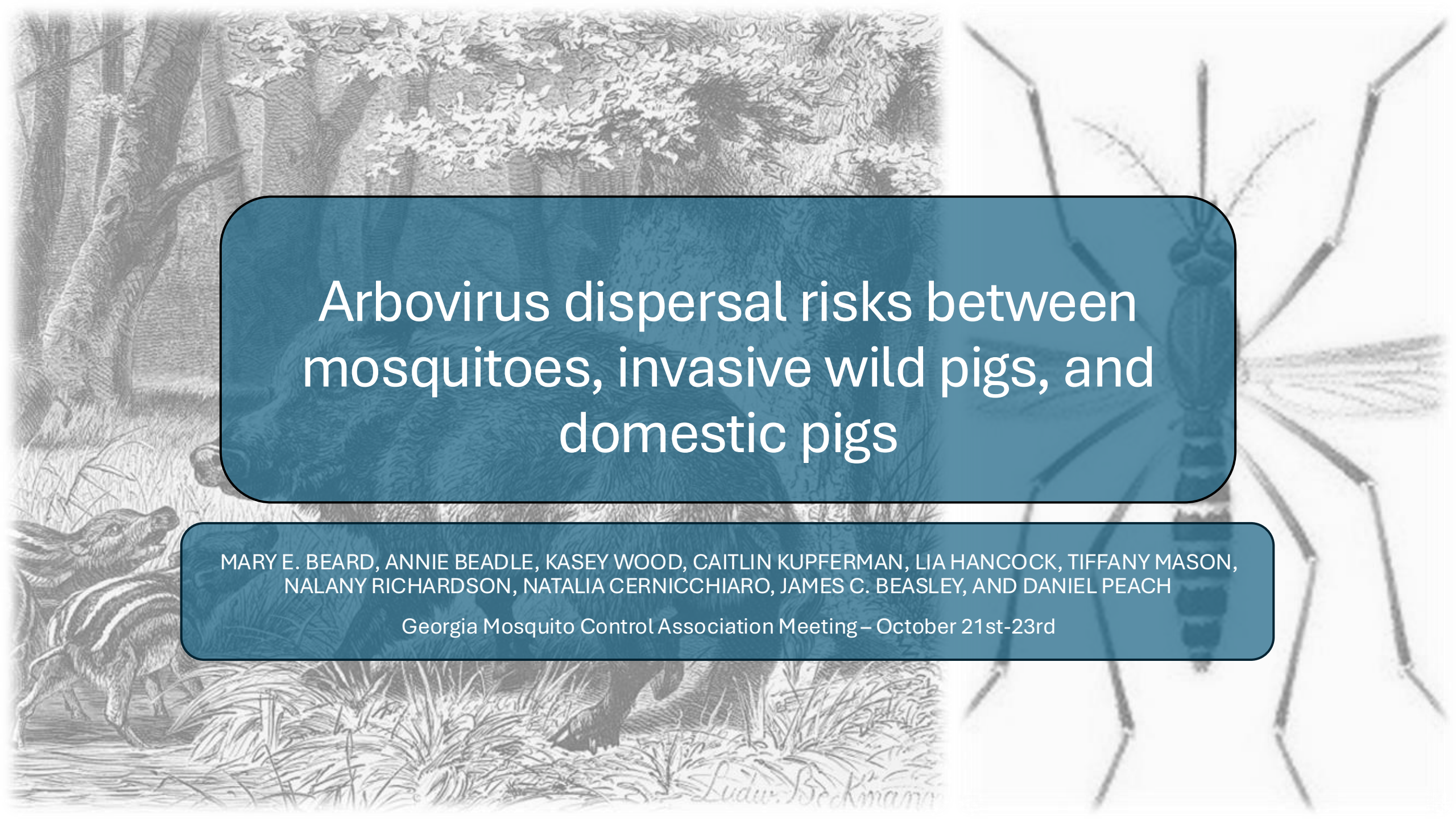


Arbovirus dispersal risks between mosquitoes, invasive wild pigs, and domestic pigs

MARY E. BEARD, ANNIE BEADLE, KASEY WOOD, CAITLIN KUPFERMAN, LIA HANCOCK, TIFFANY MASON, NALANY RICHARDSON, NATALIA CERNICCHIARO, JAMES C. BEASLEY, AND DANIEL PEACH

Georgia Mosquito Control Association Meeting – October 21st-23rd

Wild Pigs (*Sus scrofa*)

- Invasive species
  - Population range and size increasing across the U.S.
  - Disruptive to the environment and human activities
- Ecosystem engineers
  - Rooting, spread invasive plants, alter runoff/watershed quality, **wallowing**
- **Wallows**
  - Filled with nutrients from pig excrement, and other organic materials from the disturbed earth
- Pathogen reservoirs
  - **Japanese encephalitis virus (JEV)**



Courtesy of the Biodiversity Heritage Library

Pig wallow site, Savannah River Site

Wallows as Mosquito Habitat

- Pig blood for female mosquitoes; pigs as pathogen reservoirs and amplifiers; wallows provide nutritious nurseries… a problematic **"bed-and-breakfast scenario"**
- This interaction may increase the spread of vectored pathogens



Mosquito larvae in Savannah River Site pig wallow

Impacts of Pig and Mosquito Interactions

- Invasive wild pigs and *Culex quinquefasciatus*
  - Hawaiian honey creeper bird population decline
    - Avian malaria

- Gaps in understanding pig and vector mosquito interactions and related impacts on human, wildlife, and domestic animal health

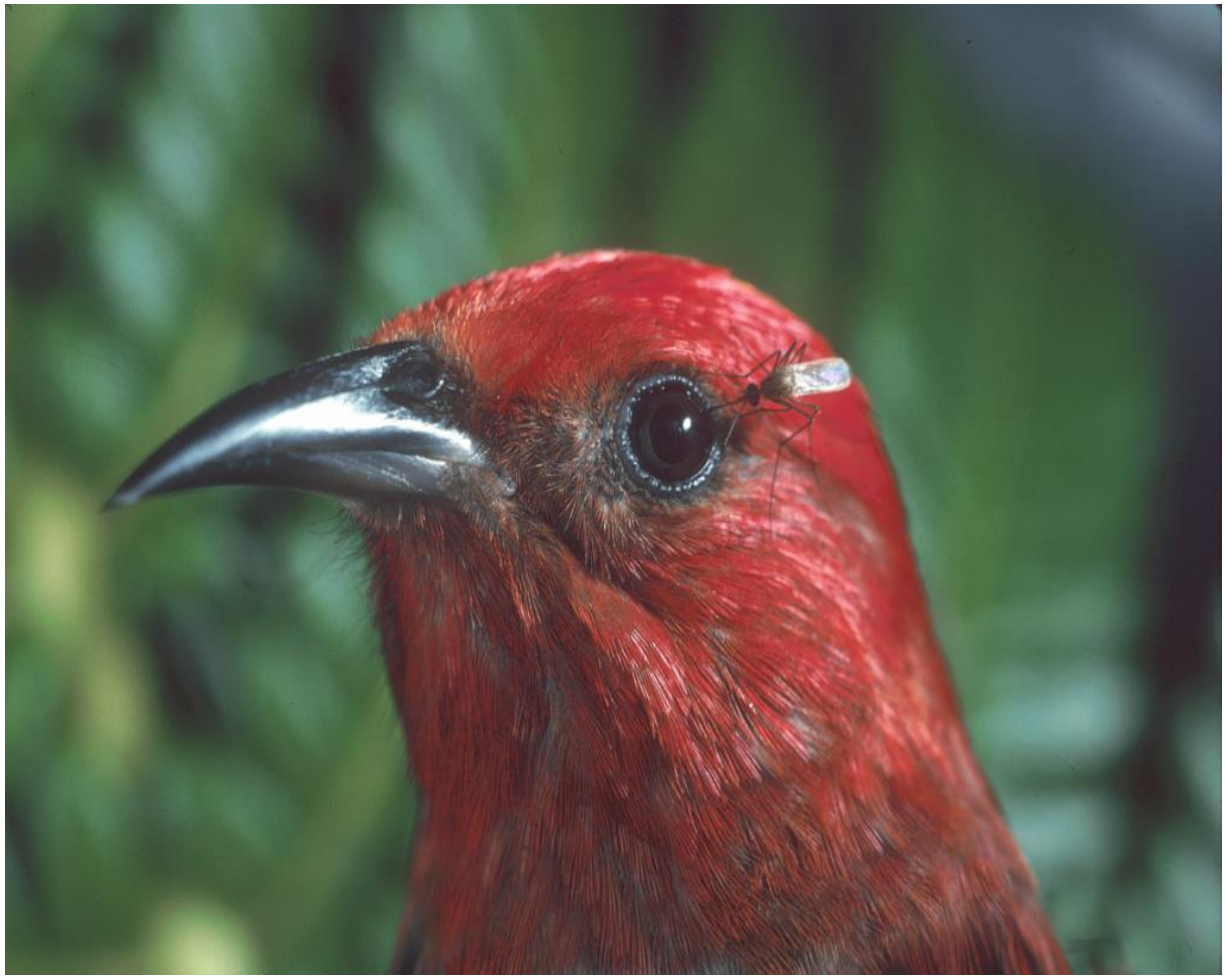


Photo: Jack Jeffrey

Mosquitoes of Interest

- *Culex* spp. (Primary)
  - Japanese encephalitis virus (JEV)
    - South-East Asia and West Pacific Region
    - Concern for emergence in North America
  - West Nile Virus (WNV)
  - Avian malaria
- *Anopheles* spp. (Secondary)
  - Historic human malaria vectors
    - Recent locally transmitted cases in United States
  - Other arboviruses currently being explored

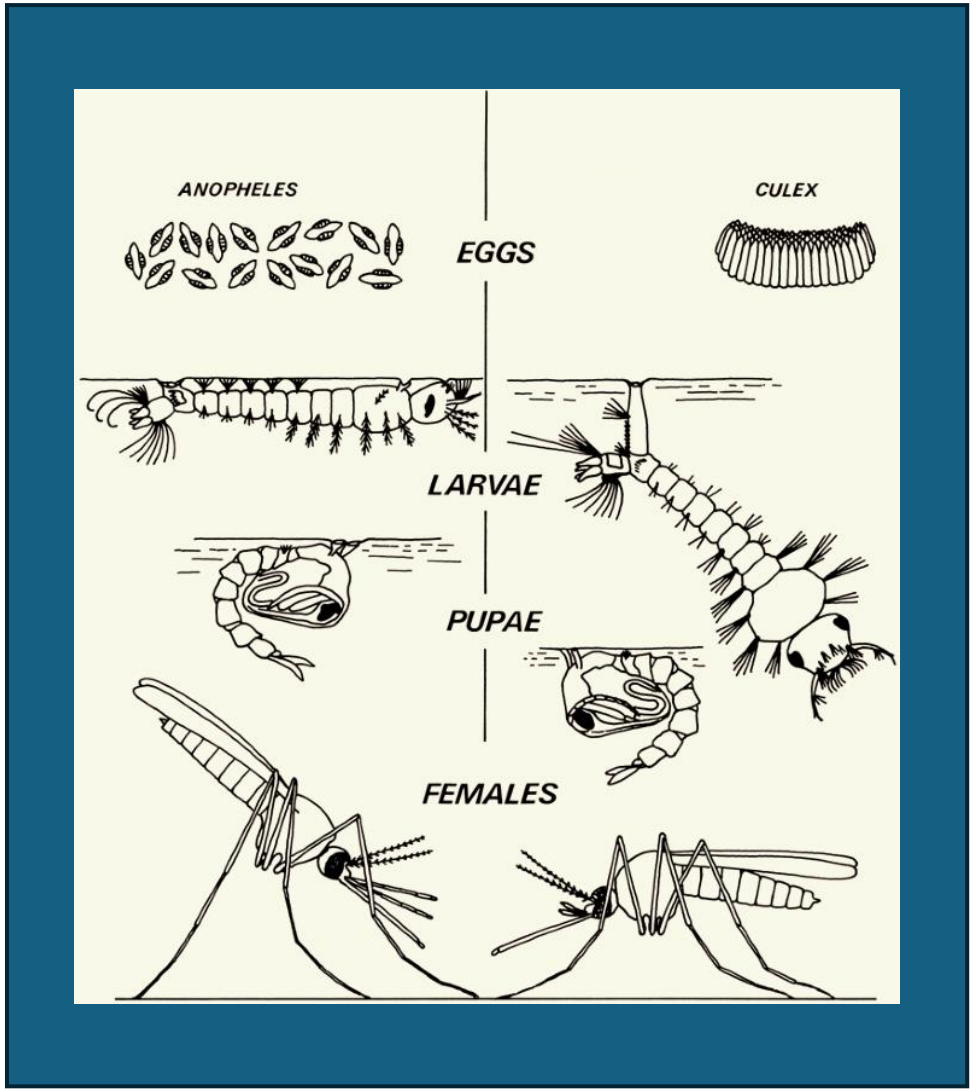
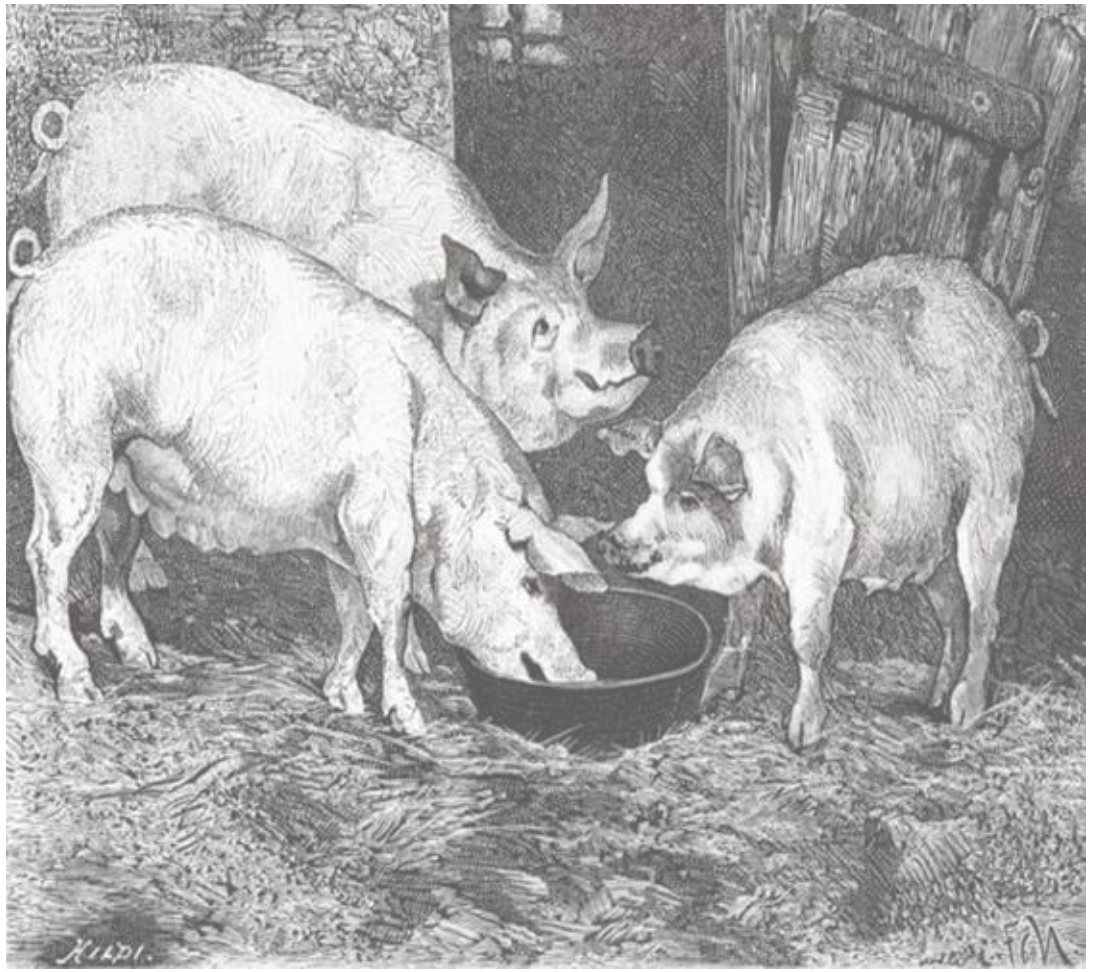


