# The Impact of Fertilizer Exposure on Caddisfly Mortality and Behavior and Leaf Litter Decomposition

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**Abstract**

I experimentally determined fertilizer exposure effects on caddisfly (*Trichoptera*) mortality, behavior and leaf decomposition. Few studies have specifically examined fertilizer impacts on caddisflies and none have conducted experimental manipulations within a controlled laboratory setting. Caddisfly survivorship decreased as fertilizer concentration increased. Contact frequency between caddisfly larvae and larvae position on the leaf, followed a unimodal curve in relation to a fertilizer concentration gradient. Findings also confirm the findings of previous studies on leaf decomposition in relation to caddisfly mortality. The removal of the experimental setup from a natural site is a novel experimental approach and has significant implications for standards of water quality monitoring using aquatic macroinvertebrates.

## Introduction

A comprehensive understanding of anthropogenic alterations to the environment is crucial to restoring aquatic ecosystems. A wide range of human impacts including agriculture, urbanization and deforestation are detrimental to the ecological integrity of rivers and streams because they alter crucial factors such as pH, sediment, and oxygen levels and ultimately, ecosystem structure and function (Couceiro *et al.* 2007). These changes can impact the abundance and diversity of aquatic organisms (Pinnel-Alloul *et al.* 1996).

From golf courses to cornfields, synthetic nitrogen fertilizers are excessively applied on artificial landscapes capable of only absorbing small quantities (Gilley and Eghball 2002, Wong et al 1998). Housing and recreational areas are often established near streams and lakes, potentially exposing these waterways to elevated levels of nutrients (Winter and Dillon 2006). This development destroys forests that would otherwise absorb much of the runoff, leading to increases in harmful algal and microbe growth in aquatic ecosystems due to excessive nutrient influx (Allan 2004), which causes over 44% of water quality degradation in the United States (Biggs 2000).

This study examined fertilizer runoff to address local concerns of the Great Lakes region (Allan 2004). Northern Michigan and Wisconsin are dependent on their tourist industry, of which outdoor recreation is a large component, so fertilizer runoff threatens the quality of bodies of water that draw thousands of visitors each year. Runoff has also long been a public health concern in the Great Lakes Region because sites near agricultural centers have shown elevated fertilizer levels ( $>$ 10 mg/L) in aquifers and wells (Hill 1982). Because pesticide treatment in recreational areas is infrequent compared to nitrogen fertilizer application (personal interview, Rhinelander Northwood Golf), I examined fertilizer rather than pesticide effects on aquatic macroinvertebrates.

Previous studies of stream integrity and land use have monitored only general effects of synthetic nitrogen runoff or the impact of proximity to waste treatment centers, agriculture, and urban areas on nutrient levels within aquatic ecosystems (Laursen *et al.* 2002, Strayer *et al.* 2003, Winter and Dillon 2005). Few experimental studies have been conducted to measure the specific concentration of nitrogen that has measurable effects on stream biota populations, much less the subtler effects on behavior (Allan 2004). Studies have also had great difficulty identifying the specific sources of nitrogen, which could potentially be caused to either direct input or secondary nutrient cycling. Other potential causes of synthetic nitrogen runoff in aquatic ecosystems on a global scale include: legume and rice production, fossil fuel consumption and the Haber-Bosch process, which is an industrialized method of artificial nitrogen fixation (Galloway 2003).

The health of benthic macroinvertebrates can be studied to indicate nonpoint-source pollution, making them ideal subjects for monitoring environmental

contamination (Borgmann 1994, Couceiro et al. 2007). Low contaminant levels in stream ecosystems are most accurately detected by monitoring individual organisms because environmental pollutants are cycled through stream ecosystems, predominantly by detritivores and primary consumers, and can be difficult to detect by other methods (Galloway 2003).

Methodology for examining the effects of synthetic nitrogen in aquatic ecosystems has been highly debated (Smith et al 2007). Most studies have relied only on field experiments because of concerns that the laboratory setting fails to replicate *in situ* effects because the organism is exposed to fewer environmental stress variables (Bonada 2004, *Mann et al.* 2010**).** Field experiments, though ideal, are problematic for identifying contamination in water ecosystems because it is difficult to differentiate between natural and anthropogenic stressors, especially on larger scales (Allan 2004).

Caddisfly larvae (Trichoptera) are designated by the Environmental Protection Agency as Sensitive Benthos (EPA 2011). Comprising over ten percent of all stream invertebrates, they are detrivorous insects that function as invaluable nutrient cyclers, and reduced abundance and diversity of caddisflies can lead to a significant reduction in secondary production (Graca 2011). Caddisflies are also crucial to the population health of microflora communities that aid in leaf litter decomposition (Bonada and Williams 2002, Cummins 1974).

Leaf litter consumption rates of aquatic macroinvertebrates have long functioned as prime indicators of an aquatic system's ecological integrity (Gessner and Chauvet 2002, Hagen et al. 2006). Predominant methods for analyzing leaf litter decomposition

rates in the field, such as leaf bags, are not consistent due to experimental design variances (Graca 2001). In some studies, survey sites are so contaminated that a baseline of comparison cannot be established for monitoring caddisfly diversity and abundance (Hopkins et al. 2011). There is also conflicting evidence over the predominant cause of leaf litter decomposition: it may be due to either macroinvertebrates like caddisflieds or to the fungal and microbial communities that live on detritus surfaces (Arsuffi and Suberkropp 1985, Graca 2001, Hagen et al 2006). Because it is impossible to remove all contaminant sources and variables within a stream habitat, laboratory experiments can be a more accurate method of measuring the effects of a single pollutant. Reduction in environmental variance more accurately determines the source of leaf decomposition in relation to shredder abundance and water quality (Graca 2001).

