

# Life in Urban Environments: The impact of Urbanization on Life History Traits in Amphibian Species



The purpose of this lesson is for students to learn how urbanization influences the community composition of species. In order to do this, students will be analyzing the impacts of urbanization on life-history traits in two amphibian species within the rural-to-urban gradient. By completing statistical analyses in R, informed decisions can be made in regards to conservation and resiliency efforts for managing amphibian communities in urban areas.

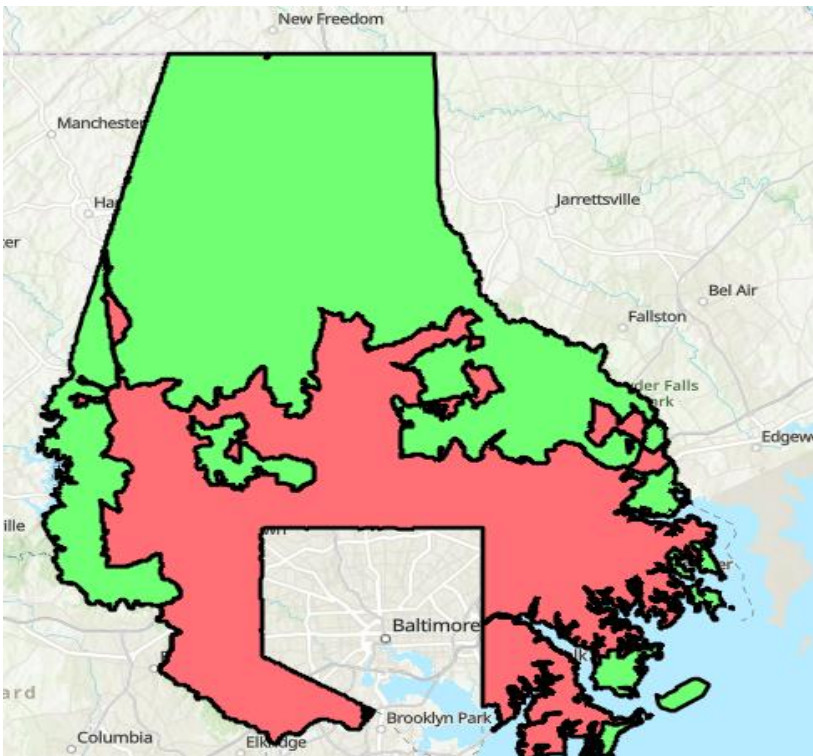
This QUBES lesson is intended to follow a specific pattern. Individuals are supposed to first, read the introduction on urbanization and how it influences wildlife. Following the introduction, a study should be read and discussed to prepare for the data analysis. After the paper discussion, an interactive data analysis lesson focuses on analyzing a subset of data obtained from the paper. The data analysis section of this lesson will allow for a more in-depth understanding of how certain statistical methods (two-way ANOVA) are used in research studies. Finally, there will be questions that need to be answered that help relate the ecological and quantitative concepts together and stimulate discussion on conservation resiliency efforts.



- 1.) Learn about urbanization and its effects on wildlife, specifically amphibians.
- 2.) Read a study on the variation of life history traits among urban and rural populations of amphibians.
- 3.) Analyze a subset of the data from the study to determine if frog and toad populations from different populations are different in size at age through the use of a two-way ANOVA
- 4.) Answer questions relating to the ecological and quantitative concepts that have been learned throughout this lesson. Relate statistical results to conservation and resiliency efforts.

## Introduction

As the world's population is increasing, a rising number of people are moving from rural areas to more urban settings. This global phenomenon is called urbanization. It is the transformation of unoccupied or sparsely occupied, rural land (an area with 2500 residents or fewer) into densely occupied urban areas (a central city and surrounding area, a population exceeding 50,000 people). The extent of urban areas is increasing drastically around the world. The United Nations has predicted that by the year 2050, 68% of the global human population will be living in urban areas (Kondratyeva, 2020). Economic, political, and social issues merge with circumstances of modernization to make people want to migrate from rural to urban areas. This increase in urban population induces a number of environmental problems.



