

BIOS 569: Practicum in Field Biology  
The Effect of Increased Variance of Dispersion on Zooplankton

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***..Abstract:***

The following is an experiment designed to test the effects of dispersion rate variance on communities of zooplankton. Using artificially created ponds and local samples of zooplankton, I ran a real-world simulation of different dispersion variances, and took initial and final recordings of the zooplankton to see how communities would develop. Results showed that there was no significant difference between any of the identified taxa across treatments in terms of either counts or estimated total biomass. In addition, there were no trends in the pools at the beginning of the experiment, and no significant trends developed by the end of the experiment. The chlorophyll showed significantly different levels of growth between treatments, but the distribution of different chlorophyll levels were not linear as the hypothesis suggested.

***..Introduction:***

Vernal ponds are unique and significant bodies of water found in woodland areas. There are many different definitions of what constitutes a vernal pond; and vernal ponds can be identified by examining different traits expressed by the body. For instance, a vernal pond can be defined by quantifiable traits such as its geographic range of occurrence. To extrapolate further, vernal ponds occur mainly in woodland areas or in close proximity to forests. They are isolated basins with no inlets or outlets and have no connections to any other body of water (Colbum 2004). The other traits by which one can define a vernal pool lie in its hydrology and biological community. The former of these contexts defines a vernal pond as a body of water, which fills seasonally and dries up annually. The latter context, biological community, speaks strongly to the ecological role vernal ponds play in forested areas. Vernal ponds lack fish and thus, provide a unique habitat for creatures that require no fish predation for reproduction purposes, as well as

providing habitat for small animals that would not be able to flourish in a fish-rich environment such as insect larvae or certain species of zooplankton (Rainis and Russell 1996).

Vernal ponds house a cornucopia of different creatures, yet some of the most numerous and prevalent are zooplankton (Colburn 2004). Zooplankton are highly evolved and extremely diverse creatures, and the species within this taxon have inhabited almost every imaginable type of aquatic ecosystem (Shurin 2001). The most abundant micro-crustaceans most likely be encountered in the vernal ponds are Copepods, Daphnia, Cladocera - water fleas, and Branchiopoda - fairy and clam shrimp (Colburn 2004; Dole-Olivier and Galassi et al. 2000).

Mesocosms are wildly important assets to ecological research. While single-species studies of the past have provided insight into the effect pollutants have on specific taxa, mesocosms have given researchers a much more realistic glimpse into the ecological implications of these factors (Graney et al. 1994). While most mesocosms are used for larger limnology or permanent water system studies in reference to pollution, they can be good analogs for almost any kind of water system (Coull and Chandler 1992; Meester et al 2005). It has been argued that pools and ponds offer great opportunities to study patterns of biodiversity in small aquatic habitats and provide glimpses into greater theories that cannot be reasonably tested in large-scale systems (Meester 2005). In addition to this, mesocosm studies provide the insight of a real-world study combined with the controllability and longevity of in-lab work, giving researchers who take advantage of them results that may not have been abstracted from the other two options (Coull and Chandler 1992).

Not much is known about how variance in the dispersion of species through time affects community assembly in a new environment. Although the rate of dispersal has been shown to be very important for community dynamics, variance through time around a particular rate has

never been studied. Specifically, it has been shown that high dispersal rates create communities that are dominated by the best competitors, thus decreasing species richness, whereas intermediate rates allow species that would not normally be able to survive continue to live (Mouquet and Loreau 2003). It can be inferred that a more dense community is an indicator of health and vitality of the population that composes the community (citation?). A simulation model found that increased temporal variation in dispersal rates could affect community assembly in a way that is similar to higher fixed dispersal rate, and this idea was supported by studies that date back to the theory of island biogeography (Mouquet and Chase Unpublished).

While the Mouquet and Chase model is suggestive, empirical evidence is necessary to test the validity of their theory in the real world. Therefore, I will test their model prediction that there will be significant differences between the varying dispersal treatments. I will use zooplankton communities in ponds as a model system because of their rapid generation time and ease to maintain.

I expect to see an increase in community development with an increase in variance, an increase in the heterogeneity of the artificial pools, which would indicate an increase in a few top competitors, and a decrease in all other zooplankton species. In addition, I expect to see a decrease in chlorophyll within the variance treatments, due to the increase in top competitors.

