**SALT LAKE CITY MOSQUITO ABATEMENT DISTRICT SITE PROTOCOL**
**on**
Surveillance Trap Quantification and Data Management

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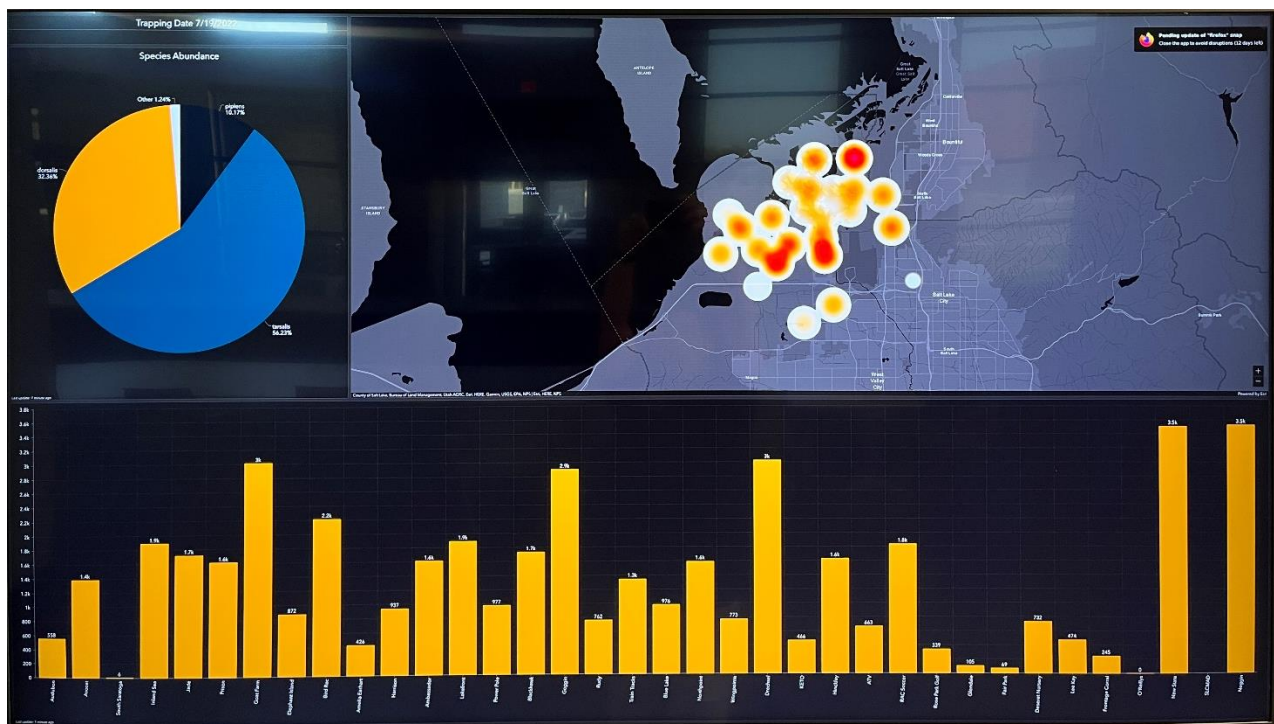Salt Lake City Mosquito Abatement District (SLCMAD)
2215 North 2200 West
Salt Lake City, Utah 84116-1108

**Protocol Inception Date:** 03 June, 2013

**Salt Lake City Mosquito Abatement District, Utah (SLCMAD)**

1.0     Title:
        Surveillance Trap Quantification and Data Management

2.0     Purpose:
        Procedure to estimate the quantity of adult mosquitoes collected in traps deployed and
        recovered for operational surveillance at SLCMAD

3.0     Scope:
        Operational estimation of adult mosquito numbers collected as part of routine trap surveillance
        and subsequent understanding of relative abundance of mosquitoes

4.0     Responsibility:
        4.1     Principally, the SLCMAD Laboratory Director is responsible for ensuring the digital
                handling system for estimating mosquito collection quantity abides SOP# OS-01 and
                implements procedures when remaining caretaker staff are absent.
        4.2     Secondarily, SLCMAD Biologist supervises auxiliary staff and implements digital handling
                procedures in the absence of auxiliary staff.
        4.3     Seasonal employees, selected staff from other programs, and any other associates
                estimating adult mosquito abundance collected from adult trap surveillance without
                specific assignments coordinated with the Laboratory Director or Biologist for whatever
                reason are responsible for implementing the procedures described in this protocol or an
                approved study protocol.

5.0     Test Systems Currently Documented:
        Three processes are employed by SLCMAD for digital handling of mosquito collection data.
        Photography process, ImageJ estimation tool, and Python handler for data summaries are
        included within SOP#: OS-01.

6.0     Preparation:
        **Camera:**
        currently using Canon EOS REBEL T1i, wire connected to computer via USB cable for A/V out
        digital port
        **Lens:**
        18 – 55 mm lens, Auto focus on, Stabilizer on
        **Zoom:**
        ▪   ~34 mm optical zoom (no digital zoom)
        ▪   Make sure not to adjust camera zoom as this will impact the ImageJ macro used to
            estimate mosquito counts
        **Height:** 73.5 cm (29 in) between counter top and tip of lens (cap off)
        **Other:**
        ▪   Additional Fluorescent lighting to improve image capture
        ▪   Computer installed with onboard Canon live-capture software, ImageJ macro ( ), Python
            interface (currently using PyCharm)
        ▪   1 tbsp scoop for sub-sampling mosquitoes
        ▪   Full sized 17 x 23 in sorting pans and 17 x 13 in half pans
        ▪   Removable window screen with standard fitted bug screen

*Figure 1: Canon EOS Rebel T1i with 18-55 mm lens set to ~34 mm optical zoom.*

7.0    Procedures:

    7.1    **Sorting (Fig. 2):**

1. If convenient, apply static guard aerosol to all sorting pans to reduce interference from static.
2. Remove trap collection jar lid and pour out trap contents on window screen.
   - Seat window screen on 17 in x 23 in full sized collection pan.
3. Remove non-mosquitoes by sieving out smaller insects through the mesh and then hand-picking large insects for removal.
4. Once sieved, transfer filtered contents into 17 in x 13 in half pans.
   - Make sure remaining contents are well mixed to make sure sub-sampling is accurate.
   - Make a transfer label of the trap site and label the pan.
   - Rack labeled half pans for inventory with camera.

