Visual ANalysis of timE SerieS dAta - *Drosophila* Activity Monitors (VANESSA-DAM) is a collection of useful tools to visualize and analyze Time series data obtained from *Drosophila* Activity Monitors (https://www.trikinetics.com/). The first in the series of tools is a shiny app for sleep analysis and visualization - VANESSA-DAM for sleep analysis (VANESSA-DAM-SA). For any suggestions, questions, troubleshooting or customization, please contact arijitghosh2009@gmail.com.

VANESSA-DAM-SA

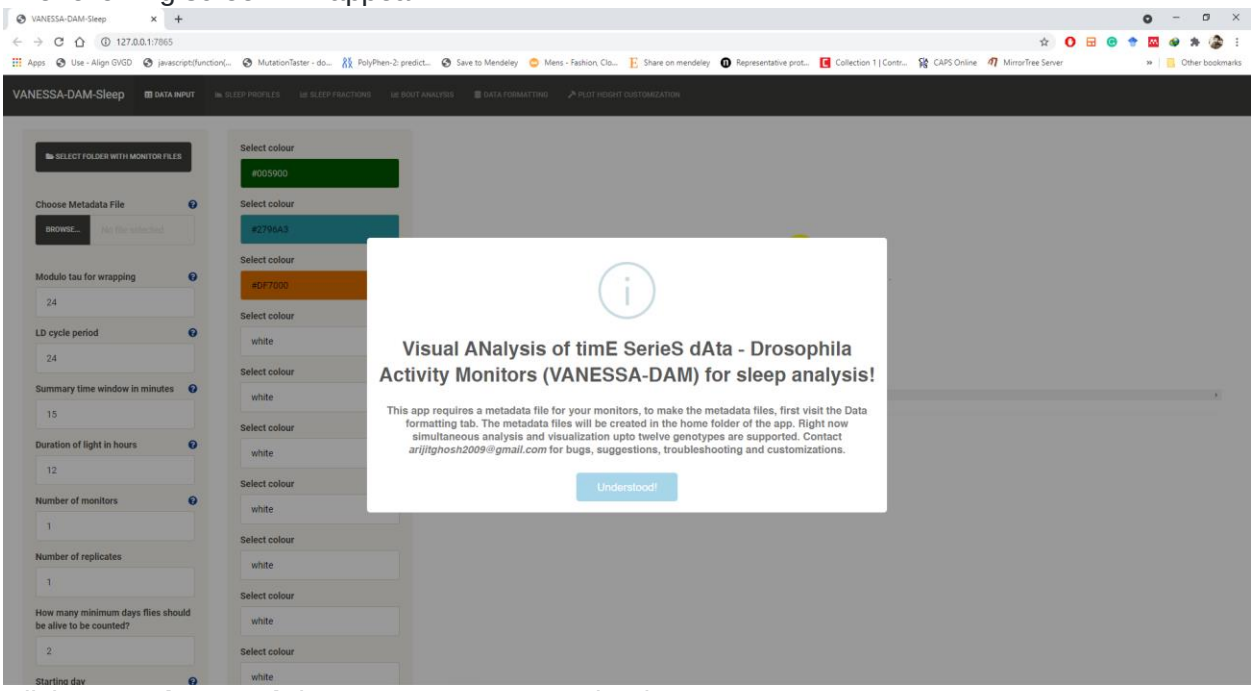
VANESSA-DAM-SA is dependent on Quentin Geissmann's rethomics family of packages - behavr, damr, ggetho, sleepr, for some analysis and visualization options. It offers several advantages over existing tools for sleep analysis from DAM systems, some mentionable ones are -

1. Analysis and visualization of multiple monitor files, genotypes, replicates together in a high-throughput manner.
2. Creating metadata files for information about experiment and better reproducibility.
3. Producing high-resolution publication-quality figures with a plethora of customization.
4. Data curation - automatic user-defined parameter-based removal of dead and arrhythmic individuals.
5. Sleep profile analysis, various sleep parameter estimation and quantification, bout analysis, latency analysis.
6. Visual comparison among genotypes, replicates.
7. Dynamic plot resizing and recoloring.
8. Reproducible code report so that you can generate the figures and analysis without the shiny app from RStudio directly.
9. Minimizing human errors – no need to tinker with raw data to accommodate analysis tools.

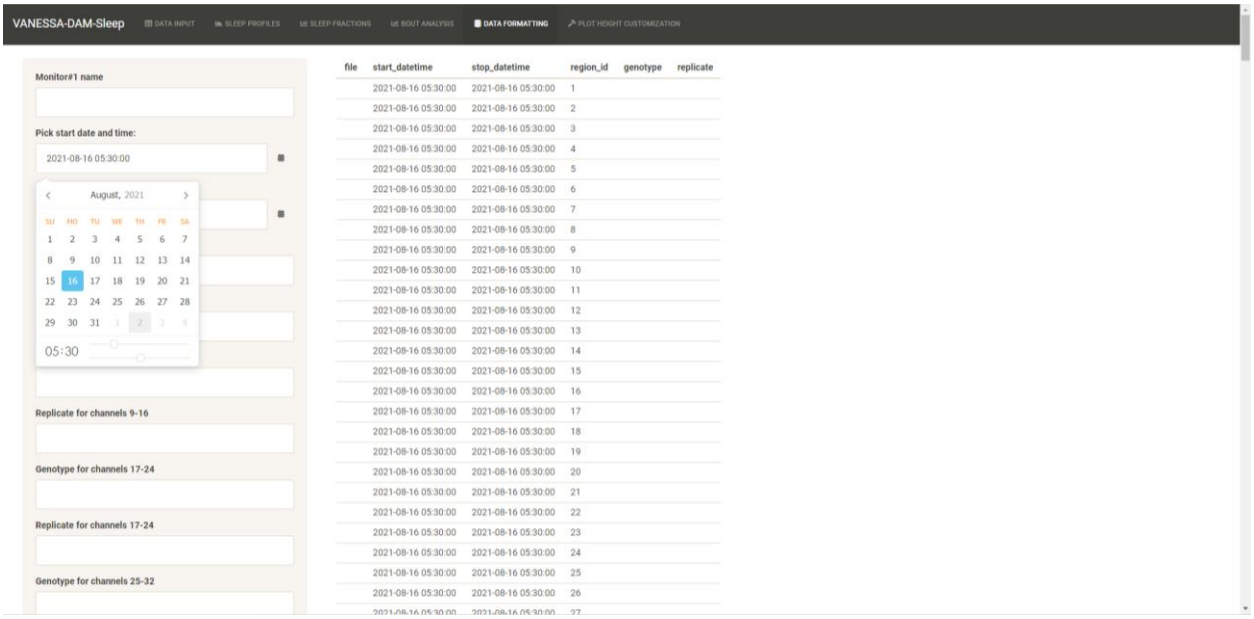
This short tutorial provided (*Easy tutorial to start using VANESSA-DAM-SA.pdf*) here should be self-explanatory and should help beginners start using the app right away.

