

BIO 342 Animal Behavior, Observing, Recording and Analyzing Behavior.

Before lab, read Martin and Bateson Ch 3 and at least these first 2 pages.

Ethograms for Observing Behavior.

An ethogram is a primary tool in ethology. The ethogram is a catalog of the discrete behaviors typically employed by a species. The included behaviors should be discrete and described with sufficient detail such that an observer can accurately identify each in order to record the number of acts, or the amount of time engaged in the behaviors. The ethogram may include drawings (or photographs) in addition to a written description of each discrete behavior. In an ethogram, behaviors should be described without explicit reference to their purpose. For example, although a specific movement may represent a putative threat display it should be described with neutral, structural characteristics that do not require subjective interpretation. The definition of the display as a component of antagonistic interactions arises from the context and surrounding behaviors in which the display is observed. Ethograms often represent an early stage of investigating the behaviors of a species and the contexts in which they occur, but may also be the basis of formal scientific research. In the early years of ethology, the primary goal of many studies was the description of behavior. Although complete ethograms are rarely published anymore, they are often used by zoos to describe normal behavior and monitor captive behavior in order to identify pathology due to illness or poor animal care. Ethograms also serve as a valuable tool for many lab and field experiments on both model organisms and ecological studies. As a tool, the “working ethogram” usually describes only a small portion of an animal’s complete behavioral repertoire. You will have additional practice creating an ethogram at the zoo in a couple weeks. But this week we will work with “simple organisms” with predictable behavior in order to learn how to use an event recorder to collect behavioral data directly to the computer including timing information.

Constructing a Working Ethogram

1. Each student pair will be assigned “a simple organism”
2. Watch the organism perform a few bouts of “behavior” and discuss with your partner how these behaviors can be broken down.
3. In your lab notebooks, devise a good working ethogram with 5 – 10 behavioral categories.
4. Provide a neutral name for each behavior
5. Provide a concise description.
6. Decide which behaviors should be recorded as events and which as states.
7. With your ethogram in mind, watch a few more bouts of behavior and discuss with your partner.
8. You may want to do a quick tally mark observation and quantify inter observer reliability.
9. You might consider asking another student to try to use your ethogram and see if they are able to accurately interpret your descriptions.
10. You may need to revise the ethogram, lumping together behaviors that are difficult to distinguish operationally or splitting behaviors that appear to have different “functions”.
11. You may need to revise whether they are recorded as states or events.

Understanding Jwatcher for behavioral analysis

Today you will use an **event recorder** to quantify the behavior of your chosen organism. **Event recorders** are computer programs (or dedicated pieces of hardware) that record keystrokes as they occur over time. In our case, keystrokes will represent behavioral transitions. For instance, when your animal is foraging, you might type an "f", when your animal is grooming, "g", etc.

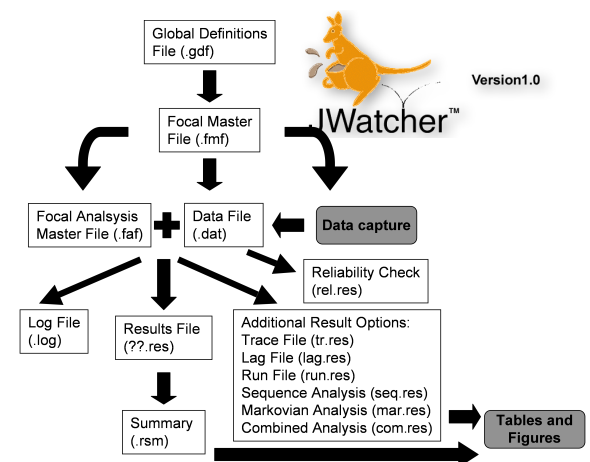
We will use an event recorder called **JWatcher** to score your observation. The JWatcher program combines some analysis algorithms along with the power of the event recorder. With these tools, you will be able to calculate not only the number of events but also the time allocated to each of the behaviors in your ethogram. This can be used to obtain an accurate time budget.

OVERVIEW of JWATCHER WORKFLOW AND FILE STRUCTURE.

This chart describes JWatcher work flow.