*Figure 2: Sorting trap contents by sieving with window screen, hand removing large insects, and transferring to half pans for camera imaging.*

7.2     **Camera (Fig. 3):**

Use camera to take pictures of any collections with over 300 mosquitos (~1 tbsp).

1. Turn on light, turn on camera, and remove lens cover.
    a. Program will open automatically on computer (EOS REBEL T1i).
2. Click "Live View Shoot" towards the bottom of the window. This will open a second window showing the camera view.
    a. The camera should focus automatically but you can manually focus by double clicking on the live view image which will open a third window with a zoomed view. Double clicking within the zoomed view will focus the camera.
3. Retrieve labeled half pan and transfer the label to an identification tin.
4. Pour contents into full sized imaging pan.
    a. Spread no more than 900 – 1,000 mosquitos on the tray (~ 3 tbsp.)
        i. Program accuracy drops off and undercounts if more than 1,000 mosquitos are placed on the tray at a time.
        ii. If trap contents exceed 900 – 1,000 mosquitoes, take multiple image captures in sequence until resolving all contents.
    b. Spread mosquitoes around pan to create white space around individuals.
        i. Manually break out dense clumps of mosquitos.
        ii. Avoid mosquitos around the edge of the tray to ensure accuracy.
    c. Ensure tray is centered in the capture window.
        i. No countertop showing and include as much of the tray as possible.
        ii. The outermost edges of the pan will not be captured in the image.
    d. Press the capture button on the REBEL program. This will take and automatically save the image.
    e. Images will save to automatically generated local folders (**trap pickup dates**) found under C:\Users\Lab\Pictures\2022\07\2022_07_21\
        i. Desktop has shortcut (Picture Desktop Shortcut > Year > Month > Date)
    f. Rename the image file with the name of the trap.
        i. If multiple pictures are being taken of the same trap contents, number the pictures consecutively (Avocet_1, Avocet_2, etc.)
5. **Image capture does not identify mosquitoes; it only quantifies.**
6. For species composition (see SOP#: OS-03), take a sub-sample of the trap by transferring 1 leveled/unpacked tbsp into the previously labeled identification tin.
    a. Tin is lined with white, pleated, paper muffin sleeves to prevent sticking to the can.
    b. Mark the transfer label with red sharpie to indicate counting is completed (usually a red P).
    c. Only one sub-sample needs to be taken per trap site (usually first pan) regardless of number of pictures.
7. If taking long series of pictures, the camera may overheat. If this occurs, turn off the **camera and light** for 5 minutes before resuming.

*Figure 3: Top left) Light and pan setup; Top Right) Spreading ~900 mosquitoes for counting; Bottom Left) Sub-sampling into muffin-paper lined candle tin after imaging completed; Bottom Right) Labeled tin.*

7.3     **ImageJ (Fig. 4):**

1. Once all pictures have been taken the ImageJ macro can be run to estimate the number of mosquitos in each picture.
2. Open ImageJ (Program pinned to taskbar).
3. Go to Process > Batch > Macro.
   - The default is the correct macro.
   - For the input put the dates picture folder C:\Users\Lab\Pictures\2022\07\2022_07_21\
4. Press "Process."
   - A new window will open containing several columns of numbers.
   - **Image totals will be organized alphabetically by site name.**
   - The "Count" column should be used and contains the estimated number of mosquitos.
5. Each image processes separately.
6. If taking multiple pictures of the same trap collection, the count will need to be manually summed across all consecutively labeled image totals.
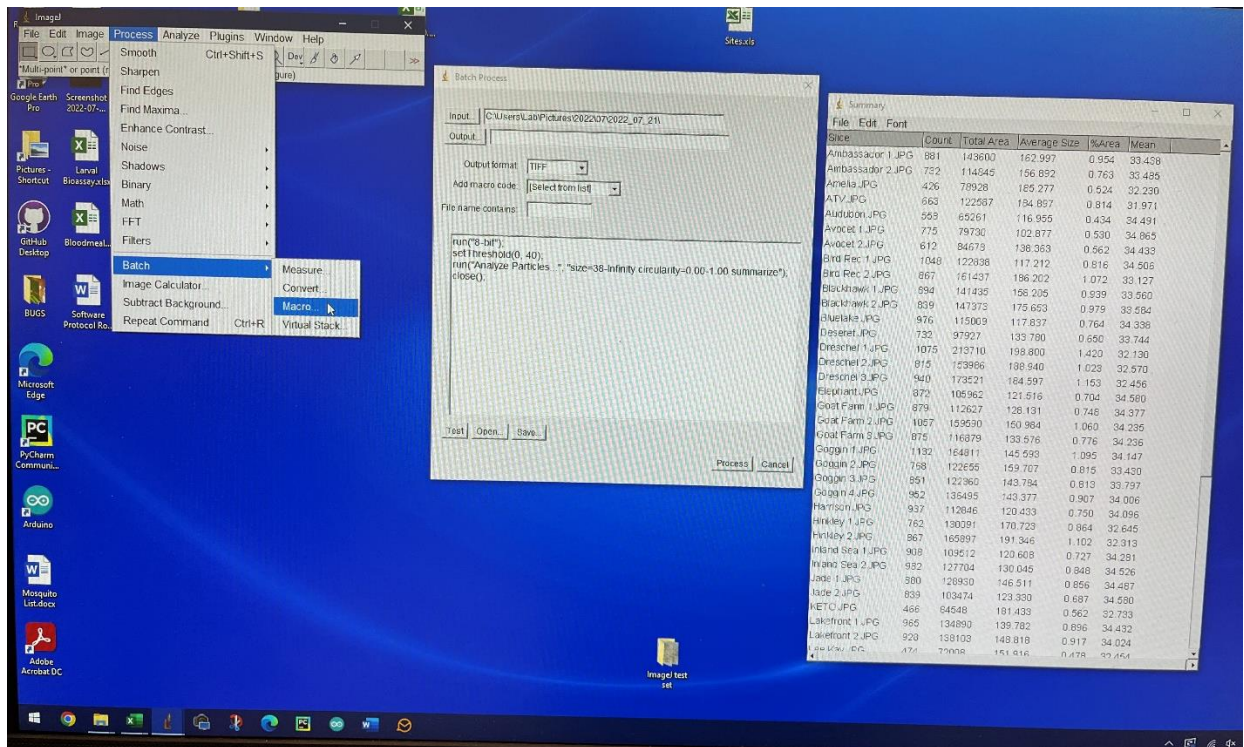


