DAY 1: PPM Research	
Date:9/21/23	**Length of Research Period(s):75 Mins**

Goal(s)/Purpose:
- Find avg wastewater PPM to get appropriate concentrations for solutions

Materials Required:
- Computer

Procedure:
N/A

Data and Observations:
- Found this review paper on coastal pesticide contamination in India
- Pesticides in soil measured at 10 ppm- heavy contamination
- Ranges of concentration vary widely depending on type of pesticide used
- Anything under 5 ppm would be impossible to accurately replicate in a solution with lab materials available to me

Procedure Reflection and Future Endeavors:

Pesticide runoff concentrations vary widely depending on a multitude of factors. These factors include the concentration at which the pesticide solution is applied, the rate at which the pesticide metabolizes, and the ecosystem in which the runoff accumulates. Generally, a concentration ranging in the low parts per million represents a moderately/heavily contaminated ecosystem. Studies following mine may seek to hone in the concentration at which these novel pesticides are effective along with the concentration at which they are going. My Planarians will have arrived, and I'll now begin the transfer and housing portion of the procedure.

DAY 1: Planarian Reception	
Date:9/21/23	**Length of Research Period(s): 75mins**

Goal(s)/Purpose:
- Receive Planarians From Carolina Biological
  - Locate a Suitable Location for Planaria Base
  - Loosen caps on planaria containers

Materials Required:

- 5 Brown Planaria, Living
- EZ BioResearch Petri Dish with Lid, 100 mm/15 mm, Sterile, 25/Pack
- Carolina® Springwater, 1 gal
- 20 1 mL plastic pipettes

Procedure:

Care & Handling

1. Tie back any loose hair and put on a lab coat, safety goggles, and a fresh set of nitrile gloves.
2. Take the container the Planaria arrived in when shipped (and should still be in) and unscrew the lid. Rest the lid on top of the container, allowing air into the container.
3. ~~Take 3 10 cm Petri dishes and label their sides with "Habitation" using a sharpie.~~
    a. ~~*Note: These dishes will serve as the Planarians' living area between testing.*~~
4. ~~Fill each habitation dish with 25 mL of spring water, measured with an approximately 100 mL beaker.~~
5. ~~Cut the tips off of 10 1mL pipettes 1 cm from the tip, measured using the 100 cm ruler.~~
    a. ~~*Note: All pipettes with their tips cut off in this step will be referred to from now on as "Transfer Pipettes."*~~
6. ~~Using a 1 mL transfer pipette, transfer the Planaria from the jar into the habitation dishes. (Distribute them across all 3, with up to 30 in each dish. Prepare more dishes following steps 3 and 4 if needed).~~
7. Transfer the ~~habitation dishes~~ containers the planaria shipped in (with Planaria inside) into a dark quiet area, with relatively stable temperatures between 15 and 25 degrees Celsius.
    a. *Note: The dark quiet area chosen in this step will act as the main storage area for the Planarians for the duration of the experiment.*
    b. *Note: A cupboard or closet that lets in little to no light is an ideal location.*
    c. *Note: From now on, the location chosen in this step will be referred to as "Planaria Base"*
8. If you have limited time to complete this step, prepare however many habitable dishes you can, leaving any leftover planarians in their shipping containers alongside the habitation dishes in Planaria Base for later sorting.

Data and Observations:

- Received 5 groups of Planaria

- None dead on arrival
- Sizes ranging from ~1 cm to ~4 cm
- Located Planaria base in locked closed in stock room
- Should be relatively dark and temp stable as long as it's not left open

Procedure Reflection and Future Endeavors:
I've received and housed all five groups of Planaria. Procedure edits won't carry over into procedure, as there were no flaws and the transfer portion was simply cut due to time constraints. The transfer will be performed as part of the feeding process. Planarians all seem to be in good condition, will feed them and transfer to habitation dishes next period.

Day 2: Feeding and Placing in Habitation Dishes	
Date:9/27/23	**Length of Research Period(s):75**

Goal(s)/Purpose:
- Complete initial care and handling and feed Planarians
  - Feed planaria with boiled egg yolk
  - Transfer them into habitation dishes
  - Return habitation dishes to planaria base

