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The Effects of Induced Interaction with *Dreissena polymorpha* on *Limnephilidae* Larval Substrate Choice

Abstract

In many freshwater ecosystems of the Northeastern United States, the invasive *Dreissena polymorpha* (zebra mussels) are causing a wide range of both biotic and abiotic changes. We inquired into the potential interactions between caddisfly larvae (order Trichoptera, family *Limnephilidae*) and zebra mussels at the mouth of the Maple River in Douglas Lake (Cheboygan County, Michigan). Specifically, we wanted to determine if the presence of *D. polymorpha* had an effect on substrate preference of *Limnephilidae* larvae in a laboratory setting. The experiment involved recording the position of caddisfly larvae within an aquarium. Two aquarium setups were arranged so that the larvae could move between two different substrates; one tank type contained sand and live zebra mussels attached to rocks (treatment tanks) and the other (control tanks) contained sand and rocks. This experiment was performed at a low and high larval density. We found that the *Limnephilidae* larvae had a statistically significant preference for substrates containing live zebra mussels and that over time *Limnephilidae* larvae increasingly preferred the live zebra mussel substrate (at high larval density). Furthermore, in the low-density control tanks we found that over time *Limnephilidae* larvae tanks preferred the sand substrate more than the rock substrate.

Introduction

A species, if introduced into a new ecological niche, has the potential to exploit many resources and alter ecological relationships of native species. Often times this can be disadvantageous to native species. This invasive species (frequently introduced by humans) lacks the natural checks and balances to keep their effective population size at a level where it does not impinge considerably on other species. Native species may be able to adapt to the new conditions but often are out-competed for resources. Aquatic invasive species can be transported via boat bilges or other vessels when traveling between bodies of water.

The presence of *Dreissena polymorpha,* commonly known as zebra mussels, has caused and will cause significant changes in the aquatic ecosystem of Douglas Lake since its introduction in 2001 (R. Vande Kopple , pers. comm.). Caddisfly larvae from the family *Limnephilidae* exist in a niche in Douglas Lake where we observed an encroaching population of *D. polymorpha.* We drew this conclusion because zebra mussels were present at the mouth of the Maple River, where we observed the caddisfly larvae, but not yet in high densities.

D. polymorpha was first identified in Lake St. Clair in 1988 (GSMC, 2005). Studies have been performed in order to understand some of the effects zebra mussels have on native species (Berkman et al., 1998). As an invasive species, it exploits new habitats and out-competes native species by filter-feeding effectively, forming dense aggregates on hard surfaces, and having toxic pseudofeces and high fecundity (Haltuch et al., 2000; Horgan & Mills; GSMC, 2005; Westbrook, 2003). An adult *D. polymorpha* can filter up to a liter of water a day, which causes aquatic ecosystems to suffer from decreasing levels of phytoplankton and chlorophyll, both of which are important resources for fresh water ecosystems (Horgan & Mills; Madenjian, 1995). As a result, an increase in water clarity is a well-recognized effect the zebra mussel has on abiotic factors within the ecosystem (MacIsaac, 1996). When filtering, they only utilize a portion of the ingested seston particles and eject the unused portion as toxic pseudofeces (Stanczykowska and Planter, 1985). The pseudofeces may cause harm in some benthic organisms by increasing acidity levels and decreasing dissolved oxygen levels (Westbrook, 2003). Other studies have suggested that the pseudofeces may increase nutrient levels and aid other benthic organisms (Hamburger et al., 1990 in Botts et al., 1996). *D. polymorpha* also has the ability to vary filtering rates to accommodate both energy expenditure and energy conservation, making them a hardy species and gives them the ability to thrive in diverse types of communities (Horgan & Mills, 1997; Haltuch et al., 2000; Berkman et al., 1998; Garton, Payne, Montoya, 1998).

D. polymorpha lives about four to five years and begins reproducing at age 2 (nationalatlas.gov, 2006). Females release around 30,000 eggs each year once they reach reproductive maturity (Westbrook, 2003). Within one month, fertilized eggs have hatched, pupated, and matured to the point where they settle and attach to hard substrates and tend to settle on or near the shells of adults (GSMC, 2005). Natural population densities from 5,000 to 30,000 individuals per square meter are not uncommon (GSMC, 2005).

