The Role of Bullfrog Tadpoles (*Rana catesbeiana*) on Stream Ecosystem Dynamics

BIOS 35502: Practicum in Environmental Field Biology

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Abstract

Amphibians, widely studied as environmental indicators, facilitate the transfer of nutrients between aquatic and terrestrial habitats. To examine the role of amphibians in nutrient cycling, I studied bullfrog (*Rana catesbeiana*) tadpoles in an artificial stream environment. I hypothesized that the presence of tadpoles would decrease algal stock biomass and sedimentation but increase chlorophyll *a* production. To test these predictions, I subjected artificial streams to three treatments: live tadpoles, dead tadpoles, or no tadpoles (control), and monitored their effects on algal biomass, sedimentation and chlorophyll *a* concentration over time. I found that the streams containing living tadpoles had significantly higher chlorophyll *a* content when compared to the other treatments. However, neither the sedimentation nor algal hypotheses were supported, partly because nonlinear dynamics affected the analysis and understand of the variables. I discuss multiple scenarios that suggest the environment's ability to affect the role of tadpoles in stream ecology.

Introduction

The decline of amphibians in Central America, the United States, and southern Canada may be correlated with increased logging, climate changes involving increases in ultraviolet B radiation, and habitat destruction (Kiffney and Richardson 2001, Green 2003). Amphibians, specifically frogs and toads (anurans), are prime indicators of ecological changes due to their high

susceptibility to pollutants but the effect of their decline is not widely studied. This role as suitable indicators is derived from the amphibians' inability to synthesize alkaloids in their skin; rather, alkaloids are accumulated in the sensitive skin from external means (Daly et al. 2002). Highly permeable skin allows for easy penetration by harmful substances, affecting the development of anurans and the ability to interact with the environment naturally (Daly et al. 2002).

Part of the reason amphibians constitute suitable indicator species is their dependence on both aquatic and terrestrial habitats across their lifetime; indeed, they shift from aquatic egg and larval stages to a terrestrially-based adulthood. Given these habitat and life stage changes, amphibians typically change their feeding preferences, as well, converting from algal feeders to insectivores after metamorphosis (Ranvestel et al. 2004, Whiles et al. 2006). These alterations allow for energy transfers between many different ecosystem components; egg-laying distributes energy into the aquatic system whereas metamorphosis leads to energy returning to the terrestrial habitat (Davic and Welsh 2004). In the larval stage, anurans consume energy and nutrients from algae and detritus (Flecker et al. 1999). The cycling continues onto land as the adult amphibian emerges into its terrestrial ecosystem (Whiles et al. 2006).

Anuran effects on interactions within aquatic ecosystems have not been explored sufficiently (Altig and Johnston 1989, Kiffney and Richardson 2001).

Streams underlying closed canopies rely heavily on terrestrially-derived organic matter to initiate the aquatic food web (Crowl et al. 2006). In small, temperate streams, tadpoles may constitute up to 90% of primary consumer biomass (Kiffney and Richardson 2001). Therefore, a strong negative correlation exists between these grazers and periphyton standing stock in streams (Hart and Robinson 1990). Tadpoles are responsible for decreasing algal abundance by as much as 50% (Ranvestel et al. 2004). The presence of amphibian larvae sometimes alters other stream grazers, presumably through competitive interactions (Lamberti et al. 1992), while some species are facilitated by the presence of tadpoles; some insect species benefit from the reduction of sediment exposing and increasing underlying food sources (Ranvestel et al. 2004).

The primary food source of tadpoles, periphyton, is most likely limited by light and inorganic nutrients, specifically phosphorus and nitrogen (Wetzel and Likens 1991, Kiffney and Bull 2000). Phosphorous, often the limiting nutrient in aquatic systems, can control and stimulate productivity (Schindler and Fee 1974). It plays a major role in metabolism, especially considering its relatively diminutive concentrations in the environment; this accounts for the wealth of information available pertaining to phosphorous distributions in fresh water systems (Wetzel 2001). Nitrogen dynamics in flowing water ecosystems is not well understood, as it is not often the limiting nutrient; therefore, nitrogen budgets of only a few streams have been estimated (Wetzel 2001).

