Stymying a persistent Northwood's pest: Investigating *Pimephales promelas* and *Epitheca spinigera* as potential biocontrols of *Culicidae* larvae in northern Wisconsin and Michigan's Upper Peninsula

BIOS 35501: Practicum in Environmental Field Biology
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Abstract

In the Northwoods, where tourism is paramount to local economies, mosquitoes (*Culicidae*) have a drastic impact on the financial stability of area inhabitants. Additionally, mosquitoes are vectors of harmful zoonotic diseases and pose a health risk to human populations. To combat the spread of these pests in the region, this study investigated easily-implemented, cost-effective, and sustainable mosquito larvae biocontrol options. The two native organisms upon which this investigation focused were Fathead minnows (*Pimephales promelas*) and odonate naiads (*Epitheca spinigera*). These organisms were purchased from nearby bait shops and trials were run in aquatic mesocosms to compare the number of consumed mosquito larvae. Mosquito larvae were collected in the field on property administrated by the University of Notre Dame Environmental Research Center. Results, although not statistically significant, showed strong trends towards significance, with both organisms showing biocontrol potential, and Fathead minnows being the most voracious predators of mosquito larvae. Minnow mortality played an unexpected, yet integral, role in the study, and more research must be done to investigate the predation capabilities of the two biocontrol organisms and to assure the viability of minnow populations when transplanted to new environments. However, initial results show promise, and with continued investigation, Fathead minnows and odonate larvae could be valuable tools in the fight against encroaching mosquito populations.

Introduction

Negative effects of mosquito population on humans

Tourism is vital in the Northwoods, with estimates noting the industry supports almost 15% of the region's workforce (Figure 1). In Wisconsin alone, tourism has a 20-billion-dollar impact on the state and supports nearly 200,000 jobs (Malina 2017). However, the substantial mosquito populations associated with the region's marshy ecosystems limit the area's attractiveness as a tourist destination, leading some tourists to turn away (Moberg 2014). Wisconsin and Michigan are home to over 50 species of mosquito, and these mosquitoes are often more than merely a nuisance. Many species common to the region can transmit zoonotic diseases, including West Nile Virus, La Crosse Encephalitis, and Jamestown Canyon Virus, among others (Paskewitz 2017). The acceleration of climate change, which warms global temperatures and allows poikilothermic mosquitoes to expand their habitats northwards, has increased the prevalence of these diseases in

Wisconsin markedly over the past several years (Figure 2). In fact, *Aedes albopictus* mosquitoes, which are capable of spreading the Zika Virus, were recently found in South-Central Wisconsin (Shastri 2017). To protect their health and to improve the value of their properties, many home and resort owners pursue various mosquito-control practices; most have endeavored to do so by contracting extermination services to eliminate their mosquito problem with chemical pesticides.

Problems with chemical pesticides

Despite the widespread use of chemical pesticides in the Northwoods, few studies have quantified their effectiveness. Additionally, several researchers have pointed out the inherent flaws in chemical pesticides, including their negative effects on human safety and on the health of the ecosystems into which they are deployed (Kim et al. 2017; Carson 1962). Insecticides have been shown to negatively affect the biodiversity of the environment, which, if combined with biological or manmade stressors, can lead to catastrophic ecosystem collapse (Brittain et al. 2010; Geiger et al. 2010).

Mosquito exterminators spreading pesticides is a widely applicable solution, as it is easy for individual landowners to utilize. With minimal labor on the part of the individual, pesticides can drastically alter mosquito populations. However, the simplicity of chemical pesticides is offset by the long-term ineffectiveness of the solution. Although these engineered solutions can rapidly reduce mosquito numbers, chemicals will ultimately wash away and the pests will return, often stronger than before. The short lifecycle of the mosquito coupled with the population bottlenecks caused by the application of pesticides lead to mosquitoes rapidly developing resistance to the chemicals (Coleman and Hemingway 2007). Biocontrol methods, relying on naturally evolved relationships between species, are generally more long-lasting (Hoffmann and Frodsham 1993). As such, this study endeavors to investigate the viability of natural biocontrol methods for mosquitoes of the Northwoods.

Potential biocontrol candidate organisms

The study examines well-known and easily available species of biocontrol organisms. The most notable family of minnows which fulfill these characteristics is the Mosquitofish family (*Gambusia*), which are noted for having a particularly ravenous appetite for mosquito larvae (Willems et al 2005). So much so, in fact, that many communities around the country deploy these fish in pools and ponds to reduce the spread of mosquito-borne diseases (Benton County Mosquito Control District 2017; Marin/Sonoma Mosquito and Vector Control District 2017; County of San Diego Vector Control 2017; Greater Los Angeles County Vector Control District 2016; York County Government 2017; Seminole County Mosquito Control Program 2017). Unfortunately, these fish are aggressive predators not only towards mosquito larvae, but also towards other species of invertebrates and towards native fish (Tomelleri and Eberle 1990; Irwin and Paskewitz 2009). Therefore, these fish are not appropriate candidates for transplant into habitats where they are not already native. Hence, they would not be effective predators in the Northwoods. In this region, the Fathead minnow (*Pimephales promelas*) holds the most promise. Fathead minnows are common throughout the United States, are frequently available at bait shops, and are quite resilient of a variety of water conditions (Tomelleri and Eberle 1990). The species is also documented to be a mosquito predator, making Fathead minnows a potential biocontrol candidate worth further investigation (Irwin and Paskewitz 2009).