Caddisfly larvae, a benthic organism in the order of *Trichoptera* (class *Insecta*), are aquatic and can be found in freshwater habitats with various types of substrates such as rocks, mud, gravel, sand, debris and vegetation (Pennak, 1978). Waterfowl feed on the immature stages of the caddisfly, while a significant portion of many fish diets are caddisflys from all life stages, showing their importantance in the energy transfer at several trophic levels (Williams and Feltmate, 1992).

Adults are terrestrial insects, generally living for 30 days (Pennak, 1978). During this period, females usually enter the water to oviposit (Pennak, 1978). Her eggs can be enclosed in gelatinous masses in the bottom debris or glued to substrates such as rocks or logs (Pennak, 1978). For most organisms of the order *Trichoptera*, their life is spent as a larva found in both lentic and lotic waters (Williams and Feltmate, 1992). *Trichoptera* usually winters over as a larva and reaches the pupal stage, which lasts only two weeks, in late-spring to early summer (Pennak, 1978). During this life stage, *Trichoptera* form large discrete groups and become immobile before maturing to adulthood (Dixon and Wrona, 1991).

Limnephilidae, the northern case-making family of *Trichoptera*, construct their cases

from materials in the substrate and glue them together with silk produced from an anterior gland (Pennak, 1978; Cave, 1998). The case, produced shortly after hatching, is used for protection and respiratory assistance. The larvae undulate in their cases which increases current and allows oxygen to pass across their gills at higher rates (Williams and Feltmate, 1992). As grazers, they consume detritus, fungi, diatoms and other algae and vegetation at random as it rests on the substrate or is loosely attached (Williams and Feltmate, 1992; Pennak, 1978). Larvae feed voraciously in order to store energy reserves for the pupal stage when they do not eat at all (Lloyd, 1922).

As *D. polymorpha* move in, we suspect they may disturb *Limnephilidae*'s habitat because of its feeding habits, substrate alterations, and toxic pseudofeces. Zebra mussels have the capability to remove organic matter from the water column and decrease oxygen levels, due to their rapid filtering rates. If this organic matter is removed before it settles to the ground it could potentially inhibit larval access. Their aggressive feeding habits can have other indirect effects on the flies due to interacting trophic levels in aquatic ecosystems. Since *Limnephilidae* are grazers and feed off algae and other nutrients clinging to rocks, zebra mussels may slow down this mechanism due to the thick density in which they cover substrates. Pseudofeces can be toxic to other organisms if the acidity content of the water reaches high levels, and could possibly affect homeostasis of larvae. Testing interactions between zebra mussels and *Limnephilidae* larvae in a laboratory environment eliminates some extraneous factors, and allows us to ask some specific questions:

1. Will *Limnephilidae* respond to *D. polymorpha* by choosing to associate or avoid *D. polymorpha* presence in a laboratory setting?
2. Will *Limnephilidae* respond negatively to the presence of *D. polymorpha* by preferentially selecting areas where *D. polymorpha* are not present?

Materials and Methods

Caddisfly larvae were caught with dip nets, using glass bottom buckets, in the southwest corner of Douglas Lake in Maple Bay at the mouth of the Maple River (Cheboygan County, Michigan). We collected 64 caddisfly larvae and transported them back to the laboratory (University of Michigan Biological Station) in a bucket filled with lake water. To keep the specimens alive, a flow-through tank was set up to mimic their natural habitat. This set up provided them with a slow current of fresh lake water, a sand bottom, rocks, small sticks, and natural vegetation. We identified the larval family as *Limnephilidae*.

From research we found that *Limnephilidae* require lotic conditions for survival. As a results, we were able to use nine 38-liter aquariums filled with approximately 26.5 liters of lake water. Each aquarium contained two oxygen stones (artificial rocks that re-oxygenate water) connected to a steady source of air. One rock was anchored on either end of the tank. The oxygen set up was a necessity to ensure that the water remained oxygenated for the specimens throughout the experiment. All aquarium bottoms were filled with a layer of sand similar to the substrate in which they were found, approximately 2 cm deep. Nine tanks were set up: three treatment tanks containing live

zebra mussels, three reference tanks containing dead zebra mussels and three control tanks containing rocks without zebra mussels.

