Hydroxyurea does not significantly limit regenerative abilities for planarians while high concentrations of dimethyl sulfoxide prove to be an uninhabitable environment

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Introduction:

Planarians are one of the only known organisms to be able to completely regenerate their body, thus making them a widely used model for learning about regeneration in the scientific field (Gonzalez-Gonzalez, 2025b). Because of the planarians regenerative ability, they become "immortal" and are even able to reproduce asexually (Gonzalez-Gonzalez, 2025b). Planarians cut anywhere on their body can completely regenerate through the use of stem cell replication (Gentile et al, 2011). Also in planarians are specific cells called organizers, or neoblasts (Gonzalez-Gonzalez, 2025b), which instruct nearby cells to a specific state which allow the planarian to regenerate the correct cells when split (Arias et al, 2018). Contrary to artificial pond water (APW), Hydroxyurea (HU) limits and damages the cells that regenerate in a planarian, though a solution of 20mM of HU has shown to have a low level of toxicity on planarians incubating in it for less than 5 days and reduces regeneration of the posterior (Planques et al, 2019).

Dimethyl sulfoxide (DMSO) is widely used in research to prevent cell damage and ice crystal formation, also known as a cryoprotectant (Tekade, 2019), and allow a hydrophobic drug to be dissolved in water (Pagán et al, 2006). Understanding the toxicity of a drug is done by measuring the EC_{50}, the concentration of the compound, DMSO, that shows a 50% effective rate at a specific time interval (Gonzalez-Gonzalez, 2025b). In prior experiments, a solution of DMSO over 5% was found to cause a planarian significant harm and lead to death (Pagán et al, 2006).

Objectives:

From this experiment, planarian regeneration in HU was compared to APW. Reviewing planarian anatomy and their toxicology for DMSO were studied and measured. Post experiment, the results were compared to other teams results to determine the planarians EC_{50} for differing solutions of DMSO.

Hypothesis:

Cutting a triangle out of the right central side of a brown planarian will produce another fully regenerated planarian and black planarians will die 3 times faster in a 15% solution of DMSO than a 5% solution of DMSO.

Methods:

Brown planarians were taken from a solution of APW (a predetermined mixture of salt and spring water) provided by the laboratory and were cut laterally with a scalpel into thirds, with sections being the trunk and the tail. Contrary to the standard 3 trials for every experiment, only 1 trial was conducted due to time constraints and was compared with the class upon completion of the experiment. 3 tails were placed in 5mL of APW, 3 trunks were placed in 5mL of APW, and 3 trunks were placed into a 5mL of 10mM of HU solution (Gonzalez-Gonzalez, 2025b). Additionally, a fourth dish incubated a planarian with a triangle cut from its right central side and put in a solution of 5mL of 10mM of HU. All 4 dishes were left in a temperature-controlled cabinet for 7 days left unsupervised (Gonzalez-Gonzalez, 2025b). The 3 trunks placed in 5mL of APW were the control while the 3 trunks placed in the HU solution was what was measured for regeneration. The fourth dish for the planarian with the central right cut was purely experimental but was still measured for regeneration.

The regeneration standard was determined prior to the experiment and was applicable to the entire class for uniformity. A planarian was said to have been completely regenerated if it possessed two distinctly separated ocelli (Gonzalez-Gonzalez, 2025c). The HU solution was measured through the formula for finding a dilution given a stock solution (Gonzalez-Gonzalez, 2025a).

$$\boldsymbol{C_i V_i = C_f V_f}$$
$$(1M)(V_i) = (10mM)(10ml)$$
$$V_i = 0.1mL \; or \; 100\mu L \; of \; HU$$

For the toxicity determination, solutions of 5%, 10%, and 15% solutions of DMSO were given to the group already measured. Each solution along with 2 black planarians taken from a predetermined solution of APW were placed into different dishes and observed via a microscope over time intervals of 3, 5, 15, and 30 minutes (Gonzalez-Gonzalez, 2025b). The control for the experiment was the dish with 2 planarians in only a solution of APW and the independent variable was the varying solutions of DMSO. The standard for planarian life was set before the experiment and said that a planarian was deemed to be alive if it responded to external stimuli such as air of light. For the same reasons in the regeneration experiment, only 1 trial was conducted. EC_{50} was calculated after the experiment concluded and class averages were found.

Results:

Not all planarians left in the solution of hydroxyurea regenerated according to the regeneration standard (**Figure 1**). In the hydroxyurea solution, no group had complete 100% regeneration of their planarian trunks. The combined class average for regeneration in APW compared to HU is 87.5% to 41.25% (**Figure 2**). Performing a t-test on the regeneration experiment yielded a p-value of 0.0064 which was deemed not statistically significant. The smaller experimental design regenerated completely.

