

# Freshwater Communities: Using Leaf Packs to Explore Community Assembly



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## Abstract

Leaf packs are essential in creating new habitats and food sources for communities, making them an excellent research tool. Using leaf packs, this study set out to determine how abiotic factors affect the community assemblage of Macroinvertebrates that inhabit Red Bug Slough in Sarasota Florida. In these experiments, 10 leaf packs were deployed into two sites in Red Bug Slough. After 2 weeks the bags were collected and data on specimen abundance was collected. Abiotic factors were recorded from both sites. A Shannon's diversity index was used to calculate the diversity of site A and B as well as Red Bug Slough as a whole community. Diversity scores of the individual sites showed a H value nearly twice as large at site A than that of site B, which would indicate more species richness at site A ( $H(B)=3.49$ ,  $H(A)=6.28$ ). A two sample T-test was performed to assess the significance of the scores, and no significant difference was found ( $p=0.25$ ). Future improvements include a bigger sampling size and possibly comparing more than two sites.

## Introduction

Allochthonous inputs in the form of deposited leaves from nearby trees provide much of the basis for many food-chains in forested, low order, streams (Fargen et al. 2015). The deposited leaf material builds up into what are known as leaf packs, which can be considered



Figure 1: Research team collecting abiotic data for site B  
Photo credit: Emily Saarinen

"new habitats" and "new food sources" for a wide range of different stream organisms to inhabit (Saarinen 2016). This means that leaf packs potentially provide a reliable and easily manipulated medium to test a defining question in ecology: what factors determine the amount of different types of organisms found and their relative abundance at a given location?

A review paper by Belyea and Lancaster delve into the answers for this question by highlighting what they call "assembly rules." Belyea and Lancaster define that these "assembly rules" are determined by three principal constraints: dispersal, environmental, and internal dynamics. Dispersal constraints are defined as the ability for an organism to get to a new habitat. Environmental constraints are defined as the abiotic conditions and resources that are available for an organism to survive, while internal dynamics are defined as the biotic interactions that an organism needs to survive such as presence of food and competitors (Saarinen 2016). Benthic invertebrates are generally used for bioassessment due to their sensitivity to taxa in diverse communities and is why they were utilized within this study (Nelson 2000).

The purpose of this study is to determine the effects of abiotic resources on the community assemblage of Macroinvertebrates species that inhabit Red Bug Slough Nature Preserve of Sarasota, Florida. We selected two separate locations for testing, site A was along the south side of the slough and site B was in the wetlands restoration site. We predicted that site A would have less favorable abiotic factors due to its closer proximity to residential areas compared to site B, and as a result of that there would be more diversity seen in the leaf packs in site B.

## Methods

A total of 10 leaf packs were prepared by filling out an oyster bag with dead leaves and a rock. The rock was used to submerge the bag in the water. We tied the bags off with a zip tie and labeled bags 1 through 10. The bags were divided into 2 groups, for each location, bags 1-5 for site A and 6-10 for site B in the wetlands. Using rope, the bags were tied off to either a tree or an infrastructure near the water and a yellow flag was put down to mark the location. It was marked on a map where the bags were deployed in the Red Bug Slough.

After 2 weeks, we went back to Red Bug Slough to collect the bags. Each bag was pulled out of the water and immediately placed in a garbage bag to prevent losing any specimen. The bags were transported back to New College of Florida. We randomly chose 4 out of 5 bags from each group and each bag was dumped into a white container and a timer was set for 30 minutes. Each bag was looked through for 30 minutes and specimen picked out of the leaves. Once the time was up, specimen were separated by species and counted to determine species richness within the community.

A water sample of each location within the Red Bug Slough was collected to do water quality test. Water quality test included pH, dissolved oxygen, nitrate, phosphate, total dissolved solids, salinity and conductivity.

Data collected from the Red Bug Slough was separated into two groups of site specific data. The south (site A) dataset was collected in an area close to a residential community, leading to increased possibility of human influence of water conditions. The wetland dataset was collected in an area with less residential development compared to that of the other location (site B).



Figure 2: Leaf packs  
10 leaf packs were put together from an oyster bag packed with dead leaves and a rock to submerge the bag.  
Photo credit: Alexander Witter