The bottom of all of the aquariums were divided into six equal rectangles, with dimensions of 16.67cm x 12.5cm. For the three control tanks medium sized rocks (2-6 cm in diameter) were used to cover about 75% of three altering rectangles while the remaining three were strictly sand, creating a checkerboard pattern on the bottom of the tank. Two pieces of vegetation were placed in the middle of each quadrat to assure that each one had approximately the same amount of food for the caddisfly larvae. The size of the rectangles, rocky substrate and amount of vegetation were held constant across all tanks.

For the three treatment tanks, live *D. polymorpha* were collected from Douglas Lake and used as the rocky substrate. After washing, the zebra mussels were placed into the 3 quadrats in the same manner as the other 6 tanks.. The three reference tanks contained rocks covered in dead *D. polymorpha.* These *D. polymorpha* specimens were placed in an oven at 50 degrees Celsius for two days to ensure desiccation. The dead *D. polymorpha* were washed with clean water to minimize confounding factors such as toxicity from the build-up of pseudofeces. The three control tanks contained plain rocks collected from Douglas Lake which were washed and placed into the 3 quadrats. Just before the *Limnephilidae* larvae were put into the aquariums, the water was replaced. We replaced the water daily throughout the experiment to replenish seston particle concentrations to ensure that live zebra mussels were not deprived of their food source (Nida and Ford, 1992).

For the first part of the experiment low density tanks containing five *Limnephilidae* larvae were placed randomly in each tank and were given an hour to adjust to the microhabitats before we began recording our observations. This density equates to 4 caddisfly larvae per square meter. After the first hour, we recorded the position of each larva in each tank. The *Limnephilidae* larvae location was recorded as in a quadrat either with or without the rocky substrate. We observed and recorded the larvae positions every hour for six more hours. On the second day, we increased the amount of time in between observations to three hours because the *Limnephilidae* larvae did not appear to be overly active. We observed and recorded their positions every three hours to complete five observations. On the third day, we observed and recorded the larvae positions every five hours. For the second part of the experiment, on the 4th day high density tanks were established by increasing the density of the *Limnephilidae* by adding six more larvae to each tank. This new density equated to 8.8 caddisfly larvae per square meter. We observed and recorded the larvae positions in the same manner of hourly, three-hour and five-hour recordings for three days.

Within the first hour of Day 1 we noticed a difference in the water of the reference tanks. After hour two, the water in the reference tanks appeared to be turbid and a white, bubbly, foam had collected around the edges of the tanks. Furthermore, oxygenation was accidentally turned off over night and observations the following morning revealed that *Limnephilidae* larvae had died only in these tanks. We observed the water on day two to be more turbid than day one and suspected that bacteria decomposing the dead zebra mussels depleted the oxygen levels in the water, so we ran an additional experiment using

an oxygen meter to test the levels of dissolved oxygen in an aquarium, which contained approximately the same amount of desiccant *D. polymorpha*. We desiccated the zebra mussels in the same manner as before and began the experiment at night. The oxygen measure before the introduction of the desiccant *D. polymorpha* was 8.08mg/L (at about 9:30 p.m.). Another reading was taken at 5:30 a.m. the following morning to ensure that oxygen levels had not yet been significantly altered by photosynthetic phytoplankton. The last oxygen reading was taken at 11:15 a.m., which was the approximate time of day that the *Limnephilidae* larvae were observed dead. Dissolved oxygen levels were 5.20mg/L at 5:30 a.m. and 0.39mg/l at 11:15 a.m. (figure 1). These results suggest that the *Limnephilidae* larvae died because the oxygen levels were too low for survival (S. Francover, pers. comm.).

