Drinks Like a Fish - Student Handout #2

Acoustic Startle Test (to be run the day AFTER treating the fish)

Zebrafish embryos have a startle response that allows them to react to vibrations in the water. This usually occurs around 5 days of development. Changes in the startle response may be indicative of neuromast (neuron) damage.

Before testing

Before testing any fish, take a look at the wells. Make sure the fish are alive. A dead fish will usually be floating upside down. Discard any dead fish with a pipette before starting the experiment. Record if there are any dead fish in your data sheet.

Performing the Acoustic Startle Test

1. Keep a beaker of egg water in the incubator to keep it warm. Keep a second beaker of egg water (room temperature is OK) for "waste".
2. Prepare a 10 cm petri dish with 25 ml of egg water prewarmed to 27º-28ºC in the incubator. Put the petri dish over a piece of white paper so you can visualize the fish easily.
3. One student (who is not blind to the treatment conditions) should transfer one of the fish from a well to the 10 cm petri dish using a plastic transfer pipette. Wait approximately 30 seconds to 1 minute for the fish to acclimate to the new dish.
4. During the acclimation period, observe the fish to see that it's wiggling/swimming in the petri dish. Take notes on your observations.
5. Using your pen or pencil, Tap ONCE on the edge of the dish. This is the stimulus for the acoustic startle reflex. Make sure to tap with the same part of the pen each time you administer the stimulus. Very important--you want to do it the same way every time! DO NOT tap more than once.
6. Observe the fish immediately after the tap. The response to the tap may range from no movement (a 0 on the rating scale), to a tail wiggle (1 on the rating scale) to darting across the dish (3 on the rating scale). Each observer should write down a score for the movement on their Student Worksheet using the movement scale below. Also record any other observations.

   The movement scores are:
   0 = no movement (it could be dead--if so, record that as well)
   1 = wiggles
   2 = swims 1 body length
   3 = swims more than 1 body length

   After recording the movement rating, move the fish with a plastic pipette into the waste beaker containing egg water

7. Repeat steps 3 – 6 for each fish (that means 60 of them!).
   Replace warm egg water as needed.

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**** Important ****

If the fish and egg water are left out too long, the water gets cold. As a result, the fish won't move as much. Keep a beaker of egg water and the zebrafish to be tested in the incubator until you're ready to test them.

8. What do I do with the fish when I'm done?

   Fish should be euthanized in a solution of Tricaine (a potent anesthetic). This will be prepared by your teacher. After the experiments are over, using a plastic pipet, move the fish from the "waste" beaker and any other beakers or plates, into the Tricaine beaker. Once the fish have been euthanized, you can safely dispose of the remains in the sink. Euthanasia is complete in approximately 10 minutes.

Acoustic Startle Test Data Worksheet

Make your own data sheet like the one below. The first line is just an example as to how you would fill it in. You may need 60 lines! (6 wells x 10 fish per well) Remember - You won't fill in the actual treatment code or treatment until after your teacher breaks the code!

New Code	Movement Score	Actual Treatment Code	Actual Treatment	Other observations during Acoustic Startle test
Q-2*	2	B	0.1% ethanol	

*this means the 2nd fish from well labeled "Q"

Data Analysis (Tables or Graphs)

Once you are done with the experiment, re-organize your table by Actual treatment code. Put all A's and D's first (the controls), etc.

Then, plot your data in a graph or list in a table.

