THE EFFECTS OF AMPHIBIAN PRESENCE AND PREDATION ON MOSQUITOES

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Abstract. Due to their importance as vectors of human diseases such as West Nile, dengue, and malaria, extensive scientific research has been done to examine the factors regulating mosquito populations. Mosquito larvae are aquatic and often share habitat with other species, including amphibians. However, it is not well known whether amphibians regulate mosquito populations. Amphibian populations are declining globally due to many factors, including habitat destruction, infectious diseases, and climate change. If amphibians influence mosquito population growth, for example, by reducing the rate at which mosquitoes lay their eggs or through direct predation of mosquito larvae, then the decline of amphibians worldwide could increase mosquito populations. We conducted a series of experiments to examine whether amphibian presence and predation of mosquitoes has a significant effect on mosquito populations. Specifically, I investigated whether the presence of Salamander tadpole species, *Ambystoma maculatum*, frog tadpole species, *Lithobates clamitans*, affected the rate at which mosquitoes lay their eggs, mosquito oviposition**.** I also examined whether amphibians prey upon mosquito larvae at different rates and whether the sizes of the amphibians affect consumption rate. *Ambystoma maculatum* consumed mosquito larvae at a much higher rate than *Lithobates clamitans*. Based on the number of mosquito eggs laid in containers with salamanders, salamander larvae do not contribute to lowering mosquito population densities in areas where mosquito habitats are predominated by vernal pools. Frog tadpoles also consumed mosquito larvae, although at a much lower rate. Though predation rates were very different between salamander and frog larvae, both frog tadpoles and salamander larvae could have significant effects on the survivorship of mosquito larvae, and thus may play an important role in regulating mosquito population density in vernal pools.

INTRODUCTION

Global amphibian populations have been decreasing due to invasive species, over exploitation, infectious diseases, and more (Collins & Storfer, 2003). While the number of studies focusing on the causes of amphibian declines increases, more research is needed to determine the effects of declining amphibian populations on other organisms and ecosystem functions (Blaustein, Sadeh, Blaustein, 2013, Collins & Storfer, 2003). Mosquitoes are well known for the diseases they carry and the nuisance they pose to humans. If mosquito-amphibian interactions are prominent in ecosystems, the global decline of amphibians could have an adverse effect on the abundance of mosquito populations. Therefore, our study was used to determine whether the presence of and predation by amphibian species, *Ambystoma maculatum* and *Lithobates clamitans* had significant effects on oviposition and larval survival of the northeastern invasive mosquito species, *Aedes japonicus*.

Previous studies have suggested that mosquito populations can be controlled most effectively during the aquatic juvenile stages (Service, 1985). Though aquatic and terrestrial amphibians both coexist with mosquitoes, the aquatic amphibians are of the most importance for this study because aquatic amphibians inhabit and feed in the pools where mosquitoes lay their eggs. A past study found that *Culiseta longiareolata* mosquito larvae and mosquito eggs are highly susceptible to salamander larvae predation and that salamander affected the rate of adult *Culiseta longiareolata* oviposition (Blaustein, Keisecker, 2002). The study by Blaustein and Keisecker (2002) did not compare mosquito larvae consumption rates in the two amphibians, Ambystoma *maculatum* and *Lithobates clamitans*, but did compare the effects of pre-metamorphosing salamanders versus metamorphosing salamanders on mosquitoes. Before metamorphosis, salamander species are aquatic suction feeders, but once they undergo metamorphosis, they are less efficient at capturing prey due to morphological changes (Lauder, Shaffer, 1986). Taking into account that metamorphosis has been proven to decrease the ability of salamander species to effectively capture prey, this study evaluates whether there are changes in rate of consumption between different sizes of pre-metamorphosing *Ambystoma maculatum.*

In this paper we describe experiments that evaluate how two prevalent amphibian species in northeastern vernal pools, including the frog tadpole (*Lithobates clamitans*) and salamander larvae (*Ambystoma maculatum*), influence oviposition and survival of immature mosquitoes.

MATERIALS AND METHODS

Field Surveys, Amphibian Collection, and Ovitraps

Field Samples: I conducted amphibian and mosquito community field surveys at vernal pools and used plastic cups (350 ml) with a leaf-water infusion (leaf tea) as ovitraps to collect mosquito eggs. I identified a representative sample of what amphibians and mosquitoes to use for my experiments by studying the patterns in which they coexisted at vernal pools. Individual amphibians for experimental use were collected from the Crear Pond at the Cary Institute of Ecosystem Studies property in Millbrook, NY. The species of amphibians found at Crear Pond were observed to coexist with several mosquito species at different vernal pool and pond locations on Cary Institute property. I collected a total of 12 salamander (*species*) larvae and 12 frog tadpoles (*species*) with IACUC approval.

