

DRAFT FINAL VERSION TO BE HANDED OUT IN LAB
OBSERVING AND QUANTIFYING BEHAVIOR

For this lab you will conduct focal sampling and continuous recording using the Jwatcher software package. This tool may be useful for independent projects.

While the second week of this lab will familiarize you with Jwatcher, the first week will be spent researching different hypotheses that could be tested, and establishing a protocol for behavioral observations to test one hypothesis.

- There will be both male and female cichlid fish.
- Fish can be starved for 24 hours prior to lab (requires pre-planning).
- Fish can be paired 24 hours prior to observations (requires pre-planning).
- Morphological data (total body length, standard length, fin length, body mass, etc.) may be collected.
- Additional objects could be added to the arena.
- Visual stimuli can be used during testing.
- For independent projects pharmacological manipulation can be arranged.

PART 1 – DESIGNING THE EXPERIMENT (week 1)

1A) Use Web of Science through the Reed College Library website to identify primary literature regarding cichlid behavior and develop a hypothesis to be tested.

1B) Create an EndNote library from Web of Science for efficient referencing.

1C) Observe the fish & develop an ethogram.

1D) Develop your hypothesis & an experimental plan to test it.

1E) Set up your animals for your experiment.

PART 2 – RECORDING BEHAVIOR (week 2)

2A) Familiarize yourself with JWatcher's workflow and file structure.

2B) Create the files necessary to record behavior.

2C) Perform the planned experiment and record behavior.

2D) Create files necessary to analyze your data.

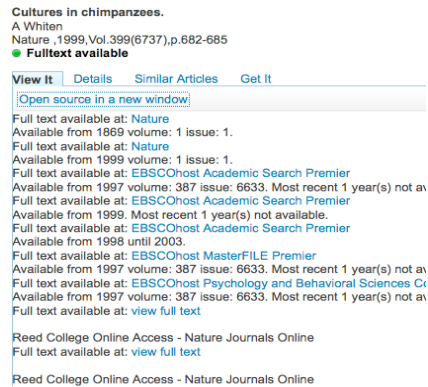
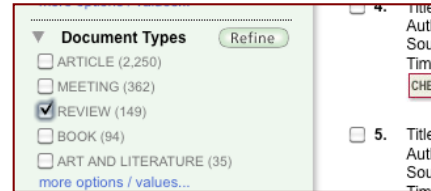
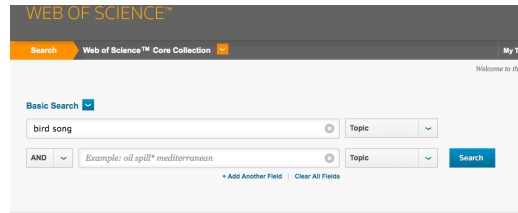
2E) Analyze and summarize your data.

PART 3 – OPTIONAL - ADVANCED ANALYSES

If you are interested, look into the sophisticated analyses that can be done.

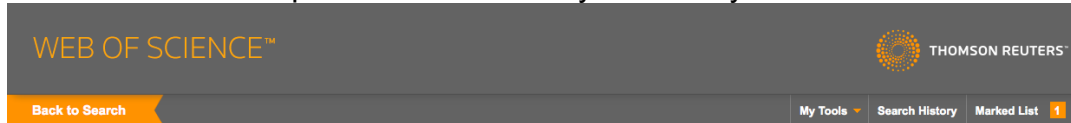
1.A.) WEB OF SCIENCE, GENERAL INSTRUCTIONS

1. Working with your partner, go to the Reed College Library Website (<http://library.reed.edu/>)
2. From the **Research** menu select **Databases**. From there select “**w**” and select “**Web of Science**”
3. Generate a list of words to describe the behaviors you are interested to study in cichlids. The more specific the terms, the more restricted the search will be. It is OK to begin very broad.
4. Choose a few of your key words and type them into the space for “**Topic**” and click “**Search**”
5. The search strategy can use Boolean Logic terms AND & OR.
6. By default, the articles are sorted according to publication date with the most recent first, but you can also sort by times cited etc.
7. When beginning a new research topic, it can be useful to find a **review article**. Use the “**Document Types**” menu to refine your search to include only reviews.
8. By clicking on the title of this article you can read the article abstract as well as other information about the article to help determine if it is relevant.
9. If this is a paper you want to read, click the “**Check Reed Holdings**” link to see if Reed has online access to this article.
10. If there is a green dot with the words “Fulltext available” Reed should have access and you can click on that to get the link to access the article. Clicking “**Get It**” will take you to Reed’s print holdings, if any.
11. If the library does not have access to this journal article (perhaps it does not have a subscription or perhaps the subscription does not cover those years) you can “**Request from ILL**”.
12. Find a few articles on your topic that are available online
13. Either download the .pdf file or open the .html version in your browser.
14. Also, save the citation to your EndNote library (see next section) you will use these to design your experiment.

