

A Macroinvertebrate Survey of Sandy Creek in Durham County, NC: A Comparative Study of  
Post-Restoration and Pre-Restoration Surveys

by

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**ABSTRACT:**

Sandy Creek, located within the Cape Fear watershed within the Triassic Basin of North Carolina, is a first order stream within a tributary watershed that feeds into Jordan Lake reservoir. A biological assessment, following the NCDENR Benthic Standard Operating Procedure, was performed and all macroinvertebrates were identified at three sites over three months within the upper Sandy Creek watershed within Duke University Wetland Center's Stream and Wetland Assessment Management Park (SWAMP). This was done three years post-restoration in 2008. Mud Creek, Sandy Creek's reference stream, was also sampled in 2008. This bioassessment was performed as a follow up to the baseline macroinvertebrate survey completed pre-restoration in 2004/2005 and to determine the restoration's effect on its macroinvertebrates and water quality. The biotic index values calculated from the pre-restoration and post-restoration macroinvertebrate tolerance and abundance levels indicated a decrease in biotic index value or an increase in water quality (6.7 pre-restoration to 6.4 post-restoration). The biotic index values calculated for the three post-restoration sites show a water quality improvement as the water flows through the restoration (6.58 input to 6.434 midpoint to 6.42output) which may indicate that the restoration is increasing stream function. The macroinvertebrate orders and feeding types that were collected provided additional information on the difference between pre- and post-restoration Sandy Creek.

## INTRODUCTION:

Sandy Creek is located within Duke University Wetland Center's Stream and Wetland Assessment Management Park (SWAMP) (see Figure 1 for location). The second phase of the restoration was completed in 2005. It is a restored stream within an ongoing restored wetland project. The upper portion of Sandy Creek is a state-designated Natural Heritage Program Priority Area (NCDENR 2001). It also is a headwater for the Cape Fear watershed in the Piedmont of North Carolina. Wetlands located within the headwater of a watershed are an important component of improving water quality. They also play a vital role in reducing runoff of pollutants and sediment. (Richardson 2007)

Water quality within Duke University Wetland Center's Stream and Wetland Assessment Management Park (SWAMP) had a low level of water quality which drove the decision for restoration. The poor water quality was attributed to the high concentration of development within its watershed (impervious surface > 20%) and the soil characteristics (low infiltration rates and high runoff potential).

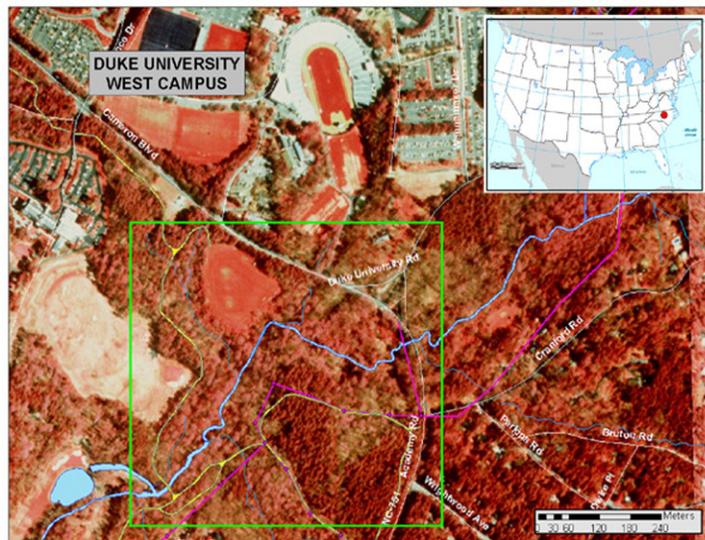


Figure 1: Location of Sandy Creek. Aerial photo taken pre-restoration (1998). The green box encloses the future location of the SWAMP restoration project. The pink line indicates the adjacent sewer line. (Richardson 2007)

I have attempted to assess the effectiveness of Sandy Creek's and its adjoining wetlands' restoration within the Duke Forest in Durham County, North Carolina by evaluating the macroinvertebrate populations within the stream/wetland system. The state of North Carolina includes assessing macroinvertebrates in stream assessment projects. This reach of Sandy Creek has not been assessed through the collection macroinvertebrates since pre-restoration.

Aquatic macroinvertebrates serve as bioindicators for environmental quality. Certain species are sensitive to a range of low to high levels of pollutants including high nutrients, industrial pollutants, and sediment. It is important to provide evidence that the restored wetland is carrying out its intended functions of filtering out chemicals and sediments to improve the quality of local water systems (Gaufin 1973). Cooperating and granting agencies will be particularly interested in this information as a follow up to the restoration project to which they have devoted so much time and money. These agencies include: Clean Water Management Trust Fund, Duke University, Durham Soil and Water Conservation District, National Science Foundation, New Hope Creek Corridor Committee, North Carolina Department of Environment and Natural Resources, North Carolina Ecosystem Enhancement Program, US Environmental Protection Agency, and US Department of Agriculture. (Richardson 2007)

### **Macroinvertebrates and Water Quality**

The macroinvertebrates that have been used to assess water quality are strictly aquatic benthic macroinvertebrates, although macroinvertebrates can exist in varied ecosystems. These benthic macroinvertebrates are used as an indicator of water quality because water quality is usually the factor limiting macroinvertebrate biodiversity (Beavan et al. 2001). Water sampling

is not as affective at taking into account the water quality between samplings. For macroinvertebrates to exist in a sample would mean that those benthic macroinvertebrates have had to survive all the water quality conditions that the stream has endured. Therefore, benthic macroinvertebrate sampling combines both short-term and long-term water quality conditions that may be missed by solely sampling water (NCDENR 2006).

There are several known techniques of sampling macroinvertebrates for the purpose of a biological assessment. O'Neil (1992) is known for creating a biological assessment method to attempt to determine whether the structure of benthic macroinvertebrate communities are significantly impacted downstream of a discharge site. Gaydos et. al (2001) employs a method similar to NCDENR with biological index numbers, with a scale that ranges from 0-5. The NCDENR (2006) employs four different methods to complete a biological assessment through macroinvertebrate sampling. These four different methods are as follows:

1. The standard qualitative method - Used to assign water quality ratings to wadeable streams and is applicable for between-site, and/or between-date comparisons and should be used for all evaluations of impaired streams.
2. The EPT method - Used to quickly determine site differences in water quality and is useful for larger basin wide studies and quick emergency sampling. Just the pollution sensitive EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera) are counted. This can provide too little information for a bioassessment of water quality.
3. The Qual 5 or Qual 4 method - Uses the same method as EPT method, but adds one rock/log wash, and is used for very small streams.
4. The boat sampling technique - Used for deeper nonwadeable waterways and is an adaptation of the standard qualitative method.

The standard qualitative method was used for this study because it is the best method to be used for between-site and between-date comparisons. The pre-restoration data was collected using this method as well.

Benthic macroinvertebrates can also be categorized by functional feeding group. The abundance of a certain feeding type can indicate a disturbance or something else about the conditions of the stream. The six feeding type categories are gathering collectors (GC), predators (PR), filtering collectors (FC), shredders (SH), and scrapers (SC). Collectors obtain nutrients from fine particulate organic matter (FPOM) and are separated into two groups: filtering collectors and gathering collectors. The filtering collectors obtain food from the water column with filters and fan-like structures. The gathering collectors feed off of FPOM that they have gathered along the stream bottom. Predators prey on other macroinvertebrates. Shredders shred leaves in order to feed and are responsible for the conversion of 30% of coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM). Scrapers are herbivores that feed by scraping periphyton off surfaces. (NCDENR 2006). Gatherers and filterers are generalists and are less sensitive to pollution because they can easily adapt. Shredders and Scrapers are specialized feeders and are more sensitive to pollution (Rawer-Jost 2004).

## **Site Description**

Located within the Cape Fear watershed and within the Triassic Basin of North Carolina, Sandy Creek is a first order stream within a tributary watershed that feeds into Jordan Lake reservoir. This reservoir is a major source of drinking water for the Triangle area. Sandy Creek is also a tributary of New Hope Creek. New Hope Creek flows from north to south within Durham County. When it enters north Durham it passes all North Carolina's pollution standards but often

does not pass these standards by the time it flows out of Durham County. The stream then continues on to Jordan Lake reservoir in this degraded state. (Richardson 2007)

Stormwater and runoff drains from approximately 1,400 acres of Durham, North Carolina into Sandy Creek. This includes most of Duke University's campus. Please refer back to Figure 1 for a visual of the location. Before Sandy Creek's restoration as part of the SWAMP project, its waters were impaired by urban pollutants and sediment from its watershed. The sources of these problems included: nutrient-rich stormwater inputs from urban/suburban development and West Campus of Duke University; an incised channel which results in a disruption of the stream's natural hydrology; and fecal coliforms originating in sewer lines that have the tendency to overflow during storms. (Richardson 2007)

As a response to Sandy Creek's importance within the watershed and its degraded state, a three phase restoration of the wetland and creek was developed by Duke University Wetlands Center, Duke Forest, and the Department of Civil and Environmental Engineering. Phase one was made up of the stream recontouring and floodplain re-establishment. Its goal was to promote overbank flooding during storms which results in increased biogeochemical reactions that filter the water and re-establishes natural hydrology. Figure (2) shows a photo of the pre-restored Sandy Creek and Figure (3) a photo of the post-restored Sandy Creek. (Richardson 2007).Phase two was made up of the dam and impoundment construction. Its goal was to reduce nutrients and sediment by slowing down and withholding the water to promote sedimentation and biogeochemical processing.