To collect mosquito larvae for the experiment, I created ovitraps by collecting 5-gallon buckets, filling them ¾ of the way full with untreated well water, and placing 2-3 pieces of seed germination paper (Nasco) around the top of the buckets. Six buckets were placed behind Bacon Flats and six were placed around the Fern Glenn, which were both on the Cary Institute property. The seed paper was checked for eggs daily and removed if eggs were present. After seed paper was collected, it was dried for 24-48 hours, and then placed in a tray full of leaf tea to hatch them. Leaf tea was made a day in advance by adding untreated well water and leaves to a container and placing it in the sun for a full day. A majority of the eggs collected from ovitraps was identified as *Aedes japonicus*, which was also present in our field samples with amphibians from vernal pools.

Amphibian Predation of Mosquitoes: Rate of Predation and Size Differentiations

Mesocosm experiments were used to test for rate of predation between two species of amphibians and to determine whether amphibians consume different amounts of mosquito larvae as they approach metamorphosis.

We conducted two predation experiments, one experiment with frog tadpoles and another with salamander larvae. Based on the field surveys and personal observations, the amphibians used for the experiments were identified as Spotted Salamanders (*Ambystoma maculatum)* and Green Frogs (Lithobates clamitans). For both the frog tadpole and salamander larvae predation experiments there were 10 controls without amphibians and 10 treatments with amphibians. All of the amphibians were fed fish flakes before the experiment commenced. Each 5-quart container for the controls and the treatments had 1 liter of leaf tea, 2 liters of untreated well water, and a mesh lid that was made to prevent any animals or insects from disturbing the experiments. The containers were placed in kiddie pools filled with cool water, which was replaced every day to prevent the amphibians from overheating outside. Mesocosms were placed outside under shade cloth from July to August 2015.

The frog tadpole experiments were checked twice a day for one week, once at 11:00 am and again at 4:00 pm. The number of surviving mosquito larvae was recorded for each mesocosm. Initially the frog tadpole and salamander larvae predation experiments began at the same time, but some of the salamanders ate all 30 mosquito larvae over night. Therefore, restarted the salamander predation experiment and recorded surviving larvae after 10 minutes and then every 20 minutes up to a total 150 minutes. Standing over the containers did not seem to have an effect on the rate of consumption of the frog tadpoles or the salamander larvae due to the fact that the amphibians seemed to entirely focus on the mosquito larvae.

Effects of Amphibians on Mosquito Oviposition

In this experiment we determined whether the presence of salamander larvae affected rates of mosquito oviposition over 8 days. The salamander larvae were used for the experiment due to their rapid consumption rate in comparison with the frog tadpoles. The salamander larvae were fed mosquito larvae before beginning the experiment, and were fed blood worms once during the experiment. Seed germination paper (11inches * 4.25 inches) was clipped on two sides of all containers for adult mosquitoes to lay their eggs. Every day, it was recorded whether there was eggs laid on the seed paper for each container. Every two days, papers containing eggs were replaced with new seed paper. Due to a shortage of seed paper, on days 2-5, 2 pieces of seed paper were used per container and only 1 piece of seed paper was used on days 6-8. The changes to the amount of seed paper used per day are accounted for in the results. The paper surface available for oviposition was kept constant across all mesocosms. The seed paper that was replaced every two days was taken into the lab, dried and rewet in a tray of leaf tea to hatch. The seed paper was left to hatch for 2-3 days and the amount of larvae and eggs hatched were recorded. A subset of seed paper was hatched in two different incubators to see if there were differences in the amount of eggs hatched. The mean temperature of incubator 1 was approximately 24 degrees Celsius (+/- 4 degrees Celsius), while incubator 2 was approximately 29 degrees Celsius (+/- degrees Celsius).

RESULTS

Frog Tadpole Predation vs. Salamander Larvae Predation

After conducting separate frog tadpole and salamander larvae predation experiments, it was evident that the salamander larvae consumed more mosquitoes per unit time than the frog tadpoles. While the frog tadpole experiment was conducted over the course of one week, the salamander predation experiment was run over 150 minutes.

The maximum amount of mosquito larvae consumed by frog tadpoles was 10 relative to the maximum amount of mosquito larvae consumed by the salamander larvae, 30, which was the maximum amount fed to them initially. Taking into account that the frog tadpole experiment was conducted over a week while the salamander predation experiment was done over 150 minutes, the salamander larvae consumed more mosquito larvae at a much faster rate than the frog tadpoles.

