INFLUENCE OF DRAGONFLY LARVAE ON MOSQUITO DEVELOPMENT AND SURVIVAL

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Abstract. Mosquito-borne diseases have become of great concern in the world today, as many are re-emerging. This has prompted an interest in better understanding what factors drive or control mosquito populations. One promising bio-control agent of mosquitoes is through the use of mosquito predators as a way of controlling the population. A predator of mosquitoes that has not been researched fully is the dragonfly. The following research examined the influence of dragonfly larvae on several different aspects of a mosquito's lifecycle. This study was done in two parts, first by performing biweekly field surveys of semi-permanent, intermittent, and ephemeral pools. Secondly, experimental mesocosms were done with mosquito larvae both with and without dragonfly larvae present. Mesocosm experiments were done to test the survival, development rate, and reproductive behavior of mosquito larvae (*Aedes* and *Culex*) in the presence of dragonfly larvae (*Tetragoneuria*). Dragonfly larvae were only found to be present in the semi-permanent and intermittent ponds. It was also found that the semi-permanent and intermittent ponds had significantly lower adult mosquito emergence. The results of mesocosm experiments identified dragonfly larvae being able to eat large numbers of mosquito larvae in very short periods of time, but dragonfly larvae did not seem to have a significant effect on the development rate of mosquitoes. Ovipositing behavior of mosquitoes did not seem to be affected by the presence of dragonfly larvae. These results suggest that dragonfly larvae could play a role in the regulation of mosquito populations.

INTRODUCTION

In the following study the relationship between mosquito species and dragonfly species will be examined through the use of broad pond surveys and controlled mesocosm experiments. Influences on mosquito populations have recently been of great interests due to increases in mosquito-borne diseases. In order to understand the reasons behind this growth further research needs to be conducted on what could control mosquito populations. Mosquitoes make up a large portion of dragonflies diet and therefore, dragonflies could play an important role in the regulation of mosquito populations (Kenny and Burne, 2001). Research is needed to better understand how dragonflies might influence mosquito production.

Mosquito-borne diseases, such as West Nile Virus, and pest concerns have initiated an interest in understanding the factors that drive or constrict mosquito production (Leisnham and Slaney, 2009). One major part of understanding influences on mosquito production is to look at the influence of mosquito predators. Predators can strongly influence populations of mosquitoes both by disturbing their development rate and also by consuming mosquito larvae and adults (Stav et al, 2005). Knowledge of predator-prey relationships is pivotal in order to identify mosquito population trends (Juliano, 2007), as the density of mosquitoes is very much affected by predation (Juliano and Lounibos, 2005). The relationship between prey and predator is a classic example of how nature works to regulate the increase and decrease of species. These relationships are what keep many ecosystems in balance. Understanding the relationship between specific prey and predators can also tell you a lot about how the species function in an ecosystem.

There have been a few studies that have found evidence that the presence of predators can influence mosquito development rate. One study found that the mosquito *Ochlerotatus triseriatus* would actually perform metamorphosis at a smaller size when a predator is around (Kesavaraju and Alto, 2007). A recent study on the population dynamics of mosquito populations suggested that predators that kill late stage mosquito larvae might be the best choice when it comes to biological control of mosquitoes (Juliano, 2007). Understanding which predators play a key role in the mosquito lifecycle is therefore important.

The order Odonata includes dragonflies and damselflies and regarded as "very beneficial insects" (Thorp and Covich, 2001). This is due to their large size as larvae, which allows them to prey on many different smaller types of larvae found in lentic pools and ponds. Adult species will prey on many types of flying insects, with its main diet consisting of mosquitoes. Lifecycles of dragonflies can last up to 1 to 6 years, and usually will go through 11 to 12 larval instars. Increases in temperatures can cause the development rate of larvae to increase (Thorp and Covich, 2001). Dragonflies are known to consume mosquitoes and they are often referred to as "mosquito hawks" (Kenny and Burne, 2001). However, consumption of late-stage mosquito larvae by dragonfly larvae has the greatest potential to control biting adult populations (Juliano, 2007).

