Effects of Duckweed on Mosquito Oviposition and Larval Survivorship

Abstract

Invasive species are those non-natives to habitat and that negatively impact the environment or human health problems. Invasive mosquitoes threaten the environment and humans, as they transmit 17% of infectious diseases including malaria, West Nile virus and yellow fever. *Aedes japonicas* and *Aedes albopictus* are invasive mosquitoes that often deposit eggs in artificial containers. Chemical pesticides, such as *Bacillus thuringiensis israelensis* (BTI), utilizes endospores to release toxins into the larvae. However, BTI creates negative consequences for the environment and other species. Many plants are being examined to see if they can act as natural pesticides. Duckweeds are native to North America and commonly found in freshwater creeks and rivers. Duckweeds present unfavorable mechanisms for survival. It forms thick mats on the surface of the water, which decreases total sunlight and nutrient available for larvae. This study examined the effect of duckweeds (*Spirodela polyrhiza* and *Lemna minor)* on the oviposition and larval survivorship of invasive mosquitoes and the possibility of duckweeds being a natural method of mosquito population control. A total of 16,490 *A. japonicas* and *A. albopictus* with significantly more eggs laid in non-duckweed treatments ($\bar{x}$= 419) versus those with duckweed ($\bar{x}$= 124). *Aedes* mosquitoes tend to avoid sites with duckweed as these plants greatly decrease larval survivorship as 75% survivor in the control treatment compared to 10% in duckweed treatment. The presence of *S. polyrhiza* and *L. minor* also decreases oviposition and larval survivorship in other species. Duckweeds could act as a natural pesticide to better reduce invasive mosquito populations, and thus improving disease transmission to humans.

Overview

Invasive species are harmful species that pose negative consequences due to their introduction (Hill, 2021). There are two types of invasive species: those that are enter into the environment naturally and those that are caused by human modification (Lavergne, 2007). Natural occurrence happens through evolution compared to those that are brought into environments through human actions, such as trade ships (Simberloff, 2010). In total, there are over 6,500 invasive species in the United States that cause unwanted effects on native species and the ecosystems (Invasive Species Program, 2023).

Invasive mosquitoes pose significant threats to human health and the environment as they carry infectious diseases, including West Nile virus and malaria (Benedict et al., 2007). Mosquitoes develop through a multi-stage life cycle before transitioning into the terrestrial adult stage (Crans, 2004). Females prefer to lay their eggs in artificial containers as these tend to be safer environments for larvae as there is a lack of predators and abundant food source (Gasper et al., 2012). Once hatched, immature larvae develop in the same environment until progressing into the adult stage. The larval phase is when the mosquitoes are most vulnerable to methods of population control. Natural solutions to mosquito population control have been examined in attempts to prevent additional damage to the environment and other species living in it (Crans, 2004).

Duckweeds are aquatic floating plants native to North America and are commonly found in lakes, rivers, and ponds. Due to its individual stems and oval shaped leaves, duckweeds are able to form thick mats on the surface of the water (Eid et al., 1992). This causes a decrease in oxygen and nutrients in the water, along with a higher water temperature. These conditions are not ideal for anything living below the surface of the water. It is these characteristics that suggest duckweeds to be an option for use as a natural option for mosquito population control (Eid et al., 1992).

This study examined the effects of duckweeds on mosquito egg laying rates and the larval survivorship. Eight blocks consisting of two treatments were established in two forested areas at Arcadia University (Montgomery County, PA, USA). Blocks consisted of two black plastic cups filled with creek water. One cup also had duckweed added, while one remains just creek water. On the sides of the cups, wooden tongue compressors covered in a brown paper towel were secured to the side of the cups for females to lay eggs on. The sticks were collected to count the total amount of eggs laid in each treatment, and then left to dry for two days before being returned to a tub a creek water to hatch. Larvae were identified based off hairlike structures. Asian tiger mosquitoes were kept, while all others were discarded. This entire process was repeated later in the year with 15 blocks. Larvae were then divided into ten cups, five with duckweed and creek water and five with only creek water. Nets were placed over each cup to contain any adults. Larvae were monitored to determine how many were able to fully develop into an adult and how many did not survive.

The results demonstrated that the presence of duckweeds reduced mosquito egg laying and larval survivorship. Treatments that did not contain duckweeds saw a greater number of eggs laid indicating that female mosquitoes tend to avoid duckweeds. Other studies suggest that chemical, visual, or olfactory cues can all contribute to why there are less eggs laid in duckweeds. The results of this study can be used to further the research into natural population control methods for invasive species. Future studies can be done to examine duckweeds effects on other invasive species and the effects other plant species can produce. A complete understand of duckweeds against invasive mosquitoes is critical to addressing the global problems stemming from mosquito borne illnesses and the environmental consequences of invasive species.