Though some studies have already used caddisfly larvae behavior as a pollution indicator, they mainly examined the effects of heavy metal contamination (Lefcort et al. 2000). Other studies have used morphological variances in caddisfly populations as an indicator of water quality (Bonada and Williams 2002). Caddisfly case and net construction can also be monitored as a response variable to elevated environmental stress (Becker 1987, Plague and McArthur 2003). However, caddisfly growth, activity and mortality are not always correlated in response to an environmental variable (Gallep 1977). No studies were found to have investigated caddisfly movement and interaction as a response variable to environmental stress.

I studied the impact of varying fertilizer treatments on leaf breakdown and caddisfly behavior and mortality. Rather than only surveying macroinvertebrate leaf shredding in freshwater streams, I expanded upon the work of previous studies by exposing the samples to a specific byproduct of land use in a controlled setting.

Specifically, I tested the hypothesis that elevated concentration of fertilizer will increase frequency of stress-induced behavior, mortality and leaf decomposition in caddisfly microcosms in a laboratory setting. The null hypothesis was that increasing the concentration of fertilizer will have no effect on frequency of stress-induced behavior, mortality and leaf decomposition in caddisfly microcosms in a laboratory setting.

## Methods

### Site Description

Both experiments were conducted on the University of Notre Dame Research Center (UNDERC) property in Vilas County, Wisconsin and Gogebic County, Michigan from May 29 to July 16, 2011. Common genera of caddisfly in this region include *Pycnopsyche*, *Lepidostoma* and *Hydropsyche.* Collecting caddisflies at a significant distance from major sites of human development ensured that they were sourced from habitats with high water quality and not experiencing ongoing stress from environmental contamination, providing a comparative baseline for other studies monitoring caddisfly response to environmental contamination.

**Field Sampling**

I collected specimens on three different occasions from three sites (see below) during the week of June 19th – June 25th, 2011. Samples were gathered from Brown Creek and three sites along Tenderfoot Creek on UNDERC property. Some caddisflies were also gathered from a site on Tenderfoot Creek in the Ottowa National Forest just north of the UNDERC property. They were collected at the same time each day, between 9:00 am and 1:00 pm. Caddisflies were collected using a handheld 500 micron sieve and hand-picking samples from woody and vegetative substrates. The majority of caddisflies collected were *Pycnopsyche* and *Leptoceridae* genera.

**Lab Experiments**

Two experiments were conducted to measure fertilizer effects on caddisfly mortality and behavior and leaf decomposition. Both experiments used well water and the same type of fertilizer. Lake water was not used because temperature and pH are not consistent. Generic 46-0-0 industrial fertilizer was used because it is a ubiquitous plant care and animal feed supplement and contains one of the highest nitrogen concentrations of any commercially available fertilizer while still containing nitrogen levels within range of products commonly used for domestic properties. 46-0-0 is also a suitable variety because it does not contain additional chemicals common to fertilizer brands, such as phosphorous. The lack of additional chemicals ensured a more direct correlation between nitrogen levels and macroinvertebrate behavior and mortality.

I allotted a total volume of 2.5 liters fertilizer treatment per container for each experiment. All individual caddisfly larvae were randomly assigned to their containers

and were examined prior to treatment exposure to ensure that they were active, responsive and healthy specimens. For both experiments, I allotted two caddisfly larvae per replicate. Caddisfly larvae samples were identified to genus after each laboratory experiment.

**Experiment 1**

The objective of the first experiment was to determine the fertilizer concentration and time duration at which changes in behavior or survivor rate are observed in caddisfly larvae All samples were transferred into holding tanks for 24 hours prior to the experiment to acclimate the organisms to laboratory conditions. I used senescent red oak leaves (*Quercus rubra*) because they have a lower breakdown rate than fresh leaves and more commonly preferred Alder (Alnus spp.) and Birch (Betula spp.) species, so a more gradual decomposition rate was anticipated (Bjelke and Herrmann 2005, Irons *et al.* 1988, Kochi and Kagaya 2005).

Samples were designated to containers stocked with 0.50 grams of leaf matter. Five different concentrations of fertilizer were used - 7%, 5%, 3%, 1%, 0%. The control had a 0% solution of fertilizer and no larvae present.

Each treatment and control was replicated five times. Weight measurements of the samples were taken before and after fertilizer exposure. The environmental conditions of pH, temperature, and the number of live specimens were monitored on the first day and every other following day for one week during the experimental trial. Caddisflies were collected as they died and were stored in a solution of 95% ethanol.

**Experiment 2**

The objective of this experiment was to monitor leaf decomposition, mortality rate and caddisfly behavior as a function of fertilizer concentration. For this experiment I ensured that the laboratory setup would not expose the caddisfly microcosm to potentially significant increases in sunlight or temperature. Seven fertilizer concentrations, each with three replicates stocked with two caddisfly larvae, were used: 0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0%. Each microcosm was stocked with 1.00 grams of fresh speckled alder leaves (*Alnus incana*) in order to eliminate food source rejection as a potential stress factor. I also added a layer of fine grain sand to the bottoms of each container (approx 0.5 cm thick) in order to give the caddisflies surface traction for movement. To make sure that proximity to laboratory walls or windows did not cause a significant variance in microcosm temperature, the microcosms were situated in the center of the room, away from direct sunlight.