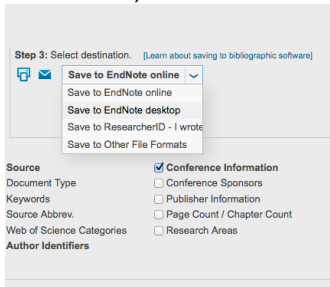


1.B.) ENDNOTE, GENERAL INSTRUCTIONS

15. In Web of Science, any citation that you want to download to EndNote must first be added to the “marked list” by checking the box near the word “Title”.
16. Web of Science keeps track of how many citations you have marked.



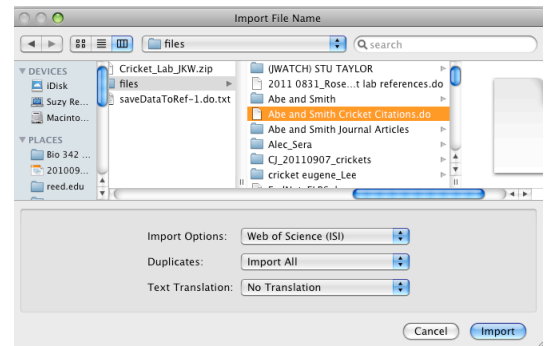
17. When have finished searching for articles export your citations by going to the “Marked List” page.
18. Here, select the EndNote button on step 3



19. These citations should automatically be saved to your download folder.
20. Move the downloaded document (ends with .ciw)
21. Open EndNote by clicking the icon in the dock or application folder.
22. From the **File** menu, select New.
23. In the popup window, name your new library and **Save** it in a logical location.

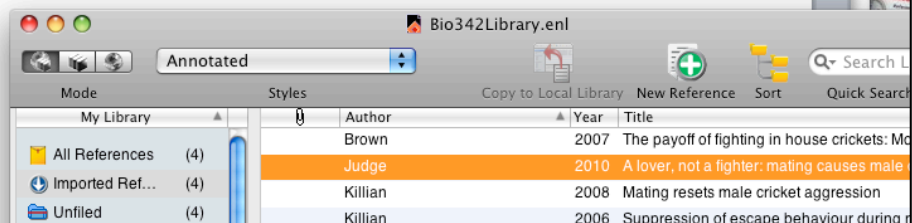


24. Use the **File** menu and select **Import...**
25. Use the popup window to navigate to the downloaded file (ends with .ciw)
26. Be sure that “**Import Options:**” is set to **Web of Science (ISI)**.
27. When you click **Import**, the citations you found should populate the endnote library.
28. In Word, you can add citations to your protocol document or write up.
29. When you get to a point for a citations in your text, navigate to EndNote & highlight the appropriate citation in the library.
- 30.



Biology lab writeup needs to have references to demonstrate where your information comes from and which research articles lead you to your hypothesis.

If your logic comes from published work about cricket lovers and fighters cite it



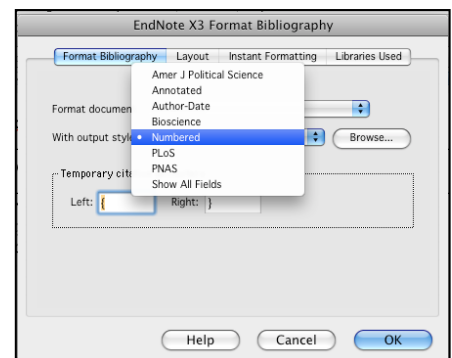
31. Return to your word document.
32. From the **Tools** menu, select **EndNote X7>** and choose **Insert Selected Citation(s)**.
33. The citation will be inserted in your word document wherever your cursor is.
34. The full citation will also be added at the end of your document.

Biology lab writeup needs to have references to demonstrate where your information comes from and which research articles lead you to your hypothesis.