**Figure 1.** Map of Baltimore County, MD. The green shows rural areas and the red shows urban areas. You can clearly see the urban-rural demarcation line that separates rural and urban areas

For the remainder of this lesson, when discussing urbanization, we will be focusing on one specific location, Baltimore County, Maryland. Baltimore County is the third most populous county within the state of Maryland. It is also a part of the Baltimore metropolitan area. Baltimore County has an urban-rural demarcation line that divides the county into rural and urban areas. This makes this county a very good space for looking at how urbanized areas affect the environment and wildlife differently than rural areas. It is also important to note that Baltimore County is the area of study for the research paper discussed further in this lesson. All the data used for the data analysis is from rural, urban, and suburban areas in Baltimore County, MD.

## URBANIZATION ON THE ENVIRONMENT

Humans are intrinsically associated with the urbanization process. On the continuum of anthropogenic activities, urbanization is the most irreversible and human-dominated form of land use. The urban habitat is created by us to meet our species-specific requirements. Urbanization is a significant form of land take that has various impacts on the pattern, functionality, and dynamics of natural landscapes, and thus also on the ecosystems it touches (Haase, 2019). The process of urbanization is dramatically changing the environment, modifying not only abiotic elements such as habitat structure or connectivity but also biotic elements. It is changing and modifying almost every system on Earth. There is mounting evidence suggesting that anthropogenic landscape changes modify different components of biodiversity, including taxonomic, functional and evolutionary diversity, and other aspects of the biology of an organism, like animal behavior or life-history traits (Ibanez-Alamo et al., 2017). This can have serious effects on species, it can alter their reproductive success, growth rates, mortality, energy allocation patterns, and many other aspects that influence and determine their survival within an environment.

