# A Study of Macroinvertebrates in Carp Creek: A First-Order, Cold Water Stream

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# Abstract

We examined the first order, cold-water stream Carp Creek for macroinvertebrate diversity and biomass. The Creek springs naturally from the ground, filtering out macroinvertebrates before forming the headwaters. Our hypothesis is that macroinvertebrate diversity and biomass will increase on a gradient as distance from the headwaters increases. We sampled the entire length of the Creek for macroinvertebrate specimens as well as the abiotic variables: nitrates (NO<sub>3)</sub>, dissolved oxygen, temperature and flow volume. We tested whether there were any relationships between macroinvertebrate mass/diversity and distance from the headwaters or abiotic factors. Our analysis indicates that there was no relationship between either diversity or biomass and any of the variables measured. However, there was a significant relation between nitrate concentration and distance from the headwaters. Furthermore, we found that nitrates were positively related with species richness, but are unable to establish any direct correlations because of the multiplicity of nitrate sources in water (Grimm 1988).

# Introduction

Carp Creek is a first order stream on the University of Michigan Biological Station (UMBS) property in northern Michigan, just south of Douglas Lake (Nadelhoffer 2009). The spring that feeds Carp Creek originates in Douglas Lake and passes underground, emerging at a site called The Gorge, forming the headwaters of the creek (Nadelhoffer). The creek is about 2.75 km long, and empties into Burt Lake. The creek runs through a series of habitats, ranging from narrow, somewhat rocky areas at the source, to a cedar swamp with beaver dams closer to the mouth (Figure 1).

In 1986, UMBS researchers injected dye into the sediments of Douglas Lake. Two months later the researchers observed this dye at the headwaters of Carp Creek, proving that the lake feeds the creek (Nadelhoffer). Most streams in northern Michigan are fed through a mixture of runoff, groundwater, and throughflow (Wiley 1997)—however, Carp Creek is primarily fed by groundwater from this underground aquifer—creating a relatively closed, cold-water ecosystem (Nadelhoffer). Groundwater rivers and streams, because of their stable temperature and flow rate, harbor many unique organisms that are normally outcompeted by habitat generalists who thrive in runoff streams that have variable temperature and flow rate (Wiley). For example brook trout, cold water specialists, inhabit Carp Creek because its cold running water creates a more suitable environment with less competition than warmer running streams in northern Lower Michigan (Creaser 1930).

Researchers use macroinvertebrate indices to measure the water quality and complexity of lotic (moving water) systems (Wallace 1996). Macroinvertebrates are subject to both "top-down and bottom-up forces and serve as the conduits by which these effects are propogated [sic]." (Wallace). Macroinvertebrates are good indicators of stream vitality and diversity, in addition to being easily identified; we decided to use them as the foundation for an analysis of the diversity and species richness of Carp Creek.

We expect that the macroinvertebrate diversity and species richness at the headwaters should be relatively low because the water—due to its subterranean journey— is expected to be relatively free of organic matter. We expect that the organic content of the creek will increase, because the diversity and biomass of macroinvertebrates in Carp Creek should increase as the water moves away from the source due to changes in water chemistry and accumulation of organic matter. We tested this by gathering data through detailed sampling at various locations along Carp Creek from the headwaters to Burt Lake.

## **Materials and Methods**

In order to assure a proper systematic approach to sampling Carp Creek, a Geographic Positioning System (GPS) was used to mark and trace the stream. The GPS data was then input into a Geographic Information System, where the creek was divided into 20 sites for sampling (Figure 1). On May 29-30, we sampled stream macroinvertebrates every 100 meters from the headwaters to a large clearing containing a beaver dam, and thereafter every 200 meters to the mouth, crossing

over Hogsback Road. We sampled from the mouth to the headwaters to avoid contaminating later samples (Mantel 2009).

The macroinvertebrates were collected using a D-net with a fine mesh sieve (1mm²), which was walked across the creek from bank to bank with its open end facing upstream. The riverbed was disturbed by jumping and kicking, releasing the macroinvertebrates from the sandy substrate. The mesh was then combed for macroinvertebrates, which were then stored in 150 ml glass jars filled with water from the stream.

At each sampling site we collected additional data. Water temperature was recorded using a field thermometer. A water sample was collected in a 250ml lexan plastic vial. A dissolved oxygen meter was used to sample oxygenation of the water in mg/L. We also measured flow rate by measuring five meters downstream from the sampling site and timing how long it took for a buoy to travel from the sampling site to the five meter mark. We also recorded the width of the stream at the sampling site, and took stream depth at the midpoint.

In the lab, the glass jars with macroinvertebrates were emptied of their water and then filled with a 70% denatured ethanol solution to prevent decomposition of the specimens through examination. Specimen jars were individually emptied onto a fine mesh sorting plate and the macroinvertebrates were sorted and counted. We used guides from Needham and Needham (1962) and Pennak (1989) for identification of the specimens to the highest identifiable level; this usually was the order of a specimen. Furthermore, these groupings were then weighed with a scientific balance to a ten-thousandth of a gram to obtain biomass.

