**The impact of sun and shade on mosquito egg laying location**

**Introduction:**

Temperature, precipitation and relative humidity all interact to influence mosquito growth and development in complex ways[1,2]. Mechanistic models predicting mosquito abundance from these weather variables have improved in recent years[3,4], as have predictions of temperature-driven changes to mosquito-borne disease risk[1,5,6]. Differences as small as 1°C can more than double transmission risk[5], highlighting the importance of using accurate temperature measures in models predicting mosquito abundance and disease risk. The temperature experienced by a mosquito depends on behavioral and microclimatic preferences that are poorly understood. Improved understanding of the most appropriate temperatures for use in mechanistic models is crucial for enhancing the predictive capacity of mechanistic models. Here, we use citizen science data to assess broadly whether mosquitoes prefer to lay eggs in sunny or shady habitats, and whether this preference is dependent upon weather variables such as temperature, precipitation or relative humidity measured during the collection period.

**Methods:**

As part of the Invasive Mosquito Project, participating classes at schools around the United States were asked to place oviposition traps in full sun or full shade for 7-14 days between May through October, beginning in August, 2015, 2016, and 2017. Traps consisted of a plastic cup filled with water and lined with sticky paper that trapped any eggs laid in the container during the collection period. Holes near the top of the cup prevented overflowing, while participants were asked to refill cups daily to prevent evaporation. Students counted the eggs at the end of the collection period and uploaded data to www.citizenscience.us.

We obtained data on temperature, total precipitation, and relative humidity during the collection period at each of the locations based on the zipcode that was entered into the database for the sampling location. The weather times series were bilinearly interpolated from the 1/8th degree (~ 15 km) from the North American Land Data Assimilation System (NLDAS) forcing dataset V2. This dataset is produced from the Land Information System (LIS) software for land-surface modeling and data assimilation developed in the Hydrological Sciences Laboratory at NASA.

We used a generalized linear mixed effects model with a negative binomial distribution (glmmADMB package in R) to predict the total number of eggs per day in each oviposition trap as a function of total precipitation, mean relative humidity, and mean temperature during the collection period. We also included an interaction between temperature and the position (sun vs. shade) of the oviposition trap. Zipcode was included as a random intercept term to account for the non-independence of multiple samples collected by the same classroom of students. Non-significant predictors were sequentially removed from the model until only significant predictors remained.

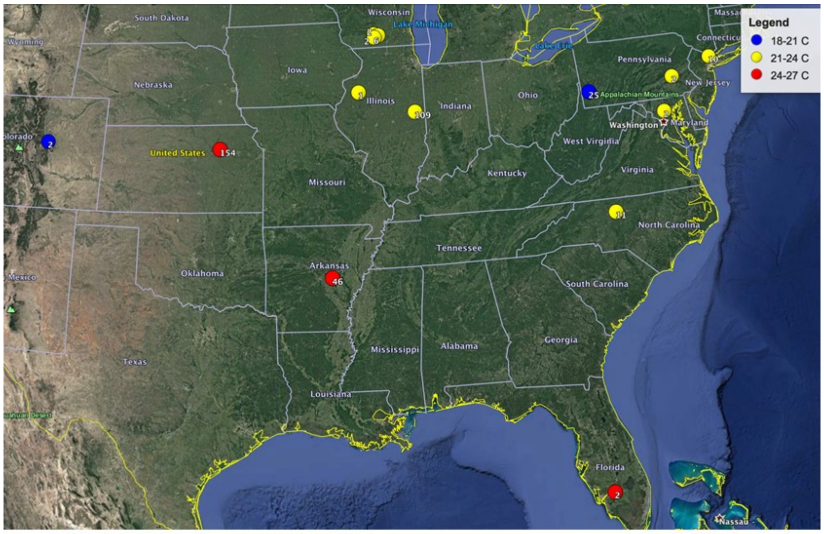


Fig. 1. Location of oviposition trap data sources. Colors represent the mean temperature during the collecting period, while numbers give the total number of samples submitted from each location.

**Results and Discussion:**

Samples were collected from 12 different school districts over varying periods of time at different times of the season (*N* = 242; Fig. 1). The position of the container (sun vs. shade) was the only variable that significantly predicted the number of eggs per day inside each ovitrap (Z = -4.1, $P < 0.01$), with shaded ovitraps having an average of twice the number of eggs as ovitraps placed in the sun (Fig. 2). This effect of ovitrap positioning was not dependent on the temperature during the time of collection, nor were the total number of eggs affected by any of the weather variables included in our analysis.

Collectively our results suggest that habitat use by egg-laying mosquitoes does shift. We found more eggs in the ovitraps placed in shaded positions; however this effect was not mediated by the environmental temperature during the collection period. Thus, our results may reflect simple behavioral preferences of mosquitoes, rather than explicit thermoregulatory responses to high temperatures. Mosquitoes may prefer shaded habitats because they are more likely to find refuge locations and/or food sources in those regions. Nonetheless, our limited sample size and inability to differentiate amongst species suggests it would be premature to conclude that mosquitoes are not thermoregulating (e.g., seeking out shaded ovipositing habitats to avoid high temperatures).

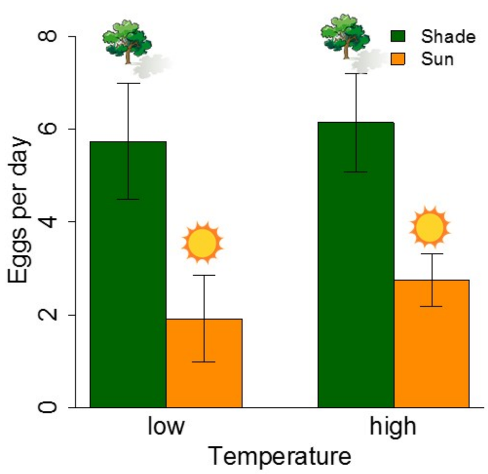


Fig. 2. Mean +/- SE of eggs laid per day in ovitraps that were placed in the sun (green) or shade (orange). Low temperatures were below the median collection period temperature of 25°C, high were above.

Analyses were complicated by the patchy spatial as well as temporal distribution of the dataset. Some school districts collecting weekly data each season for several years, with others collecting large amounts of data from the same location at the same time. A mixed effects analysis was used to account for the non-independence of the datapoints from similar locations. Future analyses accounting for latitudinal differences in mosquito responses would also be of interest. For instance, collections from lower latitudes may show less of an effect of temperature than those from higher latitudes. Controlling for the time of season during which the collection was made is another future direction that will be important to consider as the dataset becomes large enough to support more detailed analyses.

This preliminary analysis has offered substantial insight into the strengths and weaknesses of citizen science data for scientific inquiry. Prior to using this data, we had to implement certain quality control algorithms to eliminate unreasonable estimates and repeat entries of the same observations. Additionally, care must be taken to account for the non-independence of samples collected in similar locations or by the same groups of students. Larger datasets should help to counter-act some of these issues and allow for the testing of more nuanced hypotheses. Having the citizen scientists implement an experimental (e.g., placing traps in both sun and shade), rather than observational protocol is a particularly helpful component of this study, by circumventing some of the issues arising due to patchy distributions of samples. Future citizen science experiments could include using different types of containers as ovitraps, requesting sequentially collected samples over longer time-periods, and requesting samples during and after extreme weather events.

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**References:**

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