Introduction

Invasive species are non-native to a habitat and cause negative environmental and human health problems due to their introduction (Hill, 2021). In the United States, there are over 6,500 invasive species impact native species, ecosystems, and human health (Invasive Species Program, 2023). They are brought to new areas through human actions, such as global trade ships (Simberloff, 2010). Other invasive species are introduced to control other species. For example, during the 1800s, sugar cane ships brought rats to the Virgin Islands, which ended up leading to substantial crop damage (Hill, 2021). In an attempt to control the situation, farmers brought in predator mongooses in an attempt eradicate the rats. Here, the rats are an invasive species that was not intended to be introduced to a new habitat, and the mongoose was introduced purposefully by humans as a control measure. However, the mongoose was unable to eliminate invasive rats and therefore became another invasive species (Hill, 2021).

Invasive species pose negative consequences to their new environment (National Invasive Species Information Center, 2023). They often use up available resources relied upon by native species and pose a predator threat to others. In contrast, they lack predators of their own in the area since most predator-prey relationships evolve in a co-evolutionary arms race (National Invasive Species Information Center, 2023). For example, cheetahs and antelopes are consistently evolving with each other, becoming faster than one another in order for the cheetahs to capture prey and antelopes to avoid their predator (Hill, 2021). This provides an advantage to these invasive species, allowing them to flourish and reproduce rapidly without having to prioritize predator avoidance. They tend to utilize unusable resources for native species in the habitat, which lead to them having a dominance in the environment (Hill, 2021). Other contributors to rapid spread are available resources that native species do not utilize. Invasive predators are able to exploit prey due to tactics not previously seen by prey. Unfamiliar mechanisms, such as venom and speed, can be utilized by predators to exploit prey (Hill, 2021).

There are two categories of invasive species: those that are introduced to the environment naturally and those that enter through human modification (Lavergne, 2007). Invasive species that emerge naturally occurs when a species which are limited in their habitat range are introduce to a new genotype or polyploidy. This can eventually lead the species becoming invasive in their native habitats. For example, invasive weedy plants often come about through disturbances like fires or droughts, which favors natural selection on the non-invasive, native species (Lee and Gelembiuk, 2008). Native to North America, *Phalaris arundinacea,* commonly called reed canary grass, became an invasive species in its wetland habitat after human modification caused environmental changes. They can also arise through human adaptations to the environment, often for human activities (Lavergne, 2007). For example, beavers were introduced to Brazil from Canada for recreational hunting; however, they destroyed forests, which reduced native populations due to habitat loss. Trees were not adapted this new predator, so they were unable to regrow after being gnawed (Hill, 2021). Invasive species do not just affect the environment and predator-prey relationship. It is estimated that they cost the United States $120 billion a year in containment, prevention measures, and loss of resources (Crowl, 2008).

There are invasive mosquito species in North America, including the genus *Aedes*. *A. albopictus* and *A. japonicus* are the two main species that are dominate in the region. *A. albopictus*, commonly known as the Asian tiger mosquito, is native to the tropical and subtropical regions of Asia (Benedict et al., 2007). Today, it is found on all continents with the expectation of Antarctica (Versteirt et al., 2009). It was introduced to the United States by the means of tire transport ships arriving in Hawaii and then moving on the United States mainland in 1985 (Moore and Mitchell, 1997). The Asian tiger mosquito is distinguishable through its skinny black body with scattered white stripes on the leg (Moore and Mitchell, 1997). Its larvae are identified through the lack of comb-like structures on the siphon (Figure 1) (Farajollahi and Price, 2013).

Figure 1: *A. albopictus* is a predominant type of invasive species in North America. Eggs are a dark black and oval shaped. Larva hatched in water and are distinguishable through the lack of comb-like structures on its siphon. Adults have a black body with scattered stripes scattered on its legs (Farajollahi and Price, 2013).

A. japonicus, commonly known as the Asian bush mosquito, is native to much of Asia. It has been spreading across the global for over 15 years and is now reported on multiple continents (Kaufman and Fonseca, 2014). *A. japonicus* adult mosquitoes can be distinguished for their large sized and black appearance with white spots on the body and legs (Verstet et al., 2009). Its larvae are identified through the presence of comb-like structures on the siphon (Figure 1) (Farajollahi and Price, 2013).