Dragonflies are nearly ubiquitously regarded as mosquito predators, as their moniker "Mosquito Hawk" surely attests. These hemimetabolous insects are voracious predators in both their nymphal and adult stages of development. Young dragonfly naiads, with specially adapted mouthparts, are some of the most successful predators in the aquatic ecosystem, and adult dragonflies, as fast and agile fliers, are well-suited towards capturing a wide variety of airborne prey. Consequently, one community- Wells, Maine- has taken advantage of dragonflies to help reduce mosquito populations (Morrison 1994). The odonate species which was examined in this study was the Spiny Baskettail (*Epitheca spinigera*). This dragonfly species is common throughout Northern Wisconsin and Michigan, and several sport shops around the region offer the larval forms as bait (Mead 2003).

It was expected that Fathead minnows and odonate larvae would have statistically significant consumption rates when compared to a control group lacking a mosquito predator. Also, statistically significant differences in consumption rates between the two predators were anticipated. Given the Fathead minnow's larger size, these organisms were hypothesized to be the more effective predators of mosquito larvae than *Epitheca spinigera* larvae. However, the minnows were not anticipated to be as well-adapted as the Mosquitofish.

Materials and Methods

Environment surveyed

All mosquito collection took place on the property of the University of Notre Dame Environmental Research Center- East (UNDERC). This property covers approximately 7,500 acres of land on the border between Wisconsin and the Upper Peninsula of Michigan. A combination of several factors, including relatively recent glaciation, a humid climate, and undulating terrain, has led to the widespread proliferation of lakes and wetland habitats in this region (Martin 1916).

Mosquito population in the area

Wisconsin is home to more than 50 unique species of mosquito, including those of the families *Aedes, Anopheles, Coquillettidia (Mansonia), Culex, Culiseta, Ochlerotatus, Psorophora, Uranotaenia,* and *Wyeomyia* (Paskewitz 2017). Previous study has found the most common mosquitoes on property to be *Mansonia peturbans, Aedes punctor, Aedes excrucians*, and *Anopheles walkeri* (Dodds 1995). *Mansonia perturbans*, the most abundant species, is particularly aggressive and persistent in its feeding (Barr 1958). This species overwinters in the larval stage and the larvae are unique in that they obtain their oxygen by siphoning from the roots of aquatic plants. These mosquitoes are challenging to survey in their larval stages due to their tight attachment to wetland flora and due to their preference towards living in deeper areas of vernal pools.

Aedes sp. are abundant in both their larval and adult stages, with populations of adult swarms reaching into the thousands and abundances of larvae being recorded in the area to be as large as 20,000 per square foot of surface area. These species overwinter as eggs, typically emerging in late May (Barr 1958). In contrast to *Mansonia* larvae, *Aedes* larvae do not attach to aquatic plants to obtain their oxygen, but float with their siphon sticking upwards at the surface of the water. As such, these larvae are generally easier to capture.

The *Anopheles* family is infamous throughout the world as it is the principal vector for Malaria. *Anopheles walkeri* is generally not considered to be an influential pest species in this region though, as it seldom achieves substantial population size near human settlements (Centers for Disease Control and Prevention 2015; Barr 1958). In southern regions, this species has been known to overwinter in its adult stage, whereas in Northern Wisconsin, *Anopheles* overwinter as eggs, emerging in mid to late May. Larvae of this species lack a respiratory siphon, so these mosquitoes generally float at the surface of the water in a horizontal alignment (Alameda County Mosquito Abatement District 2017).

Field Work

To collect mosquito larvae, two different surveys were made during the summer, one which took place from June 12-16 and one which took place from July 5-8. Mosquito larvae are found in stagnant pools of warm water, as well as in heavily vegetated edges of swamps, lakes, and streams. In the earlier survey period, vernal pools near the Hank Dormitory were surveyed to find several potential sites for larger-scale collection. Late in the summer, mosquito larvae populations were not as concentrated, so a second survey was necessary. In this more comprehensive survey, several vernal ponds and other bodies of water were found to be potential candidates for collection (Figure 3).

Two sampling techniques were used to collect mosquito larvae at the surveyed locations. During the first collection period, in order to gain more insight into the preponderance of mosquito larvae in particular microhabitats, collections were made using a dipping technique. Researchers dipped the lid of a mason jar 1 to 2 inches under the surface of the water, then examined the collected water for mosquito larvae. These organisms were collected with a pipette, before being stored in mason jars for trial or identification.

During the second collection period, narrow mesh aquarium nets were utilized. These nets were dipped about 3 inches under the water and skimmed along the surface. The collected material, including vegetation, was placed into metal trays, from which a pipette would be used to collect the larvae. Again, the larvae would be placed in a mason jar for use in trials or identification.

Odonate larvae of the species *Epitheca spinigera* were ordered from a The Reel Thing Bait and Tackle in Green Bay, Wisconsin. These dragonfly larvae were stored in a refrigerator to induce a state of anabiosis. Fathead minnows (*Pimephales promelas*) were purchased from Dewey Catchem and How Bait Shop in Arbor Vitae, Wisconsin and Northern Highland Sports in Boulder Junction, Wisconsin. The minnows were stored in an aerated 110-gallon tub filled with water from Tenderfoot Lake and were feed fish flakes twice a day.

