

**IMPACT OF BULLFROG TADPOLES ON WATER CHEMISTRY,  
MACROINVERTEBRATE POPULATIONS, AND BENTHIC COMPOSITION  
OF TENDERFOOT CREEK**

BIOS 569 – Practicum in Aquatic Biology

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**Abstract:**

This is a study of the effects of *Rana catesbeiana* larvae on the Tenderfoot Creek ecosystem in the Upper Peninsula of Michigan on the UNDERC property. Tadpoles randomly were placed into three enclosures in the stream, while three other enclosures served as control runs. For all six of the runs, we tested a total of eight variables in order to measure the effects of the tadpoles' presence on water chemistry, chlorophyll *a* production and algal biomass, and benthic sediments and macroinvertebrates. The results from the total nitrogen, total phosphorus and dissolved organic carbon tests were not available at the time this paper was written; however, none of the results for the other variables showed that the presence of grazing tadpoles had any effect. Algal biomass, chlorophyll *a* production, and the average amount of particulate organic carbon significantly increased when measured over time. In addition, we conducted an isolated experiment in order to measure chlorophyll *a* production using eight artificial streams, in which four treatment streams contained tadpoles and four controls lacked tadpoles. In this experiment the presence of tadpoles proved to be significant in limiting the chlorophyll *a* production. Although one would predict bullfrog tadpoles would have a significant effect on the Tenderfoot Creek ecosystem due to their high density based upon biomass, our results did not reflect such expectations. Nevertheless, many of our variables did change over time, demonstrating the natural dynamics of a low velocity stream. Ideally, this continued study of the bullfrog tadpole population over a longer time scale will yield significant results that will lead to a better understanding of how densely concentrated species may affect a stream ecosystem.

## **Introduction**

The bullfrog (*Rana catesbeiana*) is the largest frog species found in temperate regions of North America. As the most abundant species present in Tenderfoot Creek, Michigan, we proposed that its presence directly relates to nutrient composition, algae abundance, and macroinvertebrate populations within a stream habitat. Reproduction occurs during June and early July, and typically this species remains in the larval stage for two or three summers in northern temperate locations. Bullfrogs feed on algae and other aquatic plants and scavenge decaying animal matter in the benthos (Harding 2000). They are generally found in areas of standing water such as lakes, ponds and bogs, and experimental studies on the roles of single species are lacking for stream ecosystems. Few studies have investigated the role of macrobiota on detrital composition within the water column and the benthos, as well as nutrient availability and primary productivity of algae (Covich et al. 2001). Thus, this study is original since bullfrogs are the most abundant macrobiota in Tenderfoot Creek based on our density surveys and thus are the most likely species to have an impact on the ecosystem.

It is generally accepted within the scientific community that macro-biota, such as the bullfrog tadpoles, both physically and chemically alter their environments, which may in turn alter ecosystem structure and dynamics (Covich et al 2001). Because bullfrog tadpoles are the dominant consumers of algae in Tenderfoot Creek, we believe that their consumption will not only limit the net primary product of algae in the stream's benthic region, but their abundance will also influence the nutrient richness within the water column. Nitrogen, phosphorus, and dissolved organic carbon are limiting factors for algal growth and in turn are measures of the aquatic health of a stream (Hauer 1996). By limiting nutrient availability within the stream,

the bullfrog tadpoles may have an impact on food web dynamics and essential ecosystem functions such as nutrient cycling, sediment mixing and the biodiversity of macroinvertebrates (Covich et al. 2001).

While many studies have been conducted assessing the importance of tadpoles as bioindicators, we proposed to examine the impact of bullfrog tadpoles on the nutrient richness of a streambed and water column, which in turn limits the resources available for other vertebrates and invertebrates. Indeed, Covich et al. (2001) conducted a study on the effects of freshwater shrimp on detrital processing and nutrient composition in a tropical headwater stream in the Luquillo Experimental Forest in Puerto Rico. Our study similarly tested the impact of a numerically dominant species on the detrital processing and nutrient release within the producer-consumer food web, and we assessed the impact of tadpoles on this temperate stream ecosystem as a whole. In addition, we measured the influence of bullfrog tadpole grazing on the primary production of algae in a controlled environment of eight artificial streams.

## **Methods and Materials**

### *Tadpole Density Study*

Before beginning our experiment, we found it necessary to measure the tadpole densities in systematic locations along Tenderfoot Creek in order to determine how many tadpoles within our enclosures would be representative of the natural populations along the stream. Visual snorkelling counts over 35 1 m x 2 m plots were conducted beginning 100 m from the southern main bridge over Tenderfoot Creek on property and were continued at regular intervals in order to maximize the replicates. As one can see in Fig. 1, the distribution varied greatly from one location to another

along the stream. This is most likely due to the fact that the tadpoles tended to cluster around reeds and vegetation, and thus it is completely logical that areas of dense tadpole populations were followed by counts containing few tadpoles. The average number of tadpoles per visual count was 7.2, but this value was obviously on the low end as one can verify during a visual survey. Ultimately, we decided to allocate 40 tadpoles to each of the three enclosures containing tadpoles (as described on the subsequent section of *Methods and Materials*) since many tadpoles swam away while conducting the visual count and no doubt even more were hiding in the streambed's sediment.