To quantify caddisfly behavior, I simultaneously monitored the three microcosms within each treatment level and recorded how much time the caddisfly spent moving during a 30 second interval, which was replicated twelve times per container over the course of the experiment. I then categorized the position of each caddisfly in relation to the other larvae within the microcosm, their activity level and their location within the microcosm. If one caddisfly had attached its case to the other caddisfly case in the microcosm, then this behavior was marked as one frequency point of contact for that trial. If a caddisfly was positioned on top of the leaf but not necessarily consuming it, they were marked as one frequency point for leaf position (on) in that trial. It is not likely

that monitoring multiple containers simultaneously resulted in inaccurate measurements because the microcosms were small, the caddisflies did not move rapidly and there were only two caddisflies per microcosm.

As specimens died, I removed them from the microcosm and stored them in 95% ethanol. At the end of the fourth day, I collected and stored the remaining samples in 95% ethanol solution and then removed each specimen from its case to identify larvae to genus, measure length (mm) and weight to the nearest milligram. The leaves were collected, dried at 60° C for 24 hours and weighed to the nearest milligram.

**Analysis**

All data set variables for Experiments 1 and 2 were tested for normalcy using a Shapiro-Wilke test. All data was proven to be normally distributed for each statistical analysis and did not require transformation. There was no mortality for any caddisfly microcosm exposed to the 0% fertilizer treatment.

**Experiment 1**

To measure the effect of microcosm location on microcosm temperature, I conducted a post-hoc 2-Way ANOVA analysis of microcosm number versus average temperature for each day of the experiment. I then conducted a Bonferroni post-hoc test. Microcosm number correlated with fertilizer treatment level and shelf position of the microcosm in relation to a window in the laboratory. Microcosm A was located at the greatest distance away from the window and microcosm E was located closest to the window.

**Experiment 2**

To ensure that temperature variance would not be a statistically significant factor in my analysis of Experiment 2, I computed a post-hoc 2-Way ANOVA of temperature versus microcosm X day to determine the effect of time and microcosm position on microcosm temperature.

A post-hoc 2-Way ANOVA of survivorship versus concentration X time was computed to measure the average number of caddisfly larvae remaining in each microcosm each day. A Bonferroni post-hoc analysis of the results was conducted. I then evaluated a regression analysis of survivorship versus time for each concentration in order to more easily examine trends across time for each concentration. An alpha value of 0.10 was established prior to statistical analysis due to small sample size and outlying values for the 2% fertilizer concentration level. To summarize the general trend of caddisfly larvae survivorship as a function of fertilizer exposure, I conducted a regression analysis of average survivorship versus fertilizer concentration with an alpha value of 0.10.

Leaf consumption was measured as a behavioral variable under the assumption that leaf breakdown would closely correlate with larvae survivorship and behavioral trends. I determined the relationship between leaf consumption and fertilizer treatment by conducting a 1-Way ANOVA of leaf dry weight versus fertilizer concentration. A regression analysis was then used to measure the relationship between summed seconds of caddisfly movement against fertilizer concentration to measure caddisfly activity. Because an ANCOVA could not be used due to limited data replications, three

Chi-Squared tests were conducted to determine the statistical relationship of Leaf position (on), Leaf position (off), and contact frequency to fertilizer treatment.

## Results

### Experiment 1

Average temperature was significantly different between microcosm position and between days, indicating that the change in atmospheric temperature during the experiment potentially influenced mortality rates and behavior ($F_{12, 388} = 13.8$, $r^2 = 0.334$, $p < 0.001$). (Figure 1), A Bonferroni post-hoc test supported significant differences between the first day and days two, four and six ($p < 0.001$ for all values, Table 1). There was no significant difference in temperature between days two, three and four ($p > 0.300$, Table 1). After examining these results and reviewing the unanticipated flaws in experimental design, I did not continue statistical analysis of my findings in Experiment 1.

### Experiment 2

The post-hoc 2-Way ANOVA and Bonferroni testing did not determine a significant relationship between temperature and concentration type or day, confirming that temperature would likely not influence caddisfly mortality and behavior and further analysis could be conducted ($F_{1, 503} = 2.207$, $p = 0.138$).

Significant interaction between larvae survivorship and concentration treatment over time was confirmed by post-hoc 2-Way ANOVA ($F_{18,475} = 1.646$, $r^2 = 0.304$, $p = 0.046$) (Figure 2). Bonferroni post- hoc testing found significance in mortality in relation comparisons between all days ($p < 0.001$) except for days 2 and 3 ($p = 1.00$) (Table 2).

Additional Bonferroni post hoc testing also showed significant difference in mortality between fertilizer concentrations of 0% and all other concentrations above 1% (Table 3).

Regression analysis showed a significant decrease in average larvae survivorship over time for each level of fertilizer concentration except for 0% fertilizer treatment ($r^2 = 0.525$, df = 1.00, p = 0.065) (Figure 3). The general trend in mortality across time was significant under the alpha value of 0.10 (df = 1.00, $r^2 = 0.113$, p = 0.065) for regression analysis (Table 4, Figure 4). When values for 2% fertilizer concentration were excluded from the data set, the regression more accurately reflected the general survivorship trend (df = 1.00, $r^2 = 0.183$, p = 0.033) and supported statistical calculations that the 2% treatment level contained outlying values.