*Figure 4: ImageJ interface on the desktop showing navigation for macro (left), default macro window (middle) with file navigation, and outputs with count data (right).*

7.4 **Excel Data Entry (Fig. 5):**

1. On the drive, navigate to Share (\\SLCMAD) (S:) > Lab > Vector Surveillance > YYYY and verify the Excel sheet titled "Mosquito Count Graphs YYYY.xlsx" where YYYY is the current year.
2. On the drive, navigate to Share (\\SLCMAD) (S:) > Mansquito and verify the excel sheets used for labels in Python titled "aerial_blocks.csv," "sprays.csv," "weather.csv," and "trap_sites.csv."
   a. "trap_sites.csv" is critical and contains a list of all trap sites (currently ordered by Lat/Long).
3. The lists on these two sheets must correspond in number of traps, names of traps, and order of the traps. When adding or removing a trap location the alterations must be made in both excel sheets.
4. Python pulls the numeric data for scripted programs from "Mosquito Count Graphs YYYY.xlsx"
   a. File needs to be created annually with identical format (copy prior year file and rename it for current year).
   b. When inputting data into the Excel sheet the "date" column should be filled with the **date the traps were placed in the field**.
   c. The "location" column should contain the trap sites in the order displayed in "trap_sites.csv." Copy/paste the locations from the prior day or from "trap_sites.csv."
   d. The "total" column should be filled with the estimates from the ImageJ macro or hand count totals when ImageJ not used.
      i. This will be the column used when generating overall abundance graphs. **This must be a direct numerical value**; creating a formula (Ex: SUM) will break the functions in Python.
      ii. The organization between ImageJ (alphabetical) and the excel sheet (Lat/Long) will not match. Always verify correct site when you transfer count totals into excel.
   e. The "problems" column can be used to document trap anomalies.
      i. Entering "not set" in the column adds "not set" on the graph.
      ii. Entering any other value will add "trap fail" to the graph.
   f. Once hand counting for species composition (see SOP#: OS-02) is completed, the species totals can be input under the corresponding columns.
5. If a new site is added or order is changed, you must make additions/subtractions/order changes retroactive for prior trap dates of data if you want to compile a prior day's graphs, **in addition to updating "trap_sites.csv"** otherwise it breaks the Python script.
6. The "Gravid" tab has an additional column for the *Culex sp.* totals that will be used to generate the graphs.
7. If adding indicators (via python) for operational treatments, open "spray.csv" and add the trap date, block treated (aerial shape file), and spray date.
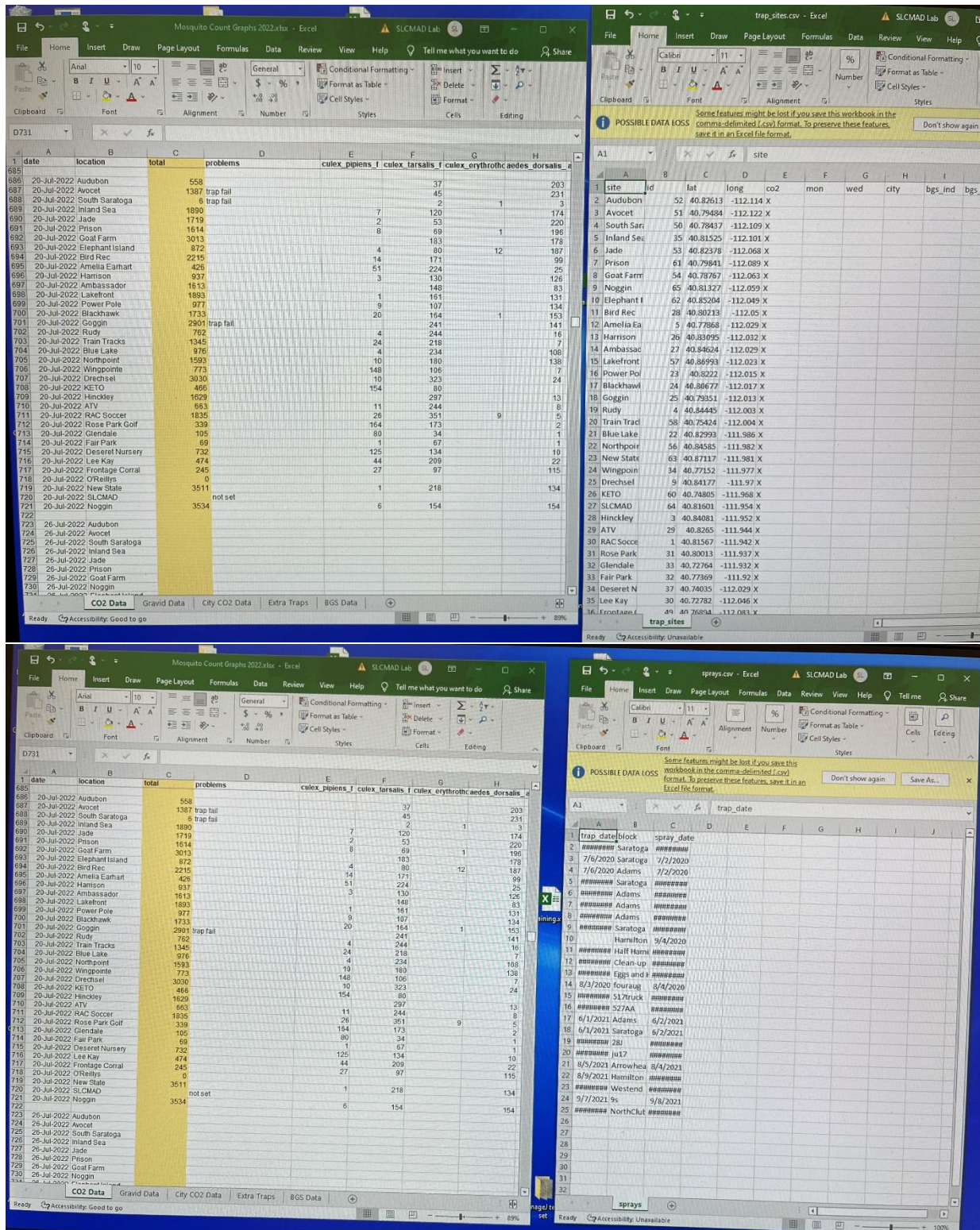
*Figure 5: (Top) Excel master sheet "Mosquito Count Graphs YYYY.xlsx" on the desktop showing ordinal correspondence with Python source file "trap_sites.csv." (Bottom) Spray arrow spreadsheet for documenting treatments corresponding with surveillance dates.*

7.5     **Mansquito Data Handler and PyCharm (Fig. 6):**
        For detailed breakdown of functions and constraints of the Python script, see Appendix.
        PyCharm is an open-source community edition software. Use this interface to generate
        total abundance graphs and convert data into a form compatible with VectorSurv.