If your logic comes from published work about cricket lovers and fighters (Judge et al. 2010) cite it

Judge KA, Ting JJ, Schneider J, Fitzpatrick MJ. 2010. A lover, not a fighter: mating causes male crickets to lose fights. Behavioral Ecology and Sociobiology 64: 1971-1979.

35. The in-text citations are highlighted in grey when your cursor is in them.
36. You can change the format of the citations and reference list.
37. Each Journal uses a different style
38. There is no “correct” style, and the biology department is not picky about what style you use, but you must be consistent. EndNote will ensure that your citations style is consistent. You can change between styles easily.
39. Go to the **Tools** menu and select **EndNote X7** and choose **Configure Bibliography**.
40. Use the **Which output style:** option to select the style you want. If you don't see the one that you want, use the **Browse** button to see all of the formats that are currently available to you.
41. If you are curious to know more about EndNote Web please consult with Linda Maddux, Reed College's Science librarian.



1C) OBSERVE CICHLIDS AND DEVELOP AN ETHOGRAM

In order to move from questioning to conducting research one must make **preliminary observations** and **formulate hypotheses**. These are actually two intimately connected steps, it is likely that you will return to web of science (Part 1A) and download additional citations (Part 1B) as you observe animals (Part 1C) and formulate your hypothesis (Part 1D) or as you develop an experimental plan (Part 1E) to test that hypothesis.

1. Observe the group housed cichlids using ***ad libitum*** recording. (This means that there are no constraints on what is recorded when. You simply note whatever is visible and “seems” relevant.)
2. Can you tell which are males and which are females?
3. Can you identify individual fish based on morphology?
4. Can you differentiate aggressive behavior and courtship behavior?
5. After you feel familiar with general fish behavior, develop an ethogram. You may also need to rely on descriptions from the articles you read earlier.

An ethogram is a catalogue of behaviors described by neutral postures and recognizable movements. Ideally, an ethogram should include objective descriptions that are not subject to observer interpretation. The particular ethogram used in any study will depend upon the question that is being asked, and also on how accurately, and with what specificity, a particular behavior can be scored. Your ethogram should be tailored to the experiments you plan to do.

- a. Describe behaviors using objective language.
- b. Categorize behaviors using quantitative measures.
- c. Avoid anthropomorphism and inference.

Your ethogram need not include all behaviors that a fish may potentially display. Your ethogram need not even include all of the behaviors that fish display in this staged interaction. Your ethogram should include all behaviors that may be relevant for testing your specific hypothesis. Spend a few minutes discussing with your lab partner, and consulting the primary literature. **Record your ethogram in your lab notebook.**

1D) DEVELOP YOUR HYPOTHESIS AND EXPERIMENTAL PLAN

Biological hypotheses are provisional and testable explanations for observed phenomena. (These are what motivate scientists in their career.). Biological hypotheses give rise to specific questions that are testable by observation or experimentation. Making preliminary observations (Part 1C) is a critical part of formulating a good hypothesis. Formulating hypotheses is a creative process, requiring imagination as well as knowledge. While a period of unfettered preliminary observation is often important, the observation period must be supported with background research (Part 1A) to ensure validity and relevance of the hypothesis.

Experimental hypotheses, more often called “alternative hypotheses” usually describe the experimental result that would allow a research to reject the null hypothesis. These hypotheses pertain to a specific testable question that may support the biological hypothesis. (These may motivate a scientist to go to work today.)

Statistical hypotheses use data to make decisions. An experimental result is called statistically significant if it is unlikely to have occurred by chance alone, according to a pre-determined threshold probability, the significance level. The null hypothesis typically corresponds to a general or default position (random assortment, lack of association etc.). (While strongly emphasized in Introductory Biology courses, these hypotheses are not highly motivating to most scientists.)

1. Working with you partner, based on your reading and your observation, develop a biological hypothesis. This may actually be a hypothesis that extends beyond cichlids to include aggression, or mate choice in general.
2. Write one paragraph to state your hypothesis and provide supporting evidence from the literature (use endnote to format your citations Part 1B).
3. Working with your partner and based on your reading, describe one experiment involving observation of cichlid behavior that is relevant to your biological hypothesis. (**Read ahead so that you understand the type of data to be collected with JWatcher.**)
4. Write a few sentences to explain the experiment and what results would support your hypothesis.

For example:

“In order to test the hypothesis that ...(*succinct biological hypothesis*).., we ...(*brief experiment description*).. with cichlids. If ...(*experimental hypothesis*)... the data would support this hypothesis.”

