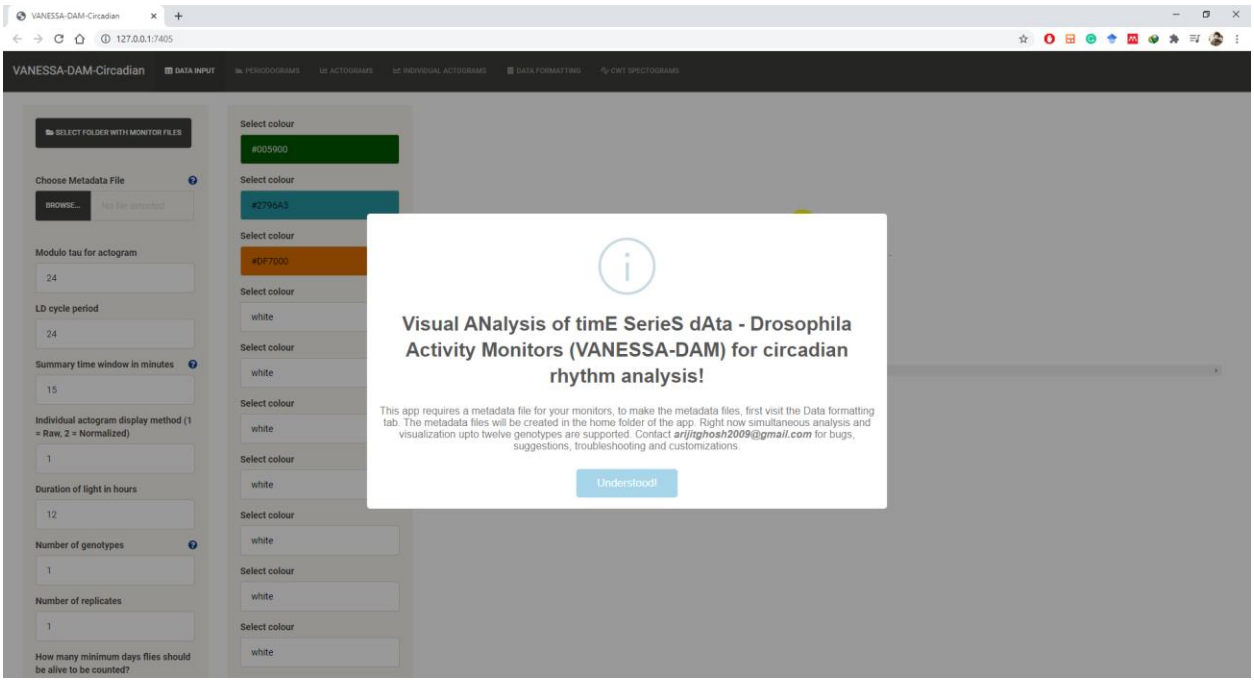
# Easy tutorial to start using VANESSA-DAM for circadian rhythm analysis

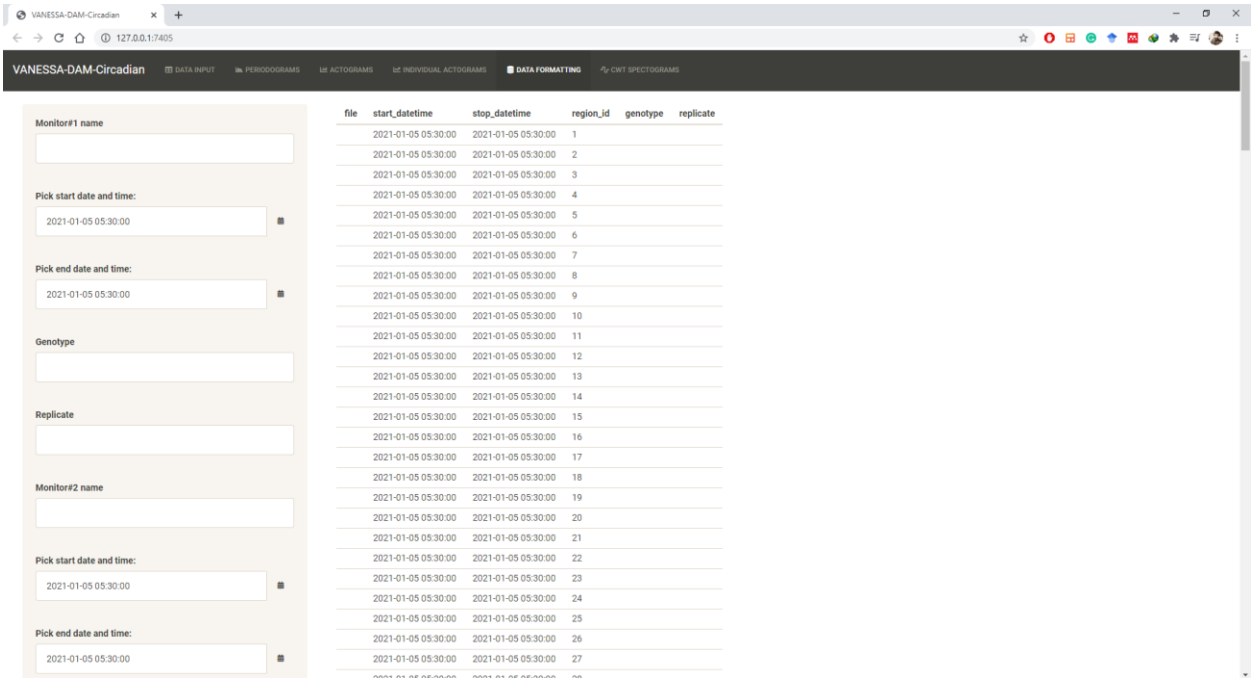
1. Install R and RStudio on your computer.
2. Download the zipped folder containing the app and unzip to a folder.
3. Open RStudio and Run the **startup.r** file to install all necessary packages to run the app.