        **Graphs:**
1.  Open PyCharm (pinned to taskbar).
    a.  Run > Run Data Handler.
2.  Enter current year when asked.
3.  Press "0" to create a graph.
4.  Choose type of graph to create. We no longer make graphs of City $CO_2$ or BGS traps.
    a.  **Run "Preliminary Graph" when all ImageJ counts have been made, but
        before species ID** (and subsequent totaling) has been performed (same day
        that traps are collected on both trap days).
        i.  If running a preliminary graph, always answer "n" to drawing spray
            arrows and two-day graph.
    b.  Otherwise, enter "n" to preliminary graph when all species identifications
        have been completed and the excel sheet is completed for the target date.
        i.  Spray arrows can be added if needed by editing "spray.csv."
        ii. If reporting on the first trap night of the week, answer "n" to two-
            day graph.
    c.  Email graph to all full time SLCMAD supervisors (Director, Assistant Director,
        Laboratory Director, Biologist, Education Specialist, Urban Supervisor, Rural
        Supervisor, Operations Manager, IT/GIS Specialist)
    d.  If reporting on the second trap collections of the week, answer "y" to two-
        day graph to create a side-by-side graph output.
5.  For all graph types, enter date(s) you want to graph. **Use date traps were set** on.
6.  Answer n to adding a note.
7.  Answer y to saving a pdf copy.
    a.  This will automatically save the graph in the correct folder in ([\\SLCMAD](\\SLCMAD))
        (S:) > Lab > Vector Surveillance > YYYY > Weekly Graph PDFs with the
        current date.
        i.   Files will mention preliminary or final graph.
        ii.  Folders for this need to be made every year or the script will crash.
        iii. Only make folders for regular $CO_2$ graphs; omit gravid and City $CO_2$).
8.  Gravid Graphs will span three-week intervals showing either the first trap night of
    the week (typically Monday route) or the second trap night of the week (typically
    Wednesday route).
    a.  When asked, enter "m" to graph the Monday route and "w" to graph the
        Wednesday route. **Use the corresponding 3 trap setdates depending on
        which route** you are graphing that day. Gravids are updated each trap day.
    b.  This creates a separate graph for each.
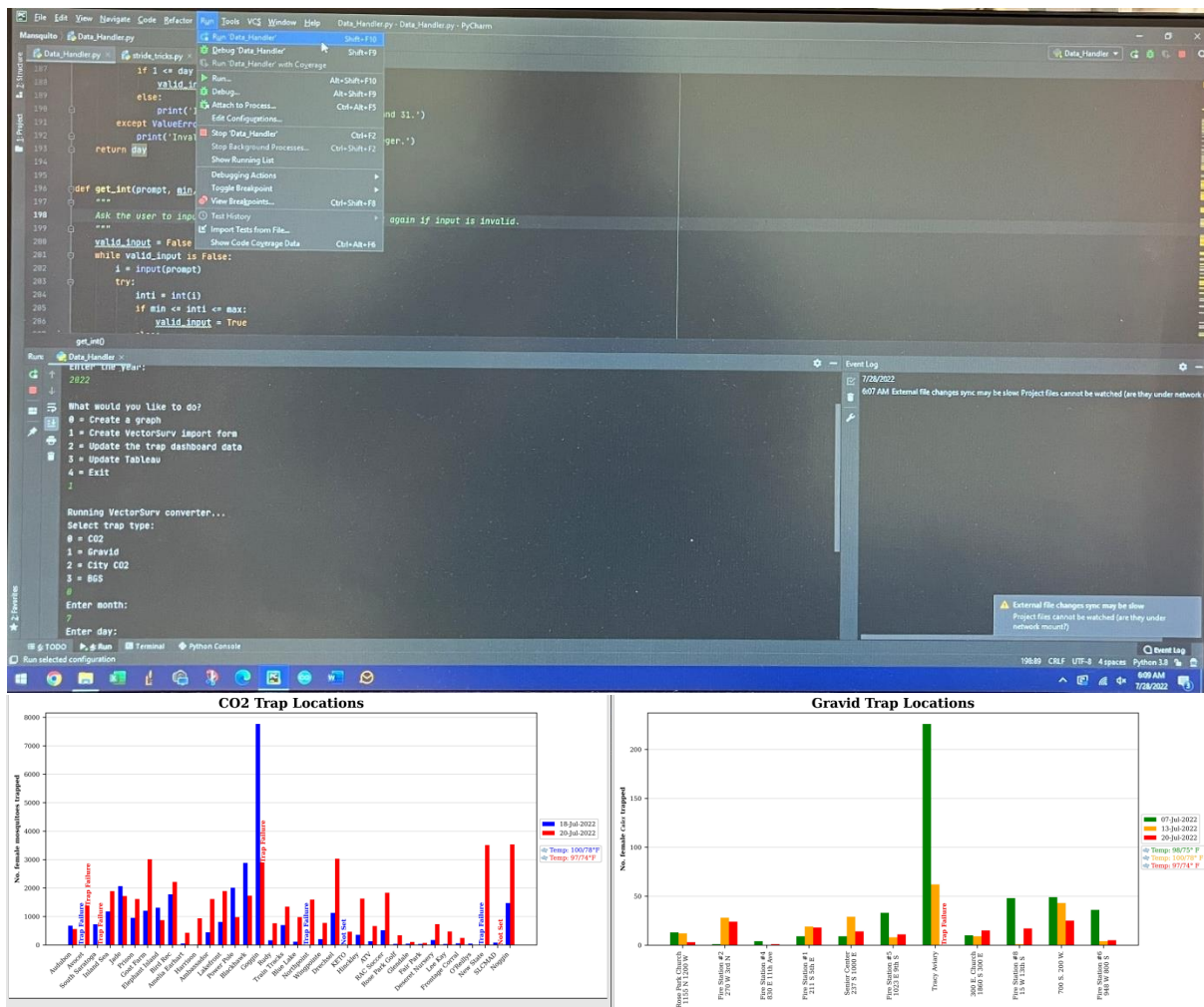    c.  Trap locations are different between the two routes.