Creek water samples were delivered to a chemistry lab onsite at UMBS, which tested them for pH, turbidity, total Nitrogen, and  $NO_3$  (measured in  $\mu g/L$ ).

We calculated both macroinvertebrate species richness and a Shannon-Wiener diversity index (Shannon and Weaver 1948) for each site. Shannon-Wiener diversity index and species richness were each separately regressed against dissolved oxygen, NO<sub>3</sub>, distance from source, and volume flow in a backwards, stepwise linear regression. This regression explores relationships between a dependent variable and multiple variables to see if any of them are significantly related to the dependent variable and/or each other.

To standardize sampling area among sampling sites, biomass was divided by stream width. This standardization was not necessary for species richness and the Shannon-Wiener diversity index because collections were considered a catch-per-unit effort (Harley et al 2001). Biomass/width was also regressed against the environmental variables in a backwards, stepwise linear regression. We also ran a Pearson correlation between each of the three dependent variables and environmental variables to determine correlation, as well as between each of the environmental variables.

#### Results

There was no significant relationship between the Shannon-Wiener diversity index and any of the environmental variables (Table 1). Species richness was positively related to  $NO_3$  (nitrate) concentration (p = .006) (Figure 2); however, there was no significant relationship between species richness and other environmental factors (Table 2).

In addition, there was no significant relationship between biomass/width and the environmental variables (Table 3). In particular, biomass/width was not significantly related to distance to source (Figure 3). There was a marginal positive relationship between biomass and  $NO_3$  (p = .077).

Temperature was positively correlated with distance from source (R square = 0.78. p < 0.00) (Figure 4), as was nitrates (R square = 0.91, p < 0.00) (Figure 5). Total nitrogen was not correlated with distance from source (R square = .448, p=.063), while turbidity and pH remained relatively constant throughout the length of the creek.

## Discussion

There is a steady increase in temperature as distance from the headwaters increases. The water is gradually heated through solar radiation and ambient air (Mohseni 1999), possibly creating a more hospitable environment for the macroinvertebrates we were sampling for. As the water moves from the source to the mouth, the temperature increases until it reaches an equilibrium temperature, roughly near Reese's swamp. The equilibrium point is achieved relatively quickly, and remains constant for most of our sampling sites. Because of this we are more interested in the relationship between distance from the headwaters and biomass.

The data indicates that there are no significant relationships between species diversity and other variables. This indicates that diversity in Carp Creek is not dependent on any of the measured variables, forcing us to reject part of our hypothesis. Changes in landscape and terrestrial influences such as fallen trees may create microhabitats and microclimates that cause sudden spikes of diversity. It

would be very interesting to see how significant these landscape changes are on the diversity and biomass of the creek.

Conceptually, macroinvertebrate diversity and species richness should increase as water travels downstream because there is an increase in flow volume, dissolved oxygen levels, and the presence of new and other organisms and nutrients (Giller 2004). The observable habitat of Carp Creek appears to remain fairly consistent after a large clearing, approximately 700 meters downstream of the headwaters. After this point, the area surrounding the creek mostly becomes a cedar swamp. Additionally, this is roughly the point where the temperature reaches its equilibrium.

A more detailed survey of Carp Creek needs to be conducted to assess a relationship between habitat complexity and biomass of macroinvertebrates.

Furthermore, more samples should be taken in closer proximity to the source—possibly with 10 or 20 meter intervals—to better examine a potential species diversity progression. It is possible that the peak for biomass in relation to distance from the headwaters was missed because our sampling sites were spaced 100-200 meters apart. This relationship may be more evident if samples were taken on a tighter interval closer to the source. This is possible because Carp Creek arises spontaneously out of the ground and reaches a substantial flow rate within the first few sites that is consistent throughout the stream.

The presence of nitrates is in part associated with macroinvertebrate excretions (Grimm 1988). This means that generally, with greater macroinvertebrate biomass, there should be greater presence of  $NO_3$ . If this were a

confounding variable, then there would be a strong relationship between biomass and NO<sub>3</sub>. Our data shows that there is marginal significance between these two variables; however, decaying plant material in the water increases along the creek, making nitrates difficult to relate to biomass. While there appears to be a marginal relationship between nitrates and biomass, there is no significant relationship between biomass and distance. This means that macroinverterbrate density does not change with the changes in nitrates; a result that was not predicted in our hypothesis. Our data shows a relationship between nitrates and species richness in Carp Creek. The richness-nitrate relationship is very ambiguous because there are many factors contributing to the presence of nitrates. We could not find enough supporting evidence that notes the excretion rates of the macroinvertebrates found. Furthermore, plants and detritus are sources of nitrates and are not inherently limited to macroinvertebrate species richness (Grimm). Because we did not measure these components of Carp Creek, it would be inappropriate to conclude that nitrates were solely present because of macroinvertebrate excretions. There is great potential for future exploration of the relationship between species richness and nitrate levels. This could explore the differences between groundwater fed and surface flowing streams, which would further explore the unique and commonplace characteristics of Carp Creek.

