

Testing how density affects caddisfly distribution along a detritus quality gradient
Keywords: Trichoptera, *Asynarchus nigriculus*, habitat preference, caddisfly, aggressive behavior, animal behavior

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Abstract

As ecosystems continue to change, the ability to find high-quality habitats is becoming a valuable skill. Caddisflies are often found in pond environments with a variety of habitat qualities, such as food (detritus) quality. *Asynarchus nigrificulus*, being highly mobile and having a unique dietary need for protein are a prime subject for testing the ability of caddisflies to find high-quality habitats when given a range of detritus qualities. *A. nigrificulus* showed a preference for high-quality habitats both individually and in groups. Density was a relatively insignificant factor in their distribution, and aggressive interactions did not increase with density. This indicates that their distribution is based more on the availability of resources than on intraspecific interference interactions.

Introduction

Many environments exhibit heterogeneity in habitat quality between different patches. As the climate continues to change, so too does the quality of habitat patches. In ponds, inundation schedules are shifting which affects the accumulation of biofilm on detritus. Being able to find high-quality habitat patches is a valuable trait for many organisms as doing so will help to better support their development and ultimately their survival. The density of conspecifics in a patch also affects the quality of habitats. High-quality patches will depreciate as more organisms inhabit it because of exploitative competition. This makes density play a role in distribution, which can be seen in the ideal free distribution (IDF) model. Ideal free distribution (IDF) is a theoretical way in which organisms disperse themselves among patches of resources. The idea is that a population of organisms will reach an equilibrium among the patches based on the patch quality and number of organisms present. For IDF to occur, the following assumptions must be met: organisms must be aware of the quality of the patches, behave in a way which maximizes their foraging efficiency, and be equally competitive. Also, an increased density of organisms in each patch must decrease the quality of that patch (Kennedy and Gray 1993).

The species of interest for this study is *Asynarchus nigrificulus* (Trichoptera, Limnephilidae). These insects spend most of their life as larvae in freshwater pond environments and emerge as terrestrial adults in mid-late summer to reproduce. They are detritivores and are key in processing organic matter in ponds to provide nutrients for other organisms in the ecosystem. Of the species of caddisflies, *A. nigrificulus* has some unique traits. This species is resource and time limited as it is normally found in temporary oligotrophic wetlands (Wissinger et al. 2003). The detritus in these ponds can lack the associated biofilms found in higher quality habitats, thus having high C:P and C:N ratios. *A. nigrificulus* is also highly mobile and has a unique dietary need for protein to fully support development (Wissinger et al. 2004). They can complete development without the protein supplement; however, development would be slower. Most of the research conducted on caddisfly distribution thus far has focused on life histories, desiccation, and permanence gradients (Wissinger et al. 1996, Wissinger et al. 1999a, Wissinger et al. 2003). Studies on this topic have yet to delve into caddisfly behavior surrounding food preferences specifically.

A. nigrificulus' unique need for protein makes it easy to control for habitat quality by adjusting detritus quality (Wissinger et al. 2004), which brings about the questions for this study. Can *A. nigrificulus* find high-quality food patches when presented with a gradient? How does density affect the distribution of caddisflies along a gradient of detritus quality? Also, what associated behaviors play a role in this distribution? Because of *A. nigrificulus*' unique dietary needs and high levels of movement, we predict that this species will be able to find high quality

patches and will prefer them over others. We hypothesize that a higher proportion of *A. nigriculus* will be present in higher quality detritus than lower quality. We also predict that as caddisfly density increases, the quality of the high-quality detritus patch will decrease due to effects of competition for resources and a higher proportion of individuals will be found in the lower quality habitats. Considering associated behaviors, we predict that aggressive behavior will be observed more frequently in the higher quality patches when overall densities are high but will be observed less in these patches when densities are low. The ability to find high-quality habitats is valuable for caddisflies in that it helps them adjust to alterations in habitats due to climate change. It allows them to find suitable habitats in ecosystems exhibiting heterogeneity and potentially perform range shifts as needed to survive. Understanding the behaviors associated with distribution will allow us to better understand why caddisflies distribute themselves the way they do.

Methods

Study site and species

This study was conducted at the Mexican Cut Nature Preserve (MCNP), Elk Mountains, Colorado, USA. The MCNP is a high elevation site with numerous ponds of varying hydroperiod. All tests were performed in a weather port at the MCNP in 1/4m² plastic tanks. *Asynarchus nigriculus* was the test species for this project, and all insects were 5th instar.

Asynarchus nigriculus distribution

The portion of this study testing distribution was conducted in 1/4m² plastic tanks, each containing 3 different detritus quality patches and filled with approximately 5 gallons of pond water from pond 8 (Figure 1). All detritus (senesced *Carex aquatilis* fronds) was collected from pond 44 (Figure 1). To achieve 3 distinct detritus qualities, we followed the procedures described by Wissinger et al. (2004). Their study showed that when *A. nigriculus* is given detritus enhanced with protein in the form of tubifex worms, the caddisflies emerged earlier, were larger in mass, and females had increased fecundity. It follows that these advantages were seen in decreasing levels with decreasing detritus quality (Wissinger et al. 2004). The medium-quality detritus was gathered from the pond and left untreated. This detritus had been inundated long enough to have accumulated biofilm of algae, bacteria, and fungi. Low-quality detritus was medium detritus that had been placed in a bucket with boiling water and left to cool twice. Boiling the detritus killed off some of the organisms in the biofilm, thus lowering the detritus' nutritional quality. High quality detritus had 0.305g (SD=0.001) of powdered tubifex added to boost nutritional quality.

