

Rates of Cannibalism in *Asynarchus* *Nigriculus* based on Sedge Nutrient Content

Abstract

Understanding the dynamic interactions between aquatic invertebrates is critical in forming an accurate illustration of mountain stream ecology. Aquatic detritivores, such as *A. Nigriculus*, form the majority of the biomass in mountain ponds and rivers around Colorado, as they are usually the primary consumers of aquatic detritus. However, certain species of caddisflies in these sub-alpine ponds, such as *Asynarchus nigriculus* larvae, feed on other caddisflies and cannibalize one another as well. This is probably a supplementation for caddisfly larvae to grow as rapidly as they do in these temporary ponds up in the Mexican Cut, at around 11500 feet in subalpine elevation, which is notorious for having a nutritionally poor environment (Fig. 4). In our study, we manipulate the number of nutrients in the water, enhancing their diet, to observe if the additional nutrients cause a shift in *A. nigriculus* larvae feeding behavior. The main nutrients we are studying, and which we are supplementing, are nitrogen and phosphorus since these are what have been studied in previous similar experiments regarding *Asynarchus nigriculus*. Through our experiment, we found the average body size, behavior, and mortality of caddisfly larvae in the function of the treatment (additional Phosphorus, additional Nitrogen, additional Phosphorus, and Nitrogen, or control) they were receiving. It is difficult to predict mortality and behavior between each of the treatments, or if the sedge detritus (or the microorganisms inhabiting their surface) have taken up the nutrients at all.

Keywords: *Asynarchus nigriculus*; caddisfly larvae; cannibalism; Mexican Cut; hydroperiod; limiting nutrients; sedge

Introduction

Caddisflies are the dominant detritivore at the sub-alpine wetland ponds and make up the dominant biomass in these ponds. Caddisflies turn over organic matter, increase the

decomposition of sedge detritus, and release nutrients into the water (Wissinger et. al 2003, Jared et. al 2018) affecting pond land ecosystems. There are many species of caddisfly and individual species can be highly specific; for example, certain species of caddisfly larvae live in ponds or streams and fill a variety of niches within aquatic ecosystems. Many caddisfly species are detritivores and play an important role in wetland, river, and pond ecosystems by consuming sedge detritus by helping turn over organic matter and recycle the nutrients contained in sedge through their excretions. Balik et. al 2018 show that there is a huge variety in the amount of nitrogen and phosphorus excreted by different species of caddisflies. They are the dominant animal biomass in many of the ponds at the Mexican Cut. (Balik et al. 2022); however, certain species can fill roles as omnivores or even carnivores and will feed on other invertebrates, even of the same species. Comparative data from subalpine wetlands in Colorado indicate that larvae of the limnephilidae caddisfly, *Asynarchus nigriculus*, are particularly abundant in temporary ponds, which dry up later in the year as snow melt decreases (Wissinger 2004).

On the other hand, other species such as *Leminiphilus externus* are more abundant in permanent ponds. This is thought to be due to an earlier phenological development (eggs hatch earlier than other caddisfly larvae like *Leminiphilus externus* in the spring, giving them a headstart) and a faster phenology and growth rate. This fast growth rate is potentially untenable in places like subalpine ponds containing sedge with poor nutrient quality without dietary supplementation from more nutrient-rich invertebrates. Thus, we concluded that there must be a limiting nutrient that limits the growth of *Asynarchus Nigriculus*. Experiments have shown (Demi et. al 2018) that *A. nigriculus* prefers sedge with the enriched phosphorus content. Further studies have revealed through behavioral observations that *Asynarchus* larvae are cannibalistic (Wissinger et. al 1996). In contrast to the IGP on *Limnephilus* larvae, *Asynarchus* cannibalism occurs among same-sized larvae and often involves the mobbing of one victim by several conspecifics, and *Asynarchus* cannibalism was not density-dependent and occurred even at low

larval densities [Wissinger et. al 2004](#). This leads us to assume that predatory and cannibalistic behavior could be a way to supplement the diet of the quickly developing larvae of *Asynarchus Nigriculus*, which in turn allows the larvae to metamorphose into flies and escape the temporary ponds before they dry up. It is interesting to note that studies such as [MAINTZ et. al 2006](#) indicate that cannibalism is more prevalent than previously considered, and is a viable way for generalists to obtain necessary limiting nutrients in their diet. [Guil-Gerrero et. al 2017](#) explores how Omega-3 fatty acids found in human brains could be a viable way for early humans to fend off fatty acid deficiency in Europe, as well as referencing stories of royalty of ancient Inca in Peru that would cannibalize others in sacrifices to develop healthily in a nutritionally poor Andes Mountain environment.

Purpose of Research

This research aims to find out what drives cannibalism in *Asynarchus nigriculus* (*A. nigriculus*). This cannibalism could be due to the previously discussed nutritionally poor diet of *A. Nigriculus* at these sub-alpine ponds at the Mexican Cut. It could also explain the quicker morphological development as *A. Nigriculus* is in a race against time to leave these temporary ponds as quickly as possible before desiccation, killing the larvae. The main limiting nutrients in sedge for invertebrate morphological development are nitrogen (for the synthesis of proteins) and phosphorus (major component of nucleic acids, RNA and DNA, and cell membranes, phospholipid fatty acids). These limiting nutrients could be key to understanding why cannibalism is prevalent in *A. Nigriculus* and not in other caddisfly species as other animals (in this case invertebrates) contain an abundance of both nitrogen and phosphorus, compared to plant detritus. A paper by [Jared et. al](#) found that larvae of *A. nigriculus* preferred sedges that contained additional phosphorus. A more detailed question would ask whether cannibalism rates in *A. Nigriculus* is affected mostly by nitrogen limitation, phosphorus limitation, or a

co-limitation of both phosphorus and nitrogen and whether these affect cannibalism rates among *A. Nigriculus*.

We hypothesize that cannibalism rates in *A. Nigriculus* is due to a co-limitation of both nitrogen and phosphorus. We further propose that if the larval diets are artificially supplemented with both nitrogen and phosphorus then cannibalism rates will fall mostly among the *A. Nigriculus* feeding on that sedge, with smaller drops in cannibalism among caddisflies feeding exclusively on either phosphorus or nitrogen enriched sedge.