A 1-Way ANOVA of leaf dry weight determined that leaf weight decreased with increased fertilizer concentration, indicating that leaf litter decomposition cannot be used to accurately monitor mortality rates or shredding behavior ($F_{1, 7} = 14.527$, $r^2 = 0.743$, p = 0.012). Regression analysis indicates, but could not determine, that the relationship between seconds of activity level and fertilizer concentration relationship approached significance ($r^2 = 0.551$, df = 1.00, p = 0.056) (Figure 6). Chi- Squared testing determined that contact frequency between caddisflies was significantly different across a fertilizer concentration ($X^2 = 54.154$, df = 6, $p < 0.001$). Chi-Square Analysis of summed leaf position (on) of caddisfly larvae across fertilizer concentration also showed significance ($X^2 = 21.414$, df = 6, p = 0.002). A Chi-Square Analysis of summed leaf position (off) of caddisfly larvae across fertilizer concentration showed no significance

($X^2$ = 9.856, df = 6, p = 0.131). Conclusively, the caddisfly larvae exhibited unimodal activity frequency in relation to fertilizer concentration (Table 7, Figure 7).

## Discussion

Experimental results support the hypothesis that caddisfly mortality and behavioral response would demonstrate a significant increase across an increasing fertilizer concentration gradient, rejecting the null hypothesis that no difference in mortality or behavior would be observed.

Comparison of behavioral frequencies with caddisfly mortality indicate that larvae activity followed a unimodal curve across a fertilizer concentration gradient, a different trend than the caddisfly larvae mortality rate. This is possibly due to an induced stress response but no significant compromise in physiological function at low levels of fertilizer exposure. As fertilizer concentration increases, the caddisfly may no longer be able to maintain physiological defense against chemical distress, resulting in decreased survivorship rate.

Diet may have also been a contributing factor to inaccurate results in the first experiment because caddisfly larvae are generally averse to senescent oak leaves. This may have been a poor diet choice that potentially increased organism stress within the laboratory environment as well, leading to distorted mortality rates. Though the results could not be used, Experiment 1 demonstrated the strong influence of even subtle changes in environmental variables. Future studies could examine the effects of abiotic

factors in relation to compromised caddisfly larvae survivorship in already contaminated environments.

Though experimental findings of mortality and behavior followed strong trends in relation to fertilizer concentration, caddisfly larvae in the 2% fertilizer concentration microcosms demonstrated persistent statistical incongruity. This might be explained by differential characteristics and feeding preferences of certain caddisfly species. Because previous studies have found that different caddisfly species can exhibit highly variable pollution tolerance even within the same genus, future analysis of my data should examine potential fluctuations in mortality and survivorship in relation to caddisfly larvae taxa (Bonada 2004, Brink et al. 2009, Smith et al. 2007). Though other studies have monitored caddisfly populations for longer periods of time to monitor abundance, the results from my own experiment were so immediate that experimental length is not a major concern (Arsuffi and Suberkropp 1995, Becker 1987).

Although larvae mortality was not a significant factor in leaf litter decomposition, my study cannot determine the exact cause of increased leaf decomposition in relation to increased fertilizer concentration. The findings support previous studies that also observed this statistical pattern, but I did not measure populations of microbes or fungi within my microcosms (Hagen *et al.* 2006). As I was conducting my experiments, I did observe that microcosm water became increasingly yellow and cloudy as fertilizer concentration increased.

Because several studies have found limitations and conflicting conclusions for both field and laboratory experimental design, future studies should conduct parallel

experiments in each setting to better understand the effects of natural conditions while still maintaining accurate monitoring of specific stress variables (Gallep 1977, Hopkins *et al.* 2011, Mann *et al.* 2010). Longer-term experiments could reveal the effects of fertilizer on reproductive behavior and larval growth and development. Future studies could determine the role of chemicals in fertilizer other than nitrogen, as some environmental toxicology studies have found that supplementary compounds, such as surfactants, play a more significant role in organism mortality (Releya and Jones 2009). The physiological consequences of exposure to fertilizer could potentially be examined by sampling caddisflies from streams known to be contaminated and placing them in environments with reduced environmental stress factors. Studying recovery time could potentially determine the permanent effects of fertilizer exposure on caddisfly health.

## Conclusion

This study supports the use of a laboratory setting for determining the specific effects of low-level environmental contamination on caddisfly mortality. Significant relationships between caddisfly behavior and fertilizer exposure were also observed, supporting future studies of caddisfly interaction as an indicator of trace contamination in aquatic environments. Increased understanding of fertilizer effects will facilitate the improvement of preventative methods for reducing fertilizer runoff, especially because limiting human development in close proximity to vulnerable aquatic habitats is not likely to occur (Allan 2004).

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# Tables

**Table 1.** Bonferroni Test post hoc indicates general significance ($F_{12, 388} = 13.8$, $p < 0.001$). Analysis reveals significant difference between average temperature on Day 1 and all other Days ($p < 0.001$). There is no significance between Days 3, 5, and 7 ($p > 0.10$) The results support the observed increases in temperature after the first day in Experiment 1.

| Day | Day | p Value |
|---|---|---|
| 1 | 3 | 0.00 |
| 1 | 5 | 0.00 |
| 1 | 7 | 0.00 |
| 3 | 5 | 1.00 |
| 3 | 7 | 0.3159 |
| 5 | 7 | 1.00 |

**Table 2.** Bonferroni post-hoc testing supports a significant relationship between survivorship and fertilizer treatment for each day of the experiment ($F_{12, 388} = 13.8$, $r^2 = 0.334$, $p < 0.001$). Significance in mortality was found between all days ($p < 0.001$) except for days 2 and 3 ($p = 1.00$) in Experiment 2.

| Day | Day | p Value |
|---|---|---|
| 1 | 2 | 0.00 |
| 1 | 3 | 0.00 |
| 1 | 4 | 0.00 |
| 2 | 3 | 1.00 |
| 2 | 4 | 0.000001 |
| 3 | 4 | 0.000002 |