Easy tutorial to start using the shiny wrapper for sleep analysis

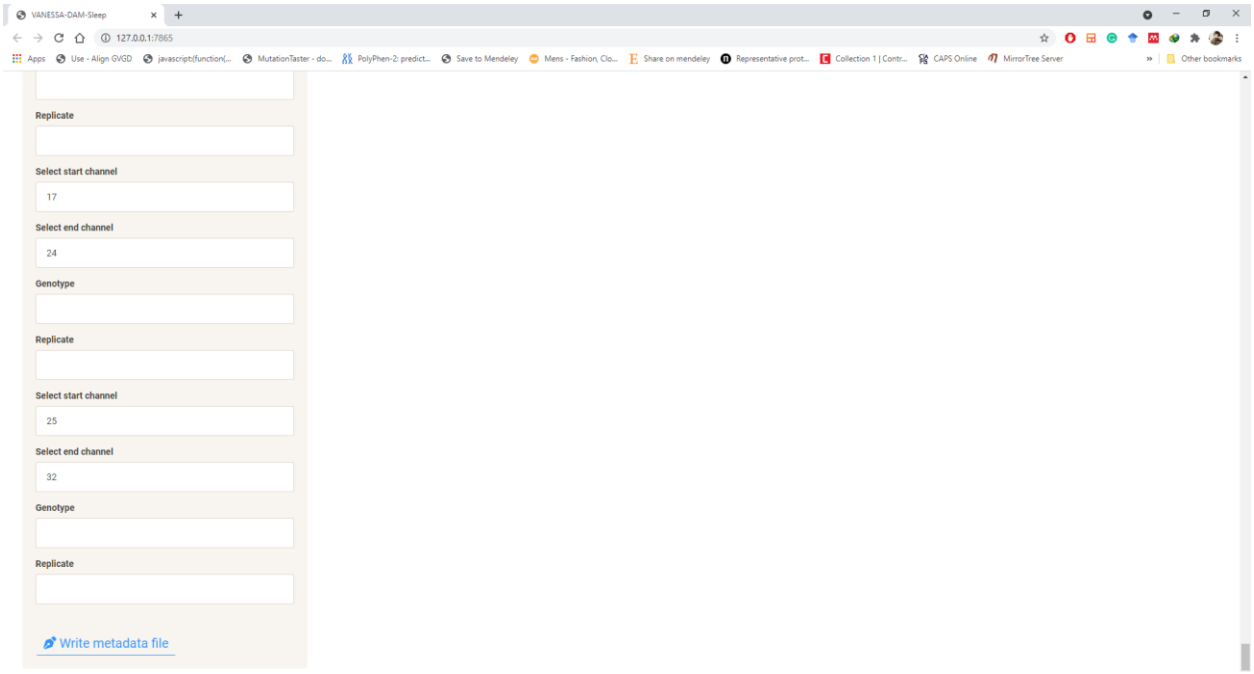
1. Install R (version ≥ 3.6.3) and RStudio (version ≥ 1.2) on your computer.
2. Download the zipped folder containing the app and unzip to a folder.
3. Open RStudio and Run the **startup.r** file to install all necessary packages to run the app.
   Note: If you have trouble installing the *damr* package, the source files are provided. Please go to RStudio > Tools > Install packages > Install from : Package Archive file and select the supplied *damr_0.3.7.tar.gz* file to install the package.
4. Set working directory as the home folder of the app (by pressing **ctrl+shift+h** OR by using the **setwd()** command) and then run the app by typing **shiny::runApp(launch.browser = T)** in the console.
5. The following screen will appear:



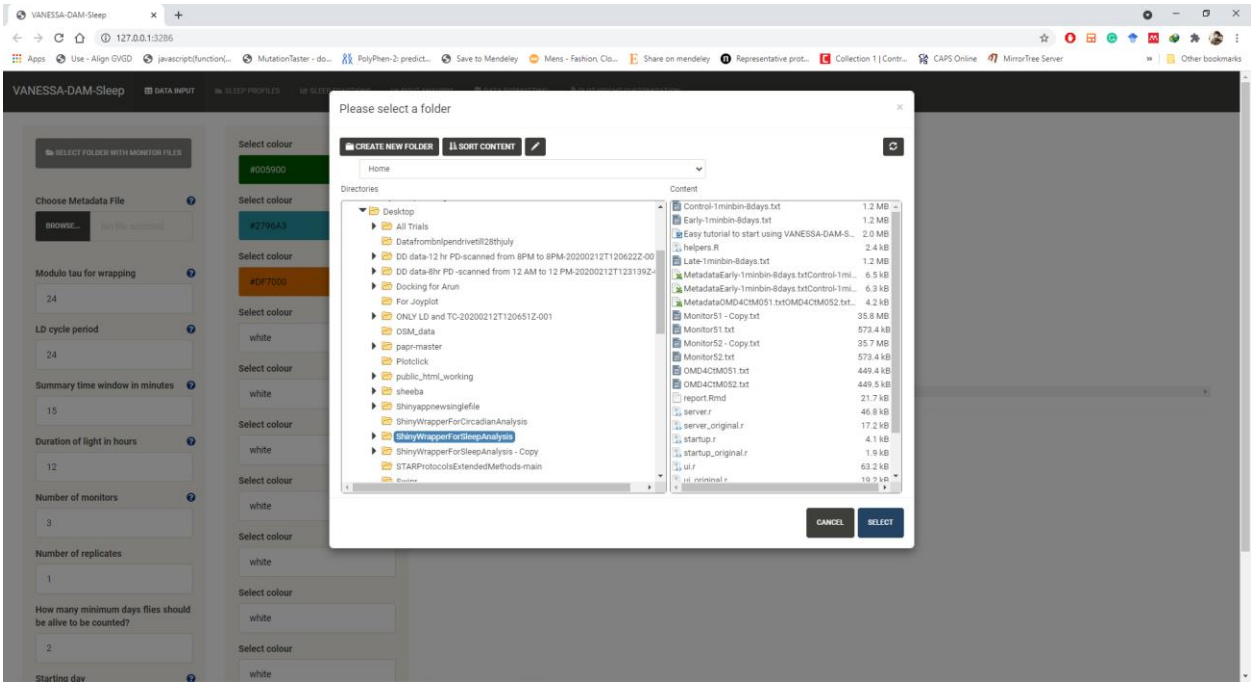
6. Click on **Understood!** button or press **Esc** to begin.
7. Assuming you have your Monitor files (*Monitor1.txt, Monitor2.txt, Monitor3.txt* provided with Genotypes *Early*, *Control* and *Late* respectively – check the ***General Notes*** section for details of the data provided) from DAMScan in the home folder of the app, first job is to make a Metadata file (Provided as *MetadataMonitor1.txtMonitor2.txtMonitor3.txt.csv*).
   Note: For sleep analysis, raw data files will have to have data in 1 minute bin.
8. In this case, we have 3 genotypes in 3 Monitor files, so enter 3 at the **Number of Genotypes** box. Then go to the **DATA FORMATTING** tab.



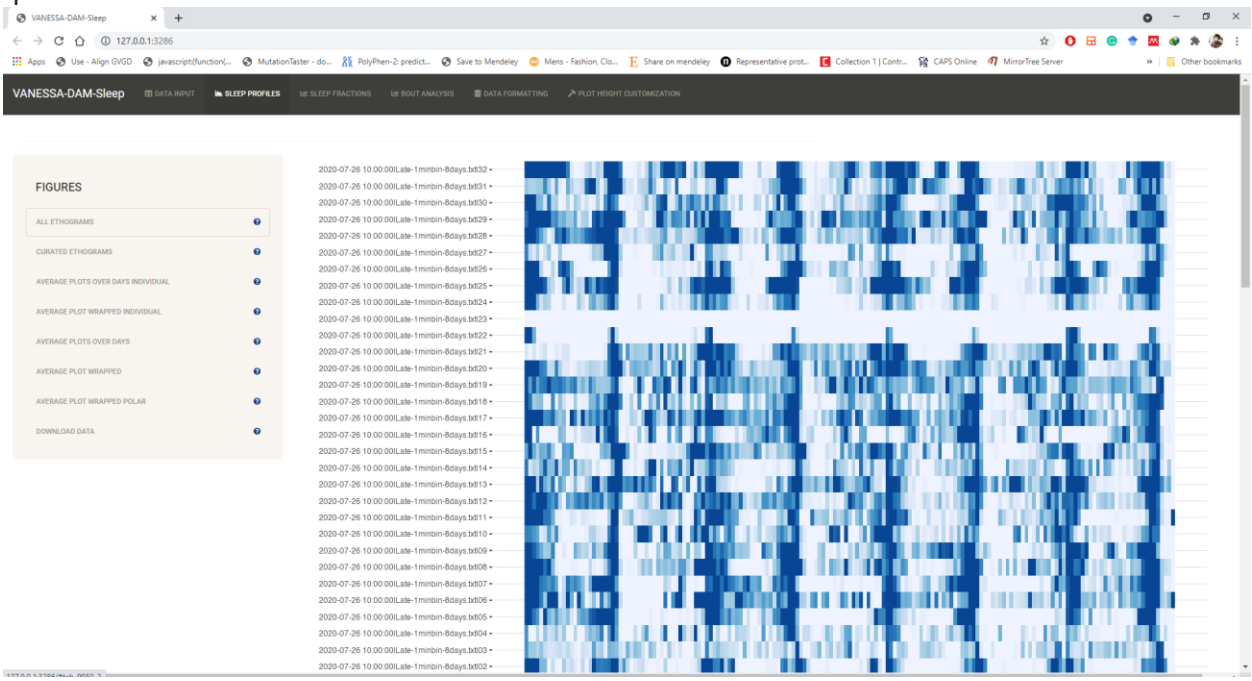
9. Enter name of your first monitor file in the **Monitor#1** name box, include extensions also (e.g., if the name of the monitor file Monitor1.txt, then enter the FULL name, including ".txt"). Fill the next boxes - **Start date and time (If your start date is 1st August 2020 and Lights-on time is 10 AM, then you write 2020-08-01 10:00:00), End date (If you start date is 10th August 2020, then you write 2020-08-10)** with your experimental details as shown above. Enter **Genotype** values and **Replicate number** (only if your experimental design has multiple replicates for all/some genotypes, else keep random number, it won't affect any calculation) in proper boxes for proper channels (marked as **Genotype for channels** 1-8 and **Replicate for channels** 1-8 etc.). A date and time picker has been added to facilitate easy entering of start and end date times without error in formatting. Each monitor can have maximum of 4 genotypes with 8 individuals.
Note: Creating the metadata file is the single most important step for using this app, any wrong information in the metadata file will wreak havoc on the results and the app may crash. DO NOT change information in the DATA FORMATTING tab once you have entered. For some unknown problem in shiny reactivity, this section does not handle deletion and change of values nicely. Alternatively, you can just crate your metadata file by changing data in the already provided metadata file in a good text editor (DO NOT USE EXCEL), like Notepad++. The metadata file serves as a base of information for your analysis too, and is good for record keeping of your experiments.