Methods

Experiment Setup:

Caddisfly Sites

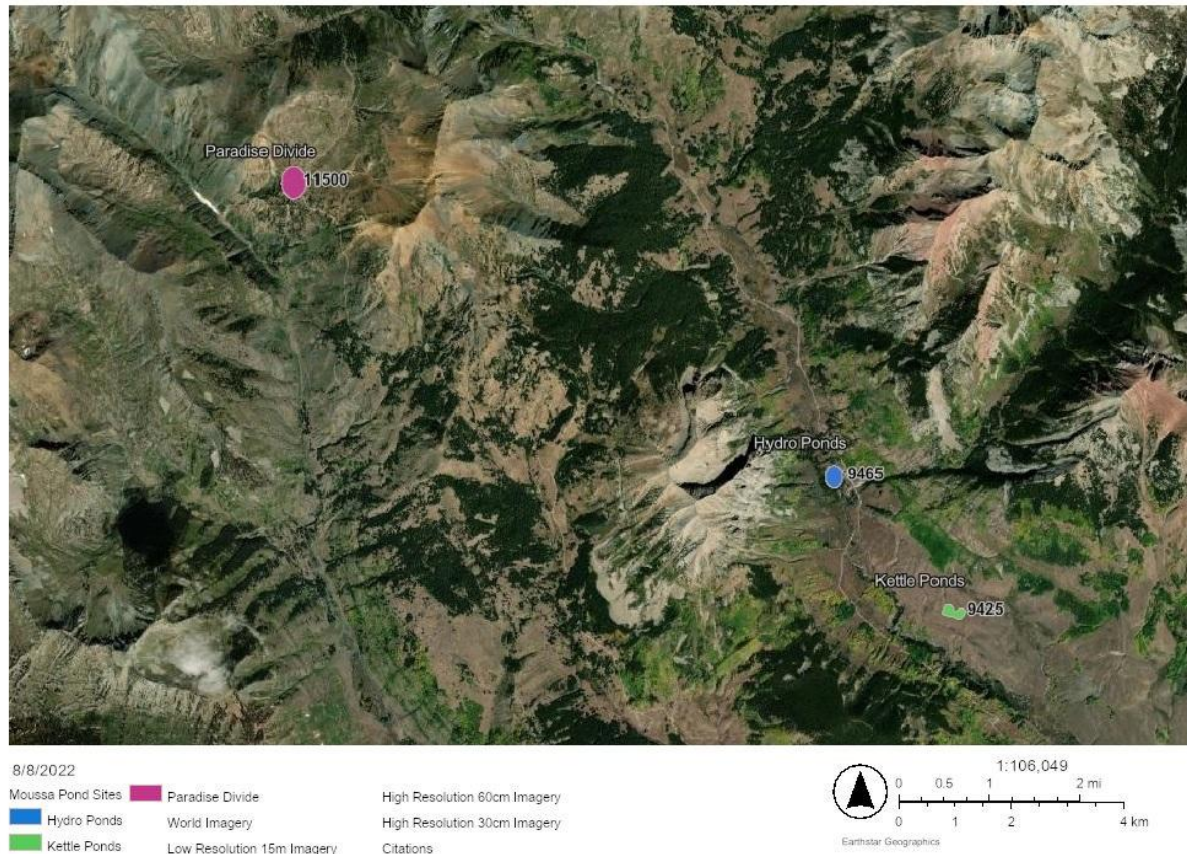


Figure 1: Geospatial map made using ArcGIS to find three sites: a) in green, and the South of the map is the place where we collected sedge detritus which we later used in the experiment as food for the developing larvae. b) in blue is the area where we set up the experiment in the Gothic townsite at the 'Hydroponds' by the East River. c) In pink is one of the few unnamed ponds where *Asynarchus* larvae were collected at Paradise Divide, at a considerable 11500 feet in elevation.

A. *Nigriculus* larvae were collected from an unnamed pond at Paradise Divide around 11500ft of elevation (Fig. 1) on June 20th, 2022 they were all around the third (of fifth) instar as they have a particularly fast phenological development. Sedge was collected from Marsh kettle pond (Fig. 1) near Gothic (on kettle ponds road) on June 20th, 2022, and placed in four tubs (incubation tanks) with water on the same day (Fig. 1). Around 2.5kg of wet sedge detritus was measured using an electronic balance, placed into the incubation tanks, and then added with

water from the East River. On the same day, around 13 grams of ammonium nitrate were placed in Tub 2 (nitrogen only enriched tub) and Tub 4 (Nitrogen and phosphorus enriched tub) following a 50% bioavailable nitrogen increase of the natural amount of nitrogen in the sedge (Fig. 9), based on data by B. W. Taylor. Around 1 gram of sodium phosphate was added to Tub 3 (Phosphorus only enriched tub) and Tub 4 following a 50% phosphorus increase of the natural amount of phosphorus in the sedge (Fig. 9). The tubs were allowed to sit for 3 days so the microorganisms living on the sedge detritus could uptake the added nutrients and incorporate them into their cellular makeup. The amount of nitrogen and phosphorus added was a calculated 50% increase from the natural amounts of nutrients in the sedge. We concluded that 50% was a good benchmark as it is a significant increase in nutrients without adding too much (as in able to be absorbed by the microbes) and not being an unreasonably large amount of phosphorus and nitrogen that could enter natural ecosystems as runoff and nitrogen deposition become an increasing threat.

On Friday, June 24th, 25 grams of squeezed wet sedge was placed into a container (microcosms) about 0.05m^2 following natural densities of sedge detritus at the Mexican Cut (Balik et al. 2022), and 15 caddisflies were placed in the container following natural densities of *Asynachus nigriculus* in these temporary ponds with a density of 300-400 per square meter based on Wissinger et al 1996. The equipment used was four large tubs, roughly 0.8 square meters each, to contain the sedge and store them as fungi and other microorganisms inhabiting the surface of the sedge incorporate the additional nutrients and provide the resource for additional nutrients for *A. Nigriculus*. Each incubation tank (large tub) contains each treatment (control, +P, +N, Control, and N+P) and is placed on a table, covered by a tent the East River (Fig. 2). We used 40 smaller plastic containers, microcosms with a unique number, about 0.05 square meters, each containing 25 grams of sedge and the initial 15 caddisflies and an adequate amount of water, with each treatment containing 10 runs each (Fig. 3). The

experimental microcosms were covered with nylon screening to prevent bugs from entering the treatments and possibly altering the experiment results. There were 2 tables used to elevate these microcosms off of the ground and a tent was used as a weather port to prevent wild fluctuations in water temperature from sun exposure, and partially protect from wind, and from further insects and debris that could harm the experiment by adding unnecessary nutrients. Two remote temperature loggers were placed in 2 different microcosms during the last week of experimentation to check the temperature fluctuations within the microcosms throughout the day. It measured the temperature every half hour starting at noon July 8th and ending at noon July 12th.



Figure 2 (left) and Figure 3 (right): Figure 2 are the 4 tubs where collected sedge detritus were stored until further use. These tubs each contain a different treatment of nutrients. From right to left: Tub N, Tub C, Tub P, Tub NP. Tub 3 on the right are four microcosms of about 0.05 m² where 25 grams of sedge were kept. In this example, we show four of the total forty replicates, each containing a different sedge treatment.

