

Using discovery for the CURE: A general biology curriculum series to promote high-level cognitive skills and an understanding of medicine from an environmental perspective

Rosier R, Bryzski J, and McAllister N

Supplementary Materials

Examples of Student Work

General Biology I Lab

- 1) Lab Meeting Presentation
- 2) Independent Research Proposal
- 3) Final Presentation Example 1
- 4) Final Presentation Example 2

General Biology II Lab

- 5) Lab Meeting Presentation
- 6) Final Poster Example 1
- 7) Final Poster Example 2
- 8) Weekly Experimental Review, Week 2
- 9) Weekly Experimental Review, Week 4

Elodea and Medicine



The Gal Pals

10/5/2023

What is Elodea?

Scientific Name: *Elodea canadensis* Hydrocharitales

Common Name: Elodea or Waterweed

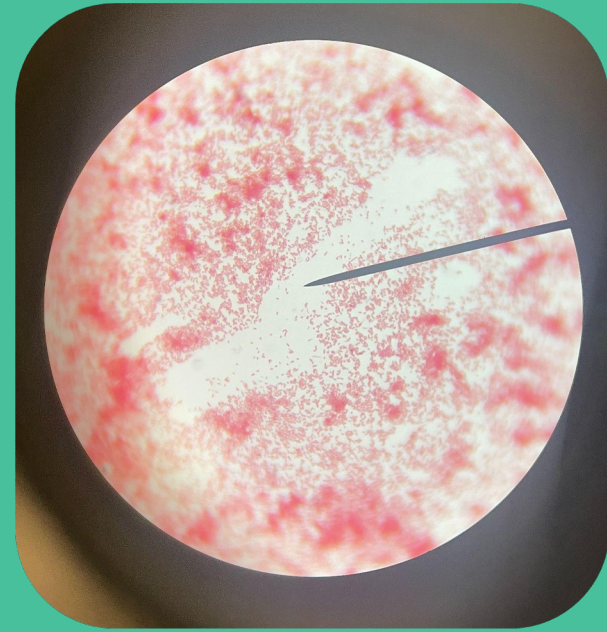
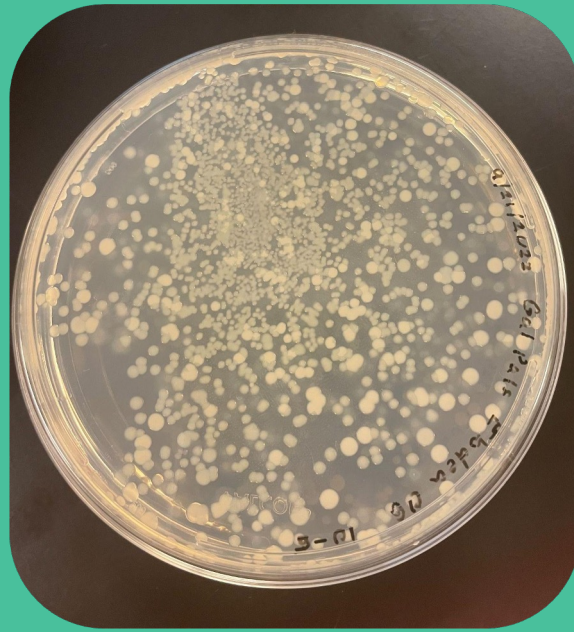
Elodea has a dark green leaf that is arranged in whorl shapes with 2 to 7 leaves that branch off the stem.



In water Elodea is used by aquatic animals and insects to provide cover. It is also provided as a food source for many different pond animals.

Elodea grows in cold and clear water. It starts to grow when temperatures are around 45-65°F. It is also found all around North America as an aquatic plant.

Summary of Elodea Microbiome Research Results



Possible Medicinal Properties



Hydrilla Verticillata: closely related to Elodea, has some promising factors medicinally

- Complete nutrition
- Improve digestion and gastrointestinal function
- Circulation
- Neurological health
- Blood sugar control
- Strengthen immunity
- Increase endurance

(Pal 2006)

Possible Medicinal Properties

- Elodea has the possibility of producing a bacterial strain that can be used medicinal
 - *Sphingomonas elodea* is a Gram negative bacterium capable of producing a gellan gum
 - Gellan gum- (exopolysaccharide) a water soluble and gelling agent that used in diverse industries; food, pharmaceutical industries
 - Used for stabilizing, emulsifying, thickening, and suspending



Possible Medicinal Properties

Gellan is one of the most widely studied microbial polysaccharides and it is a linear polymer that is produced by *Elodea*. This chain consists of a tetrasaccharide repeating unit of l-rhamnose, d-glucose and d-glucuronate. Many a lot of the studies have focused on gellan in food but since it has a unique structure and other beneficial properties, it is described as a potent multifunctional additive for various pharmaceutical products. Although gellan in pharmaceutical products is not full proof, it is seen to be useful in studies of oral, ophthalmic, nasal and other pharmacy uses.



Medicinal properties on Elodea

Gellan Gum is a common bacteria that grows on Elodea, and scientists have been studying its medicinal properties for some time. For this specific article, they noticed that gellan gum is a natural biomaterial that shows promise for tissue engineering. So, saying this, gellan gum has the medicinal properties, not Elodea.



Summary

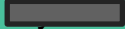
- Elodea has a bacteria that grows on it that could possibly be medicinal called gellan gum
- Elodea is not antimicrobial since so many bacterias grow on the plant
- When we looked at the bacteria with immersion oil under the microscope and gathered that the plant is gram negative

List of References

- Bacelar AH , Silva-Correia J , Oliveira JM , Reis RL . Recent progress in gellan gum hydrogels provided by functionalization strategies. *J Mater Chem B*. 2016 Oct 7;4(37):6164-6174. doi: 10.1039/c6tb01488g. Epub 2016 Aug 15. PMID: 32263628. <https://pubmed.ncbi.nlm.nih.gov/32263628/>
- Lee, S. Y., Ahn, J. Y., Kim, M., Sekhon, S. S., Cho, S. J., Kim, Y. C., & Kim, Y. H. (2017). Phenotypic and proteomic analysis of positively regulated gellan biosynthesis pathway in *Sphingomonas elodea*. *Animal cells and systems*, 21(2), 115–123. <https://doi.org/10.1080/19768354.2017.1290678>
- Osmatek, T., Froelich, A., & Tasarek, S. (2014). Application of gellan gum in pharmacy and medicine. *International journal of pharmaceutics*, 466(1-2), 328–340. <https://doi.org/10.1016/j.ijpharm.2014.03.038>
<https://pubmed.ncbi.nlm.nih.gov/24657577/>
- Pal, D. K. (2006, April 1). *Little known uses of common aquatic plant, Hydrilla verticillata (Linn. f.) Royle* . <http://nopr.niscpr.res.in/handle/123456789/7948>

Division of work

Peer-review paper #1



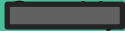
Peer-review paper #2



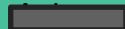
Peer-review paper #3



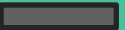
Peer-review paper #4



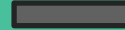
Title slide



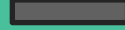
Slide 2 - Basic Info on Plant



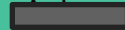
Slide 3 - Summarize Results of Plant Microbiome Research (plant stamp, bacteria conc., gram stain)



Slide 4 - Review of Medicinal Properties of Plant



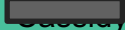
Slide 5 - Review of Medicinal Properties of Plant



Slide 6 - Review of Medicinal Properties of Plant



Slide 7 - Review of Medicinal Properties of Plant



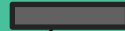
Slide 8 - Summary Slide



Slide 9 - List of References

All

Slide 10 - Division of Work



Research Proposal FINAL Instructions

General Biology Lab 1
Drug Discovery Lab Series
Fall 2023

To prepare for your team's independent investigations, studying the medicinal properties of your plant extract, you and your team will draft and revise research proposal. In the proposal, you will propose what experiment you want to conduct next with your plant extract and how you will conduct it.

The final written research proposal is due after you receive peer and instructor feedback on your draft. The final research proposal is worth 20 points.





Instructions:

1. Edit the draft research proposal you wrote with your team. If you still need to, copy and paste the following outline into a new document.
2. Follow the directions listed under each bullet point.
3. After you have added the appropriate information under each bullet point, delete the directions listed.

Research Proposal:

I. Division of Work

A. List what each person in your team is responsible for completing for the research proposal draft outline. Be equal and fair in this division of work!