Figure 2: Pre-restored incised channel of Sandy Creek. (Richardson 2007)



Figure 3: Post-restored Sandy Creek with restored sinuosity and floodplain in 2005. (Richardson 2007)

Phase Three consisted of the construction of the stormwater treatment wetland and was completed in 2007. The goal of this phase was to depress the high concentrations of nitrogen and

phosphorus running off West Campus. This is done with six constructed wetland cells that retain water which overflows out of the creek during storm events. The nitrogen and phosphorus that are retained in these cells settle out in the soil and are taken up by wetland plants, as well as, converted by bacteria. These processes reduce the high quantity of pollutants that may be carried downstream. (Richardson 2007)

### **OBJECTIVES AND HYPOTHESES:**

My overall objective in this study is to determine the biological index of Sandy Creek after the stream and wetland restoration occurred in 2005 on the main channel. By determining this, the impact of the SWAMP wetland and stream restoration on water quality can be gauged. I have attempted to do this by comparing the macroinvertebrate populations from Mud Creek, the reference site, to populations found in Sandy Creek. Mud Creek is designated as a reference site (or control) to Sandy Creek because of its similar morphology, its higher water quality, and its history of fewer disturbances. In addition, I have compared my results to a past Masters of Environmental Management (MEM) student's, Brian Roberts, pre-restoration baseline macroinvertebrate study.

I hypothesized that the biological index of Sandy Creek would be lower or of higher water quality compared to pre-restoration data. I also hypothesized that the biological index of Sandy Creek would be higher or of lower water quality compared to Mud Creek, the reference site. A protocol and guide used by the state of North Carolina was employed to assess and collect the macroinvertebrates. The official term used for this method of sampling is called sub-sampling. The macroinvertebrate and water samples were collected over a summer to match the

sampling done earlier. Three sampling events were completed in summer 2008. They were executed monthly for May, June, and July.

## **METHODS:**

### **Approach**

This study is made up a field and lab component. Specimens and water samples have been collected from Sandy Creek and were analyzed in the lab. The quality of the stream was then assessed by taking the assigned indicator weights to the species, tallying the abundances, and then computing the weighted averages. These averages were compared to the reference site, Mud Creek, and past data from pre-restored Sandy Creek. (Doberstein 2000) A study on the macroinvertebrate populations post-restoration in Sandy Creek has not been done prior to this study.

Data critical to these analyses are macroinvertebrate species identification, their assigned weights (NC Division of Water Quality 2005), macroinvertebrate data from the reference creek (Mud Creek), data from pre-restored Sandy Creek, macroinvertebrate habitat constraints, macroinvertebrate abundance data in Sandy Creek post-restoration, and water quality data from Sandy Creek over three months post-restoration. Data already available includes: macroinvertebrate species identification, their assigned weights from the state, past data from pre-restored Sandy Creek, and macroinvertebrate habitat constraints. Macroinvertebrate abundance data for post-restored Sandy Creek, macroinvertebrate abundance data for Mud Creek, and water quality data for post-restored Sandy Creek and Mud Creek was collected over May, June, and July 2008.

## **Data Collection**

The methods of biological assessment were derived from those of the NCDENR 2006 standard qualitative method, which includes the following;

- One kick net sample
- One sweep-net sample if suitable areas were present
- One leaf-pack sample if leaf packs were present
- Fine mesh rock and/or log wash samples
- Ten minute visual collection

Data was collected in May, June, and July of 2008 with three sampling sites per month. The sampling sites were chosen to best maintain the goals that sampling sites will be of similar flow speed, depth, substrate composition, and sunlight exposure to eliminate complicating factors. Sampling was done later than 48 hours after a storm event to avoid contaminating samples with an influx of water and runoff sediments/nutrients.

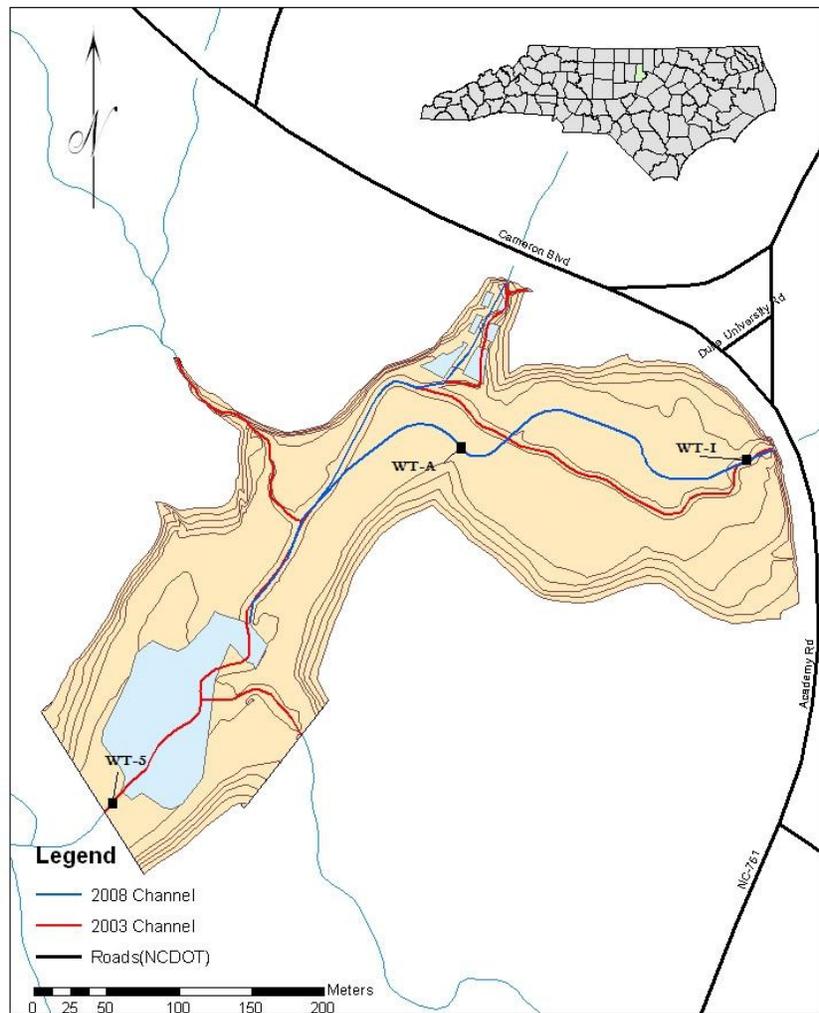


Figure 4: 2008 map of the SWAMP site with the three macroinvertebrate sampling sites (WT-1, WT-A, and WT-5).

The three sampling sites (Figure 4) were made up of a meter by meter cell. A sampling event was started at the site furthest downstream (WT-5) in an attempt to avoid contaminating the following two samples. Then sampling moved upstream to site WT-A and finally, to site WT-1. Sampling was then executed by disturbing the substrate by kicking and use a standard

aquatic insect kick net facing upstream to catch the debris and insects disturbed (net was held by a partner).

The kick net method is used to collect larger macroinvertebrates. A kick net is made up of a meter by meter double layer of flexible nylon screening stretched between two wooden poles. For sampling purposes, the net is held upright in the streambed with the bottom of the net flush to the stream bottom. The kick net used in this study did not have a weighted bottom edge. To keep the net flush with the streambed, a few cleaned large stones were used to secure the bottom edge of the net.

For the kick net sampling, if the meter by meter cell's substrate was rocky, the rocks located in the cell were rubbed vigorously underwater upstream of the kick net until the area was cleared of rocky substrate. Sampling was completed by physically disrupting the remaining sediment/sand using hands and/or feet in the rockless cell for one minute. If the cell's substrate was sandy, the substrate within the cell was disrupted upstream into the standard aquatic kick net for five minutes. Once this period was completed, the kick net was pushed forward slightly and lifted, being careful that no debris was lost. The sample within the kick net was rinsed into a sieve bucket with U.S. standard No. 30 sieve (0.600 mm openings) bottom and then was transferred to the appropriate labeled container for transport back to the lab.

Sweep net samples target edge macroinvertebrate species. This sampling is conducted by dragging a triangular net over the vegetation or under the logs on the banks. The sweep net used in the study was composed of the same size mesh as the kick net used. If submerged logs, aquatic, or bank vegetation present in the transverse from the sampling site to the shore on either side, a sweep net sample was taken once per area present. The collected sample within the sweep

net was also rinsed into a sieve bucket with U.S. standard No. 30 sieve (0.600 mm openings) bottom and then was transferred to the appropriate labeled container for transport back to the lab.

