

Effects of Nutrient Enrichment on Lentic Algal And Invertebrate Colonization

Abstract

This study sought to determine the effects of nutrient enrichment on populations of algae and invertebrates. We placed flow-through enclosures in Tenderfoot Creek; half contained commercial fertilizer, the other half served as controls. Contrary to our projections, we observed no statistically significant enhancements on invertebrate population, invertebrate colonization, algal mass, or algal chlorophyll content due to fertilization.

Introduction

Anthropogenic influences are far and away the largest factors changing our environment today. We are cutting down the forests that serve to produce a large fraction of the world's oxygen and store a major portion of the world's carbon. We are increasing global temperature by releasing greenhouse gases into the atmosphere. We are changing the dynamics of biotic interactions in every ecosystem through the destruction of species and the introduction of foreign substances.

One such human influence that has received a lot of press in scientific, agricultural, and water management circles is nutrient loading in aquatic ecosystems. Several examples are widely known, the most extreme being the Gulf of Mexico. The limiting nutrient for production has been changed from nitrogen to phosphorous in the Gulf of Mexico at the mouth of the Mississippi River due, in large part, to the agricultural runoff that travels downstream from the Midwestern states. The Great Lakes, the largest freshwater system on Earth, has also been affected by human activities. The release of wastewater from industries and treatment facilities into streams and rivers that run into the Great Lakes is also causing fish kills and great economic troubles to those who rely on fishing to make a living.

Factors controlling floral and faunal communities are more fully understood for lake ecosystems than for stream ecosystems. Streams are difficult to study because they are so dynamic. Their water is quickly and constantly renewed, discharge and water level can change greatly in relatively small periods of time, small changes within a source lake are magnified within the draining stream system, nutrients travel in more of a downstream spiral than a static cycle, and organisms can immigrate and emigrate easily. Because of their varying nature, stream studies are not easily controlled; those that are well controlled often sacrifice a high degree of similarity to natural systems and thus lose the applicability and predictability of the data in seeking better management policies. Streams also vary widely from one to another, so replicability and applicability of results is challenging in even the most well designed experiment (Hauer and Lamberti, 1996).

In order to learn more about the effects of nutrient loading on lentic ecosystems, scientists have created and implemented a number of research methods. Some have placed nutrient diffusing substrata in streams to test biotic reactions to point source nutrient additions. Others have added nutrients directly to entire streams to study large-scale effects like populations and food webs (Hauer and Lamberti, 1996). This experiment employed a more middle-of-the-road approach; we used flow-through enclosures (flumes) and an artificial substrate to test how increased nutrient loads affect rates of algal growth and invertebrate colonization in a riffle of Tenderfoot Creek. This creek is located in northern Wisconsin, USA on the property of the University of Notre Dame's Environmental Research Center (UNDERC).

Materials and Methods

Flume Construction

The first step in our manipulative project to test the effects of nutrient loading on algae and invertebrate colonization in a stream riffle was to construct the apparatus containing our flow-through enclosures (flumes) and the ceramic tiles that would serve as our replicates. We began with a rectangular wooden frame and attached six 1-meter lengths of plastic rain gutter approximately 15 cm apart to serve as the flumes. Within each flume, we attached nine 2cm pieces of thick wooden dowel rod to which we attached the ceramic tiles. These dowels allowed for a space for colonization on the underside of the tiles. Each square tile was 7.5 x 7.5 centimeters with a total surface area of 133.5 cm². We attached the tiles to the dowels using 100% silicone aquarium sealant and allowed them to dry for 24 hours. For flumes 2, 4, and 6 with added nutrients, we used Jobe's Fertilizer Spikes for Fruit and Citrus Trees. These spikes contained 10% nitrogen (N), 15% available phosphate (P₂O₅), and 15% soluble potash (K₂O). We placed half of one spike in a tube of large plastic netting attached to the front of the flume with wire twist-ties. We placed similar tubes of netting on the other three flumes for consistency of flow throughout the whole apparatus. We placed the apparatus in the stream, and added Styrofoam pieces to the bottom until it floated at the desired level, that at which water flowed over the tiles but not over the sides of the flumes. The apparatus was tethered to trees on both banks of the stream so that it would remain relatively stationary, but could still adjust with the water level.

Flume Rehabilitation

After about ten days in the creek, the silicone adhesive began to fail and numerous tiles came loose, disrupting water flow in the flumes. Our attempts to stabilize the loose tiles with wire and fishing line were insufficient, so we decided to begin the experiment again. We removed the apparatus from the creek and removed all of the old tiles. We attached 54 new tiles to the dowel rods with a different adhesive, Sportsman's GOOP. We allowed it to set up for 24 hours and returned the apparatus to its position in the riffle. The apparatus was repositioned in the stream on June 13. Every day we removed foreign objects and debris from the flumes to keep water flow consistent. The fertilizer was monitored and replaced whenever the old stakes had dissolved. New stakes were placed in each flume whenever any of them had dissolved so that each flume had equal fertilization over the summer.

Tile Sampling

Due to a loss of time after the failed initial run of the experiment, we decided to decrease the sample length from 14 to 10-day intervals. We took samples on June 23, July 3, and July 13, 2001. Stratified random selection provided us with numbers representing three tiles from each flume to be sampled at each interval. We collected from the flumes, placed them into small, labeled zipper bags with stream water, and transported them back to the lab to be processed.