Given the limited information available on the role of tadpoles in stream food web dynamics and nutrient cycling, I examined the roll of bullfrog (*Rana catesbeiana*) tadpoles in this system. Bullfrogs are abundant in the University of Notre Dame Environmental Research Center's (UNDERC) Tenderfoot Creek located in Gogebic Co., Michigan and Vilas Co., Wisconsin. American bullfrog tadpoles are also the largest frog species present and tend to remain in the larval stage for at least two summers (Rugh 1935). An unpublished survey of Tenderfoot Creek estimated the density of tadpoles to be $0.91/m^2$ (Berezowitz and Sheperd 2004). Given this information, I questioned whether the presence of *Rana catesbeiana* larvae changed stream dynamics.

I hypothesized that (H1) bullfrog tadpoles would reduce the standing stock biomass of algae (H2) and that growth rates of algae would increase in the presence of tadpoles due to nutrient input and cycling. I also predicted that (H3) sedimentation would be reduced by tadpole feeding habits, resulting in an increase in algal production (Figure 1).

Further analysis of nutrient cycling in invertebrate competition will be conducted by Dr. Todd Crowl of the Utah State University.

Methods and Materials

Artificial Streams

I filled artificial streams (10.2 cm x 2.8 m) using water pumped from Tenderfoot Lake and monitored paddles set to maintain a velocity congruent with

that of Tenderfoot Creek; ca 0.05 m/sec. To simulate natural algal growth, I incubated 54 unglazed, 10.2 x 10.2 cm (large) and 54 unglazed 5.1 x 5.1 cm (small) ceramic tiles along Tenderfoot Lake's shoreline for seven days with full light exposure. The tile sizes depended on availability and area of the artificial streams. I transferred six large and six small periphyton-colonized tiles into each of eight artificial streams and allowed them to acclimate to the streams for 48 h. I randomly placed two living tadpoles in three streams, two dead tadpoles (killed by pithing) in two streams, and the three remaining streams were controls (no tadpoles). The tadpoles were in their second year of growth and had an average biomass of 7.9 g (range 6.9 - 10.3 g). I ran this experiment from 5 June 2006 through 19 July 2006 (experimental setup began 26 May 2006), monitoring average daily temperatures through the UNDERC weather website, last accessed 22 July 2006.

On day two of the experiment, I covered all streams with 1 cm wire mesh due to the disappearance of five of the six tadpoles. I replaced all tadpoles within 24 h. Five more tadpoles vanished on day 39 but could not be replaced within 24 h. Instead, I added gray treefrog (*Hyla versicolor*) tadpoles of equal biomass to the bullfrog tadpoles to the streams for an additional 24 h until I was able to obtain more *R. catesbeiana* larvae. I then removed all gray treefrog tadpoles and continued the experiment for two days longer than originally intended.

Hypotheses 1 and 3

On days 10, 20, 30, and 44, I randomly removed one large tile from each tank. I scraped the algal growth from the tile and dried it at 55ºC for 48 h using a Fisher Scientific Isotemp® Oven. I recorded the mass of the dry product before burning it at 550ºC for 12 h in a Fisher Scientific Isotemp® Muffle Furnace. This product represented the inorganic substances or sedimentation. I subtracted this product's mass from the original dry mass to measure carbon content of the algae.

At the end of the experiment, I filtered the entire contents of the streams through 1mm screen and then through an 80 μm plankton net. I compared sedimentation and algal stock biomass in the material remaining in the plankton net. I sent the contents of the 1mm screen to Dr. Crowl for further analysis.

Hypothesis 2

I performed chlorophyll *a* extraction on one randomly selected small tile per artificial stream every week using the methods described by Clesceri et al (1989). I used a Turner® Quantech™ Digital Filter Fluorometer model FM109535 to measure chlorophyll *a* content. I set the standard curve using concentrations of 0.00, 96.08, 197.82, 395.64, and 791.28 μg/L which yielded a coefficient of determination of 1.00.

