Factors Influencing Abundance & Virus Isolation in Mosquitoes

Which environmental, ecological, and temporal factors impact mosquito populations

Andrew Ruiz

2024-04-19

# Summary/Abstract

*This project will inform the development of my dissertation prospectus by identifying significant relationships between variables and guide the methods development.* Eastern equine encephalitis (EEE) is caused by an Alphavirus transmitted to humans by the bite of an infected mosquito. Human infections are rare, but serious. About 30-50% of symptomatic cases lead to death and those who survive are left with life-long disability. The transmission cycle for EEE includes two distinct cycles: an amplifying enzootic cycle in which the virus is transmitted between the *Culiseta melanura* mosquito and birds and an epizootic cycle in which bridge vector mosquitoes transmit the EEE virus (EEEv) from birds to humans and other mammals (Go, Balasuriya, and Lee 2014). Because C. melanura feeds almost exclusively on birds, it is not considered a direct human threat. However, EEEv isolations in *C. melanura* are an early warning sign that EEEv is circulating in the ecosystem. Isolations in bridge vector mosquito species indicate heightened transmission risk to humans.

# Problem statement

Which environmental,ecological, and temporal factors influence the transmission cycles for EEE in southeastern Massachusetts and which models best describe the relationship between these factors?

**This project will lay the foundation for dissertation research that will begin in June, 2024.**

# Introduction

## Background

Eastern equine encephalitis (EEE) is caused by an Alphavirus transmitted to humans by the bite of an infected mosquito. Human infections are rare, but serious (Lindsey, Staples, and Fischer 2018). Humans and other mammals are dead-end hosts for EEE. About 94% of human EEE infections are asymptomatic. While symptomatic cases are rare, the consequences can be severe. Mortality rates associated with symptomatic infections range from 30 to 50% (Morens, Folkers, and Fauci 2019). Those who survive often experience lifelong disability that includes debilitating neurological damage (Massachusetts Department of Public Health 2024). On average, there are 11 cases reported in the US annually. Most occur in Massachusetts and Florida (Vander Kelen et al. 2014). Until recently, nearly all human EEE cases in Massachusetts occurred in two counties: Bristol and Plymouth.

Since 2000, there have been 45 human cases recorded in Massachusetts, resulting in 22 deaths (Massachusetts Department of Public Health 2024). There are no human vaccines for EEE, and treatment consists of palliative care only. Prevention measures include personal behaviors to avoid mosquito bites and decreasing the mosquito population through pesticide use and environmental modifications like removing standing water (Centers for Disease Control and Prevention 2022).

The Bristol County Mosquito Control Project (BCMCP) coordinates mosquito surveillance and testing in the county from June to October. BCMCP has used the same sentinel collection sites and trapping methods for over 40 years. Once trapped, mosquitoes are sorted and counted by species, and vector species are submitted to the Massachusetts Department of Health’s (MDPH) State Lab for PCR virus testing. Results are available within 24 hours of submission. When rates of EEE mosquito infections are above a defined threshold, MDPH notifies the local boards of health and recommends preventive measures. Recommended measures include outreach and education to increase personal protective practices . Additionally, MDPH may recommend that cities and towns in the affected areas cancel evening outdoor events and discourage outdoor activities when mosquito vectors are most active (dawn and dusk). When infection rates are high enough to indicate imminent human transmission, the Commonwealth of Massachusetts will recommend and fund aerial pesticide applications over the affected areas.

## Study area

Bristol County, Massachusetts covers an area of 691 square miles with a population density of 962 people per square mile. It borders Rhode Island to the west, Plymouth County, MA to the east, and Norfolk County, MA to the north. Naushon Island and Martha’s Vineyard are across Buzzard’s Bay to the Southeast.

Atlantic White Cedar (AWC) swamps span over 4,300 acres within Massachusetts, primarily concentrated in Plymouth and Bristol counties. These swamps serve as critical breeding habitats for *Culiseta melanura* (MEL) (Commonwealth of Massachusetts Division of Fisheries & Wildlife 2007), which is recognized as the primary enzootic vector for Eastern Equine Encephalitis (EEE). AWC trees have large root systems that form “crypts” and hold water throughout the year. These crypts serve as a main habitat for MEL larval growth and provide shelter during winter months.

Between 2003 and 2022 Plymouth and Bristol counties were the epicenters for EEE transmission in Massachusetts, accounting for 24 out of the state’s 41 human EEE cases.The Bristol County Mosquito Control Project (BCMCP) conducts mosquito surveillance and abatement activities for the county and often coordinates activities with Plymouth County Mosquito Control Project.

## Questions/Hypotheses to be addressed

*This project will lay the foundation for my dissertation research.* **The goal for this phase of the project is to answer which environmental, ecological, and temporal factors influence the transmission cycles for EEE in southeastern Massachusetts.** From prior research we know that the mosquito life cyle is influenced by a number of factors. Temperature plays a major role in the mosquito life cyle virus transmission dynamics. As temperature increases, the rate of larval development also increases and the time between when a mosquito ingests virus in a bloodmeal and when it can transmit it decreases. The result is that the mosquito population can grow at faster rate and they will have more time to transmit the virus. However, MEL larvae develop in well insulated crypts that are less sensitive to ambient air temperature changes, so the effect of temperature changes on MEL development speed may be less pronounced than other vector species that develop in open, standing water, like ditches and containers. Precipitation increases the amount of standing water and increases the chances of larval survival during the mosquito season and also during the winter months for those species that overwinter in the larval stage. For EEE, the main enzootic vector, MEL, overwinters as larvae.

While these relationships are well known, they are not well described for EEE, especially in terms of quantifying the role these two factors play in EEE transmission. Specifically, there’s a gap in our understanding of how these factors influence the number of mosquitoes caught in traps and the rate of virus infection among those mosquitoes. This gap is more pronounced when distinguishing the impacts on enzootic (mosquitoes that primarily feed on birds) versus bridge vector (mosquitoes that can transmit the virus from birds to humans) mosquito species. Even less well described is the effect the factors have on enzootic vs. bridge vector mosquito species.