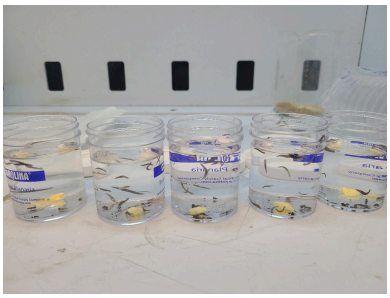
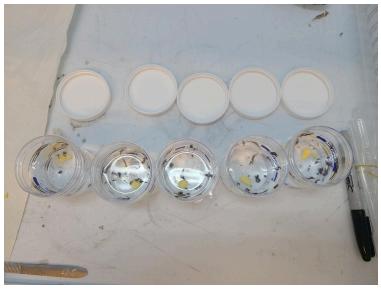
Materials Required:
- 5 1 mL plastic pipettes
- 1 hard boiled egg
- EZ BioResearch Petri Dish with Lid, 100 mm/15 mm, Sterile, 25/Pack
- Carolina® Springwater, 1 gal
- 1 100 mL Graduated Cylinder
- 1 black Sharpie

Procedure:

9. Feed planaria
   a. Place half of a pea-sized portion of boiled egg yolk into each shipment container ~~habitable dish~~.
   b. Place the dishes back into the Planaria Base, and wait 30 minutes for feeding to complete.
   c. Perform the water-changing process outlined in steps 8a through 8g.
      i. *Note: Wait 48 hours after feeding before using planarians in any of the trials outlined below.*
2. Take 3 10 cm Petri dishes and label their sides with "Habitation" using a sharpie.
   a. *Note: These dishes will serve as the Planarians' living area between testing.*
3. Fill each habitation dish with 25 mL of spring water, measured with an approximately 100 mL beaker,

4. Cut the tips off of 10 1mL pipettes 1 cm from the tip, measured using the 100 cm ruler.
    a. *Note: All pipettes with their tips cut off in this step will be referred to from now on as "Transfer Pipettes."*
5. Using a 1 mL transfer pipette, transfer the Planaria from the jar into the habitation dishes. (Distribute them across all 3, with up to 30 in each dish. Prepare more dishes following steps 3 and 4 if needed).
6. Transfer the habitation dishes (with Planaria inside) into a dark quiet area, with relatively stable temperatures between 15 and 25 degrees Celsius.
    a. *Note: The dark quiet area chosen in this step will act as the main storage area for the Planarians for the duration of the experiment.*
    b. *Note: A cupboard or closet that lets in little to no light is an ideal location.*
    c. *Note: From now on, the location chosen in this step will be referred to as "Planaria Base"*
7. If you have limited time to complete this step, prepare however many habitable dishes you can, leaving any leftover planarians in their shipping containers alongside the habitation dishes in Planaria Base for later sorting.

Data and Observations:


- Planarians all ate the egg yolk
- Transfer process was somewhat more streamlined
    - Less cleaning time b/c of transfer to diff. Container from egg yolk
- No dead planaria

Procedure Reflection and Future Endeavors:
Again, I won't be integrating the procedure reordering and edits made here into the final procedure. These alterations were solely for my specific time shortage, and all parts of the procedure should function the same when performed in their original order (relocation before feeding) with the habitation dishes instead of the shipping containers. The Planarians all seemed to be in good health, moving to their food quickly and moving around under the light of the fume hood. Planaria Base fits the dishes just as well as the shipping containers, so I will continue to use the location in the stock room closet. Next I'll set up my negative control.

Day 3: Negative Control Set Up	
Date:10/16/23	**Length of Research Period(s): 60 mins (after school)**
Goal(s)/Purpose:	

- Set up both arms of negative control
    - Transfer 9 healthy Planarians into NC dish
    - Bisect 9 healthy Planarians, and transfer the fragments into the NC-Regen dish

Materials Required:

- EZ BioResearch Petri Dish with Lid, 100 mm/15 mm, Sterile, 25/Pack
- Carolina® Springwater, 1 gal
- 18 1 mL plastic pipettes
- 1 Black sharpie
- 1 10 mL Graduated Cylinder
- Surgical grade blades and handle, pack of 10
- 1 roll of paper towels

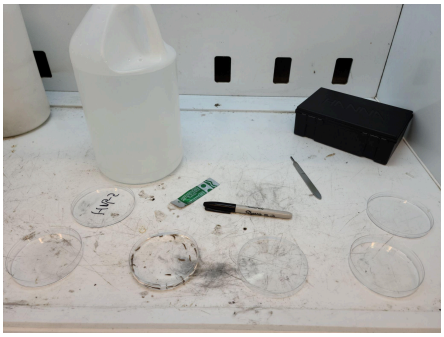
Procedure:

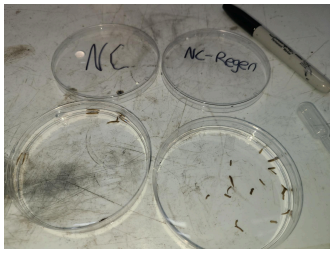
Negative Control

11. Tie back any loose hair and put on a lab coat, safety goggles, and a fresh set of nitrile gloves.
12. Take 1 10 cm petri dish, and mark the side with “NC” using a sharpie.
13. Fill the dish with 25 mL of spring water, measured using the 10 mL graduated cylinder.
14. Using a 1 mL plastic pipette, transfer 9 planarians from a Habitation dish into the NC dish.
15. Take 1 10 cm petri dish, and mark the side with “NC- Regen” using a sharpie.
16. Fill the dish with 10 mL of spring water, measured using the 10 mL graduated cylinder.
17. Take a surgical blade, remove it from its sterile packaging, and attach it to the handle.
    a. *Note: Ensure that after the blade is removed from sterile packaging, it does not come into contact with any nonsterile objects, fluids, and surfaces other than those specifically mentioned in the procedure.*
18. ~~Transfer a planarian from a habitation dish into the center of the NC-Regen dish using a fresh 1 mL plastic pipette.~~
18. Transfer a planarian from a habitation dish onto a paper towel using a fresh 1 mL plastic pipette.
19. Using the surgical blade, cut the planarian in half, separating the head from the tail.
20. Transfer the planaria fragments into the NC-Regen dish from the towel using the same 1 mL plastic pipette
21. Repeat steps 18 through 20 eight additional times.

22. Pour 15 additional mL of spring water into the NC-Regen dish, measured using a 10 mL graduated cylinder.
23. Photograph and record the condition of the planaria in each dish.
    a. *Note: The data you collect in this step should be recorded in this google sheet.*
    b. *Note: The photographs you collect in this step should be uploaded to this google drive, and retitled "NC Day 1" and "NC-Regen Day 1" respectively.*
24. Place both dishes into Planaria Base.
~~25. Once a day, for the next 14 days, remove the NC dish from Planaria Base, photograph the planaria, record their conditions, and replace the dish in Planaria Base.~~
    ~~a. *Note: The photographs you collect in this step should be uploaded to this google drive and retitled "NC Day 2" through "NC day 8" respectively.*~~
    ~~b. *Note: The data you collect in this step should be recorded in this google sheet.*~~
~~26. Once a day, for the next 14 days, remove the NC-Regen dish from Planaria Base, photograph the planaria, record their conditions, and replace the dish in Planaria Base.~~
    ~~a. *Note: The photographs you collect in this step should be uploaded to this google drive and retitled "NC-Regen Day 2" through "NC-Regen day 15" respectively.*~~
    ~~b. *Note: The data you collect in this step should be recorded in this google sheet.*~~
25. In 14 days, remove both dishes from Planaria base, photograph the planaria, and record their conditions in this google sheet.
26. Using a fresh 1 mL transfer pipette, replace all surviving NC Planarians into the habitation dishes.

Data and Observations:





- Found that cutting the planarians while they were submerged in water was difficult because of lack of friction between the planaria and the dish
- Planaria were just pushed around the dish, and caused scratching at the bottom of the petri dish

- Switched to paper towel for cutting surface, which proved to be a much easier cut

Procedure Reflection and Future Endeavors:
Process went relatively smoothly, with a few key lessons for the experimental and positive control groups. Planarians definitely cannot be bisected in solution- it's just too hard to get enough traction with the knife, and all you get is a maimed Planarian and a scratched up petri dish. Bisecting them on a paper towel is much more efficient, and transferring the fragments back into solution is easily done with a pipette. The non regenerating group should be replaced into Planaria Base before the bisection process begins, as they cramp the workspace during the most delicate process of the experiment. Rather than monitoring their conditions each day of the experiment, the data will be collected on the last day to allow for more thorough condition documentation. (Taking close ups of each subject for 14 days would be a multiple hour long process each day, and is impossible under lab and time restraints). The Planaria fragments remained mobile after bisection, and were swimming around in their dish. Next step will be to create solutions.

Day 4: Solution Creation	
Date:10/19/23	**Length of Research Period(s): 75 mins**

Goal(s)/Purpose:
- Create Orange Oil and Cyfluthrin Solutions
  - Dilute orange oil and cyfluthrin in spring water to achieve 10 ppm (by volume) solutions of each substance
  - Store solutions

Materials Required:

- Carolina® Springwater, 1 gal
- 4 fl oz Pure Orange Oil
- BioAdvanced Cyfurthin Insect killer (MSDS)
- Black sharpie
- 1 250 mL Beaker
- 1 1 L Beaker
- 1 10 mL Graduated Cylinder
- 1 100 mL Graduated Cylinder
- Micropipette
- 2 Lab grade 100+ mL chemical storage bottles

Procedure:
1. Tie back any loose hair and put on a lab coat, safety goggles, and a fresh set of nitrile gloves.

a. *Note: Each of the individual solution creation processes outlined below can be completed separately, and all glassware should be cleaned between each solution creation.*