(TURN THIS IN, BY SAVING IT ON THE COURSES SERVER BEFORE YOU LEAVE LAB!)

5. In you lab notebook, write up a protocol for the experiment that you will conduct next week.
6. Remember to include negative controls (talk with Suzy or the TAs if you are unsure about this.)
7. Consider how the animals will be housed for the week.
8. Consider how many animals will be tested in each condition.
9. Consult the articles that you read to make sure these details are realistic.

1E) SET UP ANIMALS FOR THE EXPERIMENT.

Your planned experiment likely includes one or more experimental paradigms as well as the control group. You will NOT be able to do the full experiment in one class period. Instead, plan to do one experimental observation and possibly one control observation.

1. Working with your partner, collect the animals that you will need for your experiment.
2. Each pair of students will have one 5 gallon tank.
3. The animals MUST be provided with a bubble filter.
4. The tank must be covered with a lid.
5. There are clear and opaque dividers available.
6. You may use males or females.
7. You may need to tag your animals (consult Suzy)
8. You must make a plan to check on your animals to ensure that the water is full and that they are fed every other day.
9. Though beyond the scope of the lab today, consider what molecular or physiological studies might complement your behavioral analysis.

For the behaviors you plan to observe, consider whether:

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")?

Are some behaviors modifiers of other behaviors? (such as aggression which might be "modified" by an intensity score)

Are there behaviors that change their meaning in a conditional fashion? (such as an aggressive display when an animal is in its own territory or in another territory)

Are you interested in behavioral sequences or transitions?

Are you interested in the interval between behavioral events?

Are you interested in the lag time between two specific behaviors?

PART 2 – RECORDING BEHAVIOR

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 experiments. The JWatcher program includes 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. The full JWatcher program and manual is available in class or on-line at: www.jwatcher.ucla.edu.

2A) BECOME FAMILIAR w/ JWATCHER WORKFLOW AND FILE STRUCTURE.

This chart describes JWatcher work flow.

By the end of lab today you will:

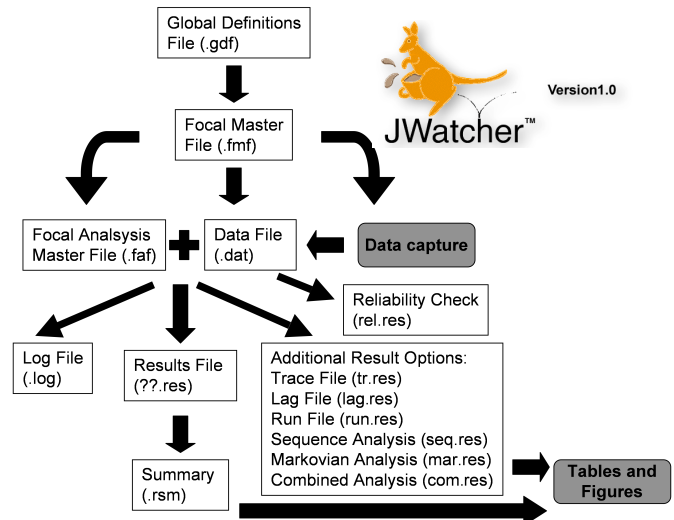
1) Create a Global Definition File (*.gdf) which will serve as the "ethogram" and assign keys to each behavior.

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 that you have recorded.

4) Create a Focal Analysis File, 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 (*.xx.res) that can be opened in Excel. JWatcher is capable of, summarizing several data files (*.rsm), quantifying conditional behaviors, testing observer reliability, and performing complex analysis (behavior combinations, sequence analysis, transitions matrices, Markovian analysis), some of which you will explore today.



As you work on the computer today, remember to use your lab notebook as a record of the steps you take, the names of the computer files that are generated, your ideas, new hypotheses, or difficulties that you encounter.

2B) CREAT JWATCHER FILES NECESSARY TO RECORD BEHAVIOR.

2B1) 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 more lengthy 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.
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.

2B2) CREAT JWATCHER FILES NECESSARY TO RECORD BEHAVIOR.

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.

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.
6. 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**.

2C) PERFORM THE PLANNED EXPERIMENTS AND RECORD BEHAVIOR.

When your animals are set and you are ready to begin scoring behavior.

Change windows by clicking on the **Data Capture** tab (upper left).