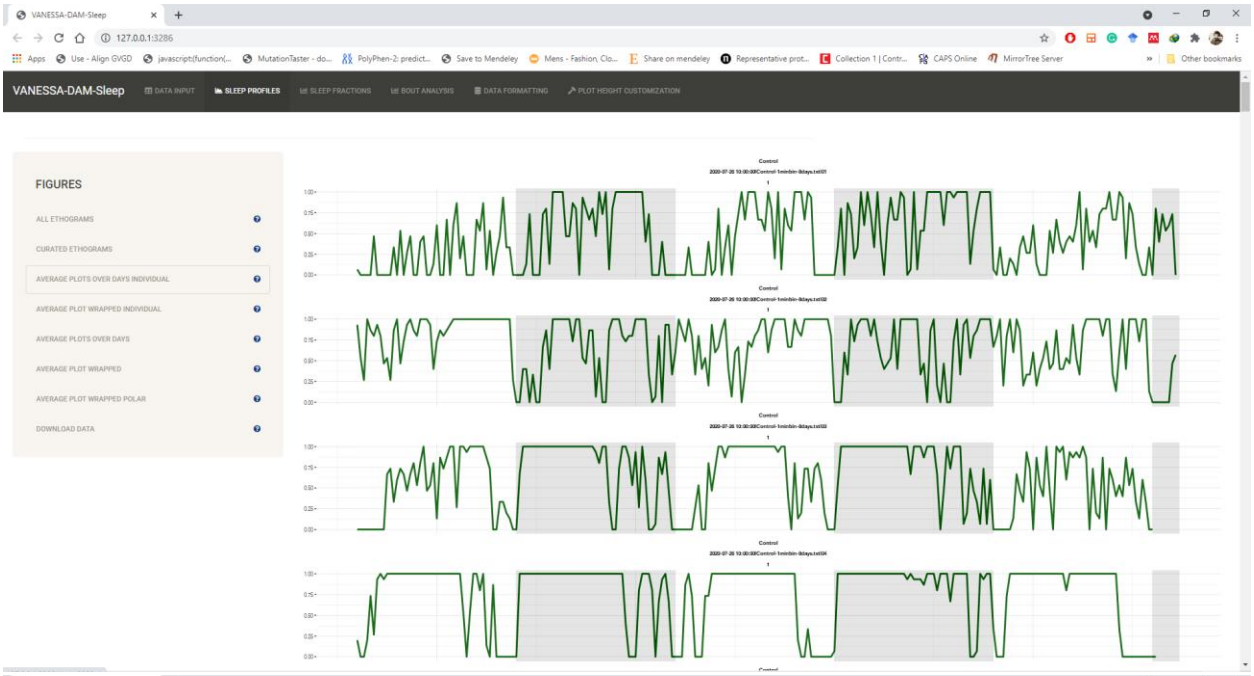


10. After filling in experimental details for all 3 monitors, go down in the page and click on the **Write metadata file** button, this will write the Metadata as a CSV file in the app home folder and is ready to be used. At this point the Metadata file will be stored in the folder set as your working directory.
11. Go back to the **DATA INPUT** tab. Enter choices one by one- **Duration of light in hours** in your experiment, **How many minimum days flies should be alive to be counted**. Also chose your **starting day** and **ending day** for analysis (**starting day** = 0 means first day in your data, so if you want to select first five days, **starting day** should be 0 and **ending day** should be 5). Enter **LD cycle period** in your experiment, **Modulo tau** for actogram can be changed for visualization purpose later, same goes for **Summary time window in minutes** and **Replicates**. You can change any of these parameters later while looking at plots, the plots will immediately update accordingly. For example, if you're looking at your actograms in 15 minutes bin, and you want to visualize them with 5 minutes bin, you just have to change the value in Summary time window in minutes box, similarly LD shading can also be changed. You are all set to start analysis now. **ONLY PROCEED FORWARD WHEN YOU HAVE FIXED ALL ANALYSIS PARAMETERS.** Select the folder where you have kept your monitor files to be analyzed by clicking the **Select Folder With Monitor Files** button and upload your Metadata file by clicking on the **BROWSE** button in the **Choose Metadata File** box. Wait for a few seconds while the metadata binds to your monitor files and a table of your metadata appears in the right panel. Press the Start **Calculations!** Button, when the calculations are done, you will be notified by a sound. Depending on how long your data is and how many monitors you are analyzing, this step (which involves curation also) will take anything between 5 seconds to 1 minute typically.
*Note: Most of the parameters have a small question mark symbol in blue near them, clicking on the symbol will show a modal with information about the parameter. This help is available is available throughout the app for different parameters, analyses and plots. In the folder selector, please click on the small black arrowheads to go to subfolders. Once you have located the folder where your monitor files are saved on the left panel, click on the desired folder in the left panel, the content of the folder will appear in the right panel of the folder selector. Make sure your monitor files appear in the right panel, and then click on **SELECT**.*



12. Go to the **SLEEP PROFILES** tab next. Explore the various analysis and visualization options by clicking on the left panel tabs. Each tab has description associated with the question mark button.