The data collected in the following study could help to determine if dragonfly larvae strongly influence the abundance of mosquitoes in wetland areas. The study will cover two areas of interest when it comes to mosquito populations: 1) a broad survey of the mosquito and macro-invertebrate species found in a wetland area and 2) controlled experiments to test the affect of dragonfly larvae on mosquito growth and behavior.

Wetlands in heavily forested areas have been found to be the ideal spot for mosquitoes to breed (Rubbo and Lanterman, 2011). Of the various wetland areas, ephemeral and semi-permanent ponds are thought to be the best breeding habitats for mosquitoes. This is due to many factors, but mainly because they hold stagnant water and they usually lack fish. Semi-permanent ponds are areas that retain water during most years except for drought years (Knight and Chase, 2003). Semi-permanent ponds will usually contain mosquito predators, as they hold water long enough for predators to complete their lifecycle. Due to regular drying, ephemeral pools usually cannot support the lifecycles of many predators (Knight and Chase, 2003). More research still needs to be done on the differences between both predator and mosquito populations in these two types of wetlands.

This research examined the following hypotheses using two approaches: 1) field survey and 2) mesocosm experiments.

1) When introduced to an environment with mosquito species, dragonflies will choose to prey on several species of mosquitoes.
2) Mosquito larvae will have lower survival rates to emergence when introduced to an environment with dragonfly larvae.
3) Mosquito species will avoid ovipositing in habitats with dragonfly larvae and will prefer ovipositing in habitats free of predators.

METHODS

Data Sampling: Survey

The survey portion of the study was performed on Cary Institute of Ecosystem Studies property, in Millbrook, NY. The area has several permanent and semi-permanent ponds, but there is also a collection of ephemeral pools that develop after consistent precipitation. The first part of the study included a broad survey of the pools to develop an idea of the dragonfly predators and the mosquito species present in the area. Three semi-permanent ponds and 7 ephemeral pools were used. Two of the ephemeral pools were later labeled "intermittent" due to the difference in drying patterns from the ephemeral pools.

Samples of mosquito larvae were collected using a standard BioQuip mosquito dipper and dipping every 5 meters around ephemeral and intermittent pools and every 10 meters around semi-permanent ponds. Dragonfly larvae were collected using D-nets and sweeping every 10 meters around each pool or pond. When using the D-nets sweeping length was standardized to approximately 1 meter in order to equalize sampling. Mosquito sampling were done at the waters edge, while dragonfly/ sampling was done 2 feet from the waters edge. Mosquito sampling was done on June 18th, July 3rd, July 17th, and August 7th. Macro-invertebrate surveys were done on June 19th/21st and July 15th. All invertebrates were counted and classified into the lowest possible level of identification. Mosquito larvae that were in their 1st or 2nd instar were placed in mosquito growing containers to allow to fully mature to their 3rd or 4th instar development stage for identification. Growth containers included de-chlorinated water and fish food and were placed in a warm (room temperature) environment to stimulate growth. All macro-invertebrates were killed using ethanol and were then identified. All samples were preserved in 70% isopropyl alcohol.

Emergence traps were placed out on each pool to catch any emerging invertebrates. Emergence traps consisted of a wood-frame pyramid (~ 0.35m^2 sampling area) covered with 200µm mesh net and floated on four plastic 1-Liter bottles. Three emergence traps were placed out at each semi-permanent pond and one at each ephemeral/intermittent pool. Ephemeral pool 712 had two emergence traps due to its size. Emergence traps were checked and species collected three times throughout the summer on June 19th/21st, July 2nd, and July 15th.

Data Sampling: Mesocosms

Survival to Emergence Experiment

The first set of experiments was conducted to test the way dragonfly larvae affect mosquito survival rate. Low mosquito density tests:

Eight 6 liter buckets were filled with 1 L of carbon-filtered water and 200 mL of filtered pond water. A single dragonfly larva was added to four buckets. The first set of reps included one bucket with 10 mosquitoes plus dragonfly, 20 mosquitoes plus dragonfly and control buckets for both. The mosquitoes for the first rep were counted after 2 hours and then 3 hours. The second set of reps included the same treatments as the first set of reps, but the mosquitoes were counted and observed after 24 hours. The experiments were done on June 24th.