### ***.:Materials and Methods:.***

Using 39 L storage buckets, a completely controlled environment was designed utilizing: well water, artificial sand, naturally occurring predators of zooplankton in vernal ponds (Chaoborus, Chironomids, Mites and Mosquito larvae) to supply light predation pressure on the population, as well as fixed starting concentration of nitrogen and phosphorus levels in the water. Nitrogen values average 1000  $\mu\text{g/L}$ , and phosphorus values averaged 100  $\mu\text{g/L}$  (Caceres et al.

2008). By controlling initial conditions, we will be able to evaluate the effect of variation in dispersal, resulting in a clear-cut illustration for how the variable rates affect resulting communities, if at all. I set up 25 of these artificial ponds, 5 levels of variation in replicates of 5.

Ten different vernal ponds were selected across the property in pairs. Each pair consisted of a clear pond and a dark, vegetation filled pond to increase the heterogeneity of the habitats that were introduced. Throughout the experimental period, weekly samples from these ponds were collected; their zooplankton density determined by sub-sampling. Due to the natural progression of the vernal ponds' hydrological cycles, some of them dried up throughout the experimental period, causing me to drop some ponds from the experiment partway through. However, I continued to keep the ratio of clear to weedy ponds the same. The information regarding zooplankton density of the natural samples was then used to determine the number of zooplankton per milliliter, and then subsequently used to disperse the zooplankton in the artificial vernal ponds according to their assigned treatment.

Inoculations corresponded to levels of dispersal variation: a control with fixed dispersal each week of 700 individuals and 4 dispersion treatments whose standard deviation increases by a factor of 100 (100, 200, 300 and 400). The ponds had four dispersal weeks where a low dispersal week was followed by a high dispersal week (Figure 1). In the end, each treatment would receive the same amount of zooplankton. In addition, I randomly distributed the variance treatments to get rid of spatial factors that could unevenly affect my artificial ponds (Figure 2). The zooplankton were sampled each week until the end of the experiment. Using Lugol's Solution as a fixing agent, I counted and identified the zooplankton, allowing me to extrapolate information regarding the internal community of the artificial vernal pools. In addition to

numerical counts, measures of length were taken from the initial vernal pond samples to estimate the biomass of the different zooplankton taxa. I used the formula described by Dumont (1975).

In addition to the zooplankton samples, I also took samples of water each week to test the amount of chlorophyll present in each mesocosm. The test required filtering the water samples through a 47 mm GF/F filter, followed by extraction with methanol and examination with a fluorometer. The resulting absorbencies would then be used as an indicator of concentration of chlorophyll present in each vernal pond.

### *Statistics*

I ran regressions comparing biomass, abundance and diversity against the different variance treatments. In addition, there was also a repeated measures ANOVA run to study the relationship between time and variance on the chlorophyll data using statistical program Systat.

### ***.:Results:.***

Both time and variance treatment significantly affected the chlorophyll concentration of each pond, but there was no interaction between time and variance treatment ( $P = 0.046$ , F-ratio = 3.45, dF = 4) significance for treatments as a factor ( $P = 0.014$ , F-Ratio = 4.11 and dF = 3) and no relationship between the two variables ( $P = 0.858$ , F-ratio = 0.559, and dF = 12).

The following regressions were run: Individual biomass of the accounted taxa across variance treatments, the total biomass of the accounted taxa across treatments, the number of individuals per liter in each taxon across variance treatments as well as the total individuals per liter, for both the beginning and end of the experimental period. Due to the high volume of material, the information regarding the p-values and  $r^2$  values can be found for the aforementioned tests in Tables 1-4, respectively.

The chlorophyll data showed significant results in terms of the day sampled and in the variance treatment; however, the treatments did not show different patterns of change through time. Figure 3 displays the significant growth of chlorophyll over time, as well as the diverging pathways of each variance treatment. The figure also illustrates the absence of relationship between the two variables, specifically. What this means is while there was different concentration of producers across the treatments, patterns did not change through time.

Results abstracted from the count and biomass data revealed no significant trends in the variance treatments, thus failing to support my hypothesis. The experiment fails to support that there is a relationship between differing variance and community dynamics. Figures 4-7 display the non-significant findings for this aspect of the experiment, while there was certainly overall growth of the zooplankton, there was no difference between the treatments.