To record and analyze data you need to:

- 1) Create a Global Definition File (*.gdf) which will serve as the "ethogram" and assign keys to behaviors.
- 2) Create a Focal Master File (*.fmf) which includes the behaviors (or a subset thereof) from the global definition file. This file also instructs JWatcher to prompt users for additional data about each observation during data collection.
- 3) Record behavior. The data file (*.dat) file is created automatically during each observation. It contains the additional notes about the observation as well as the raw data you have recorded.
- 4) Create a Focal Analysis File (*.faf), which specifies how data files (.dat) will be analyzed and specifies the results to be reported.
- 5) Quantitatively analyze each *.dat file to produce a results file (*.cd.res) that can be opened in Excel.
- 5a) JWatcher is capable of summarizing several data files into one summary (*.rsm) to obtain averages and other descriptive statistics that are probably better done in a statistical analysis software package, but this feature is handy of collecting several observation data files into one file for further analysis.
- 6) JWatcher can also be used to test observer reliability, and performing complex analysis (behavior combinations, sequence analysis, transitions matrices, Markovian analysis) that you can explore later.



As you work on the computer today, use your lab notebook as a record of the steps you take, the names of the computer files that are generated.

In your notebook record what each the purpose of each of these files.

Detailed instructions are in sections 1-6 on the following pages.

1) Defining your Ethogram in JWatcher

Based upon the written ethogram you have developed you will "define" a JWatcher ethogram. Some people prefer to use **logical key locations**, others prefer to **use logical letters** that match the behavior description. Confer with your partner and decide on a code. You will have a chance to practice and can change the code later.

1. In the **Applications** folder, find the **JWatcher_V1.0 folder** and double click. Now double click on the **JWatcher icon** (black square, with a gold J and gray W) to launch the JWatcher program.
2. When JWatcher first opens, you will be in the Data Capture tabbed window. Click on the **Global Definition tab** to change windows. The Global Definition window is where you will specify your ethogram by assigning key codes to each behavior.
3. The Global Definition window is divided into two sections: one to define behaviors and another to define modifiers. We will be defining behaviors only.
4. Click on the **Add row** button in the top section to begin assigning key codes to behaviors.
5. It is up to you and your partner to choose the key strokes that correspond to each behavior. You may use **only one single character** per behavior. Try to choose something intuitive and easy to remember, such as **a** for aggression and **f** for forage, etc. **Note that JWatcher is case sensitive:** **f** will NOT be recorded the same way as **F**. Because it is difficult to switch between upper and lower case, for this exercise, **use lower case letters**. **Record the letters that you choose for each behavior in your lab notebook.**
6. Type in the single character code in the **Key Code** box. Then either tab or click into the adjacent **Behavior** box to type in the corresponding behavior. You may also include a lengthier description of each behavior in the **Description** box but remember to avoid anthropomorphisms or "intent" in your descriptions whenever possible.
7. Click on the **Add row** button to continue adding each behavior.
8. When you are done adding all your behaviors, click the **Save** button in the lower right corner of the window. These descriptions are useful for others using your files.
9. To Save your new Global Definition file to the desktop click the **house icon** in the upper right part of the Save As window. Now double click on the **Desktop** folder icon for local user. Name your file by typing into the **File Name:** box in the lower part of the window. The extension **.gdf** will automatically be added when you click **Save** (this stands for **Global Definition File**).

NOTE: When starting a study, it is often difficult to decide how many and which behaviors to record. Your global definition file may include behaviors that you do not end up using in your observations or in your analysis. For now, include all behaviors that you think you might want to quantify.

NOTE: Generally it is good to include a key code for "out of sight" and also one for "other". Think about why these are important categories.

2) Specifying Focal Details for a JWatcher Observation Project

Based upon your global definition file you will now create the "**focal master file**" (.fmf). This file specifies what data (behaviors, and additional information) will be collected during each focal observation. This is sort of like a "protocol" for the observation.