1.  Timer, extract making, math, probe guy
2.  Extract making, lab-pro guy, set-up man
3.  First half of proposal writing, timer, extract making
4.  Proposal writing, tube management

II. Project Title -

A. If you have a couple ideas for a title, list them here!

Lavender impact on Metabolic rate

III. Purpose

- A. To see if lavender has any effect on metabolic rate. Lavender has been used as an essential oil and in general in calming so this could potentially show if it impacts breathing and digestion

IV. Hypothesis

- A. Lavender will slow down the metabolic rate of the daphnia

V. Materials and Methods

- A. Requirements for the experiment you propose:

1. **Organism: Daphnia**

2. **Testing for: Conductivity levels, which inversely correspond to the dissolved oxygen levels.**

3. Must include at least one control sample (negative or positive control).

The positive control will have no cork, while the negative control will.

4. When planning your experiment consider using different types of bacteria, various time points, different observations of your organism, concentration of samples, etc.

We will take a look at conductivity levels every 10 minutes for 40 minutes. We will note any dead daphnia.

- B. Begin outlining the protocol for your experiment. Review the protocols we have used in previous experiments. Use these as a starting point.

Materials:

-4 tubes

-4 conductivity probe

-20 daphnia

-Lavender extract

-Fresh water

-Parafilm

Protocol:

1. Sterilize all equipment
2. Put out four tubes
3. Fill up all the tubes halfway with fresh water (7mL).

4. Put 780 uL of lavender extract in one tube, and 980uL of lavender extract in the other.
5. Add 5 daphnia to a non-lavender extract containing tube. Label this positive control.
6. Add 5 daphnia to each lavender extract-containing tube. Quickly cover these with parafilm.
7. Add daphnia to the last, non-lavender containing tube, and then quickly cover it with parafilm.
8. Every ten minutes for 40 minutes, record the conductivity levels in each tube using the conductivity probe. Quickly take off the parafilm and insert the sensor. Measure the conductivity using logger pro, and then take out the probe and reapply the sensor.
9. Disassemble the setup and put the dead daphnia in the retirement tank.

C. Your protocol should include two separate sections: (1) list of materials and (2) procedure steps.

D. In the list of materials, be sure to list the samples you are using, including any controls and what those are going to be.

VI. Data Analysis

A. How will you record your data? Table? Pictures? Videos?

We will use tables and graphs in order to display our data. Perhaps we will take a photo of our set up as well.

1. If you are planning to use a table, create one and place it in this draft outline.

Table One, Conductivity(in us/cm/s) Present in Daphnia-Containing Sealed Tubes

Tube Type	Conductivity at 0 min	Conductivity at 10 min	Conductivity at 20 min	Conductivity at 30 min	Conductivity 40 min
Positive Control (uncovered tube)	277.7	278.5	277.7	274.9	273.2
Negative Control(cov)	277.7	281.3	281.0	282.1	282.0

ered tube with no extract)					
Covered Lavender Extract Tube 780 uL	281.9	284.8	284.5	279.8	279.3
Covered Lavender Extract Tube 980 uL	279.6	280.5	278.9	284.1	275.5

Table Two, Mortality Rates in Each Tube Over a Forty Minute Timespan

Tube Type	Mortality at 0 min	Mortality at 10 min	Mortality at 20 min	Mortality at 30 min	Mortality at 40 min
Positive Control	0	0	0	0	0
Negative Control	0	0	0	0	0
Lavender Extract Tube 100 uL	0	0	0	0	0
Lavender Extract Tube 200 uL	0	0	0	0	0

~Lavender made the daphnia extra sleepy, so sleepy they looked dead~

2. If you are planning to take pictures or videos, specifically list what pictures or videos you plan to take and record.

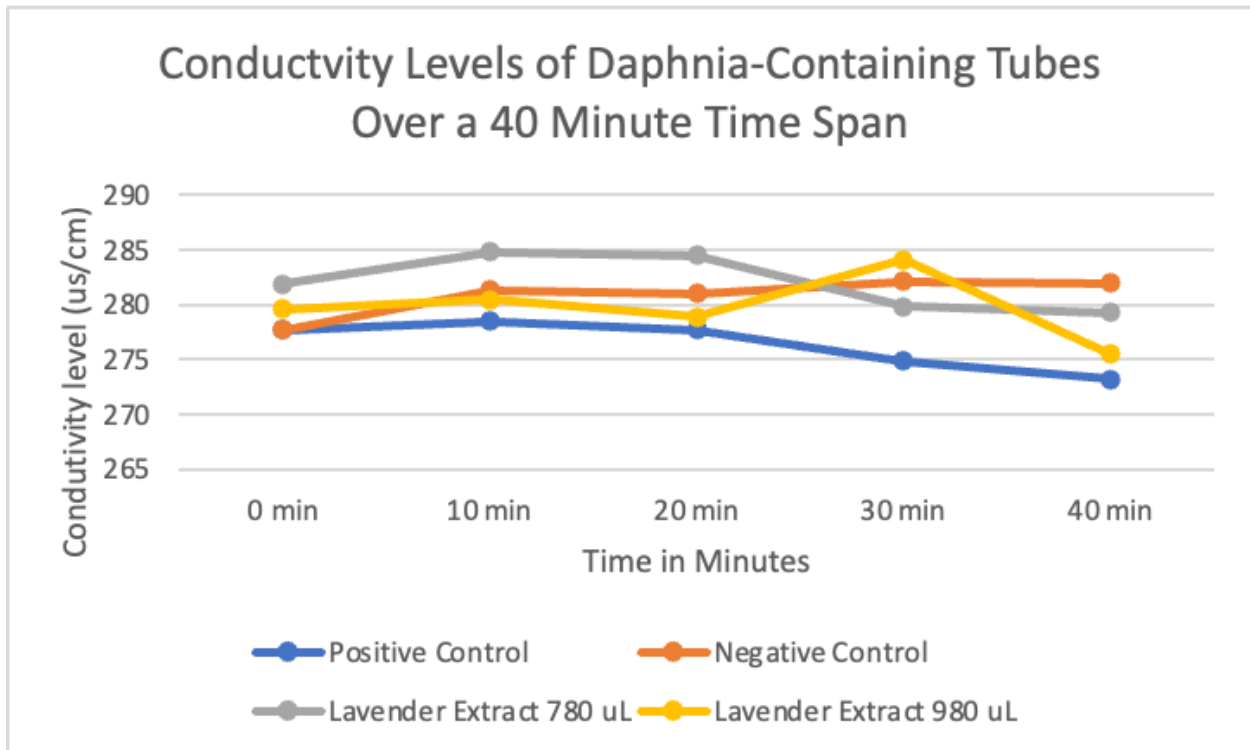
We will take a picture of our final setup, once everything is put together.

B. How will you present your data?

1. Explain how you will graph your quantitative data.
 - a) Consider what kind of graph you are going to use and what data should be included in the graph.

We are going to use a line graph. The y-axis will have conductivity levels, while the x-axis will have time in minutes. Four different data sets will be present

on the graph: The conductivity level of both lavender extract-containing tubes, the data for the positive control, and the data for the negative control.



If there are any deaths, we will also have a similar graph, but one that uses mortality rates instead of conductivity levels. The line with the lowest conductivity levels has the highest dissolved oxygen content, which means that this line would correspond to a low metabolic rate. Using this idea, we can interpret all of our data in order to figure out the effects of lavender on the metabolic rate.

2. Explain how you will show your qualitative data.

a) Are you planning to present a table? Can the table be made into a chart? Can you create a figure that includes all the pictures you have taken?

We don't have much qualitative data to show. In any case, the qualitative data is not as important as the quantitative data. But we will have a mortality table to record any daphnia deaths. This will tell us if lavender is toxic to daphnia, or if their metabolism is speed up/slow down to an unhealthy level.

C. What samples and/or controls will you compare to make a conclusion about your experiment?

We will compare our extract containing samples to our non-extract containing negative control by seeing if the conductivity levels in the extract-containing sample decreases

faster or slower than the negative control. This will tell us the effects of lavender on metabolic rate. The positive control is there to give us a baseline as to the “normal” dissolved oxygen content within the water in order to give us an idea of how much our extract decreased from this baseline compared to our negative control.

Lavender Impact on Metabolic Rate

By:



Basic Information on Lavender

- *Lavandula angustifolia*, commonly known as lavender, is an herb recognisable by its distinctive fragrance and its purple hue.
- It has historically been used since ancient Greek, Egyptian, and Roman times and was believed to purify the body and mind
- In the modern day, it is currently used in several essential oils and herbal medicines.
 - This includes:
 - Insomnia,
 - alopecia,
 - Anxiety,
 - Stress
 - postoperative pain.