If leaf packs were present, a leaf pack sample was taken as well. A leaf-pack sample consists of collecting the groupings of leaves and other floating woody debris that have collected on snags or rocks in the creek. Then the sample is deposited and washed down into a sieve bucket with U.S. standard No. 30 sieve (0.600 mm openings) bottom. This was done by filling a tray with water and pouring it over the sample inside the bucket. This process removes finer sediments, making it easier to dissect the macroinvertebrates from the debris. The remnants in the sieve bucket were transferred to the appropriate labeled container for transport back to the lab. Rarely were any of the previously mentioned potential aquatic macroinvertebrate habitats present for sweep net and/or leaf pack sampling.

Finally, a ten minute visual inspection of the general area of the stream (5 meters radius around the sampling site) was completed by picking up rocks, leaves, logs, etc. and extracting what was there with forceps. This process was halved to five minutes if there were two people completing the visual inspection.

The samples were deposited from the kick net, sweep net, and leaf packs into labeled containers by site for transport back to the lab. Once back at the lab, the debris that was collected was placed into white water filled trays and the macroinvertebrates present were extracted using fine-tipped forceps. The macroinvertebrates present in the sample were placed in an alcohol filled dish. Then they were classified and deposited in labeled vials.

## Macroinvertebrate Identification

The identification of the macroinvertebrates to the lowest possible taxonomic group was aided by NC DENR’s Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina, John Epler’s Identification Manual for the Water Beetles of Florida, and Brigham’s Aquatic insects and Oligochaetes of North and South Carolina. Some of the identifications that were more challenging were made with the guidance of Bobby Louque of the City of Durham’s Stormwater Services Water Quality Division. Tables 1, 2, and 3 each list the macroinvertebrate species collected at WT-1, WT-A, and WT-5 over May, June, and July. Table 4 lists the species sampled from Mud Creek.

Table 1: The macroinvertebrates collected over May, June, and July on Post-restored Sandy Creek.

Order	WT-1	WT-A	WT-5	MC	PRE
Trichoptera	30	33	1	21	20
Oligochaeta	14	4	3	2	3
Gastropoda	4	5	3	2	12
Coleoptera	10	5	4	26	0
Odonata	1	0	4	0	32
Diptera	30	54	34	32	38
Hirudinidae	11	11	4	1	5

Ephemeroptera	1	1	2	1	14
Bivalvia	0	1	0	1	4
Crustacea	0	0	0	22	1
Plecoptera	0	0	0	1	1

The tolerance values assigned to the macroinvertebrates were the same as those used by the NCDENR. These tolerance values are used to calculate the biological index. Tolerance values are on a scale of 0-10. The 0 tolerance value indicates that the species is the least tolerant of pollution and is an indicator of superior water quality. A tolerance value of 10 means that the species is the most tolerant of pollutants and usually indicates poor water quality. A biological index can be determined by individual sampling sites but generally the sites on one creek are combined. The formula for the biological index is as follows:

**Eqn. 1:**      Biotic Index (BI) =  $\frac{\text{Sum}(TV_i)(n_i)}{N}$

TV<sub>i</sub> = ith taxa's tolerance value

n<sub>i</sub> = ith taxa's abundance value (1, 3, or 10)

N = sum of all abundance values                      (NCDENR 2006)

The abundance values of the macroinvertebrates are not the actual number of individuals collected. If 1-2 individuals of a certain species are collected, the species is given an abundance value of 1. If 3-9 individuals of a certain species are collected, the species is designated an abundance value of 3 and if greater than 10 individuals of a certain species are present, the species is given an abundance value of 10. (NCDENR 2006)

The Shannon-Wiener Index is biodiversity indicia used to measure categorical data. It takes into account the number of species and the evenness of the species. The formula for the index is as follows:

**Eqn. 2:**

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

$n_i$  = The number of individuals in species  $i$ ; the abundance of species  $i$ .

$S$  = The number of species.

$N$  = The total number of all individuals

$p_i$  = The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community:  $\frac{n_i}{N}$

(Shannon 1948)

The macroinvertebrates were also classified by feeding type in an effort to find meaningful results. Feeding type of the macroinvertebrates present at a site can glean more information from the data than biotic index can alone. The distribution of feeding types reflects process-level attributes of the ecosystem. (Rawer-Jost 2004)

A complete list of the macroinvertebrate data sampled is available in APPENDIX I.

## **Analyses**

Once the macroinvertebrates were collected, identified, and the tolerance value, abundance value, and feeding type were determined, the data from the three months was considered individually per site and per month. This is because it is expected one will find both a difference by month as well as by site (Astin 2006). The data collected from post-restored Sandy Creek was compared to the data from pre-restored Sandy Creek by feeding type, insect order, and biological index value. The data collected from post-restored Sandy Creek was then compared to the reference site, Mud Creek, using the same components.

The biological index values of pre-restoration Sandy Creek, post-restoration Sandy Creek, and Mud Creek were calculated using the Biotic Index equation (Eq. 1). The results were compared to each other to determine the difference in overall water quality. Then the biological index values were calculated per post-restoration Sandy Creek site to determine the difference in water quality among sites.

The Shannon-Wiener Index was calculated per site (Eqn. 2) just as the biological index value was. This provided a measurement of biodiversity while taking into account species abundance and evenness.

To effectively compare the sites by the order of macroinvertebrate, fractions/ percentages of the order's occurrence were created for the combined months of post-restored Sandy Creek and Mud Creek data. Fractions were created for pre-restoration Sandy Creek data as a singular site or event. It did not make sense to split the pre-restoration data into sites because the entire morphology of the stream changed after the restoration this. Also, doing so is not necessary to answer the questions that this study poses. The three post-restoration Sandy Creek sites were

compared with the Mud Creek site and with pre-restoration Sandy Creek (Figure 4). The three post-restoration Sandy Creek sites were also compared to each other.

Then fractions of the orders' occurrences were completed per month (May, June, and July) for the three post-restoration Sandy Creek sites and for the Mud Creek site. The results of each month's fractions were compared by site. This was completed with R statistical software.

To effectively compare the sites by the feeding type of macroinvertebrate, the same method of analysis was applied to the macroinvertebrate feeding groups as was to the macroinvertebrate orders. Percentages or fractions of the group's occurrence were created for the combined months of post-restored Sandy Creek and Mud Creek data. Fractions were created for pre-restoration Sandy Creek data as a singular event. The three post-restoration Sandy Creek sites were compared with the Mud Creek site and with the pre-restoration Sandy Creek sites. The three post-restoration Sandy Creek sites were also compared to each other. The proportion of shredders to total collectors was calculated for each site as well.

Then fractions of the feeding groups' occurrences were completed per month (May, June, and July) for the three post-restoration Sandy Creek sites and for the Mud Creek site. The results of each month's fractions were compared by site. This was completed with R statistical software.

Then the probability of each macroinvertebrate order and feeding group was calculated with R statistical software. This was done by fitting the data to a multinomial log-linear model. See APPENDIX II for R scripts.

## RESULTS AND OBSERVATIONS:

### Biological Index

The biotic index calculations per post-restoration Sandy Creek site, Mud Creek, and pre-restoration Sandy Creek are displayed in the table below.

Table 2: The Biotic Index Values for the three post-restoration Sandy Creek sites, Mud Creek, and pre-restoration Sandy Creek

Location	Sampling Site	Biotic Index Value
Post-restoration Sandy Creek		
	WT-1 (input)	6.58
	WT-A	6.43
	WT-5 (output)	6.42
	Average	6.48
Reference: Mud Creek		6.90
Pre-restoration Sandy Creek		6.72

As anticipated, the biotic index values within the SWAMP site have decreased. In other words, water quality has increased since the restoration. However, the degree of decrease in biotic index value is only by 0.14 to 0.32.

The sites within post-restoration Sandy Creek decreased in biotic index value from the site at the restoration's input (WT-1) to mid-restoration site (WT-A) to the restoration's output (WT-5) as hypothesized.

The reference stream, Mud Creek, was found to have a higher biotic index value than pre-restoration and all post-restoration sites on Sandy Creek. This is unexpected because the reference is used as a model or control that is meant to have superior water quality due to its history of fewer disturbances.

## Shannon-Wiener Index

Table 3: The Shannon-Wiener Index Values for the three post-restoration Sandy Creek sites, Mud Creek, and pre-restoration Sandy Creek

Location	Sampling Site	Shannon-Wiener
Post-restoration Sandy Creek		
	WT-1 (input)	2.66
	WT-A	2.743
	WT-5 (output)	2.515
	Average	2.640
Reference: Mud Creek		3.264
Pre-restoration Sandy Creek		3.425

The post-restoration Sandy Creek sites show no trend in their Shannon-Wiener indices. According to this calculation, they are all less diverse than Mud Creek. Pre-restoration Sandy Creek has the highest Shannon-Wiener value and therefore, seemed to be most diverse. These results are the opposite of what was hypothesized. (Table 2)

## Macroinvertebrate Order Percentages

When the fractions or percentages of macroinvertebrates were calculated by month excluding pre-restoration data, the overall results for post-restoration Sandy Creek and Mud Creek (MC) varied per month per site. The most consistent trend between the months is the increase of Diptera from input (WT-1) to output (WT-5). MC contained Ephemeroptera, Plecoptera, and Trichoptera (EPT) in May (Figure 5) but only contained Trichoptera in June (Figure 6) and July (Figure 7). MC was also the only site that contained Crustacea every month. Coleoptera is consistently higher for MC in May, June, and July. The graphs of June (Figure 6) and July (Figure 7) made it more evident that the percentage of Ephemeroptera was greatest at WT-5, the site of output. Trichoptera was high every month for sites WT-1 and WT-A but varied and was lower for MC and WT-5.