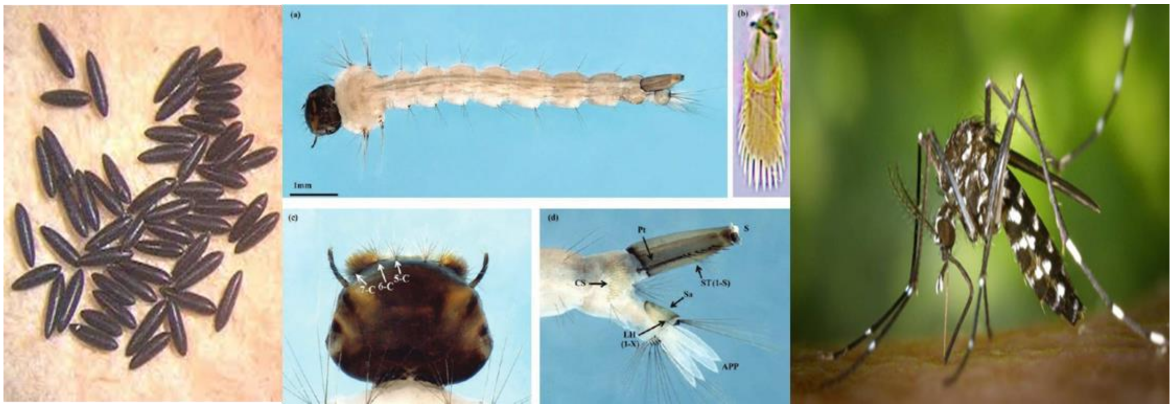

Figure 2: *A. japonicus* is one a predominant type of invasive species in North America. Eggs are black and oval-shaped. Larvae are distinguishable through the presents of dark black tail combs. Adults have a primarily black body with white stripes on the body and legs (Farajollahi and Price, 2013).

The effects of invasive mosquitoes are evolving as different species rapidly spread across the globe. A possible cause for the spread is due to the global rising temperatures. These environmental changes are fueling the rapid spread as warm temperatures are ideal for mosquito breading. For example, *A. japonicus* mosquitoes have been successful as an invasive species due to the ability to tolerate harsh summer temperatures and different compounds found in natural and artificial containers used for oviposition and larval development (Versteirt et al., 2009).

Mosquitoes are one of many types of vectors that can transmit infectious pathogens between humans or from animals to humans (World Health Organization, 2020). The terrestrial adult phase is when mosquitoes are most impactful on the environment and human health (Stevens and Lounibos, 2005). *Aedes* mosquitoes are substantial contributors to vector-borne illnesses, accounting for 17% of infectious disease (World Health Organization, 2020). Both *A. albopictus* and *A. japonicus* are harmful for not only the habitat they invade, but also humans as they carry many viruses, including West Nile virus, dengue virus, Zika virus and yellow fever (Benedict et al., 2007). Dengue has some of the highest case counts of mosquito borne illnesses, infecting more than 3.9 billion people across 129 countries annually (World Health Organization, 2020). Invasive mosquitoes present both environmental and public health dilemmas that will only continue to escalate without population control (World Health Organization, 2020).

Mosquitoes have a multiple stage life cycle consisting of five larval stages and one pupae stage (Crans, 2004). Female mosquitoes look for high quality sites to deposit eggs in the early summer. They are laid above the water and after a few days will hatch onto the water's surface. Eggs will continue hatching, as they are laid, until conditions become unfavorable for survival in the winter months (Crans, 2004). Visually, eggs are microscopic, black oval shaped deposits with a similar appearance to a football (Figure 1 & 2). Once hatched, the immature larvae will mature in the same environment and remain there until development into the adult stage (Crans, 2004).

A large span of the mosquitoes' growth and development occurs in the water. This is why females place a great importance on selecting the best oviposition site for eggs. Both *A. albopictus* and *A. japonicus* have been successful as an invasive species in regions within the United States due to their ability to lay their eggs in multiple container types, including both artificial and natural containers (Gasper et al., 2012). Females tend to have a preference towards artificial containers such as buckets, grills, rock pools, tree holes, tires, and gutters for oviposition (Gasper et al., 2012). These abundant containers provide eggs and future larvae with low predator, low competitor environments (Kershenbaum et al., 2012). Additionally, darker containers give the impression of a deeper, shaded area to females indicating more organic matter food sources (Cuthbert et al., 2019). Mosquitoes have a strong preference for shaded areas due to decreased predation and competition (Binckley and Restarits, 2003).