Statistical Analysis

Using SYSTAT 11.0 (SYSTAT Software, Inc, Point Richmond, CA), I employed a repeated measures ANOVA to analyze the results from the scrapings

and ethanol extraction using tadpole presence as the independent variable and chlorophyll *a* content as the dependent variable. I used this to detect any significant relationship between the presence of tadpoles and algal stock biomass or algal growth. I considered the sedimentation results using the same test (independent = tadpole treatment, dependent = sedimentation) to verify any relationship of tadpole presence and sedimentation.

Results

Neither algal biomass ($F = 0.850$, $df = 6$, $p = 0.552$) nor sedimentation ($F = 1.452$, $df = 6$, $p = 0.260$) had a significant relationship with the presence or absence of tadpoles. Neither factor showed any indication of a significant trend. However, the relationships of time with algal biomass ($F = 6.269$, $df = 3$, $p = 0.006$) and with sedimentation ($F = 4.560$, $df = 3$, $p = 0.018$) were both significant. As seen from figures 2 and 3, both show peaks in the third sample (day 30), followed by sharp declines.

Tadpole presence did not affect total algal biomass significantly ($F = 3.539$, $df = 3$, $p = 0.110$), but the borderline p-value indicates a weak biological trend. The least squares means (Figure 4) did not show any overlap between total algal biomass in streams containing live tadpoles and either other treatment. The relationship between tadpole presence and sedimentation was also not significant ($F = 1.805$, $df = 2$, $p = 0.257$).

Using an ANOVA, I found a significant relationship between the different tadpole treatments and the amount of chlorophyll *a* present ($F = 8.370$, $df = 8$, $p < 0.001$). Additionally, the relationship between chlorophyll *a* production and the day sampled was significant ($F = 39.467$, $df = 4$, $p < 0.001$). The relationship correlated significantly higher levels of chlorophyll *a* with tadpoles present and can be seen in figure 5. In the streams containing dead tadpoles, there was an initial increase in chlorophyll *a* production followed by a sudden drop off then gradual increase. Post-hoc analyses of tadpoles versus no tadpoles are in table 1. Insufficient replications of streams containing dead tadpoles exist to create enough degrees of freedom to conduct post-hoc analyses of the effects of dead tadpoles.

The average daily temperatures over the course of the experiment can be seen in figure 6. Temperature had a generally increasing trend with the exception of days 12 through 18; a chronically low average spanned these days.

Discussion

While no significant relationship appeared to exist between treatments and algal stock biomass, Dr. Crowl and I observed tadpole grazing predominantly on the sides of my artificial streams. Sampling the sides in addition to the artificial stream beds caused a trend towards significance, decreasing the p-value of the entire stream compared to that of the substrate alone. Independent sampling of vertical surfaces may reveal a significant relationship between algal biomass and

tadpole presence. A more extensive study may also reveal a significant relationship. The p-value was relatively low (0.110); considering the small sample size, the relationship would have to be quite strong to show significance. Therefore, this trend suggests that there might be a significant relationship if statistical power was strengthened by increasing sample size (Figure 4). This should be considered in prospective studies.

The marked decline in algal stock biomass seen between days 35 and 44 may be a result of the 24 h inhabitation of *H. versicolor* tadpoles. Their metamorphosis is much more rapid than that of *R. catesbeiana* so, consequently, the larvae have a higher metabolism (Whiles et al. 2006). Hypothetically, they would have consumed much more algae in proportion to their biomass. This inconsistency might have affected sedimentation in the same manor.

Sedimentation also had no significant relationship with tadpole presence or absence in streams. A more accurate measure of tadpole effects on sedimentation should employ still water. Even with the relatively low velocity, smaller *H. versicolor* tadpoles could not stay out of the drain, implying that small sediments would have been passed easily. Maintaining the streams outdoors left them exposed to the weather and, therefore, subject to disturbances.

The significant relationship between tadpole presence and chlorophyll *a* content supported my initial hypotheses. Treatments in which live tadpoles were present showed significantly higher chlorophyll *a* production, most likely because

tadpole grazing prompted more efficient algal growth. Pertaining to streams containing dead tadpoles, the added nitrogen and phosphorus contained in the tadpole carcass might account for the ability of the streams to initially become more productive (Wetzel and Likens 1991, Kiffney and Bull 2000). The sudden drop in chlorophyll *a* was probably caused by the exhaustion of added nutrients due to completed decomposition of the tadpoles. A different species of algae may have gradually then become the dominant species (Hart and Robinson 1990). This could have easily happened if the latter species was more tolerant of low nutrient levels (Wetzel and Likens 1991). While this theory would also explain the gradual increase in chlorophyll *a* content, testing the algal growth specifically would have to be implemented in further experimentation.