Each quality of detritus was added to its own sachet of 2.5cm² plastic mesh. 11.74g (SD=0.04g) of detritus was added to each bag along with a small stone to keep the sachet from floating in the tank. The detritus qualities were assigned randomly to either the front, middle, or back of the containers. A total of 12 containers were assembled. An example of a testing container can be seen in Figure 2. We tested three densities of caddisflies—6, 12, and 24—that represented the full range of densities found in the field, as determined by field abundance data with 4 replicates of each density. Densities were randomly assigned to test tanks.

At the start of the testing period, the caddisflies were evenly distributed among the detritus patches in each tank according to the tank's assigned density. The caddisflies were then left to distribute themselves. Counts of the number of caddisflies present in each detritus patch and in the open space between them were conducted 1, 2, 3, 6, 7, and 8 days after starting the

experiment. Only caddisflies which were visible without disturbing the tank's contents were counted. 0.304g (SD=0.003g) of tubifex was added to the high-quality detritus patches on day 6 to replenish its nutrient stores. Counts were performed before and one hour after the addition of tubifex on this day. For the final counts, the caddisflies were removed from the tank and placed into separate trays according to their location (low, medium, or high-quality patch, or open space). A total count was conducted for each location. The number of dead and pupated caddisflies was also recorded for each patch. When there were less caddisflies in the tank than were originally added, those missing were assumed to have been eaten.

We also conducted focal animal observations (FAO) on day 3 and twice on day 6 (one before and after adding the tubifex). For the FAO, an observer haphazardly selected a caddisfly from a tank to be observed. The observer watched that caddisfly for 5 minutes, recording time moving, interactions, and aggressive interactions. We followed Wissinger et al.'s (1996; 2004) description of aggressive behaviors which include foreleg wrestling, case shaking, and biting. One caddisfly was observed in each of the twelve tanks.

Detritus preference

The purpose of this portion of the study was to determine whether *A. nigriculus* is capable of perceiving detritus quality and choosing the highest quality habitat when not interacting with conspecifics. For this experiment, six testing tanks were constructed following the methods from the *A. nigriculus* distribution portion. All possible detritus arrangements were used, and arrangements were randomly assigned to tanks. The high-quality detritus was boiled in water for 10 minutes and left to cool rather than having boiling water added to it. There was 11.71g (SD=0.05g) of detritus in each of the detritus patches. 0.302g (SD=0.001g) of tubifex was added to each of the high-quality patches.

At the start of the observation period, two observers simultaneously added a caddisfly to a tank in a predetermined randomized location. Unlike the *A. nigriculus* distribution experiment, caddisflies could be placed in the open space between detritus patches at the start of this experiment. After 3 minutes, the observers each added a caddisfly to another tank, and this process was repeated one more time so that each of the six tanks contained one caddisfly. The observers then cycled through the tanks they added caddisflies to, recording the location of the caddisfly in one tank every three minutes. With this method, the observations for all tanks were staggered but occurred at three-minute intervals from the introduction of the caddisfly. Observations continued for one hour.

Data analysis

The data from the *A. nigriculus* distribution portion of the experiment was analyzed using one-way repeated measures ANOVA. We compared caddisfly densities (independent variable) with the mean proportion of caddisflies found in each detritus quality including open areas across all tanks of a particular density (dependent variable) along with elapsed time. Time and time x density interactions were tested with the error term within tanks whereas the main effect of density was tested with an error term across tanks. A chi-squared test was conducted to determine randomness of pupation distribution among the detritus patches. FAO data was analyzed using a one-way ANOVA with number of aggressive interactions (dependent variable) being compared with density and elapsed time (independent variables). Error was within tank. The total number of aggressive interactions in the observations immediately before and after adding tubifex were also compared. We used a chi-squared test to analyze the individual

preference data. As an additional method of testing habitat preference, we calculated the mean proportion of caddisflies in each detritus quality (excluding the open area) for all detritus qualities and the experiment as a whole. This data is depicted in Figure 8.

Results

Individual detritus preference

The chi-squared analysis showed that *A. nigriculus* does not choose habitats at random (X-squared₂ 148.48, $P < 0.0001$). The mean proportion of caddisflies in the high-quality habitats for the experiment as a whole were $\bar{x} = 0.51$ (SE=0.04), $\bar{x} = 0.41$ (SE=0.03), and $\bar{x} = 0.46$ (SE=0.02) for low, medium, and high densities respectively (Figure 8).