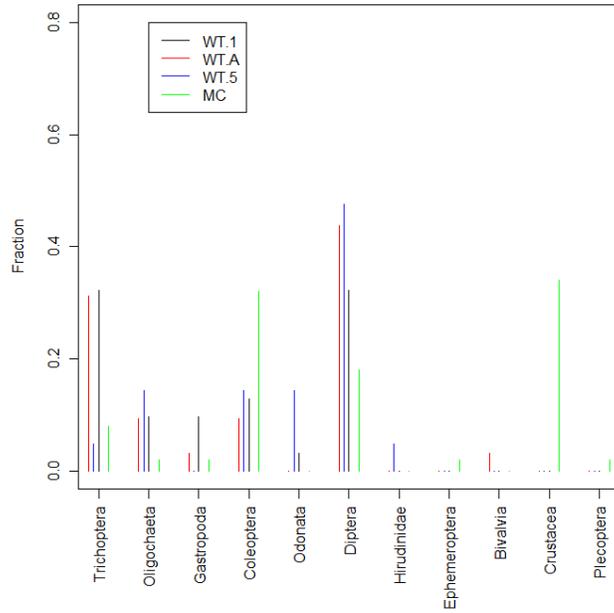


Figure 5: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output) and Mud Creek (MC) macroinvertebrate orders in fractions per site for May 2008.

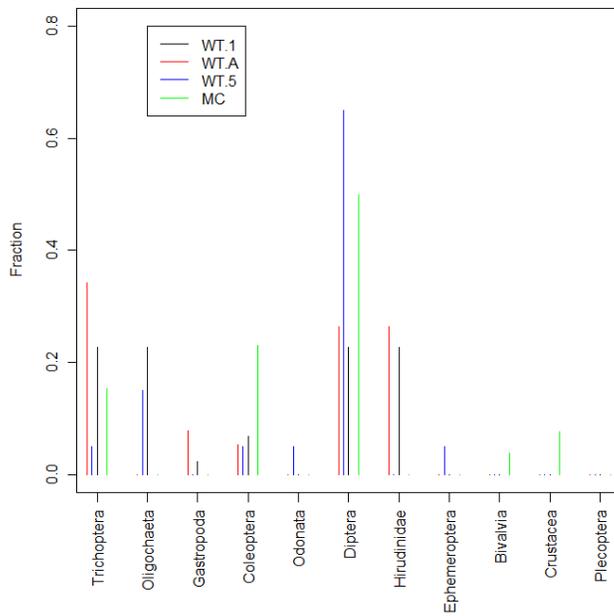


Figure 6: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output) and Mud Creek (MC) macroinvertebrate orders in fractions per site for June 2008.

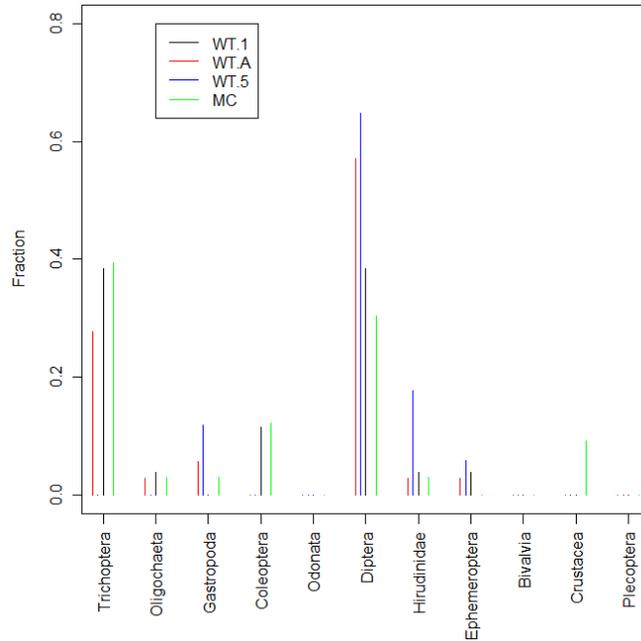


Figure 7: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output) and Mud Creek (MC) macroinvertebrate orders in fractions per site for July 2008.

When the total fractions of the insect orders for pre-restoration, Mud Creek, and post-restoration sites were compared significant differences and similarities emerged in macroinvertebrate composition (Figure 8). Out of the macroinvertebrates collected at each site, all five of the sites individually had a high percentage of Diptera than other orders present. Comparatively, the post-restoration output site (WT-5) contained the highest percentage, followed by the post-restoration mid-site (WT-A). The three remaining sites had approximately equal percentages of Diptera. Mud Creek had fairly equal percentages of Trichoptera, Coleoptera, Diptera, and Crustacea. The input site (WT-1) and mid-restoration site had the highest percentage of Trichoptera. Mud Creek had the highest percentage of Plecoptera, followed by pre-restoration samples. Pre-restoration Sandy Creek had the highest percentage of Ephemeroptera. Each of the post-restoration Sandy Creek sites was missing three of the macroinvertebrate orders. WT-1 and WT-5 were missing Bivalvia, Crustacea, and Plecoptera.

WT-5 was missing Odonata, Crustacea, and Plecoptera. Mud Creek was only lacking Odonata and pre -restoration Sandy Creek was missing Coleoptera.

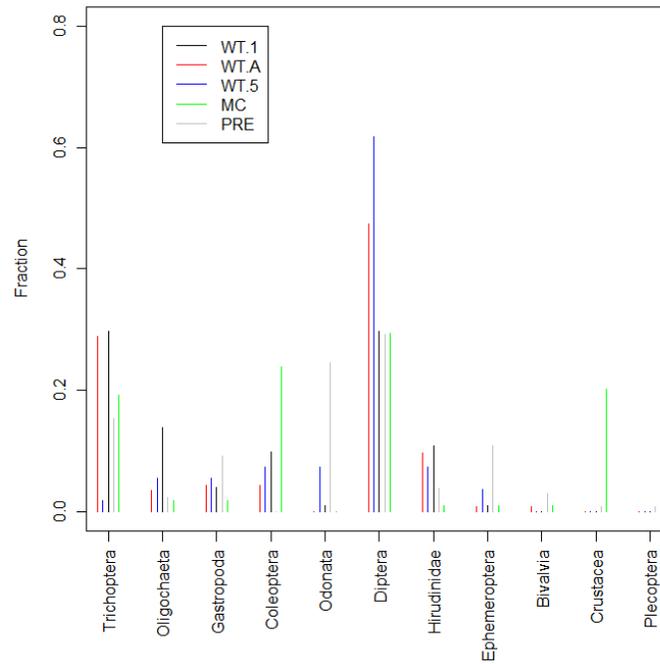


Figure 8: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output), Mud Creek (MC), and pre-restoration Sandy Creek (PRE) macroinvertebrate orders in fractions averaged per site.

## Macroinvertebrate Order Probabilities

Then I used multinomial regression to calculate the probabilities of each order per site. This analysis of probabilities further strengthens the conclusions drawn from the fraction analyses. The graph of probabilities (Figure 9) mirrors the graph of fractions (Figure 8) but is a better visual display of the trends of orders by site. Trichoptera decreased through SWAMP and Diptera increased. Oligochaetes decreased slightly as well.

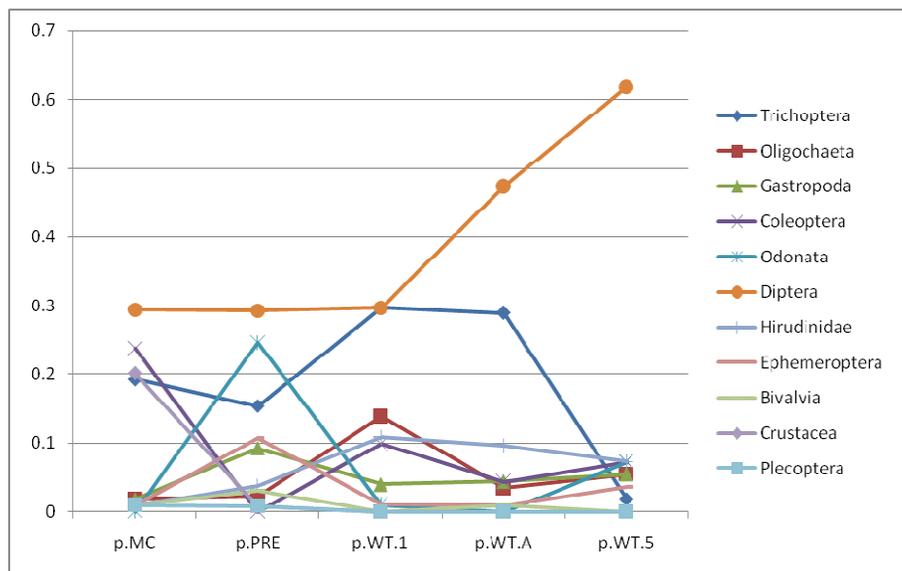


Figure 9: The post-restoration Sandy Creek (p.WT-1 input, p.WT-A, p.WT-5 output), Mud Creek (p.MC), and pre-restoration Sandy Creek (p.PRE) macroinvertebrate orders probabilities per site.