Trial design

Trials took place over an eight-hour period beginning around 11 AM. Prior to trials beginning, the 17 cm by 7 cm by 26 cm tanks were filled with water from Tenderfoot Lake, which were aerated for several hours and allowed to warm to room temperature. To replicate the natural microhabitats of the testing organisms, the minnow tanks were filled to a depth of approximately 10 cm, whereas the odonate larvae tanks were filled to a depth of about 4 centimeters. Prior to being immersed in the tanks, odonates and minnows were massed and measured. Minnow measurement extended from the tip of the snout to the beginning of the caudal fin. Odonates were measured from head to the beginning of the cerci. Minnows were fed fish flakes approximately one hour before the acclimation period began. The specimens were placed into their tanks and allowed one hour to acclimate to the environment. Water temperature was around 20□C for all trials, and the pH range of the tanks was between 7.8 and 8.4. After the acclimation period, 20 mosquito larvae were deposited into each of the tanks, including three control tanks, to which no odonate larva or minnow specimen was added. Four hours later, the tanks would be examined to make sure all the predators were still alive and to check the water conditions.

Trials ended after eight hours, at which point odonate specimens and minnows would be extracted from their tanks and the number of mosquito larvae remaining in the tanks would be recorded. A final temperature and pH reading was also made. Then, mosquito larvae were repackaged into sets of 20 for future trials. After testing, to avoid pseudoreplication, minnow specimens were placed in a new tank and odonate larvae were relocated to a nearby vernal pond.

Statistical analysis

Initially, a Shapiro Wilks test was run to test the normality of the consumption data which was obtained during the trials. Once the data was found not to be normal, a non-parametric Kruskal Wallis test was run to test for variance among the different treatments and the control group. These tests were performed in the statistical program MYSTAT.

In Microsoft Excel, a number of t-tests were performed to compare minnow mortality rates. These tests focused on comparing the number of minnows which died in the first stage of trials to the number which died in the second stage of trials. They also compared minnow mortality rates by trial number. Graphs of my data were also generated in Excel.

Results

One control data point was eliminated as it was a significant outlier, which may be attributed to the emergence of several adult mosquitoes from their pupal casings. The outlying measurement occurred in a tank with a high proportion of pupa, meaning mosquitoes which would have counted towards the control were able to escape. Since consumption data was found to not be normally distributed, the non-parametric Kruskal-Wallis test was used to compare the two biocontrol treatments and the control. Although no significant difference was found between the three groups, a trend towards significance was present (mean ± SE; Fathead, 7.269230769 ±

1.294683819; Odonate Larvae, 5.142857143 ± 0.967231161; Control, 3.214285714 ± 0.701154816, H_2 = 4.228102, dF = 2, p = 0.120748; Figure 4).

Minnow and odonate mortality observations were unexpected, yet valuable, results gathered during the course of the study. The minnows were especially difficult to keep alive. Dozens of Fatheads were killed prior to being tested, despite best efforts being given to provide them clean water, sufficient space, and adequate nutrition. Eight minnows died during the trial periods as well, which comprised approximately 31% of the total sample size. To compare minnow mortality by trial, I used several t-tests. These tests did not find statistically significant difference in the mortality rates; however, a trend towards significance was found (Mortality Rate; Trial 1, 0%, Trial 2, 0%, Trial 3, 25%, Trial 4, 66.67%, Trial 5, 100%, t-Stat = -1.796997384, dF = 8, p = 0.110056856; Figure 5). Mortality in the odonate population was not substantial, with only one odonate larva dying during the trial period.

Discussion

Although the treatments did not differ from one another in a statistically significant way, they exhibited a trend towards significance (Figure 4). Mean mosquito consumption data shows notable differences between treatments, with Fathead minnows consuming the most mosquito larvae, as hypothesized. Odonate larvae were not as voracious consumers of the mosquito larvae; however, they did appear to reduce the mosquito populations when compared to the control group. These results hold promise with continued study, as the lack of significance in the data can likely be attributed to the small sample size used. This study was unfortunately limited with regard to sample size due to a variety of factors, the two primary ones being time and the availability of mosquito larvae for testing. Additionally, mortality was an issue, as large proportions of the testing organisms, especially the mosquito larvae and the Fathead minnows, died prior to the commencement of the trial periods (Figure 5).

The issue of minnow mortality could be a crucial factor towards assessing the viability of Fathead minnow biocontrol on a large scale. The species is supposed to be particularly resilient, surviving in a variety of water conditions and with minimal human input (Tomelleri and Eberle 1990). However, a considerable number of minnows died in this study despite efforts to maintain optimal laboratory conditions for their survival. The high mortality rate could be explained by the preponderance of physical deformities, such as scarring or damage to fins and tails, in the minnow samples. These signs are indicative of poor bait farming practices (Lymbery 2002). To supply for the vast numbers of recreational fishermen who visit the Northwoods, bait shops often purchase their stock from commercial fish farms. These farms, although producing large quantities of product for consumers, are not always healthy environments for the fish (Stevenson 2007; Pulkkinen et al. 2010). Substantial population sizes invite numerous diseases and transport from bait farms to shops and then to fishermen can often overstress the fish. As a result, Fathead minnow biocontrol may not be a viable method to reduce mosquito populations unless the health of the minnow population is assured. As the research showed, merely buying Fatheads from bait shops and releasing them into mosquito infested environments may not be adequate, as a significant proportion of the predators will die off prior to affecting the mosquito population.

