

**Evaluation of *Acilius* larvae (Coleoptera: Dytiscidae) for biocontrol of mosquito larvae**

BIOS 569: Practicum in Field Biology

Nicholas Deason

Advisor: Todd Crowl

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## Abstract

Mosquitoes are common nuisance pests as well as the primary vectors for several human diseases. Current methods of mosquito control rely mostly upon expensive and highly regulated sprayings of insecticides. As a result, methods for biological control have received increased scientific attention in recent years. This report evaluates the mosquito control potential of aquatic larvae of *Acilius* dytiscid beetles collected in northern Wisconsin and Michigan. Results showed that *Acilius* were effective predators of mosquito larvae, increasing their predation rates in the presence of high densities of mosquito prey. Dytiscids were able to completely eliminate mosquito larvae from outdoor aquatic mesocosms over several days. Additionally, *Acilius* larvae preferred to prey on mosquito larvae over chironomid larvae when given a choice in laboratory experiments. These findings suggest that *Acilius* larvae have potential for biocontrol of larval mosquito populations, but further study is needed to determine the methodology for augmentative release and other potential effects of widespread application.

## Introduction

The mosquitoes (Culicidae) are a family of biting flies in which, with few exceptions, the female requires a blood meal to complete development of her eggs. This extreme nutritional requirement causes mosquitoes to be nuisance pests to human populations, and to function as vectors of infectious diseases including malaria, dengue, and West Nile virus. As a result, mosquito population control is a worldwide public health concern (Shaalán and Canyon 2009). However, current methods of control rely upon expensive and highly regulated sprayings of insecticides that can harm the environment and may only be effective for short periods while mosquito populations develop resistance and immunity (Vézilier et al. 2012). Approaches to biological control, especially at the larval stage, have received increased scientific attention as alternatives to harsh chemical applications (Shaalán and Canyon 2009).

The aquatic larvae of predaceous diving beetles (family Dytiscidae) are known to predate on mosquito larvae, but little has been done to evaluate their potential for biocontrol (James 1961, Lundkvist et al. 2003, Nilsson and Söderström 1988). Dytiscid beetles are generalists and prey on a range of aquatic species from cladoceran zooplankton (Arts et al. 1981) to anuran tadpoles (Adams 2006), but several species of the genus *Agabus* were found to preferentially predate on mosquito larvae over copepod and ostracod prey (Culler and Lamp 2009). Further exploration of prey preference is needed to ensure the effectiveness of using dytiscids for mosquito control.

In the present study, dytiscid larvae of the genus *Acilius* were collected from vernal pools in northern Michigan and Wisconsin and assessed for their mosquito control potential. I hypothesized that *Acilius* would be efficient predators of mosquito larvae in laboratory and

mesocosm experiments, and that they may be useful in future applications as biocontrol agents of mosquito populations.

## **Methods**

All research was conducted on the property of the University of Notre Dame Environmental Research Center in northern Wisconsin and the upper peninsula of Michigan. The property is primarily second-growth northern hardwood forest. Mosquito larvae of all instars were collected on 3 July from roadside ditches fed by the overflow of vernal ponds after heavy rainfall. Species of the genera *Aedes*, *Anopheles*, and *Culex* were identified using standard keys (Barr 1958) to obtain a general picture of the assemblage of diversity. *Acilius* larvae were collected by dip-netting in several vernal ponds and were also identified to genus (Gordon and Post 1965). All beetle larvae were between one and two inches in length. Collection sites were vernal ponds with both open and closed canopy and that persisted through many weeks of the summer months.

### **Predation Rate Assay**

Ten *Acilius* larvae were starved for one day after collection, then placed singly into separate small plastic containers containing 400 ml of tap water. Different initial amounts of mosquito larvae, 5, 10, 15, 20, or 25 mosquitoes, were added to each container; thus there were two containers at each level of mosquito larval density. The beetle larvae were allowed to feed for thirty minutes at which time final counts of mosquito larvae were recorded and any uneaten larvae removed. Trials were run on five successive days with the same ten beetle larvae and without additional starving between days. Rates of predation were calculated in number of individuals consumed per minute.

## **Outdoor Mesocosm Experiment**

Aquatic mesocosms simulating simple aquatic ecosystems were constructed by adding 800 ml of ditch water (filtered through a 63 $\mu$ m sieve to remove large invertebrates and debris) to 27 plastic buckets 45 cm in diameter. The buckets were placed single file outside in the shade and left open to rain water and oviposition by mosquitoes. Eighteen buckets were randomly chosen to receive initial inoculations of 100 mosquito larvae each. Nine of those buckets also received one *Acilius* larva each. A separate set of nine buckets received only ditch water and no mosquitoes or beetles. These served as controls to observe the occurrence of mosquito oviposition. Bucket mesocosms were then checked every night from 7 July to 18 July for the presence of mosquito pupae, which were counted and immediately removed.