#### *Tenderfoot Creek manipulation experiment*

For this experiment six enclosures were created, three as controls without bullfrog tadpoles and three as treatments including tadpoles. The site on Tenderfoot Creek was selected based on accessibility and suitability for constructing the six runs. Each of the three pairs of enclosures was 10 m long and 0.75 m wide and physically varied only due to water column depth. Runs one and two were 1.2 m deep, runs three and four were 1 m deep, and lastly runs five and six were 0.6 m deep. These variances in depth were simply due to the natural gradient in the streambed. Netting was wrapped around the stakes so as to prevent the tadpoles from escaping, and plastic was laid over the netting in order to prevent nutrient mixing among the various water columns; however, the upstream and downstream ends were not covered by the plastic wrap so as to allow for natural nutrient flow through the enclosures with the current. The treatments were paired based on depth, and two walkways separated the different blocks (thus, in between runs two and three and also between runs four and five) in order to allow for ease in sampling the many variables being tested in this experiment. Numerous rocks lined both the inside and outside of every enclosure in

order to hold down the netting and plastic tarp and especially to prevent the tadpoles' escape. Runs one, four and five we maintained as our control runs, while runs two, three, and six each contained forty tadpoles as the treatment.

In this part of the experiment we tested to see how the presence of the bullfrog tadpoles affected each of eight different variables as compared to the control runs lacking tadpoles. Regarding the chemistry of the water column, we sampled for total phosphorus, total nitrogen, dissolved organic carbon, and particulate organic carbon; however, only the results for the particulate organic carbon are provided in this paper because the three other variables required advanced water testing to be conducted at the University of California at Berkeley at the completion of this study. The various macroinvertebrate populations present in each of the six enclosures as well as at an unenclosed control site about 5m East of the enclosures were documented in order to note whether the tadpoles had any effect on these various populations. Furthermore, we measured the effect of tadpole grazing on the presence of algae on tiles based on both chlorophyll *a* testing and total algal biomass. The scientific processes that were followed to measure each of the eight variables are detailed in the subsequent paragraphs.

#### (a) Particulate Organic Carbon

We sampled this variable a total of four times using 480 ml glass jars that were first rinsed in the lab using deionized water. The water was collected by pointing the jars upstream and submerging them elbow-deep inside each of the runs and at a control site. We then filtered the contents of the jars back in the lab using vacuum filtration and filter paper that was previously weighed and dried. We put the filter paper (each of which was in an aluminium tin) into the dryer for a full 24 hours before weighing the contents and subsequently ashing the contents for two hours at 500

degrees Celsius. We determined that the filter paper burned completely, and thus after the final weighing of the tin and the ashed contents we calculated the particulate organic carbon over a standard volume.

(b) Benthic Macroinvertebrates

Three trays containing a leafy green plant were placed into each of the six runs, as well as at the control site, and the diversity and abundance of macroinvertebrates living in the benthic community were identified using a light microscope. Only one basket was retrieved from each run, and at each of the two remaining sampling dates one basket will be retrieved from each run in addition to the control site.

(c) Chlorophyll *a* Production

For the Tenderfoot Creek experiment, seven 15 cm x 15 cm clay tiles were placed at regular intervals in each of the six runs, starting from the downstream end and proceeding to the upstream end of the runs. Both these tiles as well as the bricks that were to collect the substrate solutes were placed onto a wire grate, two of which were pulled up at each of the two collection dates for this variable (July 5 and July 17). The remaining tiles will be pulled up on the final sampling date. In addition, chlorophyll *a* production was measured using 7.5 cm x 7.5 cm clay tiles in the artificial streams, yet we used the same method for measuring production. For each of the tiles we scraped half of the surface area into a beaker using a toothbrush and rinsed any remaining algae into the beaker as well using deionized water. The contents of these beakers were then filtered through a vacuum filtration set-up very slowly so as to prevent damage to the chlorophyll *a*. This variable required the preparation of a 90% acetone solution into which each filter was placed in a vial and kept on ice (in the dark) for a period of 24 hours. After that time, a blank vial and

each of the six experiment vials were run through a spectrophotometer at wavelengths of 665 nm and 750 nm. After acidifying each of the vials with 0.1 ml of 1M HCl per 1 ml of extract (dependent upon the volume of acetone used in each vial), we remeasured the absorbencies at both 665 nm and 750 nm. After plugging in each of the variables to a standard equation, we were able to determine the mg of chlorophyll *a* per cm<sup>2</sup> of area of tile scraped.

#### (d) Algal Biomass

This experiment is fairly straightforward in that we scraped off half of the algae from the Tenderfoot Creek tiles into beakers that we proceeded to filter through a vacuum filtration system. The filter papers were then dried in aluminium tins for 24 hours before being weighed and ashed at 500 degrees Celsius. The total algal biomass (which burned completely) was the difference between the dried filter papers with the algae (in the tins) and the ashed filter paper in the tins.

#### (e) Sediment Sample

Seven large bricks with plastic epoxy glued to the bottom were placed on the wire grates with the large tiles and placed in each of the runs along Tenderfoot stream. After the sixth and eighteenth days of this experiment, the sediment that collected in each of the three holes of the bricks (all of equal volume) was collected using a turkey baster and was transferred into 480 ml containers. Two bricks were collected at each of our two sampling dates for a total of four bricks from each run that were removed, and the remaining bricks will be removed and measured with the last tiles at the final collection date. The final measure for this variable was the total grams of inorganic sediment collected per run.