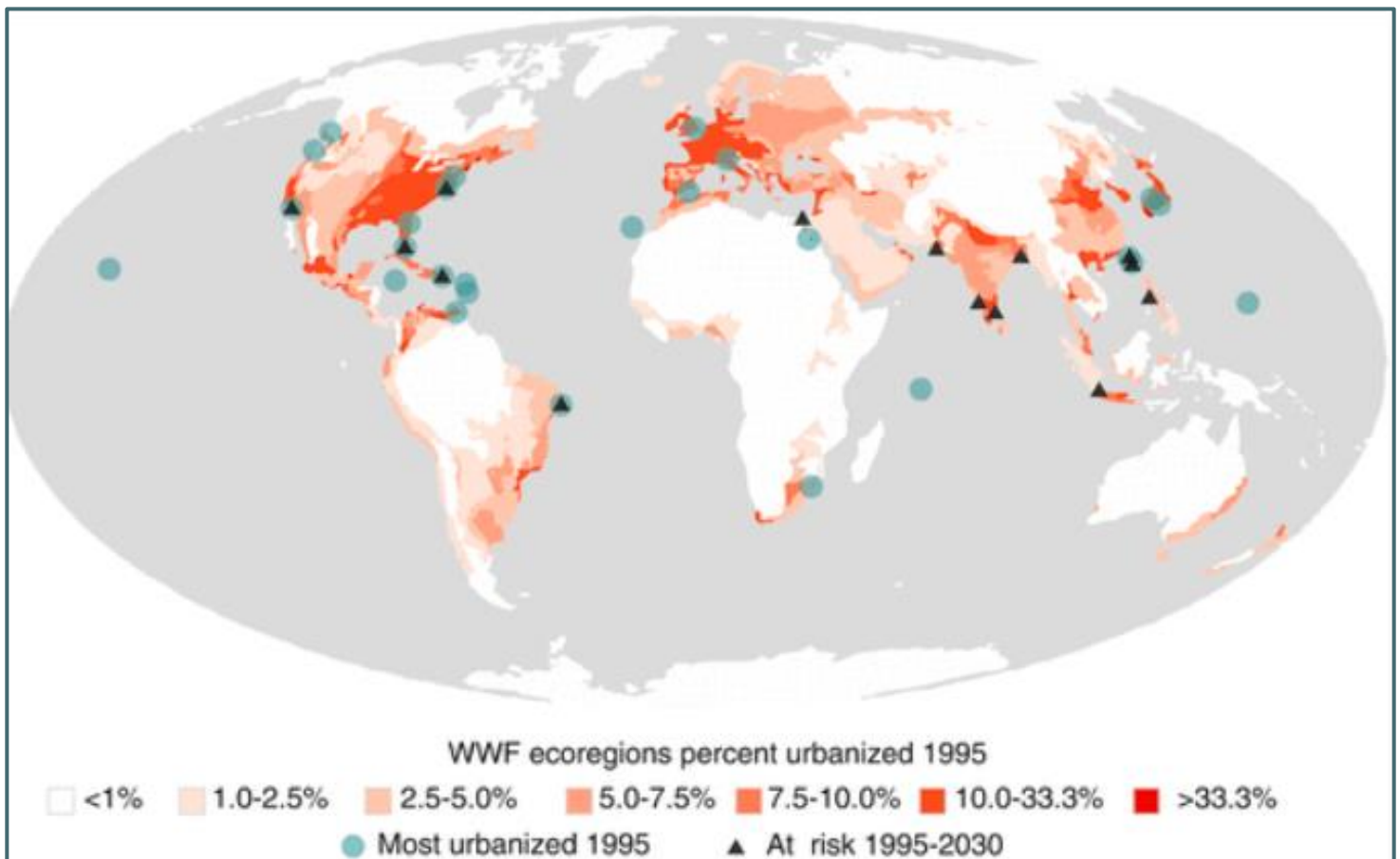


Figure 2. Percentage of an ecoregion's area that was urban in 1995. Ecoregions are major ecosystems defined by distinctive geography and receiving uniform solar radiation and moisture. Ecoregions with more than 1/3<sup>rd</sup> of their area urban are marked by circles. At-risk ecoregions, which will lose more than 5% of their remaining undeveloped area by 2030 are marked by triangles. As you can see, in developed areas, a majority of ecoregions are being touched and influenced by urbanization. Modified from McDonald et al. [2008](#), p. 198.

Urbanization influences the community composition of almost all groups of organisms studied to date. In general, many populations undergo decline as a function of increasing urbanization and may become locally extirpated, resulting in decreased species richness in urban areas (Jeanette et al., 2019). When you study organisms through a life-history framework, you assume that observed variations in their body size, reproductive traits, and age structure among populations reflect differences in their surrounding habitat quality. For example, degradation of aquatic breeding sites due to an increase in water pollution from greater amounts of urban runoff because of the increase in impervious surfaces, may have negative consequences for pond-breeding amphibians with complex life cycles. Therefore studying the differences in size at maturation, reproductive investment, and adult growth rates and body size can provide useful information on the effect of urbanization on different life stages of these organisms. Life-history theory predicts that organisms will maximize their fitness through optimal energy allocation among growth, reproduction, and maintenance demands (Jeanette et al., 2019). Understanding the life-history traits can be used to help conservation and resiliency efforts through best management practices to support these organisms within this new built environment.



Please watch this short video on [urbanization and the environment](#).

### AMPHIBIAN COMMUNITIES IN URBAN LANDSCAPES

Wetlands are frequently destroyed during urbanization, resulting in ecological communities vastly different from those in nonurban wetlands (Ehrenfeld 2000). Urbanization can impact amphibian communities through habitat loss, fragmentation and isolation, and degradation of habitat quality. Currently, an estimated 37% of amphibian species are threatened due to urbanization worldwide (Hamer, 2011). In order for amphibians to fulfill critical life-history processes, they require sufficient space and resources within their terrestrial and aquatic habitats. Aside from reductions in habitat availability and connectivity during urbanization, the viability of many urban amphibian populations are threatened due to the decreases in the quality of the remaining habitat, decreased water quality, and the introduction of predatory fish (Hamer, 2011). Therefore, the quality of habitat is an important aspect in the persistence of species within altered habitats in urban landscapes.