## **Selective Predation Assay**

Thirty glass jars containing 200 ml of tap water each received a single *Acilius* larvae (starved for one day) as well as five mosquito larvae and five chironomid larvae (collected from vernal pools). *Acilius* were allowed to feed for an hour and the final number of mosquito and chironomid larvae remaining were counted.

## **Data Analysis**

*Predation Rate Assay:* The predation rates of mosquito larvae by *Acilius* larvae were calculated in units of individuals consumed per minute at five different densities (5, 10, 15, 20, or 25 mosquitoes per container). The average consumption rates were plotted and fitted with a linear regression equation.

*Outdoor Mesocosm Trials:* The average number of pupae found each night in the buckets for each treatment (beetle + mosquitoes, mosquitoes, control) was plotted over the twelve day duration of the study. The distribution of the data failed a test of normality so treatment means

were compared using a nonparametric Friedman ANOVA test followed by Dunn's multiple comparison test.

*Selective Predation Assay:* The mean numbers of mosquitoes and chironomids consumed by *Acilius* in glass containers were compared using a paired t-test.

## Results

The average rate of mosquito larvae consumption per minute by an *Acilius* larva over thirty minutes increased in a linear fashion as the density of initial mosquito larvae was increased from 5 to 25 mosquito larvae per container. Figure 1 shows the plotted average consumption rates at each density level fitted with a linear regression model (slope =  $0.0078 \pm 0.0007414$ , y-intercept =  $0.009667 \pm 0.01229$ , r-squared = 0.9736). The highest rate of predation was seen at an initial prey density of 25 mosquito larvae per container. In this case *Acilius* larvae averaged 0.22 mosquitoes consumed per minute, or approximately one larvae eaten every four minutes.

Figure 2 shows the average number of mosquito pupae found in each treatment level of the outdoor aquatic mesocosms (*Acilius* + mosquitoes, mosquitoes, control) through the 12 day trial. The number of pupae collected from the buckets inoculated with mosquitoes but not *Acilius* was found to be significantly higher ( $p < 0.05$ ) over the 12 day period than the number from buckets in the "*Acilius* present" and "control" treatments (Table 1). Additionally, new oviposition by mosquitoes was observed in the buckets by day three, and pupae were emerging by day nine as indicated by the peak in the "control" line in Figure 2.

In the selective predation assay, a paired t-test indicated that the average number of larval mosquitoes consumed by *Acilius* larvae was significantly higher ( $p < .01$ , Table 2) than the

average number of chironomid larvae consumed. The mean numbers of prey consumed are shown in Figure 3.

### **Discussion**

Results supported the hypothesis that larvae of the predacious diving beetle *Acilius* are efficient predators of mosquito larvae. Mosquitoes of several genera (*Aedes*, *Anopheles*, *Culex*) were consumed in laboratory assays and were preferred to alternative prey options, suggesting the effectiveness of *Acilius* as agents of biocontrol.

The predation rate assay showed that *Acilius* larvae had a higher rate of predation at higher densities of mosquito larvae. This is likely due to the increased chance of encountering prey at higher densities. However, neither saturation nor satiation of the predator was observed at the highest density of 25 mosquito larvae per 400 ml of water. Higher densities of prey need to be tested to determine the limits of *Acilius* predation rate. Foraging behavior, which may vary with prey density, must also be taken into account (Formanowicz 1982).

After one week in outdoor mesocosms, *Acilius* larvae were able to eliminate mosquito populations that initially contained 100 individuals. The most voracious beetles eliminated mosquitoes by the third day, indicating a maximum predation rate of 33 mosquito larvae consumed per day per *Acilius* larva. This is consistent with the findings of Chandra et al. (2008) who observed that a single *Acilius sulcatus* larva consumed on average 34 fourth instar larvae of *Culex quinquefasciatus* per day. Additionally the *Acilius* larvae in the outdoor mesocosms prevented the appearance of any mosquito pupae after the seventh day even though the buckets were open to oviposition by new mosquitoes. This may be due do the consumption of newly

hatched mosquito larvae by the dytiscids or the active avoidance of predator-containing buckets by ovipositing female mosquitoes (Vonesh and Blaustein 2011).