The experiment began on Monday, June 28th, and was terminated when half of the microcosms had significant or total mortality unrelated to cannibalism, specifically on July 12th, and occurred a little over two weeks after the experiment and observations first began. The remaining caddisfly larvae were picked up and dried in an oven, and their dry weights were measured from each of the forty microcosms. The average weight of the caddisflies from each

tub has been taken and was used for analysis. By the end of the experiment, we had data on behavior, mortality rates, average weights of caddisflies in each treatment, and temperature. An ANOVA will be used to test the difference in means between mortality and weight, sedge and treatment, mortality and treatment, behavior and mortality, weight and number of caddisflies in each tub, mortality over time, and behavior over time.

We plan on doing a nutrient sedge detritus nutrient analysis of the detritus collected at the end of the experiment to check whether the detritus (the microorganisms inhabiting the detritus) had incorporated the additional nutrients and to what extent. The analysis will not be completed until after this paper will be turned in to RMBL.

Mortality and Behavior Observations

Sites are visited usually from Monday to Friday from 9 am-4 pm unless otherwise. While at my site I have 2 main objectives: One is to count each of the *A. Nigriculus* in half (20) of the microcosms, containing an equal amount of treatments, to determine how many have been killed and/or cannibalized. This will be done five times a week, with each half being observed every other day so we can have an accurate model of when in the experiment, and in what treatments are *A. Nigriculus* dying over time. The other observation is to measure the behavior of caddisfly in half (20) of the containers (microcosms), and this will be done 4 times a week.

Based on Scott Wissinger's 1996 method of caddisfly behavior analysis, I will choose a tub at random using a random number generator, and pick a caddisfly at random from each microcosm and observe the behavior and determine, in seconds, whether it was foraging, acting aggressive, doing nothing, or a combination within a 5-minute time frame. If cannibalism were causing the mortality, we would expect to see higher mortality in microcosms containing higher aggressiveness and less mortality in chambers containing less aggressive behavior and more foraging and no movement. In addition to this, an experiment set up in the beaver ponds set

about 200m from my experiment uses tea cups with individual *A. Nigriculus*, collected from the same location, were placed in the ponds to determine the natural mortality of *A. Nigriculus* larvae and to compare this with the mortality findings in my experiment set up.

I returned the incubation tanks to their original location. Caddisflies will be dried and weighed at the end of the experiment to determine the average mass of larva in each of the microcosms and compare between treatments. Temperature loggers will be picked up and temperature data analyzed.

Site pictures (from GIS)

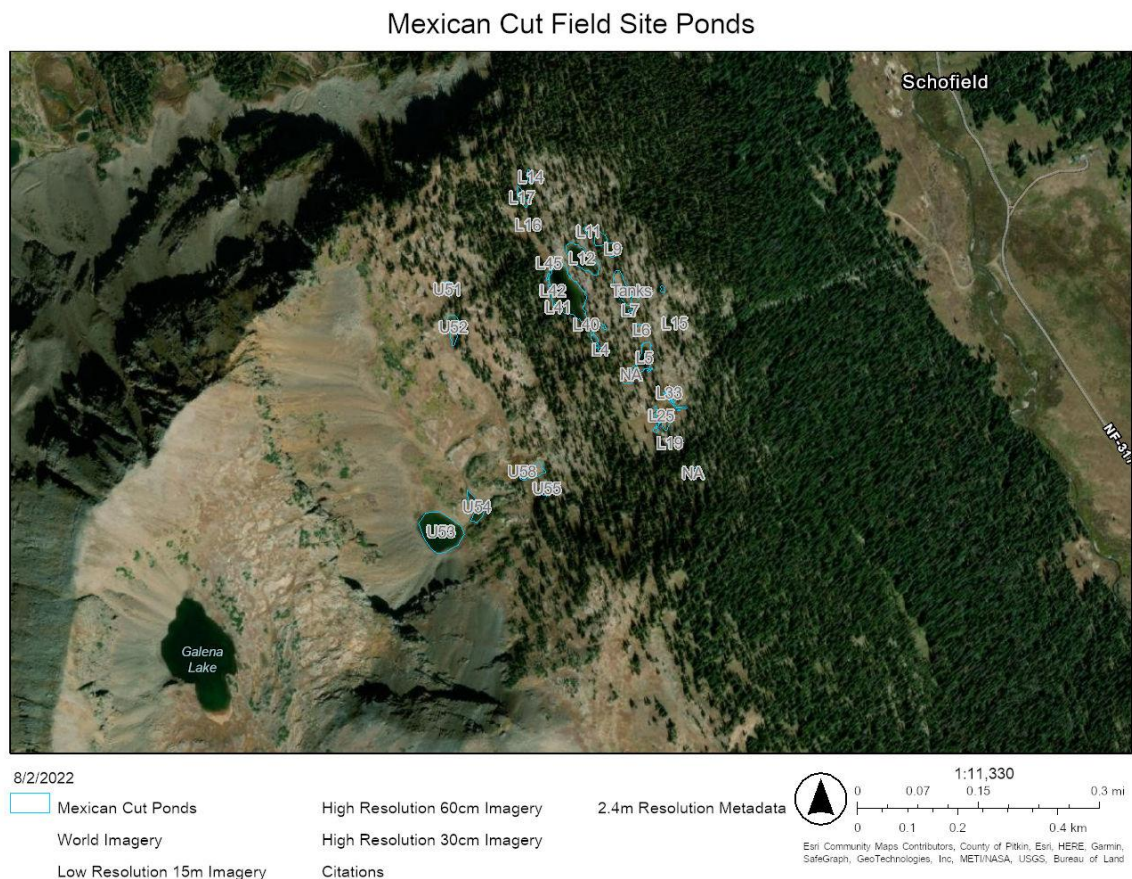


Figure 4: Map site of the Mexican Cut Ponds, at an altitude of just shy of 12,000 feet, these sub-alpine ponds are an important habitat for one of the many caddisfly species inhabiting the ponds.

Data Analysis

Data will be collected, placed in excel, and analyzed using R script. An Analysis of Variance test (ANOVA) is used to analyze the difference in means in mortality between sedge detritus treatments, behavior analysis, and average larval weight between treatments as well.