The aquatic larval phase of mosquitoes is when they interact with and impact native species (Crans, 2004). Few chemical methods have proven some success in controlling the mosquito population, such as *Bacillus thuringiensis israelensis* (BTI) (Binckley, 2017). BTI utilizes endospores to release toxins into the gut of the larvae. These toxins cause a quick death for the larvae (Binckley, 2017). It is effective in controlling mosquito population; however, there are negative factors as well. Mosquitoes seem to be avoiding breeding sites that are contaminated with BTI. This demonstrates there is an adaptation of females avoiding the BTI treated sites (Cuthbert, 2019). Negative factors started the research for a natural alternative to chemical pesticides. Many plants are being examined for pest control due to rising levels of pesticide resistance from artificial control methods. One possible natural alternative for BTI is *Lemnaceae*, or duckweeds.

Duckweeds are aquatic plants native to North America, South America, and Eurasia. It is a freshwater plant commonly found in lakes, rivers, and ponds (Illinois Wildflowers, 2021). Two species of duckweed, *S. polyrhiza* and *Wolffia arrhizal*, are small, free floating, and fast growing, in addition to being native to North America (Figure 3).

Figure 3: Duckweeds are floating aquatic plants that can form physical barriers on the surface of the water. It is thought to be an alternative pesticide to chemicals. It has a single thallus with oval shaped leaves which will form thick mats on the surface of the water (Illinois Wildflowers 2021).

Duckweeds consist of a smooth, oval shaped thallus, allowing it to float on the water surface due to tiny air pockets (Illinois Wildflowers, 2021). Floating, aquatic species, including duckweeds, can form physical barriers on the surface of water preventing mosquito larval survivorship through mechanical mechanisms. The thallus of *S. polyrhiza* and *W. arrhizal* form thick mats on the surface of the water (Eid et al., 1992). These barriers restrict anything below from being able to reach the surface of the water, including eggs ready to hatch. Additionally, this causes decreased amounts of oxygen and nutrients that are able to reach any organisms below the surface (Eid et al., 1992). These characteristics would target the larval stage of mosquito development and make it the target stage for possible control interventions (Crans, 2004). This study will examine the effect of duckweeds on the oviposition and larval survivorship of invasive mosquitoes and the possibility of duckweeds being used as natural method of mosquito population control.

Methods:

A. japonicus oviposition occurs from mid-May to early July, and *A. albopictus* oviposition occurs from mid-July to early October (Binckley and Thomas 2017). This leads to differences in species domination during the experiments. Females use varying cues, visual, and olfactory, in choosing quality oviposition site. Females prefer artificial containers due to the lack of predators. *Aedes* deposit eggs right above the water levels of surfaces, which then require submergence to hatch (Figure 4) (Binckley and Thomas 2017).

Figure 4. Female *Aedes* mosquitoes will lay their eggs right above the water line on ovisticks. Eggs were laid on an ovisticks, which consisted of a tongue compressor and brown paper towel held together with tape. There is a preference for artificial containers due to the lack of predators in the water.

Experiment 1: Effects of Duckweed on Aedes Oviposition

I established eight mosquito oviposition arrays in two forested areas at Arcadia University (Montgomery County, PA, USA) (Figure 4). The experiment ran from June 7th through June 23rd, 2021 in a wooded area near a creek. Each site consisted of four black plastic cups filled with creek water for a total of 32 cups. At each site, holes were dug in a linear array, placing one cup into each. In each array, one cup was randomly assigned the following treatments: high water (400mL) and duckweed, high water (400mL) and no duckweed, low water (125mL) and duckweed, and low water (125mL), and no duckweed. Duckweed treatments were given 3.4gs (wet mass), 0.17g (dry mass) of *S. polyrhiza* and *L. minor* species (Figure 5).

Figure 5: A set up of the control treatment with two ovisticks. Each ovistick is composed of a tongue compressor wrapped in a paper towel and secured with tape. The control only contains water while the other contained water and duckweed.

Duckweed was collected from Fire Pond at the Schuylkill Center for Environmental Education (Philadelphia, PA, U.S.A) using fishing nets (Figure 6).