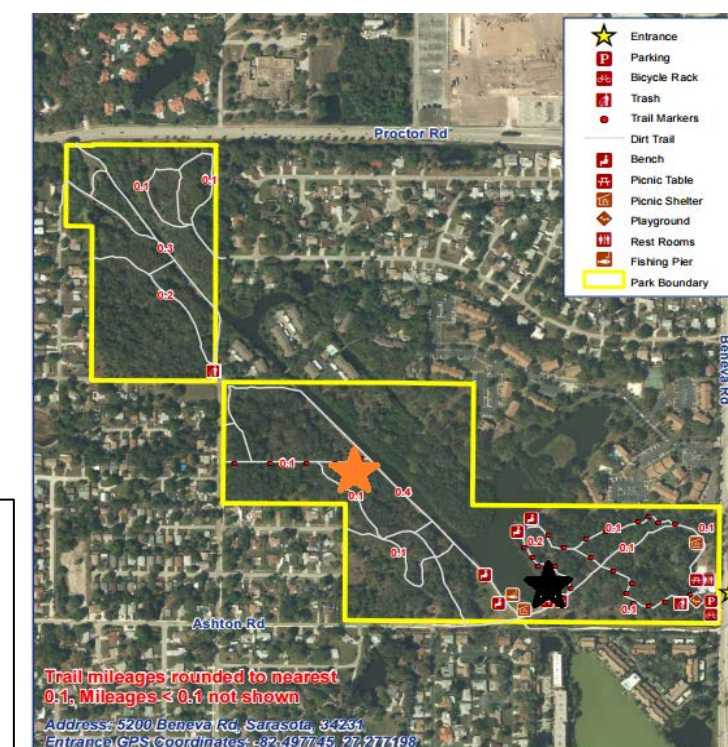


Figure 3: Map of Red Bug Slough  
Site A: ★  
Site B: ★  
Sarasota County: Nature Parks and Preserves

## Results

Data from the individual leaf packs at each site were combined into two datasets corresponding to the site location. A Shannon's diversity index was used to calculate the diversity of the individual sites as well as Red Bug Slough as a whole. The diversity score for the slough was found to be unusually high, which may be a result of a broad sampling of species, which would increase the H value ( $H_{All}=9.49$ ). Calculations of diversity scores of the individual sites showed a H value nearly twice as large at the site A than that of site B, which would indicate more species richness at site A ( $H_B=3.49$ ,  $H_A=6.28$ ). A two sample T-test was performed to assess the significance of the scores, and no significant difference was found ( $p=0.25$ ).

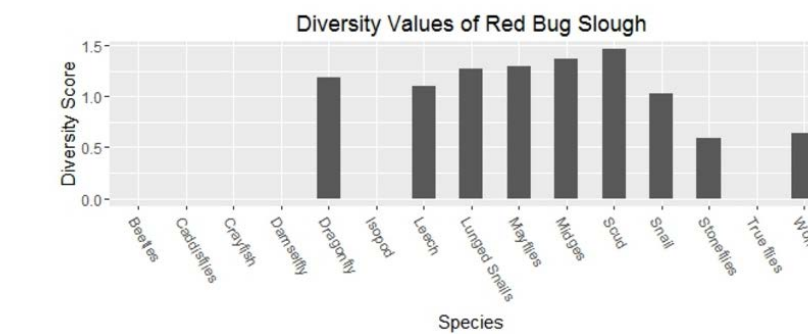


Figure 4: Shannon's diversity index for the Red Bug Slough (Site A and site B) was calculated to be  $H=9.49$ .

Abiotic data was collected at the two locations. Measurements of pH, phosphates and nitrates were identical between the two locations, both showed elevated levels of phosphates and nitrates compared to ideal environmental levels ( $pH=8$ , Nitrate=5, Phosphates=1). Dissolved oxygen measurements of the wetlands measured nearly twice as high as the southern testing site, which should favor higher populations of organisms, yet this is not reflected in the sampling of the leaf packs ( $DO_B=9$ ,  $DO_A=5.65$ ). A rank abundance curve of Red Bug Slough was made to better visualize species richness and species evenness. The curve shows a nearly linear downward slope, suggesting that there is high species richness but low species evenness among the nine species collected.

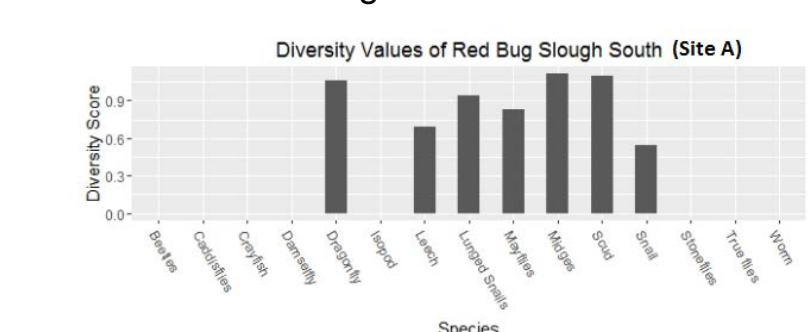


Figure 5: The diversity score per species of site A is shown above. There was a total of 7 species that collected at this site. The Shannon's index was found to be  $H=6.28$ .