*Figure 6: (Top) PyCharm interface with path for running data handler and entry prompts in lower window. (Bottom) Graph outputs generated by Mansquito Data Handler.*

## Custom Spray Arrows (Fig. 7):

- If adding spray arrows to any two-day graph report (described in Section 7.6), "aerial_blocks.csv" **AND** "spray.csv" needs to be updated for a requested spray mission.
- If referencing an existing block, open "spray.csv" and go to the bottom of the existing list.
    - Column 1 is the trap date you want the spray arrow to be on.
    - Block is the block written in "aerial_blocks.csv."
    - Spray date is the date the spray was completed.
- If referencing a custom spray block, open "aerial_blocks.csv" and go across the columns (rightwards) and find the next open column.
    - Add a name for the custom spray block (for example, the name of the experimental product, or the name of the project, like "DuetHD").
    - Underneath, list each trap location affected by the spray block, with each trap location in a new cell.
        - Make sure trap locations are the same order as "trap_sites.csv."
- Then run a normal graph report through Python and select the appropriate spray block name.
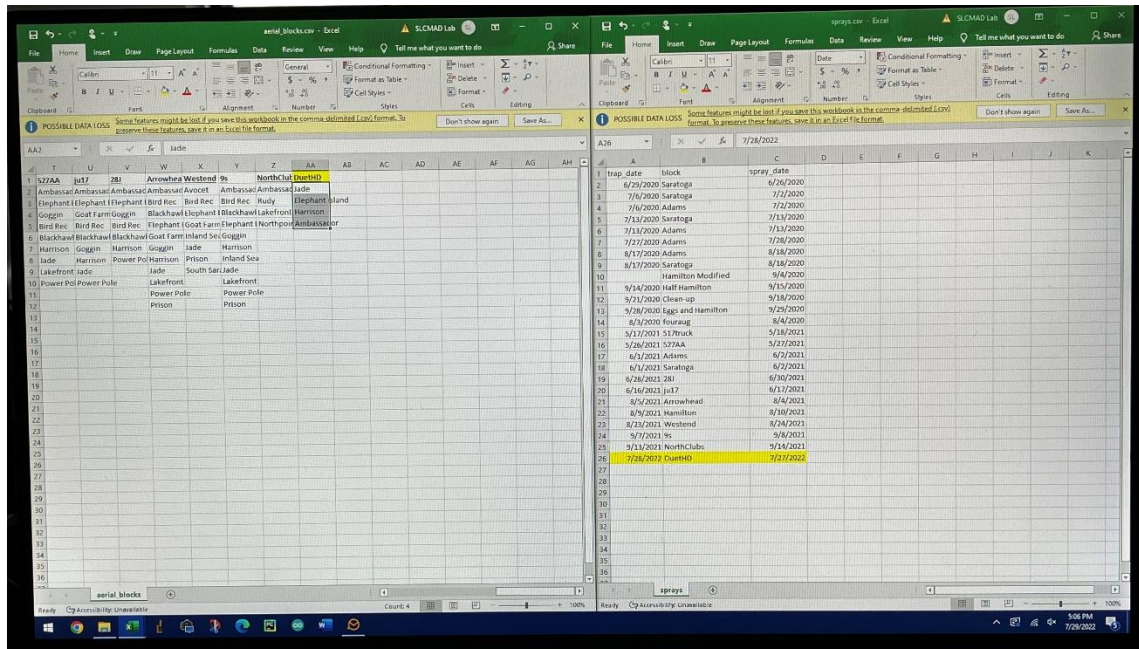
*Figure 7: (Left) "aerial_block.csv" where new blocks and affected trap locations are added to the right-most column of the series; (Right) "spray.csv" where a specific spray mission used to populate arrows on the graph report is entered into the next available row on the bottom.*

**VectorSurv Exports (Fig. 8):**

This process will upload to the California managed database on mosquito surveillance data.