Fig. Image taken from Encyclopedic Reference of Parasitology. Springer, Berlin, Heidelberg.

Concern for Agricultural Stability

- *Culex* interacting with wild pigs near domestic habitats may lead to pathogen spillover (e.g., JEV) to domestic animals
- **Pathogen spread = substantial economic damage**
- JEV limits reproductive success in boars and sows + medical complications/mortality in piglets
  - Should JEV arrive in the U.S., estimated **economic loss = $300-$600 million per year**



Drawing by Niederhausern.

Objective

- **Determine what mosquitoes are interacting with both wild pigs and domestic pigs to assess exposure risk**
  - **Aim 1: Measuring species and quantity of mosquitoes in wallows and pig barns**
    - **Hypothesis: Similar mosquitoes will be present in both site types, including primary spp. of concern like *Culex***
  - **Aim 2: Pig blood presence/absence of pathogen antibodies, primarily using WNV as a proxy for pathogens amplified in pigs, such as JEV**
    - **Hypothesis: Evidence of WNV antibodies in both wild and domestic pigs sampled**
  - **Aim 3: Testing blood meals in mosquitoes for evidence of blood feeding and presence of virus**
    - **Hypothesis: Pig DNA will be present in captured mosquito bloodmeals**

Methods

- **<u>Sites:</u>**
  - NC, SC, and GA
  - Pig barns - **similar habitat types and varying insect exclusion measures**
  - Wild pig wallows near domestic pigs and our lab (SREL)

- **<u>Capture materials</u>**
  - Standard CDC light mosquito traps and handhelaspirator (adults)
  - Dippers (larvae and pupae)

- **<u>ID and testing</u>**
  - Identification of mosquitoes via standard references, barcoding
  - Blood analysis
    - Mosquito blood-meals
      - PCR (pig DNA)
    - Wild and domestic pig blood
      - **<u>WNV</u>** SN titer test – IgG antibody

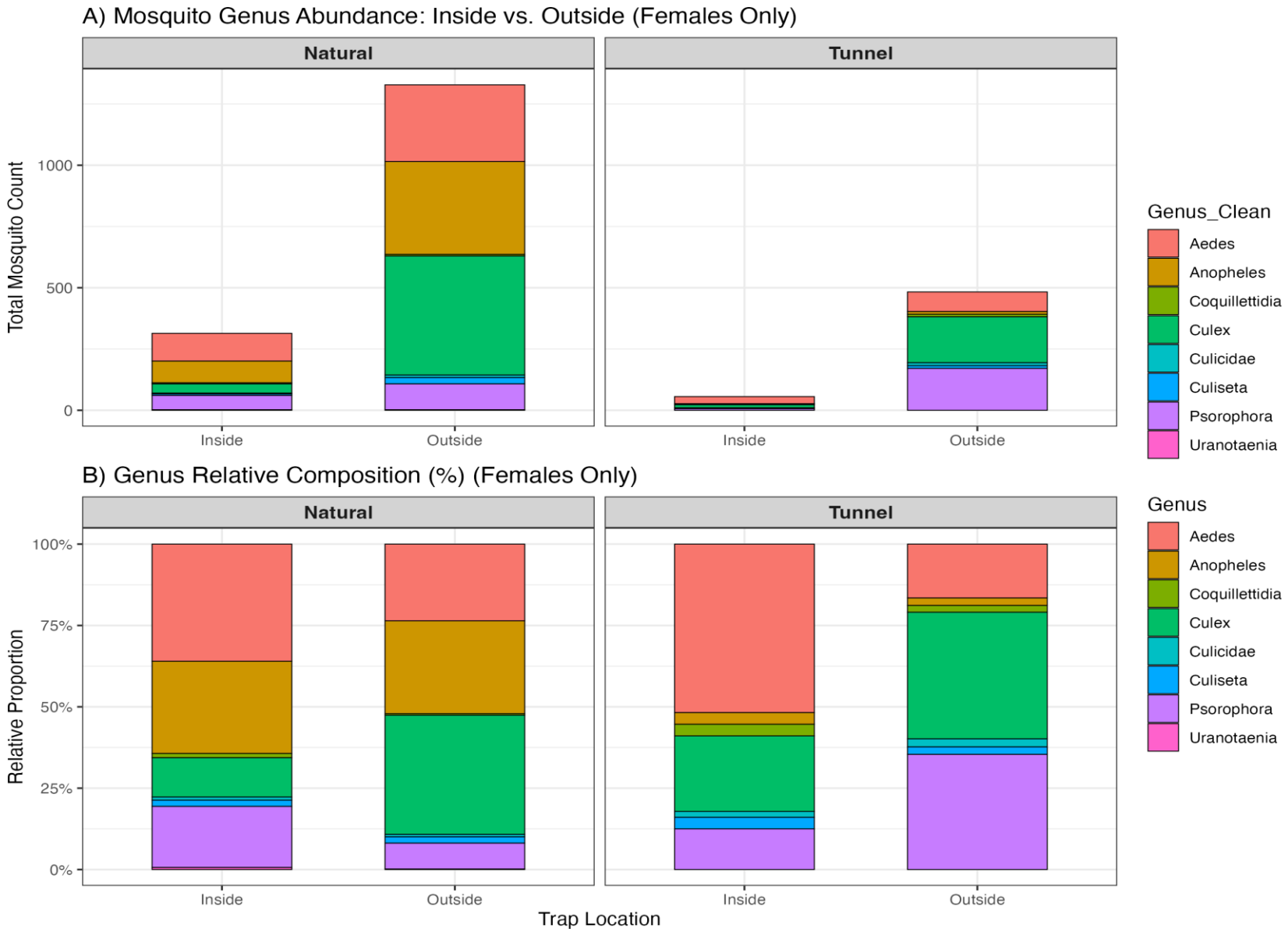






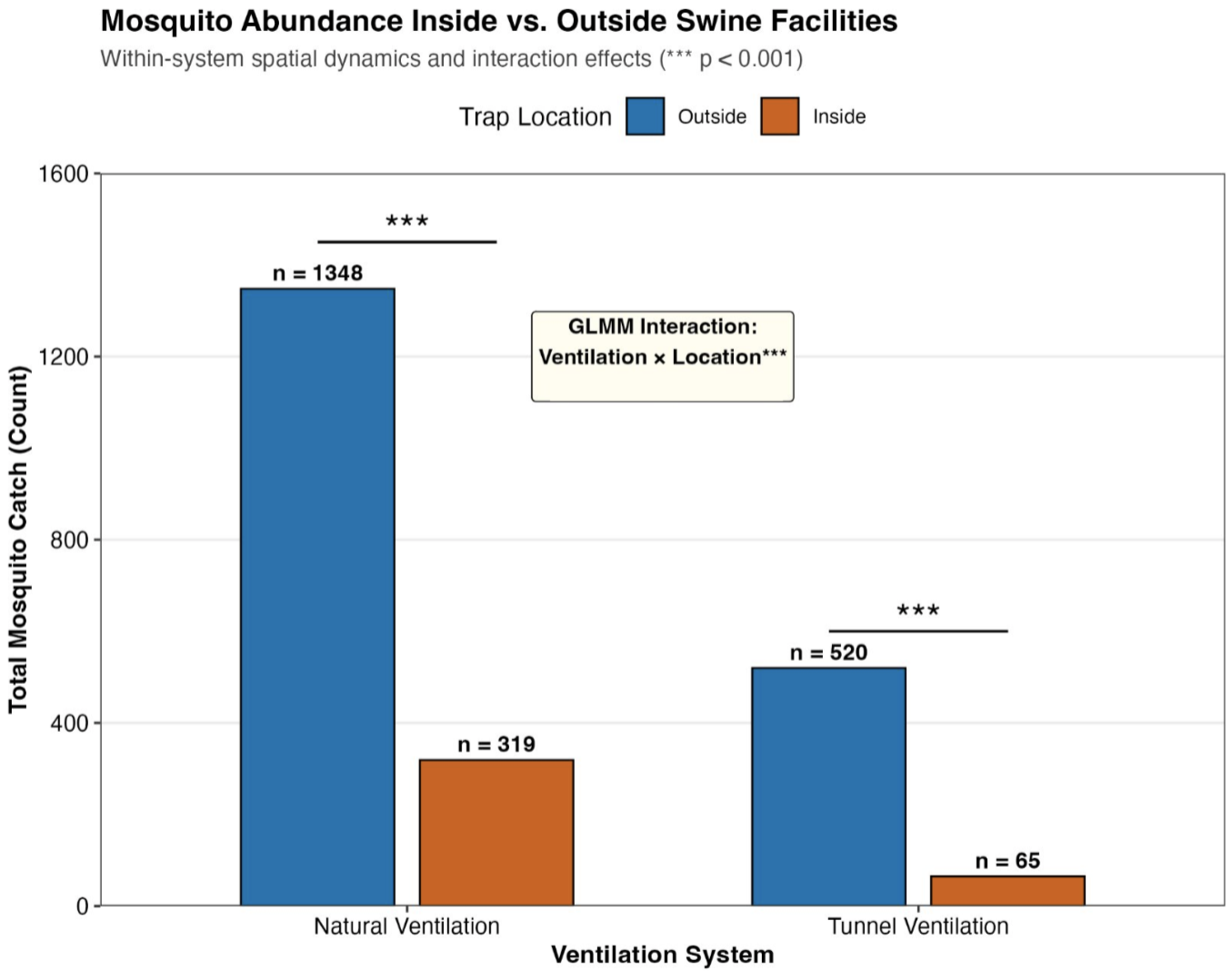


Aim 1: Species and Quantity of Mozzies-Domestic



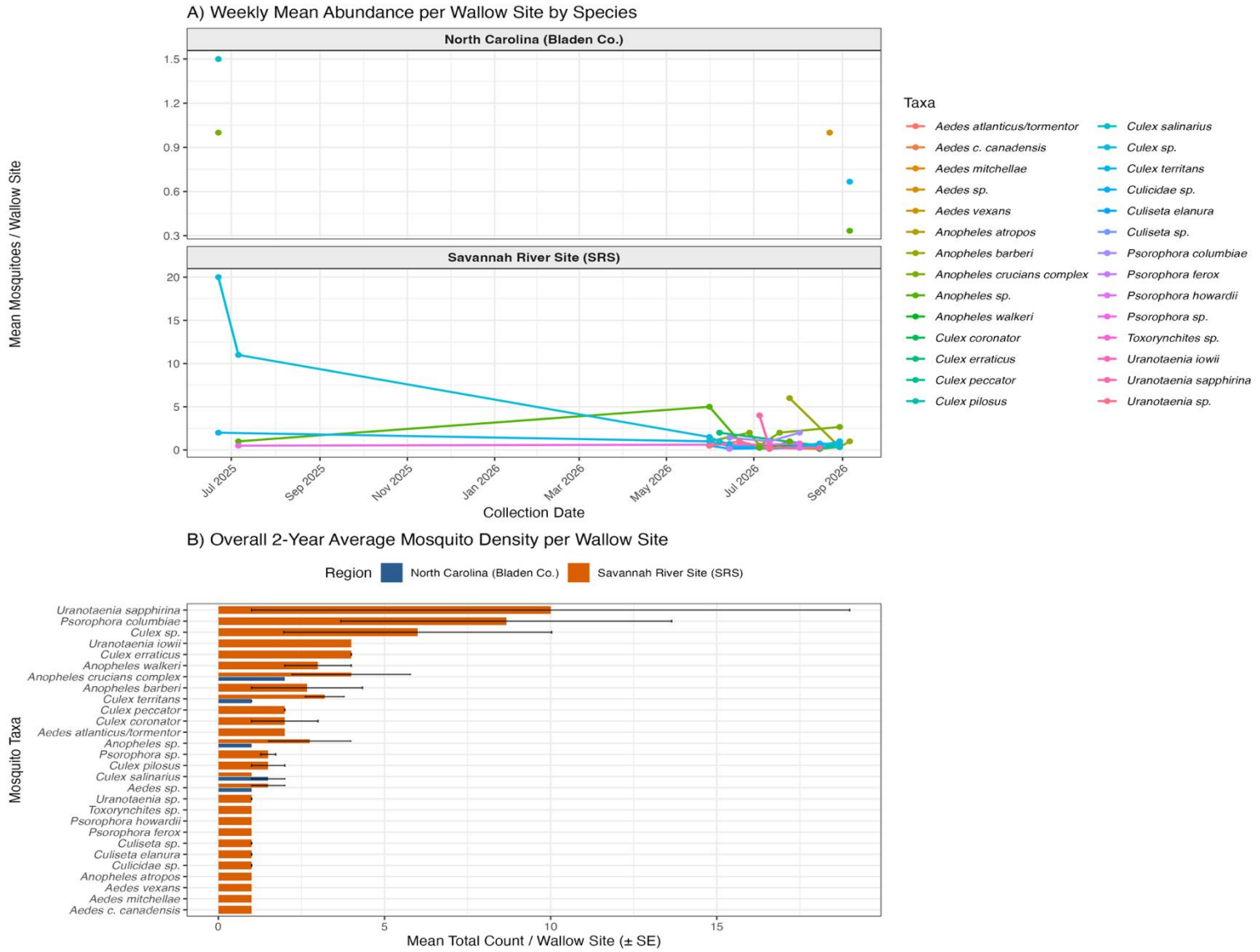
- The genera *Culex* (Kelly green) is present both inside and outside of the two ventilation types at a noticeable proportion

