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2019 grant amount: $5,561

Please answer the following questions with 1- to 2- sentence summaries:

Research Project Topic: Mosquitoes of wetland and low order streams: Identification of mosquito hosts through genetic sequencing and influential abiotic factors of north-central Illinois.

Research Project Purpose: Investigating and determining mosquito abundance compared to major abiotic factors (precipitation, temperature, and wind speed) through developing models and determining mosquito hosts through genetic sequencing and identifying mosquito species of Nachusa Grasslands within Lee and Ogle counties.

Research Project Outcomes to date:

Mosquito collections took place on Nachusa Grasslands Preserve from May 21—October 1, 2018 and May 27—October 3, 2019. Trap sites were located within the Meiners Wetland unit, including some sites near Franklin Creek and an unnamed tributary, and also in the Clear Creek unit (Figure 1). The mosquito sampling method had the addition of hanging traps using CO_2 during the 2019 sampling season alongside gravid traps and oviposition cup traps. Field sites were checked twice weekly to collect mosquito and refresh trap batteries; oviposition traps were checked once weekly to collect mosquito eggs.

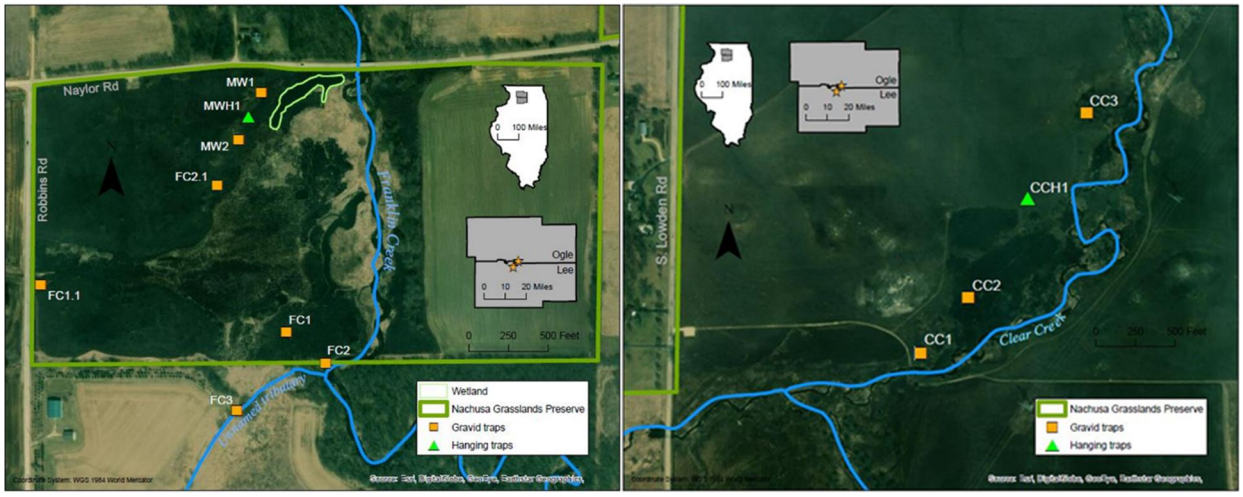

Figure 1. (Left) Gravid and hanging trap locations in the Meiners Wetland and Franklin Creek unit and adjacent agriculture land with the unnamed tributary which flows into Franklin Creek. These trap sites are located within Lee County, IL. (Right) Gravid and hanging traps located in the Clear Creek unit of Nachusa Grasslands. These traps are located within Ogle County, IL.