#### (f) Total Nitrogen, Total Phosphorus, and Dissolved Organic Carbon



For these water chemistry variables we simply collected the samples and froze them so that they may be shipped out in order to be analysed at another university. After submerging the bottles in an acid wash for at least 12 hours, we simply submerged one bottle into each run per collection date for both the total nitrogen and total phosphorus. The dissolved organic carbon required that we filter the water on the spot before putting it into the plastic bottle in order to remove everything but the DOC. We also measured the TN, TP and DOC at a control site at each of the four collection dates.

Finally, we created a timetable in order to sample for each of the variables. On June 23 we sampled for water chemistry only as our baseline before we added all 120 tadpoles. July 5 we sampled all of the variables except the macroinvertebrates in the plant enclosures. Again, on July 11 we sampled only the water chemistry (total nitrogen, total phosphorus, dissolved organic carbon, and particulate organic carbon) as well as the macroinvertebrates present on the plant traps. The final collection date for the purposes of this paper was on July 17, at which point we sampled everything but the macroinvertebrates. However, it is important to note that this study is ongoing, and two more collection dates will occur on July 31 (for water chemistry and macroinvertebrates only) and finally on August 28 (for all seven variables).

#### *Artificial Stream Study*

The last part of this study involved eight entirely artificial streams filled with continually circulating Tenderfoot Lake water and four 7.5 cm x 7.5 cm clay tiles in each of the eight individual runs. Artificial runs one, four, six and seven each contained two tadpoles, while the remaining four runs served as controls for this isolated experiment. Chlorophyll *a* was the sole variable tested for each of the four

replicates, and we tested it using a spectrophotometer during two separate time periods, namely at day one and at day 18 of this experiment.

The experimental process involved scraping the algae off of the tile and into a beaker, as well as rinsing any scum into the beaker using deionized water. Next we poured the algae through filter apparatus and let the filter sit for 24 hours at 4 degrees Celsius in a 90% buffered acetone solution. We ran the eight cuvettes as well as a blank at 750 and 665nm absorbencies both before and after adding 0.1 ml of 1M HCl per 1 ml of extract. After performing the necessary calculations, we determined the chlorophyll *a* concentrations for each of the eight artificial streams.

## **Results**

### *Tenderfoot Creek Results:*

#### A. Particulate Organic Carbon

The presence of tadpoles in the 3 experimental enclosures had no significant effect on particulate organic carbon in the water column ( $F = 3.296$ ,  $p = 0.122$ ). The POC concentration with tadpoles present was more or less the same as when they were absent (fig. 2 and 3). The *average* amount of P.O.C. taken over all the enclosures combined was found to generally increase over time (fig. 4).

#### B. Benthic Macroinvertebrates

Of the macroinvertebrate genera identified, the 6 most abundant groups were chironomids, ephemeroptera, amphipods, hirudinea, gastropoda, and trichoptera. These six were compared between the 3 treatment enclosures with tadpoles, the three without and the control sight in the open stream. Results showed that the benthic community populations varied between each of the individual enclosures for *all* genera (fig. 5). They did not vary, however, when comparing the community

populations between enclosures with tadpoles versus no tadpoles (fig. 6). This indicates that the presence of tadpoles did not have a significant effect on the benthic community.

### C. Chlorophyll *a* Testing in Tenderfoot

The chlorophyll *a* production in the experiment enclosures varied significantly over time. This means that, algae growing on the tile substrates produced a significant amount of chlorophyll *a* from the first sampling at day 6, to the second sampling at day 18 ( $F=23.75$ ,  $p=0.003$ ). The production measures did not vary significantly between the enclosures ( $F=0.671$ ,  $p=0.571$ ) indicating that the tadpoles alone did not greatly affect the amount of chlorophyll *a*-producing algae, despite the fact that tadpoles are major grazing organisms that feed mostly on algae. The *interaction* of tadpole grazing and the time elapsed did not have significant chlorophyll *a* production differences ( $F=1.156$ ,  $p=0.284$ ). This indicates that the *change* in production over time was not significantly different between enclosures (Fig 7).

### D. Algal Biomass

The production of chlorophyll *a* is an indication of the amount of algae that is growing on the substrate. These amounts were found to be significantly different over time, indicating that the algae were growing ( $F=99.014$ ,  $p=0.001$ ). However there was no significant difference in algal growth between the enclosures with and without tadpoles, reinforcing the conclusion of the chlorophyll *a* tests ( $F=3.149$ ,  $p=0.151$ ). The *interaction* of tadpole grazing and the time elapsed did indicate significant differences in algal biomass ( $F=29.151$ ,  $p=0.004$ ). This indicates that the growth of algae changed over time differently between enclosures (Fig 8).

## E. Sediment Sample

The amount of sediment collected increased significantly in the enclosures over time ( $F=133$ ,  $p=0.001$ ). The presence of tadpoles in the enclosures caused no significant differences in the amount of sediment collected ( $F=2.343$ ,  $p=0.201$ ). There was also no significant interactions between time and the tadpoles (fig 9); the amount of sediment in enclosures with tadpoles did not change significantly more or less than those without ( $F=2.322$ ,  $p=.202$ ).

