Taurine and DMSO prove an uninhabitable environment for Planarians <u>Luna, E.</u>, Peraza, A., Rivera, N., Sustaita, I., Torres, B., **Gonzalez-Gonzalez, A.**, Lab Section C: 04/8/25

Introduction:

Planarians are widely used as a model for learning about regeneration in the scientific field due to their unique regenerative abilities (Gonzalez-Gonzalez, 2025b). These planarians can reproduce asexually, giving them the ability to be immortal (Gonzalez-Gonzalez, 2025b). When a planarian is cut along their body in any direction, they can completely regenerate through the use of stem cells (Gentile et al, 2011), and other cells that have unique functions. Cells called neoblasts direct nearby cells to grow into a specific state so that the planarian may regenerate with full functionality (Arias et al, 2018).

Dimethyl sulfoxide (DMSO), a cryoprotectant (Tekade, 2019), is used in various research studies to study the effects of cell damage and ice crystal formation, as well as allowing a hydrophobic drug to be dissolved more easily in water (Pagán et al, 2006). To be able to understand the effect of a drug on an organism, it is crucial to understand the drugs EC_{50} . The EC_{50} of a drug is the concentration of the compound that gives a 50% effectivity rate at a given time interval (Gonzalez-Gonzalez, 2025b). In previous experiments, it was found that soaking planarians in any solution of DMSO over 5% for a period of over 5 minutes caused significant harm and led to the death of the planarians (Pagán et al, 2006).

Taurine acts as a mild stimulant and neurotransmitter and a neuroprotective agent to prevent injury to neurons (Wu et al, 2010). It maintains cellular structure and is essential to the central nervous system (Wu et al, 2010). There is a numerous amount of Taurine amino acids in the brain because it is important to neuron development, and because planarians are so similar to humans neurologically, the benefits of Taurine on neuron development have been previously studied (Livengood et al, 2024). Taurine is often used to protect stem cells that are used for regeneration (Livengood et al, 2024). From past experiments, data was collected of planarian regeneration in artificial pond water (APW) of 3 planarians with a regeneration rate of 100%. For this experiment brown planarians were used.

<u>Rationale:</u> Understanding the role of Taurine is important to scientific research with planarians because it implies that a planarian in Taurine would have its neurons and neoblasts covered in a protective layer. This should shield the planarian in harmful environments like DMSO, but should not affect their regenerative abilities.

Objectives:

From this experiment, both the effect of Taurine on a planarian's regenerative ability and understanding of how much longer a planarian from various solutions of Taurine should live in comparison to planarians coming from artificial pond water (APW) was hoped to be found.

Hypothesis:

A planarian living in various solutions of taurine will regenerate as normally as a planarian in APW does, but will exhibit normal behavior a 10% solution of Dimethylsulfoxide (DMSO) in intervals of 5 minutes up to 30 minutes, which the planarians from the APW will not exhibit.

Methods:

This experiment measures the percent regeneration of planarians in various concentrations of Taurine and the activity of planarians when transferred to a 10% concentration of DMSO. 2 planarians were taken from a predetermined solution of APW (an unknown mixture of salt and

spring water) and were cut laterally into thirds, with sections being the head, the trunk, and the tail, placed into the same container of a solution of Taurine. This was done a total of 5 times for the five different Taurine concentrations. Both Taurine and DMSO solutions were measured through the formula for finding a dilution given a stock solution.

$$C_i V_i = C_f V_f$$

 $(C_i)(200ml) = (0.01)(10ml)$

Solutions of 0.01mM, 0.03mM, 0.1mM, 0.3mM, and 1mM of Taurine mixed with APW were calculated, measured, and distributed into different containers as done by previous experiments (Livengood et al, 2024).

As previously stated, the independent variable was the varying solutions of Taurine and the dependent variable was the percent of planarians regenerated and the activity and behavior of the planarians coming from the solutions of Taurine. Contrary to the recommended 3 trails per experiment, only 2 trials of this experiment were performed due to time constraints.