Salamander Predation: Size Groups

The results from the salamander predation experiment were manipulated to analyze whether the differences in sizes had any effect on the rate of mosquito larvae consumption. The size groups consisted of salamander larvae that were less than 52.15 mm in length and salamander larvae greater than 52.15 mm in length. The cutoff for the size groups, 52.15 mm, was determined by finding the median size value of all of the salamanders used in the experiment.

Although it is apparent from Figure 2 that the salamanders greater than 52.15 mm in length consumed more than the size group below 52.15 mm in length, the results are not statistically significant (t= -0.9928 p=0.3509). Figure 2b shows that the larger group consumed 8.2 more mosquito larvae than the smaller size group at 10 minutes. After 30 minutes, the amount of mosquito larvae consumed by both size groups remains fairly constant with the size group greater than 52.15 mm having a larger total consumption rate over time.

Effects of Amphibians on Mosquito Oviposition

There was no significant difference between the average number of eggs laid in mesocosms (per seed paper within the mesocosm) with salamanders (59.275 +/- 57.88405) versus mesocosms with no predator (71.375 +/- 89.66396) present (t= 0.7171, p=0.4758).Though there was no statistically significant difference between the mean numbers of eggs laid in the control containers and the predator treatments, more total eggs were laid in control containers overall (Figure 4). To conclude whether the eggs laid in the control and treatment containers were viable, the eggs were hatched from the seed paper collected every two days.

Consistent with the analysis of total oviposition (Total eggs oviposited: Controls=2855, Treatment with Salamanders=2371), there was no statistically significant difference between the numbers of eggs hatched from the control (9.0 +/- 11.898) seed paper in contrast to the eggs hatched in treatments with salamanders present (0.7 +/- 2.214) seed paper, (Test statistic: t- test between control and treatment t= -0.0474, p= 0.9628). Still, more total eggs hatched from the control seed paper than the treatment seed paper (Total eggs hatched: Controls=1499.5, Treatment with Salamanders=1307). As evident in Figure 5, the numbers of eggs that hatched were variable across the experimental sampling dates, with greatest hatching success from day 6 collections, regardless of treatment. On day 6, two different incubators were used to hatch the eggs from the control and treatment seed paper, but it did not cause a difference in the amount of eggs hatched between the controls and treatments (Test statistic: t-test=-0.9291, p=0.3667).

DISCUSSION

While amphibians are not commonly thought of as a means of controlling mosquito populations, the results from our experiments showed that larvae of the salamander species, *Ambystoma maculatum*, and frog species, *Lithobates clamitans*, are capable of reducing mosquito larvae populations by direct predation. *Ambystoma maculatum* not only consumed a high amount of mosquito larvae during a short amount of time, but also was successful at deterring mosquitoes from ovipositing in their mesocosms. Considering that the amphibians in the experiment did in fact have an impact on mosquito abundance, this implies that a decline in amphibian populations could lead to an increase in mosquito populations in the future. Previous studies calculated that salamanders have such high abundances in nature and even if a salamander's diet were only 1% mosquito larvae, they would be capable of decreasing mosquito populations by a substantial amount (DuRant, Hopkins, 2008, Taylor et al. 1988). Compared to salamander larvae, frog tadpoles did not have a high consumption rate of mosquito larvae in the experiment, which may be expected due to their primarily consuming algae, fungi, and other organic matter in a natural environment (Jenssen, 1967). Therefore, it is unlikely that a decline of *Lithobates clamitans* would have a major effect on mosquito populations, a finding which parallels conclusions from other studies conducted with different frog tadpole species (Rubbo, Lanterman, Falco, Daniels, 2014).

Analyzing the differing pre-metamorphosing sizes showed that the salamanders that were larger in body length were able to consume more than the larger sized salamanders. It is logical to assume that feeding success would be lower as a salamander experiences morphological changes approaching metamorphosis in later developmental stages, which include a reduction in mouth cavity size (Lauder, Shaffer, 1986), an increase in body weight, and a decrease in body size. Personal observations showed that the salamanders with larger body lengths also seemed to have a larger body size. Therefore it would be fair to expect that as salamander larvae increase in body length their ability to consume food increases as well. We did not categorize the salamanders by Gosner's developmental stages, but categorized them by their length before reaching metamorphosis (Gosner, 1960). The salamander size group below 52.15 mm in length consumed less mosquito larvae on average than the size group above 52.15 mm. These results were expected due to the increase in body size. Considering this was not a separate experiment to determine whether the differing size groups had an effect on consumption rate, another experiment should be done with more replicates to see if the same results are obtained.