Aim 1: Species and Quantity of Mozzies-Domestic



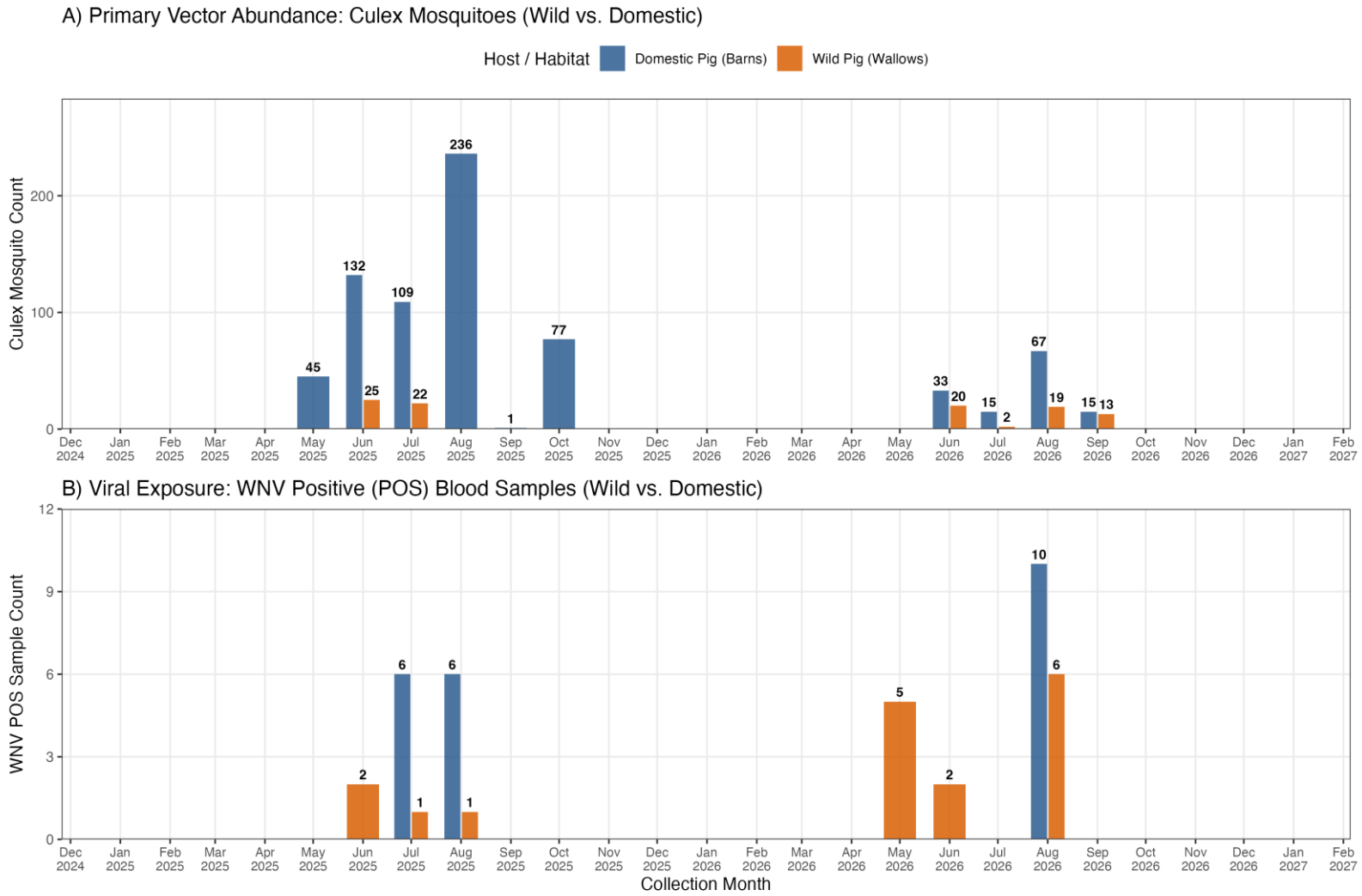
- Significant differences between mosquito abundance inside vs outside
- Decrease in overall mosquito abundance from natural (open) ventilated barns to tunnel (covered/closed) ventilated barns show less suitable habitat in/around the tunnel systems
    - **More insect exclusion measures = no more "all-you-can-eat buffet"!**

Aim 1: Species and Quantity of Mozzies-Wallows



- *Culex* present in wild pig wallows in both regions sampled
  - Bladen County, NC (n=10)
  - Savanah River Site, SC (n=12)
- Looking at mosquito taxa across all wallow sites sampled, ***Culex*** **accounts for the highest medically significant vector present**

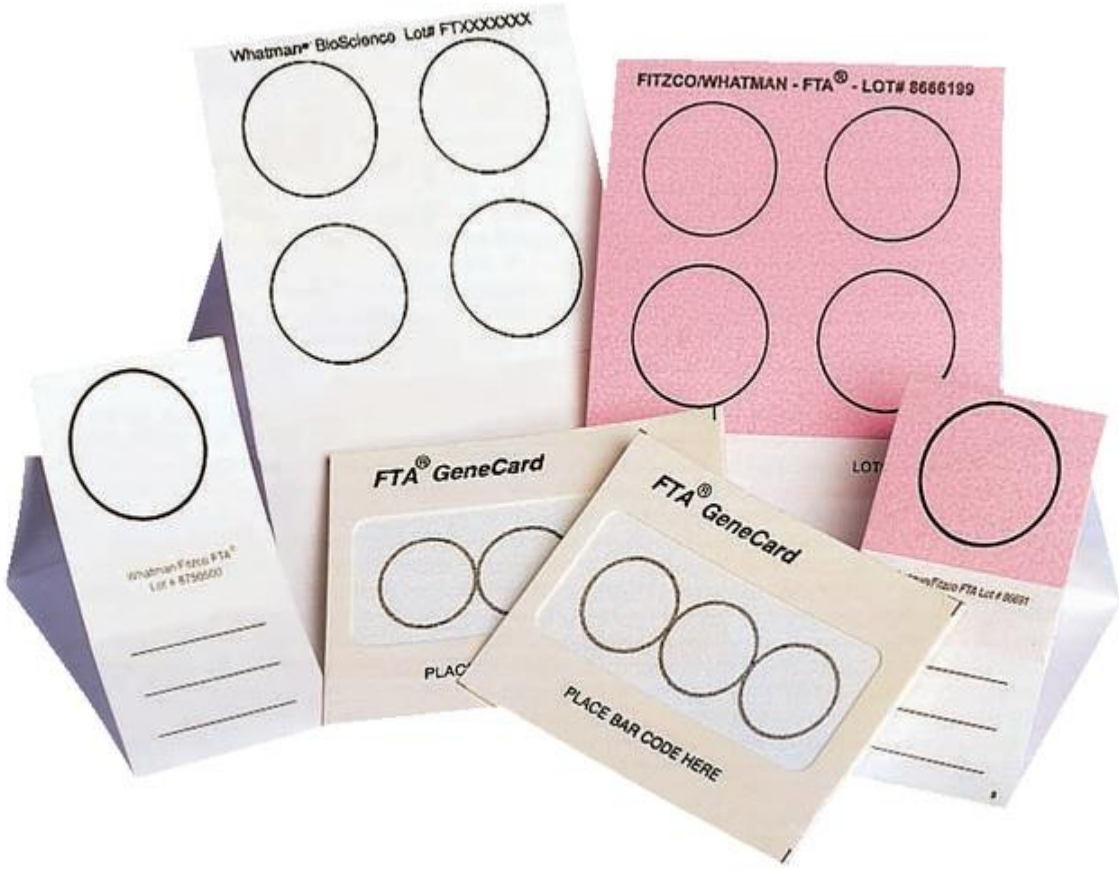
Aim 2: Blood Testing-Pig Serum



- Aggregating *Culex* mosquito counts and pig blood samples positive for West Nile virus antibodies across domestic and wild pig sites, there appears to be trends in *Culex* mosquito occurrence and positive WNV pig blood samples

Next Steps

- Blood analyses
  - Complete WNV SN titer tests on pig blood being received
  - Address aim 3 by performing PCR on mosquito blood meals
- Further statistical analyses
  - Finalize modeling (negative binomial GLMM) and statistical analyses for combined sampling years upon end of season
  - Parsing mosquito taxa in domestic and wild pig sites



Concern for Human Health

- Link between wild pigs, domestic pigs, and mosquitoes → carry over to humans
  - Wild pigs wallowing in proximity to human areas
  - Mosquito-pig interactions can lead to amplification and spread of pathogens in domestic settings

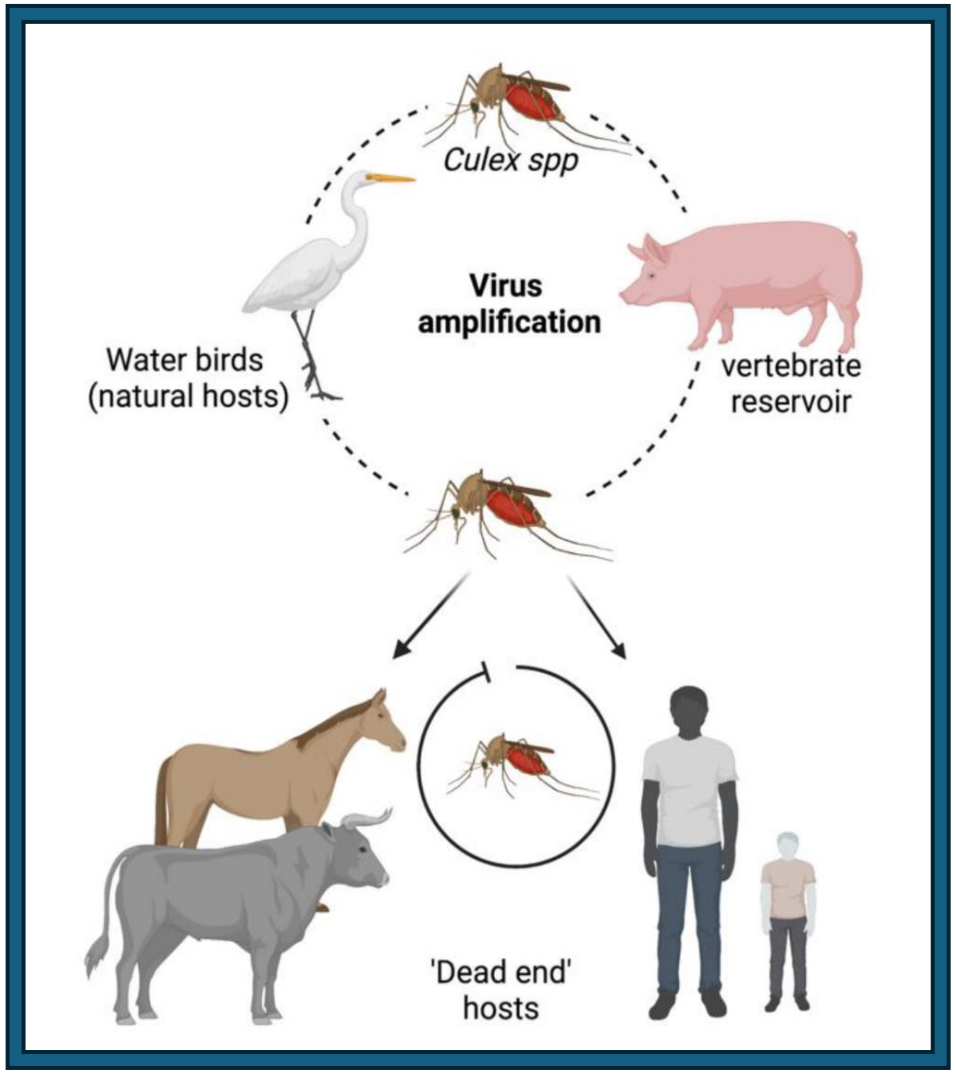


Fig. JEV cycle of infection and amplification from Srivastava KS et. al. Japanese Encephalitis Virus: An Update on the Potential Antivirals and Vaccines.