Results

Mortality Dates

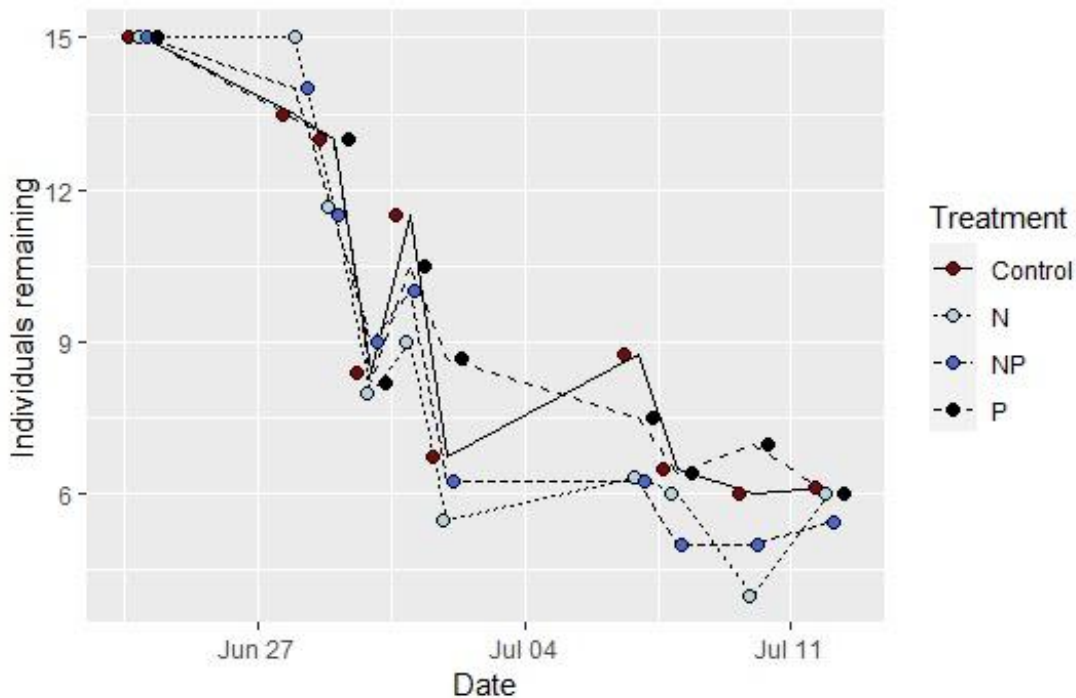


Figure 5: Graph of the average mortality of caddisflies in each of the treatments over time. We observe an overall trend from an initial fifteen A. Nigriculus larvae per microcosm at the beginning of the experiment (which we placed), down to 4-6 for each of the treatments. The overall trend could be analyzed linearly or logarithmically, however, we find no significant difference in mortality between each of the treatments. C is the control treatment, N is the nitrogen-only enriched, NP is the nitrogen and phosphorus enriched treatment, and P is the phosphorus-only enriched treatment.

At the beginning of the experiment, each of the 40 microcosms have 15 caddisflies each; however as time went on we see a sharp increase in mortality that begins to taper off towards the end of the experiment, with an average of only 4-6 caddisflies in each of the treatments.

(Fig. 5) Running an ANOVA, there was no significant difference in mortality per treatment, with a P-value of $0.736 >> 0.05$.

Behavior Data

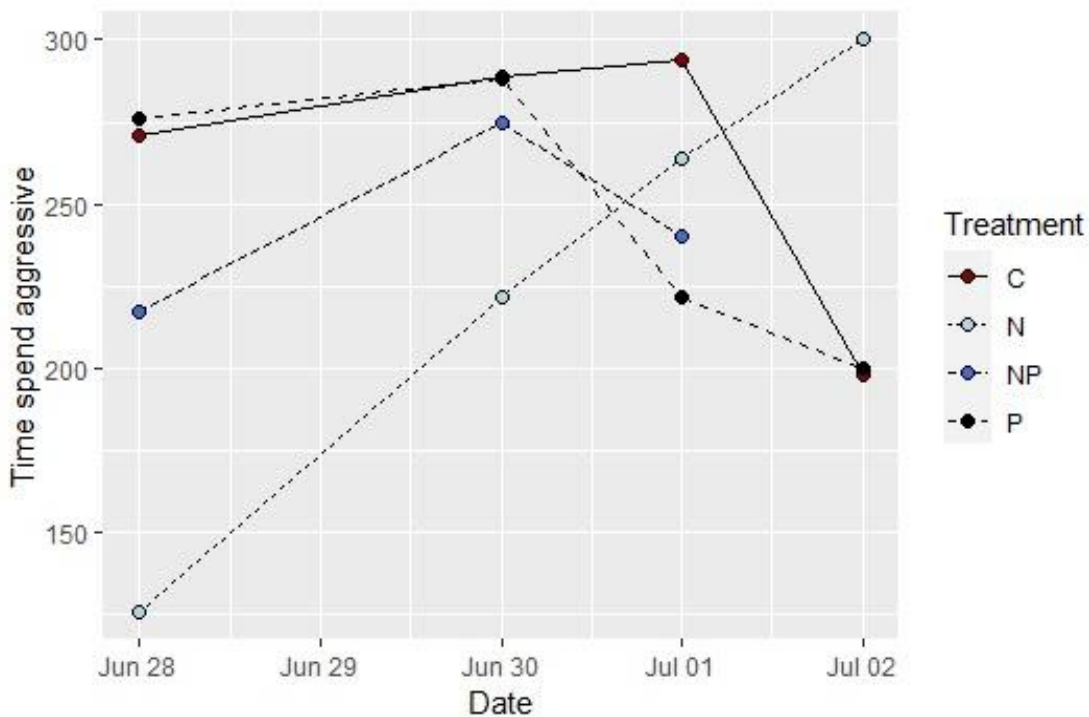


Figure 6: Graph of the average time *A. nigriculus* larvae spent aggressively for each of the treatments. Between each of the behavior analyses, we find no difference in the amount of time spent aggressively for each of the treatments we place larvae in.

Time spent aggressive per treatment was added up since there was only an average of 12.5 seconds of aggressive behavior within the 300 seconds of observation per microcosm. In that case, we still do not find significant differences in aggressive behavior based on treatment, with an average of ~250 seconds of aggressive behavior per treatment (Fig. 6). Running an ANOVA on treatment effects on behavior we find no significant effect ($p\text{-value } 0.66553 >> 0.05$)