Asynarchus nigriculus distribution

Significance of the effect density and elapsed time had on mean proportion of caddisflies in each detritus quality patch differed depending on patch quality (Table 1). In high-quality patches, elapsed time was significantly related ($F_{6,62}$ 4.69, $P = 0.00054$) to the mean proportion of caddisflies present and density in relationship with elapsed time was mildly significantly related ($F_{12,62}$ 1.94, $P = 0.0456$). Density was the only significant factor determining the mean proportion of caddisflies in medium-quality detritus patches and was only mildly significant ($F_{2,62}$ 4.44, $P = 0.016$). In low-quality habitats, elapsed time was mildly significant ($F_{6,62}$ 2.89, $P = 0.015$). There were a total of 12, 9, and 4 pupated caddisflies in the low, medium, and high-quality patches respectively across all densities (Figure 7).

A one-way ANOVA was used to evaluate the effects of density and time on number of aggressive interactions. The analysis indicated that there was no significant effect of density ($F_{2,26}$ 0.54, $P = 0.59$) or elapsed time ($F_{2,26}$ 0.92, $P = 0.41$) on number of aggressive interactions. Looking at the observations before and after adding new tubifex, there were a total of 12 aggressive interactions before adding tubifex and 4 aggressive interactions after.

Discussion

Individual detritus preference

The chi-squared analysis showed that *A. nigriculus* did not choose habitats at random. During the test, most caddisflies that were not initially placed in a high-quality detritus patch moved around until they found that patch and then stayed there. Caddisflies that were placed in the high-quality detritus patch at the start of the test did not leave the patch during the observation period. When looking at total number of times caddisflies were found in each patch type during the observation period, the high-quality detritus patch had the highest number of visits at 86 visits followed by the open space (35 visits), low-quality (3), and medium-quality patches (2) (Figure 3). These results indicate that *A. nigriculus* do not choose habitats at random and likely are able to seek out and find high-quality patches. This finding is further supported by the proportion of caddisflies that were present in the high-quality patches across the whole experiment. When open spaces are excluded, all densities had significantly more than one-third (starting distribution) of the caddisflies in the high-quality patches. This too indicates that the caddisflies were not selecting habitats at random.

The finding that *A. nigriculus* prefers high-quality detritus patches is supported by Inkley et al. (2008) who found that shredders, such as caddisflies, prefer conditioned detritus over other forms. This ability to find and choose high-quality patches is valuable for these insects especially because of how resource and time limited they can be in the temporary ponds they inhabit

(Wissinger et al. 1999b). Wissinger et al. (2004) found that *A. nigriculus* physically benefit from high quality detritus by growing to be larger, growing faster, and being more fecund. As the climate continues to change, ponds are drying quicker and resources are becoming more limited. This is putting more pressure on insects like *A. nigriculus* to find high-quality habitats to increase their chances at survival to the point of reproduction.

Asynarchus nigriculus distribution

Our second hypothesis was that as densities increase, a higher proportion of caddisflies will be found in lower quality detritus patches. This hypothesis was not supported by our experiment. Density alone was only mildly significant in medium-quality habitats and was not significant for any other detritus patch qualities. We did see that density in relationship with elapsed time was important for high-quality patches. At high densities, we saw a high proportion of caddisflies in the high-quality patches at the start of the experiment and after the addition of tubifex midway through (Figure 5). The proportion of caddisflies in these patches dropped over time, presumably in relation to the amount of available tubifex, leading to lower proportions present right before the addition of new tubifex and at the end of the experiment. This finding leads us to believe that *A. nigriculus* bases its habitat selection more so on resource availability than interference competition risk, which contrasts the reasoning for our hypothesis.

While density was not a significant determining factor for the proportion of caddisflies in each detritus quality patch, it was mildly significant for medium-quality patches. As density increased, so too did the mean proportion of caddisflies in medium-quality patches (Figure 4). The mean proportion of caddisflies in the medium-quality detritus patches was highest and closest to that of the high-quality patches in the high-density tanks. This is likely resultant from the density and time effects on the high-quality patches. As resources diminished in the high-quality patches, the caddisflies moved to the medium-quality patches (Table 3).

Our analysis also showed that elapsed time was mildly significant in determining the proportion of caddisflies present in low-quality habitats. As time went on, there was an increased proportion of caddisflies in these patches across all caddisfly densities (Figure 6). One possible explanation for this is related to pupation. The highest proportion of pupated individuals was found in low-quality detritus patches (Figure 7). It is possible that more caddisflies pupated later in the experiment and so they would have moved to the lower-quality habitat to pupate, thus raising its proportion of caddisflies present over time. The chi-squared test completed looking at distribution of pupated caddisflies showed that the distribution was not significantly different from random. While this finding was not statistically significant, the total proportion of pupated caddisflies compared to all test caddisflies was small ($n = 25/168$). With this, it is possible the trend seen in Figure 7 may be the same trend that would occur on a larger scale. A future study completed on a larger scale could determine whether pupation is the cause of elapsed time's significance for low-quality detritus patches. Performing the study on a larger scale would offer more statistical power. Another option is completing the study earlier in the year when the caddisflies are less likely to pupate as this would show whether the cause of elapsed time's significance in low-quality patches was pupation or not.