VANESSA-DAM-Sleep
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VANESSA-DAM-Sleep
DATA INPUT
SLEEP PROFILES
SLEEP FRACTIONS
BOUT ANALYSIS
DATA FORMATTING
PLOT HEIGHT CUSTOMIZATION
FIGURES
ALL ETHOGRAMS
CURATED ETHOGRAMS
AVERAGE PLOTS OVER DAYS INDIVIDUAL
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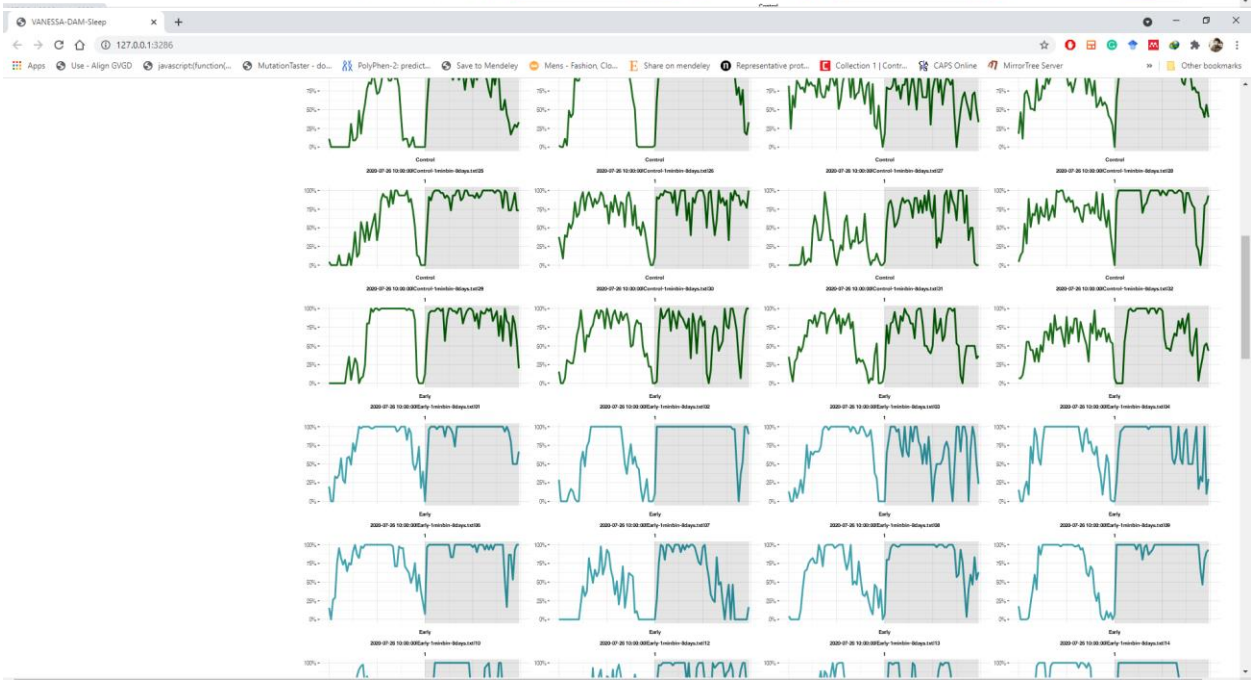
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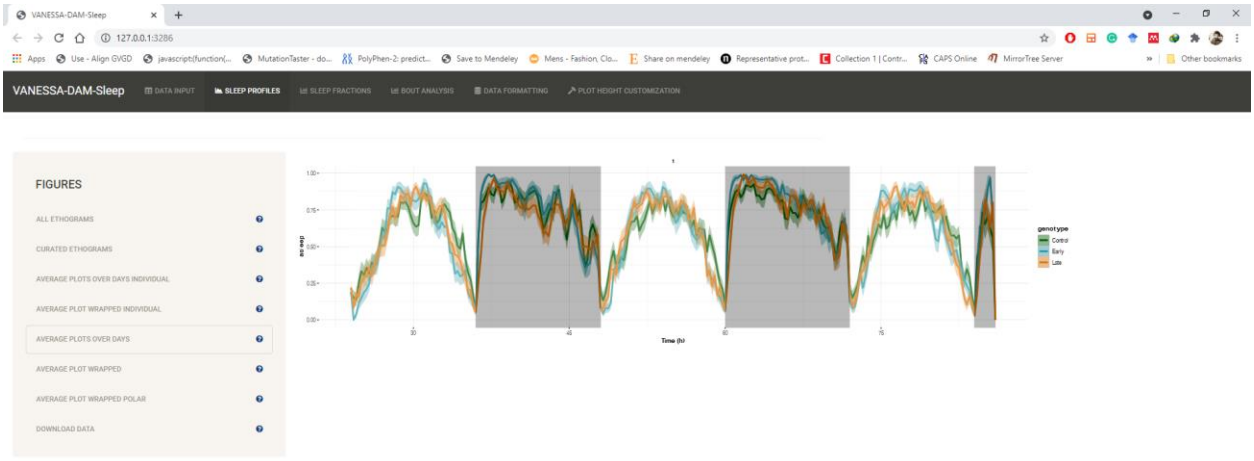
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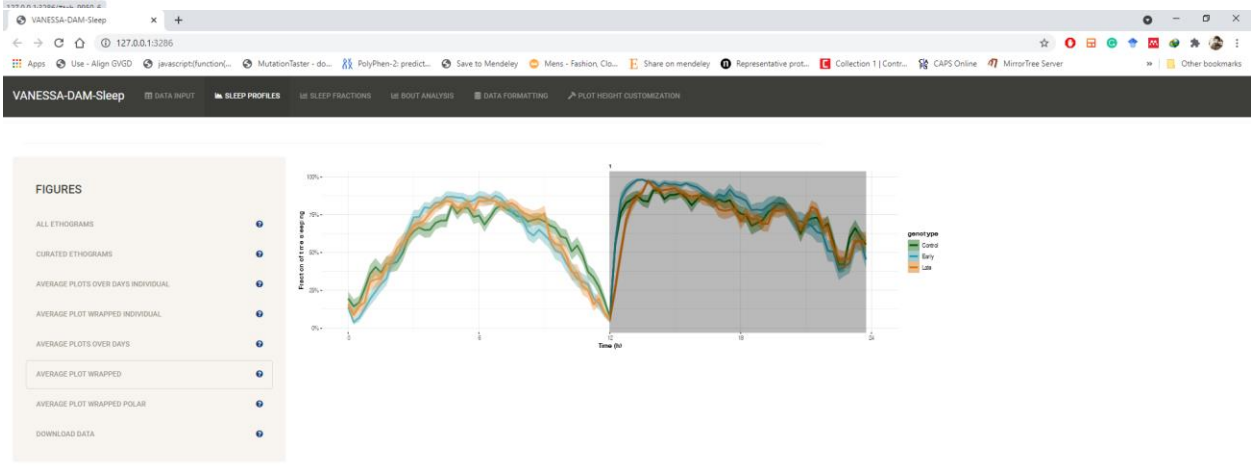