Concern for Ecosystem Health

- Ecological synergism may have greater impacts than currently understood
- Potential co-expansion and interaction of wild pigs and vectors
  - Habitat destruction
  - **Pathogen spread**



Woodland Glade by William Trost Richards, 1860

One Health Implications

- All of these elements make for an excellent example of a one health study

- Understanding pig and mosquito interactions will help better inform risks to environmental, animal, and human health

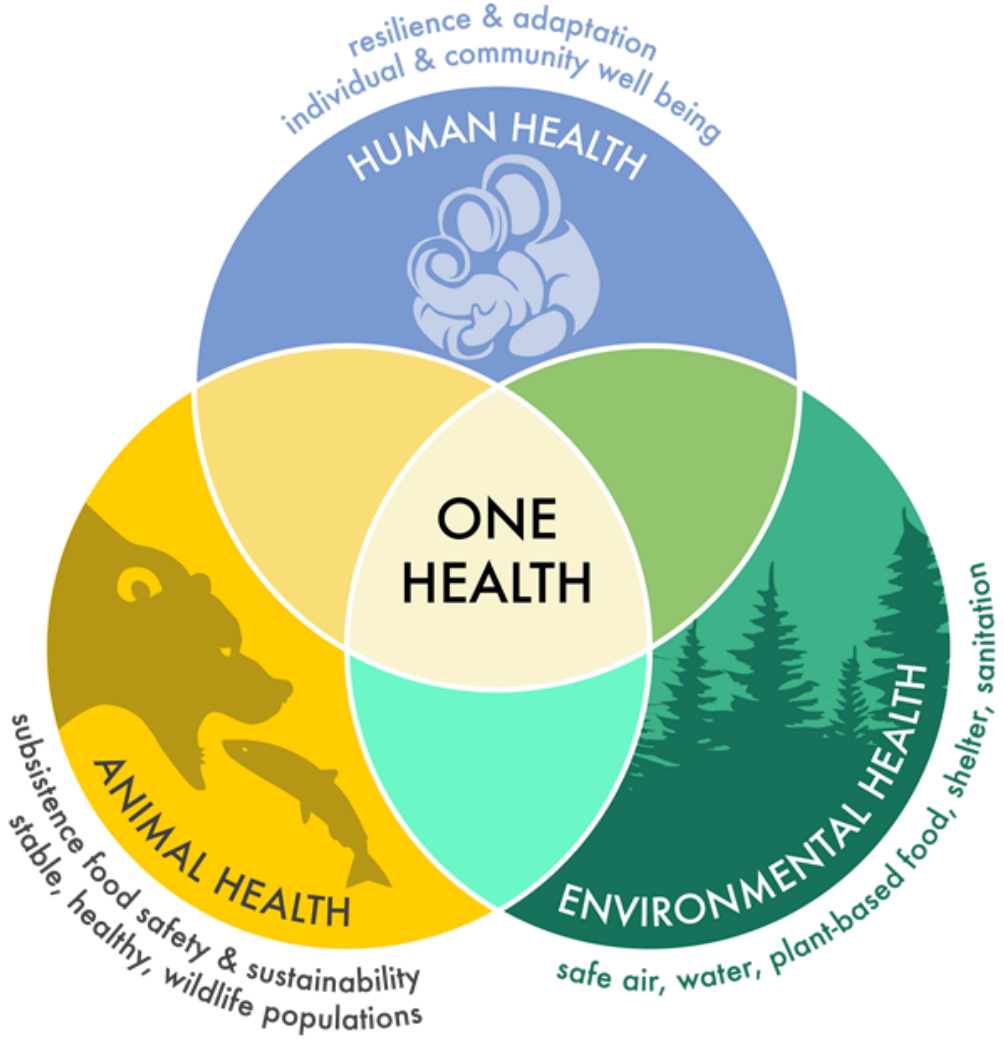


Fig. One Health graphic created by The University of Arizona

Acknowledgements:

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  - Lia Hancock
  - Tiffany Mason
  - Skyler Nichols
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  - Dr. Daniel Peach

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- Elizabeth Noblett – Smithfield
- Arkadiusz Paczkowski – Smithfield
- Wrenn Matthis - Smithfield

- Randall Pulley – USDA

Anopheles Habitat Modeling Work

"Projected Climate-Driven Shifts in Habitat Suitability for Three *Anopheles* (Diptera: Culicidae) Mosquito Taxa in the Southern United States" - Journal of Entomological Science

- Modeling historic malaria vectors *An. crucians*, *An. punctipennis*, and *An. quadrimaculatus* in the southeastern US using 2024-2026 data
- Projected models show areas becoming increasingly suitable for *An. quadrimaculatus* over time
- Highlight integration of species distribution models into vector surveillance for public health preparedness

Projected Climate-Driven Shifts in Habitat Suitability for Three *Anopheles* (Diptera: Culicidae) Mosquito Taxa in the Southern United States[1]

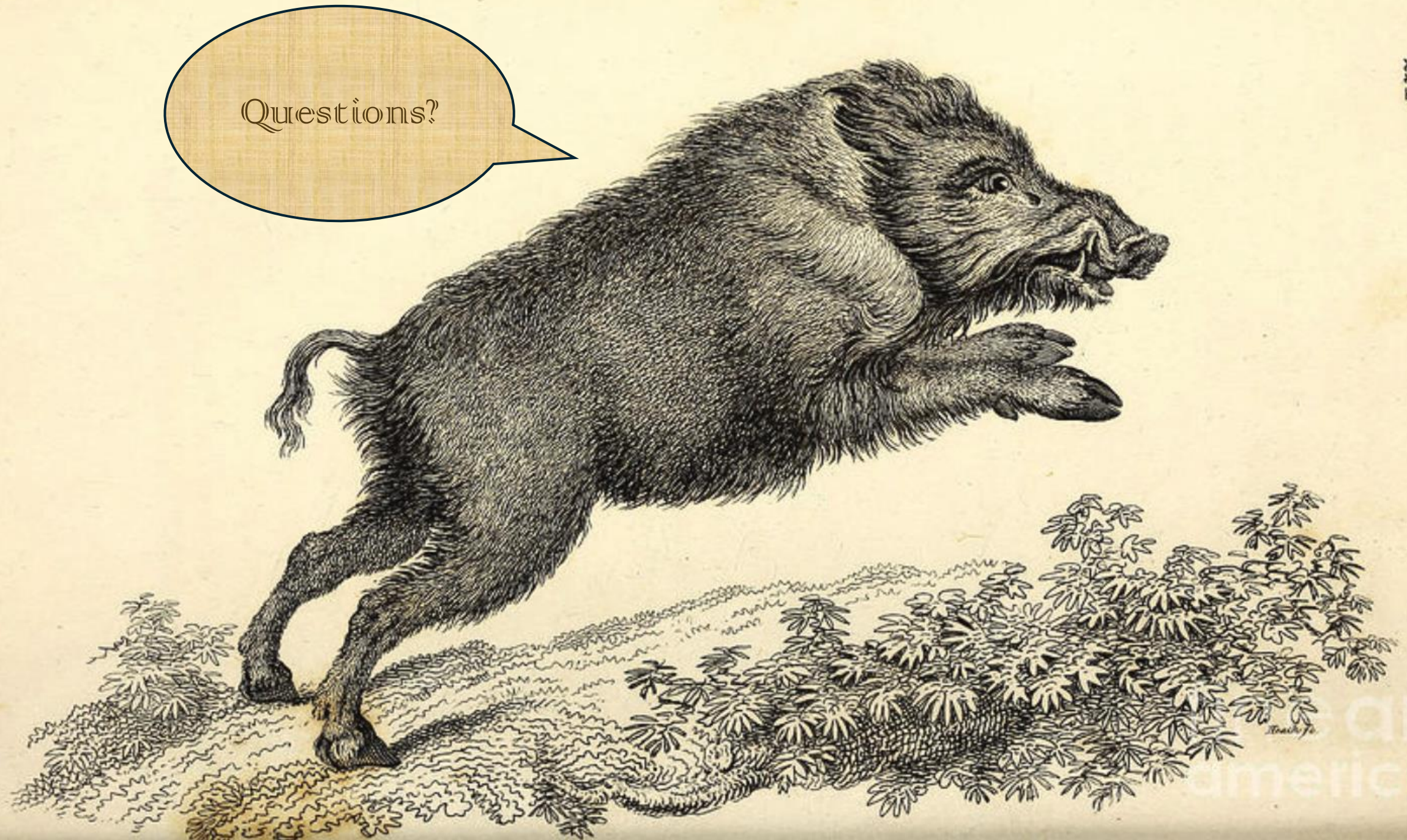
AQ:au Alan Vu[3], Mary Beard[4], and Daniel Peach[2,4]

Savannah River Ecology Laboratory, University of Georgia, Aiken, South Carolina 29802 USA

J. Entomol. Sci. 62: 000–000, 2026
DOI: 10.18474/JES26-56

Abstract In 2023, locally transmitted malaria cases in Florida, Texas, and Maryland highlighted the continued epidemiological importance of *Anopheles* spp. within the United States and the need to understand how climate change may influence their future distributions. *Anopheles crucians* Wiedemann sensu lato, *Anopheles quadrimaculatus* Say sensu lato, and *Anopheles punctipennis* Say are 3 competent vectors of human malaria found in the southern United States, a region that was a historic hotbed for human malaria within the United States. Using a Maximum Entropy approach, we modeled habitat suitability for these 3 *Anopheles* taxa across 13 southern states under historical, current to near-future, and far-future temporal scales. Final models across all 3 taxa showed distinct patterns. Model reliability was uneven and noticeably stronger for *An. crucians* s.l. than for *An. quadrimaculatus* s.l. and *An. punctipennis*. *An. crucians* s.l. showed gradual contraction of suitable habitat within the southern United States under future conditions, with habitat suitability shifting northwards. *Anopheles quadrimaculatus* s.l. showed substantial increase in habitat suitability below the fall line of the Atlantic Coast states and in Texas, Arkansas, and Louisiana. *Anopheles punctipennis* also showed rises in habitat suitability in Texas, Arkansas, and Louisiana. Projected models indicate notable changes, with many areas becoming increasingly suitable for *An. quadrimaculatus* s.l. over time. These findings support the integration of species distribution models into regional vector surveillance for public health preparedness.

Key Words climate change, malaria, mosquitoes, *Anopheles quadrimaculatus*, *Anopheles crucians*


Questions?

Pathogens

- Culex
- JEV
- WNV

- Malaria
- EEV

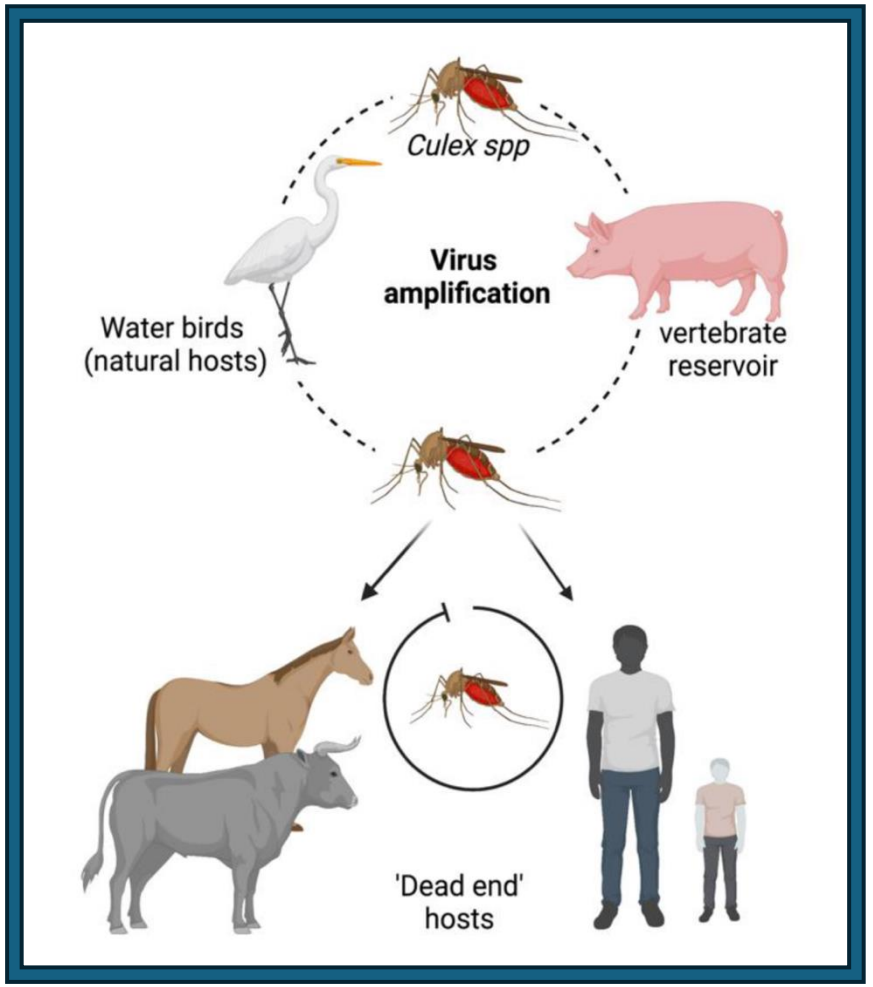


Fig 1. JEV cycle of infection and amplification from Srivastava KS, Jeswani V, Pal N, Bohra B, Vishwakarma V, Bapat AA, Patnaik YP, Khanna N, Shukla R. Japanese Encephalitis Virus: An Update on the Potential Antivirals and Vaccines. *Vaccines*. 2023; 11(4):742. https://doi.org/10.3390/vaccines11040742

Wild Pigs and Skeeters

- Wild pigs
  - Disruptive to human activities and environment
    - Disease reservoirs and amplifiers
    - Rooting and **wallowing behavior** disturbs earth
      - Depressions create nutrient-rich pools
- Mosquitoes utilize wallows
  - Breeding habitat
  - Blood meal from nearby pigs
    - **Problematic “bed-and-breakfast” scenario**