Moreover, the influence of other variables remains even less explored. Birds, especially passerine species, serve as amplifying hosts for EEEV and WNV, potentially affecting the level of virus present in an area. Despite their importance in the virus transmission cycle, the effect of fluctuations in bird populations on the prevalence of these viruses throughout a season has received minimal attention. Finally, given the migratory nature of many passerine species, it’s worth exploring whether EEEV activity in the warmer regions of the United States, during the months preceding mosquito season in Massachusetts, could serve as an early indicator of virus activity in the state. Understanding these dynamics is crucial for predicting outbreaks and implementing effective control measures.

While the following questions will not be addressed in this project, it will hopefully inform research that will answer the following questions in dissertation research beginning this summer:

1. Can an AI-driven model that leverages historic mosquito surveillance, ecological, and environmental data accurately quantify the risk of human EEEv infection in southeastern Massachusetts?

2. What is the potential of machine learning algorithms to identify early warning signals for EEE outbreaks, enabling timely public health interventions in Massachusetts to prevent human infections?

3. How can machine learning models use real-time data on mosquito activity, environmental factors, and weather to accurately predict when the risk of EEE transmission to humans is low enough to lift evening outdoor activity restrictions? \*

## Data collection and processing

### Mosquito surveillance data

The mosquito surveillance dataset encompasses counts by species from all mosquito traps deployed in Bristol County, MA, from 2007 to 2023. The raw dataset includes over 45,000 records and includes temporal and spatial variable. The BCMCP, perform mosquito surveillance from June through October annually.

During this interval, CDC light traps baited with carbon dioxide are placed at various sentinel sites each week. Designed to attract mosquitoes in search of blood meals, light traps serve as a reliable measure of the actively feeding mosquito population, thereby establishing them as the standard method for EEE surveillance. Some of the sentinel site have been used for over 50 years.

Additionally, the county employs gravid traps, which are specifically aimed at attracting and capturing gravid *Culex* mosquitoes looking for egg-laying sites. While these traps are primarily used for West Nile Virus (WNV) surveillance, they hold significant value for EEE monitoring as well. Given the role of *Culex* species as bridge vectors for EEE, their capture in gravid traps provides critical insight into the risk of virus transmission.

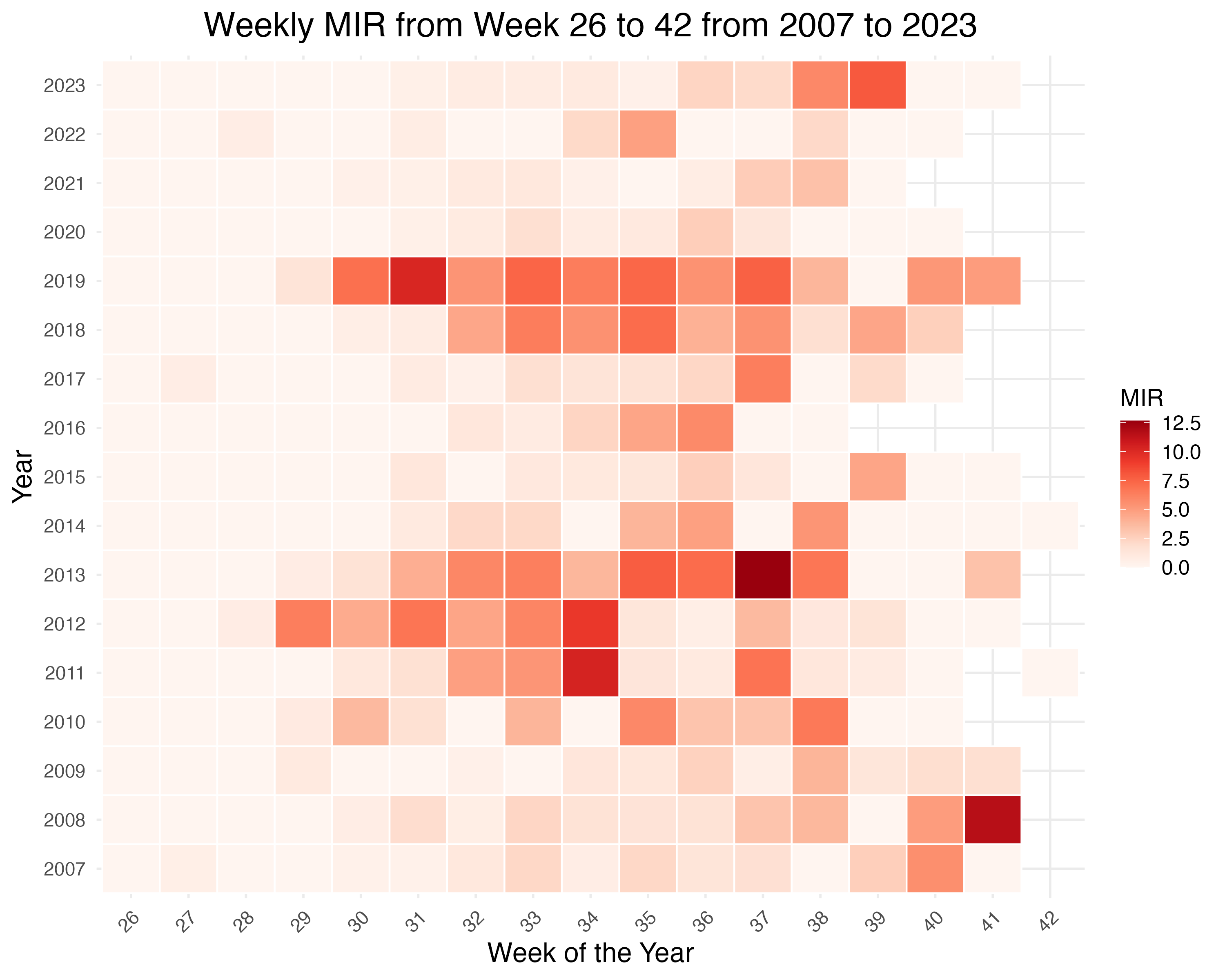
The traps are installed in the afternoon and retrieved the following morning. After the traps are collected, the next are stored in a freezer to preserve and kill the mosquitoes. The mosquitoes are sorted by species using a dissecting scope. Counts for each species are recorded in the database and a portion of the vector species are driven to the Massachusetts State Laboratory in Boston. The lab tests each mosquito pool (or batch) for WNV and EEEv using PCR. Results are shared with BCMCP on the same day of testing. Virus isolation results are recorded in the collection records.