Weight Data

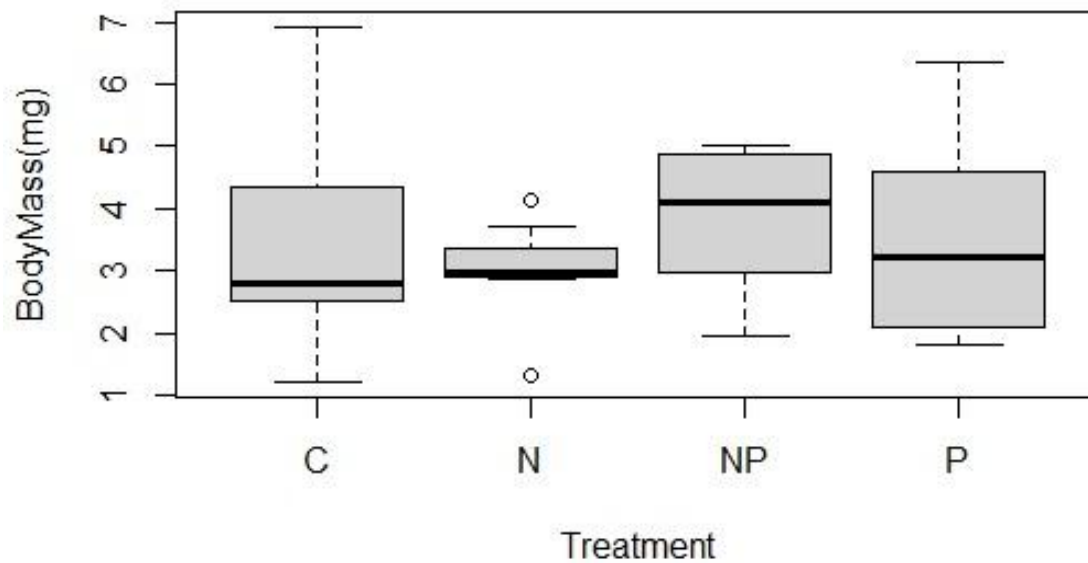


Figure 7: Box plot with a 95% regression line showing the average body mass of *A. Nigriculus* larvae per treatment. The dots represent outliers. An ANOVA did not find any difference in body mass by treatment, despite the dry weight of the caddisfly larvae varying from 2.8mg to 4mg.

To measure the average body mass of larvae, we pulled the larvae out of their case, dried them, and weighed them. Average body mass per microcosm was taken and then average across all treatments and placed in a 95% box plot. The average weight per treatment was between 2.8mg and 4mg (Fig. 7). The 95 (Fig. 7) shows that there is no significant difference in mass per treatment, using an ANOVA we find no significant difference in weight per treatment as well ($p = 0.825 > 0.05$)

Temperature Data

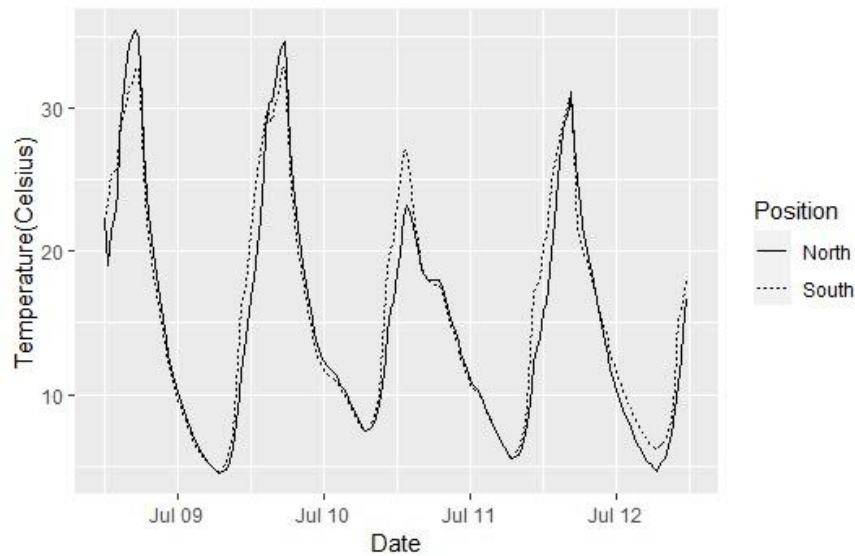


Figure 8: Two temperature logging probes, one placed on the North side of my experiment setup, the other towards the South, both show extreme variation in temperature between day and night.

Two temperature logging probes, one placed on the North side of my experiment, the other towards the South, both show extreme variation in temperature between day and night, we find temperature extremes as low as 4C at night and over 35C during the day in both probes (Fig. 8).

Discussion/Further Research

Expectations:

We expected to see a positive correlation between P limitation and N limitation, meaning that there will be higher rates of cannibalism in the control treatment with limited amounts of nitrogen and phosphorus, and the least amount of cannibalism in the treatment enriched with both phosphorus and nitrogen. Despite this, we found no significant mortality decrease in any of the treatments containing increased nitrogen and phosphorus. Another expectation is that aggressive behavior would be relatively high in microcosms containing no additional nutrients, while those with supplemented nitrogen and phosphorus detritus would become less

aggressive. Average larval mass was expected to be higher in nutrient-treated microcosms, especially with those with increased nitrogen, while larval weight would be lower in the control microcosms, as these nutrients are key for fast caddisfly growth and development and could act as limiting nutrients.

Mortality Data

There was indeed higher mortality early on in the experiment since there was a higher number of caddisflies per microcosm (container), allowing for more interactions between them. As caddisfly larvae numbers dropped, we find fewer observations of aggressive behavior due to fewer possible interactions between larvae in the microcosm. This relationship follows a standard logarithmic curve, which is predicted. However, we find it unexpected to see no difference in mortality per nutrient treatment. This could be explained by many factors. A concern with the experiment is that there was not enough time to view significant differences in mortality between treatments and that two weeks is not enough time to allow for significant differences in mortality. This probably isn't the case since there was enough time for the average number of *A. Nigriculus* larvae per microcosm dropped from 15 to around 6. A more pressing concern is that there was a considerable surge in mortality throughout the experiment due to heat and/or low oxygen levels. This heat and oxygen stress would lead to unaccounted mortality in the experiment that is not observed in the wild. An added effect of this would be that oxygen-induced stress could alter the behavior of caddisflies to become slower, and ineffective which would not be a good replicate of caddisfly behavior in the wild.

Behavior Data

Analyzing foraging, aggressive, and 'No Activity', we don't find many interesting observations in the latter two, but there is something to witness in the aggressive behavior of *A.*

nigriculus larvae. The behavior of *A. nigriculus* larvae was similar to that described in Scott Wissinger's 1999 paper, but many exceptions were evident as well. During the beginning of the experiment, we found that since all of the caddisfly larvae were of similar instar, many caddisflies would mob a weaker or smaller conspecific to overcome the overbearing shell. However as larval densities decreased, and as the number of caddisflies per microcosm dropped, there were usually a dominant 1-4 caddisflies in the microcosm that was significantly larger than others, that would overpower and consume any of the smaller larvae they encountered. It is evident, similar to that discovered in Wissinger's 2003 paper, that the larvae of *A. nigriculus* would regularly attack and consume other invertebrates when they are larger than their opponent, even of the same species. Another consequence of the lower larval densities is that aggressiveness does not accurately represent the average aggressiveness of caddisfly larvae in the microcosm, since aggressiveness is a function of the number of possible interactions between caddisflies, and as there are fewer caddisflies around, there is less opportunity for caddisflies to witness an aggressive interaction. Therefore, it might be more fitting to have a plot that represents the number of caddisflies in each of the microcosms in Figure 7. In this event, we might see a relationship between caddisfly aggressiveness increase over time, but not per treatment. This makes sense since, as observed earlier, there were larger, more active larvae roaming the microcosm which were more aggressive than others and than themselves earlier in the experiment.

