EVALUATING THE INFLUENCE OF ANTHROPOGENIC INPUTS TO BOTTOM-UP REGULATION OF THE DEVELOPMENT, SURVIVAL AND EMERGENCE RATES OF MOSQUITO LARVAE

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Abstract. In aquatic and semi-aquatic ecosystems, bottom-up effects associated with resource abundance and quality play an important role in the regulation of the development, survival and emergence rates of mosquito larvae. Studying these bottom-up effects with regard to the additive impact that anthropogenic drivers may have in both urban and rural areas may be important to explain why human exposure to mosquito-borne disease is higher in urban versus rural landscapes. This study used observational methods to evaluate mosquito resident species composition across a rural to urban gradient in the Hudson Valley in New York and used experiments to test the role that known anthropogenic inputs to potential mosquito breeding habitats (chloride and nitrogen) have on mosquito larval development and survival in temporary pools of water. Total adult emergence differed when reared in urban and rural water samples, despite no obvious differences in bacterial concentration between samples at the start of the experiment. The percent of the total population that successfully emerged as adults was over three fold greater when reared in urban versus rural water microcosms. Throughout the course of the study adult emergence was observed in both the nitrogen addition treatments and control microcosms, but was not observed in microcosms treated with road salt, although larvae in these microcosms did reach the final instar stage. This suggests that the presence of NaCl may play a role in inhibiting mosquito development from fourth instar larvae to adult mosquitoes.

INTRODUCTION

Urban areas across the mid-Atlantic United States are experiencing an increase in vector-borne diseases (Brown 2008). The presence of West Nile Virus (WNV) in urban areas is one example of a persistent vector-borne disease. Certain mosquito species act as bridge vectors, which feed on both avian species and mammalian species thereby allowing the transmission of enzootic WNV from the avian hosts to mammals. In a previous study, Turell et al. (2005) identified potential WNV vectors and their competence as bridge vectors. According to Turell's findings, nearly all of the Culex species and several of the Aedes species tested could serve as efficient enzootic or amplifying vectors for WNV. This study used *Culex pipiens* and *Aedes albopictus* which Turell's study found to be competent vectors for WNV transmission. Increased vector competence indicates increased potential transmission of WNV from avian species to mammalian species. Urban development may provide greater diversity of mosquito habitat and warmer temperatures to promote development, but anthropogenic pollutants in breeding habitats may impact larval survival and development. Here I focus on some potential bottom-up drivers of mosquito development and survival in urban environments.

In this experiment, the rate of mosquito development and survival are hypothesized to be resource limited. Mosquito larvae are aquatic and therefore need water to breed and develop prior to becoming adults. Mosquito larvae typically feed on algae, protozoa and bacteria from the water's surface microlayer by using the collecting-filtering feeding mode (Merritt et al. 1992). Environments deficient in these resources would naturally impose limits on larval growth rate and survival, while conditions that favor bacterial growth, for example, could increase mosquito abundances. It is not unusual to see this type

of bottom-up regulation occur in aquatic systems (Sweeney 1984). The bacteria that act as primary food resources for larval mosquitoes in turn rely on nutrients in the water for sustenance. There is a direct correlation between water's nutrient content and the success of aquatic invertebrates (Merrit 1992). The actual amount of high-quality or preferred food of an aquatic insect may be limiting to certain species at specific times or in different habitats (Carpenter 1983 and Grill 1996). A study by Beier et al. in 1983 showed that mosquito densities "were significantly correlated with levels of ammonia, color, and turbidity of water in tires... and water quality regulate[s] larval mosquito dynamics in tire yards." Human interference could affect bottom-up regulation of mosquito abundance if chemical or nutrient waste enters breeding water sources to impact food resources.