**Table 3.** Bonferroni post-hoc testing of the relationship between survivorship and fertilizer treatment per day determined a significant difference in mortality between fertilizer concentrations of 0% and all other concentrations above 1% ($p < 0.001$).

| Concentration (%) | Concentration (%) | p Value |
|---|---|---|
| 0 | 0.5 | 0.188938 |
| 0 | 1.0 | 0.188938 |
| 0 | 1.5 | 0.000016 |
| 0 | 2.0 | 0.000451 |
| 0 | 2.5 | 0.000052 |
| 0 | 3.0 | 0.030035 |
| 0.5 | 1.0 | 1.00 |
| 0.5 | 1.5 | 0.367359 |

| | | |
|---|---|---|
| 0.5 | 2.0 | 1.00 |
| 0.5 | 2.5 | 0.679857 |
| 0.5 | 3.0 | 1.00 |
| 1.0 | 1.5 | 0.367359 |
| 1.0 | 2.0 | 1.00 |
| 1.0 | 2.5 | 0.679857 |
| 1.0 | 3.0 | 1.00 |
| 1.5 | 2.0 | 1.00 |
| 1.5 | 2.5 | 1.00 |
| 1.5 | 3.0 | 1.00 |
| 2.0 | 2.5 | 1.00 |
| 2.0 | 3.0 | 1.00 |
| 2.5 | 3.0 | 1.00 |

**Table 4.** Values for the average and sum values for larvae survivorship across a fertilizer concentration gradient in Experiment 2

| Fertilizer Concentration (%) | Survivors per Microcosm (AVG) | Survivors (SUM) |
|---|---|---|
| **0** | 2 | 144 |
| **0.5** | 1.5 | 108 |
| **1.0** | 1.444444444 | 104 |
| **1.5** | 1.111111111 | 80 |
| **2** | 1.75 | 470 |
| **2.5** | 1.055555556 | 76 |
| **3** | 0.742857143 | 52 |

**Table 5.** Values for the average dry weight of leaf matter not consumed by caddisfly larvae (g) across a fertilizer concentration gradient in Experiment 2

| Fertilizer Concentration (%) | Dry Weight (g) of Leaf Matter (AVG) |
|---|---|
| **0** | 0.4 |
| **0.5** | 0.391 |
| **1.0** | 0.369 |
| **1.5** | 0.406 |
| **2.0** | 0.348 |
| **2.5** | 0.378 |
| **3** | 0.36 |

**Table 6.** Behavioral values based on activity frequencies and time spent moving during observation intervals in Experiment 2.

| Fertilizer Concentration (%) | Contact Frequency (SUM) | Leaf Position Off (SUM) | Leaf Position On (SUM) | Time Spent Moving (seconds) per Interval (SUM) |
|---|---|---|---|---|
| **0** | 2 | 32 | 13 | 760 |
| **0.5** | 17 | 23 | 18 | 569.7 |
| **1** | 31 | 22 | 4 | 332 |
| **1.5** | 9 | 23 | 4 | 315 |
| **2** | 22 | 37 | 6 | 470 |
| **2.5** | 8 | 26 | 4 | 245 |
| **3** | 2 | 18 | 9 | 145 |

## Figures

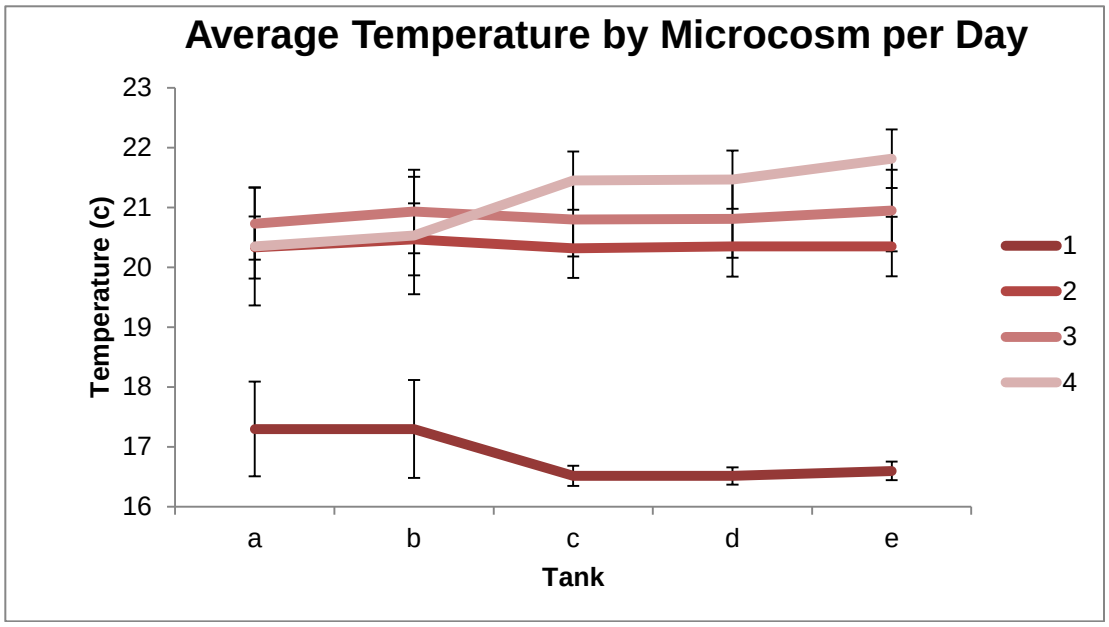


**Figure 1.** 2-Way ANOVA analysis of microcosm number versus average temperature for each day in Experiment 1. Significant difference between day one and other days is evident ($F_{12, 388} = 13.8$, $p < 0.001$).

