

**The Friends of Nachusa Grasslands  
2018 Scientific Research Project Grant Report  
Due June 30, 2019**

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**2018 grant amount:** \$3,170

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

**Research Project Topic:** Identification of *Culex* and *Aedes* mosquito microbiomes in wetland and low order stream habitats.

**Research Project Purpose:** To identify species of mosquitoes which are present in Nachusa Grasslands wetland and stream habitats while determining their microbiota. This research project also serves to investigate any overlap or differences of the mosquito microbiota and whether they may influence mosquito populations.

**Research Project Outcomes to date:**

While this research project is ongoing, the first field season yielded a total of 3,385 *Culex* (*Cx.*), 246 *Aedes* (*Ae.*), 477 *Anopheles* (*An.*), six *Coquilettidia perturbans*; two *Uranotaenia sapphirina*; and one *Culiseta inornata* mosquitoes. There were seven species of *Cx.* identified: *Cx. tarsalis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. declarator*, *Cx. salinarius*, *Cx. restuans*, and *Cx. erraticus*. Three species of *Ae.* were identified: *Ae. triseriatus*, *Ae. vexans*, and *Ae. trivittatus*. All three *Ae.* species are considered native to this area. There were also three species of *An.* identified: *An. punctipennis*, *An. crucians*, and *An. quadrimaculatus*. A total of 1,896 eggs were counted and collected from the cup traps. During 2018, 103 adults were successfully reared to adult. All 103 adults were identified as *Ae. triseriatus*, also known as the tree-hole mosquito. As of April 7, 2019, eggs which overwintered in emergence jars collected from Franklin Creek (FC1), Meiners Wetland (MW1), and Clear Creek (CC2) sites from the 2018 field season have hatched and larvae are present. Currently, 112 adult *Ae. triseriatus* have hatched in 2019 from the 2018 field season; 32 adults from CC2, 27 adults from MW1, 53 adults from FC1. Emergence jars are being observed each day for further larval and adult development for identification. Trap sites are presented in Figure 1A and 1B below.

Presently, RAMP® West Nile virus (WNV) tests have been completed on 58 pools of mosquitoes. Each pool consisted of 25 mosquitoes. The mosquitoes tested for WNV were the three most common vectors of the virus: *Cx. pipiens*, *Cx. restuans*, and *Cx. quinquefasciatus*. Out of approximately 1,450 mosquitoes, only one pool tested positive for WNV at a RAMP® reading of 176.2 units. This positive pool was located from the Franklin Creek (FC1) site between the dates of August 9, 2018 and August 13, 2018 and were *Cx. quinquefasciatus* mosquitoes.

DNA extractions and qPCR were completed on 96 mosquito samples for preparation of host blood meal sequencing. The 96 mosquitoes consisted of the three most common *Cx.* species (*Cx. pipiens*, *Cx. restuans*, and *Cx. quinquefasciatus*) from each of the eight trap sites from the 2018 field season. The 96 mosquito samples will be sent to the University of Iowa laboratory for completion of sequencing.

Further DNA extractions and qPCR will be completed on *Culex* and *Aedes* from the 2018 season. Mosquitoes will be sequenced using the Illumina MiSeq System to determine the microbiome of their guts. The Illumina MiSeq sequencing will be completed at USDA-Peoria laboratories.

Water and soil samples were taken monthly near trap sites. Soil samples will be analyzed to identify bacteria and fungi from the sites. There were five consecutive locations from which water was sampled during each month, including Clear Creek near CC1 site, Meiners Wetland near MW1 site, Franklin Creek near FC2 site, the pond near FC1 site, and on the tributary emptying into Franklin Creek near FC3 site. Water samples were tested for nitrite, nitrate, and phosphate using HACH® water test kits. Total hardness, alkalinity, and pH were also measured from each water sample. Water samples taken from Meiners Wetland had an elevated amount of phosphate ( $\text{PO}_4^{3-}$ ) compared to all other sample sites, at a reading of 1.4 mg/L. Clear Creek and the unnamed tributary had elevated amounts of nitrate ( $\text{NO}_3^-$ ) both measured at 80 mg/L. Using a global flow meter, stream velocity was measured near trap sites throughout the field season. Clear Creek had the highest maximum velocity throughout the season of 3.93 ft/s and an average of 1.27 ft/s. Franklin Creek had an average velocity of 0.38 ft/s and a maximum velocity of 2.3 ft/s, and the unnamed tributary had a maximum velocity of 0.98 ft/s and an average velocity of 0.21 ft/s.