I predict that that excess nitrate might support greater bacterial or algal growth but that chloride might decrease resources available to mosquito larvae or even have direct effects on the larvae themselves. There is compelling evidence to suggest that water that collects in temporary pools in urban and rural areas differs in nitrogen and chloride content (Fischer 2004). It is likely that the urban water samples differ from the rural water samples based on the differing number of roads, which contribute chloride from road-salt runoff, as well as the differing amounts of green space contributing nitrogen from fertilizer run-off. Additionally, urban water may differ from rural water based on the presence or absence of predatory macroinvertebrates as well as baseline resource abundance prior to any anthropogenic additions. Excess nitrogen and chloride are pollutants worthy of investigation due to their potential direct impact on the soft-bodied mosquito larvae populations, as well as their potential for indirect impacts on bacteria and protozoa food abundance.

A previous study that focused on the anthropogenic input of nutrients to mosquito breeding habitat in the form of cow dung did not show any impact on larval growth and development (Gimnig et al. 2002). In Gimnig's study there was evidence of decreasing nitrogen levels occurring with increasing larval densities suggesting that nitrogen may be a limiting resource in the larval environment. The results of Gimnig's experiment suggest the need for further investigation into how nitrogen concentrations regulate or limit mosquito development and survival.

The specific questions being addressed in this study are: 1) Do bacterial concentrations differ between urban and rural stagnant water samples? 2) Does bacteria concentration affect bottom-up regulation of the development, survival and emergence rates of mosquito larvae? And, 3) Do mosquito larvae development, survival, and adult emergence rates differ when reared in microcosms with anthropogenic inputs (i.e., chloride, nitrogen pollutants)?

METHODS

Water Sample Site selection and Preparation

The selection of stagnant water sources is important in order to obtain meaningful results from this comparative study. For each anthropogenic input that was tested, a sample of urban and rural water was used to inoculate our 'food' communities used in each treatment. In this investigation urban water sites are defined as areas containing a temporary pool of stagnant water that are surrounded by impervious surfaces (e.g. cement). Rural water sites are defined as areas containing a temporary pool of stagnant water that are surrounded by permeable surfaces (e.g. soil). Water samples were collected from seven sites in both urban and rural areas and mixed thoroughly into one rural and one urban water sample. This ensures that the water samples accurately represent the bacterial/algal/protozoan communities from the multiple urban and rural water pools and are not specific to the contents of one location.

We focused on stagnant puddles so that our food resource communities would have had time to form. The puddle must have persisted for at least 24 hours since the last precipitation, but sampling did not take

place more than 48 hours after the last precipitation event. This eliminated puddles that would disappear rapidly and therefore not sustain mosquito larvae and puddles that had extended time to collect and concentrate nutrients from being used as samples. The location of each sample was noted and photographed. The urban collection sites include; one curbside water drain, one puddle above a substrate covered manhole cover, two puddles residing in a mud and grass substrate surrounded by concrete, one puddle residing in substrate filled depressions surrounded by cracked pavement, and two puddles residing in substrate (dirt and gravel) filled potholes in driveways. The rural collection sites mirror the urban sites in number, type, and quantity of samples while instead being surrounded by permeable surfaces. Water samples were filtered to remove all macroinvertebrate organisms using a standard 45 µm sieve. Equal quantities of the urban and rural water were separately combined with carbon-filtered tap water (dechlorinated) to establish treatment microcosm containers. A 24-hour acclimation period was allowed before introducing the first instar mosquito larvae.

Microcosm Construction

We established six microcosms per trial each with the same dimensions and properties. The microcosms were constructed of clear plastic, cylindrical containers measuring approximately 3½ inches in diameter. The tops of the microcosms were covered with a fine mesh screen secured to the container to allow gas exchange but prevent mosquito escape. The microcosms were inoculated with 20 individual larvae in order to mirror average densities observed in natural populations. For the initial trial of experimental microcosm treatments, a lab strain of mosquitoes (*Culex pipiens*) was used to measure and monitor the effects of anthropogenic inputs. The subsequent trial contained lab-reared *Aedes albopictus* mosquitoes. The first instar larvae added to each microcosm were from the same egg batch so as to ensure comparable developmental stages across the treated microcosms.