Initially, we removed all invertebrates from the tiles, being careful to preserve all algae possible. They were identified to family and/or genus using the manual *Aquatic Insects of Wisconsin* (Hilsenhoff 1995) and counted to allow for the calculation of rates of colonization.

Next, we removed and quantified all algal growth from each tile. Using a hard-bristled toothbrush and a squirt bottle containing distilled water, we scraped all algae into a pan and filtered off the water using pre-weighed Whatman GF/F glass microfiber filters (catalogue number 1825 047). Using the flip of a coin, we determined if each sample would be quantified by mass or by chlorophyll content.

On the samples that would be quantified by mass, we dried the filters and samples to constant weight on aluminum weigh boats in the muffle furnace at 105°C for at least 24 hours. We then cooled the filters and samples in desiccators and massed them to the nearest 0.1mg. This mass minus the original filter mass represents the dry mass of the sample. Next, we ashed the filters and samples on aluminum weigh boats in the muffle furnace at 500°C for at least an hour. After allowing the furnace to cool down to 105°C, we cooled the filters and samples in desiccators, and massed them to the nearest 0.1mg. This mass minus the original filter mass represents ash mass of the samples. Dry mass minus ashed mass represents the ash-free dry mass (AFDM) of the organic matter collected in each sample.

On the samples that would be quantified by chlorophyll content, we allowed the filter and sample to steep in film containers filled with 7ml of 9:1 acetone buffered with MgCO₃. They were kept in the freezer in the dark for at least 24 hours. We poured the extract into 15ml centrifuge tubes and spun them for two minutes to settle out any algal cells that had transferred. We placed 3ml of extract from each sample into 1cm crystal cuvettes. Using a spectrophotometer blanked with 9:1 acetone buffered by water, we read absorbance of each sample at 750nm and 664nm. We then acidified each sample

using 0.1ml of 0.1N hydrochloric acid and agitating the cuvette. After allowing the samples to mix for 90 seconds, we read their absorbance at 665nm and 750nm.

Using these absorbance readings, we used equations from Hauer and Lamberti (1996) to calculate concentrations of chlorophyll *a* (equation 14.4) and its degradation product, pheophytin (equation 14.5), in the extracts.

$$14.4. \text{ Chlorophyll } a \text{ (}\mu\text{g/cm}^2\text{): } 26.7(E_{664b} - E_{665a}) \times V_{\text{ext}}/\text{area of tile(cm}^2\text{)} \times L$$

$$14.5 \text{ Pheophytin (}\mu\text{g/cm}^2\text{): } 26.7(1.7E_{665a} - E_{664b}) \times V_{\text{ext}}/\text{area of tile(cm}^2\text{)} \times L$$

where: E_{664b} = [{absorbance of sample at 664nm – absorbance of blank at 664nm} – {absorbance of sample at 750nm – absorbance of blank at 750nm}] before acidification

E_{665a} = [{absorbance of sample at 665nm – absorbance of blank at 665nm} – {absorbance of sample at 750nm – absorbance of blank at 750nm}] after acidification

V_{ext} represents volume of 90% acetone used in extraction (ml)

L is the length of light path through cuvette (cm)

26.7 = absorbance correction (derived from absorbance coefficient for chlorophyll *a* at 664nm [11.0] x correction for acidification [2.43])

1.7 = maximum ratio of $E_{664b}:E_{665a}$ in the absence of pheopigments

We then determined the Autotrophic Index by dividing AFDM (mg/cm^2) by chlorophyll *a* concentration (mg/cm^2) (Hauer and Lamberti 1996).

Sample Analysis

To determine if there was a significant difference in the total number of invertebrates between the control and fertilized fumes, we ran t-tests comparing both treatments on each date. We ran a t-test comparing colonization rates in the two treatments as well. Due to the disproportionately large number of *Diptera simuliidae*

(black fly) larvae in the first sampling event, we also ran all statistics excluding the *D. simuliidae* data.

We used two-way analyses of variance (ANOVAs) to examine the effect of the nutrient treatment on the ash-free dry mass (AFDM) of the algae as well as amounts of chlorophyll *a* present in the algae.

Results

Invertebrates

Our graphs showing total number of invertebrates present on the tiles over time showed two very different trends. When all invertebrates were considered, a large number of invertebrates were present after ten days, with that number drastically decreasing and seemingly leveling off at twenty and thirty days (Figure 1). When all of the *Diptera simuliidae* (black fly) larvae are excluded from the figure, a very different trend emerges. As shown in Figure 2, the numbers of invertebrates increases throughout the thirty days. The graphical representations of the data sets show very similar plots for both the control and experimental flumes. Our t-tests showed that there was no statistically significant difference on any date between the control and experimental flumes ($p=0.77$, $p=0.84$, and 0.60). Tests run excluding the black flies also showed no statistical significance between the two treatments ($p=0.55$, $p=0.94$, and $p=0.60$). Table 1 includes more complete statistical details on invertebrate populations.

