

Aquatic Macroinvertebrates and Urbanization:

Exploring urbanization's effect on leaf litter decomposition in six streams of Puerto Rico using R

What is an Aquatic Macroinvertebrate?

A macroinvertebrate is an organism that lacks a bone structure and is large enough to be seen with the eye. In the aquatic environment macroinvertebrates are usually the juvenile insects that can be found under or on rocks, however, there are other aquatic macroinvertebrates such as snails. For this lesson, we will be looking how urbanization affects leaf litter decomposition, a role of the aquatic macroinvertebrate.

Within the insect group of macroinvertebrates there are several “functional feeding groups” that are used to characterize them based on the feeding behavior (Functional Feeding Groups, n.d.). The different types of feeding categories are scrapers, shredders, collectors, filterers, and predators. The scrapers get their food from the substrate in the streams such as algae growing on rocks. The shredders break down coarse particulate organic matter (CPOM) such as leaf litter. The collectors gather their food source from fine particulate organic matter (FPOM) from the stream bottom that was provided by the shredders. The filterers also collect FPOM but from the water column where their food source remains suspended. Lastly, the predators will prey on other consumers such as those listed above.



Figure 1: A mayfly nymph
image source: <https://www.flickr.com/photos/davemedia/5789175336>

In this lesson, we will be looking at these macroinvertebrates, especially the shredders, and how their role in breaking down leaf litter is important in the aquatic ecosystem. Leaves are vital to the aquatic ecosystem as they

Click [here](#) to learn a little more about the functional feeding groups and their roles as bio-indicators of stream health

provide a large energy source to the consumers (García-Palacios et al, 2015). Also, Straka et al (2012) found that with increasing CPOM, the abundance of other functional feeding groups rose because of the litter creating microhabitats. From there the consumers who take in that organic material for food become an energy source for higher trophic level organisms such as other predator

macroinvertebrates and fishes. In this way, the shredders play a pivotal role into making sure the leaf litter decomposes in the aquatic ecosystem so that the FPOM, created from the breakdown, becomes energy for others.

Interestingly the physical characteristics of the stream are also influenced by the decomposition of leaf litter. In streams where decomposition is low, leaf litter builds up and “clogs” the flow of the stream. These pools that develop can house pollutants for a longer period of time, killing sensitive species of macroinvertebrates (Carlisle and Clements, 2005). On the other hand, when leaf litter decomposition is high, the streams can run with faster flow rates, forcing any pollutants that enter the stream to quickly evacuate a given area. Some of these macroinvertebrates are sensitive to outside influences due to their anatomical structure, such as external gills.

Urbanizations Effect on the Streams

What even is urbanization? Urbanization is when undeveloped land becomes developed land. In other words, the natural habitat is converted into roads and buildings where there is a high percentage of impervious surface area that covers the landscape. With more impervious surface areas there is less vegetation cover that provides important environmental benefits to the area. Some of those benefits being an energy source for the organisms in the streams, a natural way to prevent erosions during heavy rain events, and the ability to slow down and even absorb some of the runoff that can contain heavy metals and excess nutrients during the rain events.



Figure 2: Urbanization encroaching on a river
Image source: <https://secasc.ncsu.edu/science/urbanization-consequences/>

An increase in impervious surface causes an increase in urban runoff. With an increase in urban runoff, and little vegetation cover along the waterways, there is more pollutants that can enter the streams (Classen- Rodríguez et al, 2019). Less vegetation cover will cause there to be a shift in the macroinvertebrate assemblage for each stream. Meaning, that there may be less shredders but more predators, etc. Then, with an increase of pollutants and lower

flow rate the changes in assemblages will be exacerbated.

To accompany this change in assemblage, the leaf litter decomposition rate will change. This reduction in energy transformation, that was provided by the shredders, will reduce how much FPOM there is for the other macroinvertebrates. This will cause there to be a cascading effect to upper trophic level organisms. The waterways will have a lower flow and be a perfect habitat for the macroinvertebrates that need very little FPOM to survive while also being less sensitive to pollutants in the water due to their internal-gill filtration system they possess.

These macroinvertebrates, such as midges and black flies, are known to be pests to humans. We will now transition to a research project that studied how urbanization affected six different streams in a tropical region.

Why does this matter?

Urbanization is a process that will most likely not stop with an ever-expanding human population. This makes its affect much worse on stream ecosystems because humans like to populate near waterways due to the environmental benefits that are gained by these natural ecosystems. Urbanization can then negatively affect the species present in those aquatic ecosystems. Many of the species provide different environmental benefits to the streams and the surrounding areas. For the macroinvertebrates, they provide some interesting benefits as they spend portions of their lives inside and out of the streams.