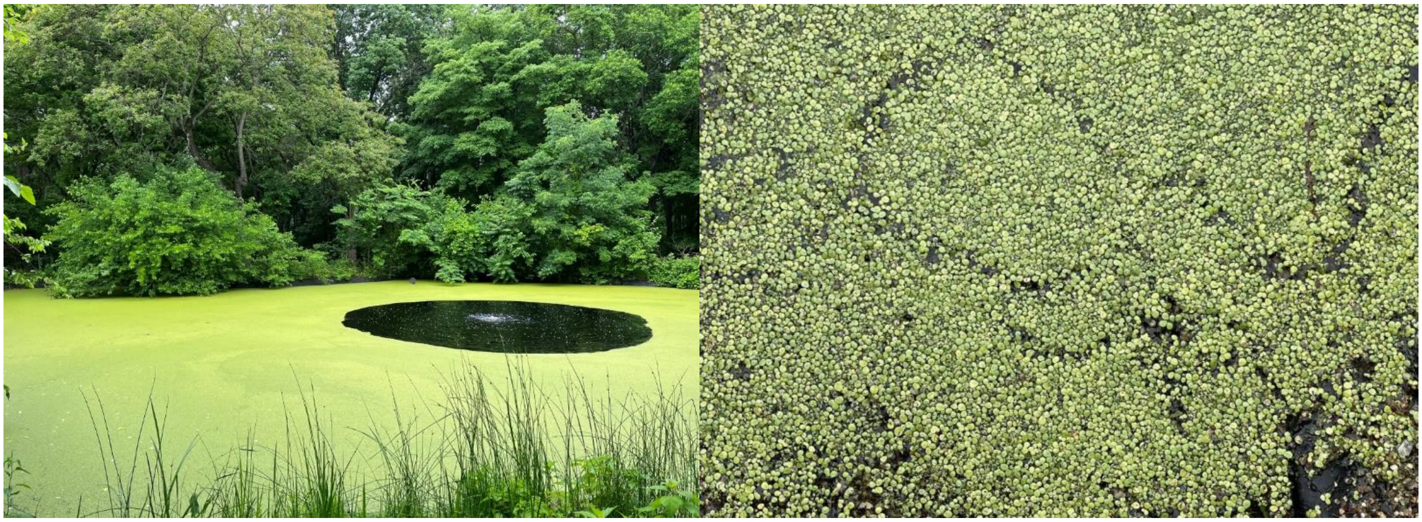

Figure 6. Duckweed was collected from Fire Pond at the Schuylkill Center for Environmental Education (Philadelphia, PA, U.S.A). Two species were collected: *S. polyrhiza* and *L. minor* species.

Storage was in a large plastic tub filled with water and was constantly aerated. All cups consisted of two ovisticks secured to the cups' sides with paperclips. Ovisticks comprise of tongue compressor wrapped in a paper towel, secured with tape. These were the deposit site for the *Aedes* eggs to be collected. Sites were checked daily for any debris and integrity of treatments. Unwanted materials, such as leaves and sticks, were removed.

On June 14th, June 16th, and June 23rd, ovisticks were collected from cups and were brought to the laboratory to count the eggs under a light microscope. Counted ovisticks were dried for two days before being placed into plastic bins filled with 2.5L of creek water to hatch eggs so to identify *Aedes* larvae (Figure 7).

Figure 7. Setup of plastic tubs filled with 2.5L of creek water. Ovisticks were submerged into tubs in order to allow for eggs to hatch before identification was able to take place.

The larvae were identified using defining hairlike structures on the dorsal side of the head and comb structures on lateral end tails of *A. japonicus* (Farajollah and Price, 2013). *A. albopictus* were saved in a fresh tub of creek water for experiment 2 while *A. japonicus* were discarded.

Experiment 2: Effects of Duckweed on Aedes albopictus Survivorship

Five mosquito larval arrays were established at Arcadia University (Montgomery County, PA, U.S.A.) on from July 12th through July 20th, 2021. Each array consisted of two black plastic cups filled with water. Within each array, one cup was randomly assigned to have duckweed or be a control. Twelve *A. albopictus* larvae were placed into each cup. Nets were placed over cups to prevent any adult mosquitoes escaping (Figure 8).

Figure 8. Experiment 2 setup included ten black plastic cups all filled with 400mL of creek water and five were filled with duckweed. Twelve *A. albopictus* larvae were placed into each. Nets were placed over the cups to prevent any adult mosquitoes from escaping.

Sets were checked daily for any mature mosquitoes or any dead larvae. After two weeks, a final count was tallied of how many larvae were able to fully develop and how many were not.

Experiment 3: Effects of Duckweed on Aedes Oviposition Part 2

I established fifteen mosquito oviposition sites in two different forested areas at Arcadia University (Montgomery County, PA, USA). The setup ran from June 14th through July 5th, 2022 near a creek in three wooden areas. Each site consisted of two black plastic cups filled with creek water for a total of 60 cups. At each site, holes were dug in a linear array, placing one cup into each. Within each array, one cup was randomly assigned of the following treatments: 0.17g (dry mass), 3.4g (wet mass) of duckweed or no duckweed. All had the same water level of 400mL. Sites were checked daily to check for any debris and integrity of treatments. On June 21st, June 28th and July 5th, ovisticks were collected and brought into the laboratory to be counted under a light microscope. Ovisticks were left to dry for two days before being placed into plastic bins filled with 2.5L of creek water. The larvae were identified uses the same features stated in experiment 1. All larvae were discarded following identification.