Figures 3 & 4 graph the trends in colonization rates both including and excluding the black flies, respectively. When the *D. simuliidae* are included, colonization rates appear to decline over time in both the experimental and control flumes, whereas, the corrected data shows an increase until twenty days which is followed by a slight decrease

in colonization rate. The t-test we ran on the colonization rate data showed no statistical difference between the rates in the control flumes and those in the experimental flumes ($p=0.91$). The other t-test run excluding all *D. simuliidae* data also showed no statistically significant difference between treatments ($p=0.97$). Please see Table 2 for further details on colonization rates

Algae

The Autotrophic Index (AI) proved very different for each of the three sampling days, but presented no consistent pattern. On June 23, the AIs for the flumes were between 100 and 600. On July 3, the AIs ranged from 1650 to 4800 except for one outlier of more than 13000 in flume 1, a control flume. On July 13, the lower AIs ranged from 1760 to nearly 3900, but this time there were two other outliers: 15760 in flume 2 and 23680 in flume 4, both nutrient-enriched. The average ash-free dry masses (AFDMs), in general, increased with each time increment with the exception of one notable decrease in flume 6 from the second to the third sampling day. For the most part, the average chlorophyll *a* content of the flumes decreased with time, but this was less universal than the AFDM pattern. See Table 3 for more information on the Autotrophic Indexes.

The ANOVA analyzing the difference between the average AFDMs for the control flumes and the treatment flumes showed that both sampling date and treatment type had a significant effect ($p=<.001$ and $p=.044$, respectively) and there was no significant effect for the interaction. See Table 4 for further details. The ANOVA examining the effect of nutrient loading on chlorophyll *a* content showed no significant

effect caused by sampling date or treatment, but did show a significant effect for the interaction of these two factors ($p=.014$). See Table 5 for further details.

Discussion

The number of all invertebrates present on the ceramic tiles did not behave as we expected. Instead of seeing an increase in numbers over time, there was a drastic decrease in numbers after the initial sampling (Figure 1). This great initial increase was composed primarily of the larvae of the species *Diptera simuliidae*, more commonly known as the black fly. Soon after this first sampling took place, we observed pupating forms of *D. simuliidae* covering the frame of our apparatus. We believe that the sudden drop off of black flies present within the flumes was due to the pupation of the larvae in preparation for emergence. When the data is then considered without the black fly data, a very different trend, and one much more similar to what we had expected can be seen (Figure 2). As time progressed and tiles became more suitable for sustaining a population (increased prey/algae), numbers of invertebrates increased. The only unexpected part of this trend is the fact that the treatment showed no significant difference from the control ($p=0.55$, $p=0.94$, and $p=0.60$).

Colonization rates showed trends very similar to the trends seen in Figures 1 and 2. Colonization rates were initially high, when there was a huge population of *D. simuliidae* inhabiting the tiles (Figure 3). During the next sampling event the rate of colonization was much lower. When the black fly population is not considered, colonization rates increase initially, and then decrease (Figure 4). This initial increase is probably due to the elimination of black fly data. The black fly larvae were initially

occupying so much space few other invertebrates were able to colonize the tiles. Once the black flies had pupated, more space was left open for new colonizers, causing a great influx to the new niches, which were probably soon filled, causing the decline in colonization rates in the final sampling. Again, there was statistically no difference between the colonization rates of the control flumes and the treatment flumes with a *p* value of 0.97 for the data excluding *D. simuliidae*.

The only overarching trend in the Autotrophic Indexes (AI) is that it increases from a level of several hundred after the first ten days to a level of several thousand after the second and third time increments. The earlier, lower numbers could mean that the algae were still in the process of colonizing the surface of the tiles and the later, higher numbers may indicate that the algae had come closer to reaching the maximum potential population that the surface area of the tiles could support. This is supported by the general increase in mass of algae collected over time. If more samples were taken at shorter intervals and over a longer time period, it is possible that their masses per time would demonstrate an S-shaped curve indicating a quick initial population increase and a gradual leveling off of colonization rate as the tiles approached their carrying capacity. Another reason for the general increase in the AI numbers could have been a decrease in the photosynthetic productivity of the algal populations. Especially in the outliers, it was not that the masses of the algae samples were much higher, but that their concentration of chlorophyll *a* was much lower. These decreases in chlorophyll *a* as the sampling period progressed may have been the result of competition for sunlight, i.e. increased shading by a larger or denser stand of nearby organisms.

We expected that both date and treatment type would have significant effects on the average ash-free dry masses. Sampling date surely made a statistically significant difference because it allowed the algae an opportunity to colonize; we were not surprised that algal colonies developed greater average mass with time. However, concerning treatment type, we expected results exactly opposite to those of our study. According to Figure 5, the flumes enriched with fertilizer maintained a significantly lower average AFDMs than the flumes monitored as controls.

There are several possible reasons that the nutrient-enriched flumes did not produce larger stands of algae than the control flumes. One, the solid fertilizer spikes may not have made their nutrients readily available in a flowing-water environment. The spikes were solid and meant to be driven into the ground in the rhizosphere of a tree. They softened and slowly broke up in the water, but they may not have dissolved into a liquid phase that was readily picked up by the algae. If bits of the spike simply broke off and bounced down the flume without being absorbed by the algae, the nutrient treatment would have had little effect. If we had dissolved the fertilizer into a solution and made the nutrients available in a drip bottle, they may have been more readily absorbed by the autotrophs.