The presence of salamander larvae reduced mosquito oviposition as measured by the number of eggs laid per container. This indicates that, in addition to controlling mosquito populations through direct predation on larvae, salamander larvae may are also contribute indirect controls by deterring adult mosquitoes from ovipositing in their presence. Previous studies suggest that adult mosquitoes may detect chemical cues coming from the salamanders (Petranka & Fakhourry 1991; Blaustein & Kotler 1993; Mokany, Shine, 2003). Chemical cues of amphibians and dead mosquito larvae have been shown to deter oviposition (Mokany, Shine, 2003, Ferrari, Messier, Chivers, 2008). Considering the salamanders were easily visible in experimental mesocosms, adult mosquitoes are unlikely to have been limited in their ability to use visual cues to detect salamander larvae. Hatching success rates of mosquito eggs from the oviposition experiment show that in addition to oviposition, egg viability was also reduced in treatment vs. control containers.

Together, these experiments showed that *Ambystoma maculatum* would be effective at controlling larval mosquito populations, in turn preventing adult mosquitoes from emerging. Considering *Ambystoma maculatum* reduced oviposition and egg viability, this could prevent new populations of adult mosquitoes from coexisting in environments with *Ambystoma maculatum* present. It is also possible that salamanders could consume the viable eggs or the mosquito larvae after they hatch (Blaustein, Keisecker, 2002). It can be concluded from this experiment that amphibians would be efficient at controlling larval and adult mosquito populations.

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APPENDIX

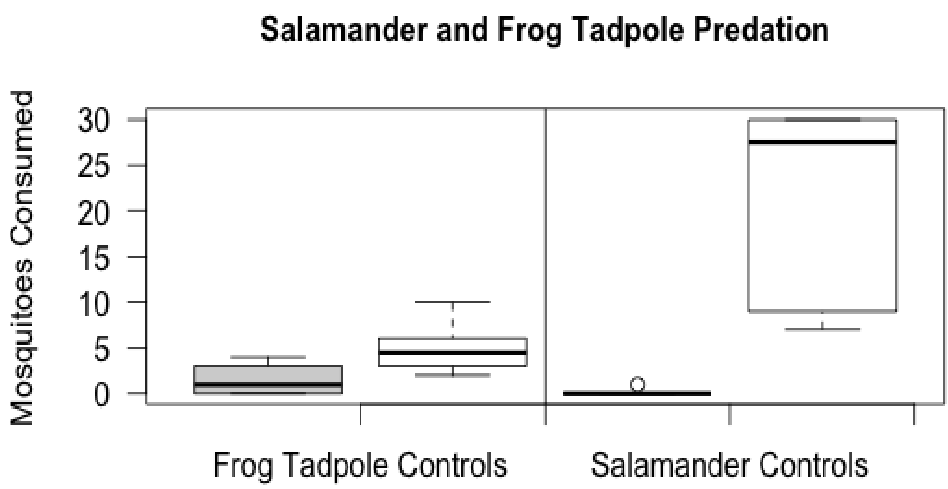
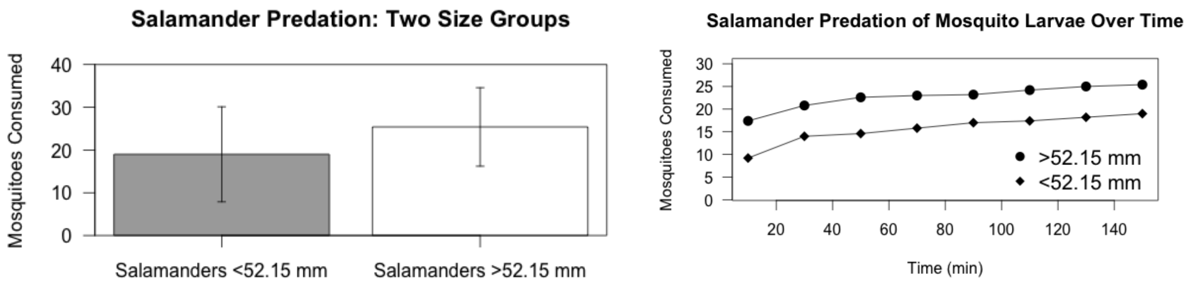


FIGURE 1. The amount of mosquito larvae lost at the end of the frog tadpole experiment over 1 week compared to the amount of mosquito larvae lost at the end of the salamander predation experiment over 150 minutes. The frog tadpole and salamander controls did not contain amphibians and thus, represent the number of mosquitoes that died naturally over the span of the experiment. Differences between the controls and the larvae numbers in the frog tadpole and salamander experiments represent mosquito larvae that were consumed by the amphibians during the experiments. Both frog tadpoles (t-test, t= -3.575, p=0.002632) and salamander larvae (t-test, t= 6.8584,p=7.334e-05) consumed mosquito larvae.



a. b.