The 2018 and 2019 sampling seasons yielded 3,385 and 555 *Cx*. mosquitoes, respectively. There were a total of seven and six *Cx*. species collected in 2018 and 2019, respectively. During 2019, there were 50 *Cx*. mosquitoes out of 555 that were collected from hanging traps, including the species: *Cx. salinarius*, *Cx. declarator*, *Cx. quinquefasciatus*, and *Cx. erraticus.*

A total of 246 and 84 adult *Aedes* mosquitoes were collected during the 2018 and 2019 field seasons, respectively. Three native *Ae*. species were collected during 2018; in 2019 a fourth *Ae*. specie was collected: *Ae. japonicus*, which is also considered an invasive mosquito specie. The appearance of *Ae. japonicus* was also a first record within Ogle County and perhaps the first record of *Ae. japonicus* on the preserve. Out of 84 *Ae*. adults collected in 2019, 58 were collected from hanging traps, which includes all species previously mentioned except *Ae. japonicus.* There were 1,896 and 3,238 *Aedes* eggs collected in 2018 and 2019, respectively. To date, all hatched adults have been identified as *Ae. triseriatus*, the tree-hole mosquito. In 2018, 103 eggs hatched and successfully developed to adult during the 2018 field season. Eggs from 2018 were observed to continue hatching in late spring and early summer of 2019 after overwintering, yielding 134 additional adults for a total of 237 adults from 2018. From 2019 egg collections, a total of 258 adult mosquitoes hatched.

Several other mosquito genera were captured including *Anopheles*, *Coquilettidia perturbans*, *Culiseta inornata, and Uranotaenia* (*Ur.*) *sapphirina. Ur. sapphirina* was a first record within the preserve and in Lee and Ogle counties. A total of 477 and 67 *Anopheles* mosquitoes were collected during the 2018 and 2019 field seasons, respectively. Three *An*. species were collected in 2018 and two *An*. species were collected in 2019. In 2019, there were 17 out of 67 adult *An*. and three *Cq. perturbans* captured in hanging traps.

West Nile virus (WNV) testing was conducted on mosquitoes captured from Lee and Ogle counties in 2018 and 2019 using the RAMP® method. Only one positive pool was detected out of 66 pools in 2018 at a RAMP® reading of 176.2 units and a minimum infection rate of 0.61. The positive pool consisted of *Cx. quinquefasciatus* mosquitoes from the site FC1 between August 9 and August 13, 2018. Ten pools were tested for WNV from 2019, however all pools were negative.

Atmospheric data were collected during 2018 and 2019, including temperature, precipitation, and wind speed. These are considered major abiotic factors in Illinois in regards to influence on mosquito abundance. Relationships and models were developed between the abiotic factors and mosquito abundance. Accumulated thermal time was calculated and compared to peak *Culex* collections. The 2018 sampling season observed the *Cx*. peak to occur at week 32, while the 2019 sampling season *Cx*. peak occurred at week 30. While there was no significant relationship between *Cx*. collected and accumulated thermal time at the start of the sampling season or *Cx*. collected and annual accumulated thermal time, there was a direct linear relationship with annual *Cx*. collected and accumulated thermal time during the sampling seasons (r^2=1). Based on nonlinear regression analysis, there was a first order relationship between *Cx*. collected and average daily precipitation over the 2018—2019 sampling

seasons, r^2=0.744 and r^2=0.936, respectively. This trend is consistent with past data from other sampling sites in west-central IL and also significant since this occurred in two separate geographic locations. Wind speed was examined with mosquito abundance and did not demonstrate much of an influence; 2018 and 2019 seasons yielded r^2=0.518 and r^2=0.548, respectively, but should still be considered in future studies.

While there were a total of 240 mosquito samples sent for sequencing to determine the host source from mosquito blood meals, only 45 samples returned reliable results. From these samples, hosts identified included human, livestock, and small rodents. Some possible explanations the sequencing did not yield greater results: mosquitoes took blood meals from more than one organism, which the Sanger sequencing method is more sensitive to this, or there was environmental contamination.

Water samples were conducted monthly from May—October in both field seasons to measure nitrates, phosphates, and pH levels in water bodies near trap sites. Stream velocity measurements were also recorded at Franklin and Clear creeks and an unnamed tributary. Phosphate (PO_4^{-3}) levels were greatest from samples taken at Meiners Wetland in both sampling seasons, reading at 1.1 mg/L and 2.1 mg/L in 2018 and 2019, respectively. Nitrate (NO^{-3}) levels were greatest from Clear Creek samples both years, reading between 59.4—80 mg/L. pH measurements ranged from 6.27—7.58 from wetland and stream sites. The greatest velocity measurements were taken from Clear Creek at 1.3 m/s.