This theory, however, would not explain the corresponding chlorophyll reductions in the other streams. An external climatic event is therefore a more likely explanation. Days 13 through 17 had consistently low temperatures in comparison with the entire time period. As I did not monitor water temperature because the same source supplied for all streams, the streams' temperatures may have collectively dropped, causing a marked reduction in chlorophyll *a* production. Likewise, tadpoles, being ectothermic, would have decreased metabolism during a period of lower temperatures. Therefore, their foraging would be reduced, causing a trend towards production mirroring streams lacking anuran larvae (Whiles et al. 2006). Consequently, chlorophyll *a* content would

drop more drastically in tadpole-inhabited streams with decreasing temperatures compared to my results. Future experiments exploring the relationships between temperature, algal growth, and anuran metabolism would help to distinguish the effects.

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Table 1: Post-Hoc Analysis of Chlorophyll *a* Content in Streams Containing Live Tadpoles vs. No Tadpoles Using a Paired T-Test

	Day 14	Day 21	Day 28	Day 35	Day 44
t-statistic	-3.640	-1.984	-1.126	-3.934	-4.051
p-value	0.048	0.124	0.332	0.017	0.295

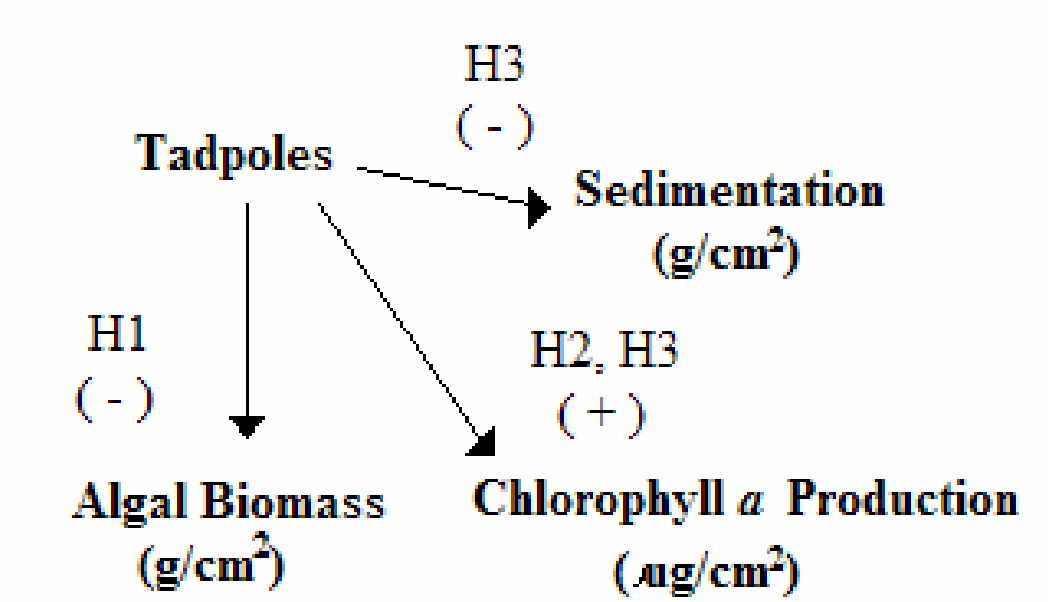

Figure 1: Hypotheses examined in this study, examining bullfrog tadpole effects on stream function. Tadpoles were expected to decrease sedimentation and algal biomass but increase the production of chlorophyll *a*.