Medicinal Properties Of Lavender

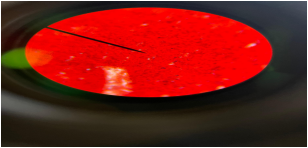



- Lavender or *Lavandula* belonging to the Lamiaceae (mints) family, is one of the most commonly researched medicinal herbs.
- Various studies certainly indicate the antimicrobial property of lavender mainly due to its chemical composition. (The main constituents of lavender are linalool, linalyl acetate, 1,8-cineole, β -ocimene, terpinen-4-ol, and camphor.)
- Anti-inflammatory and Antinociceptive Properties The lavender essential oil shows a significant anti-inflammatory effect. Lavender oil has been used in dermatitis and eczema.
- Toxicity may accompany the use of marketable essential oils. Factors involved may be product management, ingredients, excessive use or inappropriate use, sensitization/anaphylaxis, and lack of scientific evidence. It is wise to know the adverse effects along with their uses. Some of the studies have shown additional side effects of lavender essential oils like contact dermatitis, acute eczema, and facial dermatitis allergic reaction.



Methods & Results: Antimicrobial properties



Bacteria sample	shape	Gram status	species
Reference Sample: A 	Rod	+	<i>Bacillus megaterium</i> or <i>Bacillus cereus</i>
	Vasillas	-	<i>Escherichia coli</i> (<i>E. Coli</i>)
Unknown strain from plant sample	Rod	+	n/a
Unknown Pt.2	Circular	-	n/a



Methods: Toxicity

4 solutions were labeled with

- 20ul plant extract
- 100ul plant extract
- Positive control (Neem Oil)
- Negative control (Methanol)

10 brine shrimp were transferred into jars and incrementally observed to

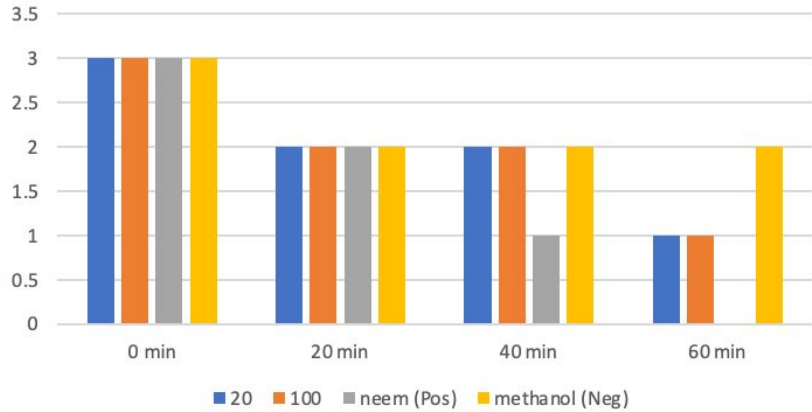
Observe if the **rate of movement** and **mortality**



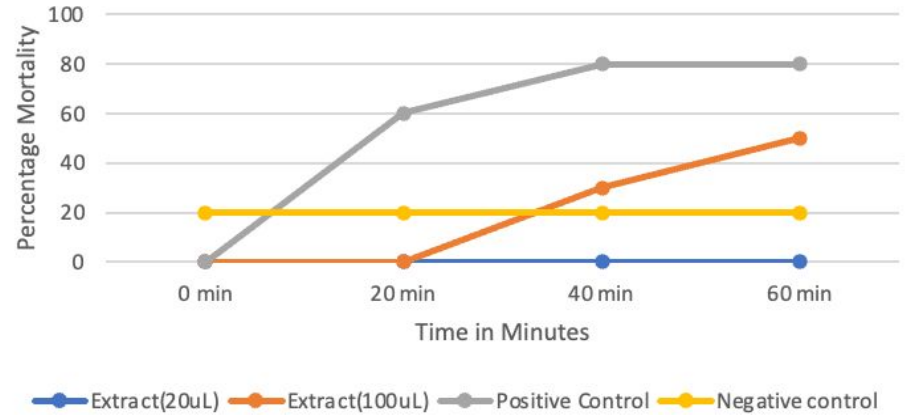
Results: Toxicity



Movement



The Effects of a Lavender-Menthol Extract on Mortality Rates of Brine Shrimp over Time



Methods: Stimulatory properties



8 1.7 microcentrifuge tubes total tubes were needed.

4 1.7 microcentrifuge tubes tubes per pair

Each tube is either positive control negative control or plant extract

8 Daphinna

4 Daphinna per group

2 Daphinna in the control positive and negative 2 in the plant extract

Use a microscope under 40x lens to watch their BPM for 15 second periods then multiplying that by 4



Results: Stimulatory properties

*BPM = beats per minute (heart rate)

		Methanol		Plant Extract	
		Daphnia 1	Daphnia 2	Daphnia 1	Daphnia 2
Count 1	15 sec count	84	64	25	31
	15 sec count x 4 (BPM)	336	256	100	124
Count 2	15 sec count	78	64	30	29
	15 sec count x 4 (BPM)	312	256	120	116
Count 3	15 sec count	72	61	23	24
	15 sec count x 4 (BPM)	288	244	94	96
Average heart rate (BPM)		312	252	105	112

Results: Stimulatory properties Continued

Table 2. Average heart rate of *Daphnia* in methanol.

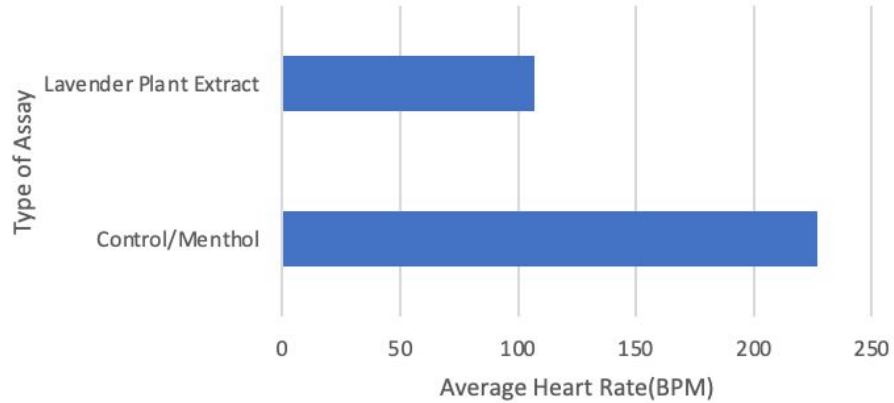
Condition:	Methanol			
	Daphnia 1	Daphnia 2	Daphnia 3*	Daphnia 4*
Average heart rate (BPM) from Table 1	141	207	312	252
Average heart rate (BPM) of both Daphnia	227			

Table 3. Average heart rate of *Daphnia* in plant extract.

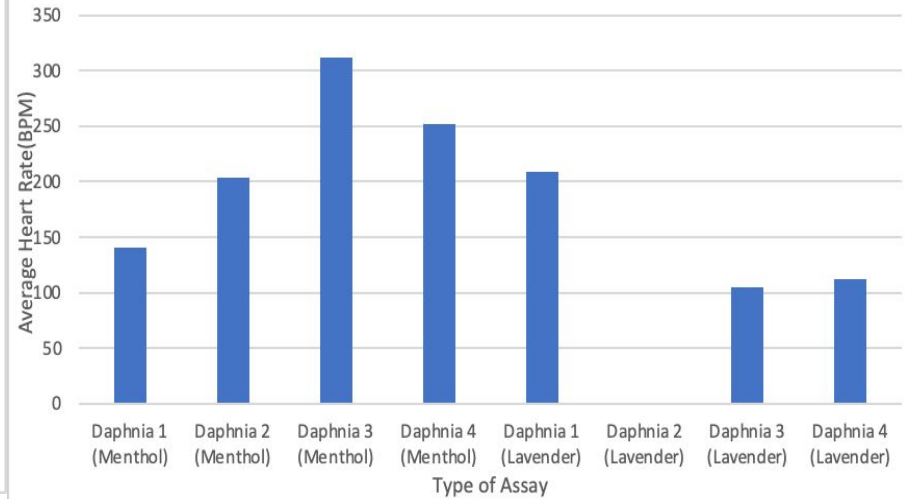
Condition:	Plant Extract			
	Daphnia 1	Daphnia 2	Daphnia 3*	Daphnia 4*
Average heart rate (BPM) from Table 1	209	dead	105	112
Average heart rate (BPM) of both Daphnia	107			