Anthropogenic Water Treatments

For each trial (one trial with *Culex pipiens* and one trial with *Aedes albopictus*) we used three microcosms inoculated with urban water and three inoculated with rural water. Each inoculated microcosm received nitrogen, chloride, or no anthropogenic treatment addition. Two microcosms received 23 mg/L of chloride in the form of sodium chloride, while two microcosms received 1.2 mg/L of nitrogen in the form of potassium nitrate. The remaining two microcosms did not receive a chemical addition. These concentrations were determined by averaging the concentrations of chloride and nitrogen found in water samples across the entirety of the Fishkill Creek which spans from out urban to rural test sites (Stainbrook 2004).

The anthropogenic treatments should inherently change the composition of resources available in the original urban or rural samples. The treatments are intended to represent what might actually be present in the environment 24 hours after a precipitation event.

Data Collection

Bacterial concentration in all of the experimental microcosm treatments acts as a measure of the water sample's larval resource availability. Bacterial counts were performed on the initial urban and rural water mixtures and for each of the microcosms following the last data day of experimental observation. Bacterial counts were performed in the Cary Institute for Ecosystem Studies laboratory following standard procedures for acridine orange staining and counting (Hobbie 1977; Pace 1992).

The microcosms were placed into a temperature-controlled incubator maintained at 26 degrees Celsius for optimal larval development and adult emergence (Rueda 1990). UV lights in the incubator were set on a

timer to replicate the light-dark cycles in a natural environment. Data collection was performed every two days to record larval developmental and survival, as well as observations on water condition. Data collection was to continue in each trial until the last larvae matured to adult or died. Data collection lasted 49 days in the first trial (*Culex pipiens*). Due to time constraints data collection for the second trial (*Aedes albopictus*) was only able to last for 18 days. This was enough time for one microcosm to be completely depleted of larvae either by maturation to adult or death and for half of the microcosms to have only one larvae remaining.

in situ Mosquito Sampling

The microcosm experiment described above was supplemented by *in situ* sampling of local mosquito species via oviposition container traps. Oviposition traps contained seed paper and water with a hay infusion to provide a suitable egg-laying environment for adult mosquitoes. Eggs obtained from traps indicated what mosquito species were present in the urban and rural areas where we collected water samples. Each trap was left outside for 7 days, during the 4th week of June 2010. The collected eggs were allowed to hatch under optimal developmental conditions in an incubator in order to quantify the number of larvae present and for later identification to species.

RESULTS AND DISCUSSION

To address the first focal question, total bacterial load in the urban and rural water samples was determined prior to the addition of larvae as well as directly after the end of each trial. Differences in total bacterial load between the initial urban and rural water samples resulted in a t-test value of -0.274 and a p-value of 0.790 and thus were not statistically significant (Figure 1). Additionally, differences in total bacterial load between urban and rural post-experimental water samples were not statistically significant (p value > 0.05). This indicates that the initial bacterial loads of the water samples are not responsible for the differences seen in development between urban and rural control microcosms. This begs further questions of whether a measurement of total bacterial load is an accurate portrayal of available food resources for mosquito larvae. However, determining what portion of larval mosquito diets are bacteria, as well as whether mosquitoes prefer certain strains of bacteria is necessary information in order to draw further meaning from the findings of this experiment. Due to very similar bacterial concentrations across urban and rural water samples in both trials we are not currently able to address the second focal question of how bacterial concentration may affect bottom-up regulation of the development, survival and emergence rates of mosquito larvae.