Macroinvertebrates are important to the stream ecosystem because they are ideal indicators for stream health water quality. Many states implement practices to collect and analyze the presence and diversity of the macro's in differing portions of the stream to get a well-balanced water health indicator. They are also important to the streams due to their primary role of cycling nutrients from organic matter talked about earlier. This process ensures that energy moves through the ecosystem in differing forms, building on the supply for other wildlife to use. Another great thing that is accomplished by many of these aquatic critters is that they help filter sediment and substrate from the water column. They essentially clean and purify the water in the streams. As pollutants enter the stream, different organizations will find it difficult to find a healthy stream and the nutrient cycling will come to a halt or be severely slowed down. This is detrimental to other life that depend on this nutrient cycling process and can cause a cascading affect up the food chain to many of the fishes that are present in that ecosystem.

Aquatic insects, when they mature and leave the water, are also important in that they create a bridge between the aquatic and terrestrial ecosystems. For the brief span of their lives out in the terrestrial world they have one function: to breed. During their breeding cycle they make easy targets for much of the wildlife. Birds, amphibians, and reptiles are a few of the organisms that take advantage of their breeding season to eat them as a food source. In this, the insects cycle nutrients from the aquatic realm to the terrestrial realm through their own bodies. This passive form of nutrient cycling is important for much of the terrestrial wildlife to stay alive at times of the year when food sources are limited. When urbanization encroaches, not only will pollutants limit the amount of macros that even make it to adult hood, but they urbanization will also reduce their chances of breeding due to extermination practices such as electric bug-lights and chemical spraying.

Meet the Scientists of Our Study

There have been numerous studies done on how urbanization has affected stream health, but there is very little information on how it affects the decomposition of leaf litter. In the study ***Leaf litter decomposition and macroinvertebrate assemblages along an urban stream gradient in Puerto Rico*** three scientists attempted to understand this relationship. Meet the scientist who performed the study that our Qubes lesson is founded on!



Leticia Classen- Rodríguez, at the time, was a PhD student at Saint Louis University. Her interests include behavioral ecology, urban soundscape and wildlife ecology, sensory pollution, and trophic interactions. Most of her research involves behavioral ecology, urban and noise pollution, and predator-prey interactions.

Image source:

<https://www.fowlerfinnlab.com/blog/2019/2/10/leticia-classen-rodriguez-is-now-a-phd-student>

Pablo Gutiérrez-Fonseca is an Assistant Professor at the University of Costa Rica and is part of Alonso Ramírez's lab at the university. He is a freshwater ecologist and his interests lay in community and Ecosystem ecology. Check out his [twitter account](#) to learn more about the work he does!

Image source: <https://twitter.com/pabloguticr>





Alonso Ramírez is a professor at North Carolina State University. His interests lay in trophic streams and how their diverse and complex ecosystems are inhabited by many different types of organisms. Within the diverse topic that he's interested in, he aims to study urban stream ecosystems. His work is primarily done in the tropical region of Puerto Rico, but his research program is transitioning to urbanization along streams in North Carolina. His lab has its own twitter page [The Ramirez Lab](https://twitter.com/TheRamirezLab) where some of his work gets noticed by the public eye!

Image source: <https://cals.ncsu.edu/applied-ecology/people/laramir2/>

Their Study

The scientist above wanted to discover how leaf litter decomposition rate would be affected by urbanization and how the macroinvertebrate assemblage diversity would change. They hypothesized that there would be a decrease in decomposition rate with an increase in urbanization and that there would be a decrease in the shredder functional feeding group diversity. Their study area consisted of six 2nd-3rd order streams, of the Rio Piedras watershed, each having different levels of urbanization nearby shown in the **Figure 3**. Within this watershed there is a major metropolitan area of San Juan. Urbanization was quantified based on the percent of cover that was impervious.

At each stream three pools were selected far enough from each other to account for any spatial variation. Within each pool six leaf litter bags were placed with coarse mesh to allow any shredders to enter the bags freely while still holding the leaf litter inside.