Results: Stimulatory properties Continued

The Average Heart Rate of Daphnia Placed in Two Different Assays

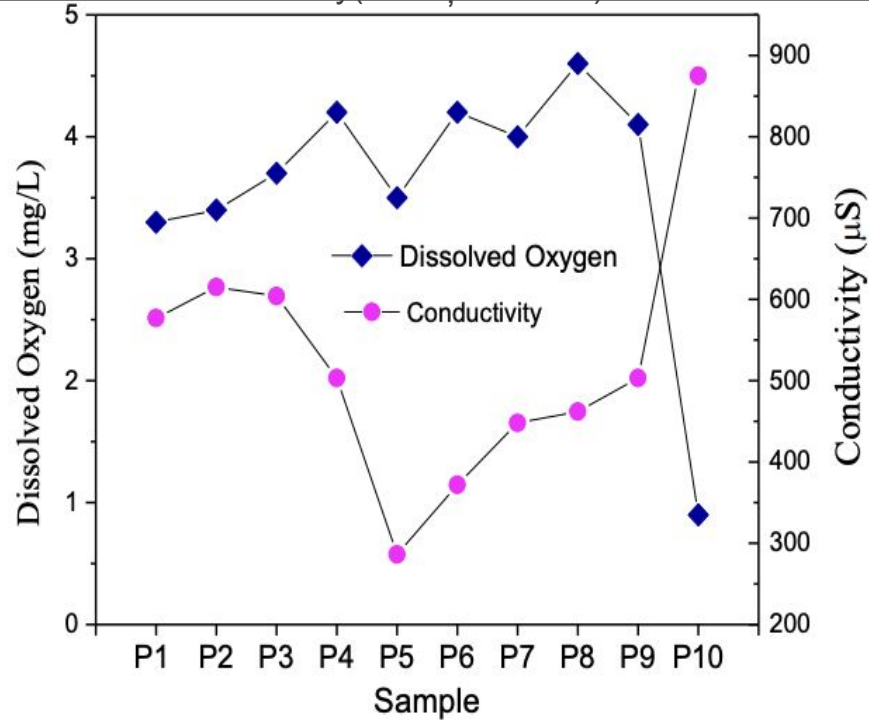


Average Heart Rate

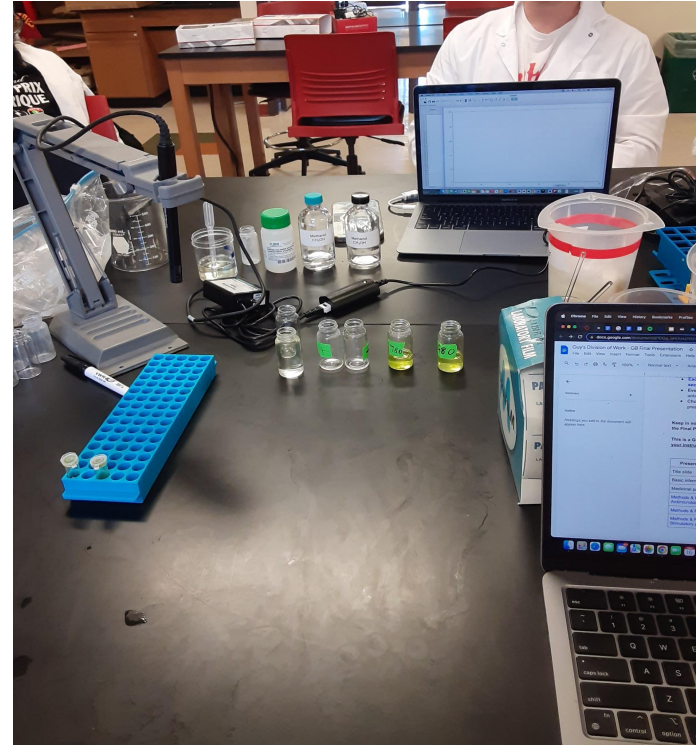


Methods & Results: Independent experiment

The Relationship Between Dissolved Oxygen and Conductivity (Moroşanu et al)



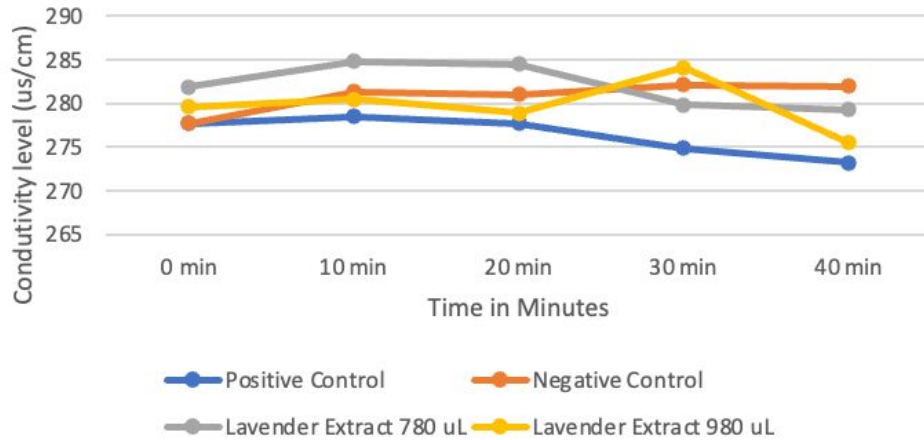
Our Independent Experiment



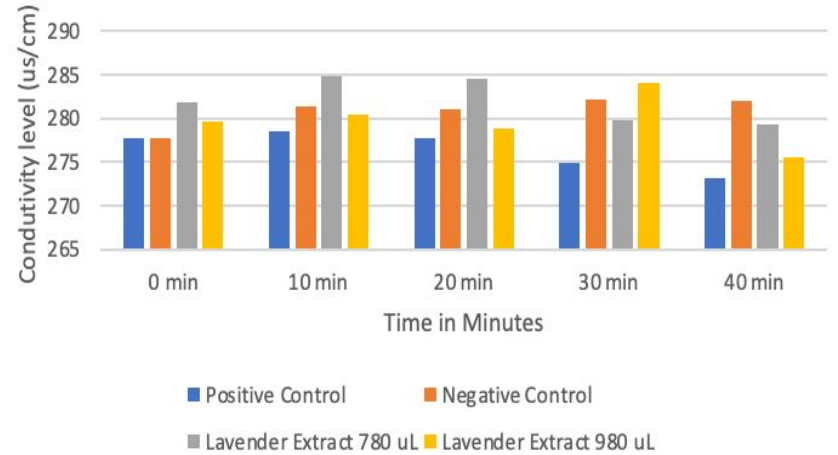


Our Results

Conductivity Levels of Daphnia-Containing Tubes Over a 40 Minute Time Span



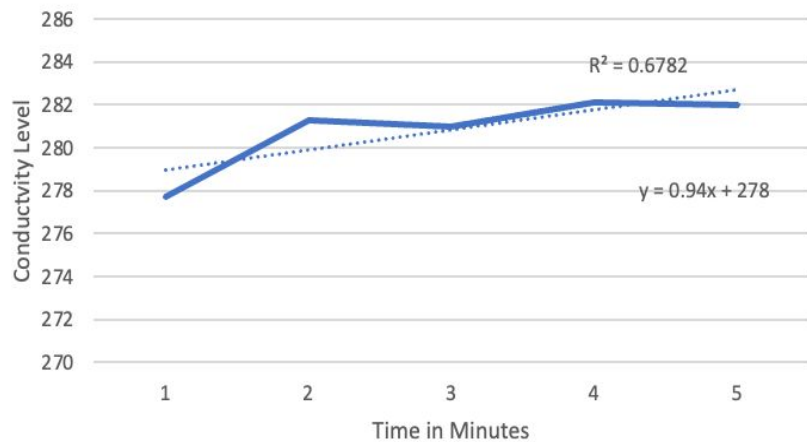
Conductivity Levels of Daphnia-Containing Tubes Over a 40 Minute Time Span