There is also the possibility that the riffle environment is already so nutrient-enriched by the outpouring of Tenderfoot Lake and/or local runoff from the watershed that the algae in Tenderfoot Creek is not nutrient limited. Therefore, the fertilizer would have either had no positive effect or perhaps even have had a depressing effect on algal growth. Despite the fact that there was no significant difference in invertebrate populations between control and treatment flumes, it is possible that grazers more

effectively depleted nutrient-enriched algal populations because of higher nutrient content.

We expected sampling date and treatment type to significantly affect the algal chlorophyll *a* content as well, but neither did so according to our ANOVA. Figure 6 has no discernible trend to show any type of consistency between the treatments. The control flumes seem to stay fairly constant between the first and second sampling day and then jump up by the third day. The treated flumes start out high, make a big drop for the second sampling day, and then stay level. The only time that the treatments even remotely coincided was on July 3, and the control treatment had more chlorophyll than the nutrient enriched treatment. The graph is erratic and does not facilitate useful hypothesis. Following from the idea that grazers more effectively feed upon the nutrient enriched algae, it is possible that algae damaged early by invertebrate grazing were trying to grow back but were not as healthy as the untreated algae that experienced less damage. To check these proposed hypotheses, it may be useful to set up a study in which an equal amount of grazers are placed in artificial environments with equal amounts of algae. The treatment would be that one population of algae would be grown in natural creek conditions and the other grown under artificial fertilization. Any difference in biomass eaten over time may indicate a preference for fertilized or natural algae.

For similar future studies, we would make one suggestion as far as algal sampling is concerned. The algae samples from the tiles were used for either biomass sampling or chlorophyll content, not for both; but we propose that studies with a limited number of replicates could use each collected sample for both mass and chlorophyll analysis. Extraction of chlorophyll *a* with acetone probably would not have any significant effect

on measures of mass, so AFDM could be determined after the chlorophyll extraction and analysis was complete. This would allow for maximum replication within the study and therefore more representative average measurements. Since we took only three tiles from each flume at each sampling date, two were used for mass and one was used for chlorophyll analysis or vice versa. Therefore, some of our averages for either mass or chlorophyll consisted of data collected from only one tile. It is highly unlikely that these measurements were accurately representative of the averages of whole populations. Increased replication would improve this situation.

Conclusions

This experiment proved to be a tribute to the fact that lentic ecosystems are complicated, dynamic, and difficult to make predictions about. We were unable to draw any confident conclusions about the relationship between nutrient enrichment and effects on algae and invertebrate populations in a stream context. As discussed earlier, there were many possible confounding factors that could have made the flora and fauna behave differently than we expected, but a more thorough knowledge of the ecosystem before we began the experiment may have facilitated more accurate hypotheses. Some preliminary research about things like average flow, species makeup of algal populations, and background nutrient levels will have to be done on Tenderfoot Creek if further studies are to be done more effectively.

Other streams throughout the country are loaded with topsoil and fertilizers eroded from nearby fields or wastewater and by-products released from industry. Hopefully, knowledge gained from studying nutrient enrichment in pristine environments like UNDERC will be most useful in the experimentation and restoration of ecosystems

that have already been altered by eutrophication. These studies will allow us to gain insight into how streams react to the initial episodes of nutrient enrichment and perhaps how any damage can be reversed at an early stage. It would also be good to document the gradual changes in the ecosystem that come with prolonged fertilization, i.e. changes in species makeup of populations of invertebrates and algae, changes in riparian communities, and changes in the use of the stream as a resource by local wildlife. Repairing long-polluted ecosystems and maintaining healthy ones should always be at the front of the mind of scientists doing stream research. New-found wisdom needs to be used for the purpose of practical application in our degenerating environment in order to reach its full potential.

Literature cited

- Hauer, F. Richard and Lamberti, Gary A., eds. Methods in Stream Ecology. San Diego: Academic Press, 1996.
- Hilsenhoff, W. L. Aquatic insects of Wisconsin, keys to Wisconsin genera and notes on biology, habitat, distribution and species. University of Wisconsin-Madison Natural History Museums Council Publication, 1995.

TABLE 1 – Number of Invertebrates Data (* minus *D. simuliidae*)