Urban infrastructure can reduce the ability of amphibian communities to function effectively. Resource availability and environmental conditions, due to the stress of urbanization, constrains the amount of energy an organism can acquire (Jeanette et al., 2019). Therefore, tradeoffs must occur among competing pathways of allocations. Poorer-quality sites associated with habitat loss and degradation because of anthropogenic causes may provide fewer resources for amphibians. This has the ability to result in reduced adult body size when compared to other amphibians occupying forested habitats. It has also been found that maternal investment and wetland quality work together to influence growth rates of larvae and post-metamorphic body size, which ultimately influences adult body size (Rasanen et al., 2008). It is clear that modifications of landscape structure by humans negatively affect amphibian communities and may result in local extinctions of populations.

The majority of studies on the impact of urbanization on amphibians show that the urban environment affects them negatively, amphibian species diversity decreases opposite to the urbanization gradient (Mazgajska, 2020). This means that significantly fewer amphibian species occur in city centers than in their peripheries, where urbanization pressure is lower. Despite this generally negative trend, the response of amphibians to urbanization depends on the species. Some species occur only in the relatively unaltered outskirts of the cities, like the wood frog which is known to be sensitive to urban environments. Whereas, some amphibian species, like the American toad, are relatively resilient to urbanization as they tend to be habitat generalists or they possess specific life-history traits that make them less susceptible to habitat changes found within cities (Jeanette et al., 2019). This is important when trying to understand how urbanization can influence certain amphibian species more than others.



*Wood Frog*



*American Toad*

## Study Overview (Jenette, Snodgrass, & Forester (2019). )

*Note: In order to complete this lesson, you should read the full research article. It is included as a PDF on the QUBES website and it is linked below.*

### STUDY TITLE: VARIATION IN AGE, BODY SIZE, AND REPRODUCTIVE TRAITS AMONG URBAN AND RURAL AMPHIBIAN POPULATIONS

#### Study Objectives:

To study the impacts of urbanization on life history traits in amphibian species with different sensitivities to urbanization. To examine variations in adult body size, maternal reproductive investment, and ages of breeding adults across multiple populations of two pond breeding anurans occurring in landscapes exhibiting varying degrees of urbanization.

#### Methods:

Two anuran populations were sampled, the wood frog and the American toad, along the urban-rural gradient in Baltimore, Maryland. They each have different sensitivities to urbanization. Their life history characteristics, body size, weight, age, and reproductive traits, were compared among urban, rural, and suburban populations. Statistical analyses were then completed to determine how and if urbanization had an effect on these two populations.

#### Significant Findings:

There was variation in age at maturity among populations but ages of breeding adults did not differ among populations. Adult wood frogs and American toads from more urbanized landscapes were significantly smaller at age than conspecifics from rural landscapes. The results suggest that in the landscapes studies, adult habitats were similar in quality but larval and juvenile habitats were of lower quality in urban areas, affecting their growth.

#### Pseudoreplication:

It is important to note that while this paper focuses on the effects of urbanization on amphibian populations, in some ways this is not a logically strong aspect of their paper. To effectively test the effects of urbanization, you would want good replication across all your treatments, ie. multiple urban sites, multiple rural sites, etc. There should be an ideal sample size N of 10 sites. The **key point** is that if the researchers wanted to study the effects of site characteristics on frogs, the study unit would have been the site, not the frog. What they have in this study and what we will be analyzing in the data analysis section, due to the nature of this study, is one urban site versus one rural site versus one suburban site. We can say that “these sites are different” but in truth, there are a variety of reasons why these sites are different. Without site replication, it is hard to know if the differences between sites are due to their urbanization level. **This is an issue of pseudoreplication.** Pseudoreplication is one of the most common errors in the design and analysis of biological research, it can and should be avoided if at all possible. When creating your study designs, you should consider how many replicates you have to avoid pseudoreplication and to make sure you accurately address your ecological questions.

#### Read the reference paper:

Jennette, M., Snodgrass, J., & Forester, D. (2019). Variation in age, body size, and reproductive traits among urban and rural amphibian populations. *Urban Ecosystems*, 22(1), 137–147.  
<https://doi.org/10.1007/s11252-018-0801-7>

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### Data Analysis

The data that students will be using for this lesson comes from the previously mentioned study completed in 2019 by Jennette, Snodgrass, and Forester. In their study, they sought to determine if there was variation in age, body size, and reproductive traits among urban and rural amphibian populations. They studied the impacts of urbanization on life-history traits in two amphibian species with different sensitivities to urbanization. Essentially they were investigating whether or not urbanization had an effect on these amphibians.