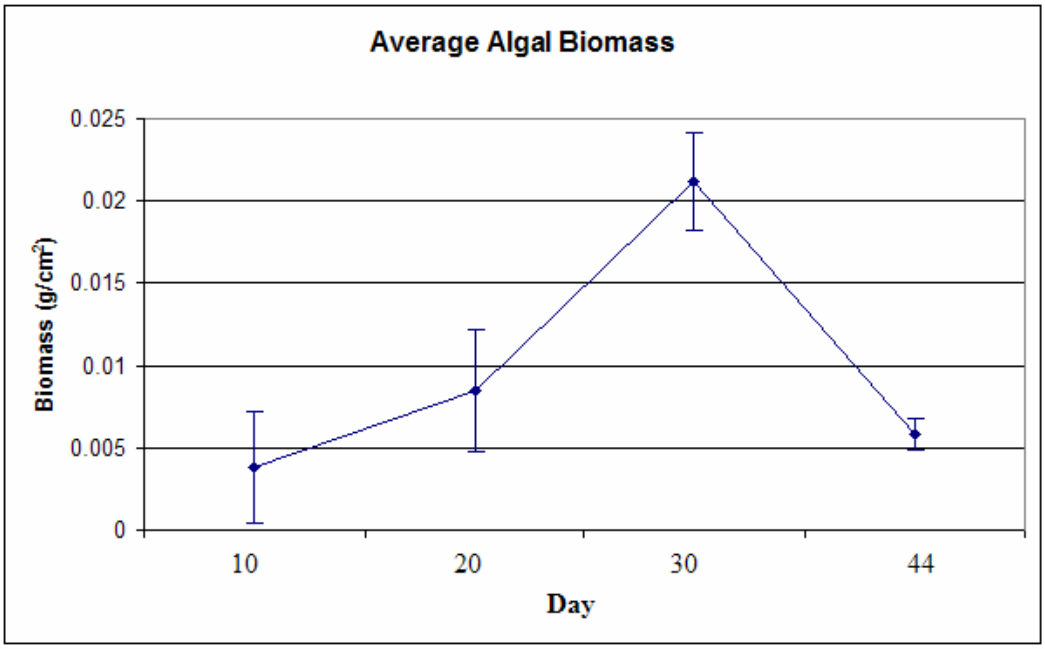

Figure 2: Average algal biomass (+/- SE) over time, showing a gradual increase in the first 30 days before declining during the final 14 days of the experiment.

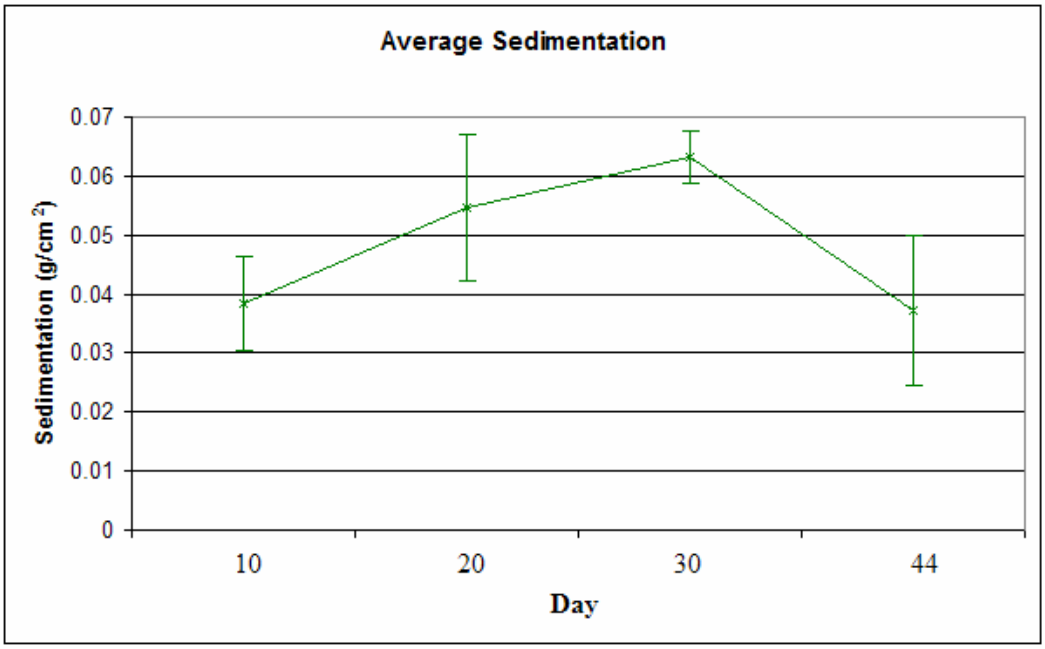

Figure 3: Average sedimentation (+/- SE) of all streams had a general trend of gradually increasing until sometime in the final quarter of the experiment when it abruptly dropped.