We run into the same issues when trying to analyze the behavior data compared with that of the mortality. There might have been a temperature effect causing the caddisflies to not act as aggressive or forage as much, and to instead remain inactive. Unfortunately, there isn't a study except for Wissinger 2003 to compare *A. nigriculus* behavior.

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Sedge Analysis

Another major concern with our experiment is that nutrient enrichment of sedge detritus did not work according to plan, and there were zero, or negligible amounts of additional nutrients supplemented in the larvae diets to observe different behaviors within each treatment. As stated earlier, there was only a 50% increase in phosphorus and nitrogen within each of the treated incubation tanks. Many flaws could have occurred: the most plausible being that the microorganisms inhabiting the sedge did not uptake the nutrients in the water, and therefore *A. Nigriculus* larvae within each of the treatments did not have the additional nutrients supplemented by their diet, and we find no significant deviation in their behavior from that of the control. Another concern is that a 50% phosphorus and/or nitrogen increase is not enough for *A. Nigriculus* larvae to significantly alter their behavior. We find this plausible, however, in an environment where getting as many nutrients as possible is imperative for fast phenological development and survival, a 50% increase should be significant enough for our experiment.

After the end of the experiment, we collected samples of sedge from each treatment and will perform an analysis in the coming months to check whether the microorganisms inhabiting the sedge detritus had uptaken the nutrients, and to what extent. A future rerun of this experiment could have us experimenting with an increased amount of nitrogen and phosphorus, and performing nutrient sedge analysis at the beginning and end of the experiment to look at the nutrient content of sedge over time.

Temperature Data

As discussed in the results section earlier, we found large fluctuations in temperature throughout the day and night cycle (5C-35C), but not much variation in temperature between the two microcosms. The minuscule variation between the two microcosms consequently leaves us

in the dark on whether the large temperature fluctuations affected *A. nigriculus* larvae behavior and cannibalism rates. One consequence of this large temperature difference is oxygen stress, and this is what we believe ended up causing significant or total mortality in some of the microcosms throughout this experiment as cooler, more shaded microcosms ended up surviving during warm, bright days, while those facing the sun in the afternoon ended up with significant mortality. Microcosms containing significant mortality were not further studied for behavior and were not counted towards mortality in our analysis.

Overall, there are still mixed results on whether additional nitrogen and phosphorus do end up affecting *A. Nigriculus* larvae at all. Despite no difference in activity, mortality, or average weight per treatment, as shown above, *A. Nigriculus* larvae that acted extremely aggressively, and are expected to have eaten many of their conspecifics, did end up having increased mass compared to those that weren't. Either the sedge detritus was not properly enriched with nitrogen, phosphorus, or both, or there may be another limiting nutrient found in *A. nigriculus* larvae that I did not account for.

Temperature and Oxygen (Future research)

Further studies are proposed for exploring oxygen limitation in sub-alpine pond invertebrate communities. One problem with our experiment is oxygen and/or temperature-induced stress caused by warming water. Oxygen limitation is further exaggerated by a thinning atmosphere with an increase in elevation and lower partial oxygen pressures.

Crojmans et. al 2021. This may put a stop to the so-called “elevation escalator” as species move up in elevation to escape to cooler areas. We'd be very excited to do further research on the thermal threshold and oxygen solubility of invertebrates in sub-alpine ecosystems, and turn our experiment's detriment into inspiration.

Literature Cited

References/ Citations:

- 1) Balik, J. A., Taylor, B. W., Washko, S. E., & Wissinger, S. A. (2018). High interspecific variation in nutrient excretion within a guild of closely related caddisfly species. *Ecosphere*, 9(5). <https://doi.org/10.1002/ecs2.2205>
- 2) Palavesam, A. (2005). *A Method for the Estimation of Detritus Energy Generation in Aquatic Habitats*, 5(49), 49–52.
- 3) Wissinger, S. A., Brown, W. S., & Jannot, J. E. (2003). Caddisfly life histories along permanence gradients in high-altitude wetlands in Colorado (U.S.A.). *Freshwater Biology*, 48(2), 255–270. <https://doi.org/10.1046/j.1365-2427.2003.00997.x>
- 4) Wissinger, S. A., Brown, W. S., & Jannot, J. E. (2003). Caddisfly life histories along permanence gradients in high-altitude wetlands in Colorado (U.S.A.). *Freshwater Biology*, 48(2), 255–270. <https://doi.org/10.1046/j.1365-2427.2003.00997.x>
- 5) Wissinger, S. A., Sparks, G. B., Rouse, G. L., Brown, W. S., & Steltzer, H. (1996). Intraguild predation and cannibalism among larvae of detritivorous caddisflies in subalpine wetlands. *Ecology*, 77(8), 2421–2430. <https://doi.org/10.2307/2265743>
- 6) Wissinger, S. A., Whiteman, H. H., Sparks, G. B., Rouse, G. L., & Brown, W. S. (1999). Foraging trade-offs along a predator-permanence gradient in subalpine wetlands. *Ecology*, 80(6), 2102. <https://doi.org/10.2307/176681>
- 7) Wissinger, S., Steinmetz, J., Alexander, J. S., & Brown, W. (2004). Larval cannibalism, time constraints, and adult fitness in caddisflies that inhabit temporary wetlands. *Oecologia*, 138(1), 39–47. <https://doi.org/10.1007/s00442-003-1397-y>

