Effects of N-Enriched Leaf Litter and Decreased Temperatures on *Aedes aegypti* Mosquito Larvae Development Time and Biomass

Oleksandr Yardas

Hye Sun Kim

Brandon DeBella

Grinnell College

November 5th, 2016

With the current trends of anthropogenic climate change indicating severe changes to the global ecosystem within the next century (IPCC), knowledge of the physiological responses of organisms in key ecosystems to rapidly changing climatic variables such as temperature and CO2 concentrations can give insight into measures to reduce the imbalancing effects on organisms and the ecosystems. The responses of mosquitoes (Culicidae), which are an extremely prolific cosmopolitan family of heterotrophic species, are of particular interest as they are effective vectors for diseases with high mortality rates. The breadth of the family allows mosquitoes to infect over 700 million people per year with such diseases, and over 1 million people per year die due to infections or related maladies transferred by mosquitoes (Caraballo 2014). Mosquitoes are also crucial parts of the food chain as a quickly reproducing species, and they serve as a nutritional source for small vertebrates and larger insects. Climate change will undoubtedly have an effect on mosquitoes, and thus an effect on infectious disease as a whole and many ecosystems around the world.

The effect of changing CO2 and temperature on microbial life is also of significance. Under increased CO2 concentration, rates of terrestrial microbial respiration are unaffected in soil communities (Randlett et al. 1996), however in aquatic microbial communities, leaf litter is a main source of nitrogen and under increased CO2 concentration the C:N ratio of leaves increase due to photosynthetic carbon fixation. As leaves senesce, this effect is amplified, and when litter of these plants cycle into the aquatic ecosystem, overall nitrogen availability for aquatic microbial communities decreases due to decreased N-availability (Tuchman et al. 2002). This has been shown to negatively impact the growth and survivability of aquatic detritivores like mosquito larvae (Ferreira et al. 2010) Mosquitoes are an integral component of their ecosystems, and as such have been previously examined by scientists in order to determine the effects of CO_2, temperature, and carbon to nitrogen ratios within the food of the mosquitos (C:N). Previous studies have found that leaf detritus grown in elevated atmospheric CO_2 decreases the rate of development of mosquito larvae (Tuchman et al. 2003), and that decreased temperatures increases the duration of mosquito larval development (Rueda et al. 1990). However to our knowledge, no study has been conducted that investigates the relationship and effect of these two variables on *Aedes aegypti* mosquito larvae[1].

Our study will address how the interactions between temperature and the C:N ratio within leaf litter affect *Aedes aegypti* larval development in a controlled environment. Studies that have already been conducted on mosquito larvae and the effects of elevated CO_2 on leaf litter have returned conflicting results; Strand et al (1999) found that there was no significant direct effect of elevated CO_2 levels on mosquito performance, while simultaneously finding that light levels and tree species had a more significant effect due to decreased photosynthetic activity in leaves and litter inhibiting growth of leaves and microorganisms that process litter. Contrastingly, Tuchman et al (2003) found that larval development rate was significantly delayed in replicates who fed from leaf litter originating from trees grown in elevated CO_2 levels (760 ppm) due to decreased microbial activity in and on the leaves. Additionally, Rueda et al (1990) found that time of development for *Aedes aegypti* from egg to pupae decreases as temperature increases, while comparing eight mosquito species and found a general trend of decreased development time from egg to pupae as a result of increasing temperatures and that light availability had no

[1] Due to time restrictions, we were unable to acquire leaf litter grown in CO_2 enriched environments, so to simulate the inverse opposite effect, we instead enriched ash leaf litter with nitrogen in order to achieve a similar C:N ratio that would be found within leaf litter grown in CO_2 reduced environments and using ambient levels of CO_2 and C:N as controls. The reasoning is that by determining the response of mosquitoes to past environmental conditions we can predict the response of mosquitoes to future environmental conditions of increased CO_2 and increased temperature, assuming that the curve of [CO_2] and temperature are calculable functions.

direct effect on larval development. Ferreira et al (2010) studied the effects of leaf litter grown under elevated CO2 on a different aquatic detritivore under increased temperature and found that overall survivability was affected by temperature more so than litter quality or composition. The different effects of the variables on mosquitoes prompted us to question the how the intersection of the two variables would affect mosquito larval development.

We hypothesize that the development time of mosquito larvae developing in decreased temperatures will be negatively affected, and that the biomass and development time of mosquito larvae fed nitrogen enriched leaf litter will be positively affected. We hypothesize that time of mosquito larval development and biomass of mosquito larvae developing in decreased temperature while being fed nitrogen enriched leaf litter will be negatively affected, but we assume that it is also possible for a positive effect to occur.

Hence, we predict that an increase in consumable elemental nitrogen in the leaf litter will significantly increase mosquito larval survival rate and biomass but will not have a significant effect on larval development time. We also predict that a decrease in temperature will increase mosquito larval development time, decrease survival rate, and decrease biomass as previously demonstrated. We predict that these two variables will interact as follows: decreasing temperature on developing mosquito larvae feeding on nitrogen enriched leaf litter will decrease rate of larval development, biomass, and survival rate because the metabolisms of the mosquitoes and the microbes within the litter will be slowed down. This will cause the leaf litter to decompose at a slower rate, which will in turn restrict the availability of the food for mosquitos.