1. Click on the **Focal Master** tab at the upper left to change windows. Be careful, as it is right next to Focal Analysis Master. You may find yourself in the **Test Details** page (see bottom left tabs). If so, change pages by clicking on the **Define Codes** tab.
2. The **Define Codes** page shows the ethogram to be used when scoring data for this particular project. Currently it should be blank.
3. To import your pre-existing ethogram from your .gdf file, click on **New** (bottom center of the window) and a new selections window will open. You should see your .gdf file that you just made. If not, you will have to navigate back to the desktop by clicking the house icon and then the desktop icon. **Select** your global definition from the list in the top part of the window. Click **Open** to load your ethogram. If you decide not to use specific behaviors in your global definition file, use the delete_row button to eliminate those. While JWatcher allows you to add rows here, it is important to remember that your global definition file is you recorded ethogram with the concise behavioral description. It is good practice to keep the focal master file consistent with the global definition file and make any required changes directly to your .gdf file. For good record keeping, the .gdf file should not be saved under the same name if it is changed, rather use a new name (ver1.1 or the date).
4. Next, using the lower left tabs, change to the **Test Details** page. You will score each staged interaction for **the same amount of time**. In the **Duration** boxes, type 0 in the hours (**HH**) box, and the number of minutes (**MM**) box, and 0 in the seconds (**SS**) box. If you expect the interactions go be of varying lengths set the time to the maximal amount of time.
5. Finally, in the lower left tabs, change pages again by clicking on **Questions**. This window is where you may define the variables that will be associated with each focal sample file, such as individual ID, age, sex, time of day, name of observer, location (if you are in the field), etc.. For today, you will decide what information you want associated with your raw data. You may specify up to 6 questions.

Click **Save As** to **save your Focal Master File**. Again, the proper file extension for a **Focal Master File** (.fmf) will automatically be added for you when you click **Save**.

3) Data Collection with JWatcher

1. When JWatcher first opens, you will be in the **Data Capture** tabbed window. If you are not there now, click on this tab to open it.
2. Click on the file navigator icon (**it looks like a sheet of paper**) to the right of the **Focal Data** box in order to name the data file you will be creating. Navigate to the desktop by clicking the **house icon** then double click the **desktop** icon.
3. Type in the name of your new file **date_exp_name_observation#** in the **File Name** box and click **Open**. Keep the names simple and logical. (Jwatcher sometimes has problems with long names and spaces in names). **In your lab notebook, record the file name you are creating.**
4. Specify the Focal Master File by clicking on the file navigator icon (**it looks like a sheet of paper**).
5. Navigate to and select the the focal master file that you just created and click **Open**.
(This file contains the same ethogram along with additional specifications such as the duration for recording the focal observation.)
6. Click the **Next** button at the bottom right to tab into the next page.
7. Answer each question and click the **Next** button to advance.

If you wish to see a list of the behavioral codes on your computer screen during the observation, click the **Behaviors** box in the upper right corner of the JWatcher screen.

8. Wind up your organism.
9. When you release your organism, click the **Start** button in the center of page.
10. Immediately type the key code for the behaviors in which your organism is currently engaged.
11. **Whenever** the behavioral state changes, type the key code for the new behavior.
12. **Whenever** a new even occurs, type that key code.
13. (JWatcher will automatically save your data in the **.dat** file named above

If you made a data entry mistake, look at the timer and make a mental note of it. You can fix it later by opening the data file from the edit data tab.

14. Repeat steps 1-13 a few times so that you and your partner both have a chance to practice recording behavior a couple times.
15. Give each new data file a new name that ties it to the appropriate observation and answer the questions to provide necessary meta-data for each .dat file.

Remember to recording critical steps, difficulties encountered, as well as the name and location of data files in your lab notebook.

If you make mistakes while recording, note those errors in your lab notebook when they occurred. It is always good practice to edit your data immediately after scoring while the details are fresh in your mind. Later, use your lab notebook to indicate which errors have been corrected in JWatcher.

Based on your difficulty here with your preliminary work, you might decide to add, subtract or change key codes before starting your research. In reality, you would expect to go through several iterations of observation, recording, and analysis before settling on the most appropriate protocol.

4) Specifying Analysis details for a JWatcher Observation Project

Once you have scored several observation periods, you are now ready to use JWatcher's analysis algorithms. Analyzing data files can be a complex process and it is necessary to carefully think through the relationships between the different behaviors you have recorded (in fact it is best to do this before collecting a lot of data).

For example:

Are all behaviors mutually exclusive, or can your organism be doing two things at once?

Are all behaviors discrete events, or are they states (such as "resting")?

