Scototaxis Scoring

This protocol describes how to score the Scototaxis assay we conduct in the lab using the logging software Cowlog.

AIMS of the study

We want to know whether a fish's anxiety and/or exploration tendencies are shaped by the social environment it grew up in. In fish, we measure anxiety and exploration in an assay called scototaxis, which was developed to study stress in zebrafish.

A fish is placed in an experimental tank, half of which is bright and light colored while the other half is dark. We measure anxiety as the amount of time they spend in the dark half and exploration as the amount of time they spend in the dark half. Further, we measure thigmotaxis, or the tendency to be near a corner or edge, as another measure of anxiety. We also want to know if this behavior is consistent and repeatable.

You will be given videos of fish who have been reared in different social environments to score. Importantly, you won't know what environment she was raised in (as it is important for you to not introduce your own assumptions when you are scoring these videos). All the videos will have super strange names, and *it is very important that you copy the exact name structure when you score these videos in cowlog* (more info in Box 1, page 3).

Each of you will be given 16 videos to score on a USB stick; and you have 1 week to finish the scoring. (Your USB stick is due back in class by the following Monday's lecture period.) Once all the data is in, you will get to do statistics on the dataset as a class and see if the fish's sociability/shoaling behavior was repeatable within an individual AND whether it differed by the social environment she was raised in.
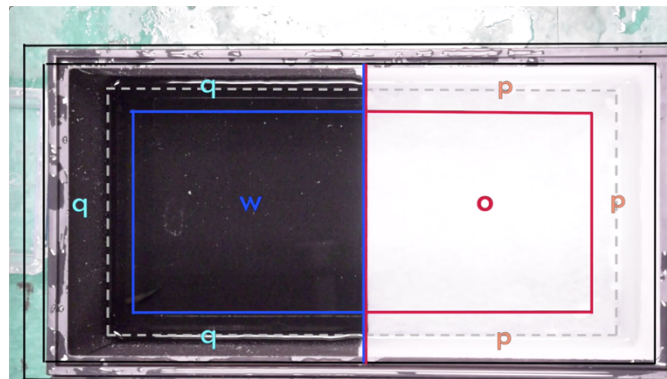
For this week

This week, you will be scoring 16 Scototaxis videos. The videos are already loaded on your USB sticks, in the folder 'Scototaxis'. Before doing anything else, access your USB stick and create a new folder called FRI_Scototaxis_YOURLASTNAME. Once you have created the folder you may proceed to the following steps.

Begin scoring Scototaxis videos by following each step.

Step 1. Open Cowlog.

Step 2. Create a new Cowlog project.

Figure 1a. Create a new Cowlog project.

a. **MAC:** Create a new project by clicking on the 'Cowlog' menu, located at the top left corner and select 'New Project' (Figure 1a). **PC:** same as for mac, except when you open Cowlog, click "Project" on the toolbar, dropdown to "New Project".
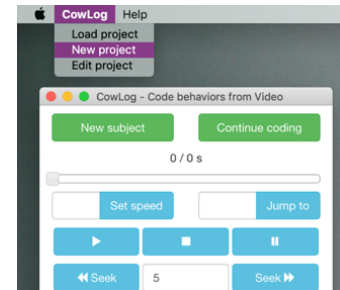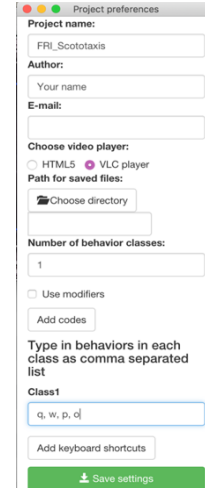
This brings up the 'Project preferences' window (*Figure 2*). Fill it out like so:

b. **Project name**: name the project 'FRI_Scototaxis'

c. **Author**: your name

d. **E-mail**: may leave blank

e. **Choose video player**: choose VLC player

f. **Path for saved files**: this tells Cowlog where to save scored files. Choose whichever directory you would like those to go in.

g. **Number of behavior classes**: type the number '1'.

h. **Use modifiers**: leave the box unchecked.

i. Now click 'Add codes'.

Figure 2. Project preferences

j. In the 'Class1' box type the following letters, each separated with a comma (Figure 2j): [**q, w, p, o**] These letters are the keyboard keys you will use to score each video. Once you have typed the letters, click 'Add keyboard shortcuts'

Figure 2j. Type in the corresponding keys in the Class1 box, as shown here.

k. Type each letter again in its corresponding box (Figure 2k). Then click 'Save settings'. You will be prompted to save your Cowlog project. Do so and you will be ready to start scoring videos.

Figure 2k. Type these letters as you see them here in the Class1 box. These will be the keys corresponding to each zone.
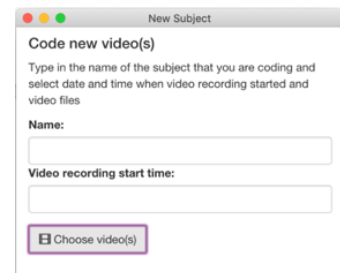
Step 3. Create new subject.

a. Now that you have created your project, each time you open Cowlog to score a Scototaxis video, you will first select 'Load project' from the top left Cowlog menu (Figure 3a) and select your Cowlog project. You only have to load the project when you first open Cowlog. Once your project is loaded continue to the next.