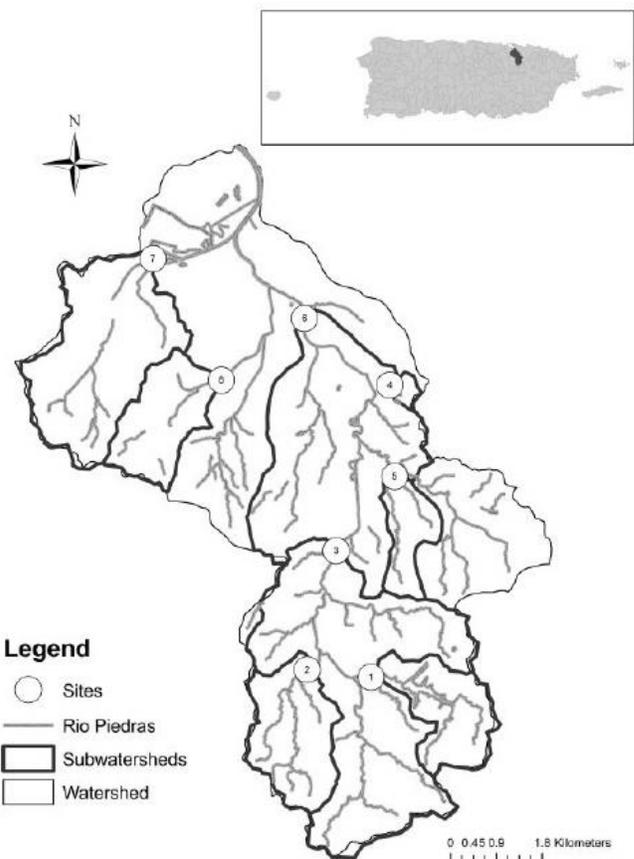


Figure 3: Rio Piedras watershed as adopted from Ramírez et al's (2014) previous study.

The experiment lasted 44 days where one bag from each pool for each stream was collected on day 2, 4, 8, 16, 32, and 44. Bags were taken back to the lab, rinsed with distilled water over a fine mesh sieve to collect the macroinvertebrates that came from the leaf litter bags. The leaf litter was then dried for a day and weighed. The bugs were preserved in 96% ethanol and identified down to their genus level, except for some of the Chironomidae (much harder to identify).

For the statistical analyses decomposition rates per stream were calculated for by using the slope of the linear regression analyses performed between the log transformed percent remaining leaf litter and the time (measured in days). Then, using the decompositions rates, they performed an analysis of variance test (ANOVA), followed by a Tukey's test to compare the slopes between the streams. They also looked at the relationship between decomposition rates and functional feeding group diversity using a second-order polynomial regression.

The Data

For this Qubes lesson, we will be using the data that was provided by the researchers from this study. The data was found on the [Dryad Digital Repository](#). Within the dataset we will be using there are four columns: Stream, Day, Replicate, and %AFDM Remaining. The streams are abbreviated with their stream identifying key. The day column would be the day that collection occurred, whether it be day 2, 4, 8, 16, 32, or 44. The replicate is a factor of 1, 2, or 3 and that is determined by which of the three pools the leaf litter bag was from per stream. Lastly, the %AFDM Remaining is a measurement of the % mass remaining from the original weight of the bag.

Impervious surface area data was taken from the authors' study (**Table 1**) and it was then related towards the decomposition rates that we will calculate in R.

What we will be doing with the data

Our Hypothesis: Impervious Surface Area, a measurement of urbanization, has an inverse relationship with the leaf litter decomposition of six streams in Puerto Rico.

In R Studio, we will recreate the original authors' analyses that were performed and from there, gain an understanding of the relationship that urbanization has on the decomposition of leaf litter. Through this lesson my goal is for the students to not only learn a valuable ecological lesson, but to also learn how to perform linear regressions and use the results of those linear regressions to do further statistical analysis to test the hypothesis of

this Qubes lesson.

For the first part of our analysis, we will be seeing if there is a significant relationship in the decomposition rates for the leaf litter bag replicates. For this analysis we will be building six linear regressions and then comparing the slopes and standard errors. This will allow us to

distinguish, in an order, which streams experience the best, and worst, decomposition rates of leaf litter.

In the second part of our analysis, we will perform one more linear regression. This regression will be looking at the relationship between Impervious Surface Area as a percent (found in **Table 1** of the authors' paper) and Decomposition rates of leaf litter. Using the slope, we will be able to find how much the decomposition rate changes per percent of impervious surface area. This shows how urbanization will affect the decomposition rate of leaves.

Make sure to have Excel open so that you can record valuable information into the two attached files included in this Qubes lesson: "Stream_Decomposition_Rates_Table" and "ImpSA_DecompRate".

There will be questions throughout the R Markdown document, make sure to answer these questions as they are important in interpreting the statistics. Once you are finished the R lesson you will navigate back to this Microsoft document and complete the "Questions" page where we relate what we did in R back to the bigger picture.