We are going to order *A. aegypti* mosquito eggs from Benzon Research, and we will grow them in 4 treatments with 20 replicates in each; these treatments will be marked as Nitrogen Concentration x Temperature (AmbNXAmbT, AmbNxDecT, ElevNxAmbT, ElevNxDecT). The ambient temperature will be 21.5°C and the treatment temperature will be 16.5°C. Mosquitoes will be hatched in water from the leaf detritus group that they will be fed. We will arbitrarily select 80 mosquito larvae from a pool of 2000 after the number of desired individuals hatch; they will then be individually transferred to 80 beakers holding 20mL of treatment solution to grow in to prevent fecal over contamination that would kill the larvae as discussed in Tuchman et al (2003). Water levels are marked in increments of 5 mL on laboratory beakers that will be used in the experiment (30 mL), and store-bought bottled spring water which will be aired out for one 24 hours prior to mosquito hatching will be used to refill the beakers as they lose water due to evaporation. The temperature in the chambers will be controlled and constant as possible, varying by no more than 0.5°C. 1 mL of $1.875*10^{-2}$ mg/mL of ground ash litter will be added to each beaker daily. 10 mL of 0.1M ammonium nitrate solution will be added to the ElevN leaf litter every day for 5 days, stopping on the day that mosquitos will be hatched, and when their food is made every day, 1.6mL of 0.1M ammonium nitrate will be added back in to keep concentration consistent. A flash combustion elemental C:N analyzer will be used to determine the C:N ratio of the leaf litter fed to the mosquitoes. Additionally, we will measure total time from hatch to pupae if possible[2] as well as the wet biomass of each larvae at the end of our experiment. To analyze our findings, we will conduct a factorial ANOVA to

[2] Tuchman et al (2003) and Strand et al (1990) found that mosquito larvae developing at 15°C can take upwards of 70 days to develop, while we will only run this experiment for ~2 weeks.

determine effects of our two independent variables on our mosquito population, returning p-values and interval plots to support or reject our hypotheses.

There is great significance to the response of mosquitoes to changing climate variables. Being a cosmopolitan species, if the climate changes to conditions that suit their lifestyle, the resultant population boom would be disastrous for millions of people in developing and developed countries. This would cause increase in the occurrence of diseases from mild infections to lethal ones. Alternatively, mosquito populations could be wiped out if microbial population decreases according to global decrease in N-availability. While this would put relief on many people in developing countries, it could have long-term consequences for ecosystems that rely on mosquitos as food sources, however it is likely that another small aquatic detritivore would take its place. A different problem arises if mosquito populations plummet, which is possible long-term lack of exposure to generalist pathogens and diseases, decreasing resistance of animal populations to these diseases. However if such pathogens are specialist to mosquitoes as vectors, their occurrence would decrease significantly.

References:

Caraballo, Hector (May 2014). "Emergency Department Management Of Mosquito-Borne Illness: Malaria, Dengue, And West Nile Virus". *Emergency Medicine Practice*. 16 (5).

Ferreira, Verónica, Gonçalves, Ana Lúcia, Gonçalves, Godbold, Douglas L., Canhoto, Cristina (2010). Effect of increased atmospheric CO2 on the performance of an aquatic detritivore through changes in water temperature and litter quality. *Global Change Biology, 16*, 3284-3296.

IPCC. (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. *IPCC*, p.26.

Randlett DL, Zak DR, Pregitzer KS, Curtis PS (1996) Elevated atmospheric carbon dioxide and leaf litter chemistry: Influences on microbial respiration and net nitrogen mineralization. *Soil Science Society of America Journal, 60*, 1571-1577.

Rueda, L. M., Patel, K. J., Axtell, R. C., Stinner, R. E.(1990). Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology, 27* (5), 892-898.

Shelton, Robert M. (1973). The effect of temperatures on development of eight mosquito species. *Mosquito News, 33*, 1-12.

Strand, Mac, Herms, Daniel A., Ayres, Matthew P., Kubiske, Mark E., Kaufman, Michael G., et al. (1999). Effects of atmospheric CO2, light availability and tree species on the quality of leaf detritus as a resource for treehole mosquitoes. *Oikos, 84*, 2, 277-83.

Tuchman, Nancy C., Wahtera, Kirk A., Wetzel, Robert G., Russo, Nicole M., Kilbane, Grace M. et al. (2003). Nutritional quality of leaf detritus by elevated atmospheric CO2: effects on development of mosquito larvae. *Freshwater Biology, 48*, 1432-1439.

Tuchman, Nancy C., Wetzel, Robert G., Rier, Steven T., Wahtera, Kirk A., and Teeri, James A. (2002).Elevated atmospheric CO2 lowers leaf litter nutritional quality for stream ecosystem food webs. *Global Change Biology, 8*, 163-170.