| | | Number of Invertebrates | | T-Test | Number of Invertebrates* | | T-Test |
|------------------|--|-------------------------|-------------|-------------|--------------------------|-------------|-------------|
| | | Control | Fertilized | | Control | Fertilized | |
| 6/23/2001 | | | | 0.768053129 | | | 0.545303195 |
| 10 | | 249 | 1375 | | 18 | 32 | |
| | | 195 | 535 | | 19 | 61 | |
| | | 180 | 116 | | 23 | 22 | |
| | | 1260 | 425 | | 27 | 7 | |
| | | 283 | 134 | | 9 | 20 | |
| | | 173 | 108 | | 26 | 31 | |
| | | 332 | 617 | | 17 | 10 | |
| | | 291 | 211 | | 13 | 17 | |
| | | 94 | 31 | | 18 | 4 | |
| AVERAGE | | 339.6666667 | 194.6666667 | | 18.88888889 | 22.66666667 | |
| 7/3/2001 | | | | 0.843176482 | | | 0.940339238 |
| 20 | | 134 | 66 | | 134 | 65 | |
| | | 141 | 117 | | 133 | 92 | |
| | | 52 | 44 | | 52 | 43 | |
| | | 51 | 52 | | 50 | 50 | |
| | | 90 | 64 | | 90 | 63 | |
| | | 34 | 141 | | 34 | 125 | |
| | | 51 | 220 | | 50 | 218 | |
| | | 94 | 33 | | 92 | 33 | |
| | | 82 | 36 | | 69 | 31 | |
| | | | 85.8888888 | | | | |
| AVERAGE | | 81 | 9 | | 78.22222222 | 80 | |
| 7/13/2001 | | | | 0.602888599 | | | 0.602888599 |
| 30 | | 100 | 103 | | 100 | 103 | |
| | | 48 | 70 | | 48 | 70 | |
| | | 57 | 83 | | 57 | 83 | |
| | | 162 | 146 | | 162 | 146 | |
| | | 198 | 123 | | 198 | 123 | |
| | | 101 | 34 | | 101 | 34 | |
| | | 40 | 58 | | 40 | 58 | |
| | | 55 | 100 | | 55 | 100 | |
| | | 110 | 50 | | 110 | 50 | |
| | | | 85.2222222 | | | | |
| AVERAGE | | 96.77777778 | 2 | | 96.77777778 | 85.22222222 | |

TABLE 2 – Colonization Rates Data (* minus *D. simuliidae*)

| DAYS | Colonization Rates of Invertebrates | | Colonization Rates of Invertebrates* | |
|------|-------------------------------------|------------|--------------------------------------|------------|
| | Control | Fertilized | Control | Fertilized |
| 10 | 249 | 1375 | 33.96667 | 39.46667 |
| | 195 | 535 | 4.05 | 4.294444 |
| | 180 | 116 | 3.225326 | 2.804074 |
| | 1260 | 425 | | |
| | 283 | 134 | T-Test | 0.915341 |
| | 173 | 108 | | |
| | 332 | 617 | | |
| | 291 | 211 | | |
| | 94 | 31 | | |
| | Colonization Rate | 33.96667 | 39.46667 | 1.888889 |
| 20 | 134 | 66 | 134 | 65 |
| | 141 | 117 | 133 | 92 |
| | 52 | 44 | 52 | 43 |
| | 51 | 52 | 50 | 50 |
| | 90 | 64 | 90 | 63 |
| | 34 | 141 | 34 | 125 |
| | 51 | 220 | 50 | 218 |
| | 94 | 33 | 92 | 33 |
| | 82 | 36 | 69 | 31 |
| | Colonization Rate | 4.05 | 4.294444 | 3.911111 |
| 30 | 100 | 103 | 100 | 103 |
| | 48 | 70 | 48 | 70 |
| | 57 | 83 | 57 | 83 |
| | 162 | 146 | 162 | 146 |
| | 198 | 123 | 198 | 123 |
| | 101 | 34 | 101 | 34 |
| | 40 | 58 | 40 | 58 |
| | 55 | 100 | 55 | 100 |
| | 110 | 50 | 110 | 50 |
| | Colonization Rate | 3.225926 | 2.8407407 | 3.225926 |

TABLE 3 - Autotrophic Index for each flume on each sampling day.

6/23/2001

| Flume | AFDM (mg/cm ²) | Chl a (mg/cm ²) | AI |
|-------|----------------------------|-----------------------------|----------|
| 1 | 0.044194757 | 0.00028 | 157.8384 |
| 2 | 0.250561798 | 0.0013202 | 189.7908 |
| 3 | 0.173033708 | 0.0003087 | 560.5238 |
| 4 | 0.079026217 | 0.0007308 | 108.1366 |
| 5 | 0.094382022 | 0.0002282 | 413.5934 |
| 6 | 0.104494382 | 0.00028 | 373.1942 |

7/3/2001

| Flume | AFDM (mg/cm ²) | Chl a (mg/cm ²) | AI |
|-------|----------------------------|-----------------------------|----------|
| 1 | 1.312359551 | 0.0001008 | 13019.44 |
| 2 | 0.843445693 | 0.0002184 | 3861.931 |
| 3 | 0.924344569 | 0.0002002 | 4617.106 |
| 4 | 0.401498127 | 0.0002002 | 2005.485 |
| 5 | 0.922846442 | 0.0005593 | 1650.003 |
| 6 | 1.421722846 | 0.0002982 | 4767.682 |

7/13/2001

| Flume | AFDM (mg/cm ²) | Chl a (mg/cm ²) | AI |
|-------|----------------------------|-----------------------------|----------|
| 1 | 1.317602996 | 0.0003402 | 3873.025 |
| 2 | 1.323970037 | 0.000084 | 15761.55 |
| 3 | 1.905617978 | 0.0007665 | 2486.129 |
| 4 | 1.193632959 | 0.0000504 | 23683.19 |
| 5 | 1.812734082 | 0.0005439 | 3332.844 |
| 6 | 0.963670412 | 0.000546 | 1764.964 |

TABLE 4 - Two Way Analysis of Variance: mass date*type (control or treatment)