High mosquito density tests:

Eight 11 liters buckets were filled with 2.75 L of carbon-filtered water, and 500 mL of filtered pond water which was used as the initial nutrition source for the mosquito larvae. In the first treatment four buckets 45 mosquito larvae were introduced as well as one dragonfly larvae (Rubbo and Lanterman, 2011). In the second treatment four buckets with only 45 mosquito larvae were introduced. Each mesocosm was covered with window screen to prevent escape of adults or introduction of other organisms. Both types of buckets were placed in a ambient environment and observed. Every 24 hours the living mosquito larvae were counted and observed for changes in development and mortality. The same was done with the buckets that did not have any dragonflies. The experiment was done over the course of two time frames with 2 buckets of treatment 1 and 2 buckets of treatment 2.The first two reps were started on June 24, 2013 and ended June 26th, 2013. The second two reps were started on July 1st, and ended July 4th, 2013.

Emergence Rate Experiment

The second mesocosm experiment run was done to determine the affect of dragonfly on mosquito larvae's emergence rate. The experiment was conducted using nine 11 L buckets, each with 3 L of carbon-filtered water and 500 mL of pond filtered water. Thirty mosquito larvae were used under three treatments: (1) dragonfly larvae loose (2) dragonfly larvae in net (3) no dragonfly. All mosquito larvae used were of the species *Culex restuans.* Mosquito larvae and any pupae were counted at 3 hours, 12 hours and then every 12 hours for 3 days. After 3 days the mosquito larvae were then counted every 24 hours. At day 4, 8, 12 and 16 500 ml of filtered pond water was added. On day twelve 50 ml of fish food slurry was also added. The experiment was started on July 11th and finished on July 29th.

Oviposition Experiment

The third experiment looked at the oviposition behavior of mosquitoes when dragonfly larvae are present. Six oviposition traps were placed in two rows, three per row. Eleven liter buckets were used with 3 liters of water and 15 dried oak leaves. Three buckets contained dragonfly larvae and three did not. The buckets were checked daily for egg rafts and eggs present on seed paper. Egg rafts and seed paper were collected and brought back to the lab. Individual eggs were counted on each seed paper.

Mosquito Species Preference Experiment

To determine the mosquito species preference of dragonfly larvae an experiment was conducted using ~11 L buckets. *Aedes j. japonicus* and *Culex restuans* were used in this experiment. Three treatments were used: (1) *Aedes j. japonicus* (2) *Culex restuans* (3) an equal mix of both *Aedes j. japonicus* and *Culex restuans.* Twenty mosquito larvae were placed in each bucket along with one dragonfly larvae on July 30th. The buckets were then treated with 20ml of fish food slurry to supply a nutrition source for the mosquito larvae. The mosquito larvae were counted after 1 hour, then after 6 hours. After that the larvae were counted and identified every 24 hours. The experiment ended on August 3rd. All dragonfly larvae were measured and identified as genus *Tramea sp.*

RESULTS

Field Survey

Larval abundance was greatest in ephemeral pools compared to semi-permanent pools (Fig. 1), however there was considerable variation among the individual pools even within each hydrology class. The numbers of adult mosquitoes sampled in emergence traps over each pool was also higher in ephemeral pools compared to semi-permanent and intermittent pools. No dragonfly larvae were found in the ephemeral pools, although they were sampled from both semi-permanent and intermittent pools. All dragonfly larvae found were from the species *Tetragoneuria* s*p.* and *Aeshna sp.* Mosquito species found were from the genera *Aedes, Anophoeles, Culex, Ochlerotatus, Psorophora*, and *Uranotaenia*. There did not seem to be any species separation between the different pool types. Dragonfly larvae were only found during the first macro-invertebrate sample on June 19th.

Mesocosms

One dragonfly larvae could consume up to 12 larvae in the first hour of exposure and up to 38 larvae in 48 hours (Fig. 3). Dragonfly larvae did not have a significant effect on ovipositing behavior of mosquito species that oviposit directly on water (P=0.5549) (Fig. 4a) or species that oviposit on dry surfaces (P=0.3133) (Fig. 4b).

When dragonfly larvae were present but restrained in a net the cumulative number of mosquito larvae that survived to pupation and the rate of development was greater than larvae held without dragonfly larvae, although differences were not statistically significant (P=0.0755).