Looking at caddisfly behavior, there was no significant effect of density or time on the number of aggressive interactions observed. As discussed earlier, it appears that resource availability had a more significant effect on distribution than competition or risks of cannibalism. Finding that density and time did not affect behavior further supports this claim, thus not supporting the hypothesis that aggressive interactions will be more numerous in higher density

tanks. Disproving this hypothesis contradicts some findings from Lund et al. (2016) however. Their study found that aggressive encounters increased with density and were more common in drying conditions. With this, aggression was also more common when there was no protein supplement. This contradiction in findings is likely due to the small sample size of our study and the possibility for confounding factors such as location within the tank. One interesting finding with behavior was that there were more accounts of aggressive interactions in the observation before adding new tubifex than the one after (12 and 4 aggressive interactions, respectively). While the difference is small, it is relatively large for the number of interactions observed. This observation supports the findings of Lund et al. (2016) in showing the effect of nutrient abundance on the number of aggressive interactions. Future studies could perform more FOAs to get a larger dataset that would provide more statistical power. It would also be valuable to have a more consistent method for selecting the observed caddisfly to eliminate potentially confounding variables.

Looking back at IDF, future studies can investigate feeding rate on tubifex by caddisflies. This would be useful in designing an IDF experiment in the future. One way to perform the IDF experiment would be to have two or three patches with known amounts of tubifex. Caddisflies can then be added to the tank one by one, and their habitat choice can be recorded. The order in which the caddisflies choose habitats will indicate whether they are following IDF because of the way a patch's quality decreases as it's number of inhabitants increases. Knowing the rate at which caddisflies consume tubifex will allow better interpretation of the habitat choice results as we would be able to calculate the quality of each patch on a continuum as it is inhabited by more caddisflies.

Conclusion

While many of the results of this study did not align with our predictions, we came across numerous unique findings. This study showed that *A. nigriculus* can find high quality habitats, and it appears that they prefer them over habitats of lower nutritional qualities. While distribution was not as influenced by density as expected, density did play a role in the depreciation of the high-quality habitats' nutritional value over time, thus affecting distribution. Time also played a role in low-quality habitats, potentially because of pupation occurring more in these habitats later in the experiment. The lacking effect of density on distribution along with its minimal effect on aggressive interactions suggests that caddisfly distribution is based more on resource availability than intraspecific interference competition. Not finding a significant effect of density on aggressive interactions contradicts the findings of Lund et al. (2016) which show an increase in these interactions when densities are high. We did see a decrease in aggressive interactions after the addition of new tubifex, which supports findings by Lund et al. (2016).

Knowing that *A. nigriculus* can find high-quality habitats offers insight into their potential adaptability to changes in their environment. The climate is continuing to change, therefore altering pond habitats. Quicker drying and limited resources put more pressure on caddisflies to find high quality habitats so they can fully develop before ponds dry. The results from this study give hope for the future of caddisflies in temporary ponds.

References

Kennedy, M., and R. D. Gray. 1993. Can ecological theory predict the distribution of foraging animals? A critical analysis of experiments on the Ideal Free Distribution. *OIKOS* 68:158-166.

- Inkley, M. D., S. A. Wissinger, and B. L. Baros. 2008. Effects of drying regime on microbial colonization and shredder preference in seasonal woodland wetlands. *Freshwater Biology* 53:435-445.
- Lund, J. O., S. A. Wissinger, and B. L. Peckarsky. 2016. Caddisfly behavioral responses to drying cues in temporary ponds: implications for effects of climate change. *Freshwater Science* 35:619-630.
- Wissinger, S. A. 1999a. Ecology of wetland invertebrates: synthesis and applications for conservation and management. *Invertebrates in Freshwater Wetlands of North America: Ecology and Management* (Eds D. P. Batzer, R. R. Rader, and S. A. Wissinger) 1043-1086.
- Wissinger, S. A., H. H. Whiteman, G. B. Sparks, G. L. Rouse, and W. S. Brown. 1999b. Foraging trade-offs along a predator-permanence gradient in subalpine wetlands. *Ecology* 80:2102-2116.
- Wissinger, S. A., W. S. Brown, and J. E. Jannot. 2003. Caddisfly life histories along permanence gradients in high-altitude wetlands in Colorado (U.S.A.). *Freshwater Biology* 48:255-270.
- Wissinger, S., J. Steinmetz, J. S. Alexander, and W. Brown. 2004. Larval cannibalism, time constraints, and adult fitness in caddisflies that inhabit temporary wetlands. *Oecologia* 138:39-47.
- Wissinger, S. A., G. B. Sparks, G. L. Rouse, W. S. Brown, and H. Steltzer. Intraguild predation and cannibalism among larvae of detritivorous caddisflies in subalpine wetlands. *Ecological Society of America* 77:2421-2430.