For analysis you must first create a file called a **focal analysis master file (*.faf)**

1. To create a **focal analysis master** file, click on the **Focal Analysis Master** tab in the upper left menu.
2. Click on the **New** box, at the bottom center.
3. You must now load your project's ethogram by choosing the same focal master file that you used to capture data. Select your ***.fmf** file and click **Open**.
4. For today you will not use the Modifiers tab, the Time tab, the Exclusions tab, the Conditional States tab or the Conditional Events tab, but all of these allow you to do more complex behavioral scoring. Instructions will be provided if you decide you need these for your independent project.
5. Next click on the **States** tab at the bottom. States are behaviors that occur over time. Some states are mutually exclusive (such as eating and sleeping). This will greatly affect the time budget calculations. Only behavioral states that are mutually exclusive will stop the timer for the previous behavior. Use the check boxes to specify which behaviors will turn each other off.
6. Click on the **State Analysis** tab (Note: there is also an Analysis tab above and a States tab below, you want **State Analysis**). If you are interested in creating a time budget for the interaction, it is appropriate to use "**all durations**" and select "**Proportion of Time**" or "**Total Time**". However if you are interested in how long each behaviors is displayed when it is displayed, then it is most appropriate to select "**natural duration**" and select "**Average**" (ask for clarification so you can make the right decision). If you are interested in the interval between specific behavioral states or events you will also select one of the interval options. While it is possible to select all options and calculate all measures you may choose to run analyses separately in order to keep output files to a manageable size. For many situations animals can be "out of sight" and then you need to include a key code for that and use the appropriate analyses so that JWatcher corrects for this.
7. The **Event Analysis** tab allows you to select those behaviors that you want to have counted as instantaneous events. Select the behaviors and select **count**. These behaviors will be counted but will not contribute to the proportion of time, thus they will not be a part of a time budget.
8. When you are done exploring your options and have settled on a basic analysis method, click on the **Save As** button. Specify the location to save your new **focal analysis file**. Again, the proper file extension **.faf** will automatically be added.

5) Data Analysis within JWatcher

1. To generate results files, click on the **Analysis** tab.
2. Use the file navigator icon open your focal analysis file **.faf**
3. Use the file navigator icon to select the *.dat data file you just created.
(To analyze an entire folder of *.dat files at once you would specify an entire folder for batch processing.)
4. Specify the location to save the results file by clicking on the folder icon and click **Open** (give the folder a name more informative than "results")
5. Select the **Print results for all behaviors** button. This ensures that all of your output files have uniform layout even if all behaviors did not occur.
6. Click the **Analyze** button at the bottom of the file window.
7. If you get a warning message telling you that there were unrecognized key strokes in your file, these will be ignored.
8. Notice that JWatcher creates a log file. This is akin to a lab notebook. It will be saved in the same folder as your results files.

JWatcher includes algorithms to provide **summary statistics** for the analyzed result files. In general, this is usually done in a statistical analysis software package rather than in Jwatcher. However, the “summarize” procedure in JWatcher combines the data from many files in a format that is easier to import to another software packages.

1. Click the **Summarize Results** tab & select the folder containing *.cd.res files.
(All files to be summarized MUST have been analyzed using the same .faf file.)
2. Choose a file name and folder for the summary.
3. Generally, **Analyze Results in Columns** provides a format that is easiest to work with in excel.
4. While you can choose summary statistics (optional) to get an average or standard error, it is likely that you would prefer to do that in a statistical package that is more powerful.
5. Click **Summarize Results Files**.
6. The resulting file is a comma delimited file that can be opened in Excel but you must first append **.csv** to the filename (filename.rsm.csv).
7. Open this file in excel to make graphs, or figures to summarize your day's work. A pie chart works well for quick view of a time budget, though a bar graph may be more appropriate to compare the average duration of different behaviors or to compare the recording for two observers or two types of organisms etc.
8. Attach the graph to your lab notebook, add a figure legend?
9. Briefly record any conclusions about your organism's behavior in your lab notebook.
10. Briefly record any thoughts about how you would set up JWatcher differently in the future.
11. **These files are all valuable protocols and data, you would want to create a .zip file archive it on the BIO342_2018 Google drive.**

There is nothing to turn in for this lab, but you should have save all of your files and have completed figures in your lab notebook.