In contrast, dragonfly larvae were found to be relatively easy to store and to keep alive. Storing the odonates in the refrigerator allowed them to stay alive without needing to be fed. Once placed in warmer water, it only took minutes for the larvae to regain full activity levels. Dragonflies as a biocontrol measure also have the added benefit of being a predator of adult stage mosquitoes, meaning the release of dragonfly nymphs will have an enhanced effect on the reduction of mosquito populations. More research must be done to ascertain which species of odonate would be the most effective mosquito predator and to determine how well dragonfly larvae are able to adapt to new environments in the long-term, such as would be the case if these organisms were adapted for biocontrol.

Another one of the limiting factors of the experiment, that is, the availability of mosquito larvae, offers clues as to the efficacy of dragonfly larvae as biocontrol agents. In late May and early June, as the first round of mosquito larvae collections began, a surplus of these juvenile mosquitoes were found around property. The same could not be said for odonate larvae, which were seldom discovered during the collection procedure. As the summer progressed, this ratio began to flip. Fewer mosquito larvae were found in the ponds where, earlier, plenty were collected. During examination of the detritus, however, far more odonate naiads were found. The reason for these observations can be traced to the unique lifecycles of the two organisms. Most mosquito species, including the four most common species on property at UNDERC, *Mansonia peturbans, Aedes punctor, Aedes excrucians*, and *Anopheles walker,* emerge from hibernation in May or early June (Barr 1958). A wide variety of odonate species emerge far later, including several which wait until August or September (Mead 2003). Mosquito populations were particularly high early in the summer as the number of natural predators, including odonates, was relatively small. When mosquito predator populations reached high numbers later in the summer, it became far more challenging to discover these pests, as many had likely already been consumed. Naturally, mosquito populations are limited by the presence of dragonflies, so odonates could certainly be used as a biocontrol organism in Northern Wisconsin and Michigan.

As with any insect extermination solution, concerns as to environmental sustainability surely arise when discussing the limitation of mosquito populations. Considering the vast swarms of mosquitoes encountered throughout the Northwoods, it is imperative to consider how the reduction of this large group of organisms will affect the broader ecosystem. Mosquitoes are a substantial source of biomass for higher-level consumers, including larger insects, birds, reptiles, anurans, and even mammals. Recent studies have noted that despite this contribution, mosquitoes are generally not a keystone species in their environments (Fang 2010). The organisms which consume mosquitoes are usually generalists, meaning the reduction, or elimination, of mosquito populations would not likely lead to adverse trophic effects.

More concerns may also arise from the chemical insecticide industry, which would surely be threatened by the widespread adoption of mosquito biocontrol techniques. These companies need not feel threatened, as environmentally sustainable insecticides can still be incredibly valuable when used in combination with effective biocontrol techniques. The combination of multiple control techniques, including biological, chemical, mechanical, and cultural controls, is known as Integrated Pest Management (IPM), and is, historically, the most successful means to eliminate detrimental organisms (Pérez-Hedoa et al. 2017). Throughout the world, success stories attributable to IPM abound, especially in the European Union, where the practice has been mandatory since 2014 (Lefebvre et al. 2015). IPM practices have also been effective more locally, and many farmers from Wisconsin and Michigan have been able to reduce their crop losses by adopting these sustainable techniques (North Central Integrated Pest Management Center 2010). Biocontrol techniques utilizing Fathead minnows or odonate larvae could easily be worked into a larger-scale IPM program, making it even more challenging for mosquito populations to achieve robust reproduction rates.

The acceleration of climate change and the concentration of human populations near waterfront areas has brought humans and mosquitoes into progressively closer contact. These mosquitoes and the host of diseases they carry pose alarming concerns not only in the Northwoods, but around the world. Fortunately, there is hope. Researchers around the globe are developing a wide variety of new mosquito control techniques, many of which utilize the tenets of IPM in an effort to be both effective and sustainable. In the seemingly unending fight against mosquitoes, Fathead minnows and odonate larvae could be two valuable additions to humanity's arsenal. With continued research, the biocontrols investigated in this study may contribute to the reduction of mosquito populations worldwide, making our planet a healthier and more enjoyable place to live.

Acknowledgements

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Wisconsin apple grower significantly reduces pesticide use through NRCS's Environmental Quality Incentives Program (EQIP). 2010. North Central Integrated Pest Management Center.