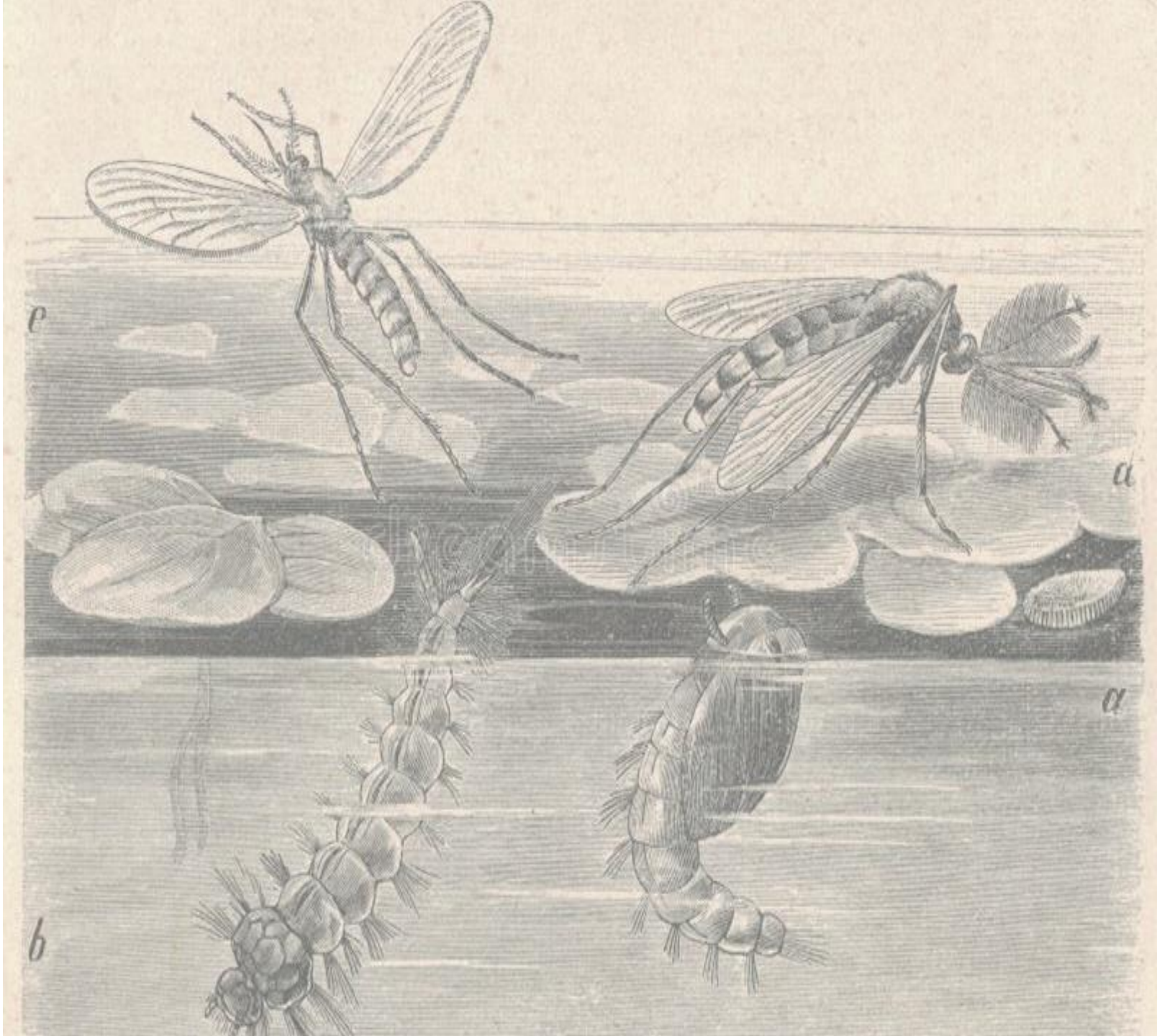
Wild Pigs (*Sus scrofa*)

- Invasive species
  - Population range and size increasing across the U.S.
  - Disruptive to the environment and human activities
- Ecosystem engineers
  - Rooting, spread invasive plants, alter runoff/watershed quality, **wallowing**
- **Wallows**
  - Filled with nutrients from pig excrement, and other organic materials from the disturbed earth
- Disease reservoirs
  - **Japanese Encephalitis Virus (JEV)**



Mosquitoes (the Culicidae)

- Deadliest animal to humans
  - >700,000 human fatalities per year globally
  - Deadly to other animals
- Vectors of infectious agents
  - Transmit pathogens via blood feeding
    - **(JEV)**
      - ***Culex* spp., of which many are invasive**
    - **Parasites that cause malaria**
      - ***Anopheles* spp.**
      - **Recent endemic malaria cases in U.S.**
- **Females require blood meals to produce eggs**
- **Mosquitoes require water to lay their eggs**



Wallows as Mosquito Habitat

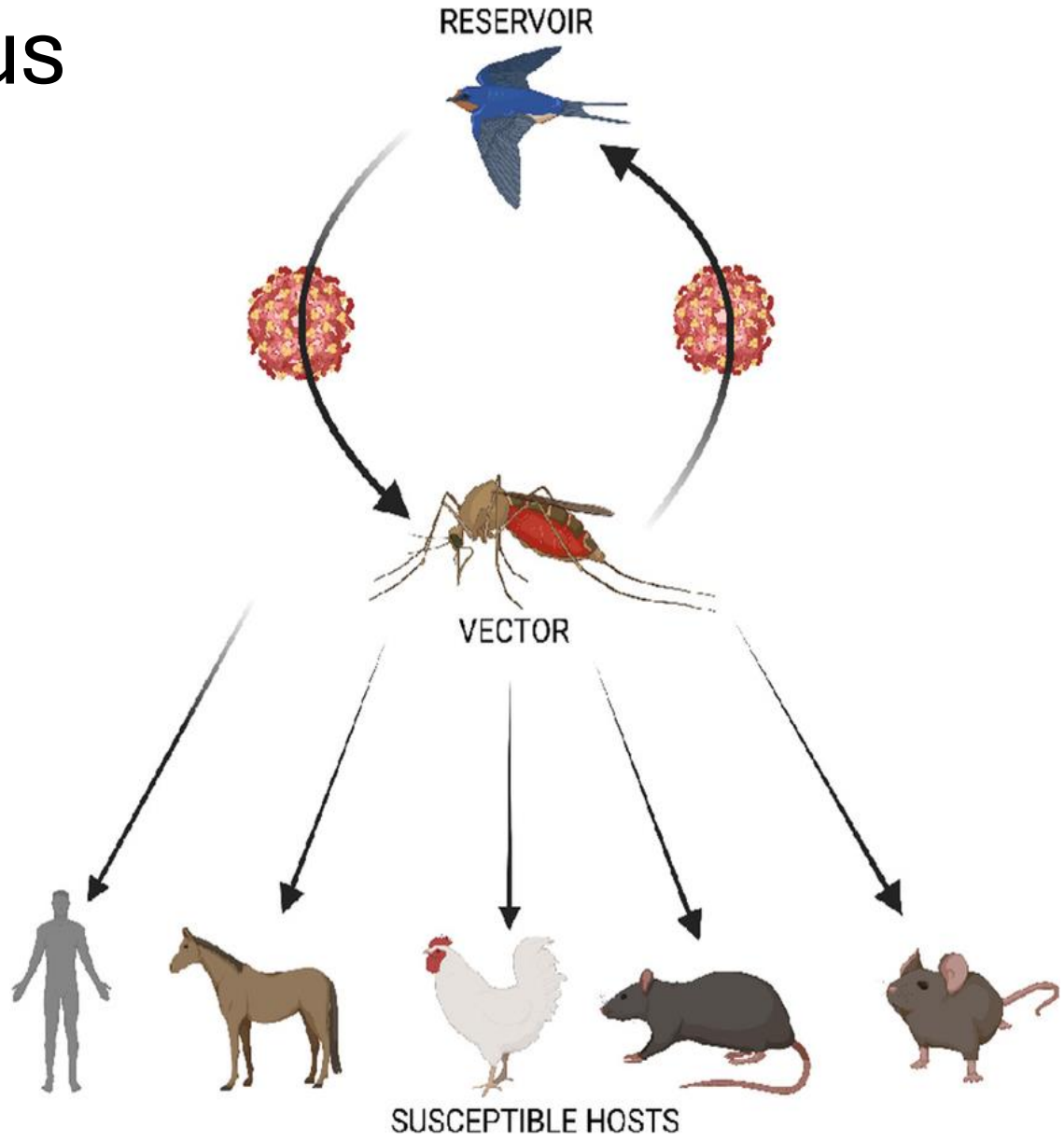
- Pig blood provides mozzie females with sustenance; JEV amplifies in pigs; wallows provide nutritious nurseries: a **bed-and-breakfast scenario**
- This interaction can increase the spread of vectored pathogens
    - Creates breeding habitat and bloodmeal source for vectors
        - Invasive mosquitoes utilized invasive pig wallows in Hawaii, led to disease-related population decline



Japanese Encephalitis Virus

- An emergent pathogen
- Asia and the western Pacific region
- Exists in an enzootic transmission cycle
- Vectored by mosquitoes and amplified in **pigs**
    - JEV can cause fatalities in humans, as well as **limited reproductive success in boars and sows.**

enzootic transmission cycle



Methods

- **Sites:**
  - Pig barns with nearby wild pig activity, similar habitat types and varying insect exclusion measures
  - Wild pig wallows within the counties associated with pig barns, as well as SRS

- **Capture materials**
  - Standard mosquito traps (adults)
  - Dippers (larvae and pupae)

- **ID and Testing**
  - Identification of mosquitoes via standard references, barcoding
  - Blood analysis
    - Wild and domestic pig blood in mosquito blood-meals
    - Test for presence/absence of antibodies
      - Using West Nile Virus as a proxy due to transmission cycle and antigenic similarities









Preliminary Data

- Mosquito species of interest, including **blood-fed**, found inside barns
  - ***Culex* spp.**
  - Blood analysis is ongoing

- Other mosquito species found inside and outside barns include ***Anopheles spp.***, though these specimens are still being quantified and further identified.

- **Collections and data analysis are ongoing for this season**

Research Design & Aims:

- **Sites:**
  - 6 commercial sow farms and wean-to-finish farms in the southeastern U.S. as study sites. We will select sites with nearby wild pig activity, similar habitat types and varying insect exclusion measures in the southeastern U.S.
- **Aim 1**: **I will establish the ecological relationship between invasive mosquitoes and invasive swine within the southeastern region of the U.S. / Establish whether wild pigs increase the habitat available to potential vectors of JEV by identifying the mosquito species that breed in wild pig wallows, in the U.S. southeast.**
  - I will determine this by locating up to 5 wallows per representative habitat type and collect larval samples within every other week from March to October using a standard sampling technique with 350 mL dippers. My study sites will be chosen based on nearby wild swine presence and comparable habitat types. I will lab rear larvae until the 4th instar and then place them in ethanol morphologically identify them using taxonomic guides. I anticipate my results determining if mosquitoes utilize pig wallows, identification and abundance of those utilizing them, and the significance and efficacy of pig wallow sites as mosquito breeding habitats.
- **Aim 2: Observe adult mosquito abundance to determine the contribution of mosquitoes developing in wallows to local mosquito diversity / Determine the extent to which mosquitoes access sow farms and wean-to-finish farms and what mosquito species are most commonly trying to enter facilities and get inside. We also will compare the effectiveness of existing insect exclusion methods for preventing mosquito access.**
  - I will accomplish this by observing adult mosquitoes caught in CDC light traps, oviposition traps, and other glue and sugar traps nearby larval collection sites. To measure visitation pressure by mosquitos to swine production facilities, we will deploy disposable mesh traps coated in sticky-trap horse fly glue to windows, airvents, and above entranceways at swine farms. To measure successful mosquito access to swine farm facilities we will survey mosquito abundance and diversity within swine operation structures. As traps baited with synthetic host odor lures are unlikely to compete successfully with the attractiveness of live pigs, we will deploy a combination of CDC light traps, oviposition traps, and attractive sugar bait traps. Captured mosquitoes will be identified via morphological analysis and via PCR and compared to sequences in BLAST or VectorBase. The results of this analysis will aid in better projections of how wallows can change overall local mosquito communities.
- **Aim 3: Elucidate potential negative contributions of mosquito breeding and feeding habits on mortality rates of native species, potential causes, and infer how wild pigs may intensify this effect / Assess how much exposure domestic swine herds currently experience to mosquito-borne pathogens present in wild pigs through blood-meal identification of mosquitoes collected in swine operations and testing samples of domestic and wild pig blood for evidence of pathogens.**
  - I will assess the specific risk involved with hematophagous insects by blood-meal mosquito identification from the study sites and compare wild and domestic pig blood, with the help of swine researchers, for the presence of similar biological risks. These blood analyses will be successfully executed by well-established bloodmeal analysis and blood testing techniques. This will aid in determining specifics of how native wildlife would be affected by projected ecological function of hematophagous insects in conjunction with swine and animal-related disease mortality mechanisms.