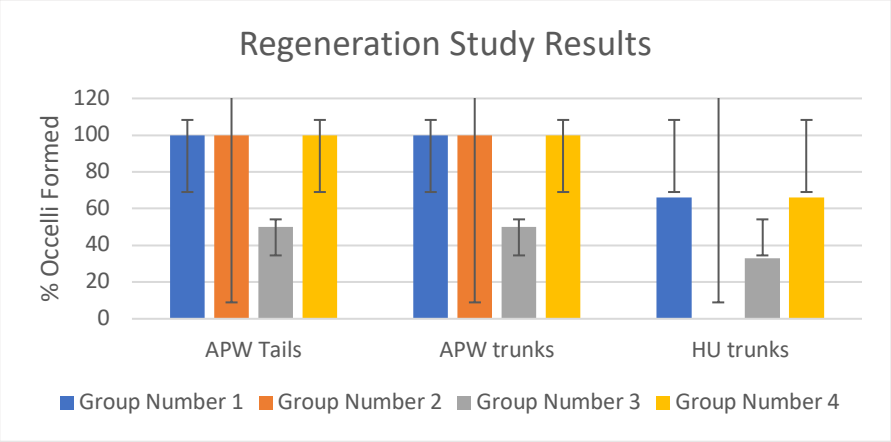

Figure 1. Individual group averages show most groups planarians following similar trends in regeneration.

Results for planarian survival rate in varying concentrations of DMSO begin declining at 10% DMSO (**Figure 3**). The groups individual EC_{50} for the DMSO was found to be 15%, 15%, 12.5%, and 10% for 3, 5, 15, and 30 minutes respectively and was a part of the class average for EC_{50}.

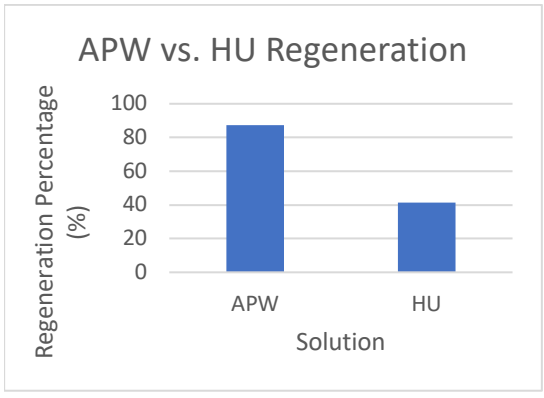

Figure 2. Combined class average for planarian regeneration in APW and HU regeneration.

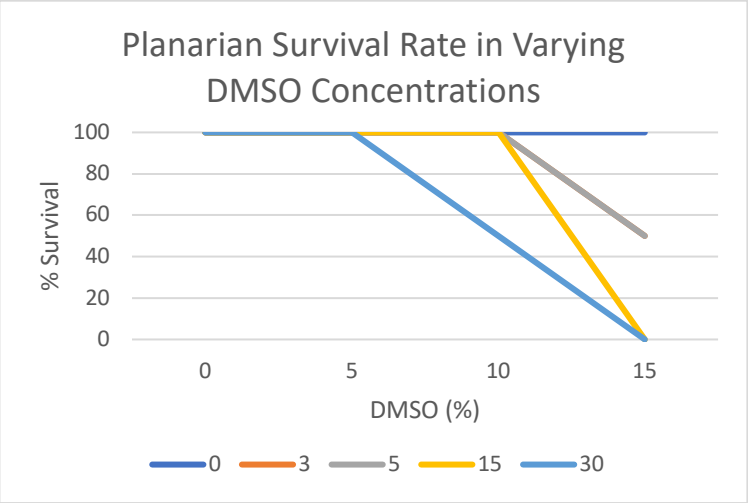

Figure 3. Legend at bottom is the number of minutes. Average planarian survival rate in several concentrations of DMSO begin declining at around 10% DMSO concentration.

Discussion:

The data for the regeneration in HU did support the hypothesis of the planarian regeneration with the central right cut. This means that the HU did not significantly affect the neoblasts in the planarian where the triangle was cut. Overall results of the trunk regeneration in HU were inconsistent with previous experiments that suggested that HU has a significant impact on planarian regeneration (Planques et al, 2019). The toxicology data is not able to support or deny the hypothesis that planarians in 15% DMSO will die 3 times faster than in 5% DMSO because no planarians died at 5% DMSO in the 30 minute time span of the experiment.

The inconsistency of results with HU regeneration could be explained by the differing solutions of DMSO that planarians were regenerating in as opposed to other experiments (Planques et al, 2019). Additionally, previous experiments conclude that a DMSO solution above 0.1% should be not be used as any concentration above that could lead to death of planarian over an extended period of time; more specifically, that exposure of DMSO above 5% for over 5 minutes was dangerous (Pagán et al, 2006). This conclusion aligns with the data found in the experiment (**Figure 3**).

Conclusion: A 10mM solution of HU does not show a significance in limiting the regenerative ability of planarians over a 7-day period. High concentrations of DMSO, such as 10% and 15%, prove to be an uninhabitable environment for black planarians after several minutes.

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