Tables and Figures

Table 1: *Asynarchus nigriculus* distribution one-way ANOVA analyses data

Response	Treatment	Degrees of Freedom	F	P
High quality patch	Density	2	0.048	0.953186
	Elapsed Time	6	4.693	0.000537
	Density * Elapsed Time	12	1.937	0.046684
Medium quality patch	Density	2	4.441	0.0158
	Elapsed Time	6	0.931	0.4792
	Density * Elapsed Time	12	0.890	0.5619
Low quality patch	Density	2	0.194	0.8245
	Elapsed Time	6	2.888	0.0151
	Density * Elapsed Time	12	1.129	0.3538
Open habitat	Density	2	0.380	0.685
	Elapsed Time	6	8.190	<0.0001
	Density * Elapsed Time	12	1.529	0.138

One-way ANOVA analyses were performed looking at the proportion of caddisflies in each habitat quality with treatments of caddisfly density and elapsed time. Elapsed time was highly significant in high quality patches with density in relationship to elapsed time being mildly significant. Density was mildly significant in medium quality patches, and elapsed time was mildly significant in low quality patches. Elapsed time was highly significant for open habitats.

Table 2: *Asynarchus nigriculus* distribution one-way ANOVA analyses data excluding open areas and first data point

Response	Treatment	Degrees of Freedom	F	P
High quality patch	Density	2	0.538	0.586
	Elapsed Time	6	1.677	0.142
	Density * Elapsed Time	12	2.097	0.030
Medium quality patch	Density	2	3.073	0.0534
	Elapsed Time	6	0.874	0.5194
	Density * Elapsed Time	12	1.273	0.2571
Low quality patch	Density	2	0.026	0.9746
	Elapsed Time	6	2.383	0.0389
	Density * Elapsed Time	12	1.149	0.3394

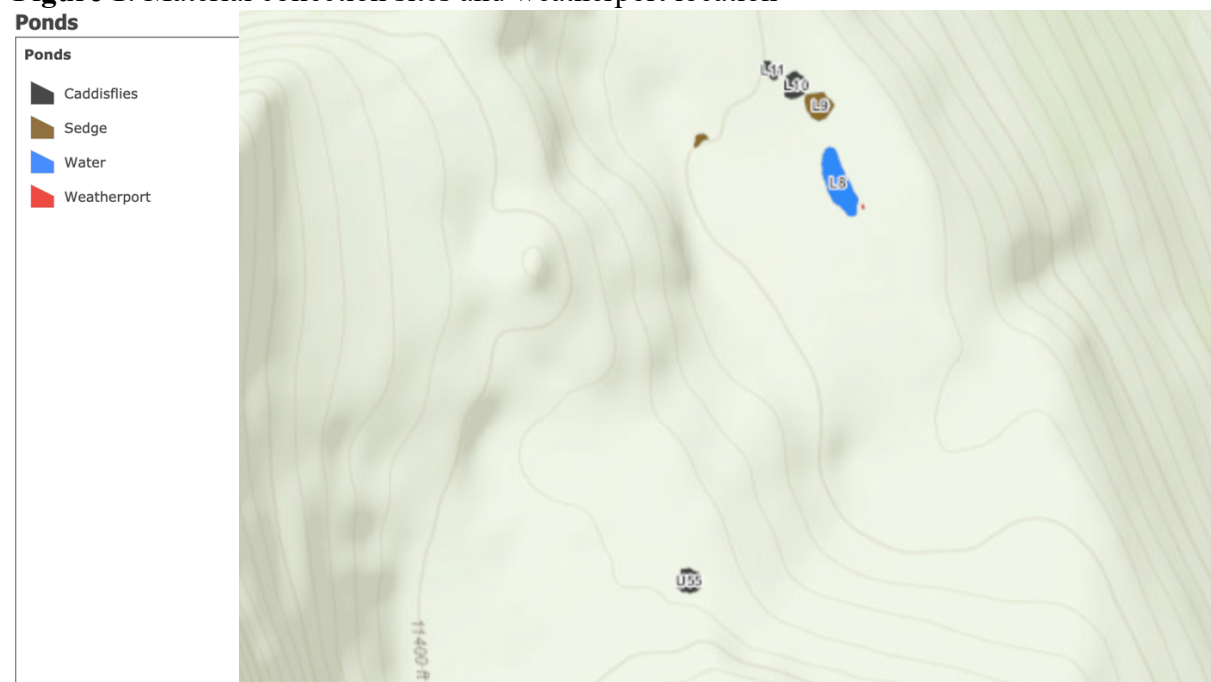
One-way ANOVA analyses were performed looking at the proportion of caddisflies in each habitat quality, excluding open areas, with treatments of caddisfly and elapsed time. Density in relationship with elapsed time was mildly significant for high-quality patches, density was mildly significant for medium-quality patches, and elapsed time was mildly significant for low-quality patches.

Table 3: Mean proportion (excluding open areas) in high density tanks over time

Elapsed Time	High-quality mean proportion	Medium-quality mean proportion	Low-quality mean proportion
1	0.51754808	0.36490385	0.11754808
20.5	0.62083333	0.24583333	0.13333333
44.83	0.47857143	0.47142857	0.05
116.25	0.17410714	0.52719156	0.2987013
119.25	0.61190476	0.18392857	0.20416667
142.68	0.44166667	0.41666667	0.14166667
166.25	0.35169363	0.41527113	0.23303524

The mean proportion of caddisflies present in each detritus patch quality, excluding open areas, was calculated for the high-density tanks. There is a correlation between the high and medium-quality tanks where the mean proportion present in medium-quality tanks increased as the proportion in high-quality tanks decreased and vice versa.