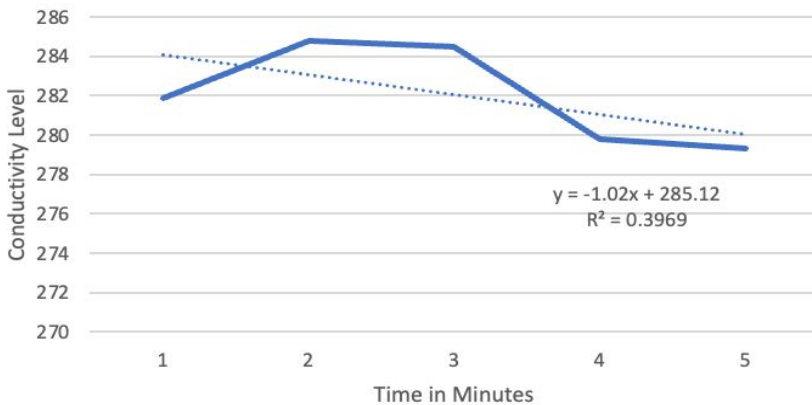
Our More Digestible Results



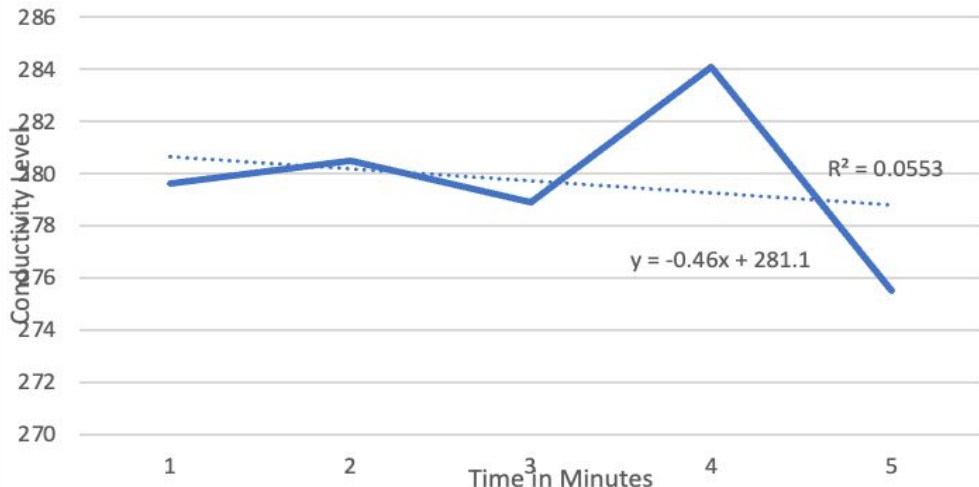
Negative Control



Lavender Extract 780 uL

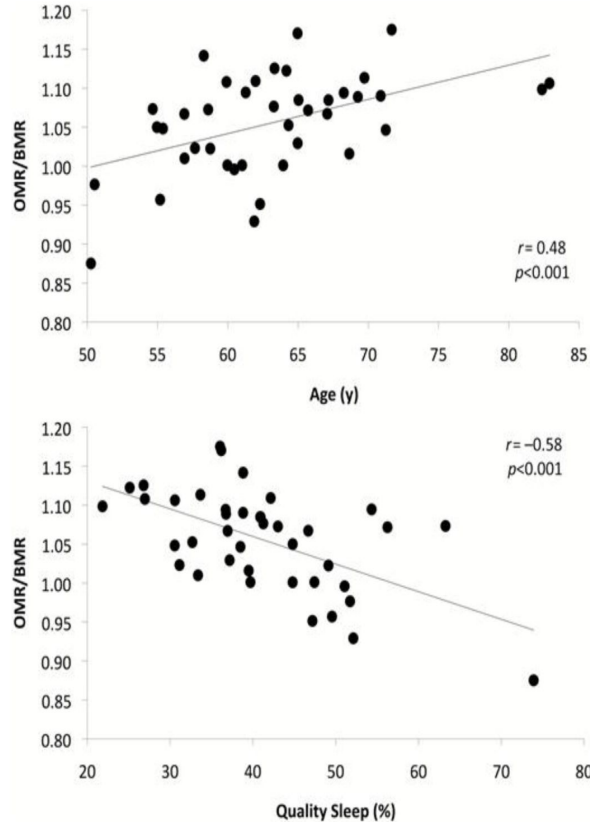


Lavender Extract 980 uL



A Surprise

Figure 2.



The overnight metabolic rates of older human adults



Crayfish



Daphnia

(Valenti et. al)

Future Directions



BMR Measuring Chamber



Dissolved O2 Meter



Conclusion

Lavender does have antimicrobial properties and should be able to be used for medicine. Research states lavender has great use for anti-inflammatory, and anxiety by slowing down heart rates. Our testing did not show these specific findings so more testing would be needed.



Reflections slide

XXXX- Enjoyed the disc diffusion assay. Thought that the micropipette was the most useful item from the lab. Otherwise, learning about all the ways lavender can be used is fun.

XXXX- Enjoyed Brine Shrimp Toxicity Assay. Thought micropipette was the most useful from the lab. Learning about Lavender was challenging but fun when completed.

XXXX- I enjoyed the gram staining and data analysis. I thought it was really interesting seeing all of our agar plates coming to fruition. Furthermore, being able to study and understand them under a microscope.

XXXX- My favorite was Bacterial Sensitivity Assay. Micropipette was most important.



Reference Slide

-Moroşanu, G. A., Simona, D. I., Gavril, V. D., Zaharia, F. A., Carstea, E. M., & Copacenaru, O. (2016, September 10). *Evaluation of the anthropogenic impact on the water quality of Dambovita River*. Research Gate.

https://www.researchgate.net/publication/329574331_EVALUATION_OF_THE_ANTHROPOGENIC_IMPACT_ON_THE_WATER_QUALITY_OF_DAMBOVITA_RIVER

-Valenti, G., Bonomi, A. G., & Westerterp, K. R. (2017). Quality Sleep Is Associated With Overnight Metabolic Rate in Healthy Older Adults. *The journals of gerontology. Series A, Biological sciences and medical sciences*, 72(4), 567–571. <https://doi.org/10.1093/gerona/glw107>

Kajjari S, Joshi RS, Hugar SM, *et al*. The Effects of Lavender Essential Oil and its Clinical Implications in Dentistry: A Review. *Int J Clin Pediatr Dent* 2022;15(3):385-388.

Rusu, A. G., Niță, L. E., Roșca, I., Croitoriu, A., Ghilan, A., Mititelu-Tarțău, L., Grigoraș, A. V., Crețu, B. E., & Chiriac, A. P. (2023). Alginate-Based Hydrogels Enriched with Lavender Essential Oil: Evaluation of Physicochemical Properties, Antimicrobial Activity, and In Vivo Biocompatibility. *Pharmaceutics*, 15(11), 2608.

<https://doi.org/10.3390/pharmaceutics15112608>



Acknowledgement slide

Dr. Rosier, with independent research experiments

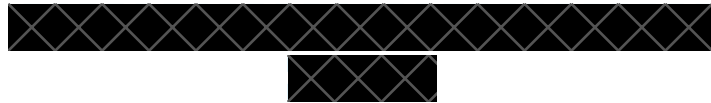
Seton Hill University, with laboratory and equipment

Dr. Hoover



GoldenRod:

Golden opportunities in medicine?



GoldenRod

Scientific Name- Solidago
Common Name- Goldenrod



Goldenrod has bunches of small, bright yellow flowers on a tall green stem.

Goldenrod blooms normally in the summer months of the year and is normally found around North America.



Goldenrod produces nectar in warm weather and when it is moist. Animals such as bees, wasps, and butterflies used goldenrod when it produces its nectar.



Medicinal Properties

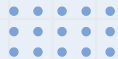
- Urological agent
 - Kidney and bladder inflammation
 - Urolithiasis
- Infections
- Anti-inflammatory
- Gastrointestinal ailments and diseases

Table 2 Mean diameter of inhibition zones (mm) where bacterial growth was inhibited by plant extracts.

Plant Species	Portion ^b	Mean inhibition zone diameter ^a			
		Gram-negative organisms		Gram-positive organisms	
		<i>E. coli</i> (PI No.336)	<i>S. typhimurium</i> (PI No.381)	<i>S. aureus</i> (PI No.4651)	<i>S. lactis</i> (PI No.525)
<u>Haudenosaunee medicinal plants</u>					
<i>A. millefolium</i>	FL	-----	8.8	9.6	-----
<i>H. pilosella</i>	FL	-----	-----	8.1	-----
	LV	-----	-----	9.0	-----
	ST	-----	9.1	-----	-----
<i>I. pandurata</i>	FL	-----	-----	7.3	-----
	LV	10.2	11.1	15.8	12.3
<i>S. canadensis</i>	LV	-----	-----	9.3	-----
<u>Plants with no known medicinal use by Haudenosaunee</u>					
<i>S. virginica</i>	LV	-----	-----	7.9	-----
<i>H. matronalis</i>	ST	-----	11.1	-----	-----
<i>R. multiflora</i>	FL	9.0	8.1	-----	-----
	LV	7.6	-----	-----	-----
Ampicillin (10 mg/mL)		35.0	29.6	37.9	33.2
Water		-----	-----	-----	-----

^a Values represent the mean of three replicates; the pooled SD of all means = 0.60 mm.