**Note: The LeafDecomp.csv file is not the author's original dataset. The original dataset can be found [here](#). The edited dataset that you will download is the cleaned-up version of it, in the format of a .csv. No data was changed. A Day=0 where percent remaining = 100% was added for every stream and its three replicates.*

Click the links below to watch a quick video on [Linear Regressions](#) and [ANOVA](#) testing

Puerto Rico Stream Identifiers	
Identifier	Stream Name
CC	Correo de Cupey
VEN	Rio Piedras
HP	Quebrada las Curias
MON	Montehiedra
LL	Las Lomas
MAT	San Patricio

Within the dataset, the authors used these stream identifiers to represent the tested streams.

*Note: The authors explicitly stated that CC is the Correo de Cupey stream. They did not explicitly label the other streams with their stream identifiers. Stream identification was done by matching the mean decomposition rates (slope) of the linear regressions performed in R to their paper's **Table 2**.*

Outline of Proposed R Lesson

Estimated time to complete: 25-35 minutes with time for questions to be answered

*Note: code has been added in the R document for those who may experience the **Stream** columns of each of the data files turning to **"i..Stream"**. Run this code chunk only if you are experiencing this issue!*

Before beginning in R, read the "Read Me" file to understand the datasets that will be incorporated into this lesson.

- Part 1: Finding each stream's leaf litter decomposition rate.
 - Build six linear regressions
 - Record the slope and standard error for each stream in the table:
"Stream_Decomposition_Rates_Table"
 - Load the table just created back into R and build a plot.
- Part 2: Linking Urbanization to leaf litter decomposition rate
 - Record the slopes from Part 1 of this exercise and transfer them to the table:
"ImpSA_DecompRate"
 - Load in the **"ImpSA_DecompRate"** data set back into R with the new decomposition rates data filled in.
 - Build a plot to visualize the data
 - Run a last linear regression to show how decomposition rate of leaf litter is a factor of how much urbanization (measured as % impervious surface area) is present in the area.

Citations:

- Carlisle, D. M., & Clements, W. H. (2005). Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. *Freshwater Biology*, 50(2), 380-390. doi:10.1111/j.1365-2427.2004.01323.x
- Classen-Rodríguez, L., Gutiérrez-Fonseca, P. E., & Ramírez, A. (2019). Leaf litter decomposition AND macroinvertebrate ASSEMBLAGES along an urban stream gradient in Puerto Rico. *Biotropica*, 51(5), 641-651. doi:10.1111/btp.12685
- Functional feeding groups. (n.d.). Retrieved April 29, 2021, from <https://dep.wv.gov/WWE/getinvolved/sos/Pages/foodweb.aspx>
- García-Palacios, P., McKie, B. G., Handa, I. T., Frainer, A., & Hättenschwiler, S. (2015). The importance of litter traits and decomposers for litter decomposition: A comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology*, 30(5), 819-829. doi:10.1111/1365-2435.12589
- Ramírez, A., Rosas, K. G., Lugo, A. E., & Ramos-González, O. M. (2014). Spatio-temporal variation in stream water chemistry in a tropical urban watershed. *Ecology and Society*, 19, 45. <https://doi.org/10.5751/ES-06481-190245>
- Straka, M., Syrovátka, V., & Helešic, J. (2012). Temporal and SPATIAL MACROINVERTEBRATE VARIANCE compared: Crucial role of CPOM in a headwater stream. *Hydrobiologia*, 686(1), 119-134. doi:10.1007/s10750-012-1003-6

Questions:

Questions from R Lesson (please answer the questions from the R Markdown document here)

- **Question 1:** Looking at the plot and the standard error bars, what can you interpret from this plot?
- **Question 2:** Based on the reading, which Stream seems to have the least urbanization? Which streams seem to be heavily affected by urbanization?
- **Question 3:** Question 3: Look at this graph. What can you infer about this plot? How can the decomposition rate be higher in ~66% impervious surface area than where the impervious surface area is ~58%?
- **Question 4:** Is there a significant relationship between impervious surface area and leaf litter decomposition rate? Utilize the r^2 and p-value along with the slope and SE.

Questions for the Qubes Lesson

- Why does an increase in impervious surface mean a decrease in leaf litter decomposition?
- What is the relationship between leaf litter decomposition and shredders (the macroinvertebrate responsible for the decomposition of CPOM to FPOM)? Even though we did not recreate the macroinvertebrate assemblage analysis that the authors had done, what does a changing leaf litter decomposition rate mean for the shredders?
- What problems could a change in leaf litter decomposition mean to the local human populations?
- What are ways that this ecological problem can be mitigated? What are ways to bring a poor aquatic ecosystem back towards a healthy, functioning one?