### ***.:Discussion:.***

While the first thought may be to say the hypothesis for this experiment is false, many mitigating circumstances may have compromised the integrity of the experimental design. My first point is that the data collected was a snapshot, a very small and brief glance into the dynamics of the artificial pools. It very well could be that given more snapshots throughout the experimental period, I could have gathered a more complete picture of how the different taxa were interacting with each other. It is important to mention that this was the original intention of this experiment to sample each week, but the weekly samples were compromised.

Another factor that could possibly be taking away from my experimental design was the failure to key out the taxa I found down to exact species. If this were done, it may have revealed some underlying inner-species competition that I may have missed by using broader groupings.

Somewhat related to this idea is the absence of total control over the species which were living in my artificial pools. While there were no inputs like those which would have been found in a natural vernal pool, my artificial ponds had major influxes of different predators like flies and beetles that may have been feeding on the zooplankton. This could mean that certain pools may have received predators others did not; causing randomization of the replicates that compromised the assumption that variance was the only community-affecting factor.

I also feel that the experiment did not run long enough. Perhaps with more time, this experiment may have revealed a more developed relationship between chlorophyll and zooplankton numbers. However, it is also important to consider that this is an experiment on zooplankton species from vernal ponds, whose very nature is transience. Perhaps a longer experimental period would have rendered unnatural results that would not be applicable to a real-world system. In addition to this notion of 'unnaturality', the artificial ponds may not have been the greatest analogs for true vernal ponds. While I tried to simulate nature as close as possible, aboveground blue, plastic tubs with gravel-pit sand and trace amounts of detritus is not a proper replication of the real vernal ponds. The water levels never dropped throughout the entirety of the experiment, and the constant exposure to sunlight on all sides of the water may have caused unnatural water temperatures that the zooplanktons were not used to. Not to mention, the water was clear, as compared to murky vernal pond water that generally contains much higher concentrations of soil, dissolved organic matter and carbon.

In conclusion, my hypotheses proved to be false for this experiment. All of the data extracted from the zooplankton samples rendered no significance, and the chlorophyll results showed only the growing concentration of producers with non-linear significant differences between the artificial ponds. It may be good for future studies to micro-manage artificial pond



sin a manner that is more consistent with nature, as well as try to gain a better grasp on how the zooplankton might be interacting with each other throughout the entire length of the experimental period.

***..:Acknowledgements:.***

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***.:References:.***

- Batzer, D.P., Palik, B.J., & Buech, R.. (2004). Relationships between environmental characteristics and macro invertebrate communities in seasonal woodland ponds of Minnesota. *Journal of the North American Benthological Society*, Vol. 23, Issue 1. pp. 50-68.
- Caceres, C.E., Tessier, A. J., Andreou, A., & Duffy, M.A.. (2008). Stoichiometric relationships in vernal pond plankton communities. *Freshwater Biology*. Vol. 53. pp 1291-1302.
- Colburn, E.A. (2004). *Vernal pools: Natural history and conservation*. Blacksburg, Virginia: The McDonald & Woodward Publishing Company.
- Coull, BC, Chandler GT. 1992. Pollution and meiofauna: Field, laboratory, and mesocosm studies. *Oceanography and Marine Biology: An Annual Review*.
- Dole-Olivier, M.J., Galassi, D.M.P., Marmonier, P., & Creuze des Chatelliers, M. (2000). The biology and ecology of lotic microcrustaceans. *Freshwater Biology*. 44: 63-91
- Graney R.L., Kennedy J.H., Rodgers J.H. 1994. *Aquatic Mesocosm Studies in Ecological Risk Assessment*. CRC Press Inc. Boca Raton, Florida.
- Meester L.D., Declerck S., Stoks R, Louette G., Meutter F., Bie T., Michels E., Brendonck L. 2005. Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 15: 715–725
- Mouquet, N. and Loreau, M. 2003. Community Patterns in Source-Sink Metacommunities. - *The American Naturalist* 162: 544-557.
- Rainis K.G., Russell B.J. 1996. *Guide to Microlife*. Grolier Publishing, Danbury, Connecticut.

Shurin, J.B. (2001). Interactive effects of predation and dispersal on zooplankton communities.

Ecology. Vol. 82. Issue 12. pp. 3404–3416

**.:Tables.:**

Table 1: The biomass readings for the first zooplankton samples. Results came from a regression which compared the biomass of the species mentioned against each variance treatment in the first week of the experiment.