Finally, *Acilius* larvae showed a preference for mosquito larval prey over chironomid larvae. These results suggest that the generalist beetle larvae may have more selective predation habits than previously believed. However, chironomid larvae reside mostly in the benthic zone of aquatic systems and thus may naturally avoid foraging dytiscid larvae near the water's surface. Nevertheless, the choice test shows that *Acilius* have prey preferences which merit further investigation. One of the main concerns of using dytiscid beetles for biocontrol of mosquitoes is the negative effect it may have on other species of aquatic invertebrates (Shaalon and Canyon 2009). However, Cobbaert et al. (2010) found that while overall biomass of aquatic invertebrate prey decreased in ponds with introduced dytiscids, diversity was not affected. Still, the effect on other aquatic organisms such as developing amphibians needs to be assessed before any large-scale introductions are to take place.

Based on the current findings, the most promising use of *Acilius* beetle larvae for biocontrol of mosquitoes is in places where dytiscids are not already present and operating at full predatory capacity. This includes many man-made water bodies such as artificial backyard ponds, roadside ditches, and newly constructed wetland habitats (Culler and Lamp 2009). This strategy would also limit the possible negative effects on other prey organisms which are not likely to be found in these artificial environments. Because dytiscids already exhibit natural control mosquito larvae in bodies of water where the range of the two organisms overlap, a policy of dytiscid conservation should be applied to the management of any ecosystem already populated by the predaceous diving beetles. In conclusion, dytiscid beetle larvae of the genus



*Acilius* show promise for use as biocontrol agents of mosquito larvae, but the appropriate strategy for release and management still demands further investigation.

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## Appendix

### Tables

<b>Friedman test</b>	
P value	<b>0.0003*</b>
Number of groups	3
Friedman statistic	16.31

\*Statistically significant

<b>Dunn's Multiple Comparison Test</b>		
	Difference in rank sum	Significant? P < 0.05?
<i>Acilius</i> present vs <i>Acilius</i> absent	-15.5	<b>Yes</b>
<i>Acilius</i> present vs Control	2	No
<i>Acilius</i> absent vs Control	17.5	<b>Yes</b>

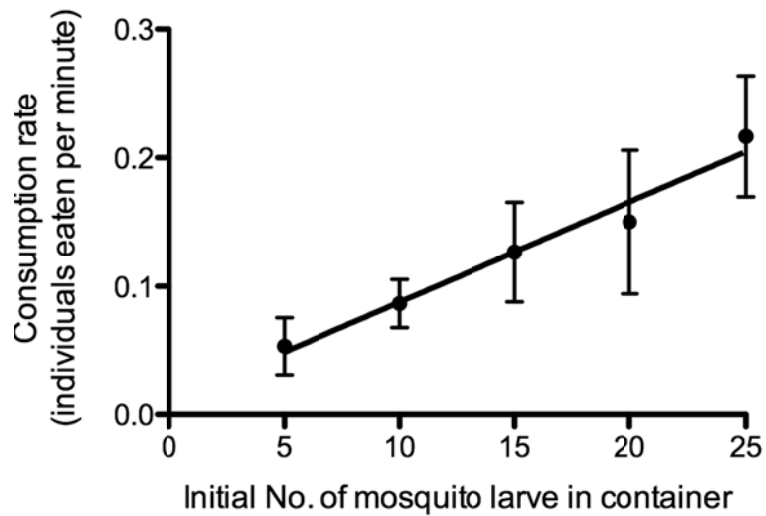
**Table 1.** Results of Friedman nonparametric ANOVA comparing the mean numbers of mosquito pupae collected from the three treatment levels (*Acilius* present, *Acilius* absent, and control) of outdoor aquatic mesocosms; followed by Dunn's multiple comparison post-hoc test indicating significant differences between mean pupal numbers of “*Acilius* present” and “*Acilius* absent” treatments, and “*Acilius* absent” vs “Control” treatments.

<b>Paired t test</b>	
P value	<b>&lt; 0.0001*</b>
t	4.64
df	29
Number of pairs	30