General Linear Model

Dependent Variable: x Value

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.141)

| Source of Variation | DF | SS | MS | F | P |
|---------------------|----|-------|--------|--------|--------|
| Date | 2 | 0.135 | 0.0673 | 45.183 | <0.001 |
| Type | 1 | 0.007 | 0.0068 | 4.589 | 0.044 |
| Date x Type | 2 | 0.006 | 0.0031 | 2.083 | 0.149 |
| Residual | 21 | 0.031 | 0.0015 | | |
| Total | 26 | 0.170 | 0.0065 | | |

TABLE 5 – Two Way Analysis of variance : chl-a,
date*type (control or treatment)

General Linear Model

Dependent Variable: x Value

Normality Test: Passed (P = 0.177)

Equal Variance Test: Passed (P = 0.034)

| Source of Variation | DF | SS | MS | F | P |
|---------------------|----|-------|-------|-------|-------|
| Date | 2 | 0.282 | 0.141 | 2.221 | 0.133 |
| Type | 1 | 0.013 | 0.013 | 0.211 | 0.651 |
| Date x Type | 2 | 0.666 | 0.333 | 5.244 | 0.014 |
| Residual | 21 | 1.334 | 0.064 | | |
| Total | 26 | 2.214 | 0.085 | | |

Figure 1 – Number of Invertebrates per sampling period

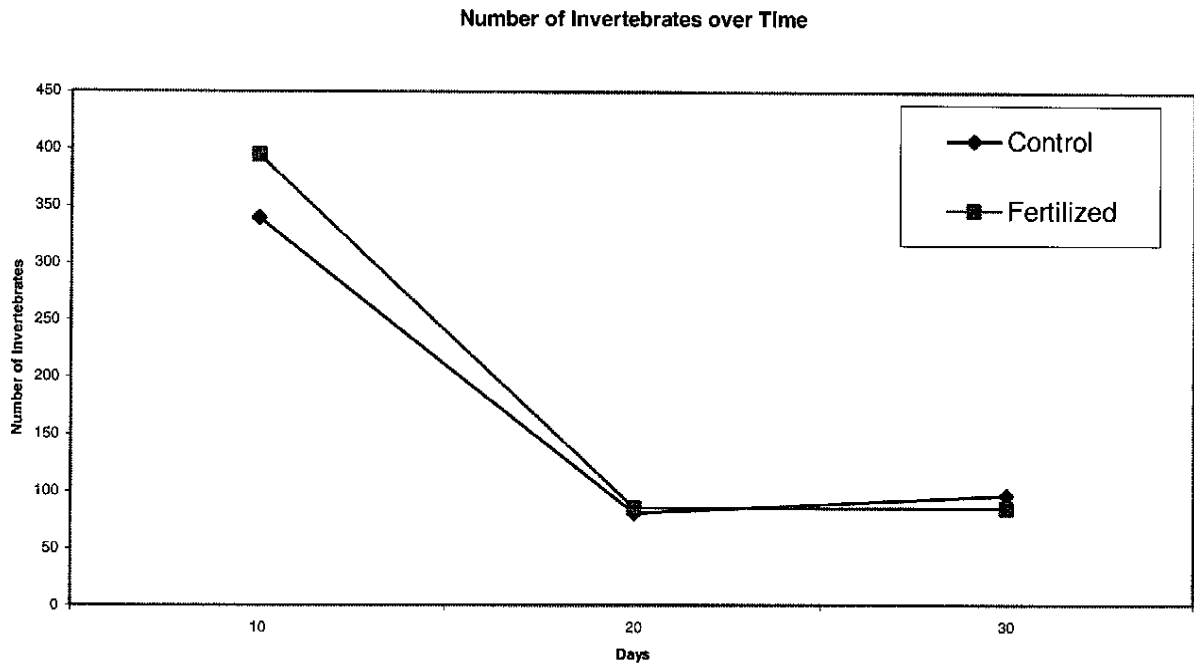


Figure 2 - Number of invertebrates per sampling period excluding all *D. simuliidae* data