   ***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 (*press ctrl+shift+h and choose the app home directory*) of the app and then run the app by typing **shiny::runApp(launch.browser = T)**.
5. The following screen will appear:

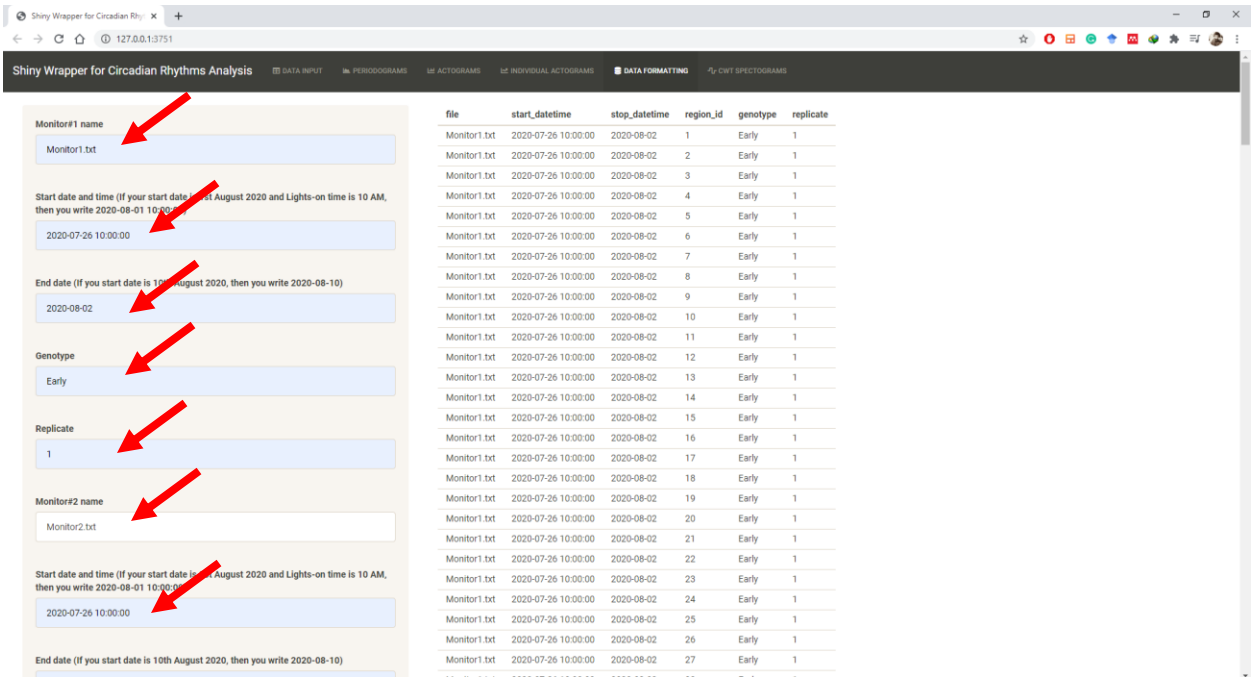



6. Click on **Understood!** Button or press **Esc** to begin.

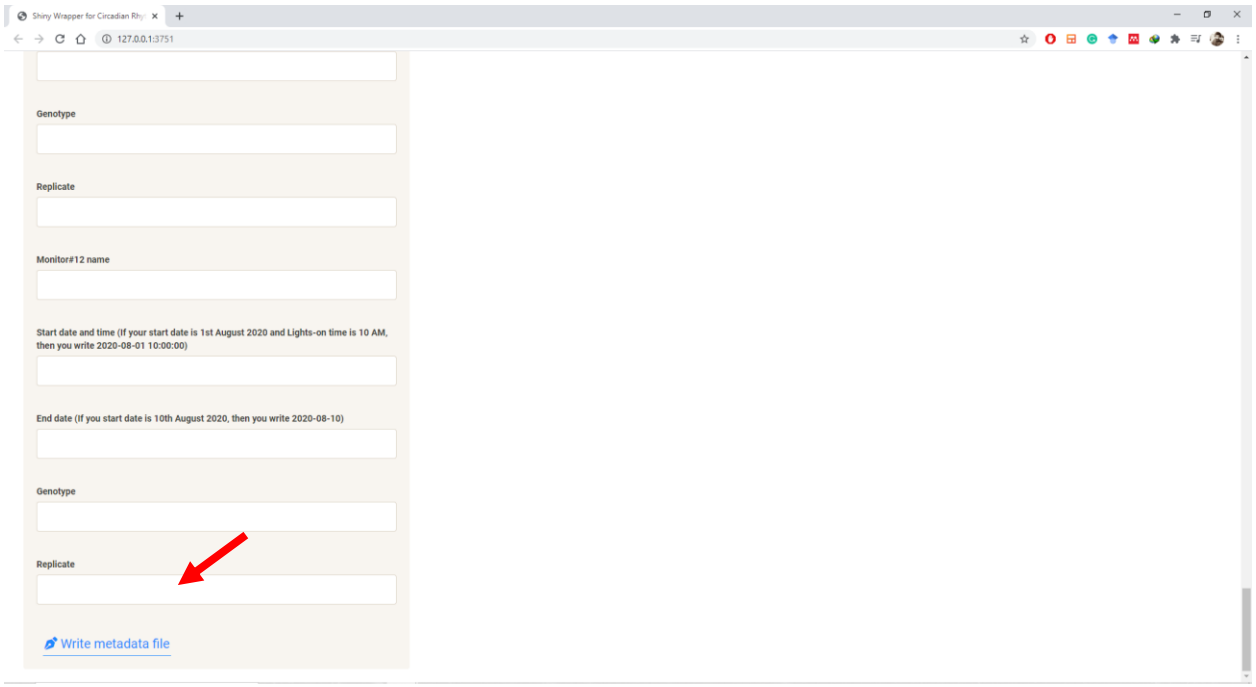
7. Assuming you have your Monitor files (*Monitor1.txt*, *Monitor2.txt*, *Monitor3.txt* provided with Genotypes *Early*, *Control* and *Late* respectively – check the ***Notes*** section for details of the data provided) from DAMScan in the home folder of the app, first job is to make a Metadata file (Provided as *MetadataMonitor1.txtMonitor2.txtMonitor3.txt.csv*).
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.



