PROPOSED STUDY PROTOCOL
on
Protective Effect of Cliff Swallows Against Mosquitoes Arising from the Great Salt Lake Area

WRITTEN BY:
Christopher S. Bibbs

TEST SITE:
Salt Lake City Mosquito Abatement District (SLCMAD)
2215 North 2200 West
Salt Lake City, Utah 84116-1108

PRINCIPLE INVESTIGATOR(S):
Charles Brown, University of Tulsa

STUDY SPONSORED BY:
Craig Wallentine, Utah Physicians for a Health Environment

Proposed Experimental Start Date: May 2026
Proposed Experimental Termination Date: TBD - September 2026

Salt Lake City Mosquito Abatement District, Utah

1.0 Study Title:
Protective Effect of Cliff Swallows Against Mosquitoes Arising from the Great Salt Lake Area

2.0 Objectives:
This study will evaluate 1) the extent to which the presence of a healthy cliff swallow colony will reduce the relative abundance of host-seeking mosquitoes and 2) the relative range of effect for the altered mosquito presence relative to ta 1 kilometer forage rage for the birds.

3.0 Test Substance:
3.1 Non-pesticidal; Surveillance only.

4.0 Test System:
4.1 Identification:
Trap Surveillance: a variation of miniature CDC style trap (3d printed, no light, Salt Lake City Mosquito Abatement District, Salt Lake City, UT).

Wild Mosquitoes: The greater salt lake (GSL) wetlands contain vast mix of palustrine floodplains, seeps, salt playas, and upland sagebrush habitat that is known to harbor nuisance floodwater mosquito species, notably *Aedes dorsalis* and *Ae. vexans*, and encephalitis vector species, notably *Culex tarsalis* and *Cx. erythrothorax*. These mosquito species are crepuscular in activity. Mosquito season can start as early as March, but early season surveillance regularly occurs starting in April to allow operational decision-making at the Salt Lake City Mosquito Abatement District (SLCMAD).

Birds: Cliff swallows migrate into wetland and urban edges annually starting in April/May, but have mixed colonization windows until mid-summer. The populations can be transient, and re-colonization of old nest sites is not always guaranteed. Cliff swallows are active from dawn until dusk, with notable bursts of foraging activity in crepuscular time windows. These birds have small body size, leading them to accept smaller sized food, and social colonies can be several dozen to several hundred strong, allowing for high density presence in ecologically relevant time and place to interact with mosquitoes.

4.2 Justification:
Trap Surveillance: Changes in relative abundance in locally occurring mosquito species will indicate if treatment successfully reduced target mosquito species within the treatment/monitoring area

Wild Mosquitoes: The most abundant species, *Ae. dorsalis* and *Cx. tarsalis* travel far 12+ miles and annually invade the Salt Lake City metroplex. With increased development in the GSL wetlands, various new risk groups have arisen among human populations working or residing in what used to be relatively distal mosquito habitat. Cliff swallows regularly occur in high numbers in the same areas, including industrial transition zones that vector mosquitoes capitalize on during the summer, affording the bird colonies frequent close proximity to human populations as well. If the presence of a robust cliff swallow colony has a protectant affect against being bitten by mosquitoes, either by reducing local numbers or deterring ingress from outlying areas, then future study can emphasize encouraging cliff swallow residence as an alternative control for mosquitoes.

5.0 Experimental Design and Control of Bias:

Mosquito sampling will focus on cliff swallow nesting sites up to 2 km^2 in diameter with allowance for 0.8 km transects through the colony foraging area. Colony locations supporting at least 200 active swallow nests will serve in a treatment group. We will select colonies that become active in June, thus ensuring overlap between bird presence and target mosquito activity. Control sites will lack cliff swallows, but preferably still be capable of supporting cliff swallow residence and will be at least 2 km from the nearest active cliff swallow colony.

Sites will be selected from the interior of the inland marsh systems west of Salt Lake City, UT that retains much of the snow melt and human diverted water for water fowl management, water rights consumption, irrigation, and other uses. Sampling will occur within and adjacent to the cliff swallow nest sites (Figure 1) across 5 plots stratified in 200-m intervals. The linear spacing will help build inference into spatial gradation of effects that cliff swallow proximity has on relative abundance of wild mosquitoes.