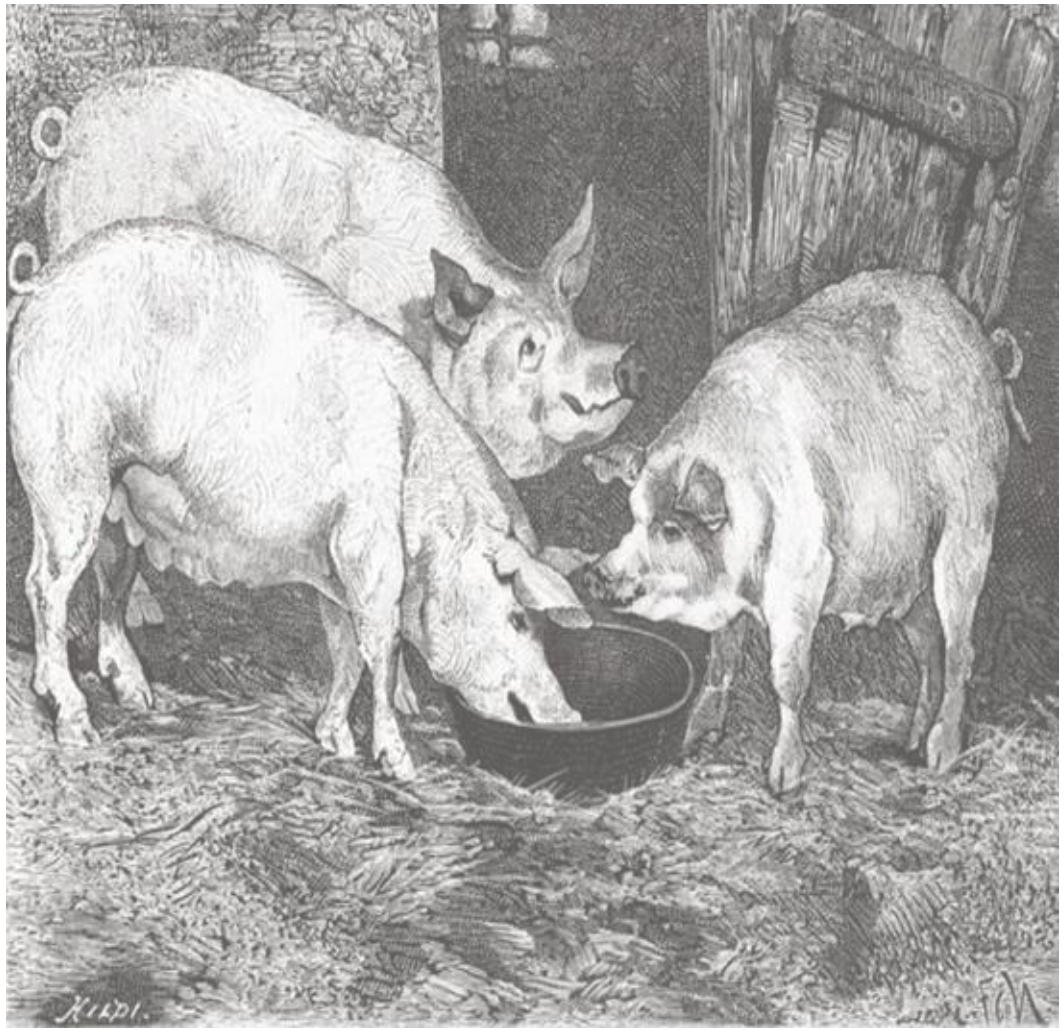


Concern for Agricultural Stability

- JEV can cause fatalities in humans and other livestock, as well as **limited reproductive success in boars and sows**.
- **Disease spread = substantial economic damage**:
  - Ex. –> In 2002, West Nile Virus (mosquito-borne pathogen) emergence and transmission caused significant economic damage to equine industries
    - $2.4 million in North Dakota, $16.5 million in Texas, and $2.75 million in Colorado and Nebraska.
- Should JEV arrive in the U.S., estimated economic loss = $300-$600 million per year

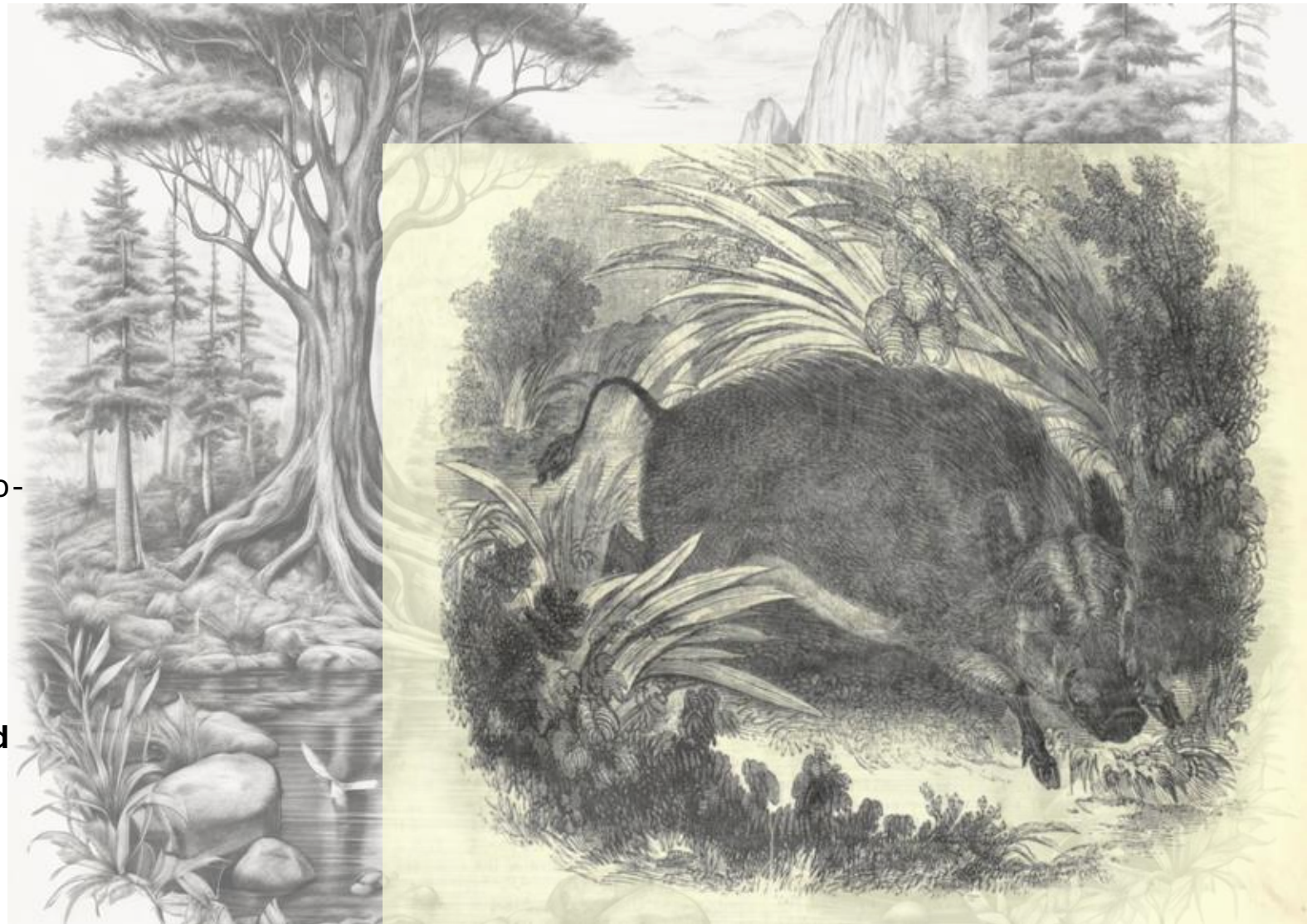
Concern for Agricultural Stability

- **JEV leads to limited reproductive success in boars and sow, and medical complications or mortality in piglets.**
- **Pathogen spread = substantial economic damage**:
  - Should JEV arrive in the U.S., estimated economic loss = $300-$600 million per year

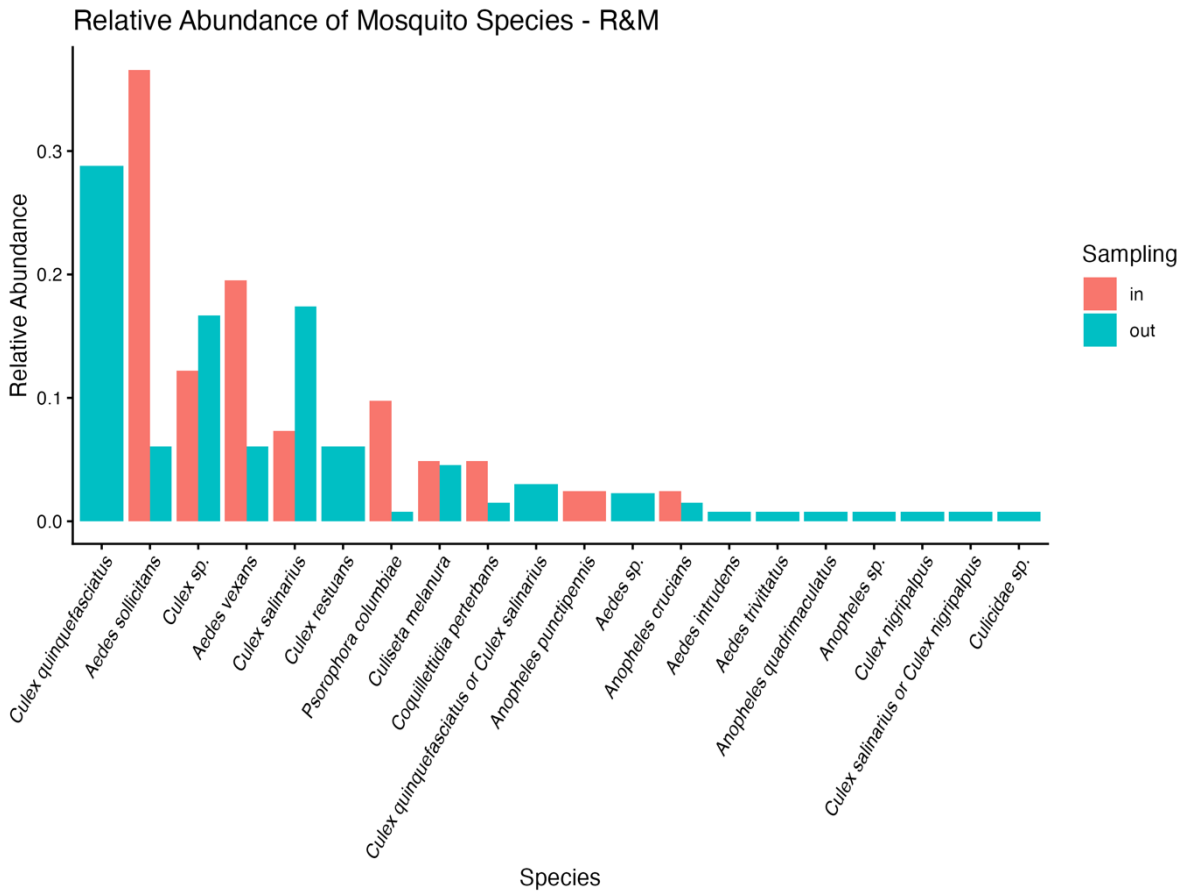


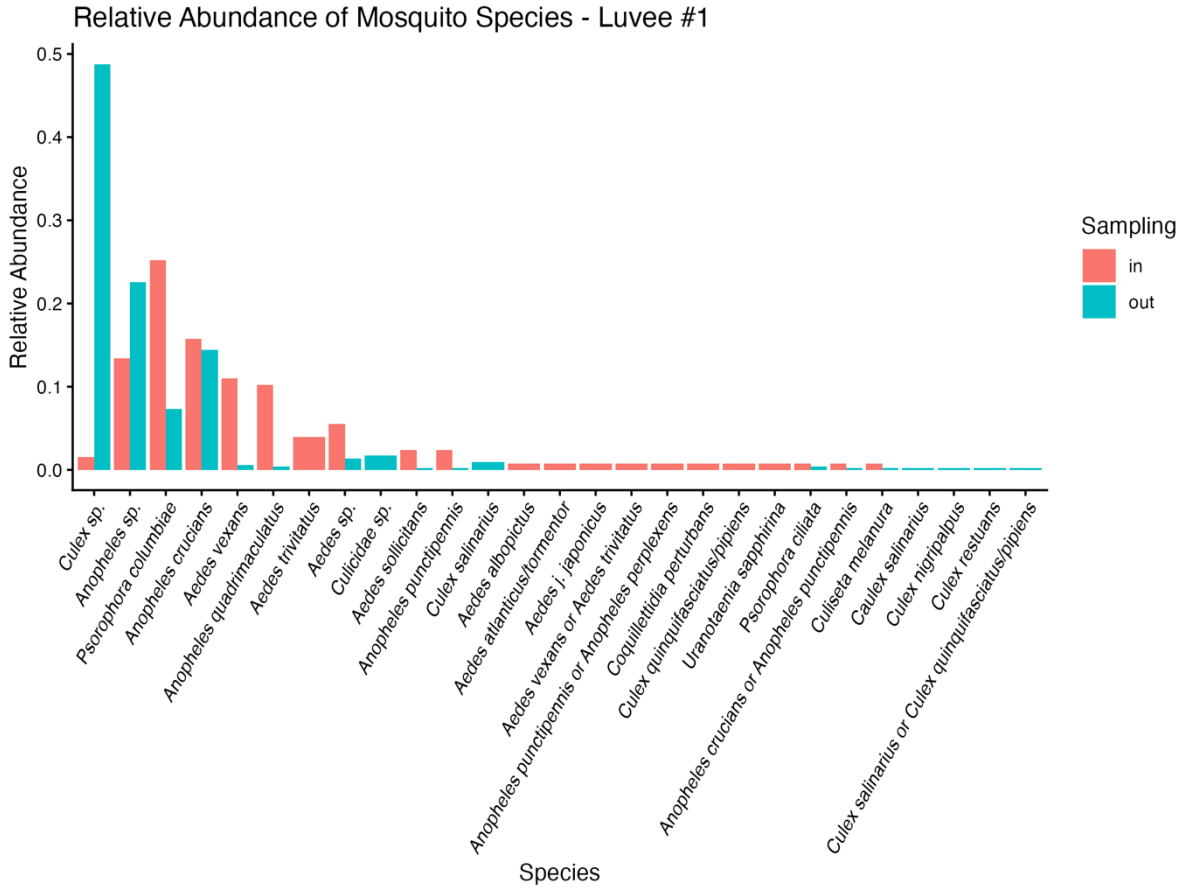
Concern for Ecosystem Stability

- **Invasive feral swine and mosquito species individually pose great threats to native ecology, but their unexplored ecological synergism may have greater impacts than currently understood and may result in a variety of disastrous consequences for local ecosystem stability.**
- Ex. -> *Culex quinquefasciatus* mosquitoes breed in pig wallows and water-filled rooting depressions in Hawaii and transmit mosquito-borne pathogens, such as avian malaria, among many critically endangered Hawaiian birds. This has led to substantial avian population decline.
- **Potential co-expansion and interaction of feral pigs and vectors may lead to local and foreign pathogen spread and habitat destruction which threatens local wildlife populations.**