Results

Chi-squared tests were performed on each of the three tank types to determine if our observations of caddisfly larvae substrate preferences were statistically significant. For each observation period a chi-squared test was performed, using the quantity of larvae on zebra mussels/rocks and on sand as our observed values. We set our significance level at $\alpha = 0.05$. To fulfill the assumptions of the Chi-Squared test for the low-density experiment, we combined the observations of the same three tanks, giving an observed total of 15 larvae and an expected value of 7.5 for the density of 5 larvae per tank per test.

For the low and high density treatment tanks 29 of the 33 chi-squared test were statistically significant (table 1). This shows a large substrate preference for live zebra mussels over sand during the entire period of the experiment. The low-density control tanks had 6 of the 17 p-values with a statistically significant preference for rock substrate (table 2). All six significant p-values occurred in the first day. For the high-density control tanks, 8 out of 16 of the p-values were statistically significant throughout the observational period with a preference for rocky substrate.

The reference tanks were observed for two days. During the first day all the observations were statistically significant, however after the second day the data could no longer be included into the analysis. The unstable conditions did not mimic the environment expected and caused an unfavorable outcome culminating in the death of the *Limnephilidae* larvae within those tanks.

Regression tests were performed to find trends in substrate preference over time. Two regressions were statistically significant. The treatment high-density tank regression showed a correlation over time with an increased preference for substrate with live zebra mussels (figure 2). The control low-density tank regression showed a correlation over time with an increased preference for sandy substrate (figure 3).

Discussion

When conducting our experiment and analyzing the results, we were required to make a few assumptions. First, we had to assume that the *Limnephilidae* larvae would exhibit

natural behaviors in a laboratory setting - otherwise our results would be of much less ecological importance. Similarly, we assumed that nothing in our experimental setup was confounding our results, such as the water we used from the shore of Douglas Lake in the South Fish Tail Bay was similar enough to the water in Maple Bay that there would be no difference in behavior. Also, we had to assume that the vegetation selection and placement was not altering larval behavior. Lastly, we assumed that our observational period was long enough to see the correct trend over time.

Our experiment shows with significant results that caddisfly larvae of the family *Limnephilidae* preferred rocky substrates containing live zebra mussels over sand substrate. This was observed at both low and high densities of caddisfly larvae. There are a number of plausible explanations for this and these results do not indicate that *Limnephilidae* larvae would prefer substrates colonized by *D. polymorpha* in a natural environment. First, the *Limnephilidae* larvae may have been scraping the zebra mussel shells for food. If this were the case, then zebra mussel shells would provide more surface area for the larvae to forage on, making foraging behavior a possible influence to caddisfly larval substrate preference. Secondly, *Limnephilidae* larvae may have preferred the zebra mussel substrate as a method to reduce the risk predation. The larger size of the zebra colonies may have attracted caddisfly larvae as a form of shelter. Thirdly, after the data recording had ended and the caddisfly larvae were removed from the tank to be returned to the lake, we noticed that five of the larvae from the treatment tanks had attached themselves head-first to zebra mussels. Our research indicates that the *Limnephilidae* larvae were entering the pupal stage of their life cycle (Dixon and Wrona, 1991). This fact may have had a large effect on larval behavior. As noted earlier, *Trichoptera* larvae aggregate into groups and attach to substrates before entering the pupate stage an this may explain in part the larval substrate preference. Lastly, it has been suggested that the pseudofeces may benefit some benethic invertebrates as a nutrient source (Hamburger et al., 1990 in Botts et al., 1996). This is an interesting possibility and the initial setup of our reference tanks would have allowed us to discuss this more. If we had prepared the mussels correctly and were able to collect data from the reference tanks, we might have been able to deduce whether live or dead zebra mussels were more preferred. As a result, we could say more about the biotic versus the abiotic effects of the zebra mussel on *Limnephilidae* larvae. The above-proposed explanations should all be investigated through further experimentation.

Our data also showed that *Limnephilidae* larvae showed some significant preference for plain rock substrate over sand substrate in the control tanks. Specifically, there was significant preference early on at low density and significant preferences were spread across the observational period at high density. Some of the explanations proposed above may also apply for the control tanks. Caddisfly larvae may have preferred the rock substrate over the sand because there was a higher proportion of algal growth on the rocks or because the larvae were aggregating in preparation for the pupal stage. Again, these proposed explanations would all require further testing.