1. Click on the file navigator icon in order to name the data file you will be creating.
2. Type in the name of your new file **dat_exp_initials_datetime** in the **File Name** box and click **Open**. Keep the names simple. (**Jwatcher sometimes has problems with long names and spaces in names so try to keep file names short and use underscores for spaces**)
3. Specify your Focal Master File by clicking on the file navigator icon Selecting your **.fmf** file and clicking **Open**.
4. Click the **Next** button at the bottom right to tab into the next page.
5. Answer the questions by typing into the box below the question and click the next button to advance.
6. Click the **Next** button.

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

7. You should be looking at the **JWatcher – Data Capture** window.
8. You are now ready to start your first staged interaction. Gently introduce your two cichlids to the arena.
9. Click the **Start** button in the center of the Data Capture page.
10. Immediately type the key code representing the behavior the focal subject is currently engaged in.
11. **Whenever** the behavior changes, type the key code for the new behavior.
12. JWatcher will time out at the time specified in your focal master file or you can end the recording.
13. JWatcher will automatically save your data in the **.dat** file that you named

If you made a data entry mistake, look at the timer and make a mental note of it. You can fix it later.

14. Stage a second interaction but have your partner record the behavior.
15. give the new data file a new name that ties it to the second interaction.

If you made mistakes while recording behavior you should have noted 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. Use your lab notebook to indicate which errors have been corrected.

10. Click on the **Edit Data** tab.
11. Use the **Open** button to select the *.dat file that contains errors.
12. On the **Key Codes** tab you can **Insert Rows**, **Delete Rows**, edit the time (with elapsed (ms) or Time) and change key codes.
13. Save your data file.

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

2D) CREATE FILES TO ANALYZE BEHAVIORAL DATA WITH JWATCHER

Once you have scored a few observation periods, you are 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 reality it would be best to do this after collecting "pilot data" before collecting a lot of data). One strength of JWatcher is that you can re-analyze behavioral data in many different ways.

Basic Analysis (For today, read, but do not execute, steps in grey.)

For a basic 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. 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. Change tabs by clicking 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.
5. 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 the most accurate time budget for the interaction, it is appropriate to use "all durations", however if you are interested in precise timing of behaviors it is most appropriate to select "**natural duration**" (this will be explained in lab, or in the manual). 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. If your cichlid was out-of-sight for some part of the observations, then JWatcher can correct for this by calculating the proportion of time in sight.
6. The **Event Analysis** tab allows you to specify which behaviors should be counted as an instantaneous event, these will be counted but will not contribute to the proportion of time, thus they will not be a part of a time budget. JWatcher permits a behavior to be simultaneously analyzed as an event and also a state.
7. When you are done, 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.
8. The **Summary** button will open a text window containing all the specification in the current focal analysis master file (*.faf). JWatcher will provide "warnings" for any parameters that are likely to cause problems.

2D optional THE FOLLOWING STEPS EXPLAIN SOME OF THE OTHER TABS, THESE WOULD BE DONE BEFORE STEP 8 ABOVE.

1. Open to the **Time** tab. This is where you determine the time during which behavior is analyzed. If all of your observations are exactly the same duration, and you always start recording at the beginning of the observation you can use the default "**Score from the beginning of the session**". However, if it takes ~ 10 seconds to start recording, you may want to use a **Fixed offset**. Or you may want to start from the first behavior recorded, or you may want to start the analysis with a specific behavioral event (such as awake). The "**Time bin duration**" specifies how the observation period will be subdivided. (0 means the entire observation period is one bin). Time bins might be useful if you were studying a response over time after a specific treatment. They may also be useful if your observation periods are not a standard duration.
2. Look at the **Exclusions** tab. This is where you could decide to ignore some of the behavioral events that you recorded, but be careful, this can produce erroneous results if you do not adjust the mutually exclusive behaviors accordingly. Here you can also subtract certain behaviors from the time budget calculation (for example you may choose to subtract all of the time that an animal was asleep and create a time budget only for awake behaviors). Likely you will ignore this for today.
3. The **Conditional States** tab allows you to specify combinations of behaviors as simultaneous streams. These behaviors cannot be mutually exclusive behaviors (as defined in the States tab). Conditional states are indicated as "a|b" which provides the statistics for a the state "a" given that "b" is already on. You can specify " a|b|c " and Jwatcher will calculate both the proportion of time that "a" is on given that both "b" and "c" are both on and also the proportion of time that "a" and "b" are on given that "c" is on. (a more clear explanation will be given in lab if you are interested in this type of analysis for your observations).
To define conditional states you can either:
 - a. **Add row** and **type** your condition
or
 - b. click **Conditional states** to see non-mutually exclusive states.
4. The **Conditional Events** tab is similar to Conditional states except that the first behavior must be an event rather than a state, and therefore a "rate" can be calculated (see Events tab). Again you can only use non-mutually exclusive behaviors. (Likely these conditional analyses are beyond the scope of your behavioral observations today but the goal is to appreciate the complexity of behavioral analysis that is possible).