1. Open PyCharm (pinned to taskbar).
    a. Run > Run Data Handler.
2. Enter current year when asked.
3. Press "0" to create a graph.
4. Press 1 to create VectorSurv form.
5. Create VectorSurv forms for $CO_2$, City $CO_2$, and Gravid traps. **Enter date traps were** **set**.
    a. This will automatically save the form in ([\\SLCMAD](\\SLCMAD)) (S:) > Lab > Vector Surveillance > YYYY > VectorSurv Import Forms.
6. Open google and go to vectorsurv.org.
    a. Click gateway and log in.
    b. Go to Tools > Data Tools > Data Import.
    c. For import type select Arthropod collections – spreadsheet.
    d. Choose the VectorSurv form you wish to upload and press import.
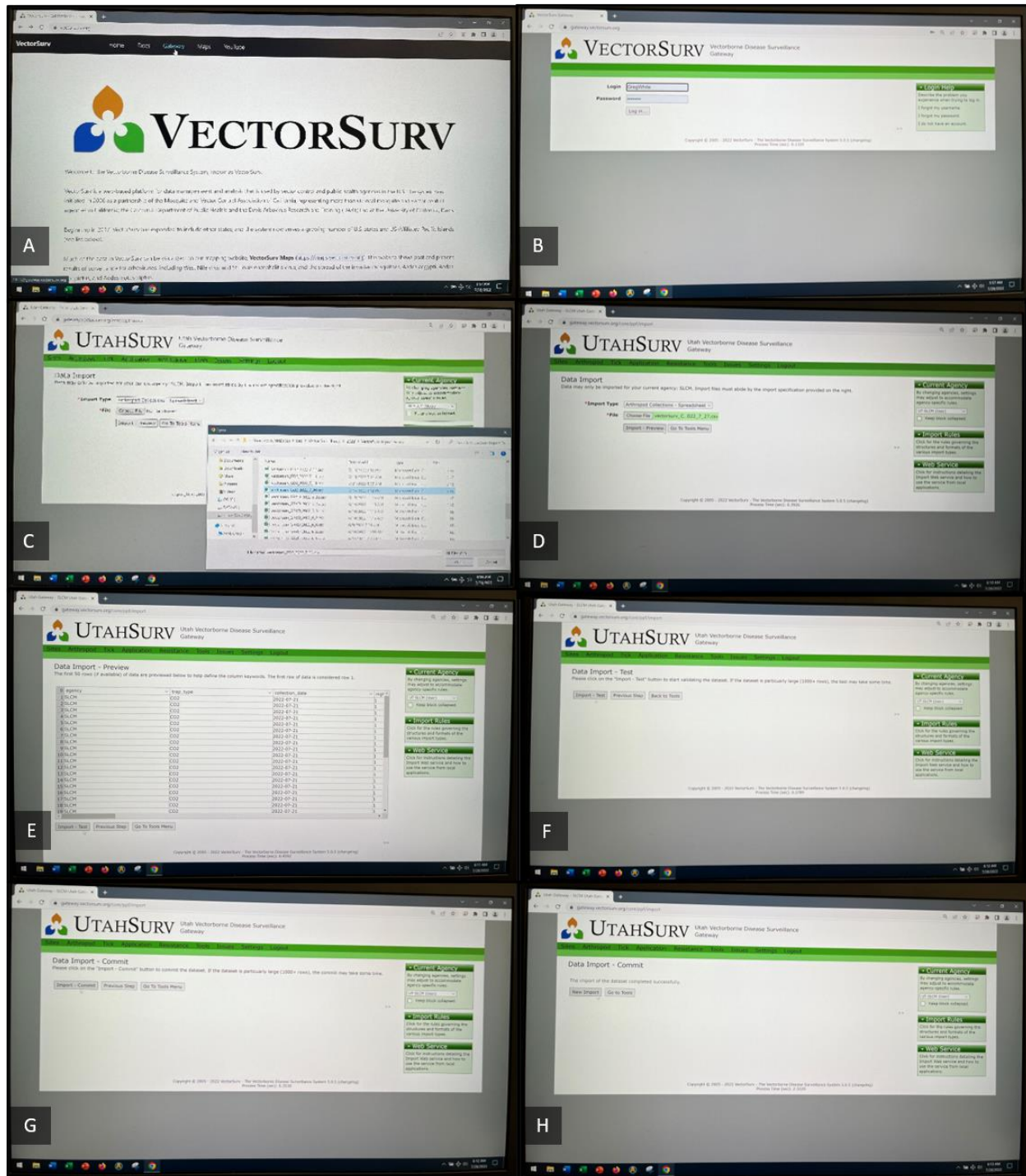7. Advance through Import – Preview/Test/Commit to verify and confirm the import.

*Figure \*: A) VectorSurv homepage; B) Login (should populate automatically); C) Tools > Data Import > Arthropod Collections – Spreadsheet; D) Import – Preview; E) Verify tables and then Import – Test; F) Tells you if any errors were detected, then Import – Test; G) Import – Commit to upload sheet; H) Landing page once import is successful.*

**ArcGIS Pro (Fig. 9, Fig. 10):**

Data entry is used to update the dashboard information on trap numbers and larval ID.

1. Software is installed on lab laptop (not the desktop).
2. Open ArcGIS Pro and select lab data entry.
3. This will open a project with four tabs.
4. Two sets of data need to be managed on the dashboard.
5. The tab labeled positive inspection sites is where larval data needs to be entered.
   a. Only rural samples are entered here.
   b. The tab labeled positive inspection sites is where larval data needs to be entered.
   c. Find the inspection site ID that matches the larval sample and use the drop-down menu to select up to three species that were found there.
      i. Duplicate inspection site IDs seem to be a common problem.
      ii. For duplicate sites, put the larval data under each duplicate.
6. To update the adult data and completed the dashboard, go to the tab labeled $CO_2$ Data\$.
   a. If $CO_2$ Data\$ does not appear, it means that either Python or another desktop is currently using the file.
      i. Close all PyCharm processes and excel openings on other computers and relaunch ArcGIS Pro.
   b. Highlight the data for the newest trap dates.
   c. Under tools, click on "append."
   d. For input, choose the $CO_2$ trap data.
   e. For output choose trap dashboard.
7. At the bottom, use the drop-down menu for "Field Matching Type" to select "use field map to reconcile differences."
   a. Check there are no 0's next to species names then press Run.
8. Go to the trap dashboard tab, select and delete old data so only the current date remains.
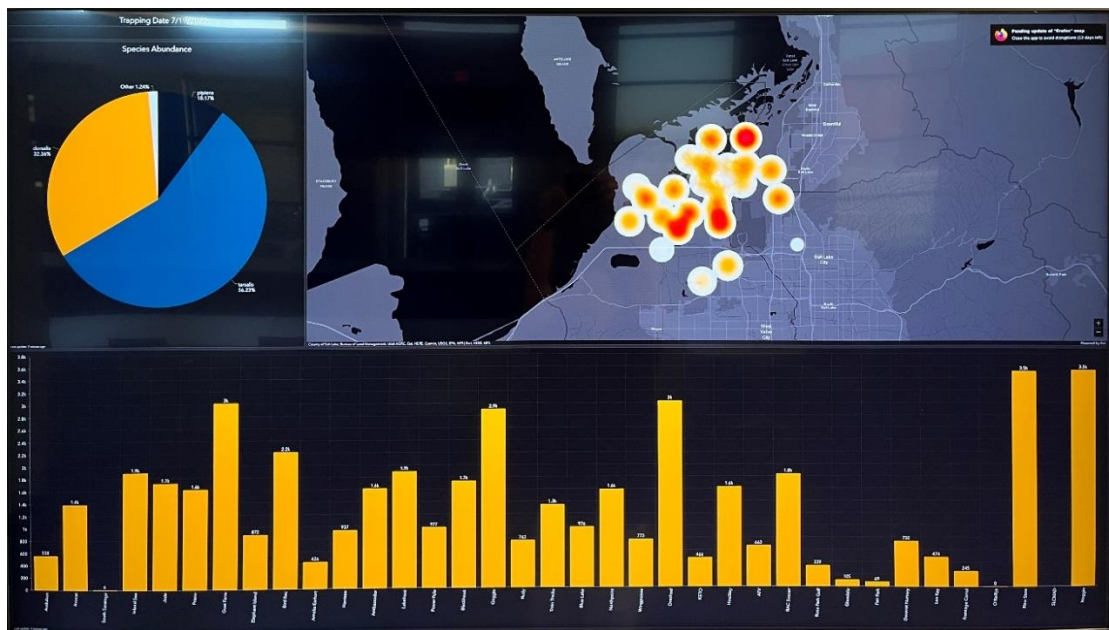   a. Edit > save