In addition to mosquito counts by species, the dataset includes the town or city in which they were collected, the collection date, and whether the mosquitoes were submitted for virus testing.

### Minimun infection rate (MIR)

One indicator of the level of virus activity present in the mosquito population can be calculated from the information contained in dataset. The minimum infection rate (MIR) can be calculated at various temporal and spatial scales from trap site over one day to all sites over an entire season. MIR is calculated as:

MIR provides an estimate of the prevalence of a specific pathogen within a mosquito population. While it does not directly measure the risk to humans, a higher MIR in mosquitoes that commonly bite humans can indicate a higher risk of transmission. A study pubulihsed in 2007 indicated that in years with human cases in Massachusetts between 1979 and 2004, MIR was ≥1.0 by week 31 (Hachiya et al. 2007). MIR allows for the comparison of infection rates across different geographical areas or time periods. This is crucial for identifying areas or times of higher risk and for evaluating the effectiveness of control measures.



Minimum infection rate by week

The surveillance dataset is structured to conform with state and federal reporting requirements. As a result, the format of the data must be transformed to in order to make it ready for analysis. Once the data structure is corrected, it can be joined to other relevant datasets using spatial or temporal attributes.

### Weather data

Weather plays a significant role in the lifecyle of the mosquito and influences the rate at which a pathogen can develop in a mosquito. Hourly and weather summaries were downloaded from NOAA’s Climate Data Portal. The datasets cover the years 2007-2023 at the Taunton Airport in Bristol County.

The hourly summaries were used to calculate weather variables, such as the daily maximum temperature and precipitation. While daily summaries provide this information will less processing, the hourly summaries provide more granular data that can be used to calculate additional variables, such as the number of hours above a certain temperature threshold or degree-days. Degree-days is a measure of the amount of heat accumulated over a period of time. It is calculated by summing the difference between the average daily temperature and a base temperature. Degree-days are used to estimate the rate of development of insects and other organisms that are sensitive to temperature. For mosquitoes, degree-days can be used to estimate the rate of larval development and the time between when a mosquito ingests a virus and when it can transmit it (Gu and Novak 2006). To simplify this variable, the number of hours the temperature was ≥50°F and ≤86°F. These thresholds were chosen based on the known temperature range for mosquito development and virus transmission.

In addition to the weather summaries, drought data will be included in the models. West Nile Virus (WNV) research suggests that drought has been a driver in WNV outbreaks. While the lack of water can lead to smaller mosquito populations, drought can lead to higher prevelance of mosquito infection (Paull et al. 2017). Drought data was obtained from the US Drought Monitor and the Federal Emergency Management Agency (FEMA). The data includes weekly drought severity ratings for the entire United States. The data is available at county and regional levels and will be used to calculate the number of weeks in drought conditions and the severity of the drought. For this project, drought data for Bristol County, MA, will be used and incorporated with FEMA East Coast regions (I-IV) drought indicators.

### Human and animal case data

While the goal of this project is to set a foundation for models that predict human cases of EEE later this year. Human case data will not be an outcome in the models for this project. However, the case data will incorporated into the models. Some have hypothesized that EEE activity in the southern United States could serve as an early warning signal for EEE activity in Massachusetts. The rationale is that birds that migrate from the south to Massachusetts could bring the virus with them. In active EEE years, more infected birds may arrive in Massachusetts and spread the virus to local mosquitoes at a higher rate. Florida is a known hotspot for EEE activity and is the only state that reports more human cases than Massachusetts. The Florida Department of Health reports EEE activity in animals and humans annually. In addition to the wild and domestic animal case reports, many mosquito control programs in Florida maintain flocks of sentinel chickens that are tested for EEE and WNV. The sentinel chicken data is used to monitor virus activity in the mosquito population.

### Bird observation data

The North American Breeding Bird Survey (BBS) is conducted annually in late spring or early summer. The United State Geological Survey (USGS) maintains the BBS database. The BBS is a long-term, volunteer-driven bird monitoring program that collects data on bird populations across North America. The data is used to track the status and trends of bird populations over time. The BBS data includes information on the number of birds observed, the location of the observation, and the date of the observation. The data is collected by trained volunteers who conduct roadside surveys along predetermined routes. The data is used to estimate the population size and distribution of bird species across the continent. The BBS data will be used to estimate the population size and distribution of bird species in Massachusetts.

Since birds are the amplifying hosts for EEE, their population size and distribution can have a significant impact on the prevalence of the virus in the environment. Additionally, the migratory patterns of birds can influence the spread of the virus across regions. By incorporating bird observation data into the models, we can better understand the relationship between bird populations and EEE activity in Massachusetts.

### Temporal scale

For most models in this project, the outcome, MIR, will be calculated by week. While the main temporal scale for this project is the week, the data will be aggregated to other temporal scales. The week is the most common temporal scale for mosquito surveillance data. The week number will be calculated according to ISO 8601 standards, and stored in a YYYY-WW format. For example, week 8 in 2019 will be displayed as 2019-08. The week number will provide a common field by which all datasets with a temporal attribute can be joined. It will also allow for grouping records and making calculations by week number. This format helps to standardize temporal scales and make comparisons between years easier.

While mosquito surveillance and weather data can be aggregated to the week, other datasets, such as the Annual BBS cannot appropriately be aggregated by week. Additionally, mosquito surveillance in Massachusetts is conducted from June to October and EEE activity in Florida occurs year-round. For these predictors, the temporal scale will be the year.

## Data aquisition

With the exception of the mosquito collection data, all other datasets are publicly available for use without restrictions.