DOWNLOAD DATA
genotype
Control
Early
Late
Time (h)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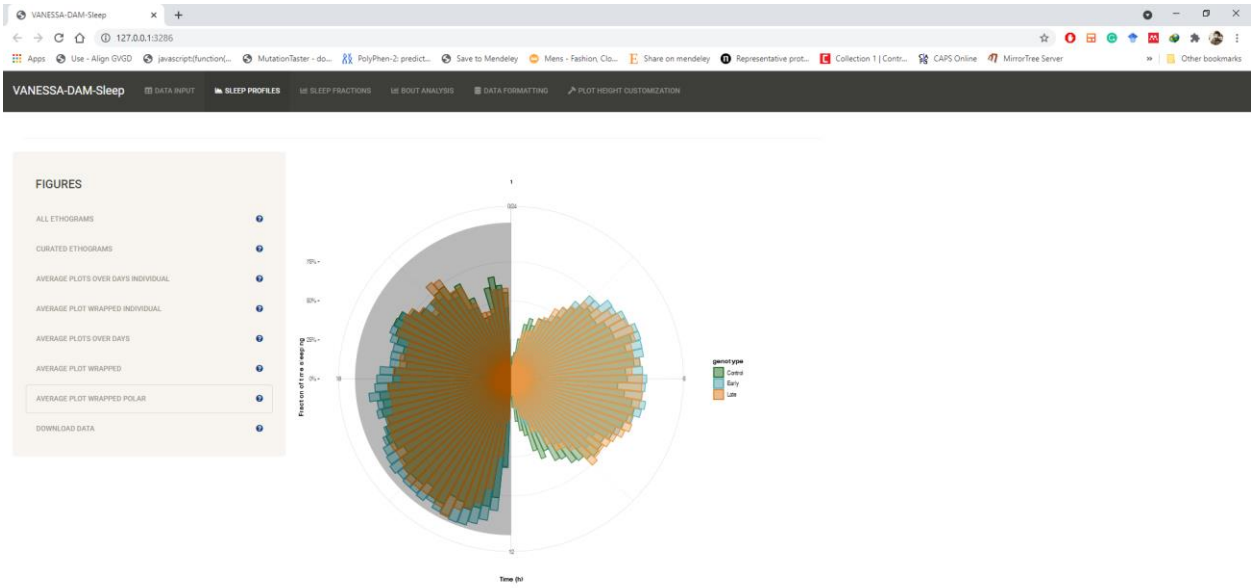
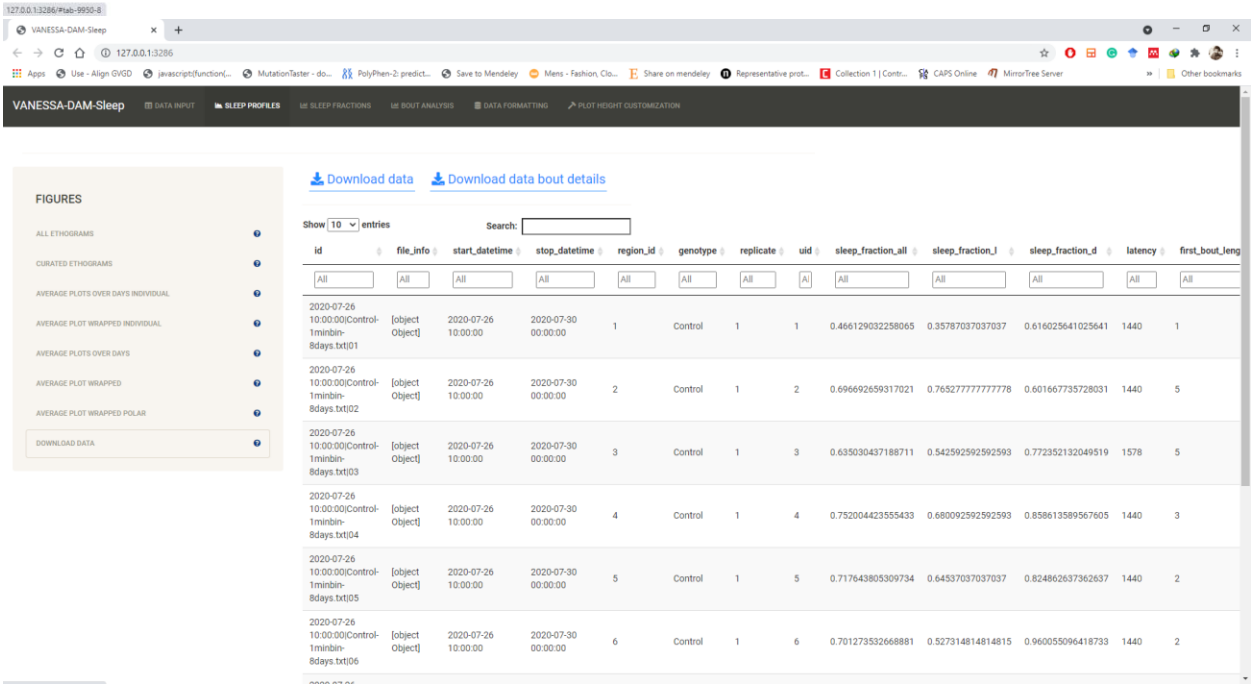
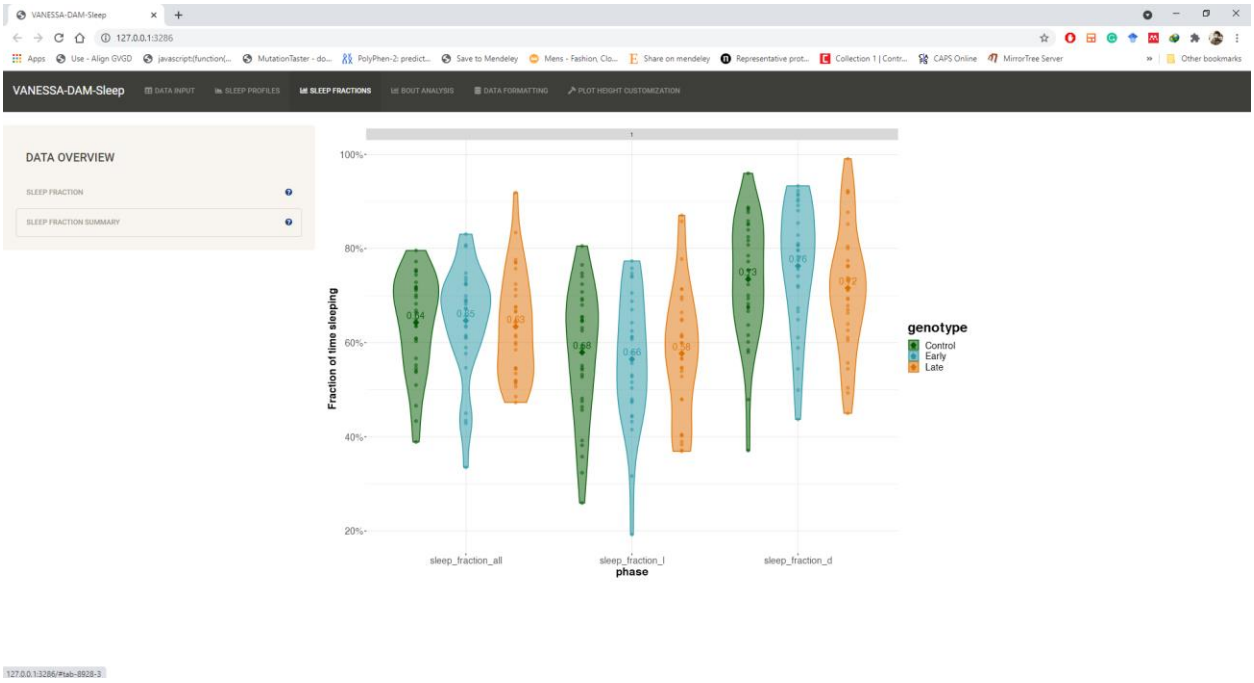
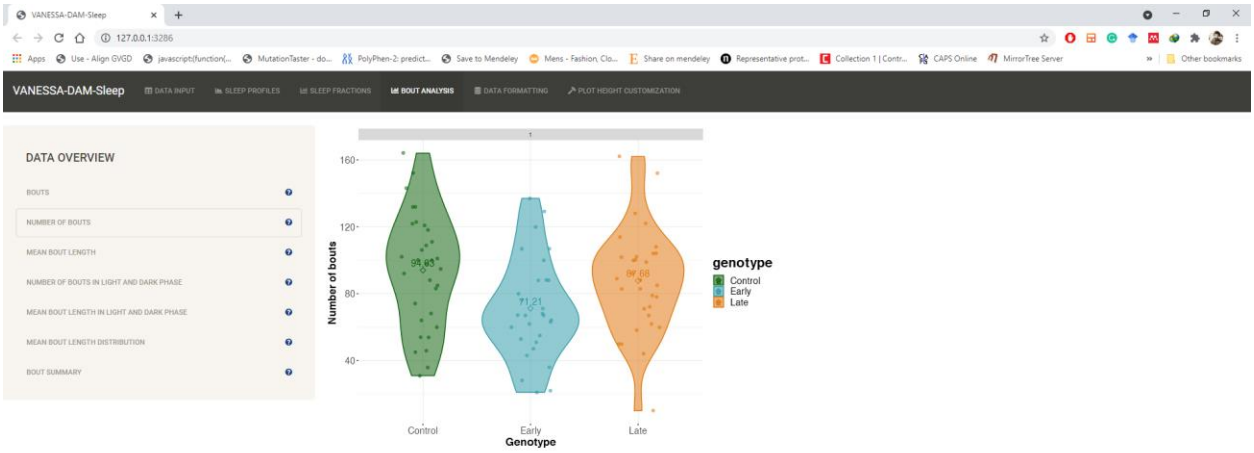