Figures

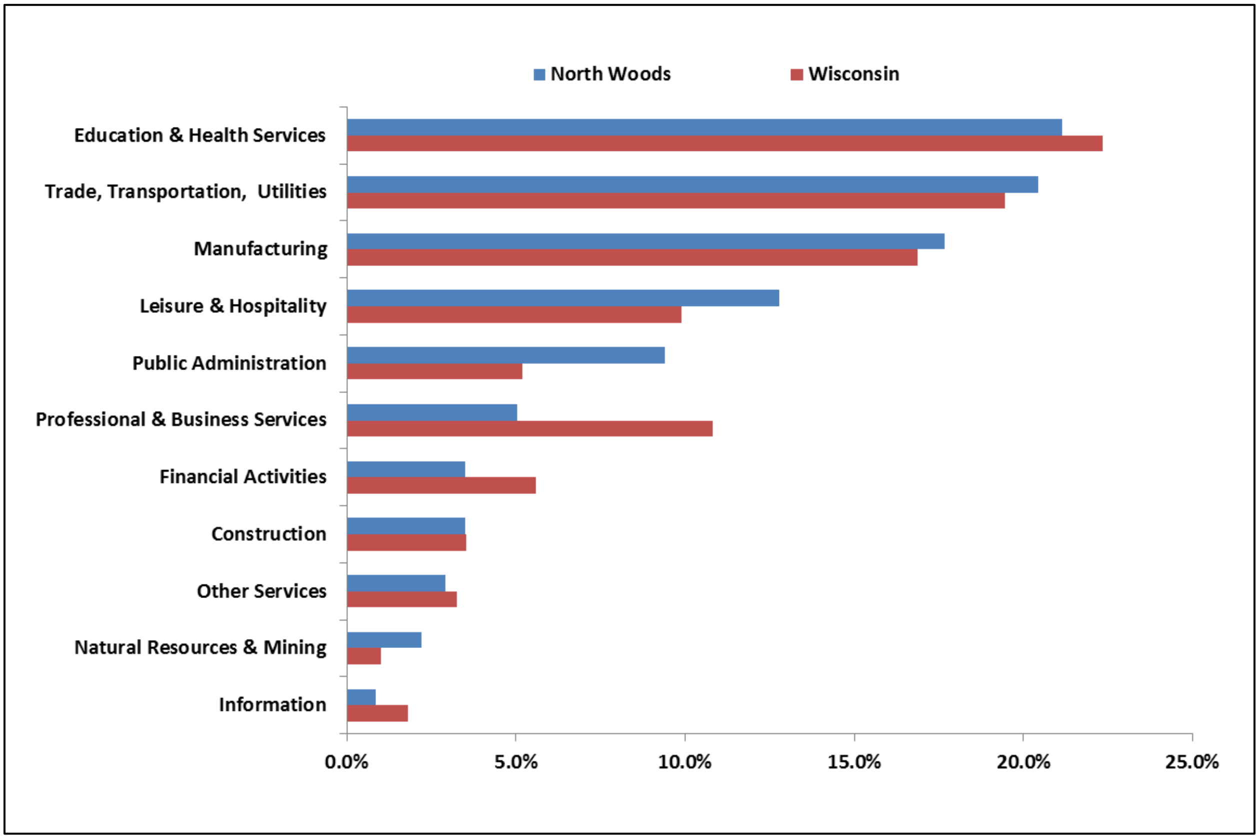

Figure 1. Employment by industry in Northern Wisconsin and the state as a whole. This chart illustrates the relative importance of the leisure and hospitality sector, which encompasses the tourism industry, in the economy of Northern Wisconsin (Bureau of Labor Statistics 2012).