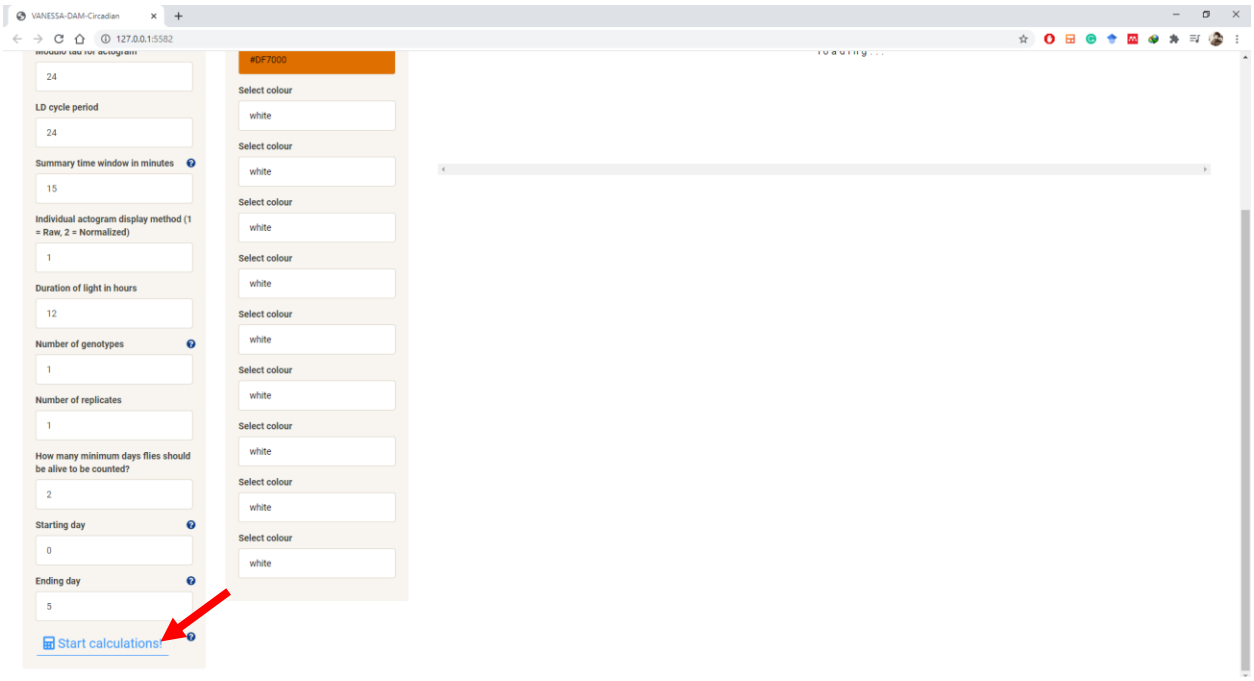

9. Enter name of your first monitor file in the **Monitor#1 name** box. Fill the next boxes - **Start date and time (If your start date is 1st August 2020 and Lights-on time is 10 AM, then you write 2020-08-01 10:00:00)**, **End date (If you start date is 10th August and Lights-on time is 10 AM, then you write 2020-08-10 10:00:00)** with your experimental details as shown below. Enter **Genotype** value and **Replicate number** (only if you have them, else keep random number, it won't affect any calculation) in proper boxes. A datetimepicker is added to the app for ease of selecting start and end date time without error.



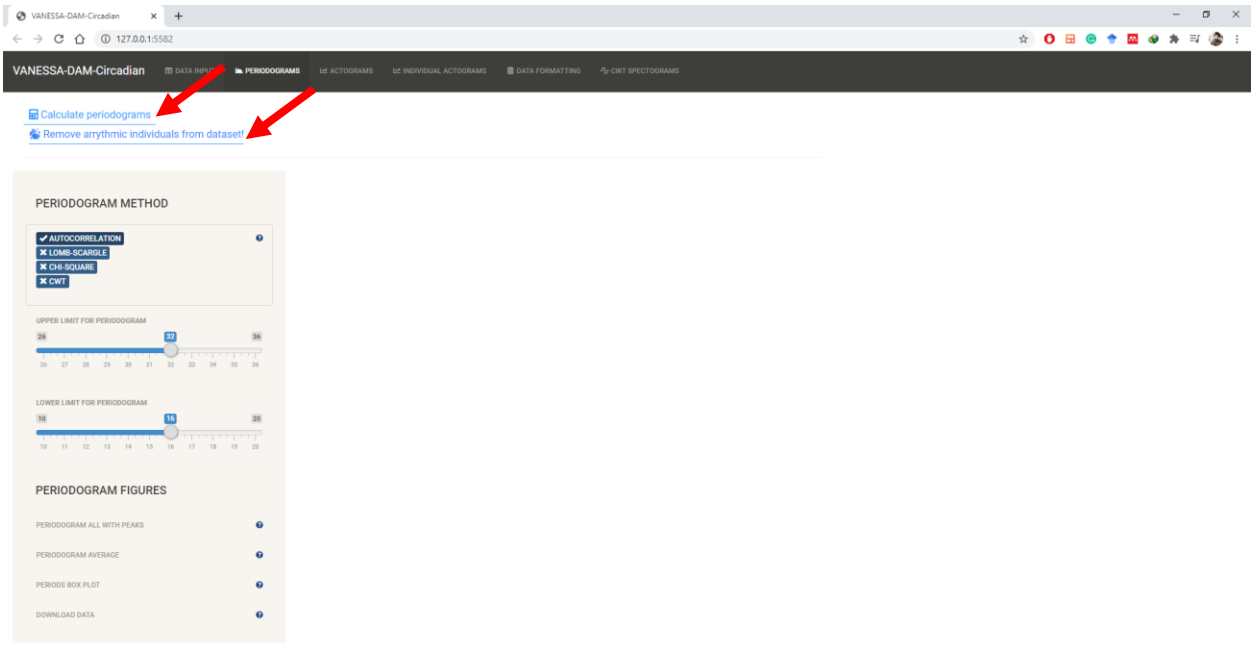

10. After filling in experimental details for all 3 monitors, go down of the page and click on the **Write metadata file** button, this will write the Metadata as a CSV file in the app home folder and is ready to be used.