*Note:* Tadpoles disappeared from cages 3 and 6 after first week, were replaced and again found to be gone in 3 days. A final replacement on day 12 marked their stay.

### *Artificial Streams Results:*

The tadpoles in the artificial streams significantly reduced the chlorophyll *a* production in the artificial streams ( $F=25.41$ ,  $p=0.007$ ). Production increased significantly over the time elapsed between sample dates ( $F=234.72$ ,  $p=0.001$ ). Also, the tadpole activity had a significant effect on how much the production changed over time ( $F=70.867$ ,  $p=0.001$ ). One control stream (5) was an outlier, which, for some reason had accelerated chlorophyll *a* production. Algal blooms were observed in streams without tadpoles where in tadpole-dwelling streams had relatively little free-floating algae. After the first six days, most of the surface around the tadpoles and on the tiles remained comparatively low.

## **Discussion**

The dominating biomass of *Rana catesbeiana* tadpoles in Tenderfoot Creek raises suspicion that these herbivores would have an important role in the ecosystem. The chemistry of the water column and subsequent effects on macroinvertebrates co-inhabiting the particular aquatic environment were tested for such effects. Previous studies on the effects of tadpoles on other aquatic habitats such as vernal ponds and permanent ponds have found that patterns of tadpole biomass fluctuate with primary production and suspended particles (Seale 1980). Through their consumption of epiphytic and epibenthic algae as well as algae in their own fecal matter, tadpoles alter the ecosystem.

*Particulate Organic Carbon:*

Tadpoles have been known to contribute to particulates greatly in vernal and permanent ponds (Seale 1980). The low significance of our POC in the water column, as may be seen in Figs. 2-4, suggests that the tadpoles do not regularly have this effect in streams. We did not conduct isolated tests for this factor so we cannot conclude that tadpoles even have slight effects in streams. The contrast between our results and that of pond studies suggests that an important factor for this variable is water flow. The constant replacement of the water steadies the local chemistry. Any additions or subtractions that the tadpoles made to the POC would be carried away in the flow. In the absence of flow (ponds) the POC effects would accumulate.

*Benthic Community:*

The significant differences in the benthic communities among all of the enclosures suggest that the streambed is not a homogeneous habitat (refer to Fig. 5 and Fig. 6). The enclosures were not of equal height, which may have been the reason

the organisms habituated them unevenly. The food web in stream environments has been studied extensively and proven to be a tight weave of autotrophs, primary productivity, consumption and decomposition. Benthic algae are said to be the dominant primary producers and insects, molluscs and crustaceans are all important herbivores in addition to the tadpoles (Lamberti 1996). All of these taxonomic groups were found to be abundant in the Benthic samples. The fact that the tadpoles had no significant effects in other tested factors was a strong indication that the benthic community would also be statistically the same. This prediction is supported by our results. Had there been drastic tadpole effects, it follows that the adjacent food web would experience disturbance as well the results would have been noticeable. The gastropods were so prevalent in the enclosures that their trails were visible in the algae on the tiles and the plastic siding. Perhaps it would be interesting to look at the competition for algae as a major food source between these two, very different organisms.

#### *Algal Biomass and Chlorophyll a Production:*

With previous studies in mind, we searched for similar findings in our stream experiment. The results of our algal biomass and chlorophyll *a* tests concur with Seale's conclusions (1980) in our artificial set up, but not the Tenderfoot creek set up (refer to Figs. 7 and 8). In the artificial streams study, the tadpoles' grazing on the algae reduced the biomass to measures significantly lower than streams with out tadpoles (see Fig. 9). In the first weeks, the difference between streams with and with out herbivores was visually conspicuous. The set up of this study was beneficial for extracting most other possible factors that would affect algae growth. In isolating the

tadpoles and algae from their natural habitat, we were able to measure their effects on the algae with more accuracy.

The results for algal biomass and chlorophyll *a* production were not as concrete in the Tenderfoot study, indicating that the tadpoles did not completely dominate over the myriad other dynamics of a natural ecosystem as rich as a slow moving stream. Experimental errors (missing tadpoles) are another possible explanation. This experiment will hopefully produce more concrete results as it continues.

*Sediment Solutes:*

Although sediment generally means inorganic particles, it has three major components: organic matter at various stages of the decomposing process, particulate mineral matter and inorganic particles of biotic origin (Wetzel 2001). The major sources of sediment are off the land via surface erosion and mass wasting. The presence of tadpoles, it seems could create more sediment by stirring the settled bottom and depositing fine particulate organic matter through their excrement. They could also conceivably lessen the sediment as tadpoles are known to consume suspended algae. Our results however showed no significant effects caused by the tadpoles. This could be explained by the flow of the stream. Anything the tadpoles stir up would be pushed out of the enclosure. Perhaps a good test would have been to take residue samples from the build up on the screens that cover the ends. Refer to Fig. 10 to see that the sediment increased with time, which follows the logic that as the brick rests on the bottom longer, more passing particles can collect.

*Comments:*

Tenderfoot creek set up proved to be erroneous. The tadpoles were harder to contain than anticipated and escaped from enclosures three and six twice. Prompt replacements with a new batch of 40 were made to minimize effects on final data. Also the sampling methods for sediment solutes and benthic macroinvertebrates need improvement. The plastic often fell off the bottom of the bricks and sediment that collected there leaked heavily. The benthic sampling had the same problem; much of the matter settled on the plastic plant and basket was very difficult to collect.