# Conclusion

Our hypothesis proposed that macroinvertebrate diversity and biomass would increase along a gradient from the source to the mouth of Carp Creek because of changes in organic matter and chemical concentrations. The biomass data and

Shannon-Wiener index did not support our hypothesis. However this opened up many new areas for future research. It would be interesting to use Carp Creek and many other streams of increasing length to conduct a study to see at what point it is possible to observe a gradient like the one we hypothesized.

The linear regression modeling showed a significant relationship between the nitrate content of the water and species richness. Because nitrate content is influenced by many factors, among which is macroinverterbrate excretion; it would be interesting to see what isotopes are excreted by macroinverterbrates and what isotopes of nitrogen are from plant detritus and other factors, in order to calculate an index of macroinverterbrate presence.

While our hypothesis may not have been supported by the data, it does not necessarily mean that a relationship between biomass, diversity and distance from source does not exist. We may not have tested the correct limiting nutrients, or at a fine enough interval to observe the relationship. There is potential for future research at the site to definitively test if any of these possibilities are correct.

**Table 1:** Shannon-Wiener has no significant relationships with tested variables.

Backwards Linear Regression		0	
Model		Standard Error	p-value
1	$NO_3$	0.013	0.738
	Dissolved Oxygen	0.013	0.159
	Volume Flow	0.123	0.534
	Distance (km)	0.278	0.355
2	Dissolved Oxygen	0.011	0.092
	Volume Flow	0.022	0.511
	Distance (km)	0.164	0.261
3	Dissolved Oxygen	0.011	0.082
	Distance (km)	0.137	0.342
4	Dissolved Oxygen	0.008	0.124

**Table 2:** Richness has strong significance with nitrate levels. No other significant relationships present.

Backwards Linear Regression			
Model		Standard Error	p-value
1	$NO_3$	0.061	0.15
	Dissolved Oxygen	0.058	0.699
	Volume Flow	0.106	0.326
	Distance (km)	1.29	0.368
2	$NO_3$	0.055	0.082
	Volume Flow	0.103	0.308
	Distance (km)	1.248	0.354
3	$NO_3$	0.028	0.056
	Volume Flow	0.098	0.424
4	$NO_3$	0.022	0.006

**Table 3:** Biomass/Width has only marginal significance with NO<sub>3</sub>. No other significant relationships present.

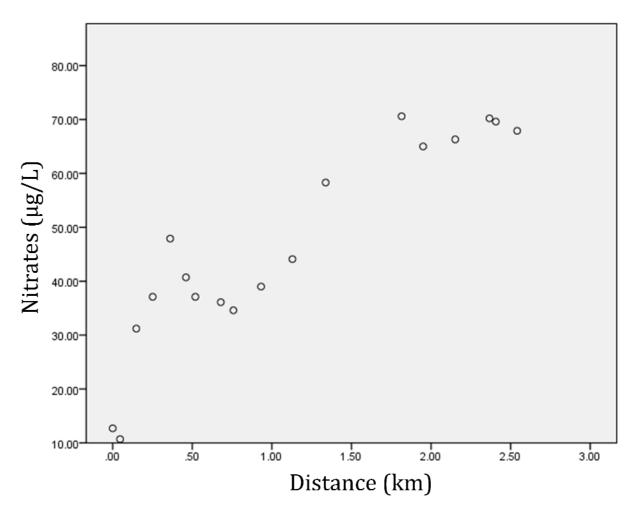
Backwards Linear	3		
Regression			
Model		Standard Error	p-value
1	Distance (km)	0.256	0.629
	Volume Flow	0.021	0.951
	Dissolved Oxygen	0.012	0.211
	$NO_3$	0.012	0.443
2	Distance (km)	0.236	0.613
	Dissolved Oxygen	0.011	0.192
	$NO_3$	0.012	0.425
3	Dissolved Oxygen	0.011	0.182
	$NO_3$	0.006	0.032
4	$NO_3$	0.004	0.077

**Figure 1:** A map of Carp Creek showing our sampling locations. Map was generated using ArcGIS software, a map of UMBS property, and GPS tagging of each site.

**Figure 2:** Scatter-plot that demonstrates the correlation between nitrate content and species richness.

**Figure 3:** Graph showing the correlation between biomass/width and distance from the headwaters.

**Figure 4:** Graph of the correlation between temperature and distance.



**Figure 5:** The scatter-plot shows the correlation between nitrate levels and distance from the headwaters

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