Two significant trends were observed over the observational period. One occurred in the treatment tanks at high density and the other in control tanks at low density. The *Limnephilidae* larvae in the treatment tank at high density were observed on the zebra mussel substrate more frequently over time. This significant trend reinforces our previous

conclusion that there is a significant preference for live zebra mussel substrate. The second significant trend, which occurred in the low density control tanks, showed *Limnephilidae* larvae residing more frequently on the sand substrate over time. This trend may be the result of newly introduced larvae "settling in" to the microhabitat. Although we waited one hour after introduction before recording larval positions, it is possible that the new larvae were still adjusting to the new environment. Another possible explanation is that the *Limnephilidae* larvae were exhibiting a form of optimal foraging behavior. The larvae were found on the rocks at highest concentrations within the first five hours. If algae were present on the rocks then the caddisfly larvae may have foraged there first, as this resource was easily excessible. After they algal concentrations decreased to a certain density, larvae may have then moved away to forage elsewhere. A future experiment on *Limnephilidae* larval foraging behavior may be able to confirm or deny this. To be complete, there were no significant trends in larval substrate preference over time in the control tanks at high density or in the treatment tanks at low density.

One error in our experiment was related to the oxygen levels - specifically in the reference tanks. The dead zebra mussels in the reference tanks decomposed and the bacteria that grew in those tanks greatly reduced the levels of dissolved oxygen. Further compounding the issue, the oxygen was accidentally left off the first night of our experiment. These errors resulted in the loss of the larvae in those tanks and forced us to remove those tanks from our experiment.

A second error with our methods may have been our choice and placement of vegetation within the tanks. A shallow-water aquatic grass was taken from the South Fish Tail Bay of Douglas Lake and placed in the flow through tank where the caddisfly larvae were held before the start of the experiment. We observed that the larvae fed on this vegetation frequently. As a food source, an equal amount of the vegetation was placed within each quadrat of each tank. The vegetation was positioned so that it stood straight up and *Limnephilidae* larvae would climb up the stalks to feed. Two problems arose from this. One, though the larvae crossed the substrate to climb up the vegetation, the larvae on the stalks were above the substrate and no were not in close contact with it. These *Limnephilidae* were recorded as in the substrate where the base of the vegetation originated. Two, sometimes the vegetation would bend under the weight of the larvae as they climbed up the stalks. When this occurred, the larvae were recorded in the quadrat that it was over and not in the quadrat where the base of the vegetation was.

A third possible error may have arose from the consistency of the observers. All four members recorded larval positions throughout the experiment and each member may have made slightly different decisions. To elaborate, if a larval case was positioned on the line between the substrate types, the individual marking the position could have made a different choice than another group member.

One large limit in this experimental setup was that *Limnephilidae* larvae were presented with only one of two substrate choices, depending on the tank type. To explain further, in the treatment tanks the *Limnephilidae* larvae were only able to choose between sand or rocks colonized with zebra mussels as substrate. Likewise, in the control tanks the caddisfly larvae were only presented with sand or rocks as substrates. A more comprehensive experiment could be performed in a much larger aquatic environment,

possibly as a cage set up in shallow lake water. A wide array of different habitats could be assembled within the enclosed area and *Limnephilidae* larvae locations could be observed. A method like this would provide the caddisfly larvae with more options and therefore give more insight into substrate preference (if present) and how the invasive *D. polymorpha* may influence habitat selection. In conclusion, we suggest that future studies be conducted in order to better understand the interactions between *Limnephilidae* and *D. polymorpha*.