We don't have time for the following today nor other complex analyses possible in JWatcher.

6) Behavioral Sequence Analysis with JWatcher.

For many behavioral questions it is sufficient to calculate time budgets or to study the frequency of events. However, these measures do not tell us about the structure of behavior. Another important way to study behavior is to look for temporal patterns in a sequence of behaviors. For example, animals often engage in a series of behaviors that are quite stereotyped (remember Niko Tinbergen's studies on fixed action patterns). Also behavior can be analyzed for structure that communicates meaning, such as bird songs, or complex ritual displays of aggression.

Open the Sequence Analysis window

You will see 6 tabs, one for **General**, in which the focal master file is specified and 5 additional tabs for different types of sequential analysis. Today you will focus on **Sequential Analysis** for simple transitions. Sequential analysis requires large datasets so the file that you have may be smaller than ideal.

The **Sequential Analysis** tab can be used to count the number of transitions between each pair of events found within each sequence. Furthermore, JWatcher will calculate the probability that an event pair occurs in the sequence as well as the probability with which a specific transition occurs. JWatcher will also calculate a z-score and p-value that tells us which transitions occur more or less often than expected by random chance. All of this information can be used to draw a kinematic diagram.

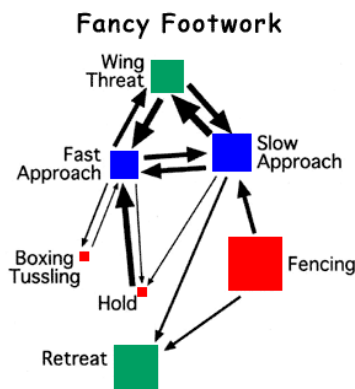


Figure 2: Example of a Kinematic Diagram (also called a behavioral state transition diagram) from *Drosophila* aggression. Aggressive (red) neutral (blue) and submissive (green) behavioral time is indicated by box size. Frequency of transitions between behaviors is indicated by arrow width.

Transition Matrix

Set the general parameters for sequence analysis.

1. Click on the **General** tab
2. open your focal master file
3. Decide which behaviors you are interested in analyzing for transitions. To keep it simple, select only events and not states. Think about how this will affect your transition matrix.

Define the sequence analysis to be performed.

4. Open the **Sequence Analysis** tab.
5. For **Focal data file(s) or folder** select your raw data file.
6. Indicate the Desktop location to create a folder with the results.
7. Specify **Lag** for analysis (for a sequence ABCD, Lag1 = AB, BC, CD and Lag2= AC, BD). It is logical to look at lag1 for most simple transition sequence analysis.
8. For the desired output select:
9. **Observed Matrix**,
10. **Transitional Probability Matrix** and
11. **Expected Z-score and p-value**.
12. Click Analyze. A file with the same name, ending in seq.res will automatically be created in a new results files.
13. The observed Matrix is a count of how many times each transition occurred.
14. The Transitional Probability Matrix reports the probability of each transition normalized to the number of occurrences of the first event of the transition pair ("row total").
15. The Z-score is a measure of the degree to which the observed transition occurs more or less frequently than would be expected by chance and is used to determine the p-value.
16. The statistical analysis necessary to compare two different kinematic diagrams is complicated and beyond the scope of today's lab.

8) Create a Kinematic diagram

1. Open the **seq.res** file in Excel.
2. Adjust the columns widths so you can view your data easily.

In PowerPoint (or another graphics program, or by hand)

3. Create a "node" for each behavior (a box or circle).
 - color code these if appropriate
 - size according to total number of times this behavior was seen.
4. Create arrows between pairs of behaviors
 - Depending upon how many behaviors you have scored, it may not be possible (or at least not pretty) to include an arrow for every transition.
 - You may choose to omit transitions that occurred rarely.
5. Use line width to indicate the observed matrix for each transition.
6. Place an asterisk next to those transitions that occurred more often than would be expected by chance. (Look at the p-value in your sequence analysis file and decide what threshold for significance you want to use.)
7. Save this file but also print a copy for your lab notebook.
8. Write a figure legend and any important conclusions.
9. You could create different kinematic diagrams for the same organism in two environments, or two similar organisms from different populations.