^b 10 μ L of each extract (100 mg/mL fresh water extraction) was used for each plant portion; FL, LV, and ST denote flowers, leaves, and stems, respectively.



Medicinal Properties

- anti -fungal
 - *M. fruticola*
 - *P.expansum*

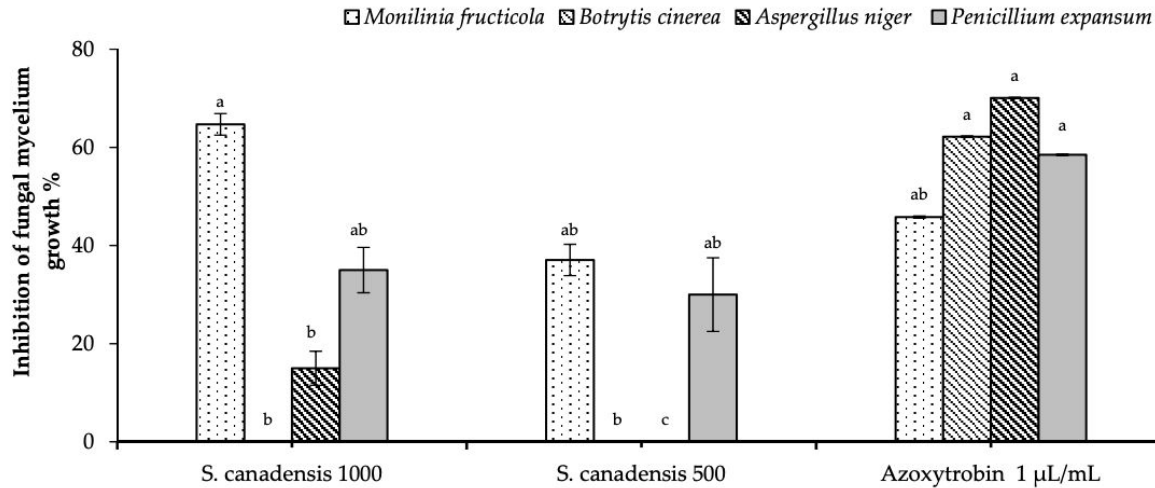


Figure 1. Antifungal activity of *Solidago canadensis* essential oil (EO). Bars with different letters for each tested fungi indicate mean values significantly different at $p < 0.05$ according to Tukey B test between *S. canadensis* EO and Azoxystrobin. Data are expressed as mean \pm SDs (standard deviations).

Medicinal Properties

- Anti- bacterial
 - *P. fluorescens*
 - *C. michiganensis*

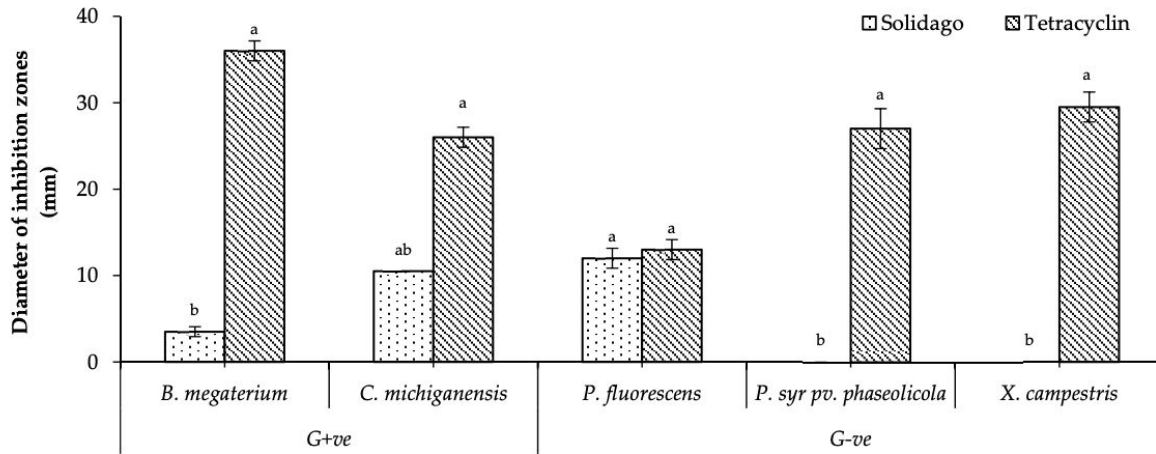


Figure 2. Antibacterial activity of *S. canadensis* EO. Bars with different letters for each tested bacterium indicate mean values significantly different at $p < 0.05$ according to Tukey B test between *S. canadensis* EO and Tetracyclin. Data are expressed as mean \pm SDs.

Antimicrobial Assay

Methods and Results

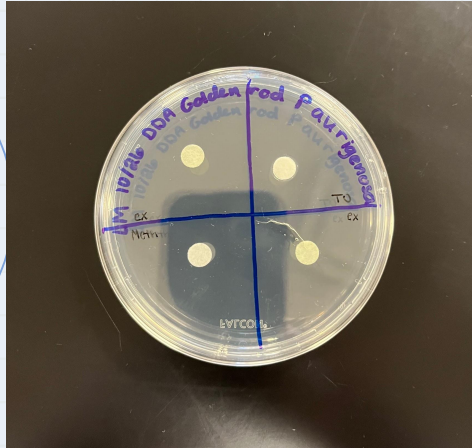
Kirby-Bauer Method



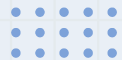
- Also known as disc diffusion assay
- Most commonly used bacterial sensitivity assay

Disk Diffusion Assay Protocol

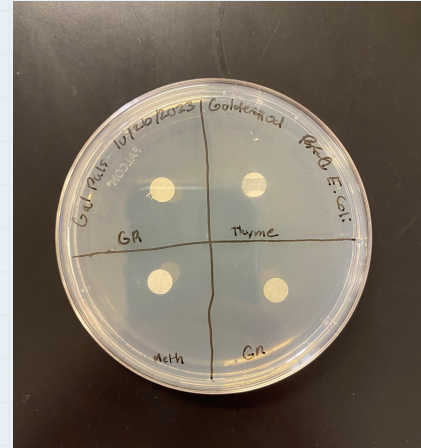
- 4 types of bacteria (100ul per of each per plate)
- Plates divided into 4 quadrants



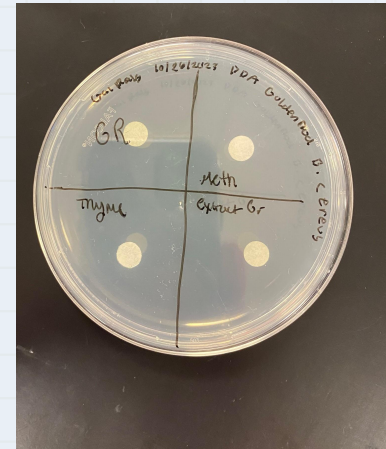
P. aurigenosia



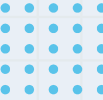
S. aureus



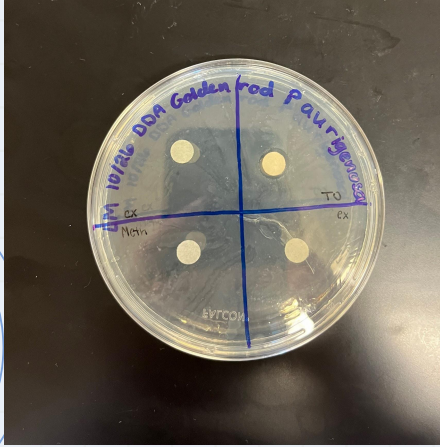
E. coli



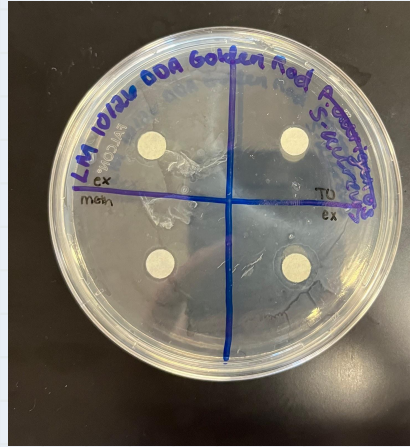
B. cereus



Results!



