________ Observer(s)____________________ Vehicle__________

Mi start______ Mi End______ Total _______ Time start______ Time End______ Total _______

Current Weather:

Precipitation: None, Light, Moderate, Heavy; Rain, Snow **Sky**: Clear, Partly Cloudy, Mostly Cloudy, Overcast

Wind: Calm, Light, Moderate, Gusty, Strong

Notes: ____

Previous Weather (last 24 hours)______________________________________

Site Code ______Time Start _______ End _______Air Temp.:_____Water Temp.:____ pH: _____ (water sample)

Rec No.	Species	Age	Sex	Repro Cond.	SVL (mm)	Micro Habitat	Remarks

VITA

Joshua Lucas Maloney was born in Wiesbaden, Germany on 11 March 1981 to Thomas and Marcella Maloney of Philadelphia, Pennsylvania. He has two siblings: one older brother Damian Maloney, and a younger sister Marcella Maloney. He was an Army brat growing up and lived such places as Germany, Colorado, Illinois, and Tennessee. He graduated with honors from Stewart County High School in Dover, Tennessee in May of 1999. After graduation he enrolled in Austin Peay State University (APSU), where he began working for Dr. A Floyd Scott as a field assistant in the spring of 2003. He received a bachelor degree in biology in December of 2006. He then entered APSU's Master of Science program under the tutelage of Dr. Scott and received a master's degree in biology in August of 2007.

He plans to continue his career in the environmental conservation field. He would like to do this as an educator with the ultimate goal of obtaining a Doctorate of Philosophy in environmental ecology.