A final experiment was done to test if dragonfly larvae preferred one species of mosquitoes over another. Different stage dragonfly larvae were used and length was included in all analyses. Dragonfly larvae (*Aeshna*) consumed more *Culex* then *Aedes* when only one genus was present (P=0.000045), however when both *Aedes* and *Culex* were available together, dragonfly larvae showed no preference. *Aedes* only pupated when *Culex* was present (P=0.0186) and *Culex* pupated more when *Culex* was the only species present (P=0.0006).

DISCUSSION

Field Surveys

These results suggest that dragonfly larvae could affect the density of mosquito larvae in natural communities. We found the greatest number of mosquito larvae found in temporary pools that did not contain dragonfly larvae (Fig. 1). More data would need to be collected in order to better observe the relationship between dragonfly larvae and mosquito larvae. Additionally, more mosquitoes emerged to adulthood from pools where dragonfly larvae were not present (Fig. 2).

This data was collected during an unusually wet summer, which resulted in the ephemeral pools filling up more often and holding water for longer periods of time. Therefore, it is proposed that many adult mosquitoes chose to lay their eggs in the ephemeral pools rather then the intermittent and semi-permanent pools. During a typical summer, without extensive rain, the adult mosquitoes would be forced to lay their eggs in the intermittent and semi-permanent pools, as these would be the only water sources available. Maintaining intermittent and semi-permanent pools that support dragonfly larvae could therefore lead to better mosquito population control.

Mesocosms

Although the data shows no significant there was affect of dragonfly larvae on ovipositing behavior of mosquitoes, interesting trends were found in the data. Therefore, future research will need to be done to clarify the influence of dragonfly larvae on mosquito ovipositing behavior. The dragonfly larvae did not appear to disturb the water at all, as they were found to have sedentary behavior. This could cause them to possibly defer the mosquito larvae from laying eggs in the mesocosms (Rubbo, 2011).

Dragonfly larvae were found to consume mosquito larvae and consume significant number of mosquito larvae in a relatively short period of time. Due to lack of resources a total of 40 mosquito larvae was the highest level of mosquito larvae exposed to the dragonfly at a given time. The dragonfly larvae did not seem to become satiated even at high levels of mosquito larvae. The presence of dragonfly larvae in a enclosed net, restricting them from consuming mosquito larvae, did not show any significant affect on the development rate of mosquito larvae. However, the presence of a dragonfly larva in the net seemed to have an effect on the survival of mosquito larvae. More mosquito larvae survived longer in the buckets that did not contain a dragonfly larva in a net.

The final mesocosm experiment tested the preference of dragonfly larvae when it came to mosquito larvae species. When *Culex* and *Aedes* were separated dragonfly larvae ate more *Culex* then *Aedes.* However, when the dragonfly larvae were placed in a treatment of both *Culex* and *Aedes*, the dragonfly consumed equal numbers of both species. *Culex* species were smaller and slower to react dragonfly larvae presence, which could be the cause of the dragonfly larvae consuming more *Culex* then *Aedes.* This does not explain why the dragonfly larvae ate the same amount during the treatment with both *Culex* and *Aedes*. Therefore, more research is needed in order to answer questions regarding preference behavior of dragonfly larvae. It was also found that *Culex* pupated more when *Culex* was the only specie present, and *Aedes* only pupated when *Culex* was present. It is proposed that both species compete with each other when in the same environment, with *Aedes* decreasing *Culex*'s pupation rate.

Overall, dragonfly larvae appear to play a significant role in the lifecycle of mosquito larvae. This relationship still requires more examination in order to define what type of role dragonfly larvae play. The data found in this study, however, provide a promising future in dragonfly larvae and mosquito larvae research.