2.E) ANALYZE AND SUMMARIZE YOUR DATA

2.E.1) JWatcher allows analysis of files one at a time or batch processing.

1. To generate results files, click on the **Analysis** tab.
2. Use the file navigator icon open your ***.faf** file.
3. Use the file navigator icon to select your ***.dat** data file to analyze.
OR
Use the folder navigator icon to specify an entire folder with multiple ***.dat** files to be analyzed with batch processing.
4. Specify the **Desktop** to save the results file by clicking on the folder icon and then click **Open** (note: this file is automatically created and named, you are just verifying that you know where it will be placed. However you can give the folder a name that is more informative than simply "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.

You can use Excel to view the results files.

There are two results files, ***.cd.res**, and ***.tr.res**.

The ***.cd.res** file has quantitative results. The ***.tr.res** is not useful for most analyses.

View the ***.cd.res** file by opening it through **Excel**. Excel will **NOT** automatically recognize JWatcher files; you will need to tell Excel do it. The ***.cd.res** file is a comma delimited text file. (ask if you need help.)

Once opened, you should see a list of the behavioral codes and several summary statistics for each behavioral code.

Each of your result files will relate to just one of your observations.

If your cichlid never went out-of-sight, then proportion of time and proportion of time in sight should be the same. The proportion of time in sight for your behaviors is the time budget.

It is a good idea to check your results files and familiarize yourself with their contents before going on to more sophisticated analyses. JWatcher includes several algorithms for summarizing and quantifying behavioral data, however, in your own research you may want to perform statistical tests that are not a part of the JWatcher package and you would use these results to do that. For analysis outside of JWatcher it may be necessary to reformat results either by cutting-and-pasting in Excel or using your own computer script (possibly written in PERL). This is beyond the scope of today's lab.

2.E.2) Summarizing Analysis Results

JWatcher includes algorithms to provide summary statistics for the analyzed result files. You may want summarize subsets of your observations, for example you might want to summarize the results for females separate from males, or experimental animals separate from control animals.

1. Click the **Summarize Results** tab.
2. Select the folder containing your results files to be summarized. All files to be summarized MUST have been analyzed using the same focal analysis master file (.faf)
3. Choose a file name and folder for the summary.
4. Generally, **Analyze Results in Rows** provides a format that is easiest to work with in excel.
5. Choose the appropriate summary statistics
6. Click **Summarize Results Files**.
7. The resulting file is a comma delimited file that can be opened in Excel.
8. Use this file to make graphs, or figures that are informative regarding your hypothesis. A pie chart works well for a time budget, though a bar graph allows you to include error bars. It is up to you to present you data in the most informative and effective format.
9. Briefly record your conclusions (if any) in your lab notebook.

2.E.3)

1. Working with you partner, open you document from last week.
2. Based on your experience today, write a one paragraph description of the methods that you used. This should be an accurate representation of what was done.
It should include:
 - a brief description of any equipment used,
 - information about how the animals were housed,
 - actual sample size that was tested and
 - citation of any relevant articles from which you drew methods.
3. Do not worry about statistical analysis!
4. Briefly describe the results and include at least one figure with an accurate figure legend.
5. In this document, state whether or not your results support you biological hypothesis and explain why (or why not).

(TURN THIS IN, BY SAVING IT ON THE COURSES SEVER BEFORE YOU LEAVE LAB!