The mortality rates of the mosquito larvae differed between trials, water source and anthropogenic treatment they were exposed to. The average mortality rate in trial one across both water types and all anthropogenic treatments was .47 mosquitoes per day as compared to .86 mosquitoes per day in trial two. Overall the microcosms treated with urban water had a lower average of mosquito mortality than the rural water treatments (average daily mortality was 2.8% of the initial population versus 3.9% respectively). The anthropogenic additions did not appear to be affected mosquito mortality as greatly as the other experimental variables. The average daily mortalities for each anthropogenic addition were 3.75% for the control with no addition, 3.4% for the NaCl addition, and 2.8% for the KNO_3 addition. This indicates that the impact of chloride and nitrogen additions to the microcosms were not a large factor responsible for increased mortality of mosquito larvae.

Emergence of adult mosquitoes differed across anthropogenic treatments in the urban water samples (Figures 3 and 4). Throughout the course of the study adult emergence was observed in each anthropogenic treatment and control except for microcosms treated with NaCl, although larvae did reach the final instar stage (Figure 2). This suggests that the presence of NaCl may play a role in inhibiting

mosquito development from fourth instar larvae to adults. It is unclear whether this potential inhibitory effect acts on the transition from larval to pupal stage or pupal to adult emergence.

Total adult emergence also differed in respect to urban and rural water samples, despite no obvious differences in bacterial concentration (Figure 1). The percent of the total population that successfully emerged as adults was over three fold greater in urban water microcosms. In experimental urban samples, emerged adults amounted to 6.7 percent of the initial microcosm population compared to 1.9 percent of the initial microcosm population in rural samples. Additionally, larval development was slower in urban water samples despite a higher percent of adult emergence Figure 2). One potential explanation for this is that a longer developmental time allows for increased survival. However, a competing explanation regarding development time states that faster development would convey higher survival due to a lesser possibility of predation. This suggests that if predation was incorporated into this experimental design, total adult emergence may have differed. This consideration should prompt future studies to more accurately mirror natural mosquito ecosystems. Predation experiments in both urban and rural environments would be beneficial to determine of this a confounding factor.

Variables that could/may be tested in follow-up experiments include; varying temperatures, performing control experiments using only carbon filtered water and the anthropogenic input, performing a control experiment in distilled water (is the effect solely due to the difference between water sample sources?), and measuring the effect of differing concentrations of anthropogenic additions.

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APPENDIX

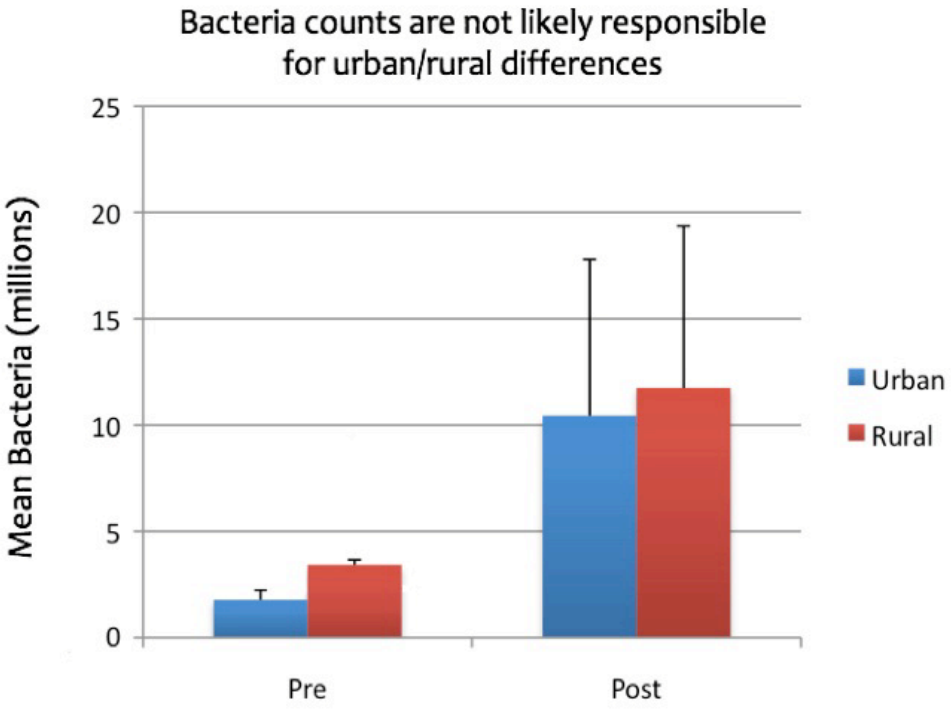

FIGURE 1. Mean number of bacteria in urban and rural water samples pre and post microcosm experiment.