Do not get average scores of the 10 fish per group. Rating scales don't lend themselves to averages (i.e., there is no such score on the scale as a 2.5). Instead, list the number of fish that have a certain score in each group. This is an example of plotting a frequency (e.g., 8 out of 10 fish had a score of 2). Here are some examples of how you can present your data (using "made-up" numbers):

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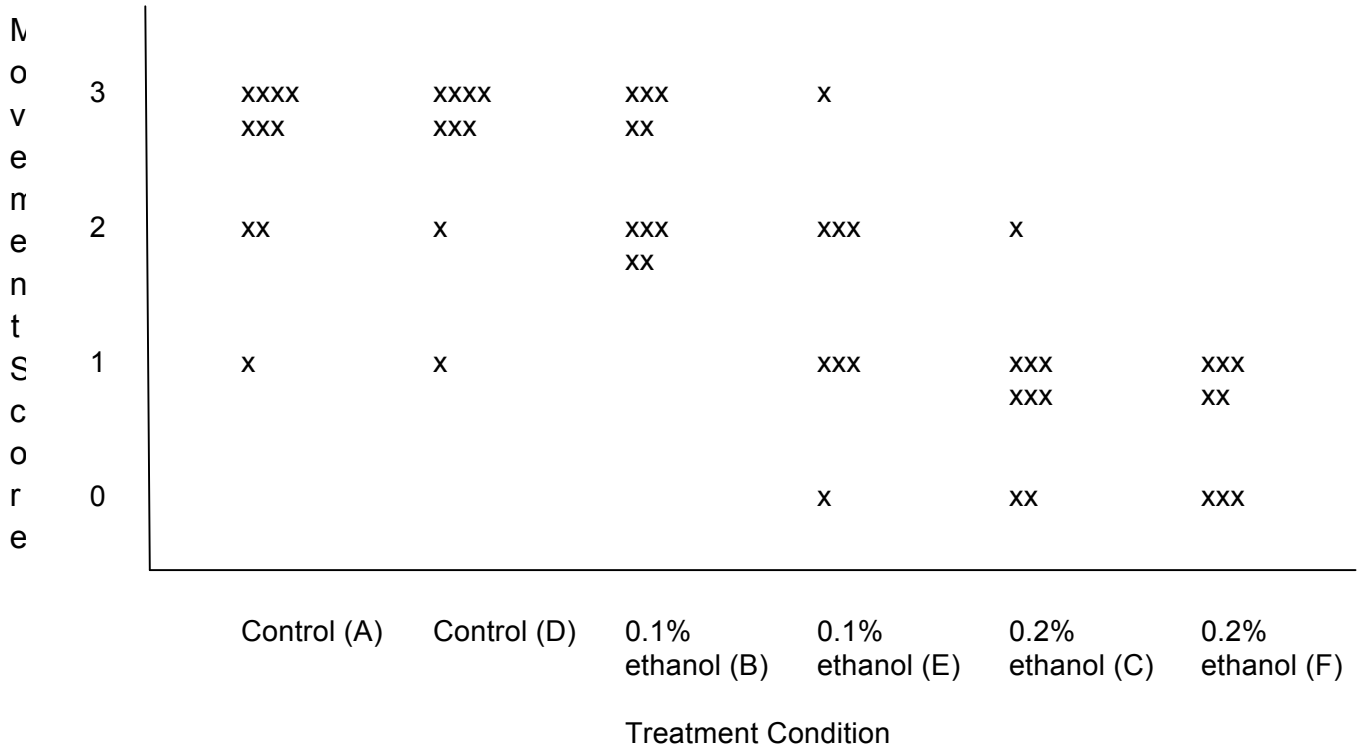
Possible table:

Code: Treatment	Score				Total fish*
	0	1	2	3	
A: Control		1	2	7	10
D: Control		1	1	7	9
B: 0.1% ethanol			5	5	10
E: 0.1% ethanol	1	3	3	1	9
C: 0.2% ethanol	2	6	1		9
F: 0.2% ethanol	3	5			8

*do not count fish that died or were not scored for some reason (dropped on the floor?)

Possible graph:

Put an "x" in the score slot for each fish as listed in the sample table above. You could also draw a line for the "median" score for each condition. Don't forget to LABEL YOUR AXES!



Once you have plotted your data in a table or graph, now you can look at it to discuss what you found from doing the experiment. Did alcohol in the concentrations tested damage the neuromast? Do your duplicate conditions agree with each other?