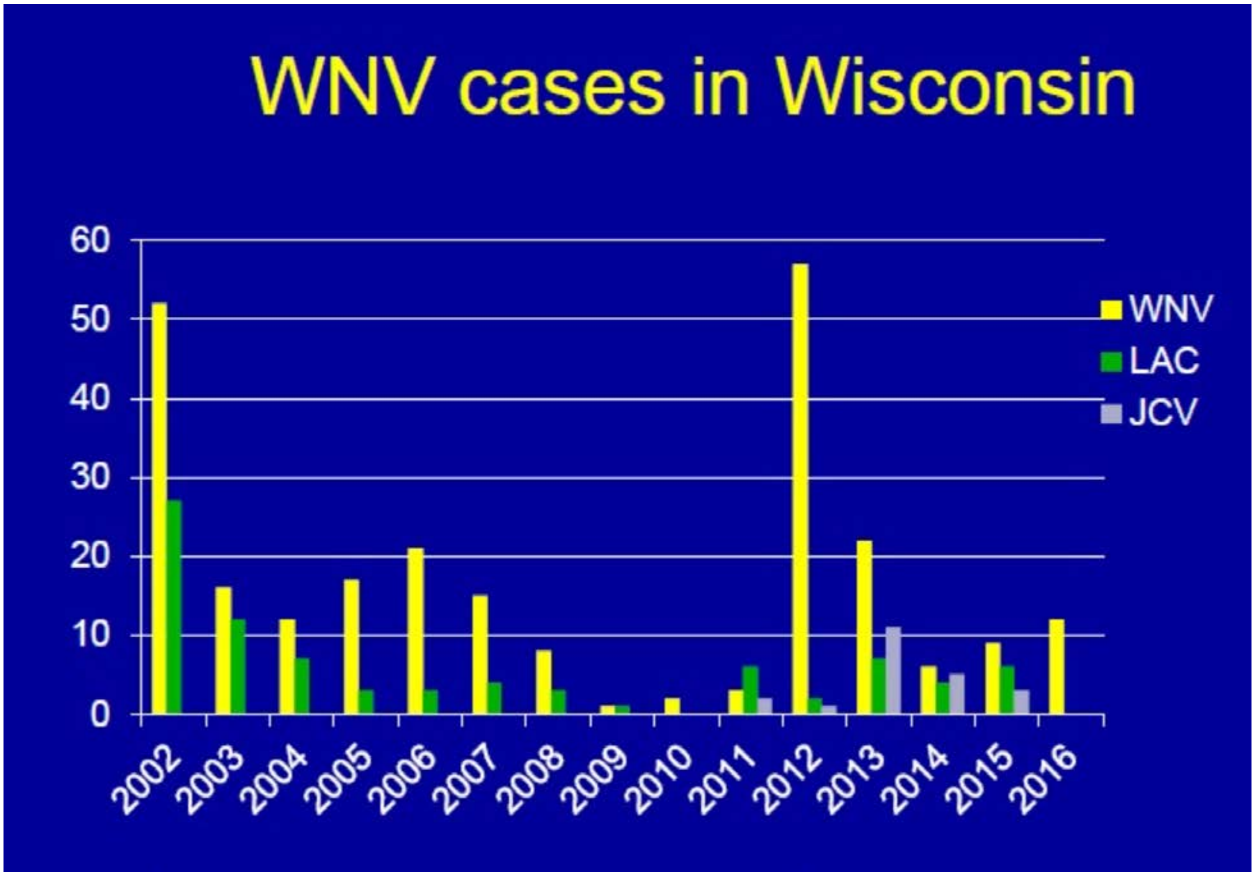

Figure 2. Prevalence of mosquito-borne diseases in Wisconsin since 2002. Although mosquito-borne diseases reached historically low levels in 2009 and 2010, this chart shows the occurrence of West Nile Virus (WNV), Jamestown Canyon Virus (JCV), and La Crosse Encephalitis (LAC) has risen considerably since then (Paskewitz 2017).

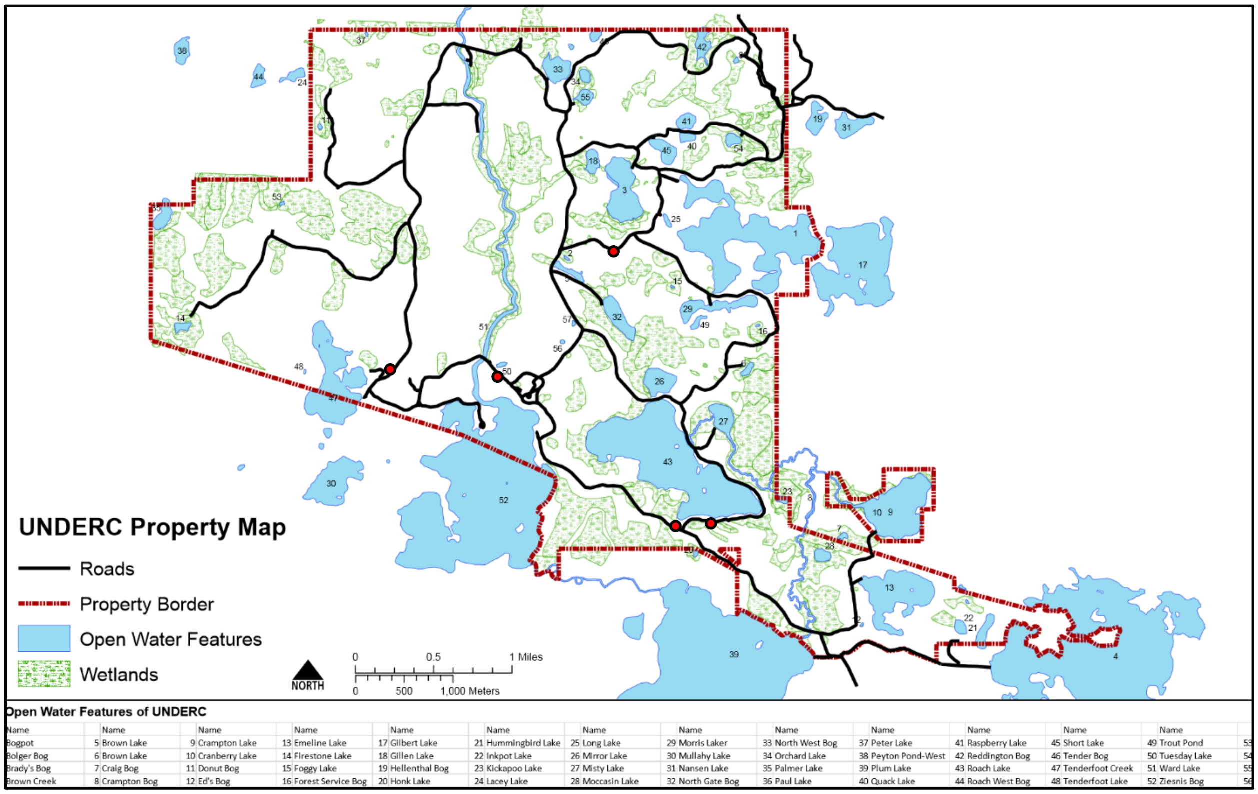

Figure 3. Mosquito collection locations around the UNDERC-East property. The red dots on this map indicate the vernal pools from which mosquito samples were collected. In the early summer, collections were taken from the vernal pool north of Tenderfoot Lake. As the summer progressed and mosquito larvae became more scare, collections expanded to include the other four locations.