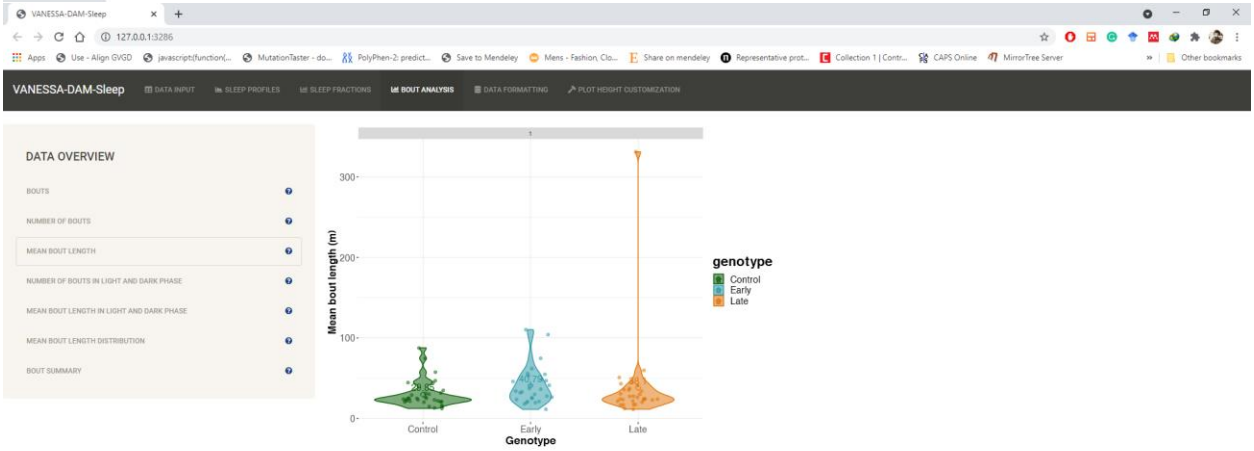


13. Go to the **Download data** tab to download a csv file for all sleep parameters extracted from your data. There are two different files to be downloaded, one with all sleep parameters, and one with specific bout analysis details.
14. Go to the **SLEEP FRACTIONS** tab to start visualizing sleep fractions as mentioned in different side tabs, feel free to explore.
    Note: sleep_fraction_l denotes sleep in the light part of the day, sleep_fraction_d denotes sleep in the dark part, and sleep_fraction_all denotes total sleep in a day.



15. Go to the **BOUT ANALYSIS** tab to see different plots from sleep architecture analysis.





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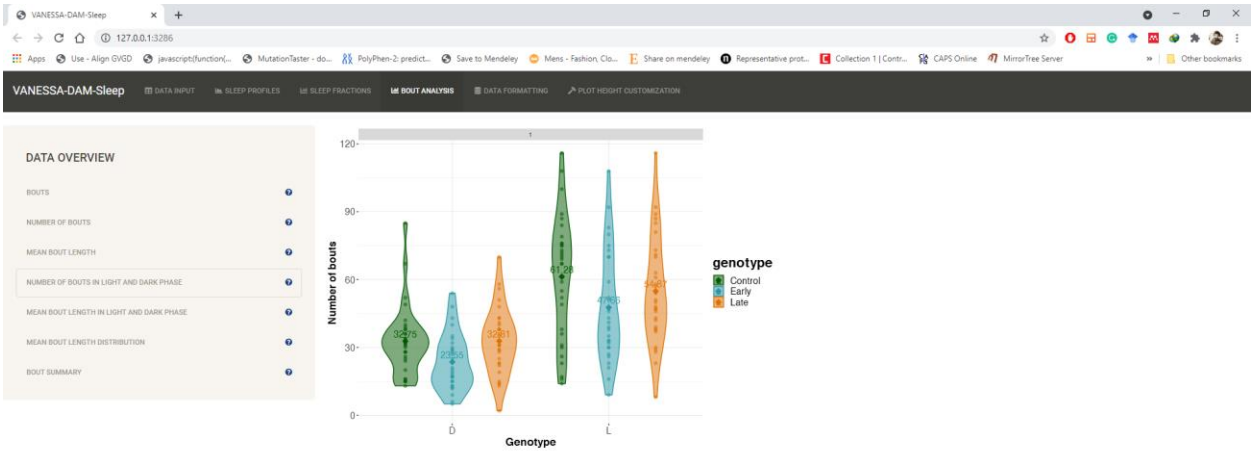

16. The last tab is for **PLOT HEIGHT CUSTOMIZATION**. The plots in the app appear in a predefined size and resolution. The plots dynamically change in response to changes in **Summary time window in minutes** and number of replicates, monitors etc. However, due to excessive deaths, sometime the plots may look stretched, you can change dimension of the plots from this tab.

GENERAL NOTES:

1. If you have different replicates, they will be plotted separately.
2. In faceted panels, the values on top of each panel are as following: 1-Genotype name, 2- serial ID assigned on raw data, 3-Replicate number.
3. All images produced are high-resolution, can be copied onto clipboard, saved as png files and directly used.
4. Cleaning up your Monitor file before using is desired, you don't necessarily have to subset your data by date, all data can be in the Monitor file, only the dates you specify in your Metadata file will be used, thus reducing hassle for the user.
5. If your run was in DD, please put **Duration of light in hours** as 23.99, as it does not 0 as input, it does not affect any calculations. Also, after you know your average period value in DD, you can change **Modulo tau for actogram** and **LD cycle period** accordingly, and plots will be updated accordingly.
6. The data provided is from a locomotor activity run with DAM2 system under LD12:12 (~750 lux light) for 8 days of three populations artificially selected for divergent chronotypes in our lab (as their names signify, early, control, and late). Each monitor file has 32 flies loaded onto it (standard format for DAM2 systems).
7. All plots can be resized from the last panel named **Plot height customization**. This is needed because when you remove arrhythmic individuals, the program won't automatically change dimensions of plot area, majorly only the "Height" parameter for the plots will have to be tweaked. It is also important to change the "Height" of actograms manually for achieving best visualization of your length of data.