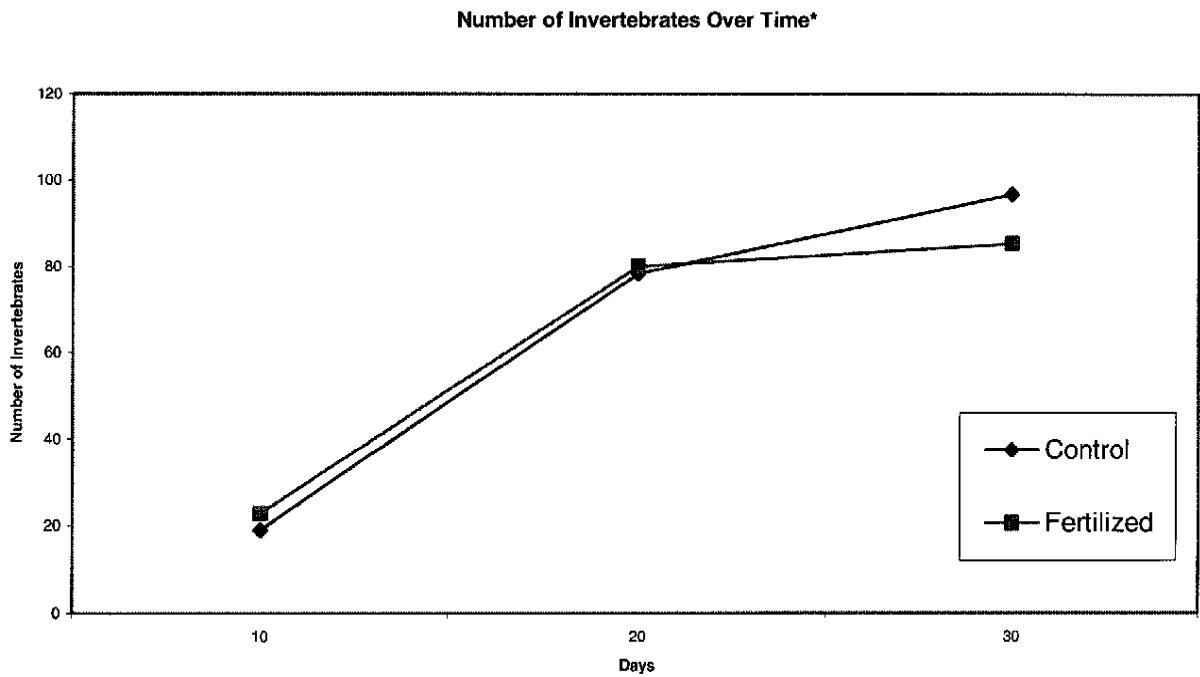


Figure 3 - Colonization rates of invertebrates per sampling period

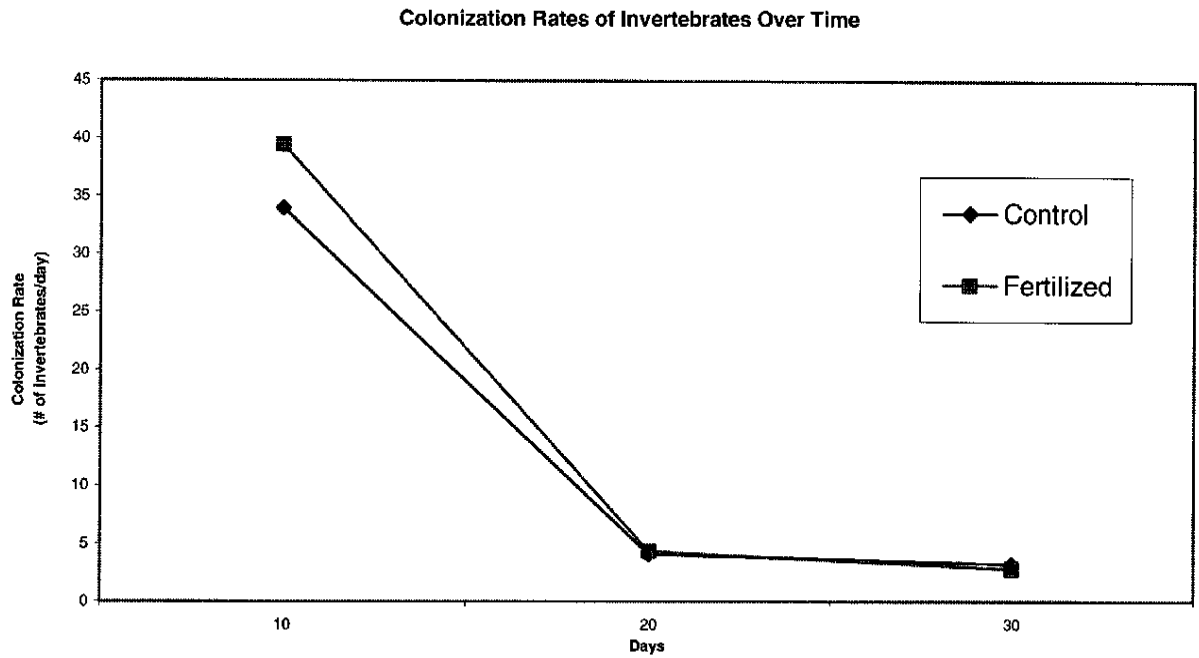


Figure 4 - Colonization rates of invertebrates per sampling period excluding all *D. Simuliidae* data