Describe how the grant funds you have received from the Friends of Nachusa Grasslands have been used in regard to the above topic, purpose, and/or outcomes:

The grant funds from the 2019 Friends of Nachusa Grasslands award have been used solely for the purpose of conducting this research. With this award, three new hanging mosquito traps were purchased along with CO_2 cartridges and accessories to use in comparison against gravid traps. The hanging traps were specifically designed to lure *Aedes* and other species due to the CO_2 bait and height of the trap (mosquitoes seek hosts at different heights). Fresh motors and fans for gravid traps were purchased to help support the field work needed to complete mosquito collections, which burn out after a season of use. A portion of this award went towards travel to and from the field sites at Nachusa Grasslands and the WIU-QC campus in Moline, IL to conduct laboratory work. Another portion of this award was used towards purchasing laboratory supplies to aid in mosquito identification, kits for DNA extractions and PCR on mosquito samples, and to help with the cost of sequencing. Please see the table below presenting the expense report.

Table 1. Nachusa Grasslands 2019 scientific research expense report.

Date	Category	Description	Notes	Amount
4/10	**Equipment**	Omni-Directional Fay-Prince Trap x3	John Hancock Co.	$567
4/29	**Lab supplies**	Safe-lock graduated tubes, tris-acetate-EDTA (TAE), tube rack, AmpliTaq gold master mix, DPBS	Fisher Scientific	$512
4/29	**Equipment**	Trap motors x10	JameCo. Electronics	$35
5/15	**Travel**	Field work/lab work	TV2716600	$33
6/14	**Travel**	Field work/lab work	TV 2741800	$32
6/30	**Equipment**	CO2 cartridges, adapters	AirGas, other	$100
10/04	**Travel**	Field work/lab work	TV2716700, 2807700, 2807900	$302
10/08	**Lab Supplies**	GeneJET Viral DNA/RNA Purification Kit x2	Thermo Scientific	$420
10/25	**Travel**	Field work/lab work	TV2833000, 2833100 2833200 2833300	$480
11/01	**Sequencing**	Sanger sequencing	University of Iowa	$1700
11/20	**Travel**	Field work/lab work	TV2832700, 2832800, 2832900	$360
12/03	**Supplies**	Gel/PCR DNA Fragments Extraction Kit (100 preps)	IBI Scientific	$100
8/14	**Supplies**	Statistics software	XLSTAT	$99
Total:				**$4,740.00**

Describe how your project has benefited the work and goals of Nachusa Grasslands:

The research I conducted at Nachusa helped contribute to the inventory of species that currently live within the preserve. Illinois contains numerous mosquito species and some expand their range each year bringing new health risks with them. Recording the current mosquito species each year helps determine what are preferred hosts, which mosquito specie populations are changing over time, and what arboviruses they can potentially vector.

Through my research I developed models and relationships between mosquitoes and several major abiotic factors, including thermal time, precipitation, and wind speed and determine which has the most influence on mosquito abundance. These models can be applied each season to observe changes in the population and weather and also through a grand unifying model of all sampling seasons. These general models between abiotic factors and mosquito abundance worked in two separate geographic locations in Illinois. The models can be applied to other natural systems, and may be of use in future science conducted at Nachusa. The relationship between thermal time and mosquito abundance can be observed and used to determine when peak mosquito abundance is during the given year. That information can be compared to peak times of other species which may be of significance at Nachusa. Precipitation and mosquito abundance was observed as a first order relationship and can be a predictor of increased mosquito presence.

I was able to establish an initial baseline of mosquito hosts at Nachusa through sequencing their blood meals. However, future work should be continued to expand this information. It is very useful in understanding what organisms have a greater risk of becoming infected with an arbovirus, such as West Nile virus, and potentially act as bridge vectors that can infect humans and other wildlife.