Several regular surveillance sites served by SLCMAD also have anecdotally confirmed cliff swallow presence. Sites can be selected with large separation in land area (Figure 1) depending on habitat identification and bird colonization success in the sampling year. Coordinates for all sites will be listed in the final report. Weekly mosquito abundance will be tracked near test sites using standardized sampling procedures. This portion of data collection is intended to answer whether the presence of cliff swallows reduces the relative abundance of mosquitoes in proximity to their nest sites.

A secondary data collection will be conducted in which three volunteers per test group will conduct human landing catches in sites adjacent to and again far away from Cliff Swallow nesting sites. This will be conducted three separate crepuscular timing windows. This portion of data collection is intended to answer whether the immediate presence of cliff swallows protects humans from contact with host-seeking mosquitoes.

A tertiary data collection will be conducted to characterize whether cliff swallow populations demonstrate direct evidence of feeding on mosquitoes. Collections will be directly with birds under Utah department of natural resources permitting using mist nets. Bird collections will center around critical nest development time points throughout the season and use metabarcoding and fecal sample collections to identify percentage of the population feeding on mosquitoes. This portion of data collection is intended to answer whether the Cliff Swallows ecologically interact with mosquitoes in a meaningful way for mosquito control.

The final data collection effort will be to characterize the risk of mosquitoes to the cliff swallow colonies. This will be carried out by sampling mosquitoes from around nesting areas and committing them to blood meal analysis, for identifying host utilization in the sub-sample, as well as screen for bird-associated viruses such as West Nile virus. This portion of data collection is intended to answer whether the Cliff Swallows are actively harmed by mosquito presence, which may be significant if the birds are implemented intentionally to intervene in wild populations.

Materials and Methods:

Trap Surveillance

1. Trapping will be conducted with a routinely employed SLCMAD miniature CDC style trap (Figure 1; Bibbs et al. 2024) (3d printed, no light, Salt Lake City Mosquito Abatement District, Salt Lake City, UT).
    a. Traps will sit approximately 1.5 m above the ground.
    b. All traps will be baited with 300 ml/min of CO_2 via a pressure regulated cylinder.
2. One trap will be located at each plot.
3. All traps will be placed at conserved locations that will remain unchanged throughout observation.
    a. Traps will be recovered after 1 trap night on a weekly basis for duration of study (ex: set trap Wednesday, collect Thursday, repeat 10-26 weeks).
    b. Mosquito collections will be returned to the lab for sorting and quantification using a combination of light microscopy and digital cataloguing (ImageJ, National Institute of Health) according to methods in Faraji et al. (2025).
    c. Species identification will be carried out with dichotomous keys relevant to the region and state.
4. There will be a minimum of 10 trap weeks for comparison between sites, but duration can extend longer, contingent on when a colony is confirmed active and the remaining mosquito season available for monitoring.
5. Direct monitoring with mosquito traps will only be conducted inside the forage ranges of the compared areas, but circumstantial information can be pulled from the routine surveillance by SLCMAD in outlying areas.

Biting Pressure Evaluation

6. Two sites will be occupied by teams of three volunteers. These sites will be allocated as treatment (containing Cliff Swallows) or control (lacking Cliff Swallows).
7. Volunteers will don loose fitting, light colored, long sleeve and hooded outer wear, but not use chemical repellents.
8. Volunteers will position in teams at their randomly allotted sites, between treatment and control, within 20 minutes of forecasted sunset.
9. Volunteers will be monitored for mosquito landings in intervals of three minutes at standing locations within the field site.
10. Once observation and data collection is concluded, volunteers will rotate positions and re-conduct landing counts.
11. After all sites are measured by each volunteer, the night will be concluded for biting pressure assessment.
12. Three nights in this design will be carried out at corresponding dusk windows.