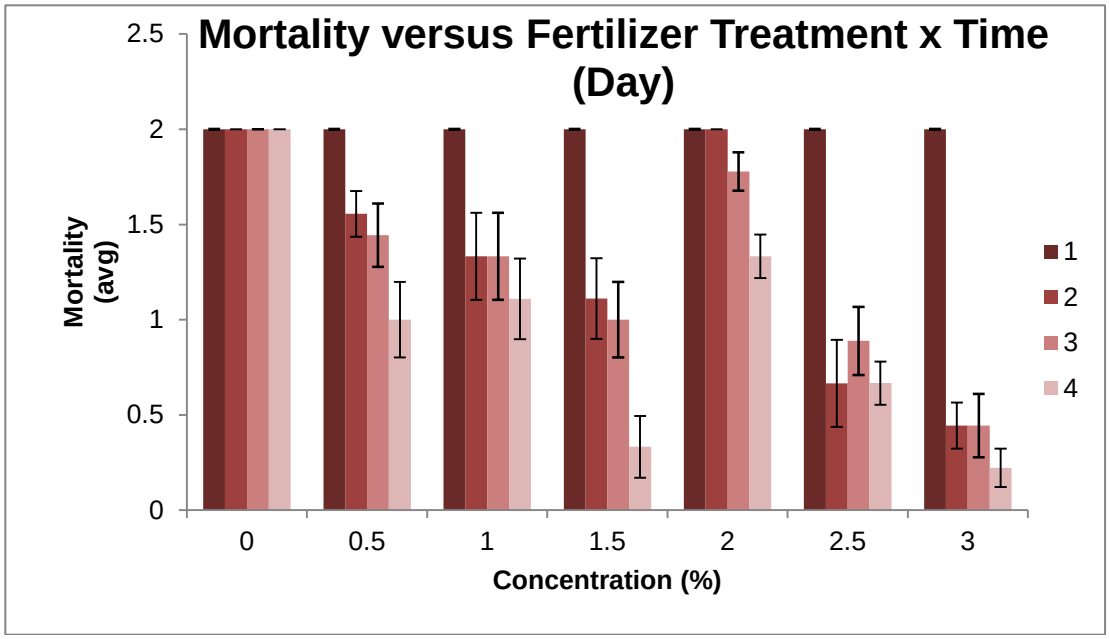


**Figure 2.** 2-Way ANOVA analysis of survivorship versus concentration X time (days) for Experiment 2. Mortality was significant with increased exposure to fertilizer ($F_{18,503}$ =5.604, $r^2$ = 0.535, $p < 0.001$).

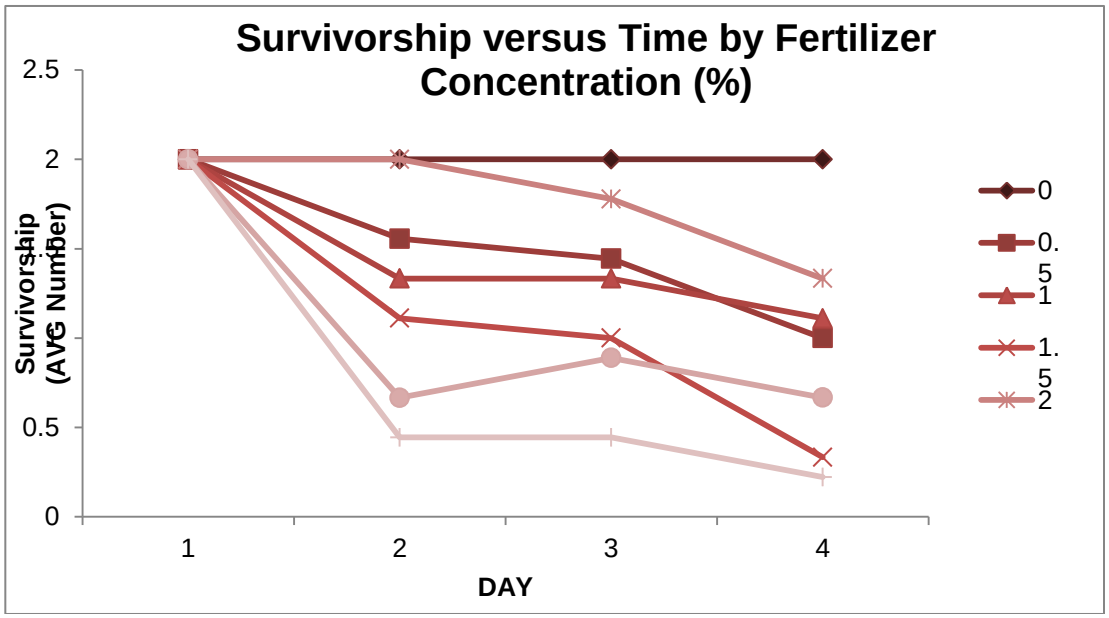


**Figure 3.** Regression analysis of daily survivorship versus time per concentration level for Experiment 2. The results indicate a significant decrease in average larvae survivorship per microcosm over time for each level of fertilizer concentration except for 0% fertilizer treatment ($r^2$ = 0.525, df = 1.00, p = 0.065).