Species	<i>P</i>	$r^2$	F
Cyclopoid	0.748	0.005	0.105
Chydorids	0.488	0.022	0.496
Daphnia	0.34	0.041	0.950
Total	0.889	0.001	0.020

Table 2: The biomass readings for the final zooplankton samples. Results came from a regression which compared the biomass of the species mentioned against each variance treatment in the last week of the experiment.

Species	<i>P</i>	$r^2$	F
Cyclopoid	0.57	0.015	0.332
Chydorids	0.364	0.038	0.861
Daphnia	0.119	0.107	2.633
Total	0.258	0.058	1.349

Table 3: The Individuals/L counts for the first zooplankton samples. Results came from a regression which compared the number of individuals/L of the species mentioned against each variance treatment. These are from the first week's samples.

Species	<i>P</i>	$r^2$	F
Naplii	0.307	0.047	1.093
Cyclopoid	0.748	0.005	0.105
Canadora	0.141	0.096	2.334
Daphnia	0.34	0.041	0.950
Mosquito	0.921	0.001	0.010
Chaoborus	0.456	0.025	0.575
Mites	0.237	0.063	1.476
Chironomid	0.595	0.013	0.291
Chydorids	0.489	0.022	0.496
Total	0.507	0.02	0.454

Table 4: Individuals/L for the final zooplankton samples. Results came from a regression which compared the number of individuals/L of the species mentioned against each variance treatment.

These are from the last week's samples.

Species	<i>P</i>	$r^2$	F
Naplii	0.836	0.002	0.0436
Cyclopoid	0.57	0.015	0.332
Canadora	0.149	0.092	2.239
Daphnia	0.119	0.107	2.633
Mosquito	0.933	0	0.007
Chaoborus	0.257	0.058	1.357
Mites	0.346	0.04	0.926
Chironomid	0.653	0.009	0.208
Chydorids	0.364	0.038	0.861
Total	0.214	0.452	2.472

*.:Figures.:.*

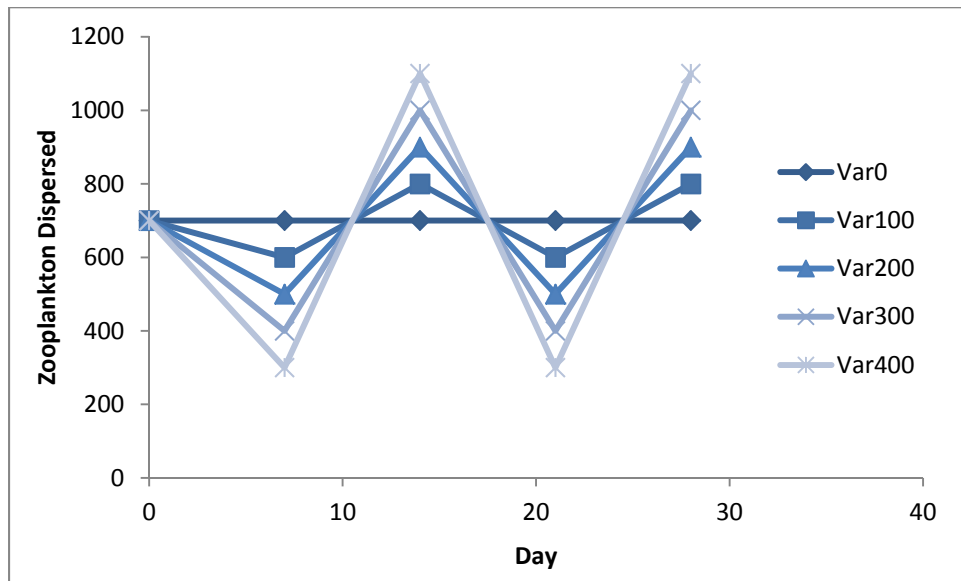


Figure 1: Visual representation of the four dispersion treatments.