## Data import and cleaning

Datasets will be loaded into R in a Quarto document to document and the processing methods will be documented.

The Massachusetts Department of Public Health requires that all mosquito control districts submit their collection data in a specific format. This format divides each trap event into separate rows based on mosquito species and whether they were submitted for virus testing. However, this format is not useful for certain calculations where a zero count for a species is not explicitly recorded. For example, even when sorted by species to calculate the average count per trap, the average could be inflated since the records will not include trap events with a zero count of that species. In order to correct this, the data was pivoted to create a column for every unique “species code” then the “pool size” for each species code is summed for each trap event. A trap event is when “town”, “date of collection”, “trap type” are all the same. For species codes not included in the orginal table for a trap event, the field will be assigned a zero value. The resulting table will have one row for every trap event.

# Methods

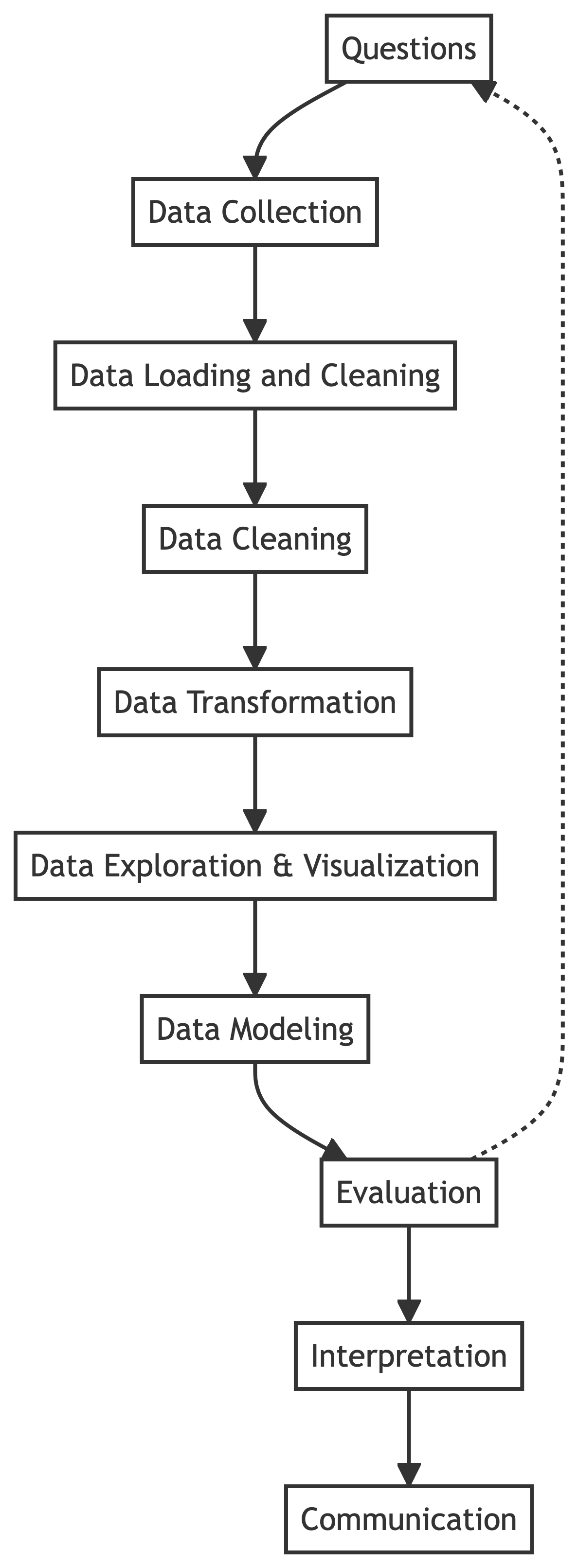
*This section is under development and will be completed after analysis is complete. The following is a general outline of the methods.*

Symptomatic EEE, and to a lesser extent WNV, human cases are rare. On average, the United States records only about 9 cases of EEE annually. Notably, Massachusetts reports more cases than any other state except Florida, highlighting regional disparities in disease occurrence. However, the incidence rate for neuroinvasive EEE cases in Bristol County, Massachusetts, is estimated to be only around 0.06 per 100,000 people (Centers for Disease Control and Prevention 2022). The low incidence of human cases poses significant challenges for traditional statistical models, making it difficult to accurately estimate human transmission risk. This difficulty is largely due to the sparse nature of the data and the variability in transmission patterns, which complicates efforts to predict outbreaks and necessitates alternative modeling approaches.

As stated earlier, MIR will be the main outcome of interest for this project.