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**. 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 **Individual actogram display method**. 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. Enter **Starting day** and **Ending day** if you want to subset your data further (if there are 10 days in your metadata file, but you want to visualize and analyze only 3 days, from $4^{th}$ day to $7^{th}$ day), instructions are provided, and can be accessed by pressing the "**?**" button. You are all set to start analysis now. Upload your Metadata file by clicking on the **BROWSE** button in the **Choose Metadata File** box. 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.

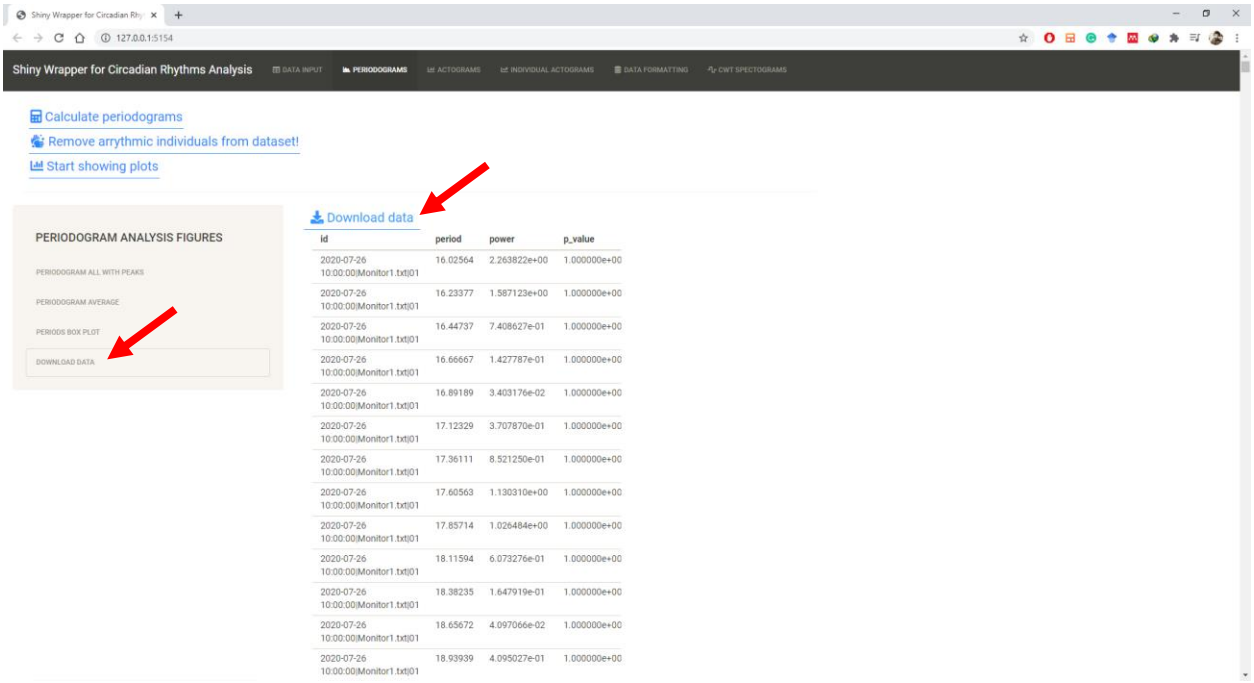



12. Go to the **PERIODOGRAMS** tab next. You will see the screen below. Select **Periodogram method** and **Upper and Lower limits for periodogram analysis**. Click on **Calculate periodogram** button to start calculating period of the individuals with the method you have chosen previously. If you click on the **Remove arrythmic individuals from dataset!** button next, all arrhythmic individuals will be removed from all calculations and plots.