## Macroinvertebrate Feeding Group Fractions

The percentages of macroinvertebrate feeding types were calculated and graphed for the months of May, June, and July for the post-restoration and Mud Creek data (Figures 10, 11, and

12). The fraction of feeding types varied greatly for each site over the three months which shows that the insect populations fluctuate with the time of year as a result of rainfall or temperature. In May, the percentage of GC was highest at WT-5 and second highest at Mud Creek. Also, FC were highest at WT-A and second highest at WT-1. In June and July, the May trends of the percentages of both collectors (GC and FC) do not continue. They do continue to be present in all four sites, however. The percentage of predators decreased throughout the SWAMP in May but this trend did not persist for June and July. The percentages of SH and SC was the least consistent between the three months. There were no visible trends for SH and SC.

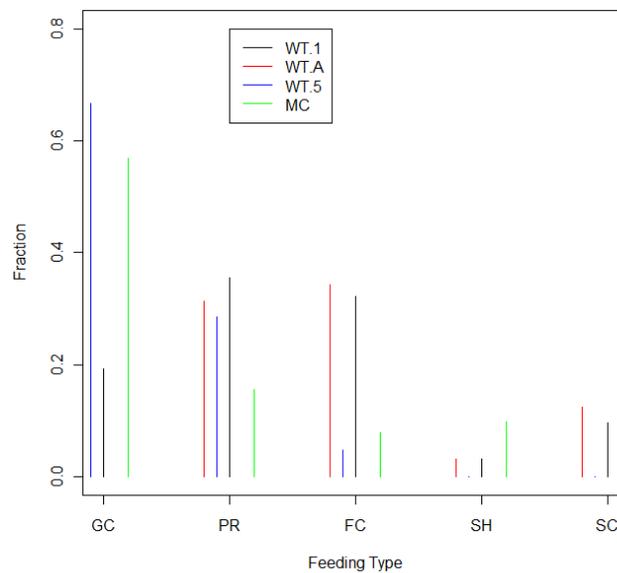


Figure 10: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output) and Mud Creek (MC) macroinvertebrate feeding groups in fractions per site for May 2008. (GC-gathering collector, PR-predator, FC-filtering collector, SH-shredder, SC-scraper)

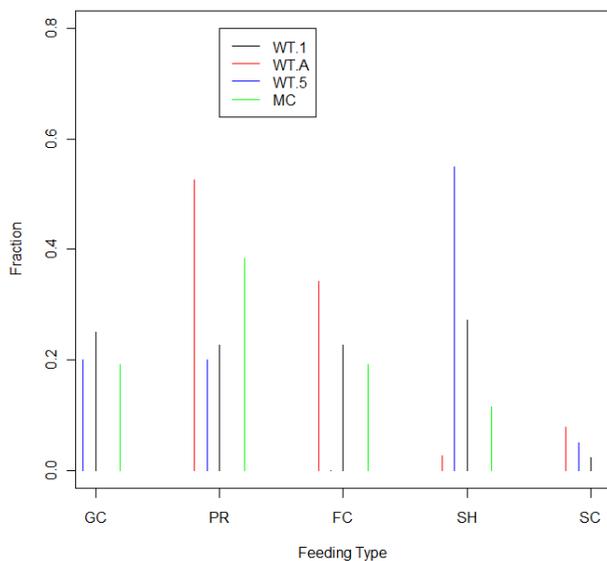


Figure 11: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output) and Mud Creek (MC) macroinvertebrate feeding groups in fractions per site for June 2008. (GC-gathering collector, PR-predator, FC-filtering collector, SH-shredder, SC-scraper)

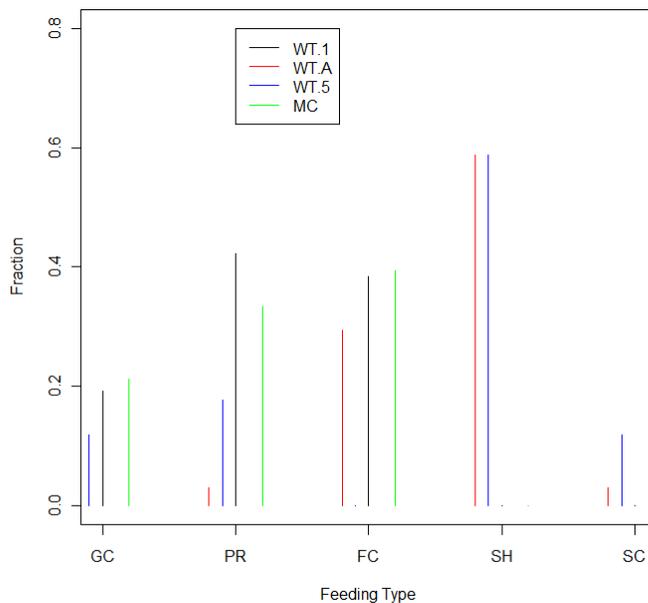


Figure 12: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output) and Mud Creek (MC) macroinvertebrate feeding groups in fractions per site for July 2008. (GC-gathering collector, PR-predator, FC-filtering collector, SH-shredder, SC-scraper)

The total seasonal percentages of the collected macroinvertebrates' feeding types for post-restoration Sandy Creek, Mud Creek, and pre-restoration Sandy Creek were calculated (Figure 13). Site WT-1, site WT-5, and pre-restoration Sandy Creek are the only sites that contain macroinvertebrates in every category of feeding type. Pre-restoration Sandy Creek contained significantly more predators than any other feeding type (55%). Post-restoration Sandy Creek sites WT-A and Mud Creek both contained four out of the five macroinvertebrate feeding types. Site WT-A was lacking gathering collectors and Mud Creek was lacking scrapers. WT-5 had low percentages of filtering collectors and high percentages of shredders. WT-5 had low percentages of filtering collectors and high percentages of shredders. Site WT-5 and Mud Creek had similarly high percentages of gathering collectors. Both WT-1 and WT-A had high percentages of filtering collectors and predators. Both Mud Creek and WT-5 had the lowest percentages of predators.

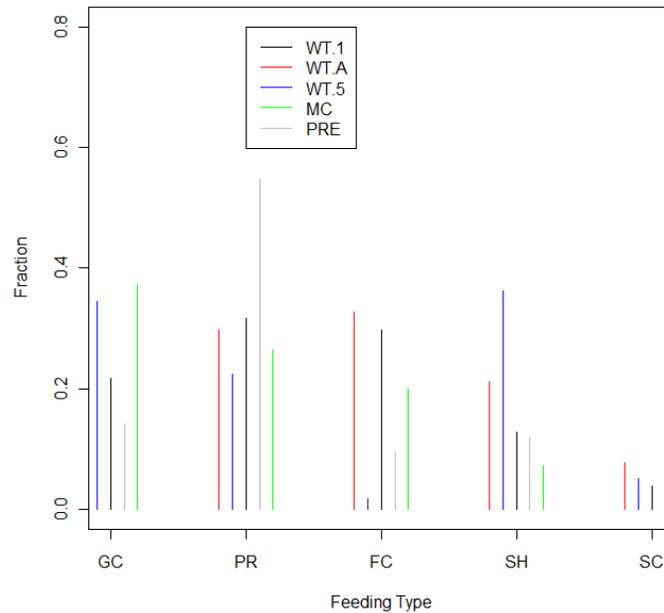


Figure 13: The post-restoration Sandy Creek (WT-1 input, WT-A, WT-5 output), Mud Creek (MC), and pre-restoration Sandy Creek (PRE) macroinvertebrate feeding groups in fractions averaged per site. (GC-gathering collector, PR-predator, FC-filtering collector, SH-shredder, SC-scraper)