Be sure that all the name of every member of the group is on it)

also

The following items should be saved by each student team in a folder on the courses server (it is ok to zip them together and upload one file)

a global definition file (.gdf)	result files (cd.res)
a focal master file (.fmf)	the summary (.rsm)
a focal analysis file (.faf)	the endnote library
all raw data files (.dat)	

EXTRA Part 3) Checking Your Reliability**Eliminate this step but consider why it is important for a research project.**

If you have several assistants collecting data for you, it would be necessary to demonstrate that they are all coding behaviors in the same way and that there is no confusion between the behaviors defined in the ethogram. If your assistants are not reliable you would want to offer further training before sending them out in the field. If you are collecting all of your own data, it would be helpful to demonstrate to yourself that you are accurately able to code the behaviors. If you are not reliable you would want to redefine your behaviors, choose different keys, or record fewer behaviors.

1. Click on the **Analysis** tab and select the **Reliability** page.
2. Select the two data files you wish to compare (simultaneous observations of the same individual).
3. Name the reliability output file and indicate where this file should be created.
4. Click **Calculate Reliability** to perform the calculations. The file extension .rel.res will automatically be added to your file name and this comma delimited text file can be opened in excel.

There are three main parts to this file:

1. The **confusion matrix** tabulates the number of agreements between two data files, broken down by key code. Relatively large values found in an off-diagonal cell would indicate a systematic bias that needs to be addressed.
2. The **statistics section** tabulates results from the confusion matrix. The table includes the percent agreement and kappa coefficient. Cohen's Kappa adjusts the percent agreement by incorporating a correction for the probability of agreement due to chance. It is based on the percent agreement for keycodes without respect to the time at which those key codes occurred. For more discussion of reliability measures see Martin and Bateson pg 120 - 124.
3. The **list by line** is a simple juxtaposition of the two data files.

If you and your partner did not score the behaviors reliably, discuss the cause and make adjustments in your ethogram if necessary.

EXTRA Part 4) 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 and 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.

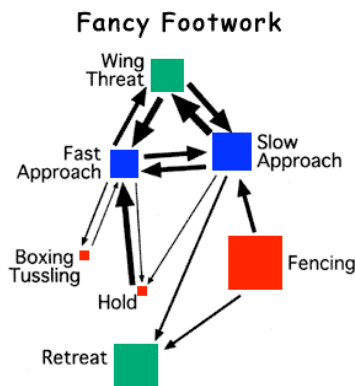


Figure 2: Example of a Kinematic Diagram (also called a behavioral state transition diagram) from *Drosophila* aggression. Aggressive (red) neutral (blue) and submissive (green) behavioral time is indicated by box size. Frequency of transitions between behaviors is indicated by arrow width.
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4A 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.

4B Create a Kinematic diagram

1. Open the **seq.res** file in Excel.
2. Adjust the columns widths so you can view your data easily.
3. Print this out to put into your lab notebook.

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

1. Create a node for each behavior.
 - color code these if appropriate
 - size according to row total if appropriate
2. Create arrows between these 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 rarely occurred.
3. Use line width to indicate the observed matrix for each transition.
4. 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.)
5. Save this file as a Powerpoint presentation but also as a jpeg file (so that you can easily insert it into a word document later)

EXTRA) Part 5) ADVANCED ANALYSIS IN JWATCHER

5A Markovian Analysis in JWatcher.

OPTIONAL

Markovian analysis examines the overall structure of a series of behaviors, as opposed to specific pairs that are analyzed with transition matrices. Markovian analysis determines whether serial dependencies exist within the sequence. For example in the English language the letter "q" predicts that the following letter will likely be "u". This is an example of a first order Markovian model: one event determines the event that immediately follows. A second order Markovian model is one in which an event is predicted by two immediately preceding events. A second order process is the limit of analysis available in JWatcher. (note: many protein structure and gene finding algorithms rely on more complex Markovian models).

5B Substituting or Combining.

After viewing your data you may decide that your ethogram was too detailed and you would like to combine 2 or more behaviors (for example you might decide to count both bite and chase as "aggression"). Note that combinations may generate a series of repeated behaviors that could affect state statistics.

1. Click the **Combinations Master** window tab.
2. Click **New**
3. Select the **focal master file** that you want to modify
4. Use **add row** in order to create a new key code and behavior name for the combination and the key codes that are to be combined (Note: this key code is not part of your global definition file. It is important to track such changes).
5. Save this new focal master file. (**newname.fmf**)
6. Click **Next** to open the **Combine Key Codes** window
7. Select the focal data files or folder for which you want to combine behaviors. This will create new data files and leave the originals in place.
8. To analyze the new combined data files you will need to create a new focal analysis master file (*.faf) using the codes defined in the newly created focal master file (*.fmf).