Bird Captures

13. Two to three sites will be selected for the presence of active, growing Cliff Swallow colonies at least numbering 80 adults.
14. Sampling windows will be designated for each of the incubation timeline of the nest (eggs present but unhatched), within 3-5 days of hatch (nestlings present), and at fledged flight (15 days post hatch).

    a. Colony establishment is expected to be somewhat asynchronous, occurring from April to June; sampling windows will be adjusted by calendar according to the detected maturity of the colony.
15. Mist nets will be deployed around flyways for the nest adjacent to nesting structures, but far enough away to intercept those birds returning from foraging; target will be for 20-30 birds per sampling window.
16. Fecal material will be collected from birds by hand with semi-contained handling in plastic bags.
17. Fecal material will be handled for DNA meta barcoding and submitted to laboratories offering appropriate extract services.
    a. Management-relevant cutoffs will be used for diagnosis of relevant bird-mosquito foraging, with 10% or less colony dietary presence of mosquitoes being insignificant and 30% or greater colony dietary presence of mosquitoes being potentially significant for mosquito reduction.

Mosquito Risk Assessment

18. Two sites will be selected for the presence of active, growing Cliff Swallow colonies (treatment) or with suitable habitat or evidence of colony history for Cliff Swallows, but with no active bird population (control).
19. Mosquitoes will be collected directly from nesting areas with intent to acquire blood-fed mosquitoes.
20. Blood-fed mosquitoes will be cold-chained at submitted for blood-meal analysis to diagnose host utilization as a percentage of collections arising from inside and outside Swallow habitats.
21. Non-blood fed mosquitoes from direct collections and from trap transects in the foraging area surveillance will be committed to viral RNA extractions to determine presence or general risk of bord-associated zoonoses.