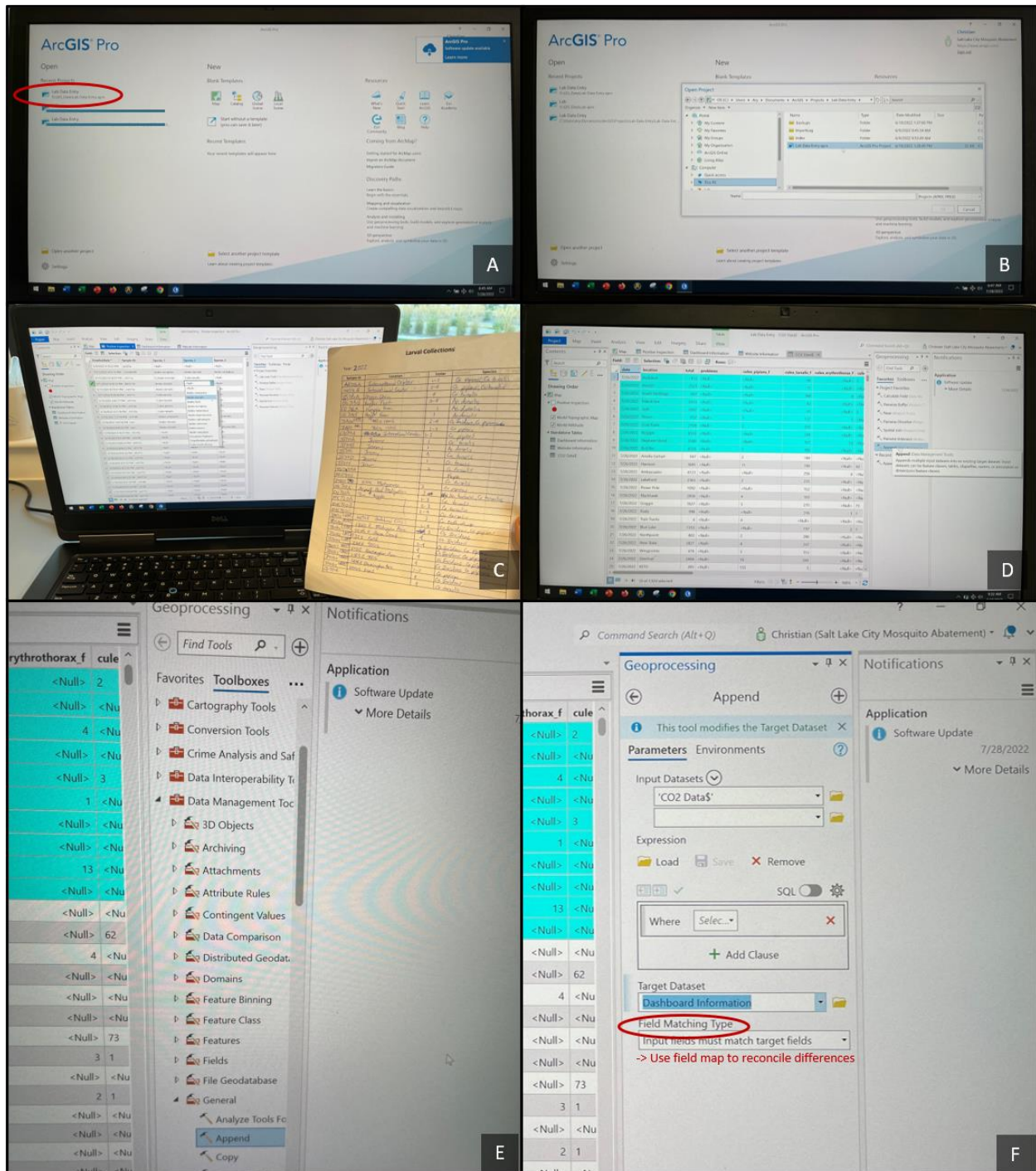*Figure 9:  ArcGIS Pro dashboard; main training room*

*Figure 10: A) ArcGIS Pro startup, Lab Data Entry can be selected from recent projects; B) Or you can navigate the file path to reach the same project); C) Larval data entry under Positive Inspection tab; D) Adult mosquito count update on CO2 Data using Append tool, in the favorites/recent in Geoprocessing E) Or you can navigate Toolboxes > Data Management Tools > General > Append; F) Select CO2 Data for input and target dataset as Dashboard information, then run the exchange before deleting old data in Dashboard Information and then Edit > Save.*

8.0      <u>Troubleshooting Comments</u>

**Sorting:** Some insects are too large to filter with the window screen but are not apparent when hand removing large insects, notable mayflies and midges. There is no procedural correction for this, it is just an inconvenience during hand identification of mosquitoes.

**Python:** If a crash/error message occurs while trying to find the weather, make sure weather.csv file is closed; try updating chromedriver.exe; check to ensure WeatherUnderground has not changed their format; double check internet connectivity. Other crashes are likely due to missing files or incorrectly formatted sheets.

Ensure files are saved in correct spots with correct names. Ensure data is entered correctly in excel sheets. Ensure files are closed when finished entering data or it will lock the files from other access pathways.

If a trap site was removed or added to the excel sheets the python code will crash when trying to generate a graph of a day before the change occurred. This can be fixed by retroactively adding/removing missing/extra traps for the target day.

In the graph formatting, the species folder is not read by the Mansquito Data Handler. The files are manually saved from Tableau.

When saving PDF outputs of graphs, it will be named for the latest date in the excel data. However, any graphs saved will automatically overwrite old versions of the graph if they share the same output date.

9.0      <u>Support Hierarchy</u>:

For items not addressed in SOP#: OS-02, contact the SLCMAD Biologist, Laboratory Director, IT/GIS Specialist, Education Specialist, Assistant Director, or Executive Director (in this order of escalation).

10.0    Appendix – Supporting Technical Write-Up:
        Mansquito is a python data handling script written by former SLCMAD biologist Christian
        Weinrich.

        **Mansquito Data Handler**
        This script is used to create graphs and reformat mosquito count data generated by the Salt
        Lake City Mosquito Abatement District Surveillance Program. The primary function of this
        program is using count data to create bar graphs showing total abundance per trap site.
        Additionally, the program is able to convert trap data from its current format into differently
        formatted Excel files which can then be used to upload data to VectorSurv, Tableau, or the
        ArcGIS dashboard.