Figure 3a. Load the Scototaxis project EVERY time you open Cowlog to score Scototaxis videos.

b. Click 'New subject'. Each video you score will be a new subject, so you will do this for every video.

c. In the 'New Subject' screen, click 'Choose video'. Now select the video you want to score from your USB stick.

d. Once you choose your video file, you will be able to see the location and name of the video (Figure 3d). Transcribe the title of the video (blue box) exactly as you see it on your screen (remember not to copy the title in this example, as it is an example and not the video you are scoring).
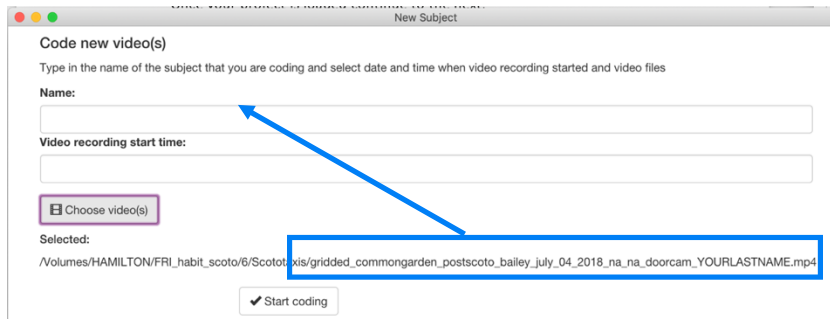

Figure 3c. Select the video you want to score.

Figure 3d. Transcribe the title of the video (red box) and transcribe it into the 'Name:' box so that it follows the format of the blue box.

VIDEO NAMING FORMAT (Box. 1)

The naming format for the Scototaxis videos is as follows:

gridded_commongarden_postscoto_bailey_july_04_2018_na_na_doorcam_YOURLASTNAME.mp4

When you name each new subject in Cowlog (Step 3d), you will have to transcribe the name of the video exactly, followed by your last name.

Original video name:

gridded_commongarden_postscoto_bailey_july_04_2018_na_na_doorcam_YOURLASTNAME.mp4

What you will name your file in Cowlog:

gridded_commongarden_postscoto_bailey_july_04_2018_na_na_doorcam_SMITH.mp4

*note that the only change is to add an underscore followed by your last name in all caps.

e. Note that after your score a video, cowlog uses this name as the name of the output csv sheet. *It also adds a string of numbers to the name you wrote. Please delete this string,* which will be anything in the filename that appears after your last name. For example, for an original video named: 'gridded_commongarden_postscoto_bailey_july_04_2018_na_na_doorcam_SMITH. mp4

Will be named as:

"gridded_commongarden_postscoto_bailey_july_04_2018_na_na_doorcam_SMITH",

but cowlog will output a csv to your usb stick with the following name:

"gridded_commongarden_postscoto_bailey_july_04_2018_na_na_doorcam_SMITH _2019-01-29T180106_7".

*Please delete everything after YOURLASTNAME, (e.g. **2019-01-29T180106_7**).*

f. Leave the box 'Video recording start time' blank. Once you have chosen the video and named the Cowlog file, click 'Start coding'.

Step 4. Scoring the video.

    a. You should now see the video loaded up in a new window called 'Cowlog video' (figure 4a). The video is sectioned into different regions of the tank: q, w, p, o.
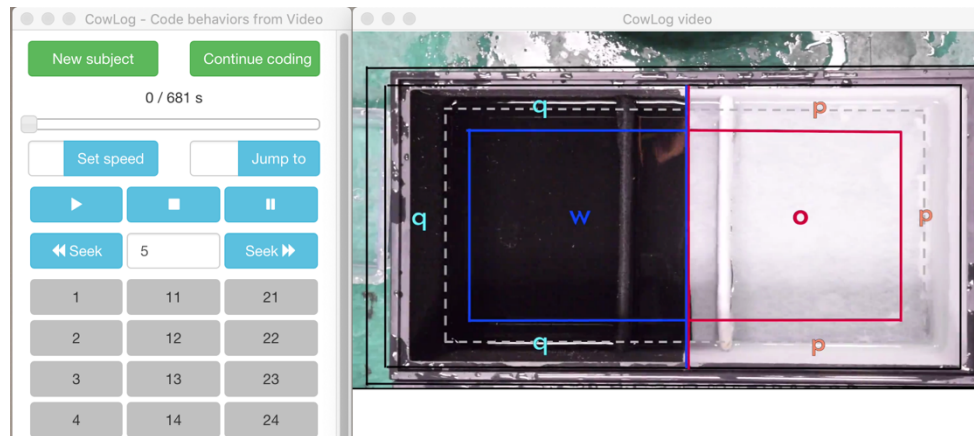

Figure 4a. There are 4 zones: q, w, p, o.

We are interested in scoring WHERE the fish is throughout the video. Place fingers on the 'q', 'w', 'p', and 'o' keys on your keyboard.

    b. When you are ready to start scoring your video, you will click the play button. This will begin playing the video. The fish will be placed in the tank at different times in each video, so play the video and find the time in which the fish is put in the tank. Go back a few seconds (right before the fish is put in the tank) and press play. As soon as the video begins to play and the fish is put in the tank, press the key corresponding to the location of the fish.

As you score, you are watching for the location of the fish, so that if it is in the area labeled 'o' (the white zone), you would press the 'o' key on the keyboard. As soon as it crosses to a new area, press the corresponding key on your keyboard.

    c. <u>If you make a mistake</u>, press the pause button and the press "undo". This will take you back to your previous keystroke and will let you start again from there, overwriting your error.

d. <u>When the video is finished, press "End Coding".</u> Nothing will appear to happen when you press this, but only press it once please. Close the video window and a .csv sheet will appear in the folder you selected when you created the project. Double check to make sure the datasheet went to where you meant it to.

e. To start your next video, simply click "new subject" again and continue as before.

f. When you are finished scoring your videos, make sure the Cowlog .csv files are on your USB stick to turn in by next Monday.