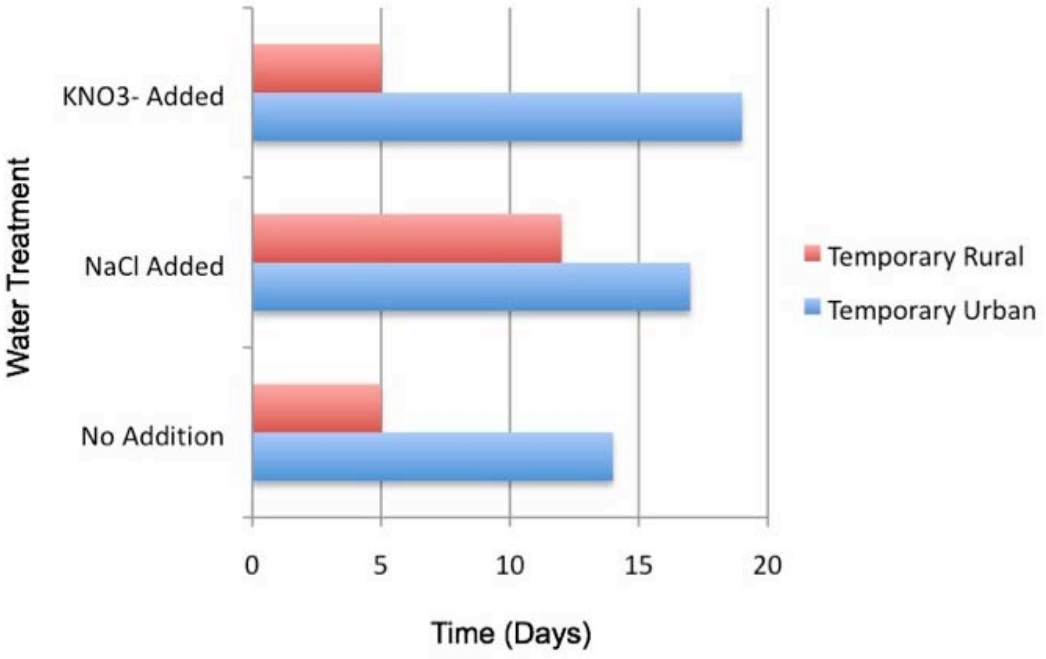

FIGURE 2. Time until first larvae reach the fourth instar.