The artificial stream procedure would have been better with more than two tadpoles per stream. After the second week there was enough algae on the walls of the streams that the tadpoles grazed there, almost entirely ignoring the tiles. Another way to avoid this could be to routinely scrape the algae off the walls to make sure the only food source is the tiles. Yet another method could be to forgo the tiles and take random scrapes from the surface of the stream.

The unique nature of this study is that tadpoles have not previously been studied in a stream environment, which is a habitat that has generally been researched extensively. The results of this experiment are important in understanding biotic and abiotic interactions of stream habitats, as well as furthering our education on this topic. Since algae is the major primary producer in streams, it is of particular interest. Pollution and human disturbance are increasing at alarming rates and it is important to understand what we are destroying in order to ensure the possibility of rectifying the damage.

### **Acknowledgments**

We would sincerely like to thank Dr. Todd Crowl and Dr. Gary Belovsky for the numerous hours they spent designing this experiment and the effort they put in to



order the necessary materials. Furthermore, we would like to commend Dr. Crowl for his much needed assistance in conducting the 35 snorkelling tadpole density counts, as well as for his patience in teaching us the necessary methods required to sample each of the various variables. In addition, thanks to Kelly Stanton for continuing this experiment for two collection dates after the completion of the UNDERC undergraduate Practicum in Aquatic Biology. Finally, we feel it is necessary to thank the entire 2004 UNDERC Class for the manual labour they put into constructing our Tenderfoot Creek enclosures. We hope that this experiment may provide the groundwork for further study of the dense bullfrog tadpole populations throughout Tenderfoot Creek.

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1980
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## Figures:

Figure 1. Densities of Tadpoles Along Tenderfoot Creek

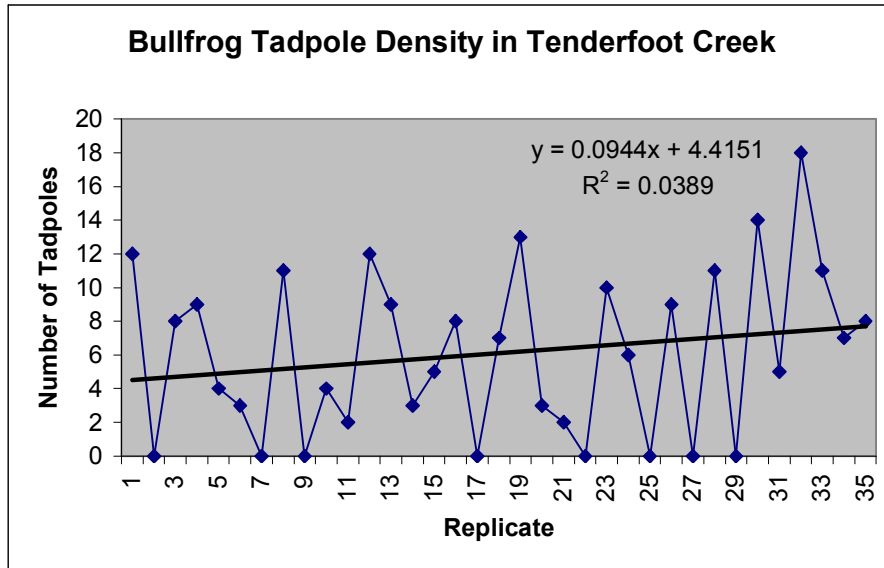


Fig. 1 illustrates the densities of tadpoles in 35 locations along Tenderfoot Creek. These visual counts were conducted over a 1m x 2m area beginning 100m from the southern bridge and continuing in regular intervals downstream.

Figure 2. Change in POC Over Time in Treated and Control Enclosures

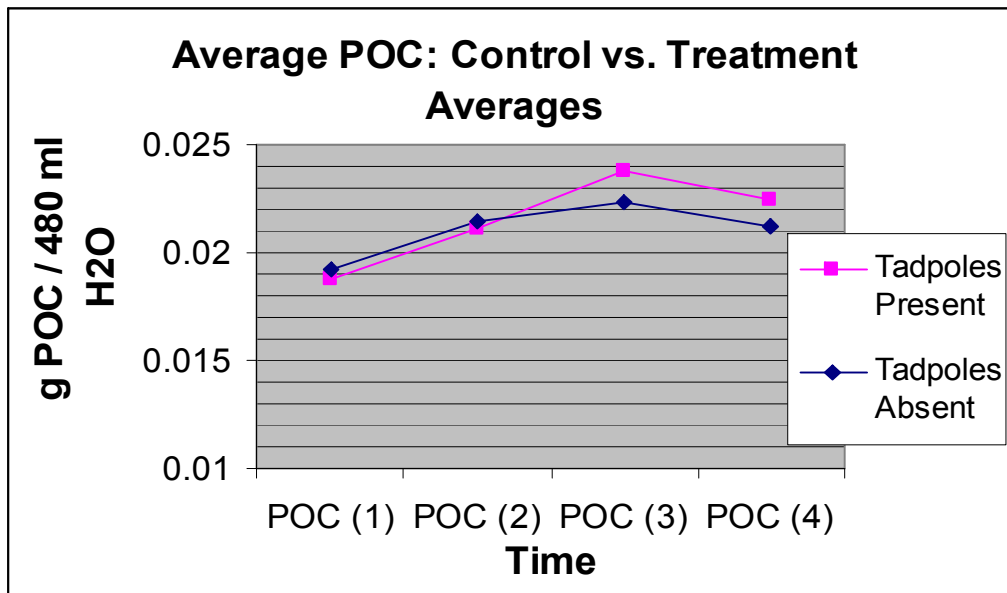


Fig. 2 illustrates the change POC concentration over time contrasting enclosures with tadpoles vs. those without tadpole. These values are presented as an average POC level taken from all treated enclosures and all control enclosures (no tadpoles).