P. aurigenosia



S. aureus



E. coli

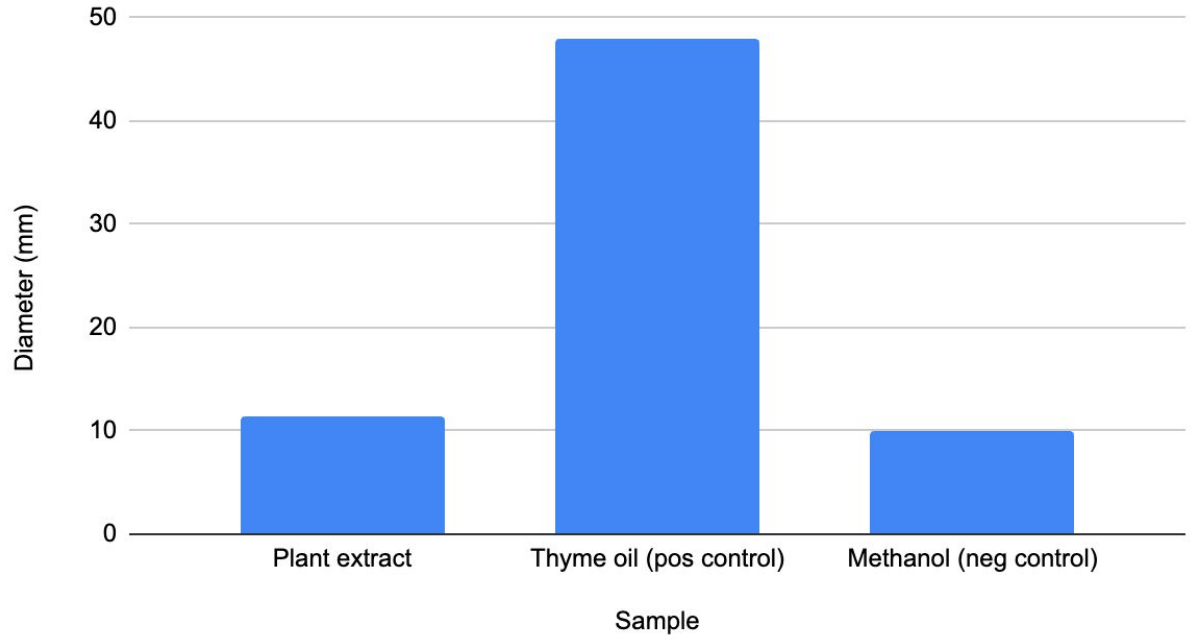


B. cereus

S. aureus

Sample	Avg. Diameter (mm)
Goldenrod extract	11.5
Thyme Oil (+)	48
Methanol (-)	10

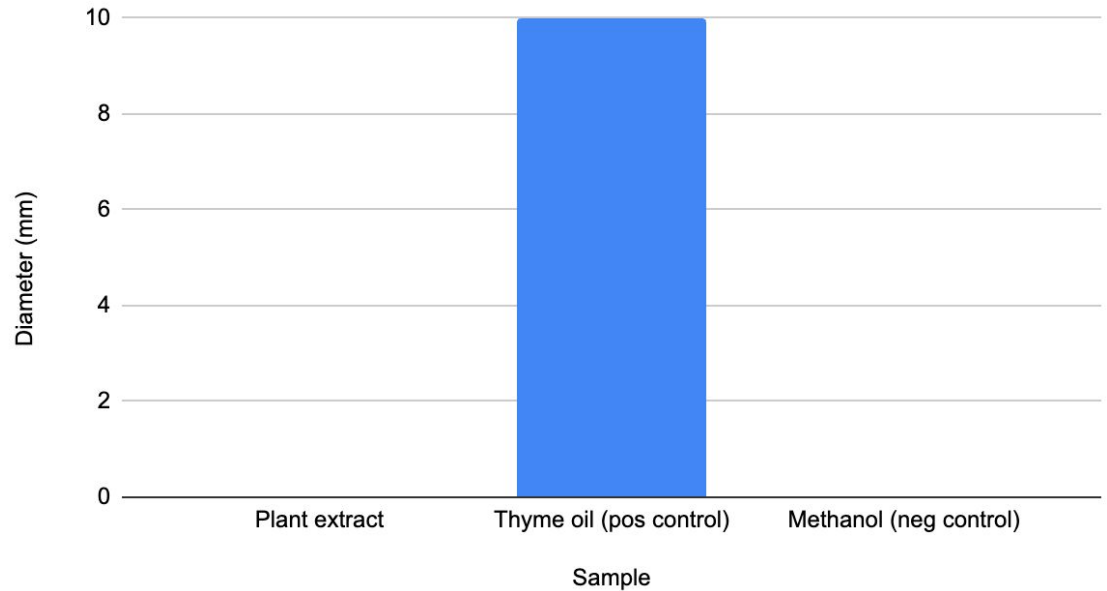
Diameter (mm) of Zones of Inhibition Against S. aureus



P. auriegenosa

Sample	Avg. Diameter (mm)
Goldenrod extract	0
Thyme Oil (+)	10
Methanol (-)	0

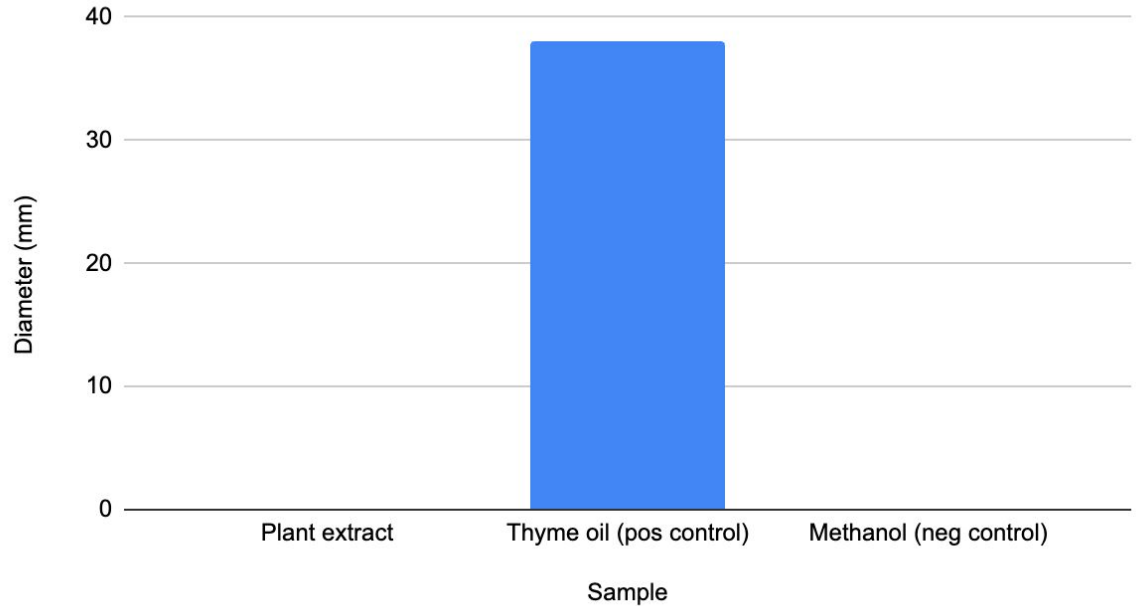
Diameter (mm) of Zones of Inhibition Against P. auriegenosa



E. coli

Sample	Avg. Diameter (mm)
Goldenrod extract	0
Thyme Oil (+)	38
Methanol (-)	0

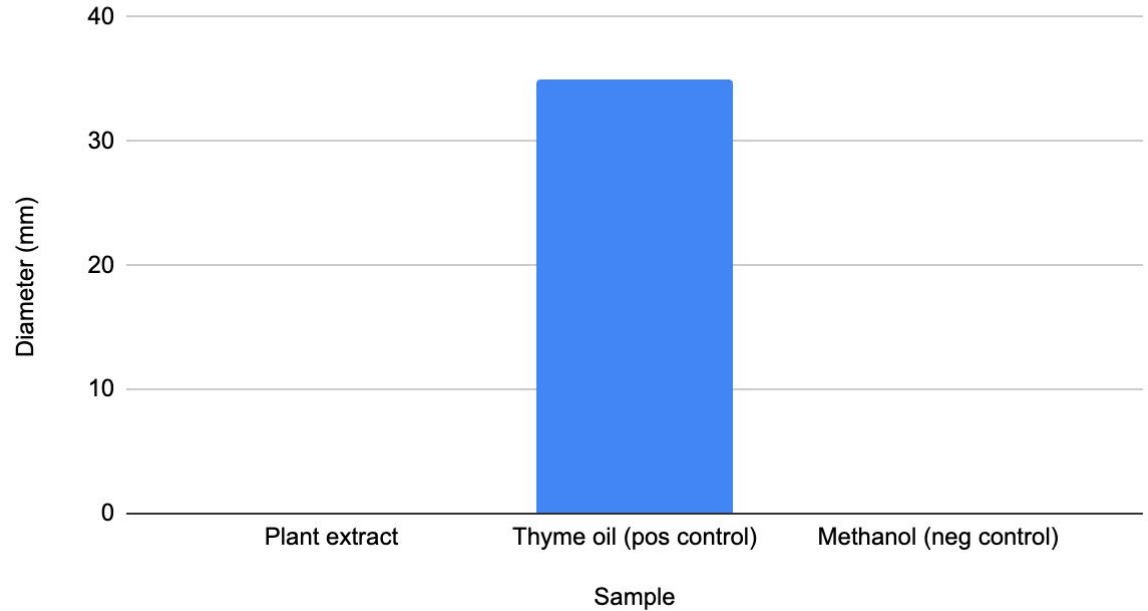
Diameter (mm) of Zones of Inhibition Against E. coli