Results

In experiment 1, there was a statistical difference between the control and duckweed treatments on *Aedes* oviposition. A t-test was performed to show that the duckweed treatment received significantly less eggs than the control (p= 0.00279) (Figure 9).

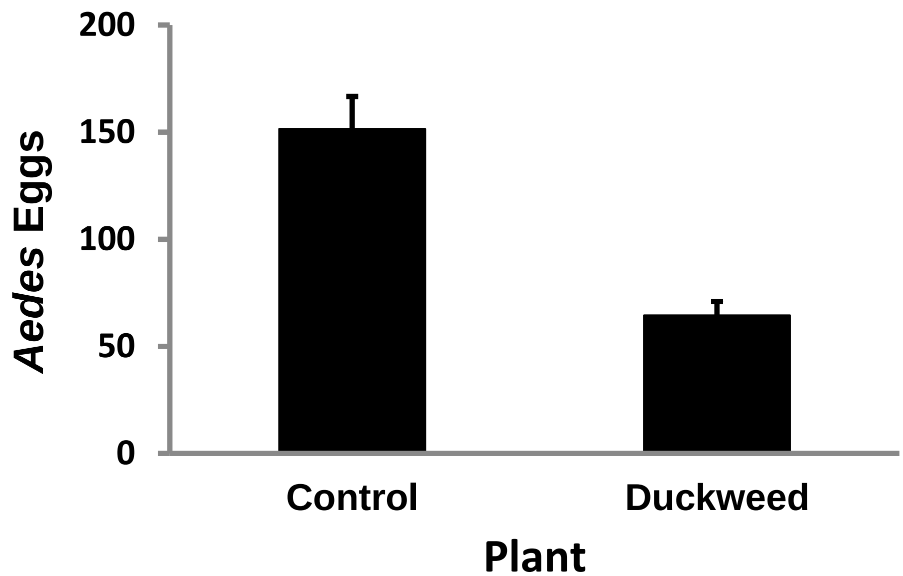

Figure 9. The average number of *Aedes* eggs in the control treatment and duckweed. The mean eggs count for the control (152) and duckweed (64) were statistically significant with a p value of 0.00278 with a preference for the control treatment.

The same analysis was performed for *Aedes* larval survivorship in experiment 2. The control had significantly higher survivorship compared to the duckweed treatment (p= 0.00499) (Figure 10).

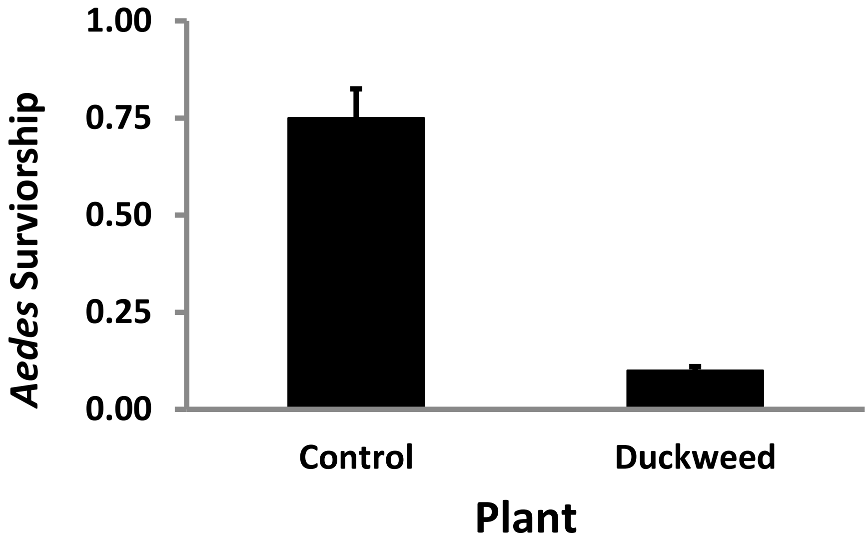

Figure 10. The average percent of *Aedes* larval survivorship. The average percent in the control (0.75) was statistically significant to the duckweed treatment (0.10) (p = 0.00499).

Experiment 3 used the same analysis method to determine a statistical difference in *Aedes* oviposition. The control had significantly more eggs laid then duckweed treatments (p= 0.00000653) (Figure 11).