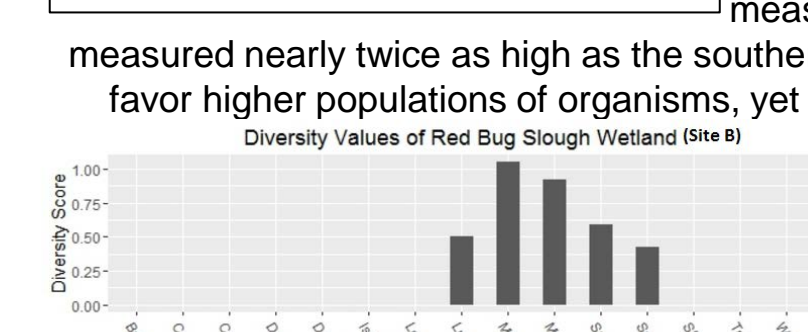


Figure 6: The diversity score per species of site B is shown above. There was a total of 5 species collected in this site. The Shannon's index was calculated to be  $H=3.49$ .

Species	Species Richness	Rank
Midges	231	1
Snail	144	2
Lunged snails	84	3
Scud	42	4
Mayflies	32	5
Dragonfly	23	6
Stoneflies	11	7
Caddisflies	5	8
Leech	3	9

Table 1: Left Table corresponding species to the rank given in the Abundance curve in Figure

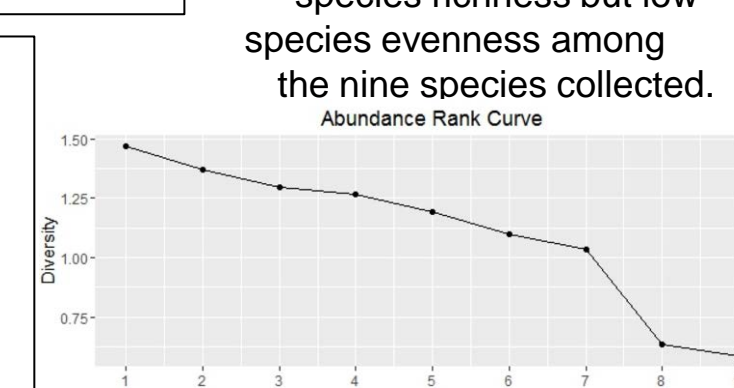


Figure 7: Above The Abundance curve shows the relationship between species evenness and richness. The downward slope shows there is a high species richness but a low species evenness.

## Conclusion

We expected the abiotic conditions that we were able to collect data for would show generally more favorable conditions in site B than in site A, as a result of human influences from residential areas closer to site A. In the end, most of the abiotic data we collected was nearly identical between the two sites except dissolved oxygen, which was higher at site B. The higher dissolved oxygen normally indicates more favorable conditions for aquatic life, which in theory would increase species richness, but the diversity scores we calculated between the two sites does not support this assumption. Site A had a diversity score (H value) nearly twice that of site B, but the performed statistical T test indicates that the difference is not significant ( $p=.25$ ), and that it cannot be concluded that species diversity is different between site A and site B within Red Bug Slough.

There are a few possible explanations for the discrepancies that could account for the conclusion made within this study. First off, the small sample size of 5 bags at each site could account for the high p-value of the statistical T-test used to determine the significance of the dataset. The small sample size could potentially create a high standard deviation that ultimately results in a higher p-value within statistical t-tests. Second, communication and field-work data taking techniques was a significant source of error within this study. The communication and data-taking errors created a significant hole in the data set that had to be accounted for in data analysis. Thirdly, it is worth noting that at site B many of the marker flags were harder to find because they had sunk into the mud or shifted position way more than at site A. This is an indication that a potentially important major factor was not included in our analysis: the effects of the differing substrates on the community assemblage of the leaf packs. Since we did not sample the substrate type, there is no way of known whether the leaf packs were affected by differing substrates.

Overall our prediction about differences in species richness in the leaf packs as a result of different abiotic conditions between our two test sites was not substantiated based on the data we collected. We could not show a significant difference in either abiotic factors or species diversity scores between the two sites, though some difference was seen in each of those categories, we could need much larger sampling efforts to be able to determine significance.



Figure 6: Left Research team collecting abiotic factors for site A.  
Photo credit: Emily Saarinen



Figure 7: Right Picture of Site A  
Photo credit: Emily Saarinen

## Bibliography

- Belyea, L. R., & Lancaster, J. (1999). Assembly rules within a contingent ecology. *Oikos*, 402-416.
- Fargen, C., Emery, S. M., & Carreiro, M. M. (2015). Influence of *Ionicera maackii* invasion on leaf litter decomposition and macroinvertebrate communities in an urban stream. *Natural Areas Journal*, 35(3), 392-403.
- Nelson, S. (2000). Leaf pack breakdown and macroinvertebrate colonization: Bioassessment tools for a high-altitude regulated system? *Environmental Pollution*, 110(2), 321-329.
- Saarinen, Emily. (2016) Freshwater Communities: Using Stream Leaf Packs to Explore Community Assembly.

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