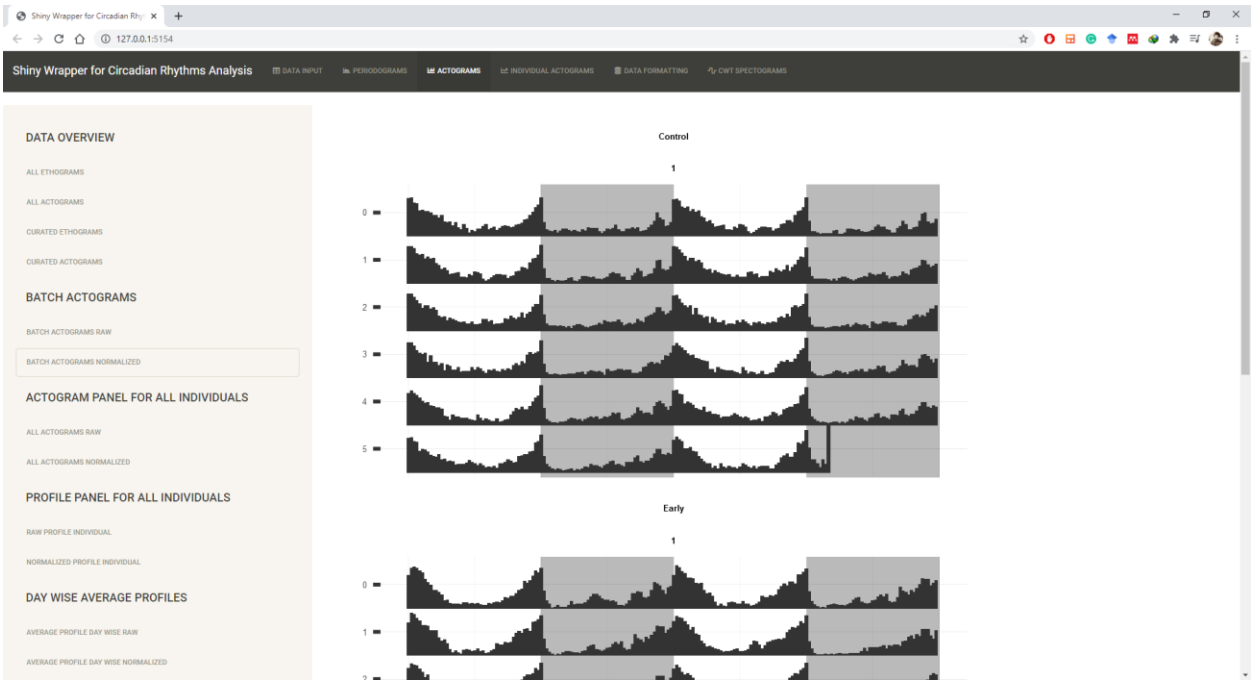



13. When you click on **Start showing plots** button, you will start seeing plots as mentioned in different side panels.
14. Go to the **Download data** tab to download a csv file for all period and power values. Different peaks of periodograms for all individuals are noted in the file. If you open the file in Excel or LibreOffice or any spreadsheet program, you can put a filter on the "peak" column which is the last column of the file. When you filter the data by "peak" and select "peak" as "1", it will show you the highest peak of the periodogram of all rhythmic individuals. You'll notice that this file will now show you these columns - a) id: start_datetime|Monitor name|Indivudual number, b) period: the period defined by the "peak" (if you select 1, it will show you the period of the highest peak in the respective periodogram), c) power: the peak value from the periodogram (in case of autocorrelation it's the peak autocorrelation value, i.e., rhythmicity index, in case of chi-sq, it's the power of the chi-sq periodogram, etc.), d) signif_threshold: significance threshold for the

