Drinks Like a Fish – Student Handout #1

Does Alcohol Disrupt the Acoustic Startle Reflex* in Zebrafish?

(Disruption of acoustic startle reflex reflects damage to the neuromast)

Instructions for ethanol dilutions

1. **Make a stock solution of ethanol (10%)**
Depending on the concentration of ethanol you have on hand (e.g., 100%, 95%, or 70%), make a 10% stock solution in egg water. Make about 10 mls. Use the table below to calculate how much of each reagent to use. The first line is filled in for you if you are starting with 100% ethanol.

Starting ethanol concentration	# of mls of ethanol	# of mls of egg water	total # of mls
100%	1 ml	9 mls	10 mls
95%	1 ml		
70%	1 ml		

2. **Make working solutions of ethanol (0.1 and 0.2%)** from the stock. Make about 100 mls of each working solution. Fill in the table.

Stock ethanol concentration	Working ethanol concentration	# of mls of stock ethanol	# of mls of egg water	total # of mls
10%	0.1%			100 mls
10%	0.2%			100 mls

It's best to prewarm the solutions to 27º-28º C if you are doing the entire experiment at that temperature. If not, just use room temperature.

Set-up your treatment plates (6-well plates)

1. Take a clean 6-well plate, labeling the lid with code letters according to the chart below (A-F). You have 3 treatment conditions: no ethanol (control), 0.1% and 0.2% ethanol. There are enough treatment wells to do each condition in duplicate. Doing duplicate conditions increases your accuracy, and is helpful in case you make a mistake.

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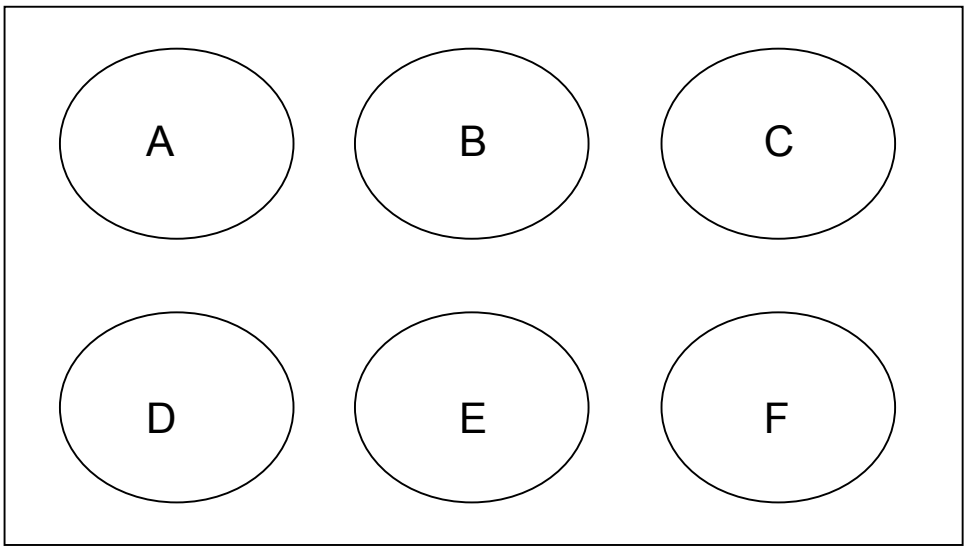
Well Label	Solution for Experimental Treatment (9 mls)
A	Control – Egg water alone
B	0.1% Ethanol
C	0.2% Ethanol
D	Control – Egg water alone (duplicate of Well A)
E	0.1% Ethanol (duplicate of Well B)
F	0.2% Ethanol (duplicate of Well C)

2. You can label the wells as follows (use a “Sharpie”)
   Label the net-inserts the same way.



3. Add 2 mls of the corresponding solutions (pre-warmed) to each well (use your table above)
4. Add the net-inserts into the wells
5. Add the remaining 7 mls of solution into the net well-insert (this helps avoid air bubbles)

<u>Expose the 4 day old zebrafish to the treatment solutions</u>

1. Transfer 10 zebrafish into the net well-inserts containing 9 mls of prewarmed egg water (with or without alcohol) using a pipette
2. Place the lid on the plate and then wrap the plate with parafilm or plastic wrap to prevent evaporation.

3. Put plates in incubator/oven at 27º-28º C for 18-24 hours. If you incubate at room temperature, it will take longer to produce the neuromast damage.

4. The next day, fill 3 more 6-well plates with fresh egg water at 27º-28º C (9 mls/well). Be sure the net wells themselves are labeled and not just the 6-well plate!! Transfer the net wells (individually) into one of the new plates containing fresh egg water. Allow them to sit for 5 minutes. Repeat two more times. This is a "wash" step and ensures that no residual ethanol remains.

5. Your fish are now ready to be tested in the super-duper acoustic startle apparatus. Go to Student Handout #2.

Attention!!

Before doing any testing, you need to be "blinded" by the treatment well. Your teacher will reassign the well designations with new letters and keep the "code" secret until your experiment is over. This will avoid any bias on your part while you are giving your fish their movement scores. After the experiment is over, your teacher will "break the code" and you can write the actual treatment into your data column.