Figure 1: Material collection sites and weatherport location



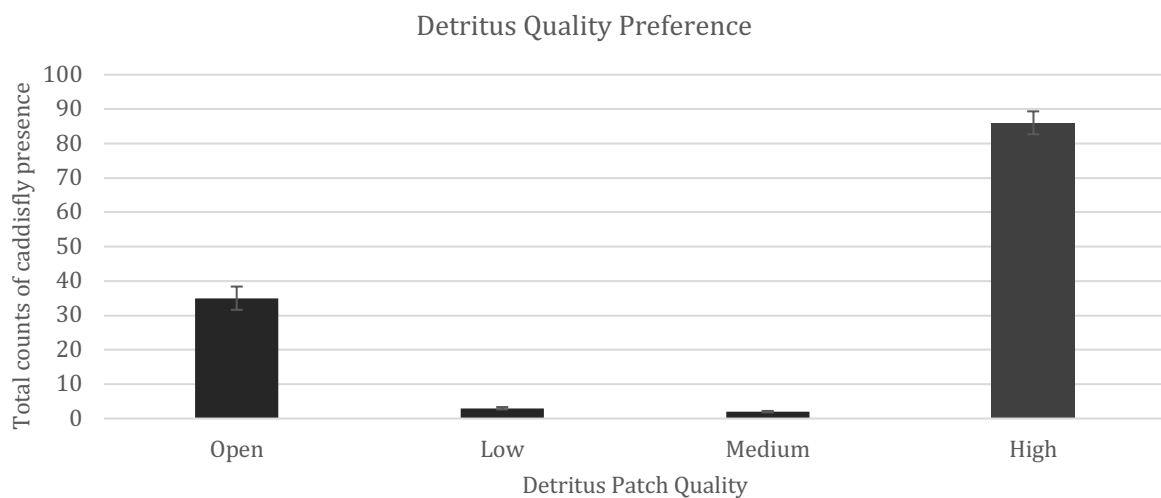
A map indicating which ponds were used to gather each material for the study. Caddisflies were gathered from ponds 10, 11, and 55. Water was taken from pond 8. Sedge was gathered from ponds 9 and 14.

Figure 2: Testing tank



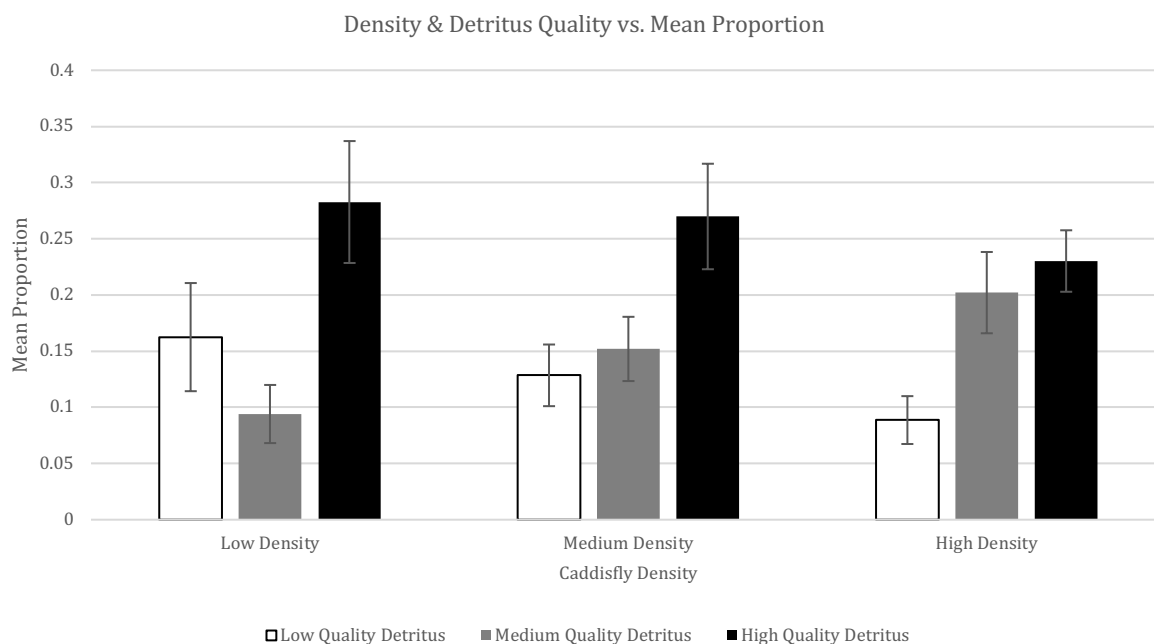
Image of one of the testing tanks with three detritus patches of different qualities. *A. nigrificulus* can be seen in various positions around the tank.