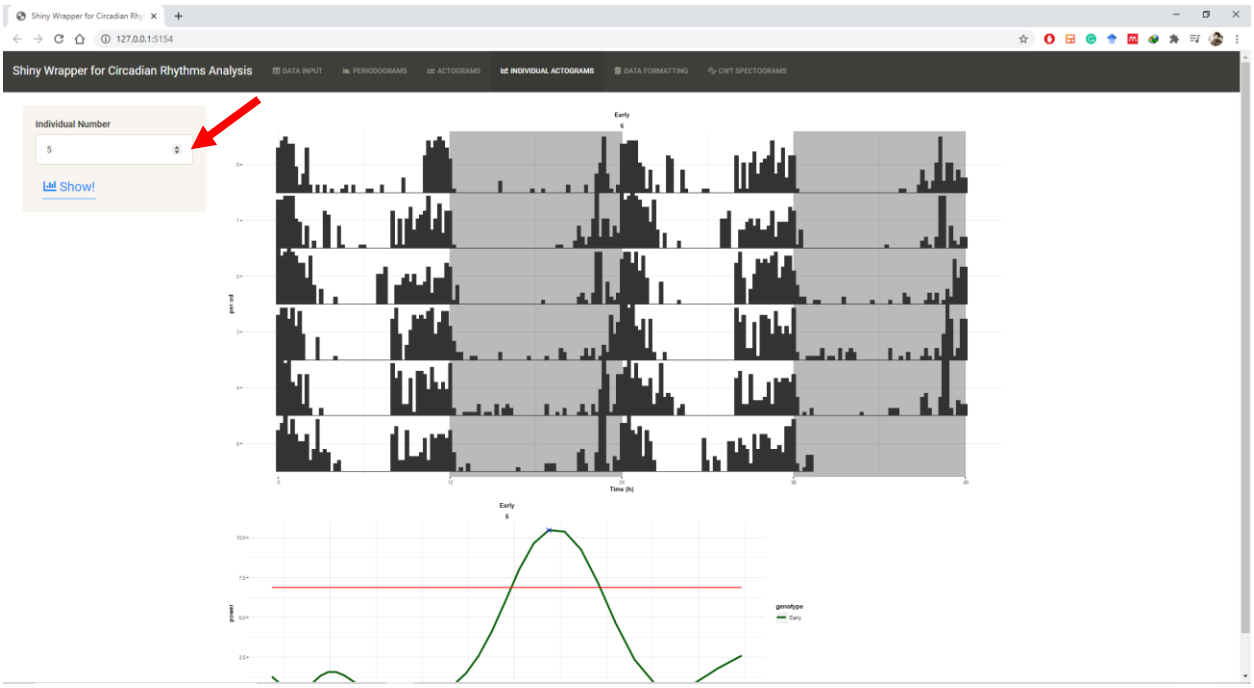
particular peak, e) p_value: the p_value is reported here for the particular peak, f) peak: different peaks in the periodogram (filter based on this).



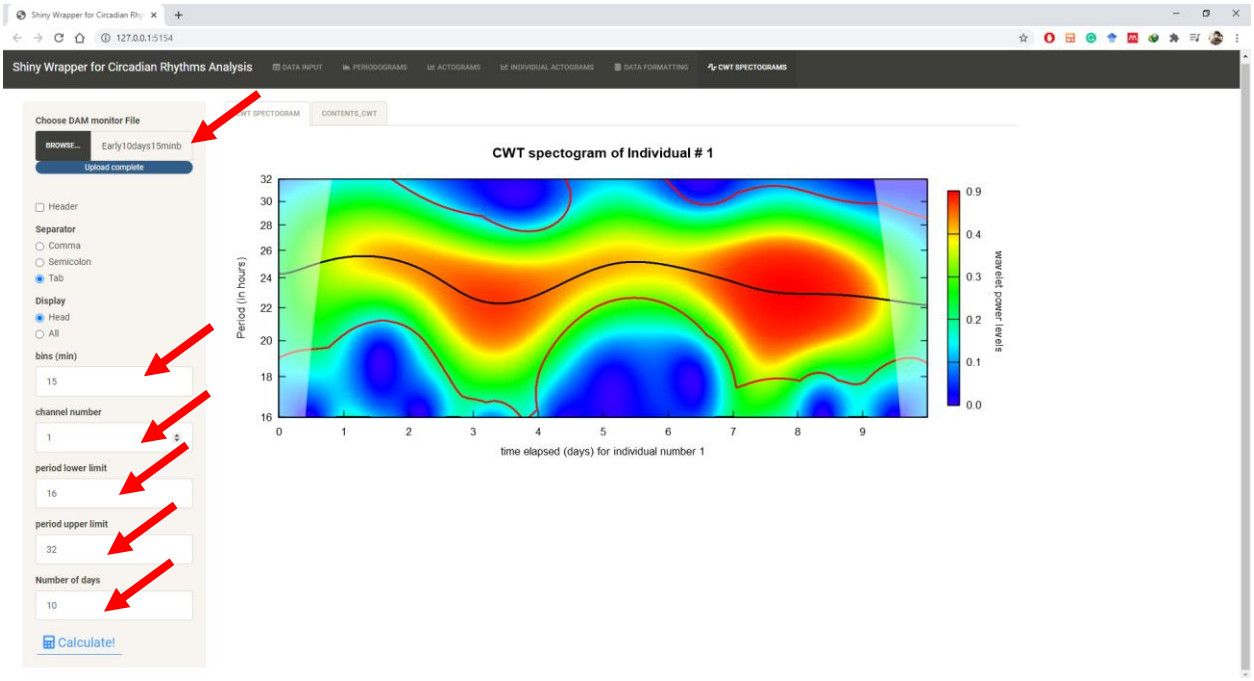

15. Go to the **ACTOGRAMS** tab to start visualizing Actograms and Profiles as mentioned in different side tabs, feel free to explore.