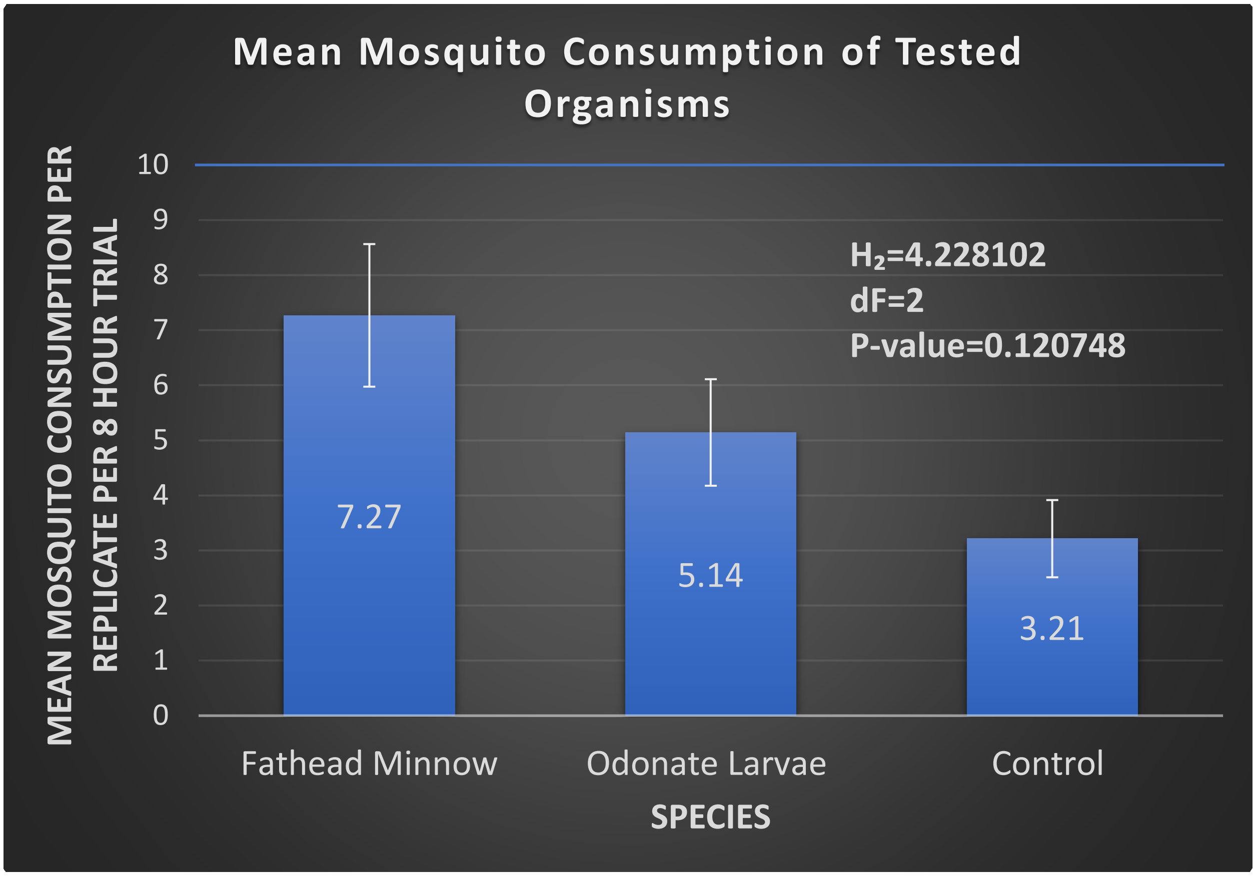

Figure 4. Mean mosquito consumption of tested organisms. This figure records the average mosquito larvae consumption of the two tested predator organisms, Fathead minnows (*Pimephales promelas*) and odonate larvae (*Epitheca spinigera*), over the course of an 8-hour trial period. Error bars represent standard error. The horizontal blue line indicates the expected mean consumption of mosquito larvae by Mosquitofish (*Gambusia affinis*) over the same time period (Willems et al. 2005). Although statistically significant results were not discovered, a clear trend towards significance is shown in the data.