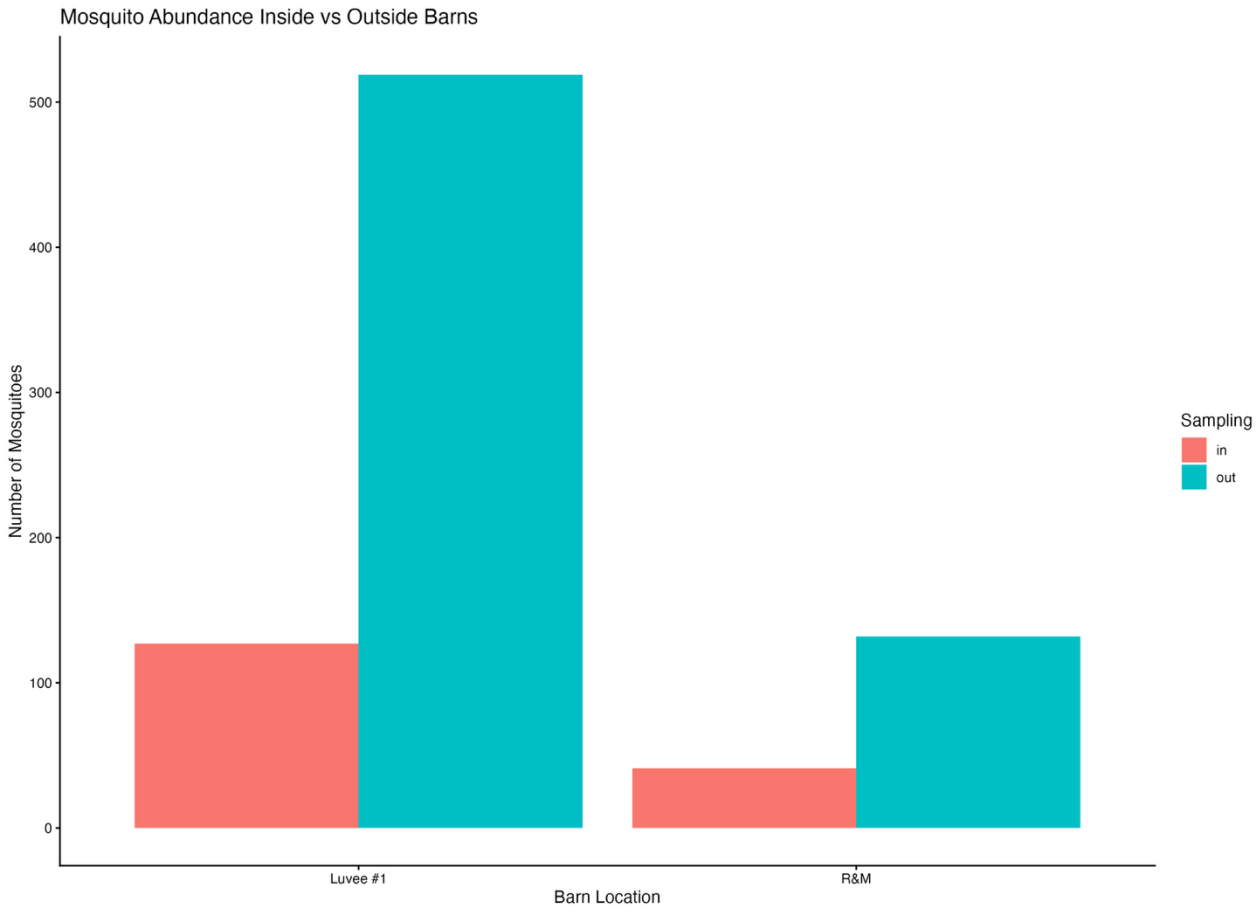


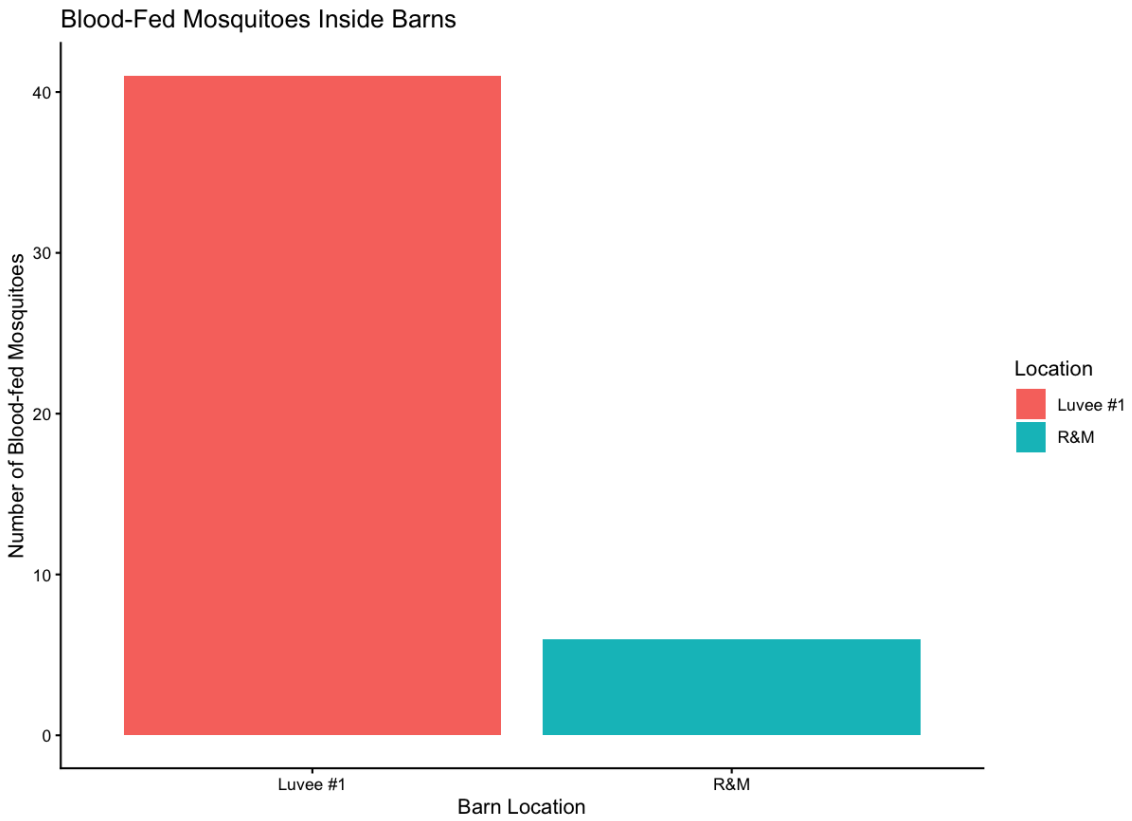
Preliminary Data





Preliminary Data





Aim 1: Species and Quantity of Mozzies-Domestic

YEAR-ADJUSTED MARGINAL MEANS (BACK-TRANSFORMED) ---

Ventilation response SE df

Natural 27.036447 14.763349 1.56

Tunnel 8.094562 2.774129 2.40

lower.CL upper.CL

0.3902042 564.4151

1.9617891 26.9260

Degrees-of-freedom method: kenward-roger

Confidence level used: 0.95

Intervals are back-transformed from the log(mu + 1) scale

STATISTICAL MODEL SUMMARY ---
Linear mixed model fit by REML. t-tests use
Satterthwaite's method [lmerModLmerTest]
Formula:
log(Barn_Total + 1) ~ Ventilation + (1 | Year) + (1 | Barn)
Data: barn_weekly

REML criterion at convergence: 220.9

Scaled residuals:
Min 1Q Median 3Q Max
-2.42552 -0.56425 -0.07963 0.78289 1.61803

Random effects:
Groups Name Variance Std.Dev.
Barn (Intercept) 0.321217 0.56676
Year (Intercept) 0.005832 0.07637
Residual 1.202707 1.09668
Number of obs: 70, groups: Barn, 10; Year, 2

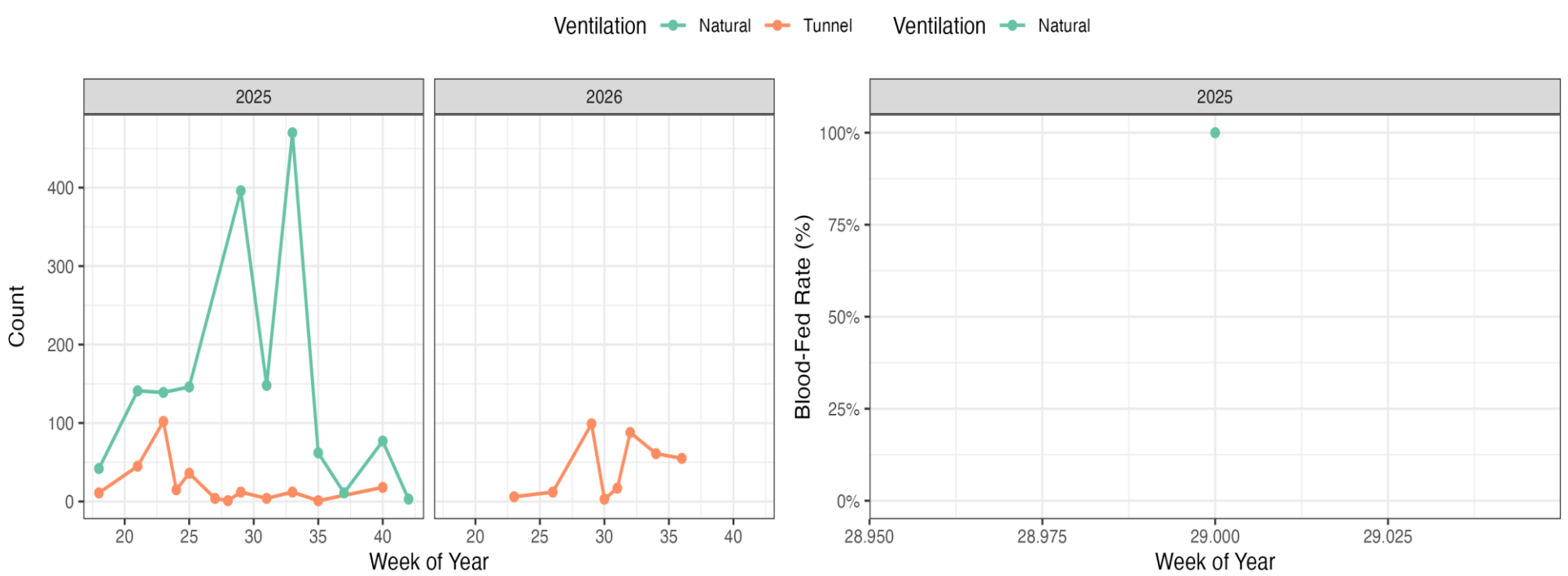
Fixed effects:
Estimate Std. Error df
(Intercept) 3.3335 0.3487 1.0575
VentilationTunnel -1.1258 0.4559 4.3212
t value Pr(>|t|)
(Intercept) 9.561 0.0590 .
VentilationTunnel -2.469 0.0643 .

Signif. codes:
0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
(Intr)
VentltnTnnl -0.747

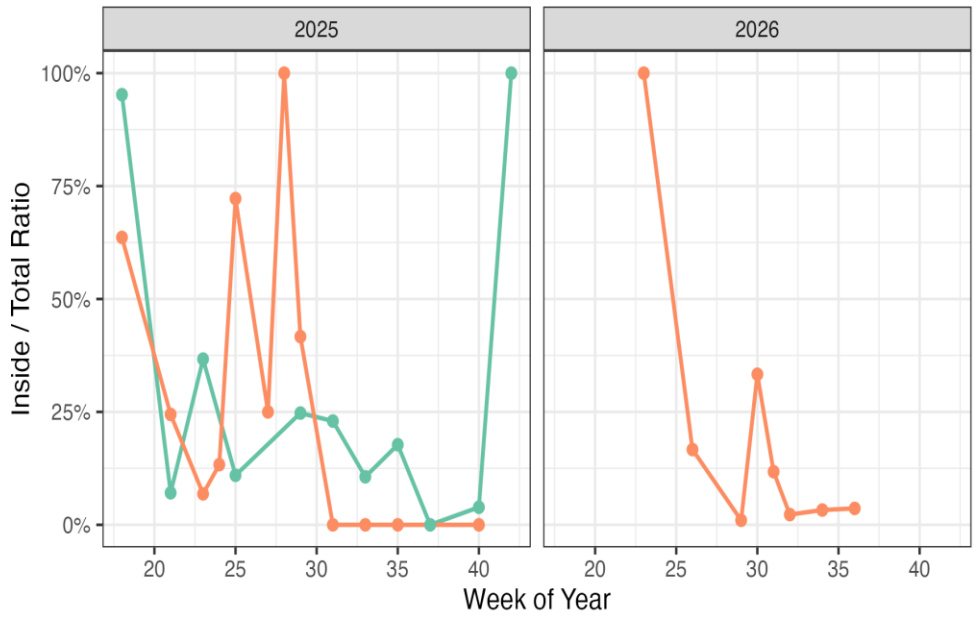
Aim 1: Species and Quantity of Mozzies-Domestic

- Negative Binomial GLMM
  - Tunnel-vent barns experience significantly lower total mosquito pressure overall compared to natural-vent barns ($X^2 = 11.01$, $df = 1$, $p = 0.00091$).
  - Inside counts are significantly lower than outside counts across both ventilation types ($X^2 = 26.14$, $df = 1$, $p = 3.18 \times 10^{-7}$)
  - Tunnel vs natural interaction term is **not statistically significant** ($X^2 = 0.39$, $df = 1$, $p = 0.535$)