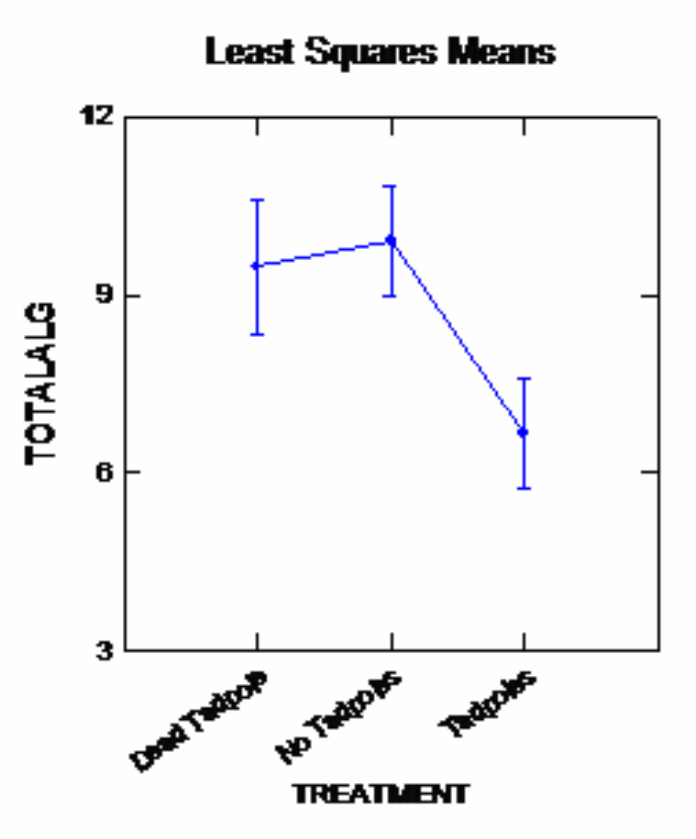

Figure 4: Lease squares means test, showing that total algal biomass in streams containing live tadpoles was lower, though not statistically significant, than other treatments ($F = 3.539$, $df = 3$, $p = 0.110$).

