The Effect of Different Food Coloring on the Development and Hatch Rates of Zebrafish

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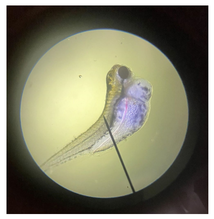


Introduction

The purpose of this experiment is to determine the effects of food dye on zebrafish development and hatching rates. Based on research by Radhika Gupta and et al in Delhi, India, Red No. 3 (erythrosine) is shown to be embryotoxic at concentrations of ≥0.05% and decreased hatching rates. Yellow 5 Lake (tartrazine) needs a higher concentration to be embryotoxic at ≥0.5% but can also increase hatching rates. The used food colorings have the following ingredients: Red: water, propylene glycol, FD&C red 40 and 3, propylparaben (preservative); Yellow: water, propylene glycol, FD&C yellow 5, propylparaben (preservative), FD&C red 40. Leading to the question of: how do food colorings impact zebrafish embryos and, thus, humans, too?

Investigated hypotheses:

1. If different food colorings are used in the environment of zebrafish embryos, then the yellow group will have the highest hatch rate while the red group will have a lower hatch rate, as previous research has shown that tartrazine has a positive effect on hatch rates while erythrosine has a negative impact.
2. If different food colorings are used in the environment of zebrafish embryos, then the orange group will have the least amount of development and the control will have the most as the orange will have too much variance from what a zebrafish natural habitat is while the control is more like its habitat.

Abstract

This experiment was conducted to see if food coloring, which is used on a daily basis in the kitchen, is harmful specifically to zebrafish (*Danio rerio*) embryo development. There were 15 zebrafish placed into each group (control, and red, yellow, and orange dye). The control group did not have any food dye, the group with red dye was placed in a concentration of 0.025%, the group with yellow dye was placed in a concentration of 0.25%, and the orange group was put into a 4:1 ratio of yellow to red at a concentration of 0.274%. The fish were observed over a period of 7 days. Quantitative and qualitative data was collected on how many fish were alive and hatching rates on days 1, 2, 3, 4, and 7. The different food dyes were demonstrated to be harmful to the zebrafish as many of them died or became deformed. If a human embryo were to be exposed to the chemicals from food coloring, deformations may occur.

Results

The control variables include temperature (28.5°C), wells, water, instant ocean, and light. The independent variable is the food coloring used (control, yellow, red, and orange). The dependent variables are zebrafish embryonic development and hatching rates.

The total count of noticeable deformations is 0 for control, 3 for red, 1 for yellow, and 2 for orange.

Data Analysis

Based on the graphs and data, it is clear that the control had the highest survival rate (100% for 7 days), however, yellow has a faster development rate as the fish in yellow were the only ones to reach 120 hpf. The control has the largest hatch rate (all 15) while orange has the lowest hatch rate (only 4 of 15). Overall, the most zebrafish hatched on day 4. Red was overall the most lethal (only 3 alive by 7th day) but orange (mix of 4:1 yellow to red coloring) was lethal the quickest (caused the death of 5 fish by 2nd day).

Conclusion

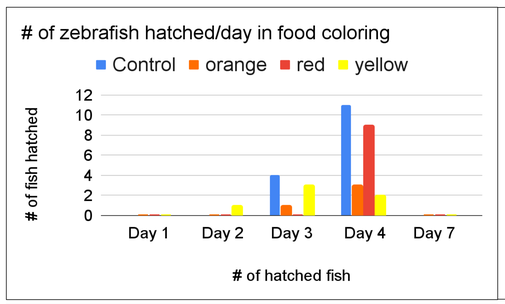
The first hypothesis was partially supported. This is shown by the graphs where the control group has the largest hatch rate, however, yellow did at least have the first and quickest hatches. Red did, however, have a slower hatch rate than the control. The second hypothesis is partially supported, shown by the graphs again as orange did in fact have the least amount of development while yellow had the most (as opposed to the control).

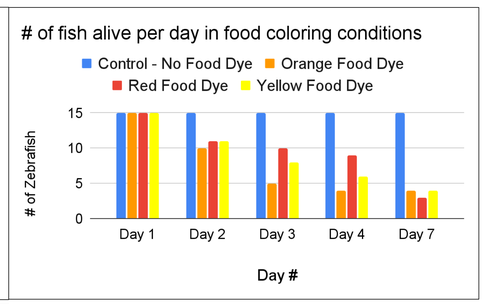
Yellow had the most amount of development and would likely have had the largest hatching rate if there were no complications caused by the food coloring itself. Yellow does have a positive impact on development (which thus includes hatching) but overall is still quite dangerous; red is very dangerous, even in very small concentrations. This can show how dangerous food colorings are on living and developing organisms, especially when applied to zebrafish living within these concentrations. Case in point, some of the fish within the food coloring conditions were hatched but had severe deformities (curving backward along their spine).

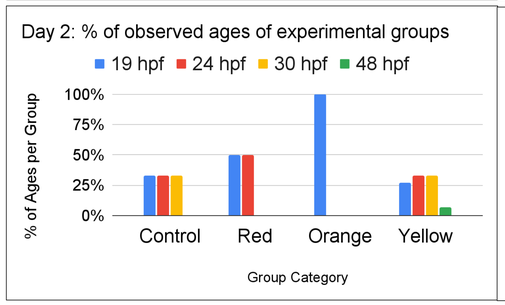
There were many uncontrollable aspects within the experiment: difference in ages of embryos when collected, stress during transport, “jumps” in data over the weekend (as data could not be collected), pure food coloring ingredients were not available, other ingredients were included in coloring, data collection stopped on the 7th day. If the experiment is repeated, a larger experimental group should be used and/or done on other types of organisms. Using the pure ingredients of the food coloring should produce more clear-cut results. Since the results semi-support the research done in the introduction, it is worth questioning whether the data was disrupted (compared to previous experiments and research) or if there are new conclusions based on the use of commercially available colorings overall.

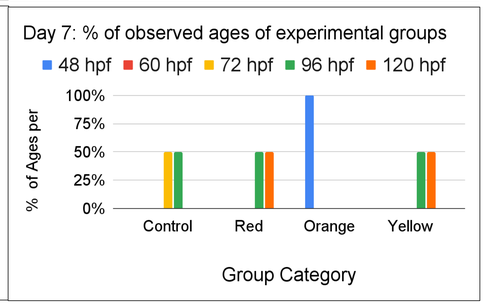
Methods

1. Gather materials:
-Ocean solution (40x stock), degassed/ deionized water, thymol blue, red and yellow (McCormick) food coloring, pipettes and micropipettes, labeled beakers, graduated cylinder, dissection microscope, compound microscope, stir stick
2. Measure 50 mL water w/ graduated cylinder and put into the labeled beakers (4 w/ 50mL)
3. Measure food coloring w/ micropipette
    -red: ≤0.025% = 12 µL/ 50 mL deionized water
    -yellow: ≤0.25% = 125 µL/ 50 mL deionized water
    -mix: 137 µL (4 yellow drops:1 red drop ratio)/ 50 mL water
4. Measure ocean stock solution and add a drop of thymol blue
5. Add 1 mL of respective solution into corresponding (labeled) well
6. Add 5 embryos per well. Collect qualitative data and observations









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