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APPENDIX

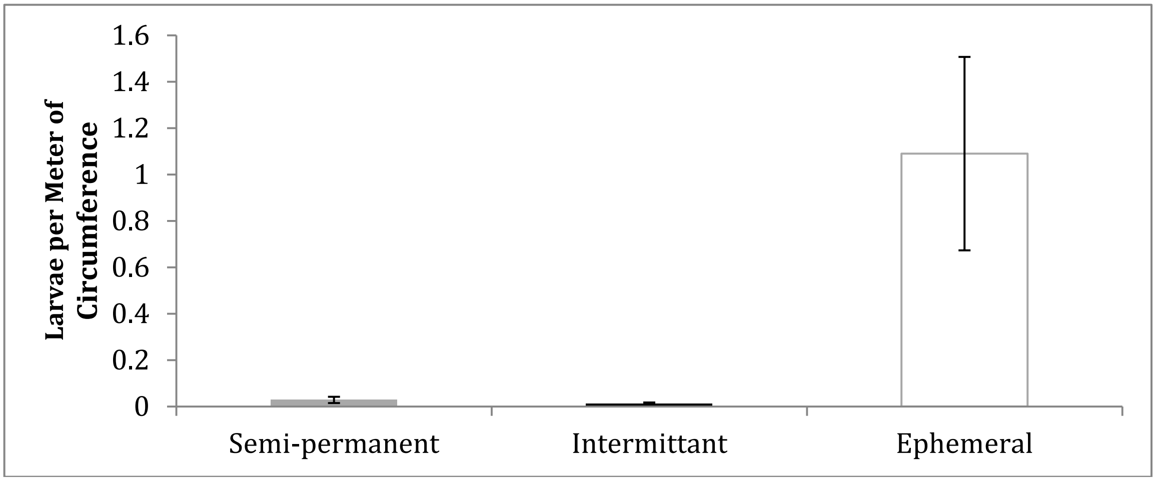

FIGURE 1. Larvae per meter of circumference across pool types.

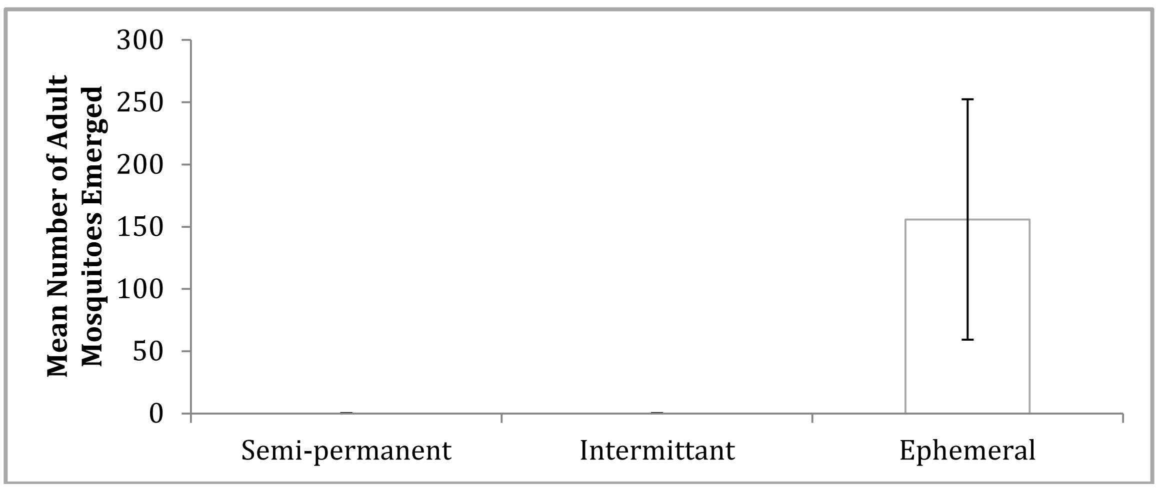

FIGURE 2. Mean number of adult mosquitoes emerged across pool types.