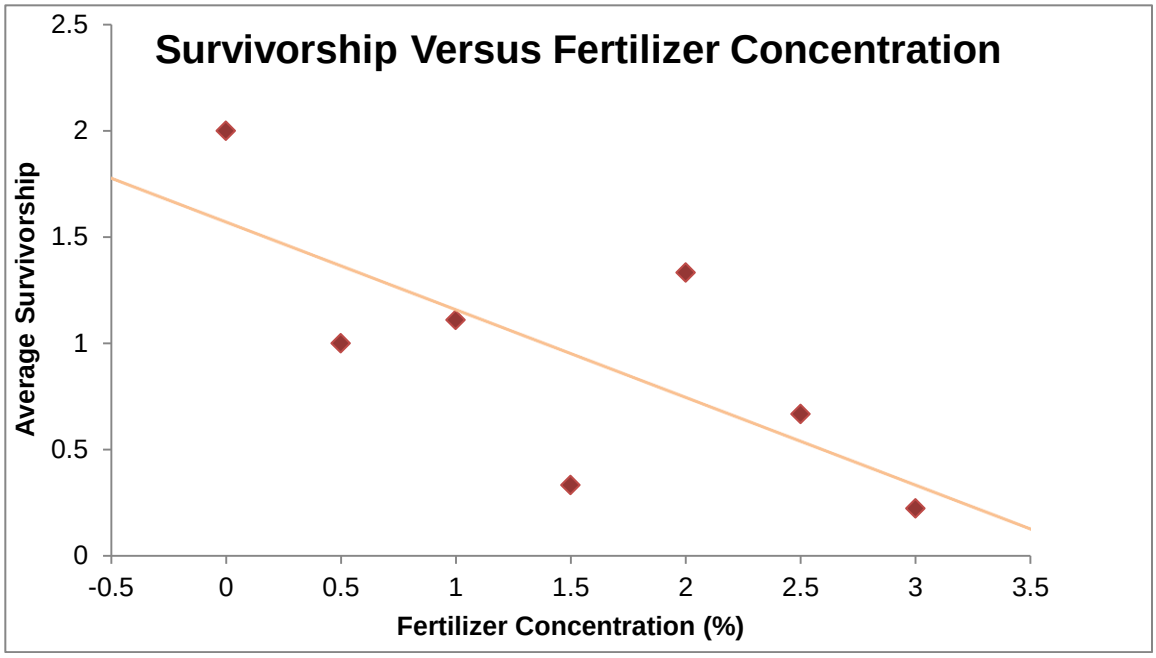


**Figure 4.** Regression analysis of average survivorship versus fertilizer concentration for Experiment 2.The results were significant using an alpha value of 0.10 (df = 1.00, $r^2$ = 0.113, p = 0.065) for regression analysis (Table 4, Figure 4).

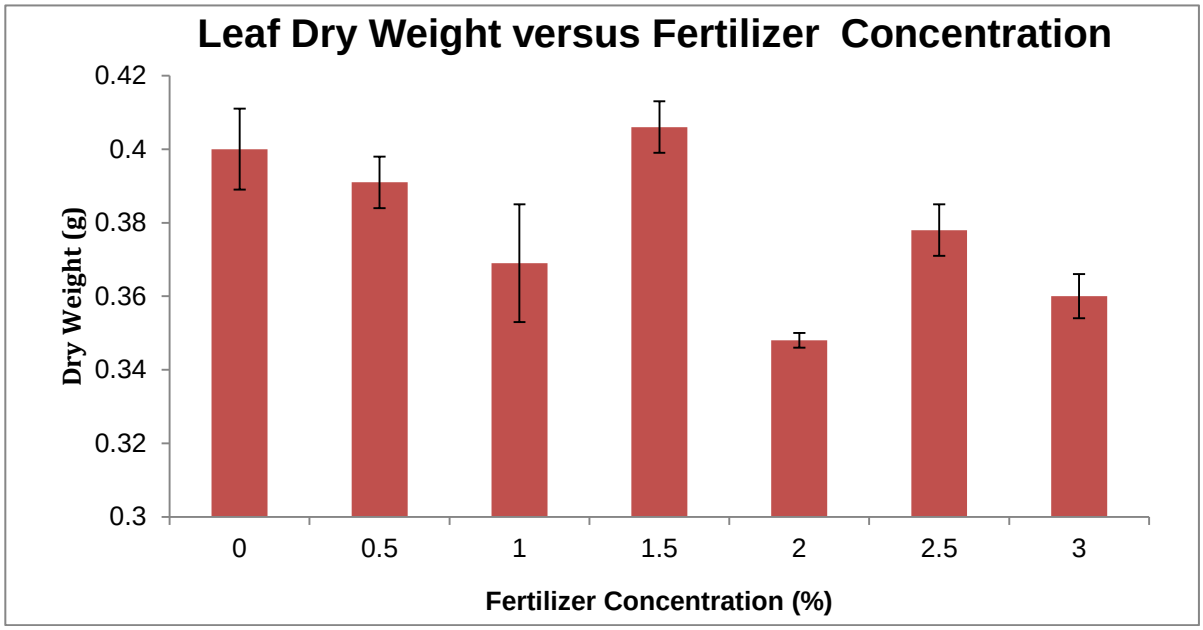


**Figure 5.** 1-Way ANOVA analysis of leaf dry weight X fertilizer concentration for Experiment 2. Leaf weight decreased with increased fertilizer concentration, indicating that leaf litter decomposition cannot be used to accurately monitor mortality rates or shredding behavior ($F_{1, 7}$ = 14.527, $r^2$ = 0.743, p = 0.012).