16. Go to the **INDIVIDUAL ACTOGRAMS** tab to see individual wise actograms (raw or normalized) for closer inspection of your data. Change **Individual Number** by pressing up or down arrow or use the buttons in the box to change individuals to look at and they'll update automatically.

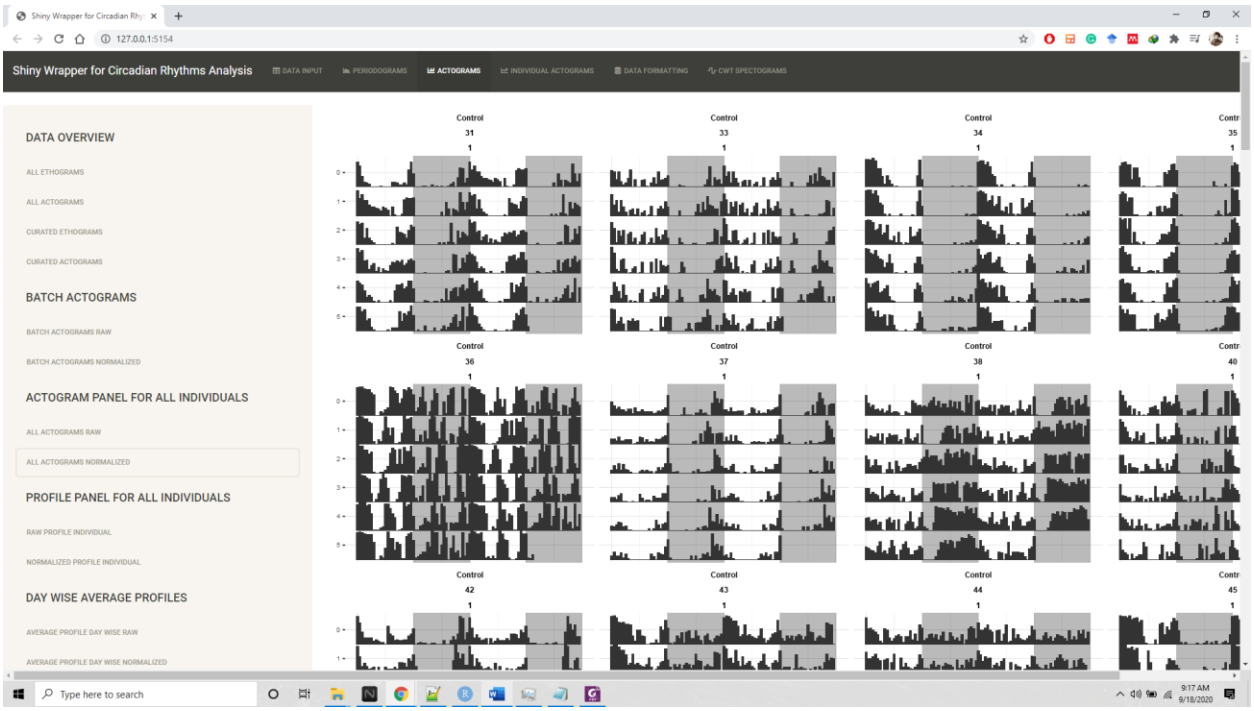



17. The last tab is **CWT SPECTOGRAMS**. This helps in constructing individual CWT spectrograms and plot ridge values and provides 95% CI. Useful for visualizing changing periods in case of long recordings and regime changes. Still in basic stage. After entering appropriate details in the **bins (min)**, **period lower limit**, **period upper limit** and **Number of days** boxes, upload your Monitor file in the **Choose DAM monitor file** box and press the **Calculate!** Button. After calculations are done for the individual chosen, a CWT spectrogram will be plotted. Change **channel number** to visualize different individual.




**NOTES:**

1. If you have different replicates, they will be plotted separately in **DAY WISE AVERAGE PROFILES**, **AVERAGE PROFILES** and **CIRCULAR AVERAGE PROFILES** tabs.
2. **RAW PROFILE ALL REPLICATE AVERAGE** and **NORMALIZED PROFILE ALL REPLICATE AVERAGE** tabs will average over ALL individuals in all replicates in a Genotype.
3. In faceted panels, like **ALL ACTOGRAMS RAW**, **ALL ACTOGRAMS NORMALIZED** etc., the values on top of each panel is as following: 1-Genotype name (Control in the following example), 2-Arbitrary serial ID assigned on raw data (31,33,34 etc. in example), 3-Replicate number (1 in example).




4. All images produced are high-resolution, can be copied onto clipboard, saved as png files and directly used.
5. For best results, use Monitor files with <5 minutes bin data. Also, 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.
6. 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.
7. 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).