## Macroinvertebrate Feeding Group Probabilities

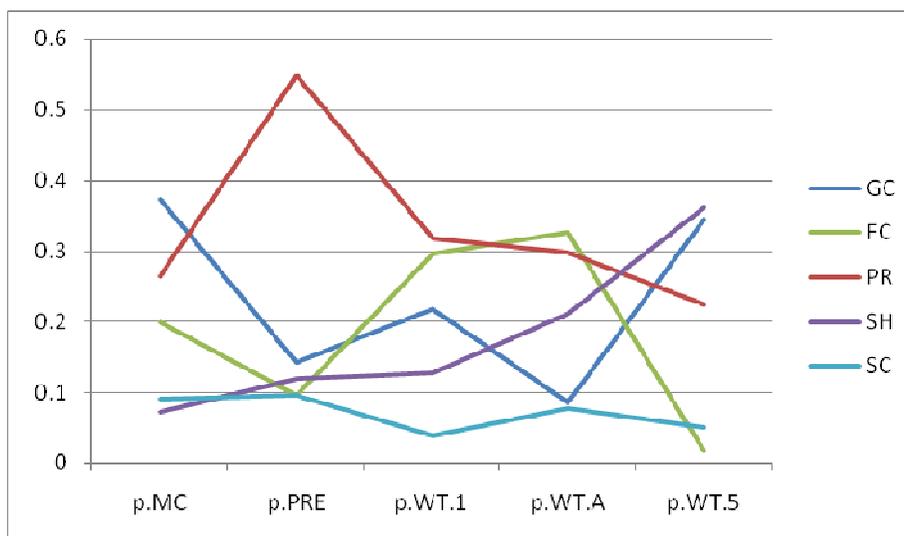


Figure 14: The post-restoration Sandy Creek (p.WT-1 input, p.WT-A, p.WT-5 output), Mud Creek (p.MC), and pre-restoration Sandy Creek (p.PRE) macroinvertebrate feeding groups' probabilities per site. (GC-gathering collector, PR-predator, FC-filtering collector, SH-shredder, SC-scraper)

Then I used multinomial regression to calculate the probabilities of each feeding group per site. This analysis of probabilities further strengthens the conclusions drawn from the fraction analyses. The graph of probabilities (Figure 14) mirrors the graph of fractions (Figure 13) and is a better visual display of the trends of feeding group by site. PR clearly decreased through SWAMP as SH increased. The occurrence of collectors increases through SWAMP. This is not immediately apparent because the GC numbers for WT-A are low because of the high amount of FC (which fills the same niche). SC show no significant trends.

## Macroinvertebrate Feeding Group Ratios

The proportion of shredders to total collectors is expected to be greater than 0.25 for a normal shredder summer in a healthy functioning riparian system (Merritt 1996). The three post-

restoration Sandy Creek sites and pre-restoration Sandy Creek had proportions greater than or equal to 0.25. WT-1 had a proportion of shredders to total collectors of 0.25, WT-A had a proportion of 0.5, WT-5 had a proportion of 1, and pre-restoration Sandy Creek had a proportion of 0.5. Mud Creek had a proportion of 0.13 and was the only site that did not have a proportion above or equal to 0.25.

## **DISCUSSION AND CONCLUSIONS:**

The macroinvertebrate populations in Sandy Creek have seen improvement since the pre-restoration study. The biotic index value has decreased slightly from 6.72 to 6.48 which may mean that the macroinvertebrate populations are more diverse and abundant since the restoration of the wetland and creek through the SWAMP project. Hopefully this means that the restoration has carried out its intended functions by allowing the water flow rate to slow down, overflow the creek during storm events, and be withheld in wetland cells and the lake, which improves water quality (Flanagan et. al 2008). If this is occurring, the water's manipulated flow gives pollutants a chance to be processed and withdrawn by bacteria and plants. For example, it has been shown that 64% of the nitrate-nitrogen has been removed and 28% of the phosphorus is retained in SWAMP during storm events (Flanagan et. al 2008). Sediment and BOD have also been shown to be reduced by the restoration. Reducing flow and flooding onto the adjacent riparian wetland would settle sediments out of the water column. All of these actions are conducive to a lower biotic index score and higher water quality.

Even though the biotic index values indicate an improvement in water quality, the improvement is small after three years. There may be a portion of the restoration that was unsuccessful or the macroinvertebrates are not a sensitive measure of restoration success in this

instance. The small improvement may also be because field observations overlooked an important component of macroinvertebrate habitat or factors such as the recent drought, beaver disturbances, or the time needed for macroinvertebrate populations to become established.

Importantly, the biotic index values decreased as the sampling sites moved down through the restoration which indicates that the water quality increased as it moved through the restoration. This parallels the reduction in storm event nitrate and nitrite loads (kg/day) which are over 20 kg/day at input into the SWAMP and are approximately 7 kg/day at the SWAMP's output (Flanagan et. al 2008). WT-1 was the input site into the restoration, WT-A was a mid-point in the restoration, and WT-5 was the output point of the restoration (Figure 4). The result of increasing water quality through the restoration was a goal of the project and current macroinvertebrate results support this finding (Flanagan et. al 2008). This is intuitive because upper Sandy Creek would likely contain more pollutants and sediment than lower Sandy Creek because one expects pollutants and sediment to settle out and be processed as water moves through the restored stream/wetland system. Upper Sandy Creek receives direct runoff from the athletic fields of Duke University and the streets and outflows from the City of Durham. The beginning of its system's waters will likely bear the brunt of the fertilizer and chemical runoff. This may be why WT-1 has the lowest water quality and further downstream, the macroinvertebrate populations indicate better health. Like the difference between pre- and post-restoration biotic index values, the difference between the biotic index values of sampling sites on post-restored Sandy Creek is small. Again, this may be an indication that the wetland system is not largely successful in filtering out pollutants, the system has not had enough time to fully recover, or the macroinvertebrates are not the most accurate measure of water quality gradient in Sandy Creek.

The biotic index value of the reference stream, Mud Creek, was the highest or displayed the poorest water quality. This was unexpected because a reference site is meant to have improved water quality due to its history of fewer disturbances. The higher biotic index value may be an indication that Mud Creek may need to be reevaluated as a reference stream. On the other hand, the biotic index value was only slightly higher. This may be due to the bioassessment not being a sensitive enough test. Also, my technique of sub-sampling is not fool-proof but often is a good indicator of water quality (Doberstein 2000).

The post-restoration Sandy Creek sites show no trend in their Shannon-Wiener indices (Table 2). It was expected for the biodiversity to increase through the restoration with the increase in water quality. They are all less diverse than Mud Creek and pre-restoration Sandy Creek. Mud Creek's higher value was expected because it was the reference site and had a history of fewer disturbances. Pre-restoration Sandy Creek's highest value was unexpected because Sandy Creek had poorer water quality before the restoration took place. The reason for these anomalies may be because the post-restoration sampling occurred three times over one summer producing a small dataset. It is also possible that other factors have impacted Sandy Creek's recovery rate. The 2007 drought, beaver dams, or short time since restoration may be to blame. (Shannon 1948)

Out of the macroinvertebrates that were collected at pre-restoration Sandy Creek, post-restoration Sandy Creek, and Mud Creek, all five of the sites individually had a high percentage of Diptera. The post-restoration output site (WT-5) was found to contain the highest percentage, followed by the post-restoration mid-site (WT-A). The three remaining sites had approximately equal percentages of Diptera. There is a positive trend of Diptera numbers through SWAMP as one goes from input to output. Individuals in the order Diptera are usually associated with poor

water quality (Whiles 2002). The results seem unusual because the biotic index values tell the opposite story. The reason for this difference is that the types of Diptera present in Sandy Creek's samples have similar tolerance values (not as high as the majority of Diptera) as the other macroinvertebrates found at the site. The majority of the Diptera found in SWAMP are not indicators of poor water quality. Therefore, the increase of Diptera may mean a change in habitat or food source, not a decrease in water quality.

Mud Creek had fairly equal percentages of Trichoptera, Coleoptera, Diptera, and Crustacea and was only lacking Odonata. This balance may attest to Mud Creek appropriateness as a reference site despite its higher biotic index number. Also, Mud Creek had the highest percentage of Plecoptera which is an indicator of good water quality (NCDENR 2006). Coleoptera was consistently higher for MC in May, June, and July. Also, MC was also the only site that contained Crustacea every month. This is due to the high number of crayfish collected.

Pre-restoration Sandy Creek had the highest percentage of Ephemeroptera and the second highest percentage of Plecoptera and was only missing Coleoptera. Each of the post-restoration Sandy Creek sites was missing three of the macroinvertebrate orders. WT-1 and WT-5 were missing Bivalvia, Crustacea, and Plecoptera. WT-5 was missing Odonata, Crustacea, and Plecoptera. This indicates that pre-restoration Sandy Creek contained more diversity of insect orders than post-restored Sandy Creek. This may be due to disturbances, extraneous factors, or the method of sub-sampling. Another possibility is that populations still haven't had time to recover since the restoration, beaver dams, or summer 2007's drought.

When the data was looked at by month for summer 2008 (post-restored Sandy Creek and Mud Creek) (Figures 5, 6, and 7), it became evident that the percentage of Ephemeroptera was greatest at WT-5, the site of output. This reflects well on the stream restoration because

Ephemeroptera is a member of the EPT group, of which the members are most sensitive to pollutants (NCDENR 2006). This may be an indication that the water coming out of the restoration system is of better water quality than the water flowing in.

It also was made more evident that Trichoptera decrease through SWAMP (Figure 15). The Trichoptera found throughout the site were in the family Hydropsychidae and are filter-collector feeders (FC). FC are not sensitive to pollutants/sediment and thrive on fine particulate organic matter. Even though Trichoptera are a member of the EPT group (which usually indicates high water quality), the decrease in the type of Trichoptera collected in Sandy Creek indicates that sediment and nutrients are accumulating and settling as the water flows through SWAMP. This indicates that water quality is improving.