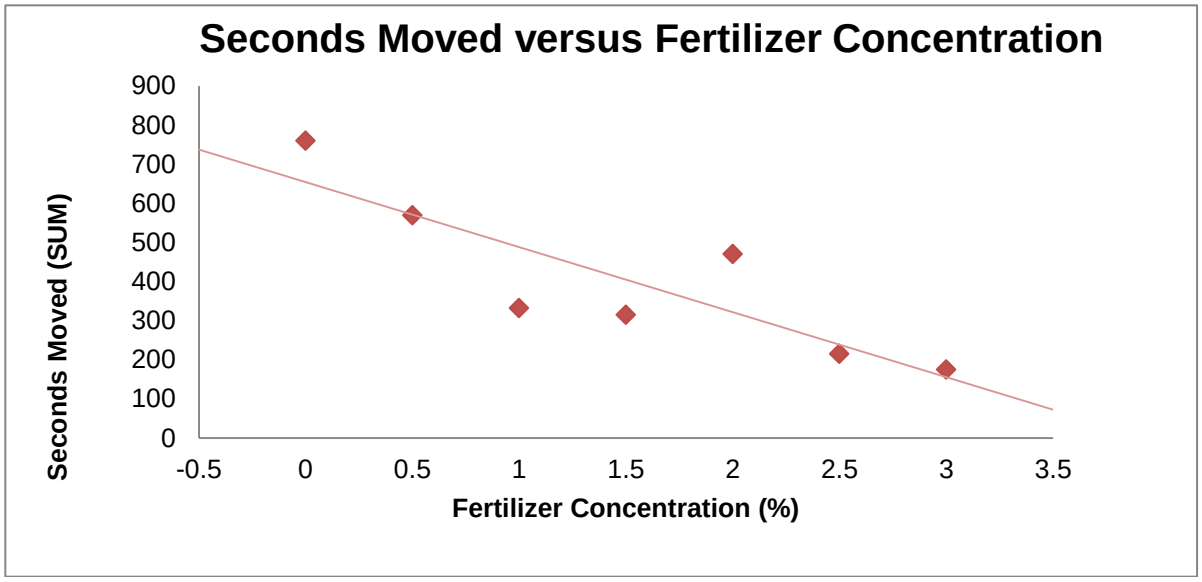


**Figure 6.** Regression analysis of summed seconds of caddisfly movement versus fertilizer concentration suggests a significance between seconds of activity level becuase fertilizer concentration relationship approached significance ($r^2$ = 0.551, df = 1.00, p = 0.056).

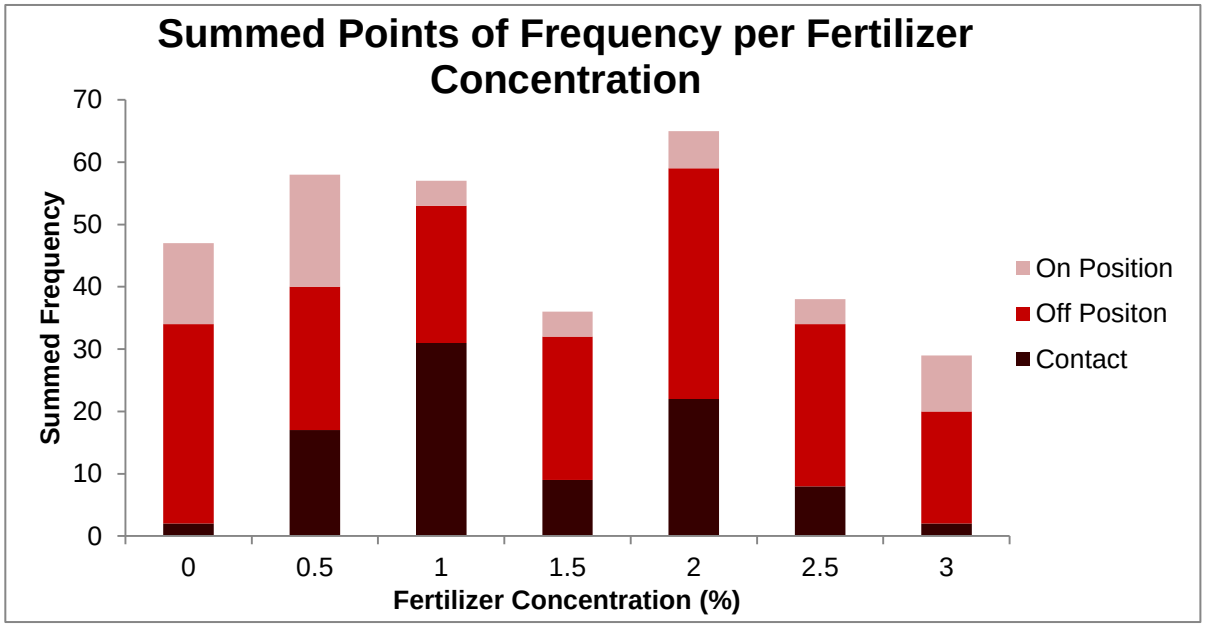


**Figure 7.** Chi-Squared Tests exhibited unimodal activity frequency of the larvae. Contact frequency between caddisflies increased with fertilizer concentration ($X^2$ = 54.154, df = 6, p < 0.001). Chi-Square Analysis of summed leaf position (on) of caddisfly larvae across fertilizer concentration showed significance ($X^2$ = 21.414, df = 6, p = 0.002). Chi-Square Analysis of summed leaf position (off) of caddisfly larvae across fertilizer concentration showed no significance ($X^2$ = 9.856, df = 6, p = 0.131).