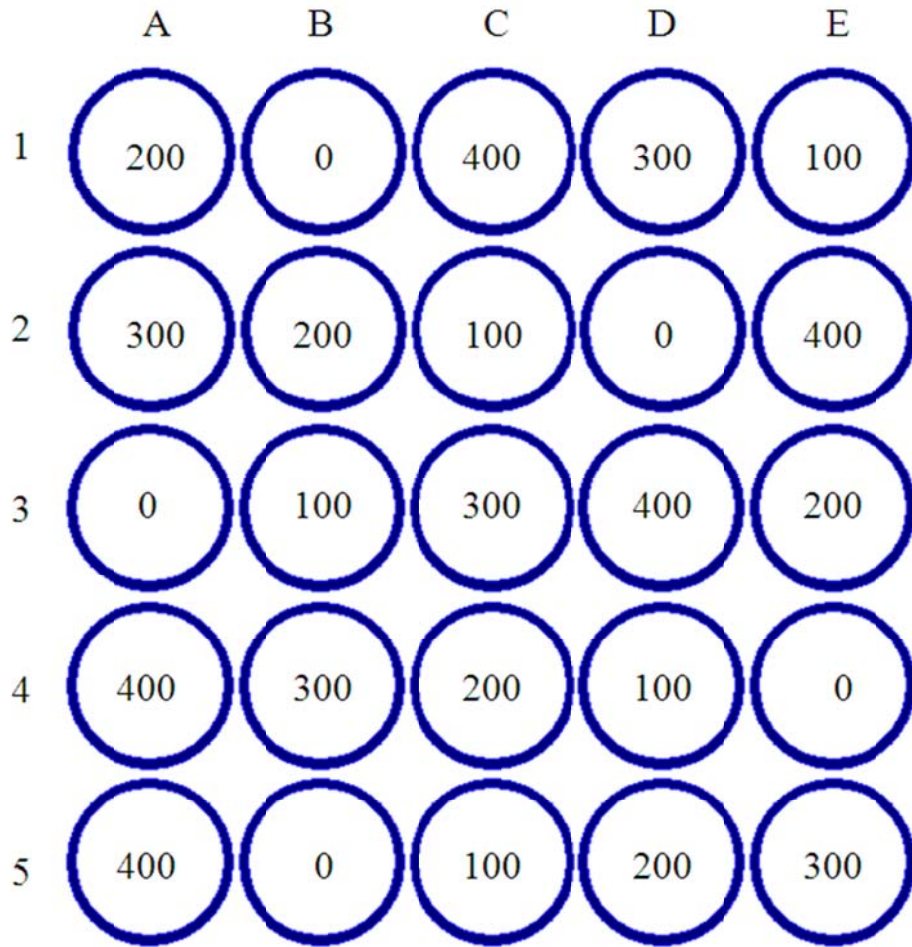


Figure 2: A figure representing the way I distributed the variance treatments. This is an aerial view of the plot with the standard deviation of the variance treatment inside of each circle.