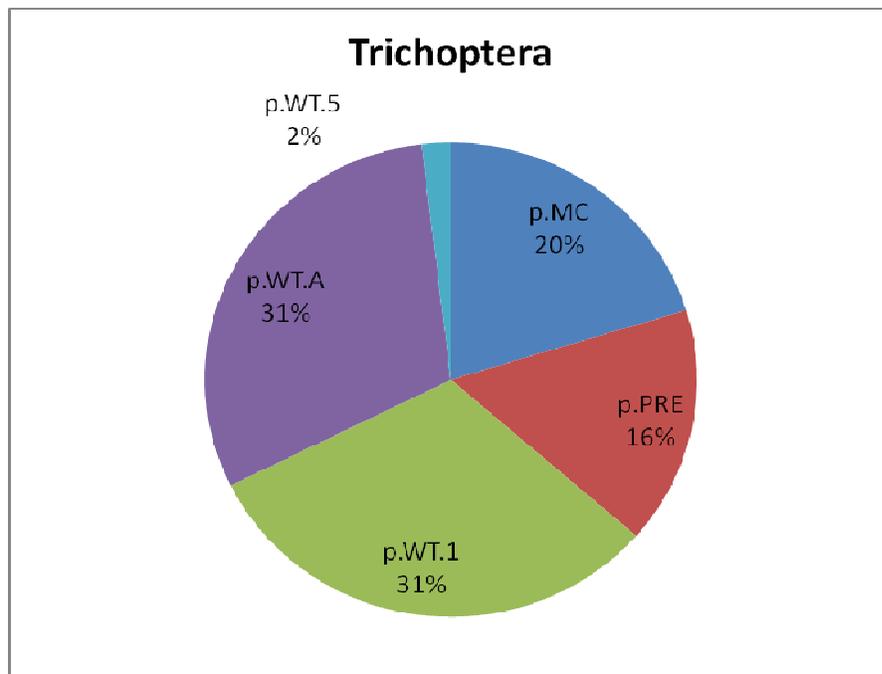


Figure 15: The probability of Trichoptera's occurrence per site (Post-restoration Sandy Creek (p.WT-1 input, p.WT-A, p.WT-5 output), Mud Creek (p.MC), and pre-restoration Sandy Creek (p.PRE)).

Site WT-1, site WT-5, and pre-restoration Sandy Creek are the only sites that contain macroinvertebrates in every category of feeding type. This is an indication of a diverse

ecosystem and usually is associated with better water quality. However, pre-restoration Sandy Creek contained significantly more predators than any other feeding type (55%) which shows that the pre-restoration system was severely unbalanced as compared to current conditions which have a more even distribution of feeding type (Figure 13). The feeding types varied greatly for each site over the three months which shows that the insect populations fluctuate as expected with the time of year as a result of rainfall or temperature (Figures 10, 11, and 12). (Rawer-Jost 2004)

Gatherers and filterers are generalists and as a result, are less sensitive to pollution because they can easily adapt. Shredders and Scrapers are specialized feeders and are more sensitive to pollution (Rawer-Jost 2004). WT-5 had low percentages of filtering collectors and high percentages of shredders. Site WT-5 and Mud Creek had similarly high percentages of gathering collectors. This may indicate a higher water quality in the restoration's output site, WT-5 and the reference site, Mud Creek. Both WT-1 and WT-A had high percentages of filtering collectors and predators which indicates a lower water quality compared to WT-5 and Mud Creek. (Rawer-Jost 2004)

Calculating the proportion of shredders to total collectors is a common way of obtaining water quality information from feeding groups. This proportion is expected to be greater than 0.25 for a normal shredder summer in a healthy functioning riparian system (Merritt 1996). The three post-restoration Sandy Creek sites and pre-restoration Sandy Creek had proportions greater than or equal to 0.25. WT-1 had a proportion of shredders to total collectors of 0.25, WT-A had a proportion of 0.5, WT-5 had a proportion of 1, and pre-restoration Sandy Creek had a proportion of 0.5. The 0.25 increase trend per site in post-restoration data from input to output provides further evidence of the restoration's ability to improve water quality. Mud Creek had a

proportion of 0.13 and was the only site that did not have a proportion above or equal to 0.25. This provides another piece of evidence to use when questioning Mud Creek's suitability as a reference creek in terms of water quality to Sandy Creek.

This study provided evidence that stream/wetland complex in the SWAMP is being restored and contributes to the health of the environment by providing a filter for pollutants and sediment, as well as, a habitat for wildlife. Also, the biotic metrics provide evidence of a successful restoration since biological assessments are integrators of water quality over time and thus mean more than simple monthly water tests. Water sampling provides a snapshot of water quality while environmentally sensitive species such as macroinvertebrates provide evidence of longer more stable water conditions. These findings support the value of restoration since SWAMP demonstrates an increase in stream and wetland habitat services on the landscape. This should provide cooperating and granting agencies information to justify their support of stream/wetland restoration projects they have devoted so much time and money. In addition, this study builds on top of the earlier baseline study and provides project opportunities for future students.

In summary, aquatic macroinvertebrates are proven to be accurate bioindicators for environmental quality. Certain species are sensitive to a range of low to high levels of pollutants including high nutrients, industrial pollutants, and sediment. It is important to provide evidence that the restored wetland is carrying out its intended functions of filtering out chemicals and sediments to improve water quality. The results from this study provide evidence of SWAMP's positive impact on water quality through an increase in macroinvertebrate populations. Hopefully, this study will provide encouragement to Duke University's Wetlands Center and the Durham community that the environment is being improved for us and for future generations.

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APPENDIX I

Macroinvertebrate Data

Site ID	Collection Date	Order	Family	Genus	Species	Tol Value	Ab
WT-5	5/20/2008	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-5	5/20/2008	Odonata	Coenagrionidae	Argia	Argia spp.	8.17	
WT-5	5/20/2008	Oligochaeta	Lumbriculidae			7.00	
WT-5	5/20/2008	Coleoptera	Elmidae	Macronychus	M. glabratus	4.58	
WT-5	5/20/2008	Coleoptera	Haliplidae	Peltodytes spp.	Hydroch	6.60	
WT-5	5/20/2008	Diptera	Chironominae	Stenochironomus	Stenochironomus spp	6.50	
WT-5	5/20/2008	Coleoptera	Hydrophilidae	Helophorus	Helophorus spp.	7.57	
WT-5	5/20/2008	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	
WT-A	5/20/2008	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-A	5/20/2008	Oligochaeta	Lumbriculidae			7.00	
WT-A	5/20/2008	Diptera	Simuliidae	Simulium	Simulium spp	6.00	
WT-A	5/20/2008	Gastropoda	Physidae	Physella	Physella spp	8.80	
WT-A	5/20/2008	Diptera	Tipulidae	Tipula	Tipula spp.	7.33	
WT-A	5/20/2008	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7.00	
WT-A	5/20/2008	Bivalvia	Sphaeriidae	Sphaerium	Sphaerium spp.	7.6	
WT-A	5/20/2008	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.40	
WT-1	5/20/2008	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-1	5/20/2008	Oligochaeta	Lumbriculidae			7.00	
WT-1	5/20/2008	Gastropoda	Physidae	Physella	Physella spp	8.80	
WT-1	5/20/2008	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7.00	
WT-1	5/20/2008	Odonata	Coenagrionidae	Argia	Argia spp	8.20	
WT-1	5/20/2008	Coleoptera	Dryopidae	Helichus	Helichus spp.	4.63	
WT-1	5/20/2008	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.40	
MC-A	5/20/2008	Crustacea	Palaemonidae	Palaemonetes	Palaemonetes spp.	7.1	
MC-A	5/20/2008	Coleoptera	Dytiscidae	Neoporus	Neoporus spp.	8.62	
MC-A	5/20/2008	Crustacea	Asellidae	Caecidotea	Caecidotea spp.	9.1	
MC-A	5/20/2008	Heteroptera	Notonectidae	Notonecta	Notonecta spp.	8.7	
MC-A	5/20/2008	Coleoptera	Dryopidae	Helichus	Helichus spp.	4.6	
MC-A	5/20/2008	Coleoptera	Haliplidae	Peltodytes spp.	Hydroch	6.60	
MC-A	5/20/2008	Diptera	Chironominae	Polypedilum	P. flavum	4.93	

MC-B	6/3/2008	Coleoptera	Psephenidae	Psephenus	Psephenus Herricki	2.4	
MC-B	6/3/2008	Diptera	Tipulidae	Tipula	Tipula spp.	7.33	
MC-B	6/3/2008	Crustacea	Asellidae	Caecidotea	Caecidotea spp.	9.1	
MC-B	6/3/2008	Crustacea	Talitridae	Hyallolella	H. azteca	7.75	
MC-B	6/3/2008	Trichoptera	Philopotamidae	Chimarra	Chimarra spp	2.80	
MC-B	6/3/2008	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
MC-B	6/3/2008	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7.00	
MC-B	6/3/2008	Diptera	Simuliidae	Simulium	Simulium spp.	6.00	
MC-B	6/3/2008	Ephemeroptera	Baetidae	Baetis	B. flavistriga	7.00	
MC-B	6/3/2008	Plecoptera	Perlidae	Perlesta	Perlesta spp.	4.7	
MC-B	6/3/2008	Gastropoda	Planorbidae	Planorbella	Planorbella spp.	6.8	
MC-B	6/3/2008	Oligochaeta	Lumbriculidae			7.00	
MC-B	6/3/2008	Diptera	Tanypodinae	Ablabesmyia	A. mallochii	7.19	
MC-B	6/3/2008	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.42	