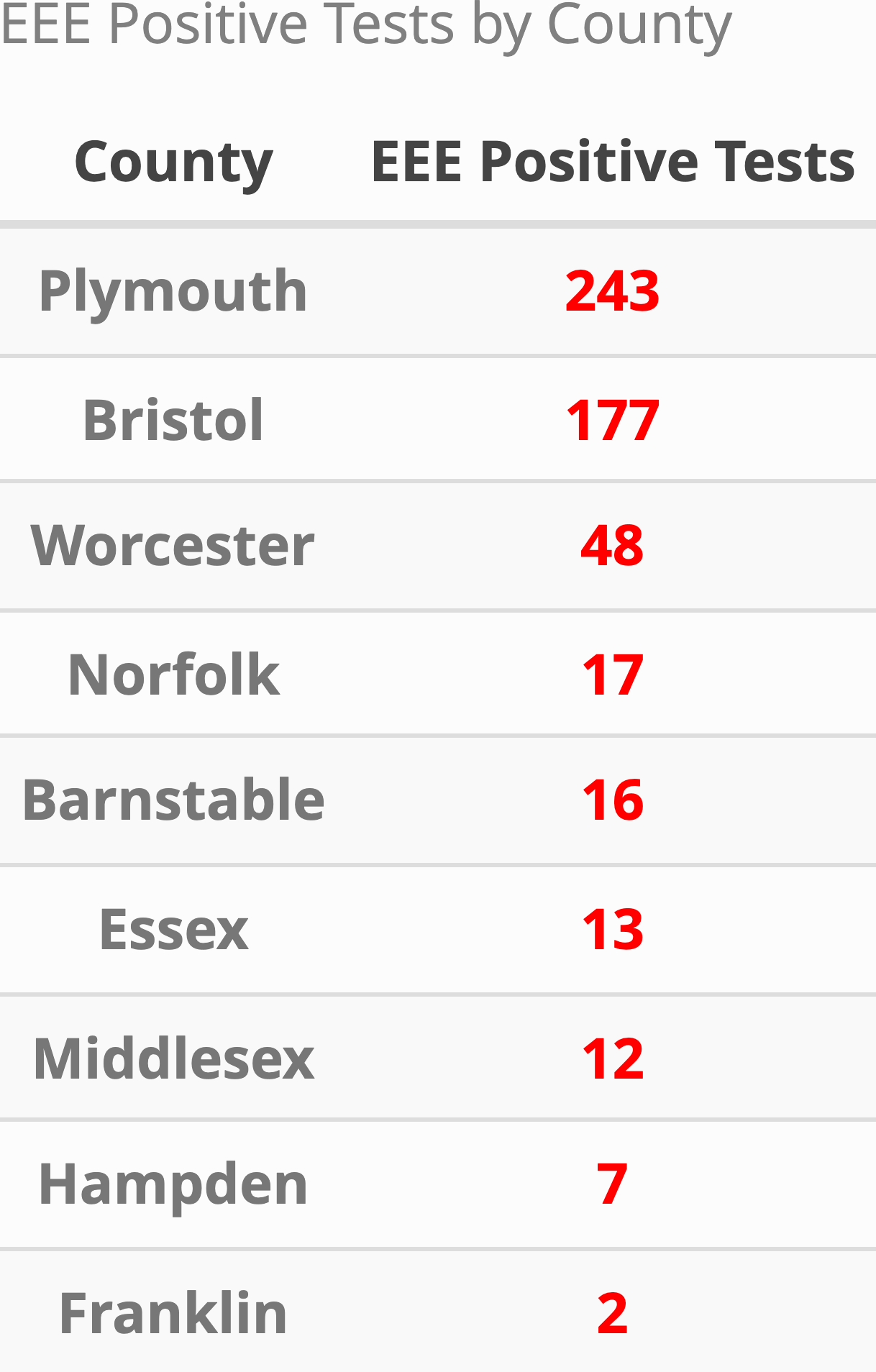
### Schematic of workflow

*Schematic will be refined in later steps. The doc will also be reformatted to move the schematic to an appendix* *The image should appear upon rendering*

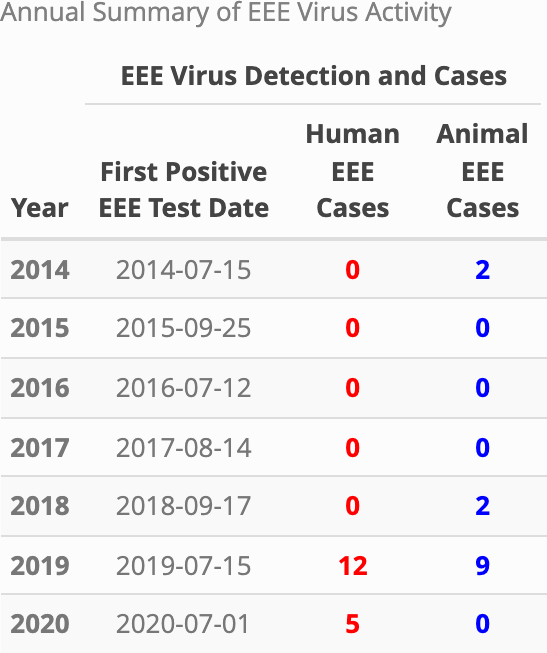


## Exploratory/Descriptive analysis

It is important to know where the mosquitoes infected with EEE are captured. Below is a table showing the virus isolations for EEE in trapped mosquitoes by county between 2014 and 2020.