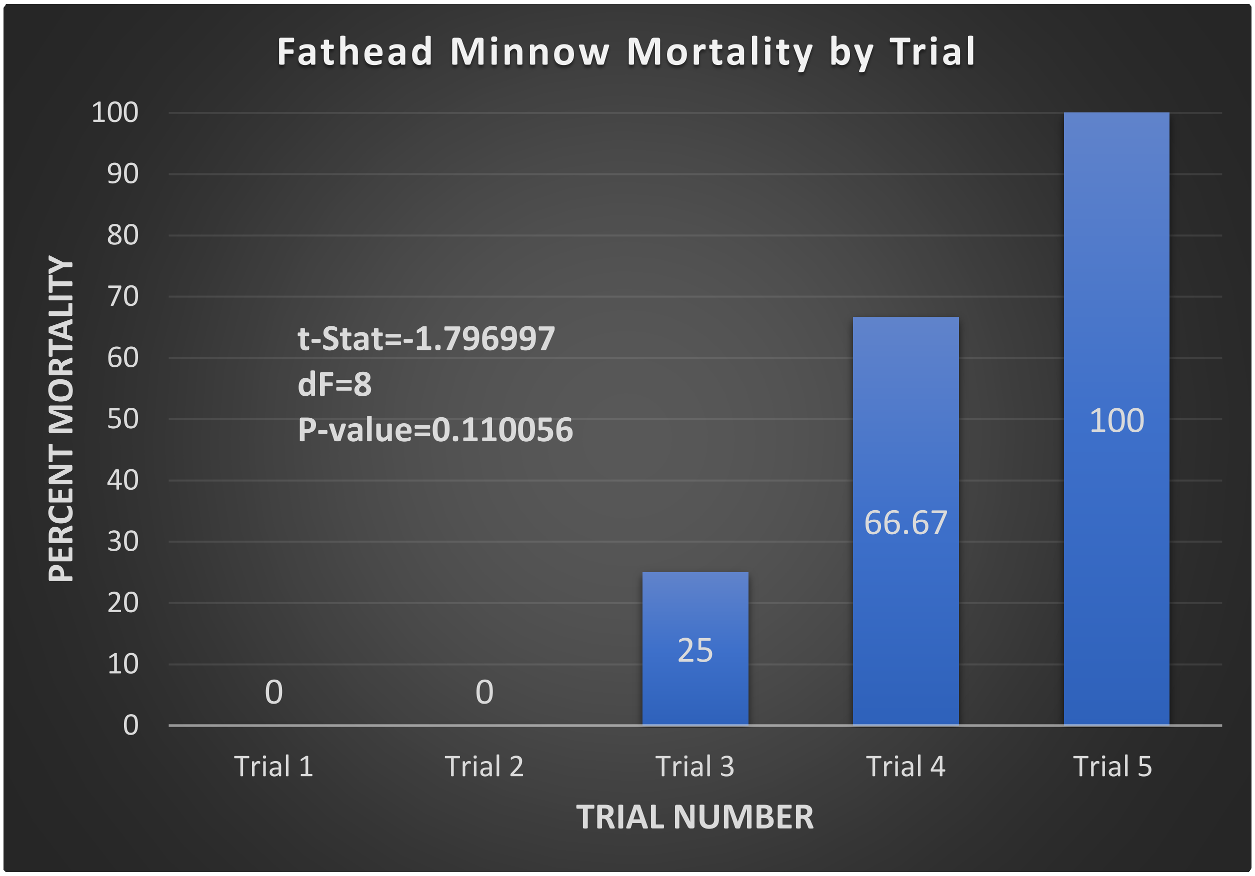

Figure 5. Fathead minnow mortality by trial. This figure records the percent mortality of Fathead minnows (*Pimephales promelas*) over the course of an 8-hour trial period, as organized by trial number. The increasing mortality rate of the minnows as the experiment progressed led to questions as to the health of the minnow population being tested.

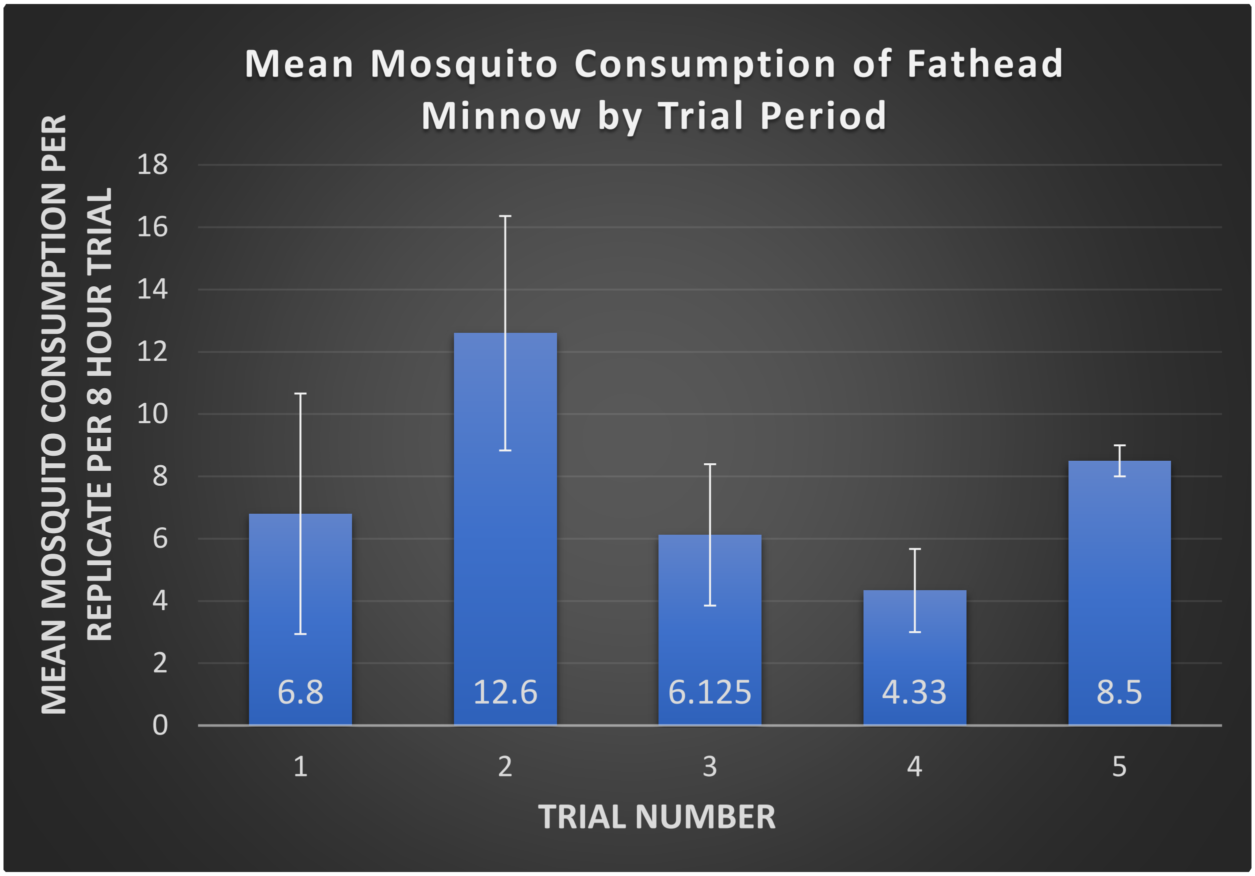

Figure 6. Mean mosquito consumption of fathead minnow by trial period. This figure records the average mosquito larvae consumption of Fathead minnows (*Pimephales promelas*) over the course of an 8-hour trial period, as organized by trial number. Error bars represent standard error.