A [starter guide](#) to downloading R and R studio if you do not have it on your system

Here is a [link](#) to download R studio

### UNDERSTANDING THE DATA

The total data set contains multiple files within its folder with different measures of life-history traits that were used for the data analysis in their paper. All of this data is available for direct download on the [Dryad Digital Repository](#). The main data files that are of importance to us are the femaleswoodage.csv and the femalestoadage.csv. Within these two sub-datasets there is a population, POP, variable that contains the population of origin for the female frogs and toads. It contains the somatic dry weight of the amphibians, the snout-vent length in mm, and the estimated age of the amphibians based on their skeletal chronology. When completing our Two-Way ANOVA, we will be focused on the population of origin, the age, and the snout-vent length. The snout-vent length is used to estimate the life-history trait of body size for these amphibians. It is important to note that we will be working with an even more

simplified subset of data from this paper. These files are called *female\_wood\_frog\_data.csv* and *female\_toad\_data.csv*. This allows for a more in-depth understanding of the statistical methods of a Two-Way ANOVA and to limit confusion while trying to teach this new technique. This simplified dataset for the female wood frogs includes only one rural, suburban, and urban population origin for the 3 and 4 year age structure. Whereas, the simplified dataset for the female American toads includes only one rural, suburban, and urban population origin for the 4 and 5 year age structure.

## ECOLOGICAL QUESTION

The main ecological question that we are trying to answer throughout this lesson is, how does urbanization affect amphibian populations?

## HYPOTHESIS

We hypothesize that the populations of urban-sensitive wood frogs will have younger age-structures comprised of individuals displaying reduced size due to an association with urbanization



## TWO-WAY ANOVA

To investigate the differences among populations from urban, rural, and suburban areas, in size at specific age structures, a two-way analysis of variance (ANOVA) model will be completed. A Two-Way ANOVA is used to evaluate simultaneously the effect of two grouping variables on a response variable. When completing a Two-Way ANOVA there are three test hypotheses: 1.) there is no difference in the means of factor A, 2.) there is no difference in the means of factor B, and 3.) there is no interaction between factors A and B. For our analysis our hypotheses would be: 1.) size does not depend on age, 2.) size does not depend on site, and 3.) the effect of age on size does not depend on site. The assumption of a Two-Way ANOVA is that observations within each cell are normally distributed and have equal variances (Beckerman, 2017). Below is a brief progression of how you would go about completing a Two-Way ANOVA.

### Extra Resources:

[Online tutorial for completing a two-way ANOVA](#)

[YouTube video "R Two-Way ANOVA"](#)



## Assignment

You have been given access to data on the life history traits of two amphibians that live along the urban-to-rural gradient. Using RStudio, you will create, run, and analyze this data to help you draw conclusions about how urbanization affects amphibian populations. To understand how to complete the analysis, please follow along with the R code in the Rmd document labelled Student Version and answer these questions as you go.

1. What is the Null Hypothesis and the Alternative Hypothesis for the Two-Way ANOVA for the wood frogs?
  - a.  $H_0$ : The effect of population type (urban, suburban, or rural) on the snout-vent length does *\*not\** depend the age of the frog.
  - b.  $H_a$ : The effect of population type (urban, suburban, or rural) on the snout-vent length *\*does\** depend on the age of the frog.
2. What are the two assumptions of a Two-Way ANOVA? What are their importance? Does the data for the Wood Frogs satisfy these assumptions?
  - a. The two assumptions are that the observations within each cell are normally distributed and that they have equal variance. The normal probability plot is a graphical tool for comparing a dataset with the normal distribution to assess whether the data is normally distributed. The data is plotted against a theoretical normal distribution in such a way that the points should form an approximate straight line. Departures from this straight line indicate departures from normality. The points on this normal probability plot form a nearly linear pattern which indicates that the normal distribution is a good model for this data set. The importance for testing for equal variance is to see whether or not the variability of the residuals is roughly constant within each group, if it follows the constant variance assumption. The constant variance assumption is that the spread of residuals is roughly equal per treatment level. There does not appear to be too much of a pattern, so it looks like the constant variance assumption is satisfied here. With this being said, we can continue.
3. What does the Two-Way ANOVA tell us? Is it statistically significant?
  - a. The ANOVA table presents a sums-of-squares analysis-of-variance table. Because we conducted and are analyzing an experiment, we have a specific hypothesis in mind: The effect of population type on snout-vent length depends on age. Testing this hypothesis is embodied in the Population:Age row. This table reveals that there is statistically significant variation in snout-vent length explained by allowing the effect of population type to vary by age ( $F = 6.4913$ ;  $df = 2$ ;  $p = 0.002346$ ).
  - b. There is statistical evidence that the population source and age are not independent of each other, they depend on one another. When looking at the figure, it can be seen that as you go along the urban-rural gradient, the mean snout-vent length changes for each of its age group, 3 & 4. In one case, when looking at age 3 frogs, it seems like the population source matters quite a lot in determining the size of the frog at its given age. You can see that the urban