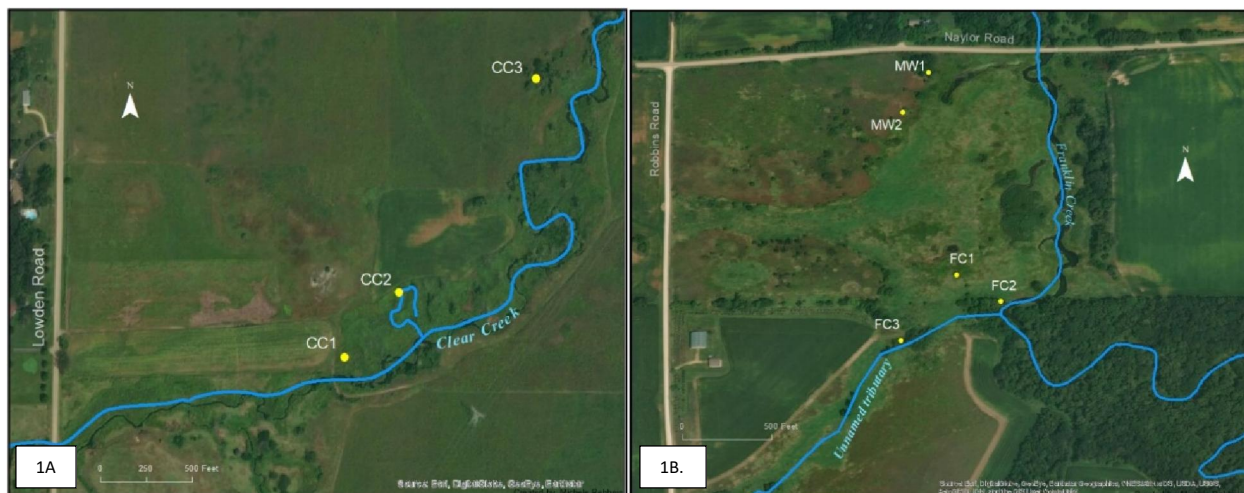


Figure 1A. Clear Creek trap sites from the 2018 mosquito field season. Figure 1B. Jay Meiners Wetland, Franklin Creek, and the unnamed tributary mosquito trap sites from the 2018 mosquito field season.

**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 2018 Friends of Nachusa Grasslands award have been used solely for the purpose of conducting this research. With this award, new mosquito traps were purchased. Without the mosquito traps, this research would not have been started and the samples most vital to this project would not have been collected. 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, completing DNA extractions, and conducting qPCR on mosquito samples. Please see the table below presenting the expense report.

Table 1. Nachusa Grasslands 2018 scientific research expense report.

| <b>Date</b>   | <b>Category</b>           | <b>Description</b>                              | <b>Notes</b>             | <b>Amount</b>     |
|---------------|---------------------------|---|--------------------------|-------------------|
| 4/10          | <b>Equipment</b>          | Frommer Updraft<br>Gravid traps                 | John Hancock Co.         | \$1,140.86        |
| 5/16          | <b>Field<br/>supplies</b> | Safe-lock graduated<br>tubes                    | Amazon                   | \$51.45           |
| 6/12          | <b>Field<br/>supplies</b> | Microcentrifuge<br>tube racks                   | Amazon                   | \$27.39           |
| 6/21          | <b>Travel</b>             | Field work/lab work                             | TV24641, 24644,<br>24648 | \$655.00          |
| 7/20          | <b>Equipment</b>          | Multi bottle rack                               | Amazon                   | \$60.07           |
| 8/3           | <b>Travel</b>             | Field work/lab work                             | TV24989, 24991,<br>25241 | \$295.00          |
| 8/7           | <b>Supplies</b>           | Safe-lock graduated<br>tubes                    | Amazon                   | \$34.67           |
| 9/21          | <b>Supplies</b>           | DPBS, AmpliTaq<br>gold mix, distilled<br>water  | Fisher Scientific        | 331.55            |
| 9/21          | <b>Supplies</b>           | Stainless steel<br>beads                        | Fisher Scientific        | \$121.89          |
| 9/21          | <b>Supplies</b>           | Ethanol, agarose,<br>tris-acetate-EDTA<br>(TAE) | Fisher Scientific        | \$239.83          |
| 9/21          | <b>Supplies</b>           | MiniElute PCR<br>purification kits (2)          | Qiagen                   | \$247.10          |
|               |                           |   | IES paid                 | -\$34.81          |
| <b>Total:</b> |                           |   |                          | <b>\$3,170.00</b> |