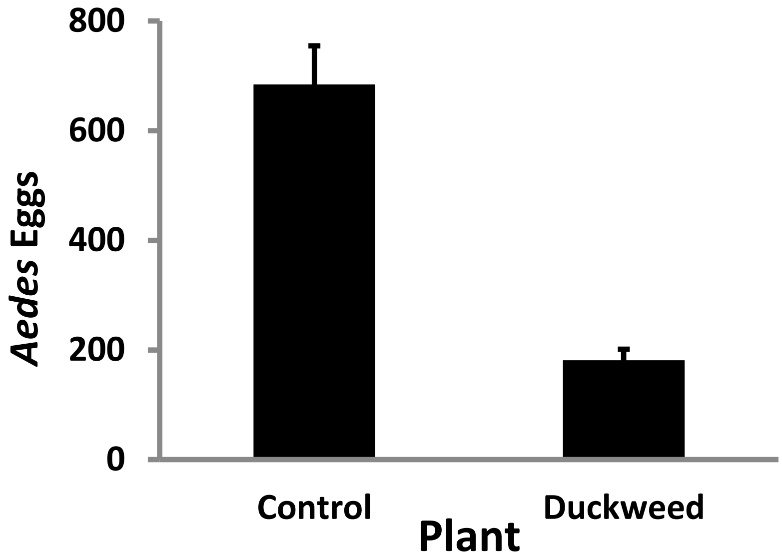

Figure 11. The average number of *Aedes* eggs in the control treatment and duckweed. The mean eggs count for the control (686) and duckweed (183) were statistically significant with a p value of 0.00000653 with a preference for the control treatment.

Discussion:

This study demonstrated that the presence of *S. polyrhiza* and *W. arrhizal* duckweed species reduced mosquito oviposition and larval survivorship. Control treatments received significantly more *A. albopictus* and *A. japonicus* eggs than duckweed treatments. Duckweed treatments also greatly reduced larval survivorship in both *Aedes* species. This presents a possible explanation of a possible preference to environments without duckweed. Females laid the most eggs in environments that lacked duckweed. Our results were consistent with previous findings that suggest duckweed can be an effective and long-term natural method for mosquito population control (Figures 9,10,11).

Other *Aedes* species exhibited similar patterns with *L. minor* (Judd and Borden, 1980). *A. aegypti*, commonly known as the yellow fever mosquito, were impacted negatively from the presence of *L. minor*. Female yellow fever mosquitoes avoided of oviposition sites containing duckweed. Females were determined to be avoidant of the methanolic extracts found in *L. minor* at concentrations of 1000-10,0000 PPM due to females using olfactory cues in selection of oviposition sites (Judd and Borden, 1980). This implies that extract levels must be at a minimum threshold in order to have an impact on mosquitoes. There was a negative correlation between the extract levels and *A. aegypti* oviposition (Judd and Borden, 1980). However, when expanding into *Culex pipiens* mosquitoes, the methanolic extract of *L. minor* did not have the same deterring effects. The extract limited *C. pipiens* at the oviposition sites, but did not completely deter oviposition, suggesting that *L. minor* only played a minor role in the deterring oviposition (Judd and Borden, 1980).

Baz (2017) examined *C. pipiens* female oviposition rates in environments with and without *L. minor* duckweed, in addition to water quality. Female mosquitoes were attracted to the environments with the highest levels of duckweed, even though it proposes a barrier for larval survivorship (Baz, 2017). Females laid the most eggs in high density, regardless of the water quality. Females choose oviposition sites based on different visual and olfactory cues, along with food availability as young survival is directly correlated to the choice of oviposition site. Baz (2017) proposes that *C. pipiens* females are strongly attracted chemical cues that are released by *L. minor*. The attraction to this chemical cue is so strong that females appear to ignore any other cues, such as water quality and food availability, leading to a negative correlation between egg survival and duckweed densities (Baz, 2017). Two proposed reasons for the negative impacts were mechanical obstruction and dryness from the thicker duckweed layers.

There were similar findings that the presence of *L. minor* duckweed increases *C. pipiens* mosquito oviposition and decrease larval survivorship as duckweed was shown to have fatal mechanisms which can be exploited and used for mosquito population control (Cuthbert et al., 2019). A significant number of egg rafts deposited in the presence of duckweed, but there was a minimal number of survivors compared to those without duckweed (Cuthbert et al., 2019). Findings agree chemical and mechanical effects of duckweed are detrimental to larval survivorship (Cuthbert et al., 2019). Chemically, *L. minor* releases cues that attract females to lay eggs in it. (Eid et al., 1992). Mechanically, duckweed forms thick mats on the surface of the water preventing larva from proper oxygen levels and being able to reach the surface of the water to hatch (Cuthbert et al., 2019). Cuthbert et al. (2019) expanded into looking at effects of commercial pond dye which is thought to increase mosquito oviposition. Dye alone had no significant effect on oviposition rates (Cuthbert et al., 2019). When used alongside duckweed, it increased female attractiveness to the site. Furthermore, the number of eggs laid was directly correlated to the addition of pond dye with duckweed (Cuthbert et al., 2019). *C. pipiens* mosquitoes were shown to have a preference towards oviposition sites containing duckweed.