population frogs are significantly smaller for their age than the other populations of frogs. But, when looking at age group 4, there isn't that much difference between the size of the frog from this age in all the different populations. With this being said, there is this support of the hypothesis that in populations of urban-sensitive wood frogs, they would have younger age-structures comprised of individuals displaying reduced size at metamorphosis in association with urbanization.

4. Please recreate this analysis for the female American Toad. Please state the  $H_0$  and  $H_a$  and the results of the ANOVA model? What does this model tell us? What could explain these results?
  - a.  $H_0$ : The effect of population type (urban, suburban, or rural) on the snout-vent length does *\*not\** depend the age of the toad.
  - b.  $H_a$ : The effect of population type (urban, suburban, or rural) on the snout-vent length *\*does\** depend on the age of the toad.
  - c. The table reveals that there is **NOT** a statistically significant variation in snout-vent length explained by allowing the effect of population type to vary by age ( $F = 0.3532$ ;  $df = 2$ ;  $p\text{-value} = 0.703639$ ).
  - d. The populations at different land uses are different sizes and different ages are different sizes, seen in the graph, but those two effects are independent of each other. The body size for the toads at a given age doesn't depend on the population source.
  - e. American toads are more resilient to urbanization and land use changes than wood frogs, this could explain these results.
5. Now that we have the results of the Two-Way ANOVA, how could you use these results be used in conservation and resiliency efforts for managing amphibian communities in urban areas?
  - a. The findings that some of the populations that we studied were unable to attain body sizes as large as others may have ramifications for individual fitness, juvenile survival, and population dynamics. Knowing this, we can develop new landscapes using best management practices (BMPs) such as forested stream buffers. The proximity of these buffers to the ponds that these amphibians breed and live around eliminates the need for them to cross roadways to reach breeding sites. This reduces the mortality of these amphibians. These buffers also allow for a decrease in pollution entering the waterways which could affect the maturation of the larva.

## Bringing it all together

We found differences in size at age among breeding populations of wood frogs. However, we did not find differences in size at age among breeding populations of American toads. The populations we studied were all associated with different levels of urbanization, heavily urbanized areas, suburban areas, and rural areas. Watersheds of populations in the urban and suburban settings were characterized by high degrees of impervious surface cover and very small amounts of forest cover. This results in these

areas receiving greater amounts of runoff from impervious surfaces that contain pollutants that have the potential to degrade amphibian habitat quality. Therefore, reduce habitat quality in these amphibian environments could be responsible for the differences in size at age that we observed. Now that we know what populations are being affected and possible explanations for how, we are able to think about ways to address these issues. Best management practices (BMPs) are one of the best ways to address the negative impacts of urbanization on the environment. Some of the best BMPs to protect amphibian populations within urban areas include stormwater retention and detention ponds and the preservation of forested stream buffers. These allow for suitable habitats for amphibians to forage and breed, connecting habitats and limiting fragmentation and the need to cross roadways. Developing landscapes using BMPs allows for the resiliency of pond-breeding amphibians, as well as other organisms with complex life-history traits, to the stressors of urbanization.



A [good video](#) on storm water retention ponds and how they manage storm water runoff in urban areas.

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