Figure 3: Detritus quality preference



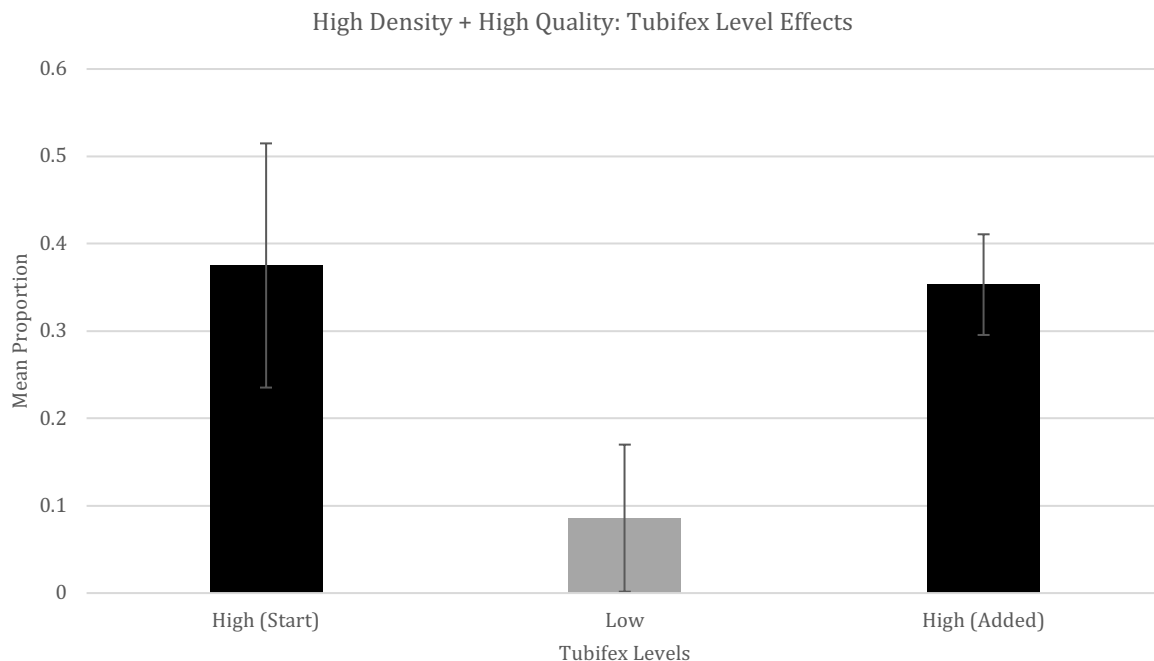
This graph shows that over the course of an hour, the high-quality detritus patch was the patch with the highest total counts of caddisfly presence. The open habitat had the second highest number of counts followed by the low-quality and medium-quality detritus patches.

Figure 4: Mean proportion present in each detritus quality patch across densities



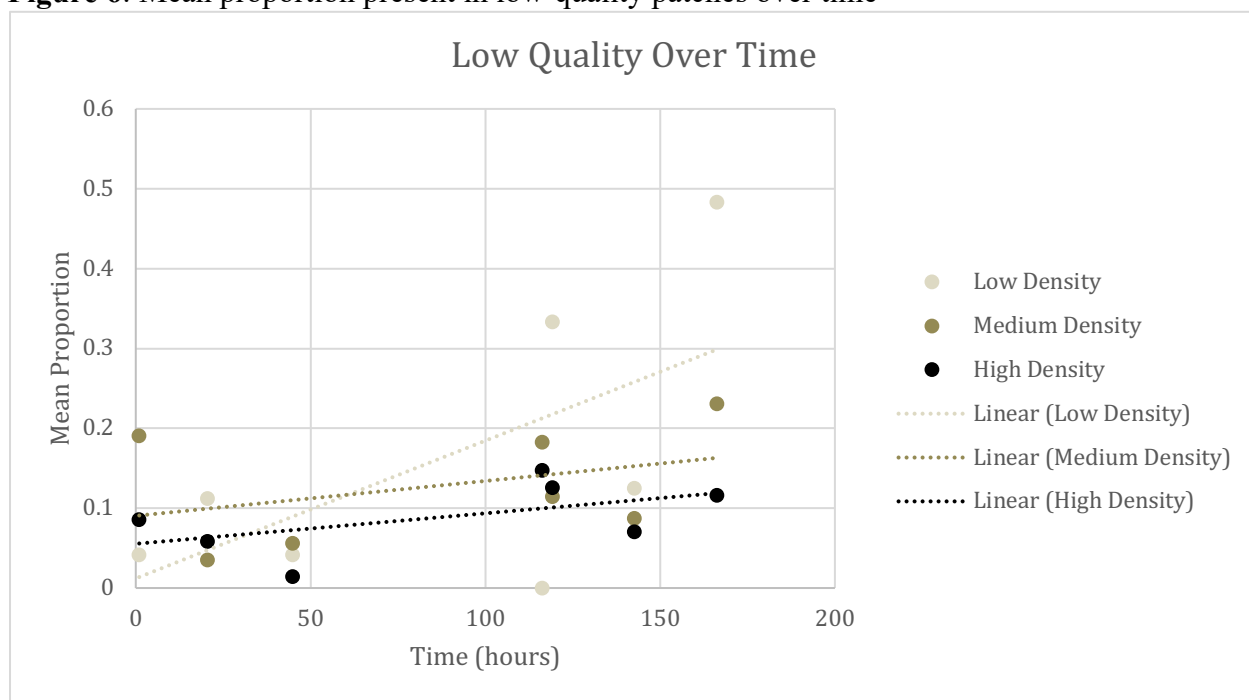
This graph shows the mean proportion of caddisflies found in each detritus patch quality at each caddisfly density. The overlapping of standard error bars indicate that caddisfly density was only mildly significant for medium-quality patches and was not significant for high or low-quality patches.