- 8) Demi, L. M. (n.d.). Food web phenology in a changing climate: a trophic dynamics and bioenergetics approach. Raleigh; North Carolina State University.
- 9) Demi, L. M., Hughes, D., & Taylor, B. W. (2022). Characterizing the role of phosphorus availability and periphytic algae in the food choice and performance of detritivorous caddisflies (Trichoptera: Limnephilidae). *Freshwater Science*, 41(1), 18–32.
<https://doi.org/10.1086/717953>
- 10) West, D. C., Balik, J. A., Owens, M., & Taylor, B. W. (2020). *Didymosphenia geminata* blooms are not exclusively driven by low phosphorus under experimental conditions. *Hydrobiologia*, 847(4), 1057–1069. <https://doi.org/10.1007/s10750-019-04168-y>
- 11) Balik, J. A., Jameson, E. E., Wissinger, S. A., Whiteman, H. H., & Taylor, B. W. (2021). Animal-driven nutrient supply declines relative to ecosystem nutrient demand along a pond hydroperiod gradient. *Ecosystems*, 25(3), 678–696.
<https://doi.org/10.1007/s10021-021-00679-9>
- 12) Croijmans, L., De Jong, J. F., & Prins, H. H. (2021). Oxygen is a better predictor of macroinvertebrate richness than temperature—A systematic review. *Environmental Research Letters*, 16(2), 023002. <https://doi.org/10.1088/1748-9326/ab9b42>
- 13) Guil-Guerrero, J. L. (2017). Evidence for chronic omega-3 fatty acids and ascorbic acid deficiency in Palaeolithic hominins in Europe at the emergence of cannibalism. *Quaternary Science Reviews*, 157, 176–187.
<https://doi.org/10.1016/j.quascirev.2016.12.016>

Tables and Figures

Asynarchus nigriscus											
Diet item	Proportion of die	%N of dry mass	%P of dry mass	Total N	Total P						
FPOM	0.15	2.5	0.075	0.00375	0.0001125						
Sedge (CPOM)	0.7	2	0.08	1.4	0.056						
Algae	0.05	5	0.1	0.25	0.005						
Animals	0.1	10	0.15	1	0.015						
Total	1			2.65375	0.0761125						

Tub	Treatment	Wet Mass (g)	Dry Mass (g)	% P of dry mass	% N of Dry Mass	Total P (g)	Total N (g)	Added N	Added P	Added Na2HPO4	Added NH4NO3
1	added N	2409.68	481.936	0.086234	1.92	0.4155926902	9.2531712	4.6265856	0		13.2195714
2	control	2683.93	536.786	0.086234	1.92	0.4628920392	10.3062912	0	0		
3	added P	3020.84	604.168	0.086234	1.92	0.5209982331	11.6000256	0	0.2604991166	1.193854796	
4	added N + P	2467.3	493.46	0.086234	1.92	0.4255302964	9.474432	4.737216	0.2127651482	0.9750923382	13.53567632
		481.936	0.129351	2.88	0.6233890354	13.8797568					
		536.786	0.129351	2.88	0.6943380589	15.4594368					
		604.168	0.129351	2.88	0.7814973497	17.4000384					
		493.46	0.129351	2.88	0.6382954446	14.211648					
										0.086234	From Demi et al. 2022
										P	N
										tub 3 added	1.1929
										tub 4 added	0.9611
										tub 1 added	13.551
											13.238

Na2HPO4	molar mass	# of atoms	g total								
Na	22.99	g/mol Na	2	45.98	32.39	%Na by mass					
H	1.008	g/mol H	1	1.008	0.71	%H by mass					
P	30.974	g/mol P	1	30.974	21.82	%P by mass					
O	15.999	g/mol O	4	63.996	45.08	%O by mass					
				141.958							
NH4NO3	molar mass	# of atoms	g total								
N	14.0067	g/mol N	2	28.0134	34.99820095	% N by mass					
H	1.008	g/mol H	4	4.032	5.037330215	% H by mass					
O	15.999	g/mol O	3	47.997	59.96446883	% O by mass					
				80.0424							

Figure 9: Excel sheet showing the calculations used to determine the amount of nutrients to add to each container.

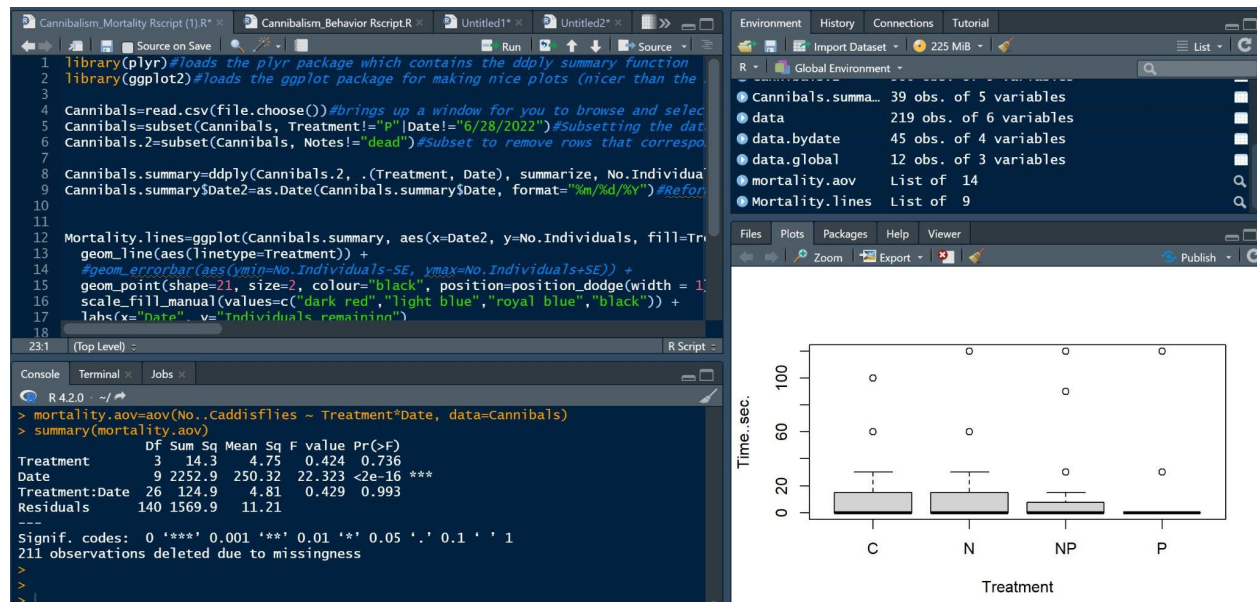


Figure 10: Example of ANOVA calculation and script used to analyze data collected from my experiment