Describe how your findings can be applied to challenges in management practices for restoration effectiveness and species of concern:

This research can be applied toward mosquito control and preventive measures to reduce their population that could otherwise affect the visitors and wildlife at Nachusa. The 2019 field season resulted in a capture of an invasive mosquito specie called *Aedes* (*Ae*.) *japonicus*. *Ae. japonicus* has the ability to carry viruses such as La Crosse virus, eastern equine encephalitis virus, St. Louis encephalitis virus, and even West Nile virus. *Aedes* mosquitoes can carry other serious diseases including dengue and chikungunya. It is significant to note the presence of *Ae. japonicus* at and around Nachusa since these mosquitoes have been known to feed on both avian and mammalian species, are more aggressive, and more hardy. Understanding the *Aedes* mosquito feeding habits and breeding preferences can help control the population while lessening its impact on other species of concern such as reptiles, birds, and even bison at Nachusa. This research started gathering surveillance data of *Ae. japonicus* that can be useful in monitoring its impacts in certain locations where other organisms may be at risk. This may also identify locations *Ae. japonicus* and other *Aedes* species are laying their eggs. *Aedes* eggs are resistant to desiccation, however at extreme temperatures may be destroyed. This opens the possibility that prescribed burns may help eliminate some eggs and thus decreasing the mosquito and the pathogens that could infect humans or wildlife in the area.

Water quality and stream velocity measurements were recorded near mosquito traps to identify baseline properties of mosquito larval habitats. Stream velocity allows us to observe likely habitats for mosquito egg and larva development. Levels of nitrate and phosphate can be used for short and long-term monitoring of stream and wetland habitats and particular species already inhabiting them on the preserve. Management practices can be used accordingly to help increase the presence of a targeted specie(s) through

those findings and also help collaborate with surrounding property owners to help manage nutrient levels in the water sources.

Please list presentations/posters you have given on your research:

Posters and presentations of this research:

1. Rehbein, M. and Viadero, R., Jr. Environmental Factors Influencing Mosquitoes in Wetland and Low Order Streams at Nachusa Grasslands Preserve. Poster: Upper Mississippi River Conference, Moline, IL. October 23, 2019.

2. Rehbein, M. and Viadero, R., Jr. Environmental Factors Influencing Mosquitoes in Wetland and Low Order Streams at Nachusa Grasslands Preserve. Poster: Nachusa Grasslands Science Symposium, Dixon, IL. October 19, 2019.

3. Rehbein, M.M. and Viadero, R.C. Identification of *Culex* and *Aedes* Mosquito Microbiomes in Wetland and Low Order Stream Habitats. Oral presentation: Nachusa Grasslands Science Symposium, Franklin Grove, IL. October 20, 2018.

4. Rehbein, M.M., Hunt, J.R., Miller-Hunt, C., & Viadero, R.C. A Mosquito Inventory and Abiotic Factors That May Affect Their Presence in Wetland and Low Order Stream Habitats at Nachusa Grasslands. Poster: Upper Mississippi River Conference, Moline, IL. October 24, 2018.

Media coverage:

1. Mosquito research discussed in relation to Mississippi River flooding and mosquito populations, interviewed and televised by WQAD: https://wqad.com/2019/09/03/fewer-mosquitoes-likely-because-of-flooding-western-illinois-university-researcher-says/.

2. Mosquito peaks and populations discussed, interviewed and televised by WQAD: https://wqad.com/2018/07/10/mosquito-season-about-to-hit-peak-months/.

Have you submitted manuscripts to scientific journals? If so, which ones? If not, do you anticipate doing so? (Please send copies of published articles to the Friends so that we can learn from your work.**)**

None have been submitted yet, but manuscripts will be submitted and shared with the Friends of Nachusa Grasslands in the future.

<u>Optional</u>: Suggestions for improving the application and award process for future Friends of Nachusa Grasslands Scientific Research Grants:

The application and award process was straight forward. I enjoyed having the chance to present my own research at the symposium each fall during 2018 and 2019, and meeting other scientists and learning about their research at Nachusa. I have appreciated the support and help from Friends and others who are involved with the scientific community at Nachusa Grasslands. It has been a great experience conducting research at Nachusa. Thank you again for this award and allowing the opportunity to conduct research at Nachusa Grasslands!