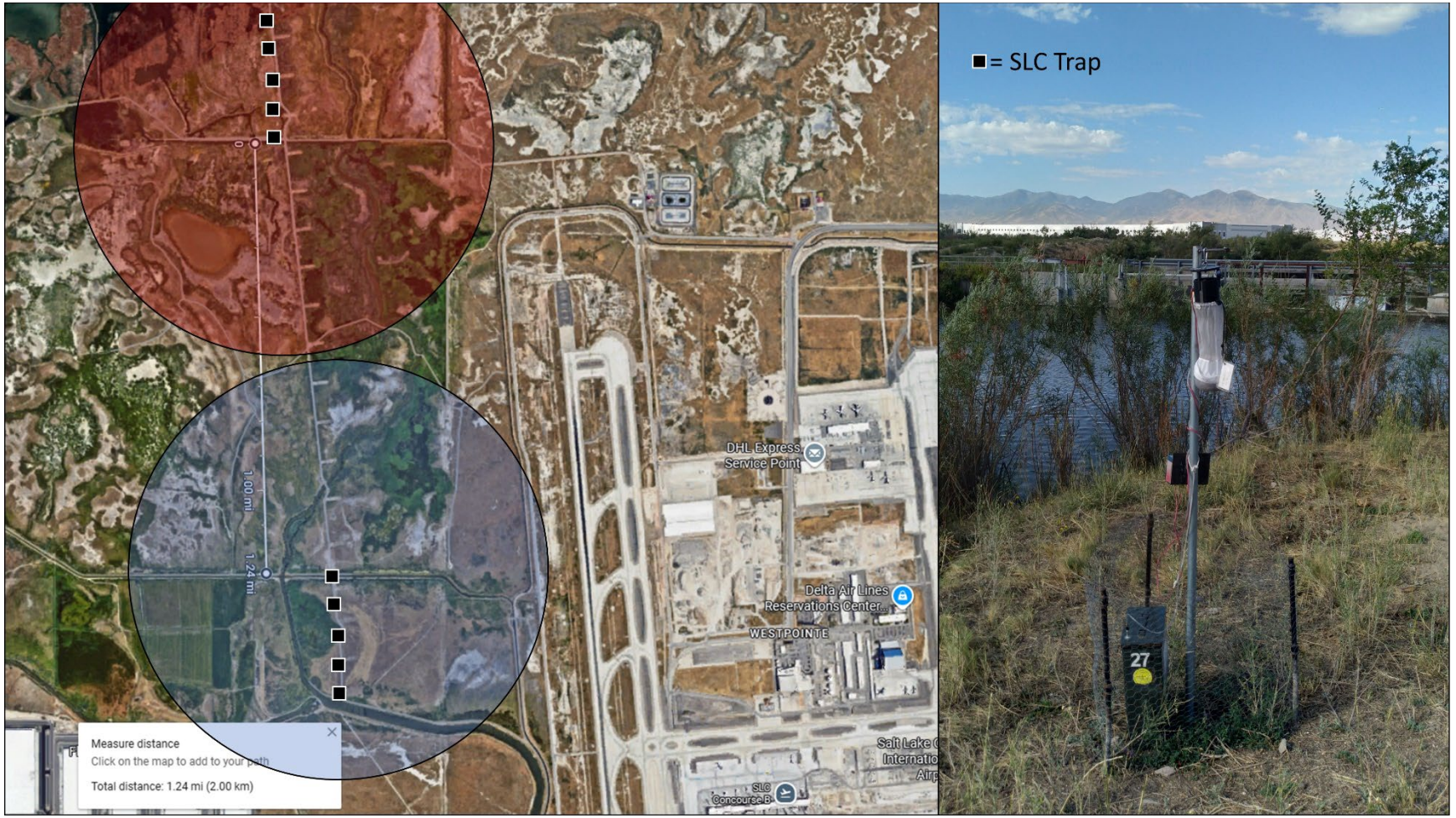


Figure 1: Left) Cliff swallow nesting sites within the same general habitat. Forage range superimposed as transparent circles. Trap site examples denoted with black squares. Right) 3D printed SLC Trap used for routine surveillance. Trap location is the southernmost square of the blue circle.

Figure 2: Cliff swallow nesting sites in widely separated areas with no possibility for interference/treatment dilution effect, but increased possibility for less comparative habitat. Forage range superimposed as transparent circles. Trap site examples denoted with black squares.

6.0 Statistical Analysis of Data:
The below statistical calculations will be conducted. Any further statistical modeling will be fully described in the final report with full statistical outputs presented. For an example of statistical analysis see Rochlin et al 2022.

6.1 *Trap Surveillance:*

6.1.1 Percent reduction can be conducted according to Mulla et al. 1971's application of the following formula:

$[1 - (C_1 \times T_2)/(T_1 \times C_2)] \times 100 =$ Percent reduction

C_1 = Mosquitoes released of target species in placebo
C_2 = Placebo count at time corresponding with treatment count
T_1 = Mosquitoes released of target species in treatment
T_2 = Treatment plot count

Any additional analyses required to understand the efficacy measure, such as Before-After Control Impacts (Rochlin et al. 2022) or standard pairwise analysis, will be described in the final report.

Analyses for human landing counts, bird sample metabarcoding, blood meal analysis, and viral detection will be described in the final report.