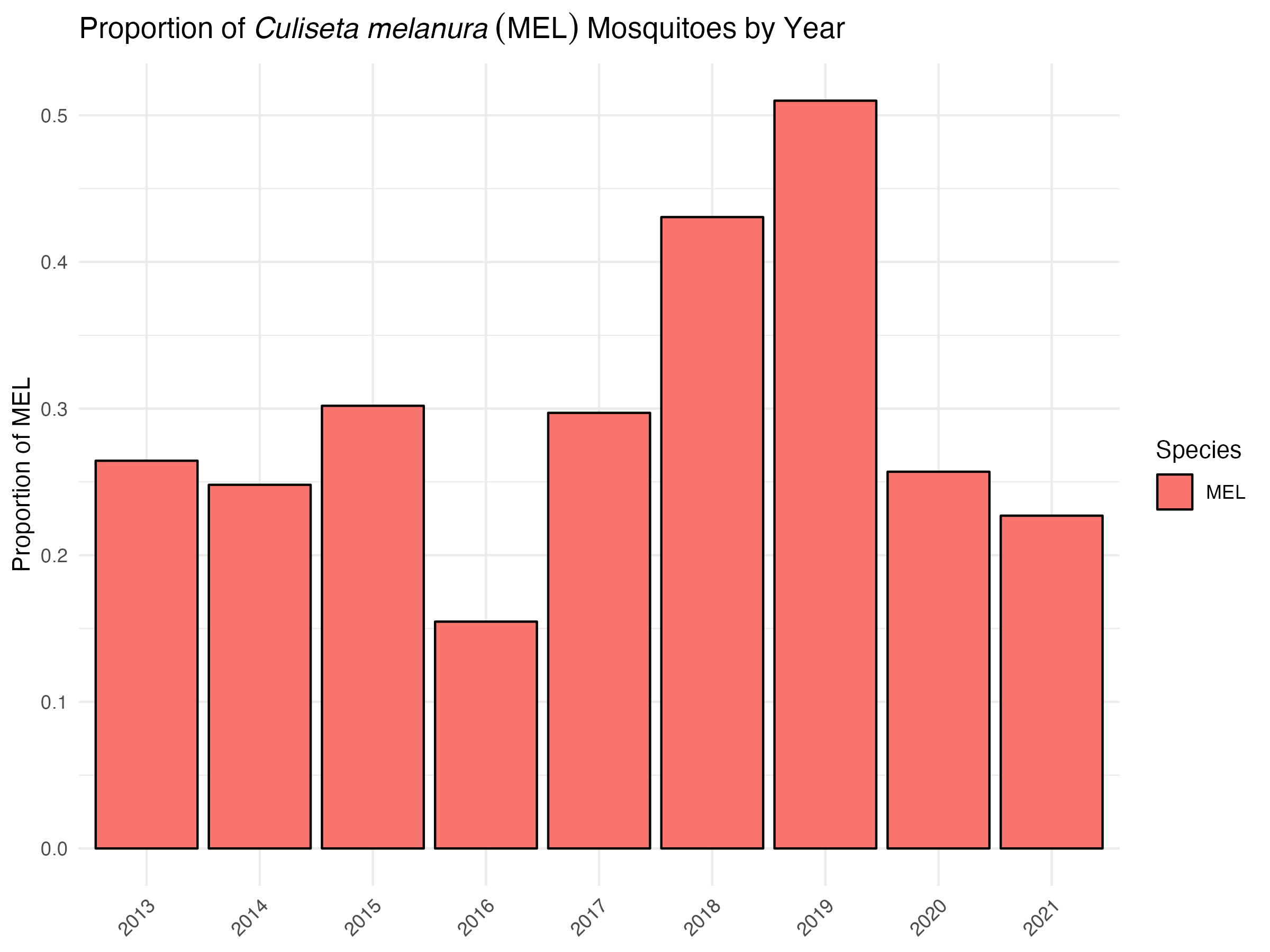
One factor to consider is if early virus isolations are associated with cases in humans and other mammals. The table below shows the date of the first EEE virus isolation in mosquitoes and the number of human of other animal cases by year.



Unfortunately, the time horizon is limited by data availability. Additionally, EEE human infections are rare, so traditional statistical methods may be inadequate to suggest any relationships. What is clear is that 2019 was an exceptional year for EEE.

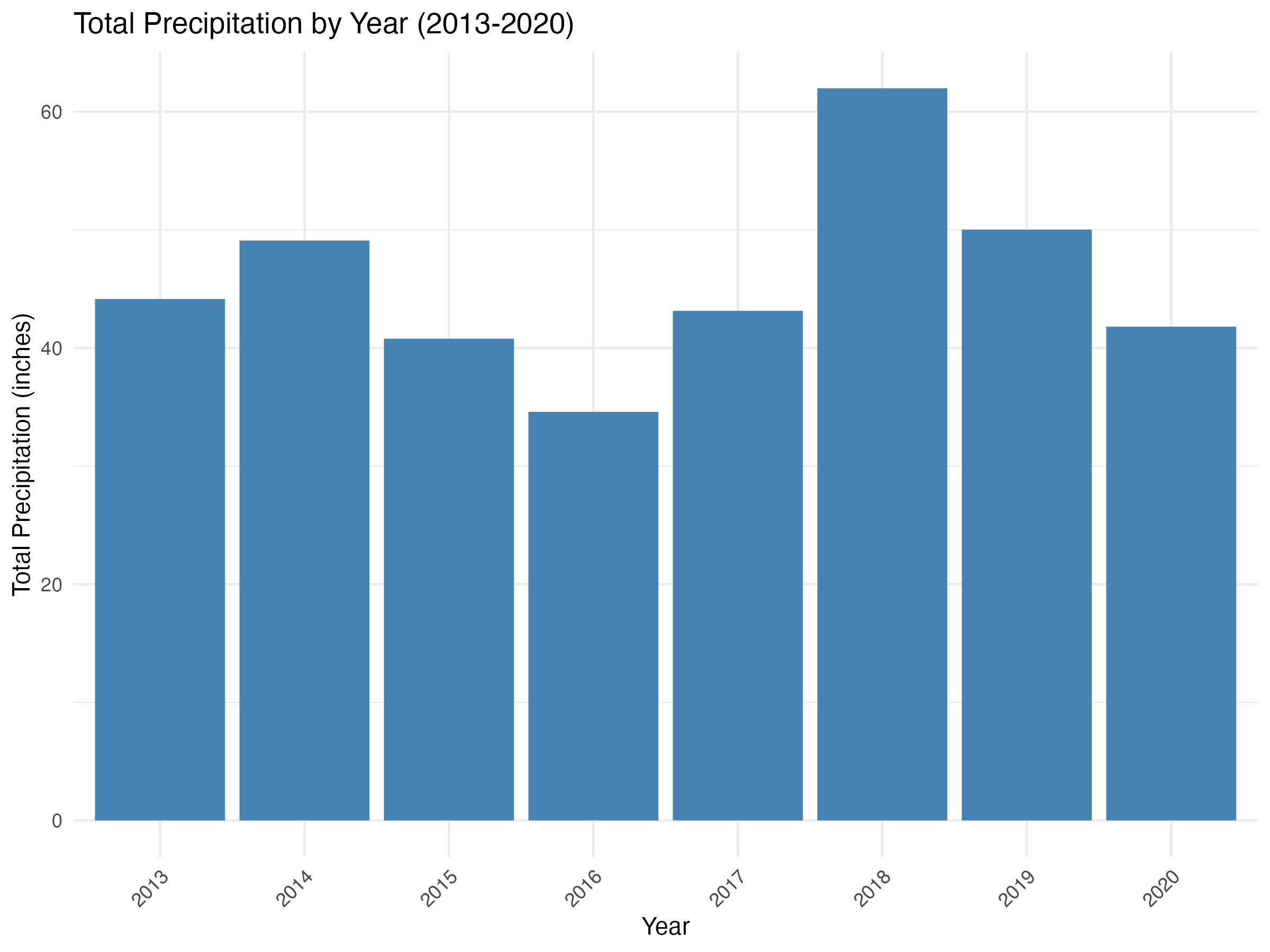
*Culiseta melanura* (MEL) plays an important role in EEE virus amplification in the enzootic cycle. Understanding the population dynamics might provide more insight into what is driving the 2019 spike.