        As of writing this document, the script and all requisite files are located on the district's server at
        S:\Lab\Mansquito\

        **Required files**
        **Mosquito Count Graphs YYYY.xlsx**
        This is file where count data is stored. The file should be saved as an .xlsx and the 'YYYY' in the
        file name should be replaced with the year. Rules about how the file must be formatted are
        listed below.

        The file contains 4 worksheets: CO2 Data, Gravid Data, City CO2 Data, and BGS Data. The file
        may contain other worksheets, but the script will ignore them.

        For each worksheet, the columns begin with 'date', 'location', 'total', and 'problems'.

        Following these first four columns, a series of mosquito species are listed in the format,
        'genus_species_f' (or 'genus_species_m' for males). If adding a new species, add it among the
        other columns prior to the final column, labeled 'comments'.

        The Gravid Data worksheet has a unique column, 'total_culex_f', which follows the 'comments'
        column.

        The 'comments' column (or 'total_culex_f') is the final column that is read by this script,
        although other information can be stored for human use in subsequent columns.

        - The 'date' column is automatically formatted by Excel to mm/dd/yyyy. Location names must
          be in order and spelled correctly.
        - Site names in the 'location column' must match the order and spelling of sites names for
          that route as they appear in the 'trap_sites.csv' described below
        - 'total' is the total estimated by ImageJ or the hand count for traps <300 mosquitoes.
        - The 'problems' column is checked for text. If the text says 'not set' then the trap will be
          marked as not set on the graph. Any other text will cause the trap to be marked as a trap
          failure.
        - The species columns contain the number of that species within the subsample, not the
          entire trap. Therefore, the total column will usually read much higher than if all the species
          columns were summed.

- The 'comments' column can be used to override the 'problems' column by starting the entry with 'text:'. This is useful if you want to specify on the graph that a trap was a partial failure, was set on a different day, etc.

**trap_sites.csv**
This file contains all the information about each trap site. The file contains 11 columns.

The first four columns are 'site', 'id' (for VectorSurv), 'lat', and 'long'. These columns are self-explanatory. The order and spelling of site names in 'Mosquito Count Graphs' must match this file. Latitude and longitude should be given in decimal degrees.

The next six columns are 'co2', 'mon', 'wed', 'city', 'bgs_ind', and 'bgs_city'. These columns are used to mark which routes include that site as a trap location. If a site is to be included on a route, simply mark the column corresponding to that route with a capital 'X'.

The final column is an address column. This column is optional and is used to add the address to the x-axis label on the graphs for that site.

**chromedriver.exe**
ChromeDriver is used to access the internet for weather scrapes from Weather Underground. It may need to be periodically updated. To do so, go to https://chromedriver.chromium.org/downloads and download the driver version which matches the current version of Chrome. (To check the version of Chrome, go to Settings, then select About Chrome from the sidebar.) After downloading, unzip the file and place it in the same folder as the script. Unfortunately, this function will break if Weather Underground changes the formatting of their web pages.

**weather.csv**
Weather data is scraped from Weather Underground and saved in a .csv file called 'weather.csv'. The file contains 'date', 'high', 'low', 'precip', and 'wind' columns. If the webscraper ever breaks, this file can be updated by hand. Be sure to save as a .csv file.

- 'date' will be automatically formatted by Excel.
- 'high' and 'low' are the temperatures in F.
- 'precip' and 'wind' hold either a 0 or 1 indicating whether or not it was rainy or windy
- respectively.

**weather icons**
To render weather icons on the graphs, a 'wind.png' and 'rain.png' must be in the script directory. 'wind.png' is 15x11 and 'rain.png' is 10x12. These dimensions and file names must be conserved if changing to a new image for the icons.

**aerial_blocks.csv**
This file holds the name of a spray block and the sites that are within that spray block. The top row lists the name of the spray block. The subsequent rows list the sites included in that block. Spelling of site names must match this script.

**sprays.csv**
This file contains the record of actual sprays. The first column 'trap_date' is the date for which you would like the spray arrows to be drawn on (e.g. if the spray happened on 6/3/2020, the arrows would be drawn onto the traps set the next night on 6/4/2020). The next column, 'block', is the name of the block that was sprayed. This must correspond to a block in the aerial_sprays.csv. The last column is 'spray_date'. This column is only for human reference to know what date the spray occured (as opposed to the date of the next trap night).

## Graphs
### $CO_2$
$CO_2$ graphs are bar graphs which display the total species abundance per trap site. Four graphs are created each week, two preliminary graphs and two final graphs. The preliminary graphs only show trap sites where the total count exceeded 300 mosquitoes, the threshold for treatment. The final graph shows all trap sites. The first prelim/final graph pair shows the data gathered on Monday night. The second pair of graphs shows the data for Monday night and Wednesday night side by side. However, this script will also support side-by-side comparisons of any two nights from the same year.

In addition to count data, the $CO_2$ graphs also display a legend showing the temperature for the trap nights and small icons indicating whether it was windy or rainy. If an aerial spray was performed, arrows indicating which sites were sprayed may also be rendered on the graph. Weather data is scraped from web, and aerial spray data is saved in a pair of .csv files (aerial_blocks.csv and sprays.csv, described above).

### Gravid
Gravid trap graphs show total *Culex* abundance per trap site. Each graph shows data for each site for the last three trapping dates. (As with the CO2 graphs, the script can graph any 3 nights from that year.) There are two separate gravid routes, one set on Monday and one set on Wednesday. Weather data is collected and displayed by the same function as the $CO_2$ graphs.

### City $CO_2$ and BGS
City $CO_2$ and BGS graphs are stacked bar graphs showing total abundance as well as species composition. This function is still unpolished and is not often utilized since the information is redundant to the Tableau maps. Previously, the maps could only be made after data was uploaded to VectorSurv and then accessed from the VectorSurv server via Tableau, creating a day long delay since VectorSurv data is only pushed to the server every 24 hours. The stacked graphs were used to present the data in the interim. However, since data can now be passed directly to Tableau, these graphs are now unnecessary most of the time.

## Data Formatting Functions
The other three major functions, andrive(), vecsurv(), and tableau() simply take the data and save it in a different arrangement as a .csv file. For example, the andrive function adds GPS coordinates for each trap site so that the species data can be displayed geographically on the ArcGIS dashboard in the conference room. The specific transformations are documented within each of the functions.