Section II: Methodology

Role of Student vs. Mentor

All work was conducted at the Massachusetts Academy of Math and Science laboratory under the guidance of Dr. Kevin Crowthers in roughly 5 months. I was responsible for designing experiments, conducting treatments, imaging planarians, and analyzing data, while Dr. Crowthers provided mentorship regarding experimental design and troubleshooting.

Equipment and Materials

The study used *Schmidtea mediterranea* as a model organism to study the effects of 0.4 mM lithium chloride (LiCl) and 10 ng/mL basic fibroblast growth factor (bFGF), prepared following standard dilution procedures. This methodology was inspired by previous studies (Farooq et al., 2021; Molano, 2024) but was adapted for exogenous application in *Schmidtea mediterranea* by modifying concentrations. A compound microscope and a Celestron digital imager were used to examine the regeneration rate. The Celestron S-Viewer Software was used to quantify the area of tissue regrowth over time, ensuring precise measurements.

Chemical Treatment and Tissue Amputation

Planaria were maintained in distilled water under standard laboratory conditions. Experimental groups were exogenously fed either LiCl or a combination of both LiCl and bFGF, while a control group was maintained in untreated water. *Schmidtea mediterranea* specimens were then transected anterior to the pharynx using a fine scalpel to induce regeneration. Post-amputation, the specimens were placed into sterile well plates containing planarian water.

Microscopy and Imaging Analysis

Regeneration was monitored using the Celestron digital imager and Celestron S-Viewer Software by measuring the blastema size. Images were captured daily for up to nine days post-amputation (dpa). The ImageJ software was then used to analyze the area of regrowth, standardising the measurements by normalising to the initial wound size.

Statistical Tests

To determine whether treatment had a statistically significant effect on regeneration, a one-way ANOVA was conducted to compare mean regeneration rates across all experimental groups. Since the ANOVA indicated a significant effect with a p-value of 0.04 ($p < 0.05$), Student's t-tests were performed to compare individual treatment groups against the control and each other.

Student's t test

A two-tailed Student's t-test was used to compare means between treatment and control groups. A significance threshold of $p < 0.05$ was applied. Data are reported as mean ± standard deviation (SD). The control group and bFGF treated group had a p-value of 0.04, the control group and the bFGF and LiCl-treated group had a p-value of 0.001, while the bFGF treated group and bFGF and LiCl treated group had a p-value of 0.005, making it statistically significant.