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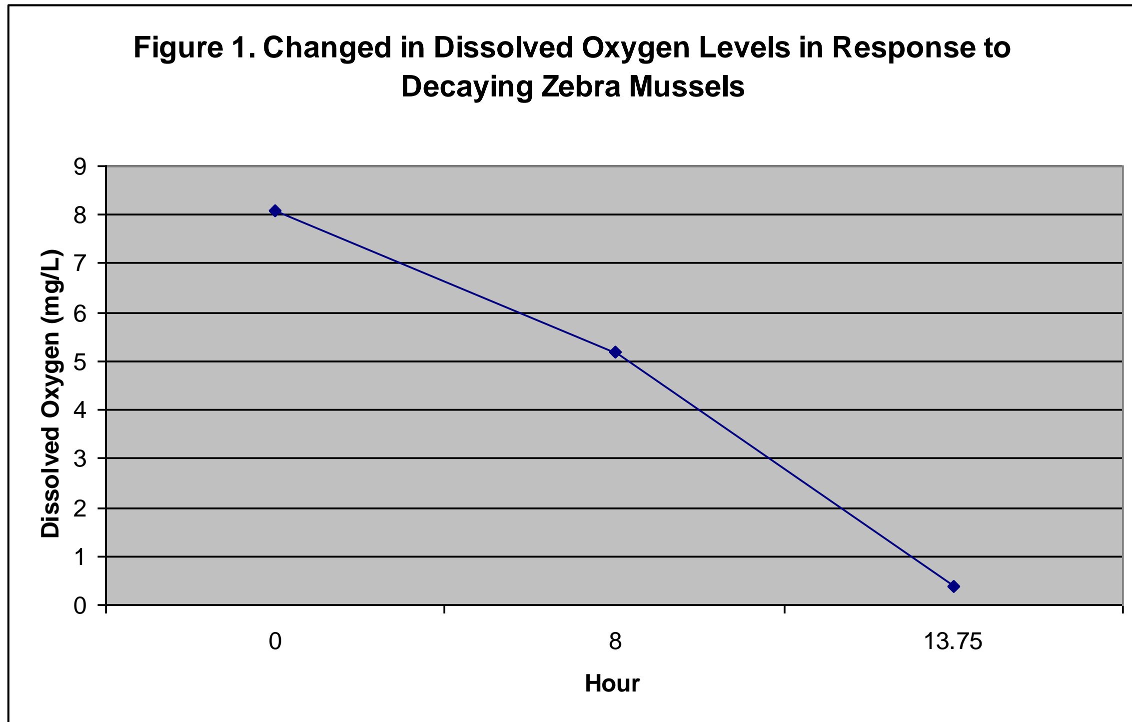
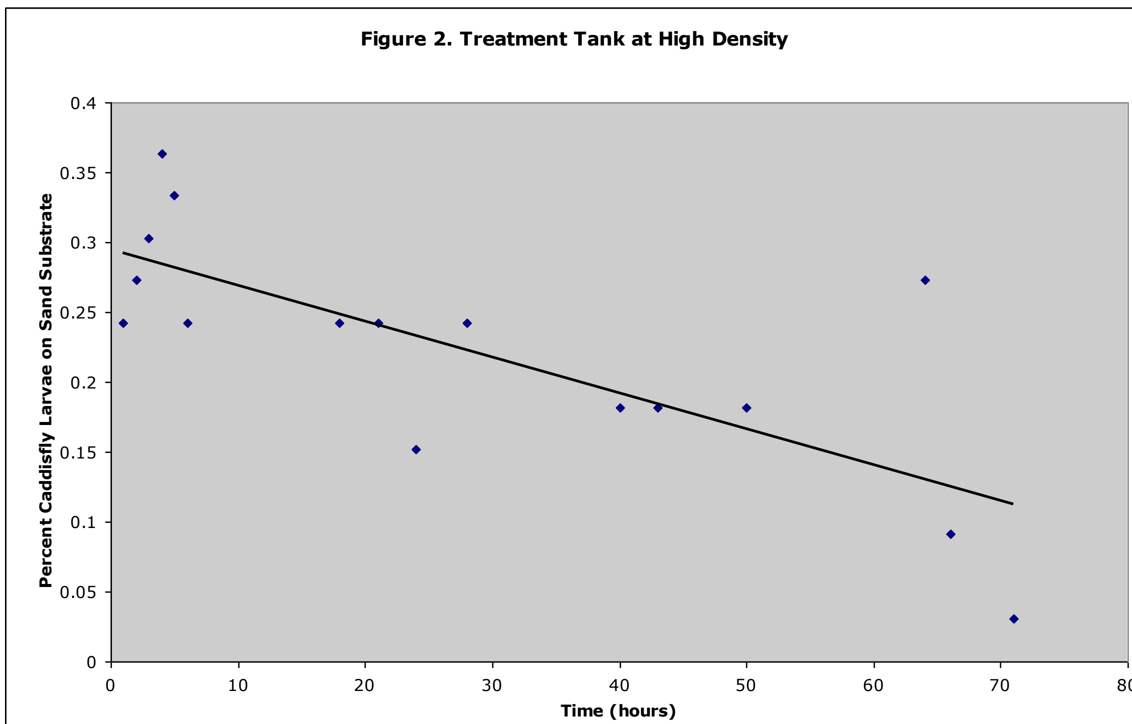
Appendix