2. Using a micropipette, place .133 mL of the Cyfluthrin-based synthetic pesticide into a 250 mL beaker.
    a. *Note: Cyflurthin is acutely toxic, and chronically harmful to aquatic life. Work with Cyflurthin only under a fume hood with proper PPE. If swallowed, call poison control immediately. If inhaled, seek fresh air immediately. If skin contact occurs, remove contaminated clothing and wash skin with water and soap. Avoid environmental release.*
3. Using a 10 mL graduated cylinder and a micropipette, place 99.866 mL of spring water into the same beaker.
4. Pour the solution into a lab grade chemical storage bottle, and seal the bottle.
5. Using a sharpie, label the bottle “Cyfurthin Solution,” and place it into storage.
6. Pour the solution into a lab grade chemical storage bottle, and seal the bottle.
7. Using a sharpie, label the bottle “Cyfurthin Solution,” and place it into storage.
8. Using a micropipette, place 5 µL of the orange oil into a 1 L beaker.
    a. Note: The pure essential oils are disruptively pungent. All portions of procedure that involve the pure oils should be done under the fume hood, with the fan on.
9. Using 100 and 10 mL graduated cylinders and a micropipette, place 499.995 mL of spring water into the same beaker.
10. Pour the solution into a lab grade chemical storage bottle, and seal the bottle.
11. Using a sharpie, label the bottle “Orange Oil Solution,” and place it into storage.

Data and Observations:

- Measuring microliters with the micropipette takes some practice
- Too much solution was created to fit in storage bottles
- Oils are incredibly pungent- fume hood should be ON for the other solutions

Procedure Reflection and Future Endeavors:
Creation process generally doesn't need any refinement. Using the 2-10 microliter micropipette is a little tricky, as the depression of the plunger needed to obtain a few microliters is very subtle. Practicing with water a few times helped a lot. The Cyfluthrin was odorless, a milky white solution that is invisible in the 10 ppm solution. The orange oil is dark orange, but also indetectable in the 10 ppm solution to the naked eye. The oils produce an overwhelming smell when their containers are opened, which turning on the fume hoods fan seemed to help with. The 2 solutions are now in storage, ready for use. Next I'll record the final data for the positive control, make the other solutions, and move on to experimental groups.

Day 5: Negative Control Data Collection	
Date:10/31/23	**Length of Research Period(s): 75 mins**

Goal(s)/Purpose:
- Collect data for negative control
  - Retrieve NC and NC-Regen dishes
  - Note successful reproductions, failed reproduction, viable reproductions
  - Note surviving planaria, dead planaria

Materials Required:
- Recording Device

Procedure:
27. Photograph and record the condition of the planaria in each dish.
    a. *Note: The data you collect in this step should be recorded in this google sheet.*
    b. *Note: The photographs you collect in this step should be uploaded to this google drive, and retitled "NC Day 1" and "NC-Regen Day 1" respectively.*
28. Place both dishes into Planaria Base.
~~29. Once a day, for the next 14 days, remove the NC dish from Planaria Base, photograph the planaria, record their conditions, and replace the dish in Planaria Base.~~
    ~~a. *Note: The photographs you collect in this step should be uploaded to this google drive and retitled "NC Day 2" through "NC day 8" respectively.*~~
    ~~b. *Note: The data you collect in this step should be recorded in this google sheet.*~~
~~30. Once a day, for the next 14 days, remove the NC-Regen dish from Planaria Base, photograph the planaria, record their conditions, and replace the dish in Planaria Base.~~

~~a. Note: The photographs you collect in this step should be uploaded to this google drive and retitled "NC-Regen Day 2" through "NC-Regen day 15" respectively.~~

~~b. Note: The data you collect in this step should be recorded in this google sheet.~~

27. In 14 days, remove both dishes from Planaria base, photograph the planaria, and record their conditions in this google sheet.
28. Using a fresh 1 mL transfer pipette, replace all surviving NC Planarians into the habitation dishes.

Data and Observations:

(more photos in sheet)

- One of the planarians in the NC dish underwent fission and reproduction over the two weeks
- Left with 10 healthy planarians in NC dish (+1!)
- 17 of the fragments in the NC-Regen dish successfully regenerated, with tails that taper to a point and heads with photoreceptors
- One fragment hasn't fully regenerated, but is alive and viable

Procedure Reflection and Future Endeavors:

The positive control yielded great results, paving the way for the rest of my experiments. All of the fragments and whole planarians survived, with one unexpected reproduction in the non-bisected group. More thought needs to be given in how to represent this unexpected reproduction in data. The bisected group was nearly all successful reproductions, with the exception of one fragment which didn't have a defined head. Next I'll move into the positive controls, and make better procedure for data collection and fitting in reproduction anomalies.