\*IES = Institute for Environmental Studies

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

Understanding which mosquito vectors exist within the Nachusa Grasslands property is significant and helpful in conducting research on other species of importance, such as bison and various birds, reptiles, and amphibians. Mosquitoes have the potential to carry very deadly and harmful infectious diseases that impact not only humans, but wildlife, too. Having further knowledge of the current mosquito inventory at Nachusa can aid in proactive steps towards managing them and other fauna around them.

Water quality is an important factor in stream and wetland management including the aquatic species inhabiting them. Nitrate and phosphate levels can affect aquatic vegetation, macroinvertebrates, and vertebrates such as waterfowl, fish, and amphibians. Conducting water quality sampling and stream velocity measurement helps both short and long-term monitoring of these habitats.

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

One genus of mosquito that is collected through this research is the *Aedes* (*Ae.*) mosquito. *Ae.* females oviposit their eggs in various locations, including tree holes that collect water, substrates in water that will soon become inundated, and also in the soil along banks of streams and other aquatic sources. Their eggs can withstand desiccation up to a certain temperature, however some research has suggested controlled burning may reduce the number of mosquito eggs by destroying them and leaving them no longer viable. Using this management practice, *Ae.* and other mosquito species populations may decline. With a decrease in mosquitoes, opportunities for harmful pathogens to spread to other animals will be reduced.

Identification and classification of bacteria and fungi residing within mosquitoes can further the knowledge of the association of microorganisms with a particular pathogen and its infection of a host. Understanding these potential associations can assist with conservation practices and management of not only the bison at Nachusa Grasslands, but also other animal species of concern within Nachusa Grasslands including endangered or threatened birds or reptiles. Through studying mosquito microbiomes, more data on microbial taxa existing within the aquatic habitats of wetlands and streams at Nachusa will be known. This research will add to existing knowledge of microbial communities within mosquitoes from other geographic locations to compare populations at Nachusa to and can be used to potentially control mosquitoes and the diseases they vector on a larger scale. By monitoring mosquito microbiomes and West Nile virus from mosquitoes at Nachusa, these data may prove helpful in maintaining native mammal species which reside on Nachusa Grasslands property through determining active mosquito species and populations actively carrying this virus.

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

Rehbein, M.M. and Viadero, R.C. 2018. Identification of *Culex* and *Aedes* mosquito microbiomes in wetland and low order stream habitats. Nachusa Grasslands Science Symposium, Franklin Grove, IL.

Rehbein, M.M., Hunt, J.R., Miller-Hunt, C., & Viadero, R.C. 2018. A mosquito inventory and abiotic factors that may affect their presence in wetland and low order stream habitats at Nachusa Grasslands. Upper Mississippi River Conference, Moline, IL.

**Have you submitted manuscripts to scientific journals? If so, which ones? If not, do you anticipate doing so? (Please keep us informed on publications.)**

No manuscripts have been submitted yet, but I do anticipate submitting manuscripts after another field season has been completed and data have been analyzed from both seasons. Copies of the manuscripts will be shared with and given to Nachusa Grasslands Preserve.

**Optional: Offer suggestions for improving the application and award process for future Friends of Nachusa Grasslands Scientific Research Grants:**

The application and award process in my experience has been straight forward and the Friends of Nachusa Grasslands are very quick to help if any questions arise during the process. Thank you again for this award and allowing the opportunity to conduct research at Nachusa Grasslands.