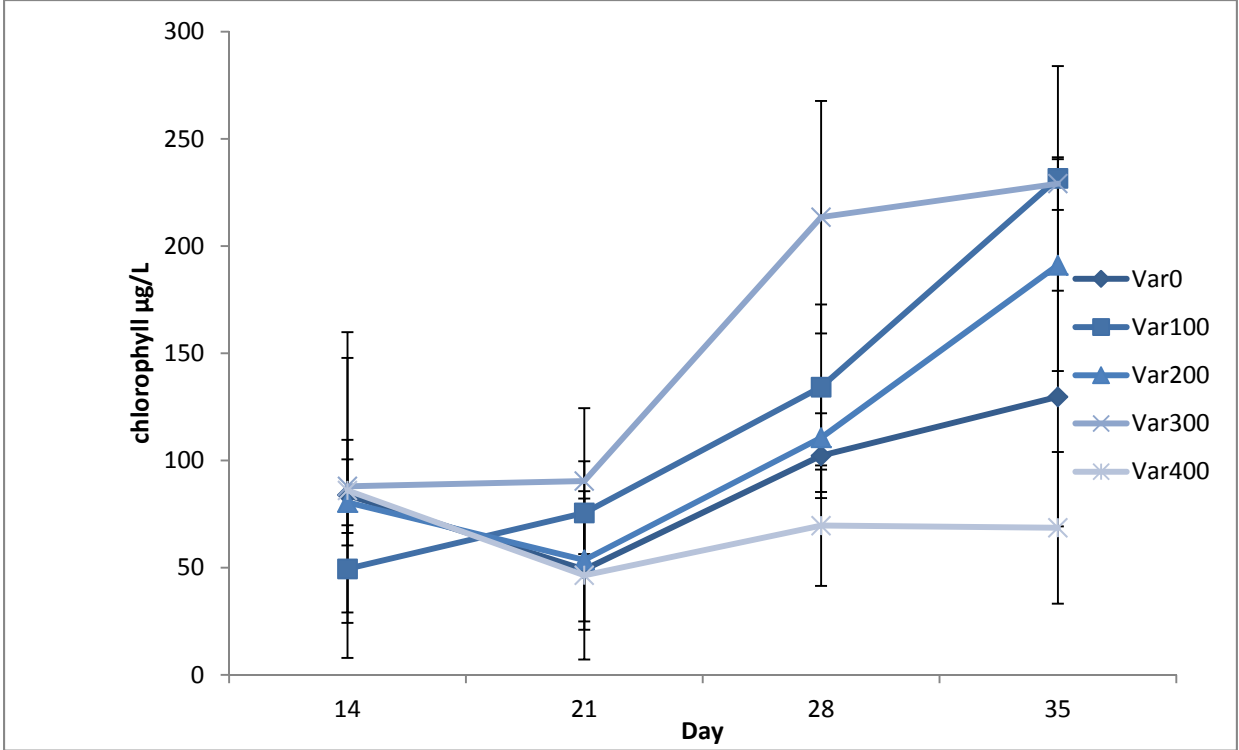


Figure 3: This graph displays the change of chlorophyll over time according to the four different variance treatments of zooplankton.