Site ID	Collection Date	Order	Family	Genus	Species	Tol Value	A
WT-5	6/17/08	Gastropoda	Physidae	Physella	Physella spp	8.8	
WT-5	6/17/08	Odonata	Aeshnidae	Basiaeschna	B. janata	7.35	
WT-5	6/17/08	Oligochaeta	Lumbriculidae			7	
WT-5	6/17/08	Coleoptera	Hydrophilidae	Helophorus	Helophorus spp.	7.57	
WT-5	6/17/08	Ephemeroptera	Baetidae	Baetis	B. flavistriga	7	
WT-5	6/17/08	Diptera	Chironominae	Stenochironomus	Stenochironomus spp	6.50	
WT-5	6/17/08	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.42	
WT-A	6/17/08	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-A	6/17/08	Gastropoda	Physidae	Physella	Physella spp	8.8	
WT-A	6/17/08	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7	
WT-A	6/17/08	Coleoptera	Hydrophilidae	Helophorus	Helophorus spp.	7.57	
WT-A	6/17/08	Trichoptera	Philopotamidae	Chimarra	Chimarra spp	2.8	
WT-A	6/17/08	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	
WT-A	6/17/08	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.40	
WT-1	6/17/08	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-1	6/17/08	Oligochaeta	Lumbriculidae			7	
WT-1	6/17/08	Gastropoda	Physidae	Physella	Physella spp	8.8	
WT-1	6/17/08	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7	
WT-1	6/17/08	Coleoptera	Dryopidae	Helichus	Helichus spp.	4.63	

WT-1	6/17/08	Diptera	Chironominae	Polypedilum	P. flavum	4.93	
WT-1	6/17/08	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	
WT-1	6/17/08	Coleoptera	Hydrophilidae	Helophorus	Helophorus spp.	7.57	
MC-A	6/17/08	no data: dried up	on sampling day.				
MC-B	6/17/08	Coleoptera	Psephenidae	Psephenus	Psephenus Herricki	2.4	
MC-B	6/17/08	Diptera	Tipulidae	Tipula	Tipula spp.	7.33	
MC-B	6/17/08	Crustacea	Asellidae	Caecidotea	Caecidotea spp.	9.1	
MC-B	6/17/08	Crustacea	Talitridae	Hyalala	H. azteca	7.75	
MC-B	6/17/08	Trichoptera	Philopotamidae	Chimarra	Chimarra spp	2.8	
MC-B	6/17/08	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
MC-B	6/17/08	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7	
MC-B	6/17/08	Diptera	Tanypodinae	Ablabesmyia	A. mallochi	7.19	
MC-B	6/17/08	Bivalvia	Sphaeriidae	Sphaerium	Sphaerium spp.	7.6	

Site ID	Collection Date	Order	Family	Genus	Species	Tol Value	A
WT-5	7/15/08	Gastropoda	Physidae	Physella	Physella spp	8.8	
WT-5	7/15/08	Gastropoda	Planorbidae	Planorbella	Planorbella spp.	6.8	
WT-5	7/15/08	Diptera	Chironominae	Tribelos	Tribelos spp.	6.31	
WT-5	7/15/08	Ephemeroptera	Caenidae	Caenis	C. spp.	7.4	
WT-5	7/15/08	Diptera	Chironominae	Polypedilum	P. flavum	4.93	
WT-5	7/15/08	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	
WT-A	7/15/08	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-A	7/15/08	Oligochaeta	Lumbriculidae			7	
WT-A	7/15/08	Gastropoda	Physidae	Physella	Physella spp	8.8	
WT-A	7/15/08	Gastropoda	Planorbidae	Planorbella	Planorbella spp.	6.8	
WT-A	7/15/08	Ephemeroptera	Baetidae	Baetis	B. flavistriga	7	
WT-A	7/15/08	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	
WT-A	7/15/08	Diptera	Chironominae	Stenochironomus	Stenochironomus spp	6.50	
WT-A	7/15/08	Diptera	Chironominae	Polypedilum	P. flavum	4.93	
WT-1	7/15/08	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
WT-1	7/15/08	Oligochaeta	Lumbriculidae			7	
WT-1	7/15/08	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7	
WT-1	7/15/08	Ephemeroptera	Baetidae	Baetis	B. flavistriga	7	
WT-1	7/15/08	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.40	

WT-1	7/15/08	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	
MC-A	7/15/08	no data: dried up	on sampling day.				
MC-B	7/15/08	Coleoptera	Psephenidae	Psephenus	Psephenus Herricki	2.4	
MC-B	7/15/08	Crustacea	Asellidae	Caecidotea	Caecidotea spp.	9.1	
MC-B	7/15/08	Trichoptera	Philopotamidae	Chimarra	Chimarra spp	2.8	
MC-B	7/15/08	Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche spp.	6.22	
MC-B	7/15/08	Coleoptera	Elmidae	Stenelmis	Stenelmis crenata	7	
MC-B	7/15/08	Gastropoda	Physidae	Physella	Physella spp	8.8	
MC-B	7/15/08	Oligochaeta	Lumbriculidae			7	
MC-B	7/15/08	Diptera	Tanypodinae	Conchapelopia	Conchapelopia grp	8.42	
MC-B	7/15/08	Arhynchobdellida	Hirudinidae	Macrobdella	M. ditetra	4	

## APPENDIX II

```
insect<-read.csv("5proborder.csv")
insect$Site<-factor(insect$Site, labels=c("WT-1", "WT-A", "WT-5", "MC", "PRE"))
t(insect)
t(insect[,-1])
tinsect <- t(insect[,-1])
tinsect
row.names(tinsect)
multinom(formula = tinsect ~ row.names(tinsect))
insect.multinom <- multinom(tinsect~row.names(tinsect))

M1.coef <- coef(insect.multinom)
xb.MC <- M1.coef[,1]

denom <- sum(exp(xb.MC))
p.MC <- c(1/(1+denom), exp(xb.MC)/(1+denom))

xb.PRE <- M1.coef[,1]+M1.coef[,2]
denom <- sum(exp(xb.PRE))
p.PRE <- c(1/(1+denom), exp(xb.PRE)/(1+denom))

xb.WT.1 <- M1.coef[,1]+M1.coef[,3]
denom <- sum(exp(xb.WT.1))
p.WT.1 <- c(1/(1+denom), exp(xb.WT.1)/(1+denom))

xb.WT.5 <- M1.coef[,1]+M1.coef[,4]
denom <- sum(exp(xb.WT.5))
p.WT.5 <- c(1/(1+denom), exp(xb.WT.5)/(1+denom))

xb.WT.A <- M1.coef[,1]+M1.coef[,5]
denom <- sum(exp(xb.WT.A))
p.WT.A <- c(1/(1+denom), exp(xb.WT.A)/(1+denom))

insect.prob <- cbind(p.MC, p.PRE, p.WT.1, p.WT.5, p.WT.A )
row.names(insect.prob) <- c("1", "2", "3", "4", "5", "6", "7", "8", "9", "10", "11")
insect.prob
write.csv(insect.prob, "ORDERPROB.csv")
```

```

feed<-read.csv("4probfeed.csv")
feed$Site<-factor(feed$Site, labels=c("WT-1", "WT-A", "WT-5", "MC", "PRE"))
t(feed)
t(feed[,-1])
tfeed <- t(feed[,-1])
tfeed
row.names(tfeed)
feed.multinom <- multinom(tfeed~row.names(tfeed))

M1.coef <- coef(feed.multinom)
xb.MC <- M1.coef[,1]

denom <- sum(exp(xb.MC))
p.MC <- c(1/(1+denom), exp(xb.MC)/(1+denom))

xb.PRE <- M1.coef[,1]+M1.coef[,2]
denom <- sum(exp(xb.PRE))
p.PRE <- c(1/(1+denom), exp(xb.PRE)/(1+denom))

xb.WT.1 <- M1.coef[,1]+M1.coef[,3]
denom <- sum(exp(xb.WT.1))
p.WT.1 <- c(1/(1+denom), exp(xb.WT.1)/(1+denom))

xb.WT.5 <- M1.coef[,1]+M1.coef[,4]
denom <- sum(exp(xb.WT.5))
p.WT.5 <- c(1/(1+denom), exp(xb.WT.5)/(1+denom))

xb.WT.A <- M1.coef[,1]+M1.coef[,5]
denom <- sum(exp(xb.WT.A))
p.WT.A <- c(1/(1+denom), exp(xb.WT.A)/(1+denom))

feed.prob <- cbind(p.MC, p.PRE, p.WT.1, p.WT.5, p.WT.A )
row.names(feed.prob) <- c("1", "2", "3", "4", "5")
feed.prob
write.csv(feed.prob, "FEEDPROB.csv")

```