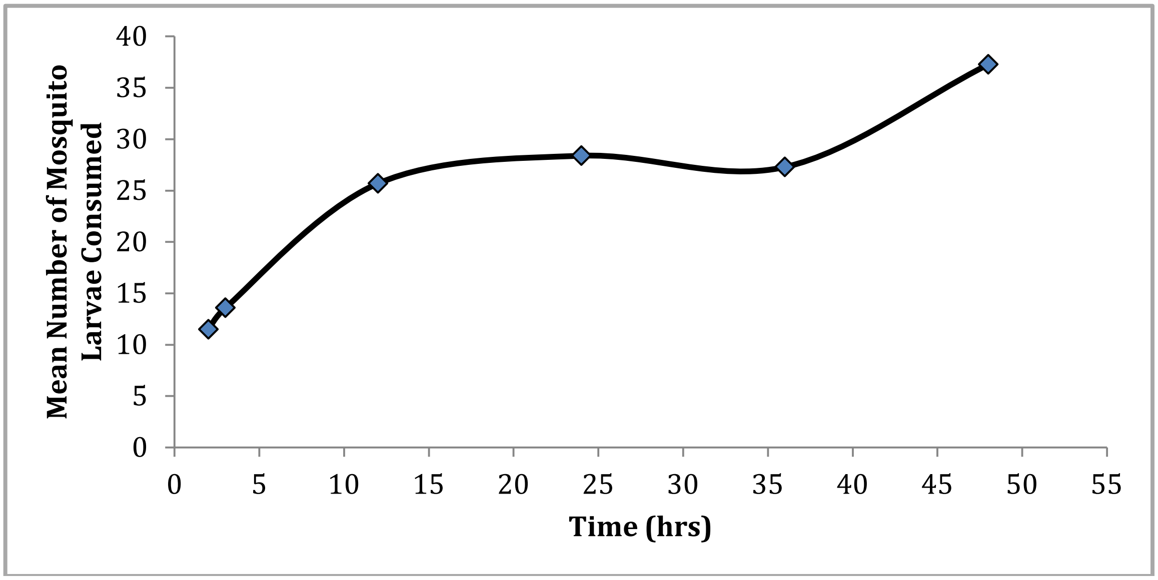

FIGURE 3. Mean number of mosquito larvae consumed over the course of time.

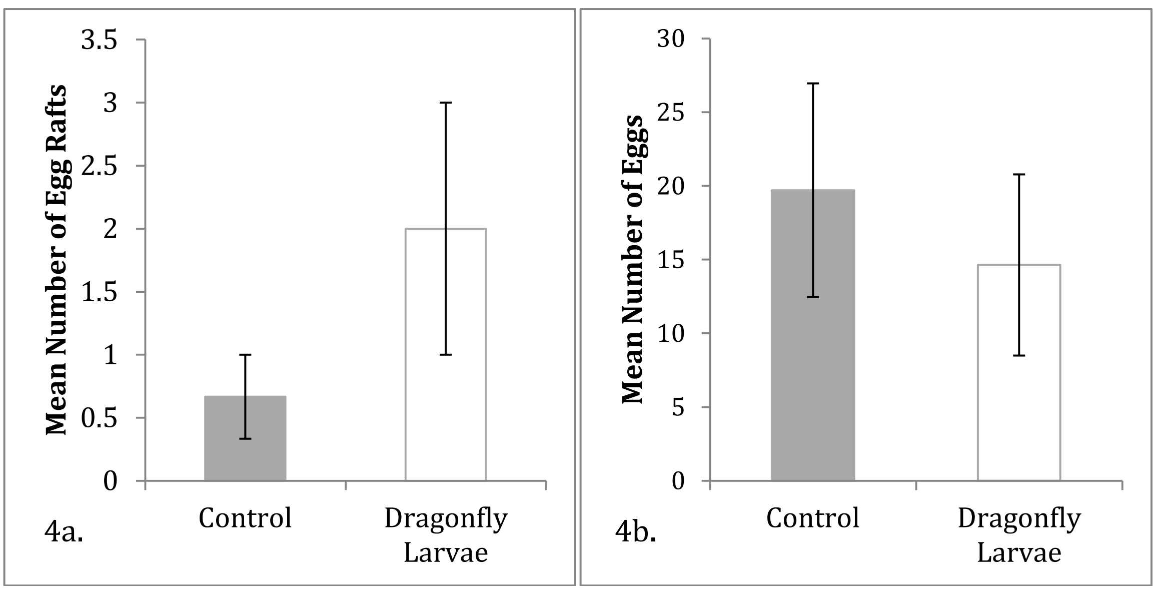

FIGURE 4A AND 4B. Mean number of egg rafts with and without dragonfly present (4a), mean number of eggs per seed paper with and without dragonfly larvae present (4b).