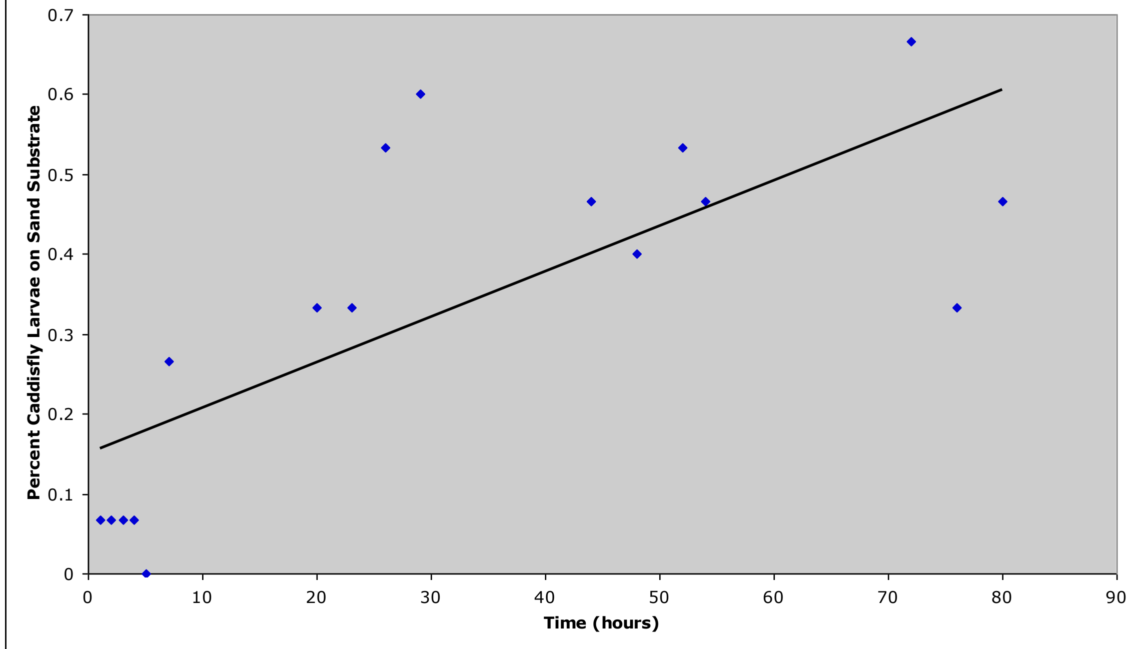


Figure 3. Control Tank at Low Density
Percent Caddisfly Larvae on Sand Substrate
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
80
90
Time (hours)

Table 1. Do *Limnephilidae* prefer sand or Live Zebra Mussel (ZM) substrate?