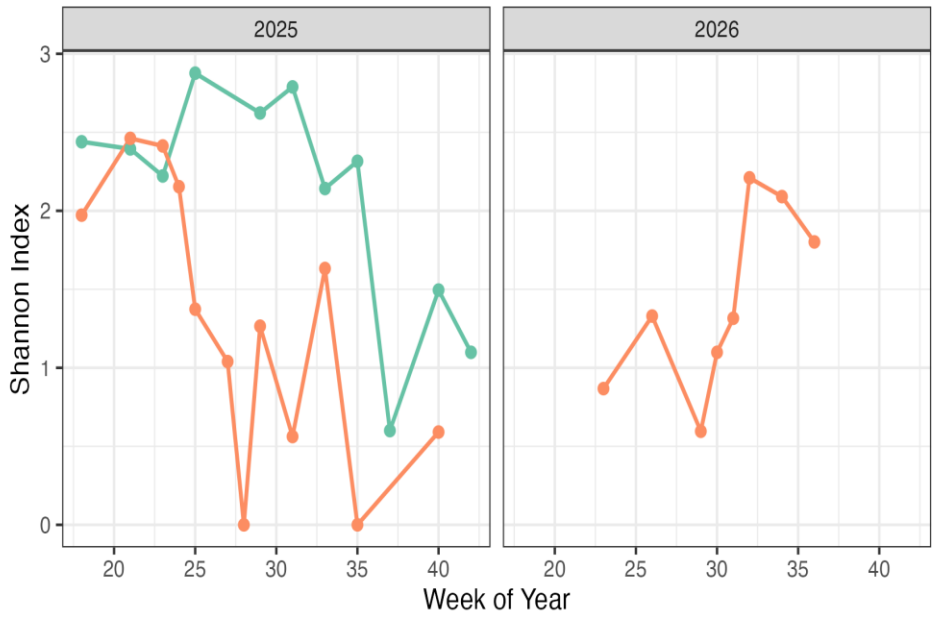


omestic

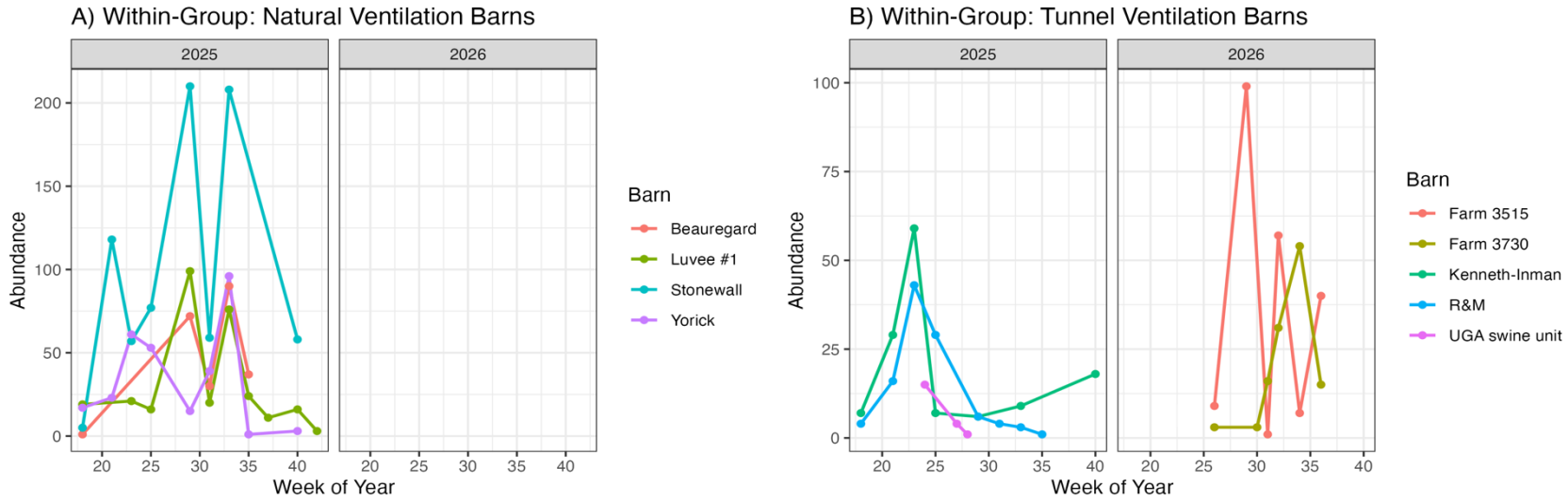
C) Indoor Occupancy Rate

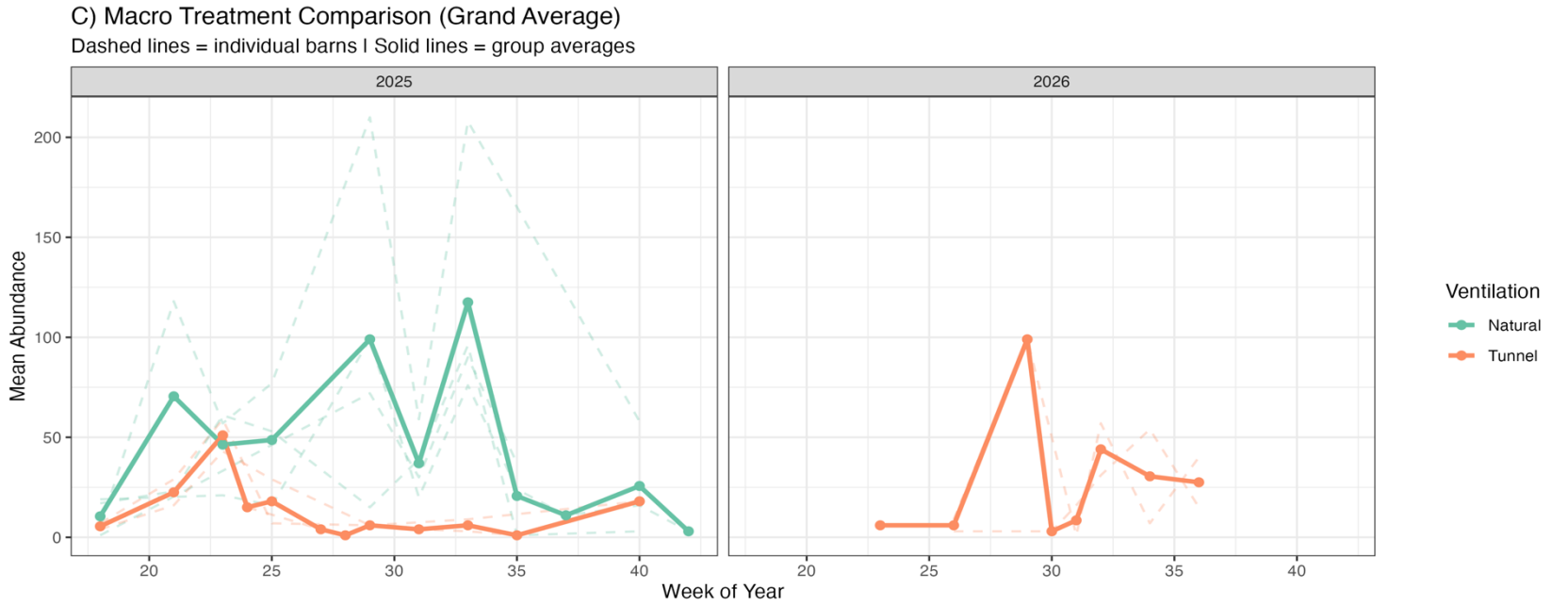


D) Species Diversity



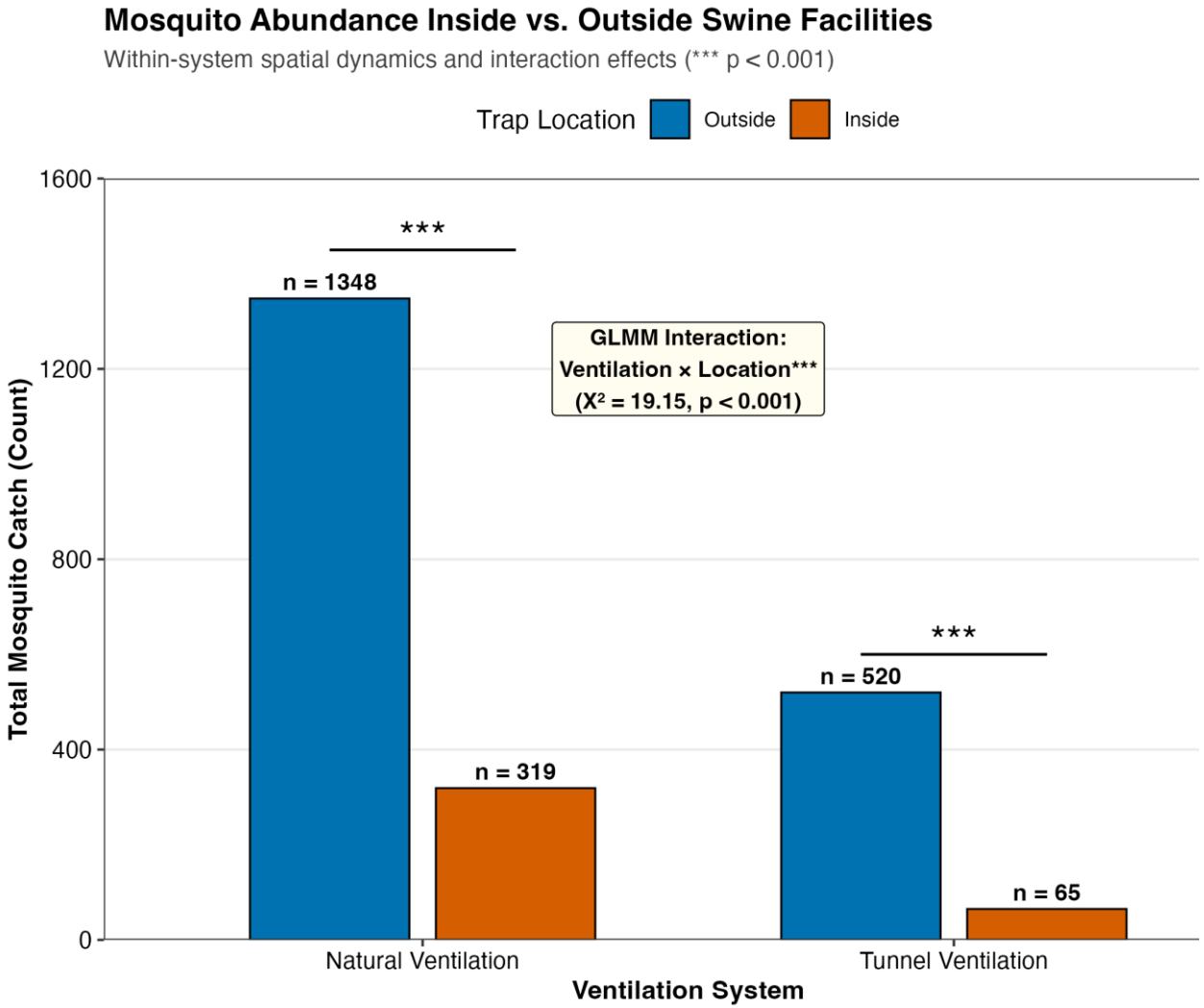
Aim 1: Species and Quantity of Mozzies-Domestic

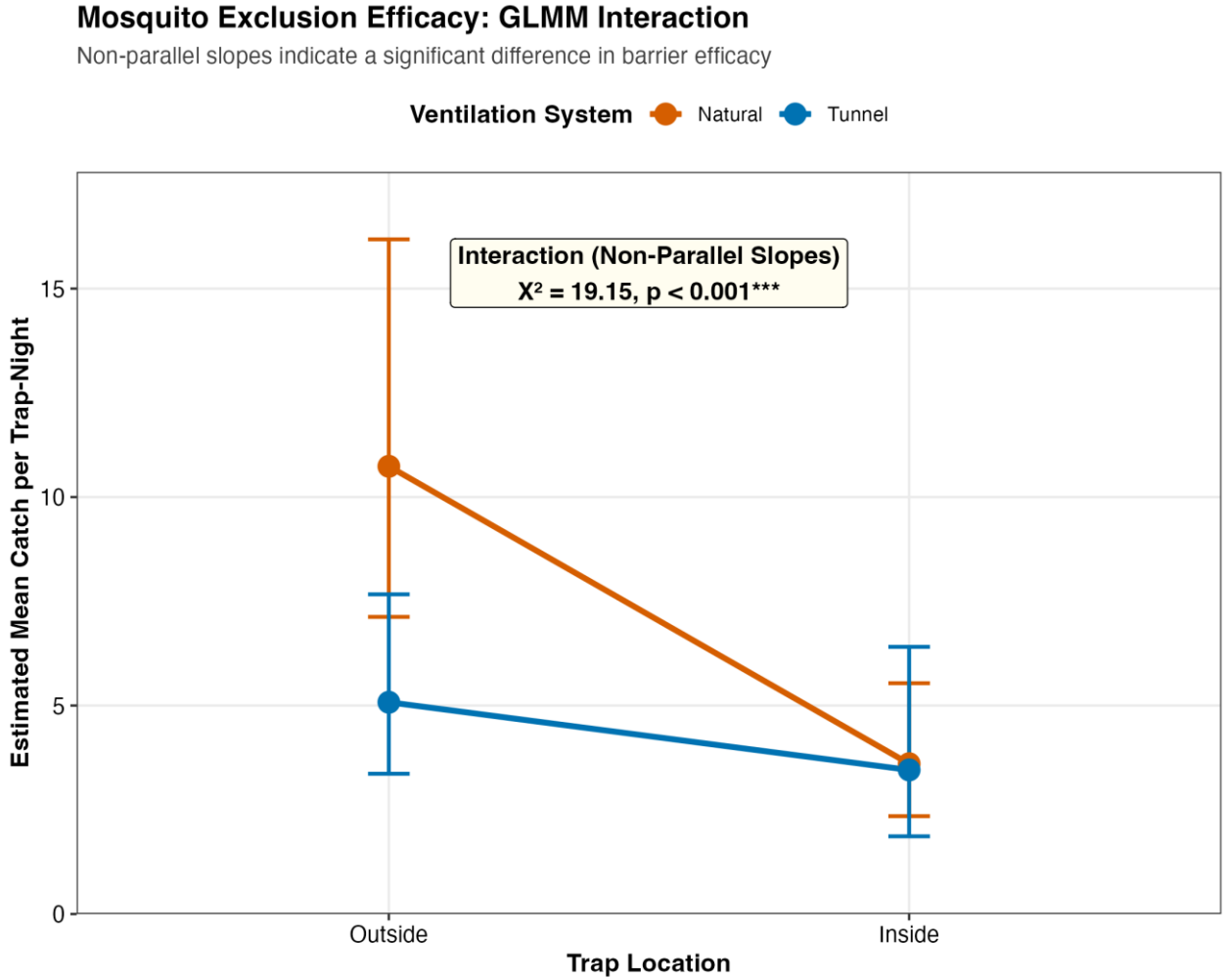




- Within-group shows similar trends throughout the season, aside from occasional spikes
- Looking at the grand average of both types, it appears that we see **greater abundance in the natural ventilated systems (n=4)** than the tunnel ventilated systems (n=5)

Aim 1: Species and Quantity of Mozzies-Domestic





Aim 1: Species and Quantity of Mozzies-Domestic