Controls were taken from previous experiments that overlapped the goals for this experiment. Given that the experiment was to last around 30 minutes, the solution of 10% DMSO was determined from the EC_{50} of 10% DMSO a previous experiment, where there was noticeable change from the beginning of the experiment to the end. A dilution from a given stock solution of 15% DMSO was calculated.

$$C_{i}V_{i} = C_{f}C_{f}$$

$$(.15)(8) = (.1)(C_{f})$$

$$\frac{(.15)(8)}{(.1)} = C_{f}$$

$$12ml = C_{f}$$

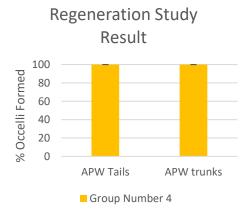


Figure 1. Previous Results. Results from previous experiment show 100% ocelli regeneration from all parts of the planarian excluding the head.

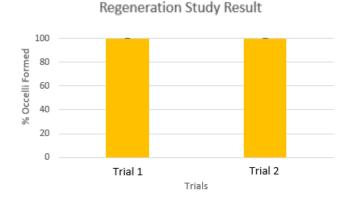


Figure 2. Trials 1 and 2 of Regeneration in Taurine. Results from current regeneration experiment. 100% ocelli regeneration for all planarians in every solution of Taurine for both Trials 1 & 2. Supports hypothesis.

Results:

The planarians that regenerated in Taurine regenerated in an abnormal way. Contrary to the hypothesis, only 6.7% of the planarians regenerated close to their original size, whereas the remaining 93.3% failed to regenerate past a few millimeters in length. Placing the planarians into their respective concentrations of DMSO led to all planarians shrinking rapidly and failing to respond to any external stimuli within the minute, leading to an immediate halt in the experiment. After the experiment was halted, it was not resumed.

Discussion:

The data collected from the experiment did not support the hypothesis. Not only did some planarians regenerate abnormally, but they also did not completely regenerate where cut. A planarian was found to regenerate according to the predetermined requirement, but it contained a cut the did not fully heal. The origin of the cut (whether it was scarred before or after the original cutting procedure) is unknown.



Figure 3. Planarians in DMSO. Photo of planarians from a 0.03mM solution of Taurine shortly after being placed in the solution of DMSO.

Even though each dish was supposed to contain 3 planarians after regeneration, one section of a dish contained 4 planarians while its neighboring section contained 2. Though each section was ultimately the same solution of Taurine, each planarian was expected to stay in its respective section. One of the dishes showed that a planarian crawled on the lid of the container, therefore drying out and failing to regenerate, leading the experiment to alter the total planarian count per solution of Taurine from 6 to 5.

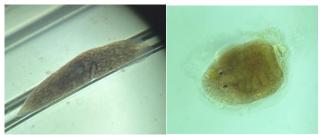


Figure 4. Planarians in Taurine. (Left image) Photo of a planarian with a scar on its central right side. (Right image) A photo of a planarian that dried out and died on the lid of the container.

During the experiment, the group was unable to check on the planarians' progress daily and to make sure all planarians were still in their respective sections and containers.

Previous research suggests that planarian activity increases with Taurine concentration (Livengood et al, 2024). This is consistent with the extended hyperactivity of the planarians as observed in **Figure 4** for the planarian that

climbed to the roof of the container, far away from the Taurine.

Results align with previous research that suggest Taurine has no effect on head regeneration in planarians (Latham). This was observed in the regeneration portion of the experiment, where the regeneration seemed to have a heavy effect on the trunk and tail portions of the body, but the head remained able to fully regenerate on each planarian. While temporal exposure to Taurine shows beneficial effects, prolonged exposure has an adverse effect on the planarians' ability to regenerate the rest of its body and causes many replicating stem cells to become unresponsive and nonworking when cut. Because of this, the DMSO instantly immobilizes and kills the planarians in their weakened state.

Planarian Lifespan in DMSO after bathing in

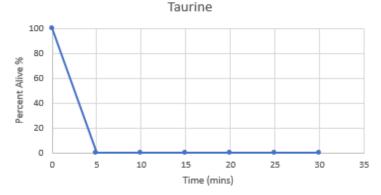


Figure 5: Lifespan Results. Planarians are taken out of the Taurine solutions and placed into 10% solutions of DMSO, after which they stopped responding to stimuli within the first 5-minute interval.

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