Figure 3. POC by Enclosure

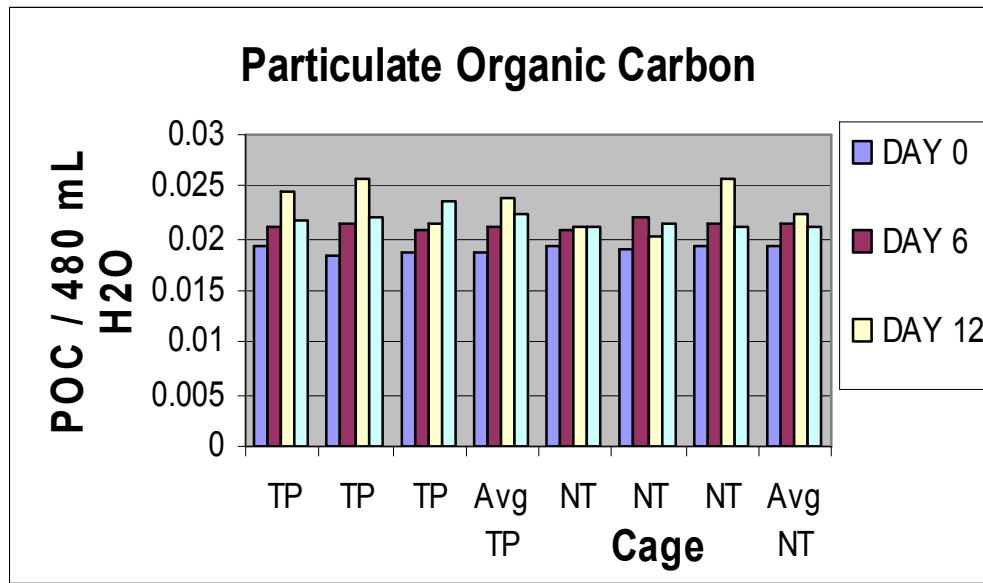


Fig. 3 represents the POC concentration in each enclosure. TP=Tadpoles Present, NT=No Tadpoles. Also illustrated are the averages for these two categories.

Figure 4. Average Particulate Organic Carbon Over Four Sampling Days

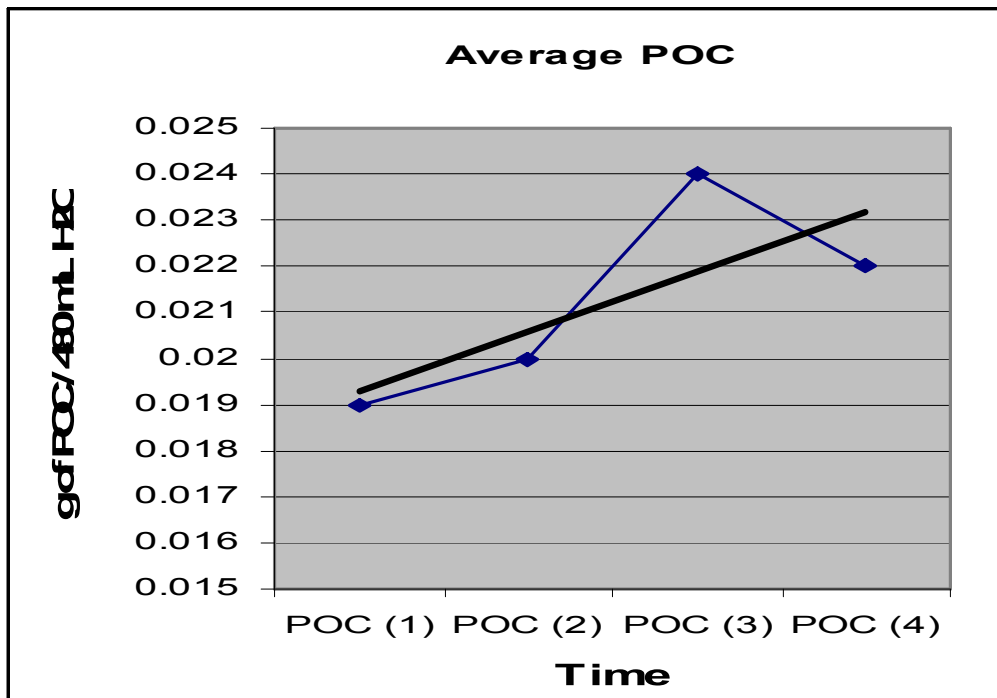


Fig.4 illustrates the average weight in grams of POC filtered from 480 mL samples of water. The average is taken across all six enclosures.