The graph below shows the proportion of MEL among all mosquito vector species trapped in Bristol County, MA.



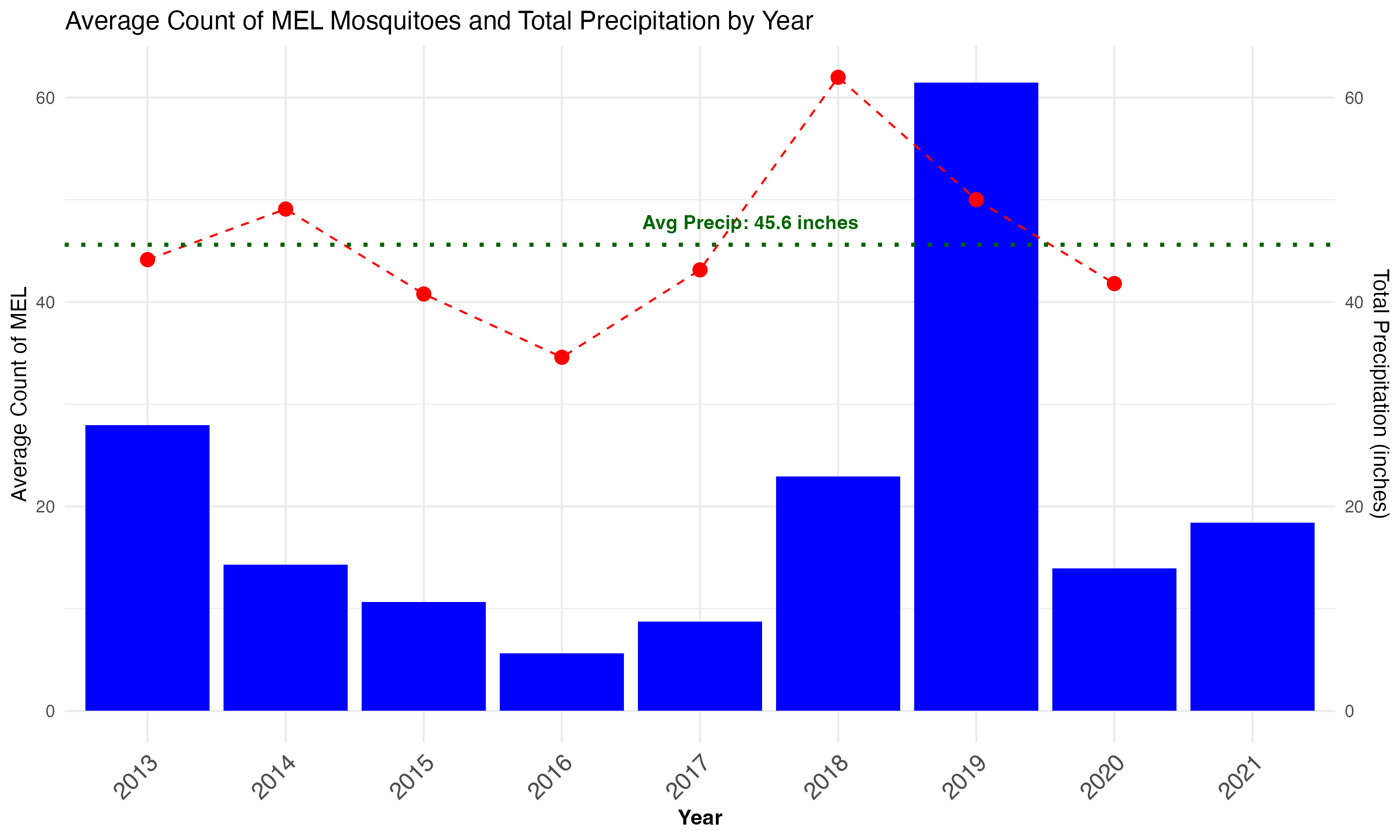
From this we see a large proportion of MEL in the traps for 2019. In fact, they comprise more than half of all vector species for that year. Another interesting aspect is the high proportion in 2018. MEL overwinters as larvae and as long as there is sufficient standing water to last the winter, MEL hatched in the previous fall would survive to emerge the next year. This suggests it might be important to look at conditions in the previous year or season for a better understanding.

Since water plays a significant role in the mosquito life cycle, let’s look at the annual precipitation.



The graph indicates that the annual precipitation total for 2018 is higher than the other years. This suggest that there was enough water to support large MEL larval populations though out the winter.

Another view plots the average annual MEL catch against total precipitation.



## Basic statistical analysis

The initial tests have been basic regression models.

The first models explored the relationship between virus isolation in mosquitoes and weather conditions (average temperature by week number and total precipitation by week). The outcome was whether there was at least 1 positive pool in a week. The predictor in the first model was the average maximum daily temperature the week before the pool week. The logistic model suggest that there was a relationship between the two. (The R code for the model can be found in the EDA-Code file: logistic\_.pos\_temp\_precip.R) The average temperature in the week before is positively associated with the likelihood of a positive result, with an odds ratio of approximately 1.069 for each unit increase in temperature (Estimate = 0.06695, SE = 0.02033, p < 0.001), indicating that higher temperatures significantly increase the probability of a positive outcome. The model’s improvement is highlighted by a decrease in residual deviance to 405.79 from the null deviance of 417.24, upon incorporating Lagged\_Avg\_TMAX as a predictor, demonstrating a better fit to the observed data. However, with a residual deviance of 405.79 on 299 degrees of freedom, it is evident that not all variability in the outcome is explained, suggesting the presence of other influential factors. The model’s AIC of 409.79 indicates a balance between model complexity and predictive power, supporting its relative utility in understanding the data. However, this relationship should be examined more closely as temperature is likely auto correlated with progressing weeks during summer months. While precipitation has been demonstrated to influence the population dynamics it did not show to have any effect on a positive result.