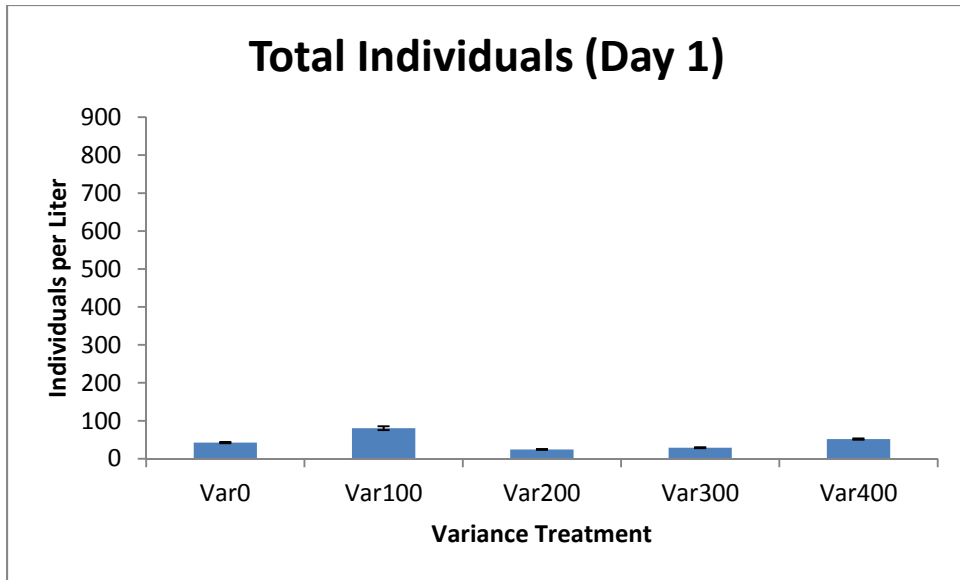


Figure 4

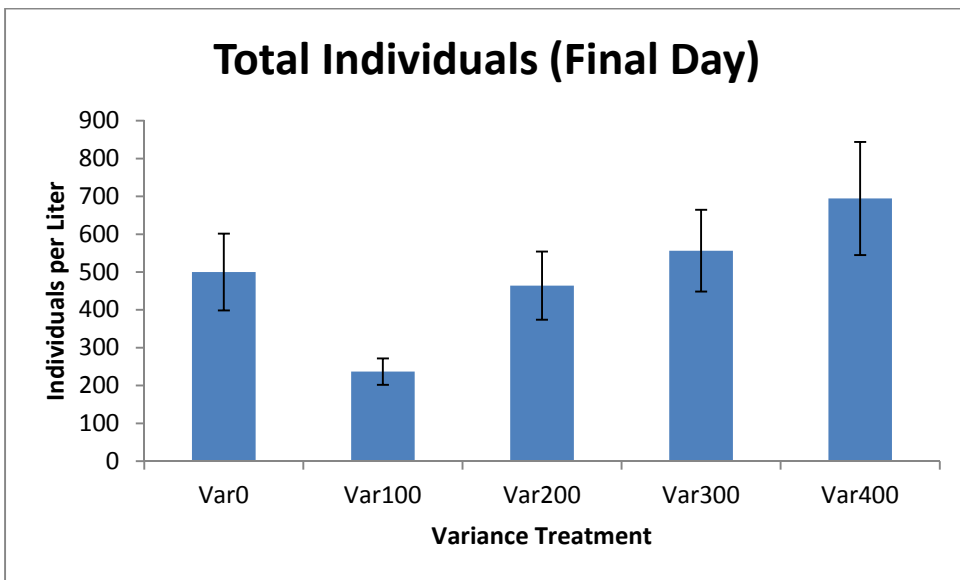


Figure 5

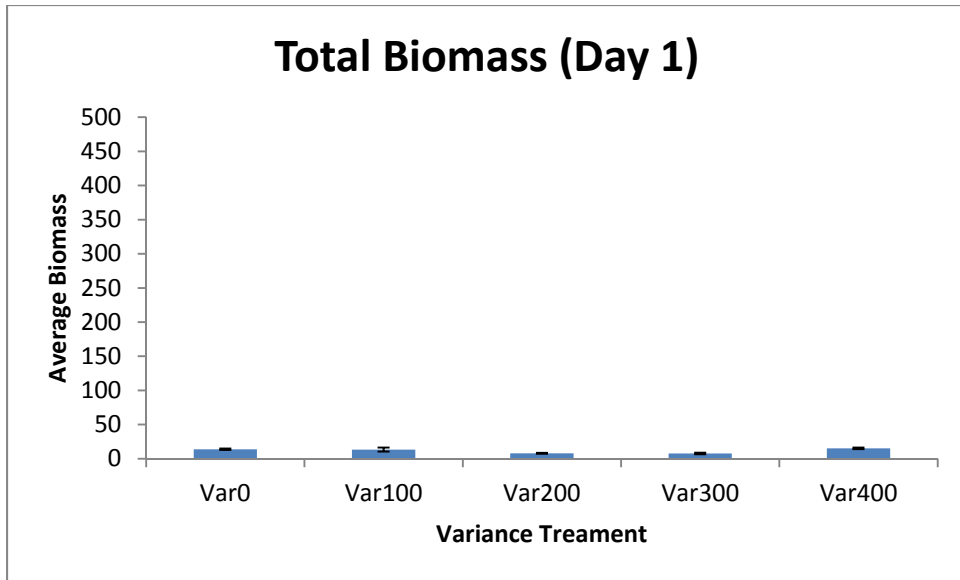


Figure 6

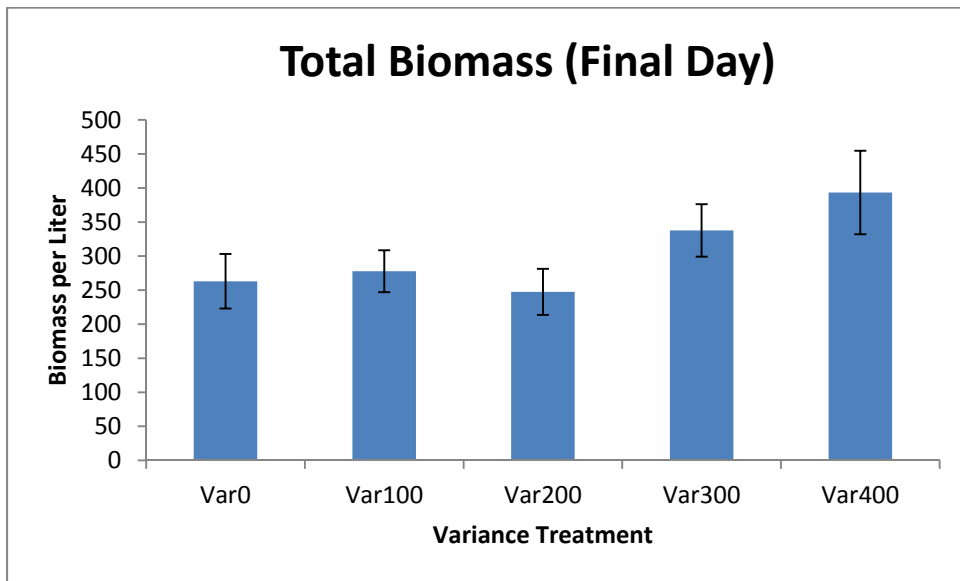


Figure 7