FIGURE 2. Figure 2a. The total amount of mosquito larvae consumed between the two salamander size groups, salamanders below 52.15 mm in length and salamanders above 52.15 mm in length. Figure 2b. The amount of mosquito larvae consumed over the course of 150 minutes for both size groups. It can be seen that the larger size group (>52.15 mm) consumed more mosquito larvae over time compared to the small size group (<52.15 mm).

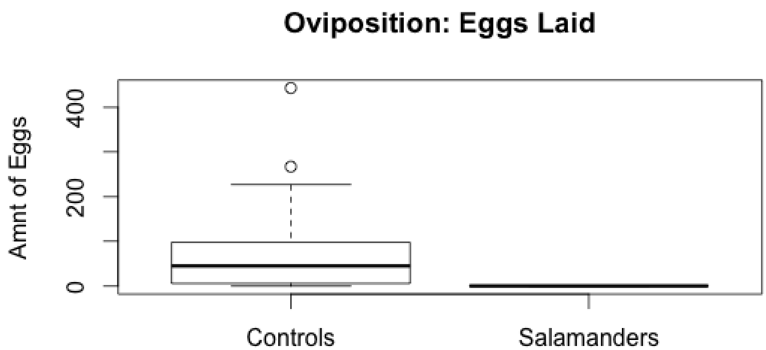


FIGURE 3. The amount of eggs laid in the control containers without salamanders in comparison to the number of eggs laid in the treatment containers with salamanders. All containers were left outside with seed paper around the rims to allow for adult mosquitoes to lay their eggs on them. It is noticeable that there were more eggs laid in control containers that did not contain salamanders.

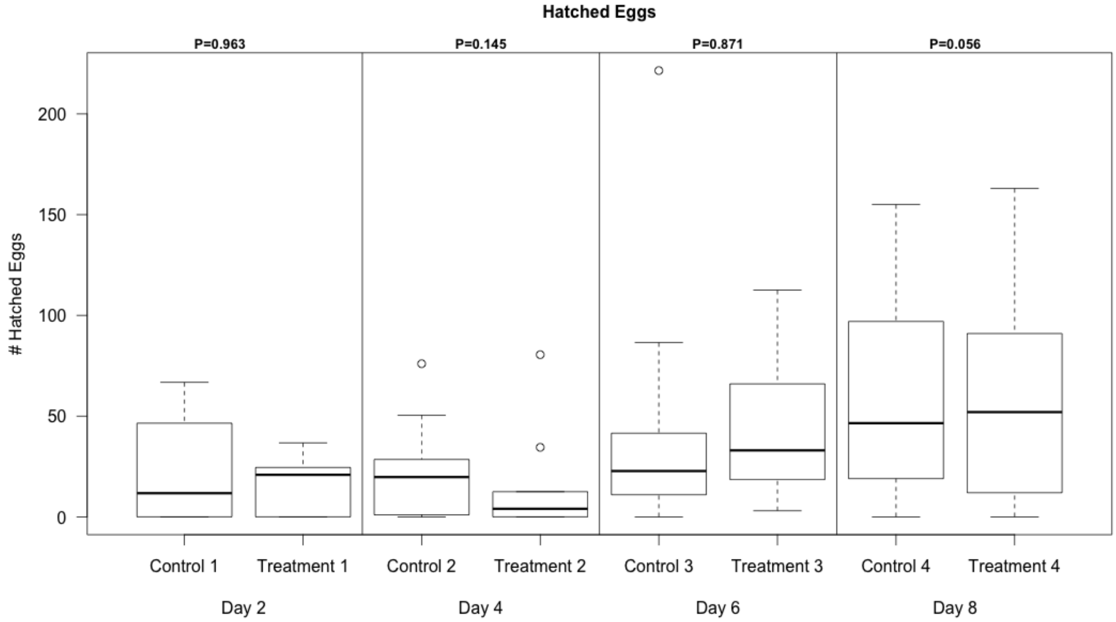


FIGURE 4. The numbers of eggs hatched every two days on the control seed paper and treatment seed paper. The controls were eggs hatched from seed paper from containers that did not contain salamanders while the treatments were eggs hatched from containers with salamanders present. Every two days, the seed paper was collected from the containers outside and the eggs were hatched indoors to obtain this data. An outlier for Treatment 3 that had 264 eggs hatched was included in the analysis but is not shown in this figure.