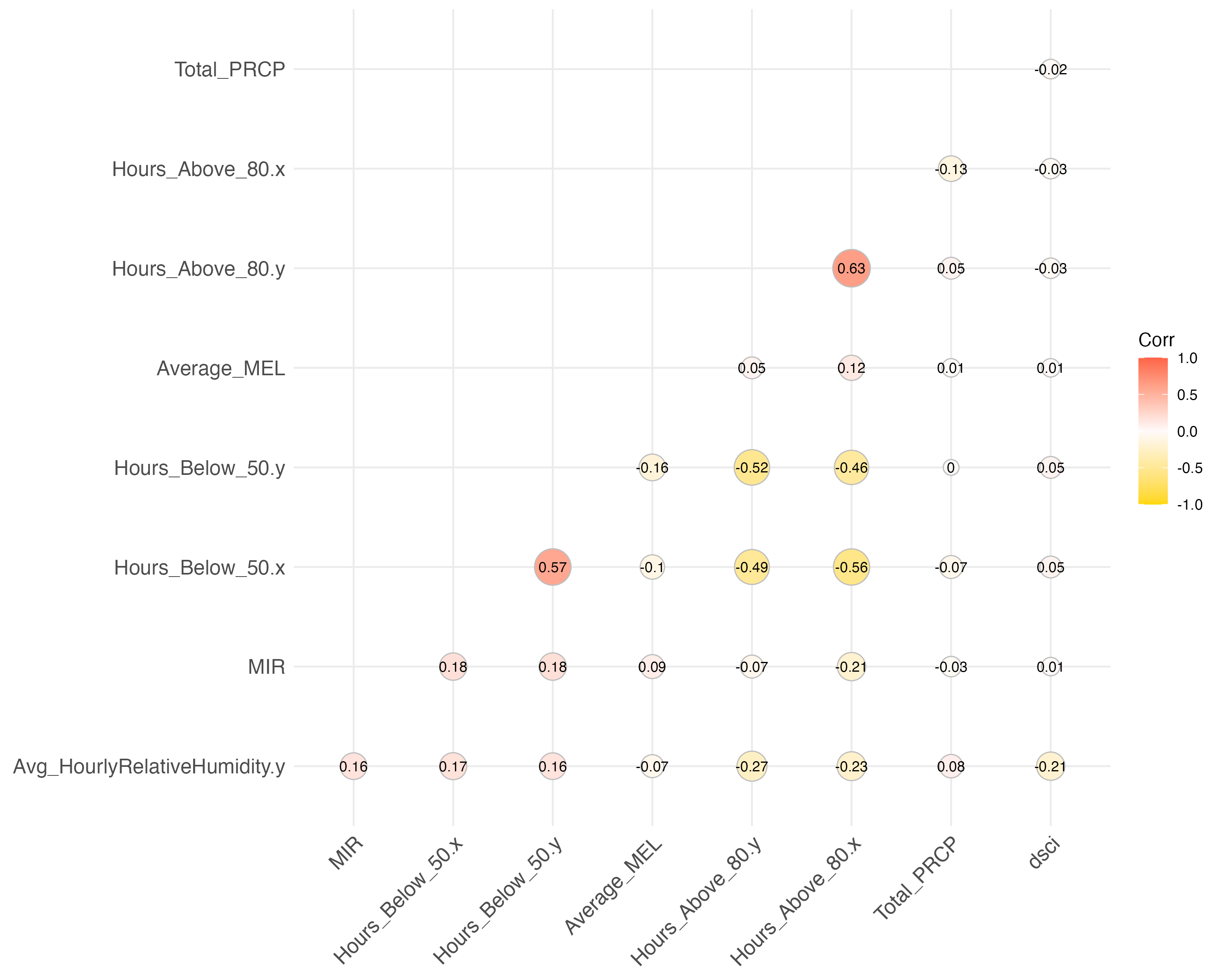
Another model assessed whether the temperature precipitation in April, May, and June of each year effected the date of the first positive pool test. The date of the first positive pool was identified and assigned a day number from January 1st of that year. The predictors in this linear model were the average maximum temperature for April, May, and June and the sum of precipitation for the same time period. None of these predictors were significant.

There was one other significant result. When MIR is set as the dependent, continuous variable in a linear regression and the average maximum temperature for the previous week is used as the predictor, the model suggests there is a significant inverse relationship. As average temperature increases, the MIR decreases (model\_mir\_temp.R). This relationship is counterintutive, especially given the result of the logistic regression and requires more examination.

More models assessing the effects of weather and other factors on positive pools by mosquito species and MIR values will be developed in Part 4 of the assignment. Future models will examine the the effects of weather conditions on mosquito population dynamics.

## Statistical analysis

*UPDATE for Pt 4* *a better source for weather data was found and it being processed.* *the analysis will be updated in the next version of the document*



# Results

*updated in later phases of the project*

## Full analysis

*These will be generated in Pt 5* # Discussion *this section will be completed when analysis is done*

## Strengths and Limitations

*this section will be updated after analysis is complete* The accuracy and reliability of the mosquito collection data is excellent. The same procedures and sentinel sites have were in place for the entire analytical horizon. Further, there were only two people responsible for sorting, identifying and counting species over the entire period.

MIR is not a perfect measure of virus activity in mosquitoes. It tends to underestimate the level of virus circulating in the environment. However, other measures require equal pool numbers for testing. There is a correction factor for variable pool numbers and I will explore this option in later phases of this project.

The spatial scale is an issue with the human and veterinary cases data. Human case data is released only on a county scale to ensure patient privacy. Some veterinary case data is available on the town-level, but most remains only on the county-level. These limitations are barriers to accurate spatial analysis. The project will rely on virus isolation in mosquitoes to model risk. The available bird data relies heavily on crowd-sourced reports. There is one standardized survey (Breeding Bird Survey) that seems to be more accurate and reliable. However, this data is not available for every year.

## Conclusions *this section will be completed when analysis is done*

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