Figure 5: Effect of time on high-quality patches at high densities



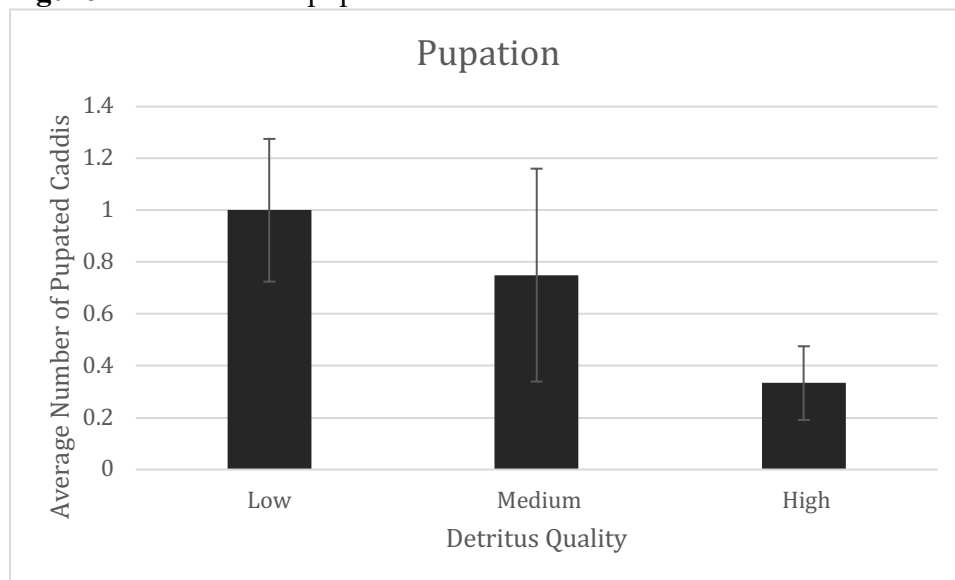
This table shows the effect of time on high-quality patches at high caddisfly densities. The mean proportion of caddisflies present in the high-quality detritus patches was high at the start of the experiment and shortly after adding new tubifex worms when tubifex levels were high. The mean proportion was low midway through the experiment before adding new tubifex when the tubifex levels were presumably low. This shows the effect of elapsed time on high-quality patches at high densities.

Figure 6: Mean proportion present in low-quality patches over time



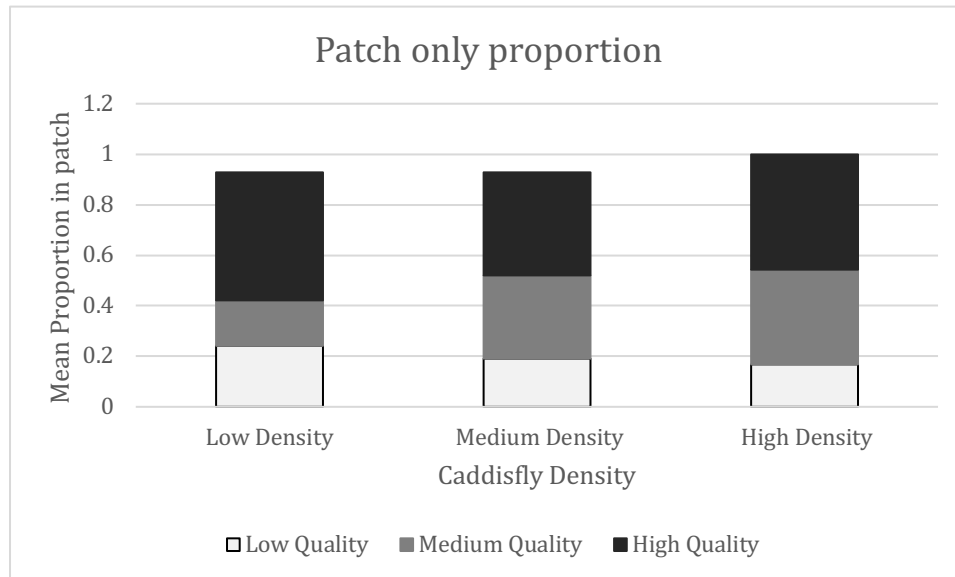
This graph shows the mean proportion of caddisflies present in low-quality detritus patches at all three densities over time. There is a positive trend for each density with the trend for low density being stronger.

Figure 7: Locations of pupated caddisflies



On average, more caddisflies pupated in low-quality detritus patches followed by medium then high-quality patches. While the standard error bars overlap, there is still a visible trend for pupation locations.

Figure 8: Mean proportion of caddisflies in each patch quality (excluding open areas) across all densities



The mean proportion of caddisflies present in each detritus patch quality was calculated excluding the open areas and first data point (1 hour after start of experiment). There is more than 33% of the caddisflies in the high-quality patches in all densities signifying a non-random distribution and a preference for the high-quality patches.