Figure 5. Comparison of Benthic Communities between the Six Individual Enclosures and the Open Water Control

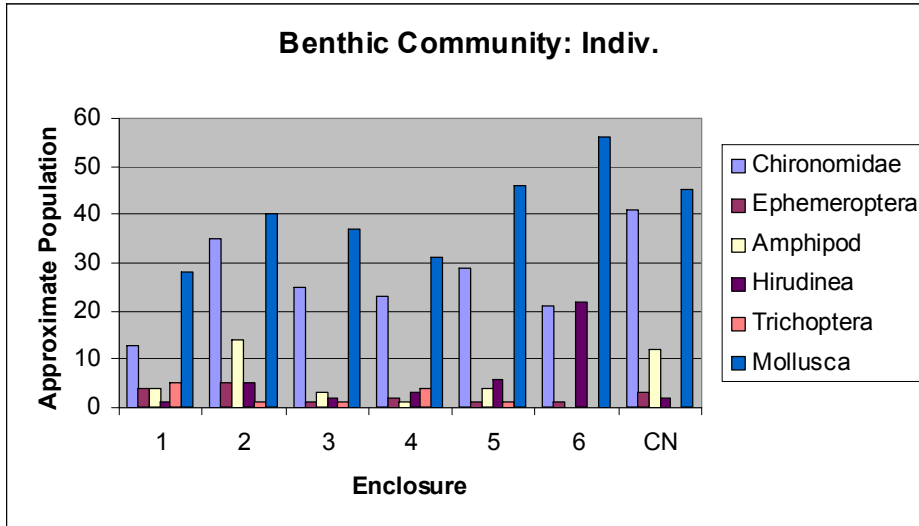


Fig. 5 illustrates the varying populations of 6 different macroinvertebrate genera across the individual enclosures in addition to the control set in the open water.

Figure 6. Benthic Community Populations Comparing Treatments

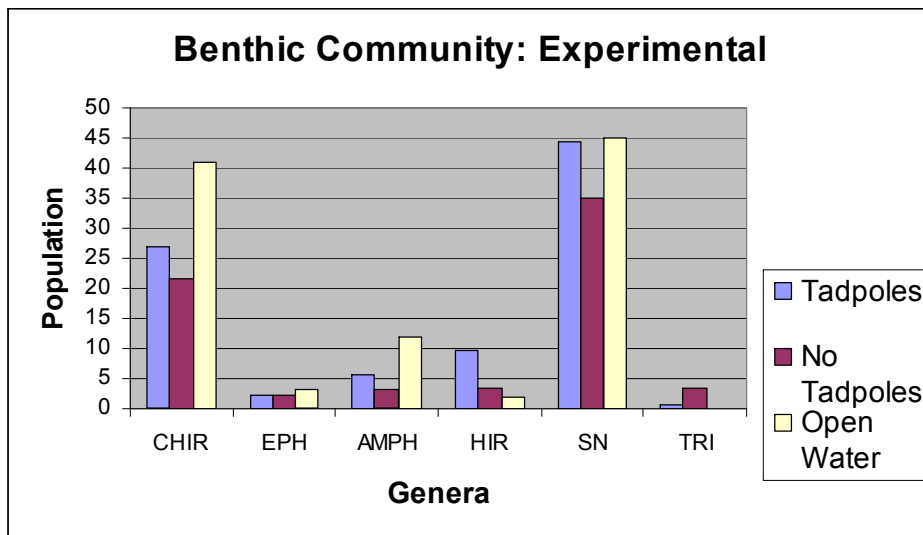


Fig. 6 illustrates a benthic community comparison between the tadpole treatment and the controls (no tadpole enclosures and open water).

Figure 7. Chlorophyll *a* Production in Tenderfoot Creek

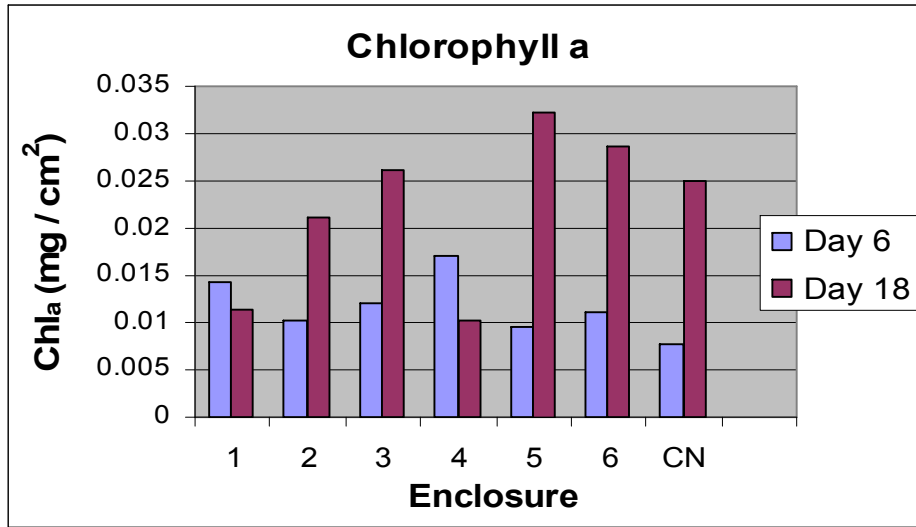


Fig. 7 illustrates the varying production of chlorophyll *a* over time and between enclosures.