7.0 References Cited

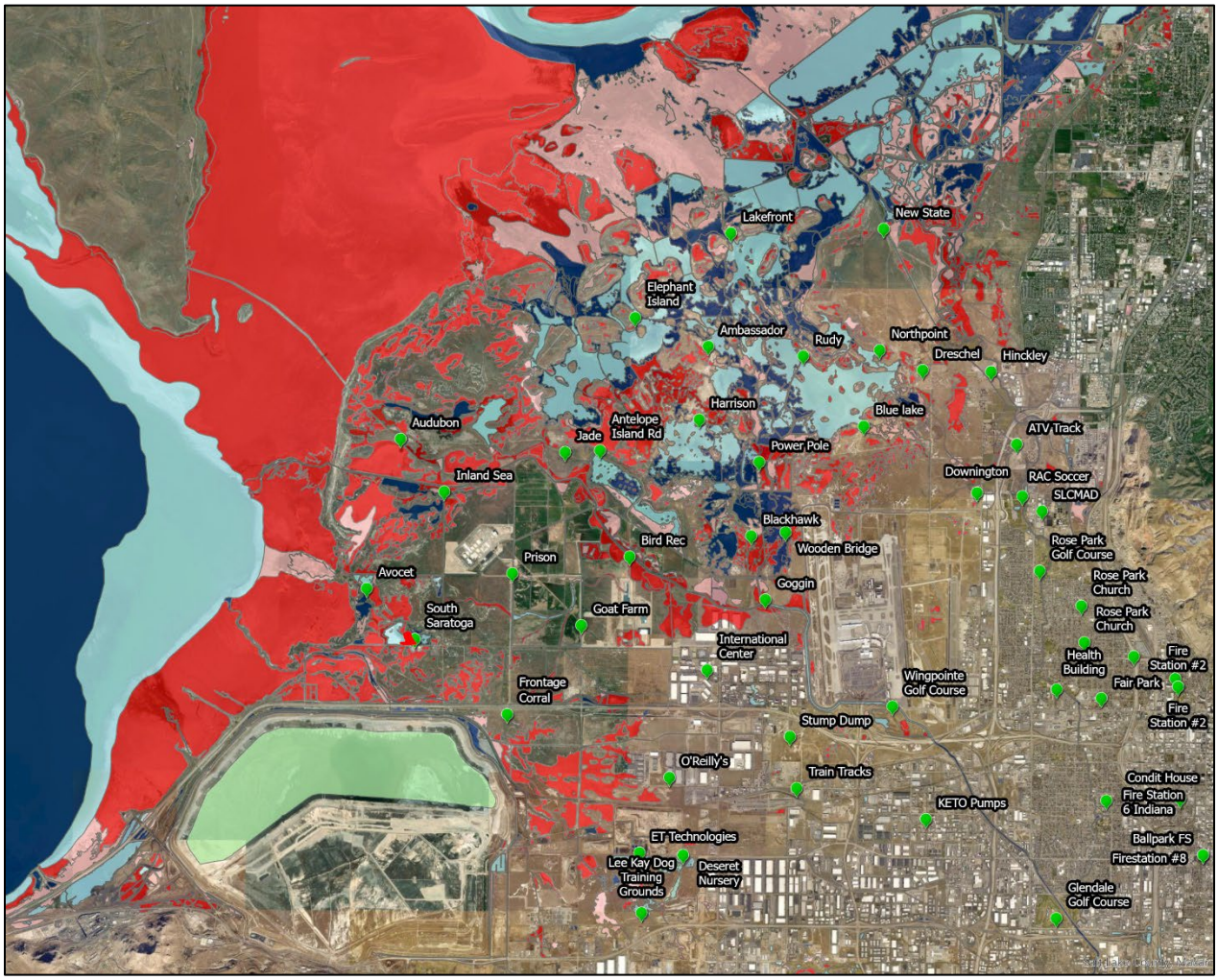
7.1 Bibbs CS, Reissen N, Dewsnup MA, Sorensen RB, Faraji A, White GS. 2024. Do it yourself: 3D-Printed miniature CDC trap for adult mosquito (Diptera: Culicidae) surveillance. PLoS Neglected Tropical Diseases, 18(1): e0011899. DOI: https://doi.org/10.1371/journal.pntd.0011899

7.2 Faraji A, Fairbanks KA, Faraji A, Byers NM, Bibbs CS. 2025. Comparative resilience and precision of digitized optical-counting using ImageJ during routine mosquito (Diptera: Culicidae) sample processing. Journal of Insect Science, 25(2): 6. DOI: https://doi.org/10.1093/jisesa/ieaf026 [Special Collection: Advancement of Methods in Insect Science: From Genes to Ecosystems].

7.3 Mulla MS, Norland RL, Fanara DM, Darwezeh HA, McKean DW. 1971. Control of chironomid midges in recreational lakes. J Econ Entomol, 64: 300–307.

7.4 Rochlin I, White G, Reissen G, Martheswaran NT, Faraji, A. 2022. Effects of aerial adulticiding for mosquito management on nontarget insects: A Bayesian and community ecology approach. Ecosphere, 13: e3896. DOI: https://esajournals.onlinelibrary.wiley.com/doi/full/10.1002/ecs2.3896

8.0 Supplemental Material:



Existing mosquito surveillance network. Dark blue regions are permanent water. Light blue denotes temporary nexus and swells that dry down with seasonal progression. Red indicates flood plans receiving inclement spring snowmelt. Light red/pink areas are drainage watersheds that percolated to the nearby retention areas colored in blue. All trap sites are employed from the beginning of April until the end of October and serviced twice weekly during the operationally engaged mosquito season.