Findings tend to agree, regardless of if there is an attraction to duckweeds or not, that larval development and survival decreased in duckweed environments. This occurred due to the lack of presence other organisms as food sources and sunlight (Baz, 2017). Conditions slowed the development of surviving larvae. The ability of those that survived to breed and reproduce was inhibited in duckweed environments due to low respiration (Baz, 2017). These results can vary when placed into nature rather than a laboratory setting due to environmental factors (Baz, 2017). Microorganisms were affected from lack of sunlight and oxygen caused by thick layers of duckweed. This created harsh living environments for microorganisms and caused many of them to die off. This is crucial since they are a primary food source for the larvae (Baz, 2017). Chemical and structural aspects of duckweed were examined as aspects of natural population control methods as both provided no protection for larvae and made unfavorable living environments (Baz, 2017).

There was a tendency across many studies that showed *Aedes* females to avoid sites that were treated with duckweed. Females are selective when selecting oviposition sites as they have additional challenges of finding the right site to accommodate both aquatic larval and terrestrial adult phases (Kershenbaum et al., 2012). Habitat selection plays an important role in survivorship for *Aedes* larvae, as it acts as the first level of defense (Binckley & Restarits, 2003). Visual cues are often utilized to select the best suited habitat (Marin et al., 2020). Colors preferences of oviposition sites were examined by comparing the total amount of eggs laid at sites colored red, black, green, blue, and orange. Oviposition sites that are colored red or black had the most eggs laid. In contrast, green, orange and blue had the lowest amounts of eggs (Martin et al., 2020). The reasoning behind the color preferences is still not fully understood, although it is thought to involve whether or not the colors absorb or reflect heat and create a warm environment for larvae to flourish (Martin et al., 2020).

In contrast to natural vector control methods, some chemical options, including BTI, have shown success in limiting *Aedes* and *Culex* mosquitoes. *A. albopictus* tended to avoid sites that had BTI present and laid more eggs in the control sites (Binckley and Thomas, 2017). The *Aedes* mosquitoes exhibited similar behavioral trends were seen within duckweed. In contrast, *C. pipiens* were attracted to the oviposition sites that contained BTI and laid more eggs in these sites (Binckley and Thomas, 2017). *Culex* mosquitoes also exhibited similar behavioral patterns as those that were exhibited with duckweeds. *A. japonicas* had no response to BTI at all (Binckley and Thomas, 2017). However, BTI's implications are not limited to only effecting mosquitoes as it causes many negative impacts on other species and the environment itself. It is for reasons such as these that natural alternatives are being looked towards. Studies examining how both duckweed and BTI affect *Culex* oviposition are needed since their combined affect might greatly reduce *Culex* populations as both have been shown to attract egg rafts but kill larvae.

There are other plant options that can be used as a natural solution to mosquito population control. *Ageratum houstonianum* is a medicinal plant whose extract has been examined as a possible natural pesticide (Tennyson et al., 2012). The effect of its crude leaf extract was shown to not have a deterrent feature in *Anopheles stephensi*, *A. aegypti* and *C. quinquefasciatus*. However, there was an overall decrease of total oviposition at sites containing the extract as compared to those without it (Tennyson et al., 2012). The methanolic extract from the crude leaves was of higher levels than that of other plants. This suggests that there must be a certain threshold needed for the female mosquitoes to be able to detect the cues and decide to not oviposit at the site (Tennyson et al., 2012).

A detailed understanding and further research on duckweed is needed in order to be used as a natural tool for vector control. Mosquito borne illnesses are expected to get worse if effective control mechanisms are not implemented. Further studies need to be done to see if *S. polyrhiza* and *L. minor* have the same deterrent effects on all species of mosquitoes. Other species of duckweed should be examined to see if there are similar effects on mosquito oviposition. Additionally, further research needs to be done into alternative plant pesticides to see if there are better options to defend against mosquitoes or to see if there is a threshold amount of the plant needed to be a deterrent and cause the negative implications. If previous studies hold true about insecticidal properties of duckweed on mosquito populations, it can have a benefit to the world regarding human health issues and environmental issues. It can propose an answer to vector control in areas which are harmful or unpractical to use artificial insecticides, like BTI. The ability to observe duckweed in natural habitats is critical to examine if these findings will hold true in nature. A full understanding of duckweed on mosquitoes can be an answer to two global issues: the public health dilemma of mosquito borne illnesses and the harmful effects of invasive species on other species and the environment.

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