Figure 8. Algal Biomass in Tenderfoot Creek

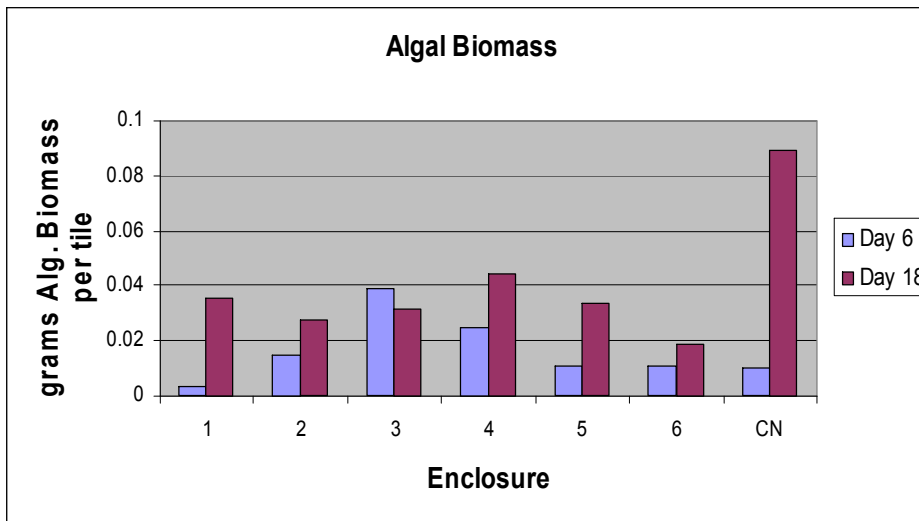


Fig. 8 illustrates the varying growth of algal biomass over time between enclosures.

Figure 9. Chlorophyll *a* Production in the Artificial Streams

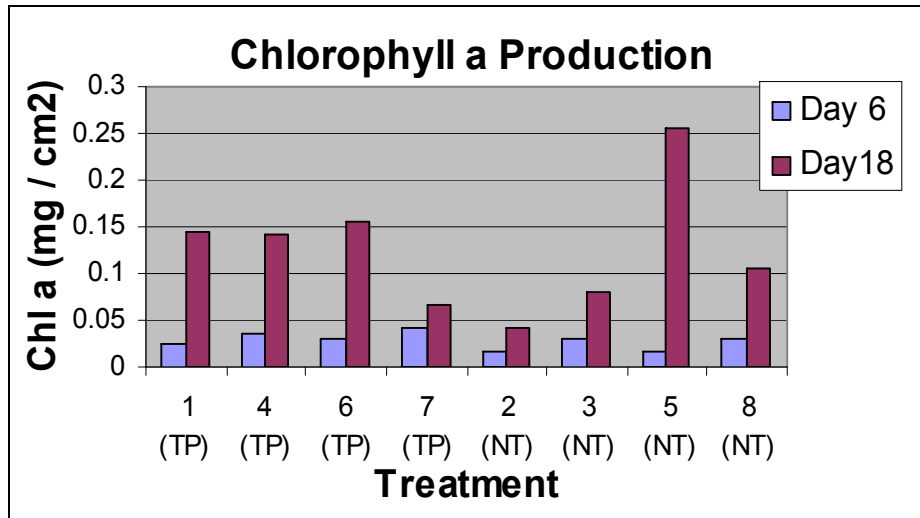


Fig. 9 illustrates the level of chlorophyll *a* production in the artificial streams after 6 and 18 days of the experiment. The data is organized by treatment.

Figure 10. Inorganic Sediment Collected Per Run

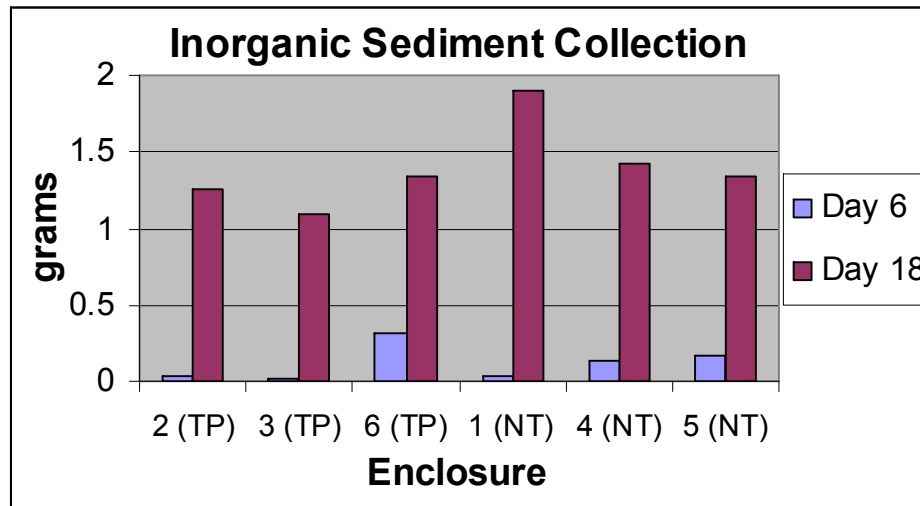


Fig. 10 displays the amount (in grams) of sediment collected in the bricks after 6 and 18 days in each of the enclosure (grouped by treatment).