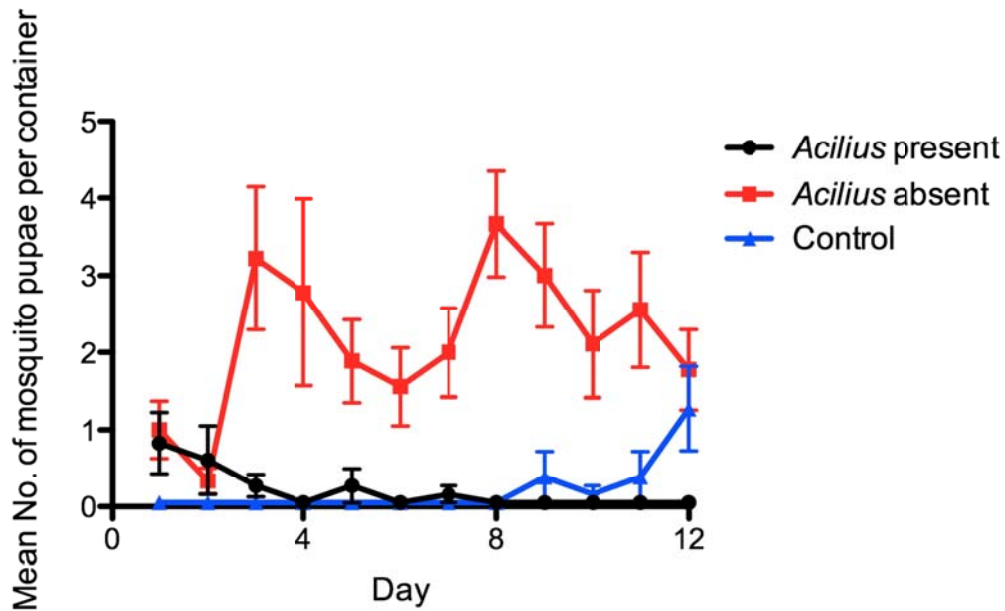
\*Statistically significant

**Table 2.** Results of a paired t-test comparing the mean number of chironomid larvae and mosquito larvae consumed (1.23 and 2.63, respectively) by *Acilius* larvae. The beetle larvae consumed significantly more mosquito larvae than chironomids.

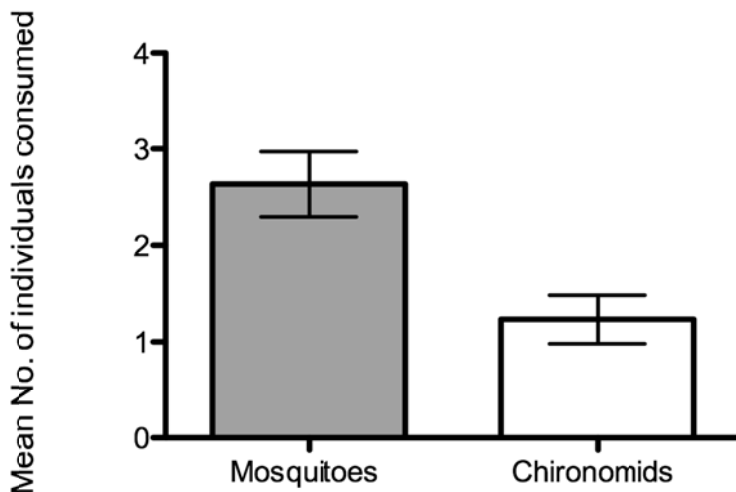
## Figures



**Figure 1.** Average larval consumption rate (per minute) of mosquitoes by *Acilius* in plastic containers ( $n = 30$ ) over a thirty minute time period. Line shows a best-fit linear regression model of the means (slope =  $0.0078 \pm 0.0007414$ , y-intercept =  $0.009667 \pm 0.01229$ , r-squared = 0.9736). Predation rate values for the different initial densities are: 5 – 0.053 mosquitoes consumed/min., 10 – 0.086 mosquitoes consumed/min., 15 – 0.126 mosquitoes consumed/min., 20 – 0.15 mosquitoes consumed/min., 25 – 0.216 mosquitoes consumed/min. Bars show  $\pm 1$  SEM.



**Figure 2.** Mean number of mosquito pupae collected from outdoor aquatic mesocosms over a twelve day period from 7 July to 18 July. There were three treatments ( $n = 9$  for each level) – buckets with an initial inoculation of 100 mosquito larvae + one *Acilius* larvae (“*Acilius* present”), buckets with 100 mosquito larvae and no beetle (“*Acilius* absent”), and control buckets with only water. The pupae collected from the control buckets from Day 9 onward are the result of natural oviposition by mosquitoes. The average number of pupae collected from the “*Acilius* absent” treatment was significantly higher ( $p < 0.05$ ) than the other two treatments based on a Friedman ANOVA (see Table 1). Bars show  $\pm 1$  SEM.



**Figure 3.** Average number of mosquitoes and chironomids consumed by an *Acilius* larvae over an hour in glass containers filled with 200 ml of water. Each container ( $n = 30$ ) contained one beetle larvae along with five mosquito and five chironomid larvae. An average of 2.63 mosquito

larvae and 1.23 chironomid larvae were consumed in the one hour period. The consumption of mosquitoes was significantly larger ( $p \ll .01$ ) than the consumption of chironomid larvae (see Table 2). Bars show  $\pm 1$  SEM.