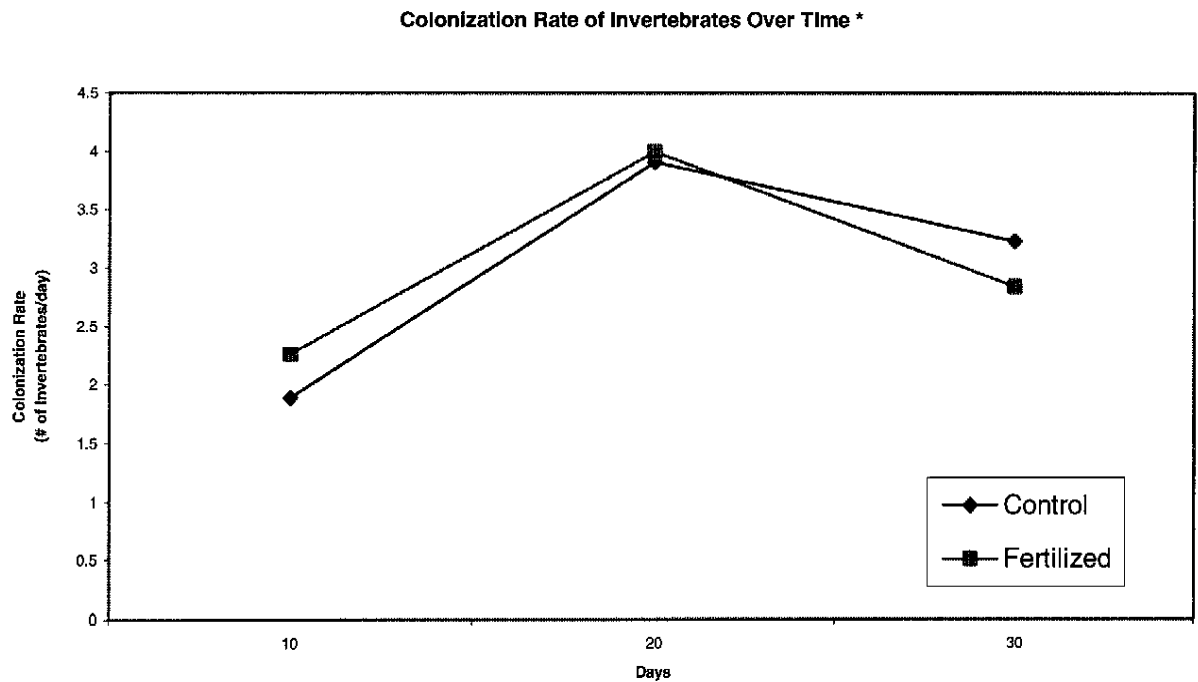


Figure 5 - Average ash-free dry masses of algae in control and treated flumes

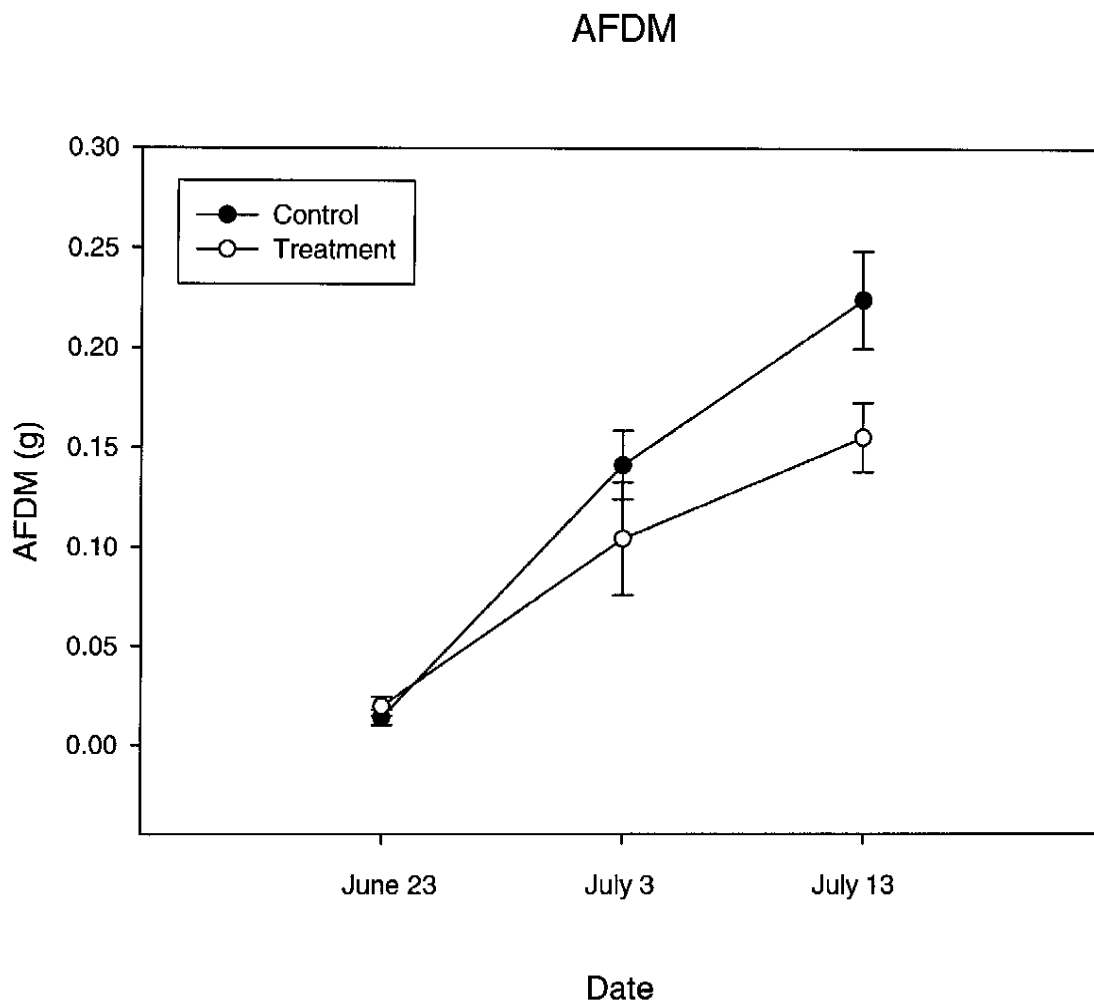


Figure 6 - Average chlorophyll *a* content in control and treated flumes