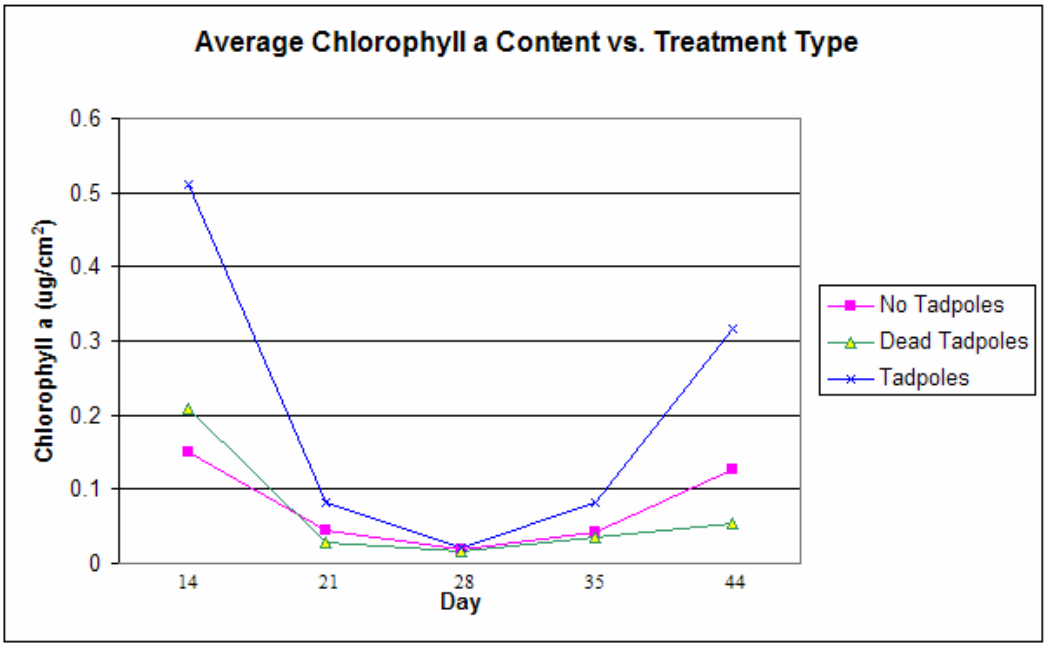

Figure 5: The average chlorophyll *a* contents of each stream treatment are plotted against time. These data represent a significant relationship between treatment type and productivity of algae ($F = 8.37022$, $df = 8$, $p = 0.00006$).

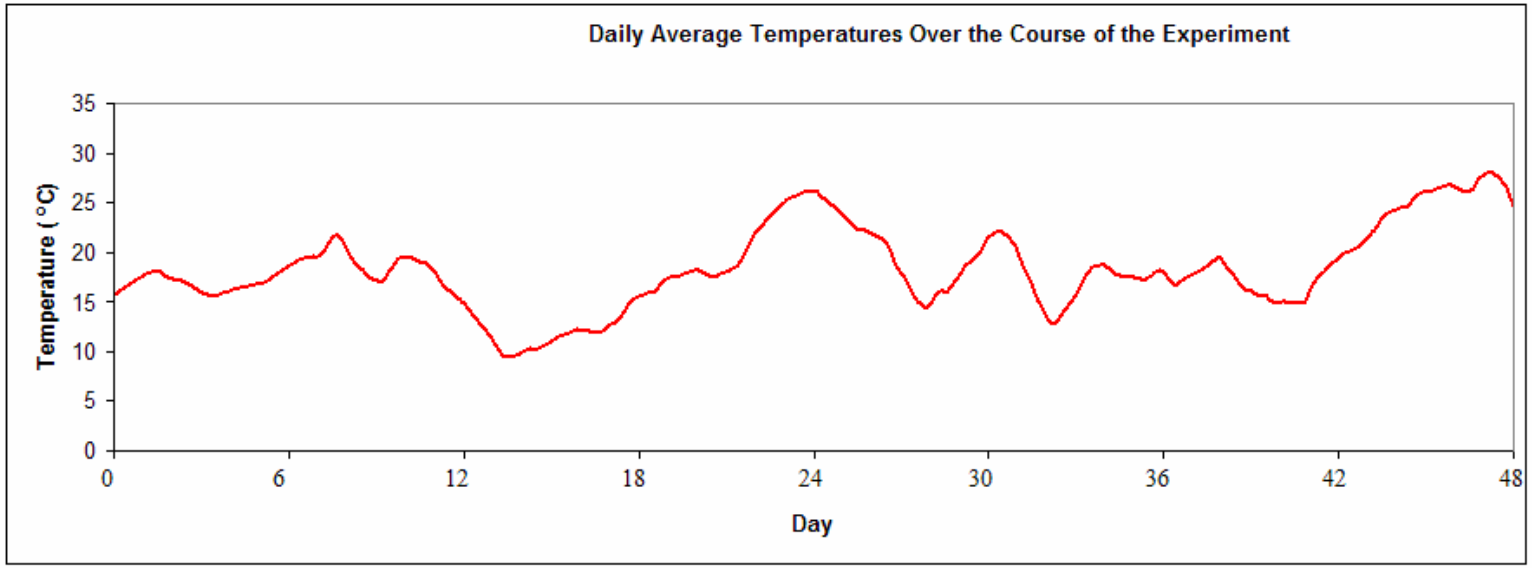

Figure 6: The average daily temperatures (ºC) of the experimental period, as recorded by the UNDERC weather station (at Grasshopper Nation). A marked drop around day 14 corresponded with a drop in chlorophyll *a* production that occurred across all treatments.