Treatment Tanks (Density- 5 *Limnephilidae*)					
hr	**Live ZM Obs**	**Sand Obs.**	**Exp**	X^2	**Pval**
Day 1					
1	13	2	7.5	8.066667	**P<.005**
2	11	4	7.5	3.266667	**.1>P>.05**
3	13	2	7.5	8.066667	**P<.005**
4	12	3	7.5	5.4	**.025>P>.01**
5	12	3	7.5	5.4	**.025>P>.01**
7	11	4	7.5	3.266667	**.1>P>.05**
Day 2					
1	11	4	7.5	3.266667	**.1>P>.05**
3	11	4	7.5	3.266667	**.1>P>.05**
4	12	3	7.5	8.066667	**P<.005**
6	12	3	7.5	5.4	**.025>P>.01**
Day 3					
1	13	2	7.5	8.066667	**P<.005**
3	11	4	7.5	3.266667	**.1>P>.05**
4	11	4	7.5	3.266667	**.1>P>.05**
6	14	1	7.5	11.26667	**P<.005**
Day 4					
1	9	6	7.5	0.6	.9>P>.1
4	11	4	7.5	3.266667	**.1>P>.05**
8	11	4	7.5	3.266667	**.1>P>.05**
(Density- 11 *Limnephilidae*)					
Day 5					
1	25	8	16.5	8.757	**P<.005**
2	24	9	16.5	6.818	**.01>P>.005**
3	23	10	16.5	5.12	**.025>P>.01**
4	21	12	16.5	2.4545	.9>P>.1
5	22	11	16.5	3.666	**.1>P>.05**
6	25	8	16.5	8.757	**P<.005**
Day 6					
1	25	8	16.5	8.757	**P<.005**
4	25	8	16.5	8.757	**P<.005**
8	28	5	16.5	16.03	**P<.005**
12	25	8	16.5	8.757	**P<.005**
Day 7					
1	27	6	16.5	13.363	**P<.005**
4	27	6	16.5	13.363	**P<.005**
8	27	6	16.5	13.363	**P<.005**
Day 8					
1	24	9	16.5	6.818	**.01>P>.005**
4	30	3	16.5	22.09	**P<.005**
8	32	1	16.5	29.1212	**P<.005**

Table 2. Do *Limnephilidae* prefer sand or rock substrate?

Control Tanks (Density- 5 *Limnephilidae*)					
hr	**Rock Obs.**	**Sand Obs.**	**Exp**	X^2	**Pval**
Day 1					
1	14	1	7.5	11.26667	**P<.005**
2	14	1	7.5	1	**P<.005**
3	14	1	7.5	4.5	**P<.005**
4	14	1	7.5	1	**P<.005**
5	15	0	7.5	6	**P<.005**
7	11	4	7.5	0.666667	**.1>P>.05**
Day 2					
1	10	5	7.5	1.666667	.9>P>.1
3	10	5	7.5	0.5	.9>P>.1
4	7	8	7.5	8	.9>P>.1
6	6	9	7.5	24	.9>P>.1
Day 3					
1	8	7	7.5	0.066667	.9>P>.1
3	9	6	7.5	16	.9>P>.1
4	7	8	7.5	8	.9>P>.1
6	8	7	7.5	4.5	.9>P>.1
Day 4					
1	5	10	7.5	1.666667	.9>P>.1
4	10	5	7.5	0.333333	.9>P>.1
8	8	7	7.5	4.5	.9>P>.1
(Density- 11 *Limnephilidae*)					
Day 5					
1	19	14	16.5	0.7575	.9>P>.1
2	20	13	16.5	1.48	.9>P>.1
3	18	15	16.5	0.2726	.9>P>.1
4	21	12	16.5	2.4545	.9>P>.1
5	16	17	16.5	0.0303	.9>P>.1
6	24	9	16.5	6.818	.9>P>.1
Day 6					
1	23	10	16.5	5.12	**.025>P>.01**
4	26	7	16.5	10.939	**P<.005**
8	20	13	16.5	1.48	.9>P>.1
12	27	6	16.5	13.36	**P<.005**
Day 7					
1	23	10	16.5	5.12	**.025>P>.01**
4	21	12	16.5	2.4545	.9>P>.1
8	24	9	16.5	6.818	**.01>P>.005**
Day 8					
1	24	9	16.5	6.818	**.01>P>.005**
4	24	9	16.5	6.818	**.01>P>.005**
8	18	15	16.5	0.2726	.9>P>.1