Asyntherus nigricollis - Cannibalism Experiment
RMBL - 2022

Start Date: _____ End Date: _____

Sedge	Tub	Treatment	Observation Date: 10/26/22		Observation Date: 10/27/22		Observation Date: 10/28/22		Observation Date: 10/29/22	
			Time	Micro	Time	Micro	Time	Micro	Time	Micro
1	1N	746	0	0	0	0	0	0	0	0
2	1N	746	0	0	0	0	0	0	0	0
3	1N	746	0	0	0	0	0	0	0	0
4	1N	746	0	0	0	0	0	0	0	0
5	1N	746	0	0	0	0	0	0	0	0
6	2 Control	746	0	0	0	0	0	0	0	0
7	2 Control	746	0	0	0	0	0	0	0	0
8	2 Control	746	0	0	0	0	0	0	0	0
9	2 Control	746	0	0	0	0	0	0	0	0
10	2 Control	746	0	0	0	0	0	0	0	0
11	3P	746	0	0	0	0	0	0	0	0
12	3P	746	0	0	0	0	0	0	0	0
13	3P	746	0	0	0	0	0	0	0	0
14	3P	746	0	0	0	0	0	0	0	0
15	3P	746	0	0	0	0	0	0	0	0
16	4NP	746	0	0	0	0	0	0	0	0
17	4NP	746	0	0	0	0	0	0	0	0
18	4NP	746	0	0	0	0	0	0	0	0
19	4NP	746	0	0	0	0	0	0	0	0
20	4NP	746	0	0	0	0	0	0	0	0
21	4NP	746	0	0	0	0	0	0	0	0
22	4NP	746	0	0	0	0	0	0	0	0
23	4NP	746	0	0	0	0	0	0	0	0
24	4NP	746	0	0	0	0	0	0	0	0
25	4NP	746	0	0	0	0	0	0	0	0
26	4NP	746	0	0	0	0	0	0	0	0
27	4NP	746	0	0	0	0	0	0	0	0
28	4NP	746	0	0	0	0	0	0	0	0
29	4NP	746	0	0	0	0	0	0	0	0
30	4NP	746	0	0	0	0	0	0	0	0
31	4NP	746	0	0	0	0	0	0	0	0
32	4NP	746	0	0	0	0	0	0	0	0
33	4NP	746	0	0	0	0	0	0	0	0
34	4NP	746	0	0	0	0	0	0	0	0
35	4NP	746	0	0	0	0	0	0	0	0
36	4NP	746	0	0	0	0	0	0	0	0
37	4NP	746	0	0	0	0	0	0	0	0
38	4NP	746	0	0	0	0	0	0	0	0
39	4NP	746	0	0	0	0	0	0	0	0
40	4NP	746	0	0	0	0	0	0	0	0
41	4NP	746	0	0	0	0	0	0	0	0
42	4NP	746	0	0	0	0	0	0	0	0
43	4NP	746	0	0	0	0	0	0	0	0
44	4NP	746	0	0	0	0	0	0	0	0
45	4NP	746	0	0	0	0	0	0	0	0
46	4NP	746	0	0	0	0	0	0	0	0
47	4NP	746	0	0	0	0	0	0	0	0
48	4NP	746	0	0	0	0	0	0	0	0
49	4NP	746	0	0	0	0	0	0	0	0
50	4NP	746	0	0	0	0	0	0	0	0
51	4NP	746	0	0	0	0	0	0	0	0
52	4NP	746	0	0	0	0	0	0	0	0
53	4NP	746	0	0	0	0	0	0	0	0
54	4NP	746	0	0	0	0	0	0	0	0
55	4NP	746	0	0	0	0	0	0	0	0
56	4NP	746	0	0	0	0	0	0	0	0
57	4NP	746	0	0	0	0	0	0	0	0
58	4NP	746	0	0	0	0	0	0	0	0
59	4NP	746	0	0	0	0	0	0	0	0
60	4NP	746	0	0	0	0	0	0	0	0
61	4NP	746	0	0	0	0	0	0	0	0
62	4NP	746	0	0	0	0	0	0	0	0
63	4NP	746	0	0	0	0	0	0	0	0
64	4NP	746	0	0	0	0	0	0	0	0
65	4NP	746	0	0	0	0	0	0	0	0
66	4NP	746	0	0	0	0	0	0	0	0
67	4NP	746	0	0	0	0	0	0	0	0
68	4NP	746	0	0	0	0	0	0	0	0
69	4NP	746	0	0	0	0	0	0	0	0
70	4NP	746	0	0	0	0	0	0	0	0
71	4NP	746	0	0	0	0	0	0	0	0
72	4NP	746	0	0	0	0	0	0	0	0
73	4NP	746	0	0	0	0	0	0	0	0
74	4NP	746	0	0	0	0	0	0	0	0
75	4NP	746	0	0	0	0	0	0	0	0
76	4NP	746	0	0	0	0	0	0	0	0
77	4NP	746	0	0	0	0	0	0	0	0
78	4NP	746	0	0	0	0	0	0	0	0
79	4NP	746	0	0	0	0	0	0	0	0
80	4NP	746	0	0	0	0	0	0	0	0
81	4NP	746	0	0	0	0	0	0	0	0
82	4NP	746	0	0	0	0	0	0	0	0
83	4NP	746	0	0	0	0	0	0	0	0
84	4NP	746	0	0	0	0	0	0	0	0
85	4NP	746	0	0	0	0	0	0	0	0
86	4NP	746	0	0	0	0	0	0	0	0
87	4NP	746	0	0	0	0	0	0	0	0
88	4NP	746	0	0	0	0	0	0	0	0
89	4NP	746	0	0	0	0	0	0	0	0
90	4NP	746	0	0	0	0	0	0	0	0
91	4NP	746	0	0	0	0	0	0	0	0
92	4NP	746	0	0	0	0	0	0	0	0
93	4NP	746	0	0	0	0	0	0	0	0
94	4NP	746	0	0	0	0	0	0	0	0
95	4NP	746	0	0	0	0	0	0	0	0
96	4NP	746	0	0	0	0	0	0	0	0
97	4NP	746	0	0	0	0	0	0	0	0
98	4NP	746	0	0	0	0	0	0	0	0
99	4NP	746	0	0	0	0	0	0	0	0
100	4NP	746	0	0	0	0	0	0	0	0

For each observation date a single caddisfly is observed for 5 minutes in each of 20 microcosms (5 reps of each of 4 treatments)
Record time spent in each behavior: foraging (min), aggression, non-active

Final body mass

Chamber	Treat.	Pan no.	Pan wt (g)	Pan + bug wt (dry)
60	N	7	119.528	
76	N	4	87.735	
43	N	10	111.784	
49	N	4	80.717	
34	N	6	140.348	
37	N	3	108.444	
38	N	8	67.097	
35	N	6	122.446	
78	C	3	91.188	
67	C	9	96.198	
62	C	8	74.493	
41	C	7	79.740	
25	C	4	141.453	
74	C	7	127.087	
71	C	3	123.562	
75	C	7	94.524	
55	P	7	107.343	
68	P	9	90.851	
52	P	7	82.477	
22	P	4	100.074	
23	P	7	161.084	
51	NP	5	101.891	
29	NP	6	185.064	
63	NP	3	105.198	
64	NP	5	101.526	
66	NP	7	158.770	

Figure 10 (left) and Figure 11 (right): Figure 10 is an example of the data sheet I used to record mortality data. Figure 11 on the right is an example of a data sheet I used to measure the average weight of caddisflies in a container, before drying at this point.