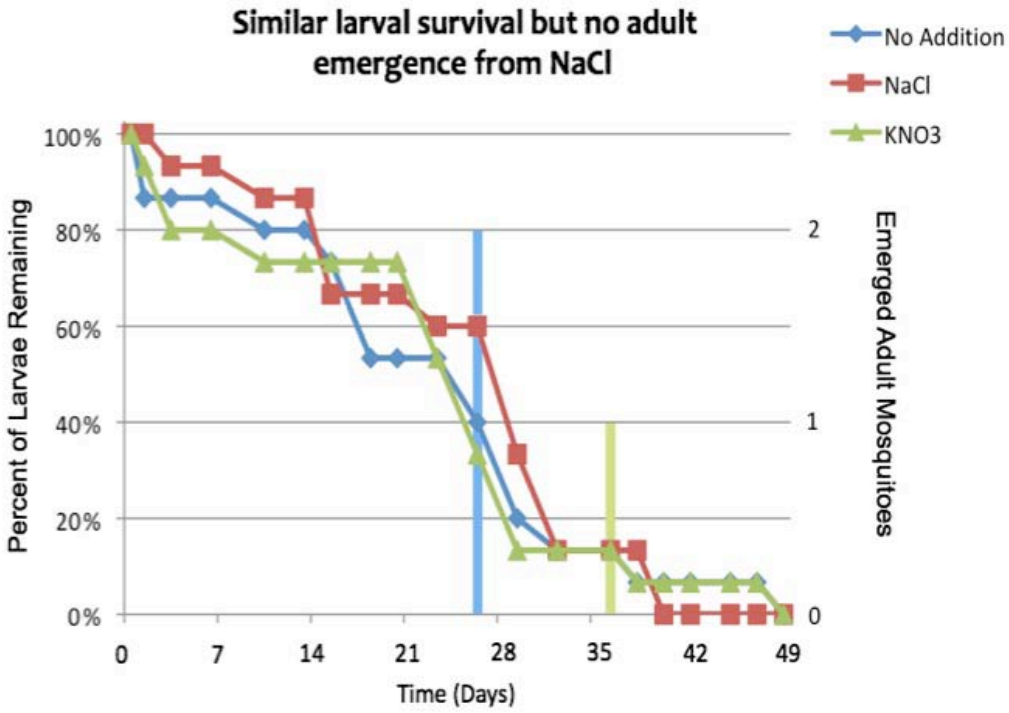

FIGURE 3. Larvae survival and adult emergence in the urban water treatment using *Culex pipiens*.

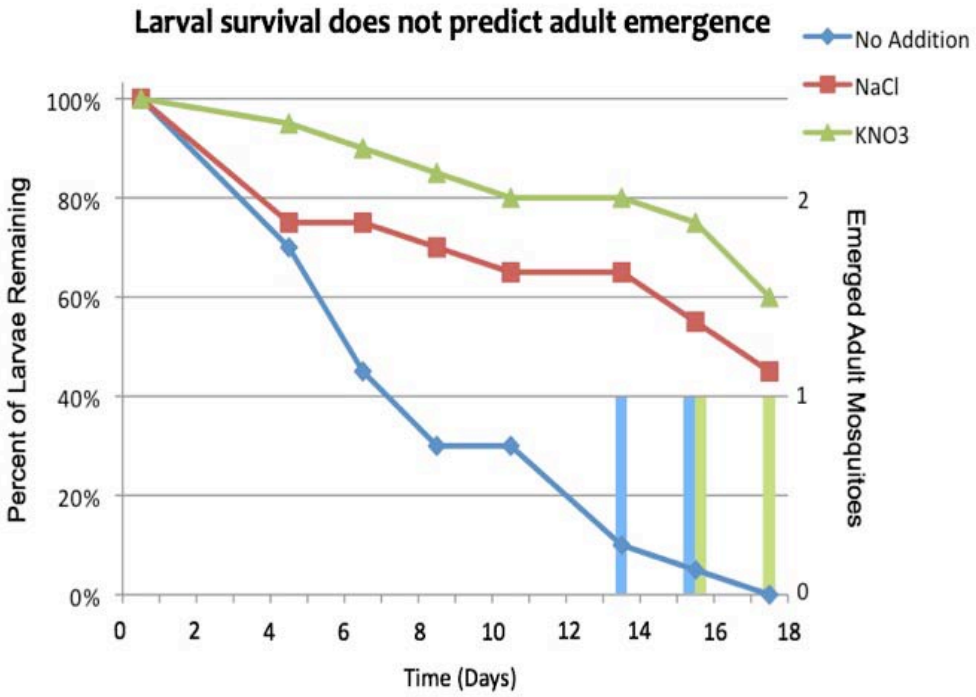

FIGURE 4. Larvae survival and adult emergence in the urban water treatment using *Aedes albopictus*.