B. cereus

Sample	Avg. Diameter (mm)
Goldenrod extract	0
Thyme Oil (+)	35
Methanol (-)	0

Diameter (mm) of Zones of Inhibition Against B. cereus



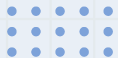
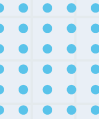


Toxicity Assay

Methods and Results

Toxicity Properties

- We tested Brine Shrimp activity after being put in our GoldenRod extract and other controls to test how many would die and how long they would live.
 - GoldenRod extract (20ul)
 - GoldenRod extract (100ul)
 - Neem Oil (positive control)
 - Methanol (negative control)
- We put **Ten Brine shrimp** in each of our four extracts
- We would check in on the Brine shrimp at certain intervals to check up on their movements and if they were still alive
 - 0 minutes (right as they entered the extracts)
 - 20 minutes
 - 40 minutes
 - 60 minutes
- When checking in on the Brine shrimp we would take into account how they were moving in the extracts. This chart is what we used to describe their activity.
 - 0 - no movement
 - 1 - slow movement
 - 2 - medium fast movement
 - 3 - fast movement



Toxicity Results



GoldenRod assay sample
100 ul at 0 min.



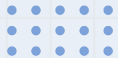
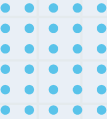
GoldenRod assay sample
100 ul at 20 min.



GoldenRod assay sample
100 ul at 40 min.



GoldenRod assay sample
100 ul at 60 min.



Toxicity Results

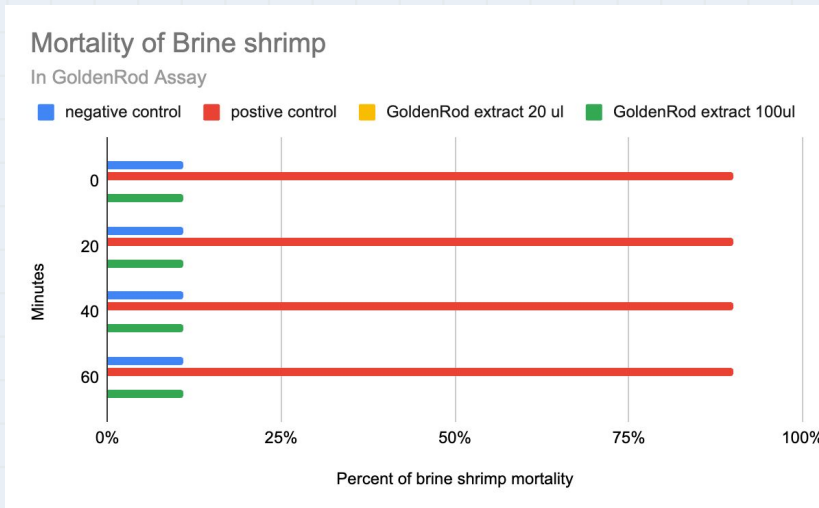
- As we watched the Brine Shrimp at each interval and recorded the activity in the substance
 - In the Positive control the start out all at a frequently moving pace, but as time goes on there movement died down
 - In the Negative control and the plant extracts they all kept moving the whole time at a similar pace

	0 min	20 min	40 min	60 min
[Golden Rod] extract (20ul)	3- fast movements	3- fast movements	3- fast movement	2- medium fast movement
[Golden Rod] extract (100ul)	2 medium fast movements	2 medium fast movements	2 medium fast movements	3 fast movements
Positive control (neem oil)	2- medium fast movements	0-1-slow/no movements	0-1-slow/no movements	0-1-slow/no movements
Negative control (methanol)	All alive. 3 -fast movement	3- fast movement	2-medium fast movement	3- fast movement, spinning

Toxicity Results

Based on the results we see that there is little to no toxicity of GoldenRod to the brine shrimp

- The positive control killed the Brine Shrimp like it was supposed to
- The negative control kept them alive like it was supposed to. (one death due to transportation issues)
- The GoldenRod extracts did not kill the Brine shrimp which means it was not toxic to them



	0 min	20 min	40 min	60 min
[Golden Rod] extract (20ul)	Live: 10 Dead:0	Live: 10 Dead:0	Live: 10 Dead:0	Live: 10 Dead:0
[Golden Rod] extract (100ul)	Live: 9 Dead:1	Live: 9 Dead:1	Live: 9 Dead:1	Live: 9 Dead:1
Positive control (neem oil)	Live: 10 Dead:0	Live: 4 Dead:6	Live: 3 Dead:7	Live:1 Dead:9
Negative control (methanol)	Live: 10 Dead:0	Live: 9 Dead:1	Live: 9 Dead:1	Live: 9 Dead:1



Stimulus Assay

Methods and Results

Stimulatory Properties

- We predicted that our plant extract would be stimulatory since the brine shrimp from the previous experiment had jerky and erratic movements.
- As a pair we were supposed to study the heart rate of daphnia , as a whole we studied 4 daphnia total.
- 2 Daphnia were studied in the presence of our plant extract
- Another 2 Daphnia were studied in methanol



Stimulatory Assay: Table 1- Heart rate

Methanol

Plant Extract

	Daphnia 1	Daphnia 2	Daphnia 1	Daphnia 2
Count 1 15 sec count	29	39	38	40
15 sec count x4	116	156	152	160
Count 2 15 sec count	35	36	42	42
15 sec count x4	140	144	168	168
Count 3 15 sec count	35	37	38	41
15 sec count x4	140	148	152	164
Average Heart rate	132	148	157	164

Table 2- Average rate of daphnia in Methanol

Condition:

Methanol

	Daphnia 1	Daphnia 2	Daphnia 3	Daphnia 4
Average heart rate table 1	132	148	=200	=201
Average heart of both Daphnia	=200			

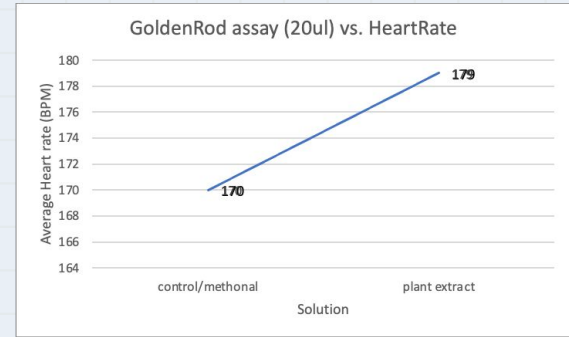
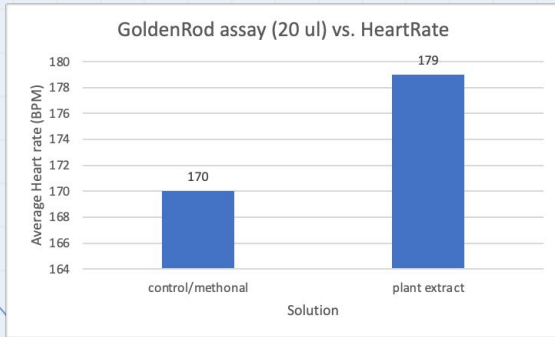
Table 3: Average Daphnia Heart Rate in plant extract

Condition:

Plant Extract

	Daphnia 1	Daphnia 2	Daphnia 3	Daphnia 4
Average Heart Rate table 1	157	164	188	208
Average Heart rate of both Daphnia	=179			

Stimulatory results



- Both Graphs show how much the heart rate increases when the Daphnia were exposed to plant extract
- Our observations match our prediction since it showed signs of an increase in heart rate
- Based on our results, Goldenrod shows favorably potential in having stimulating effects, however further testing would be needed in case we had any potential errors in our experiment

Independent Experiment

Methods and Results

Independent Investigations

Methods:

- We measured the heart rate of **Ten total**

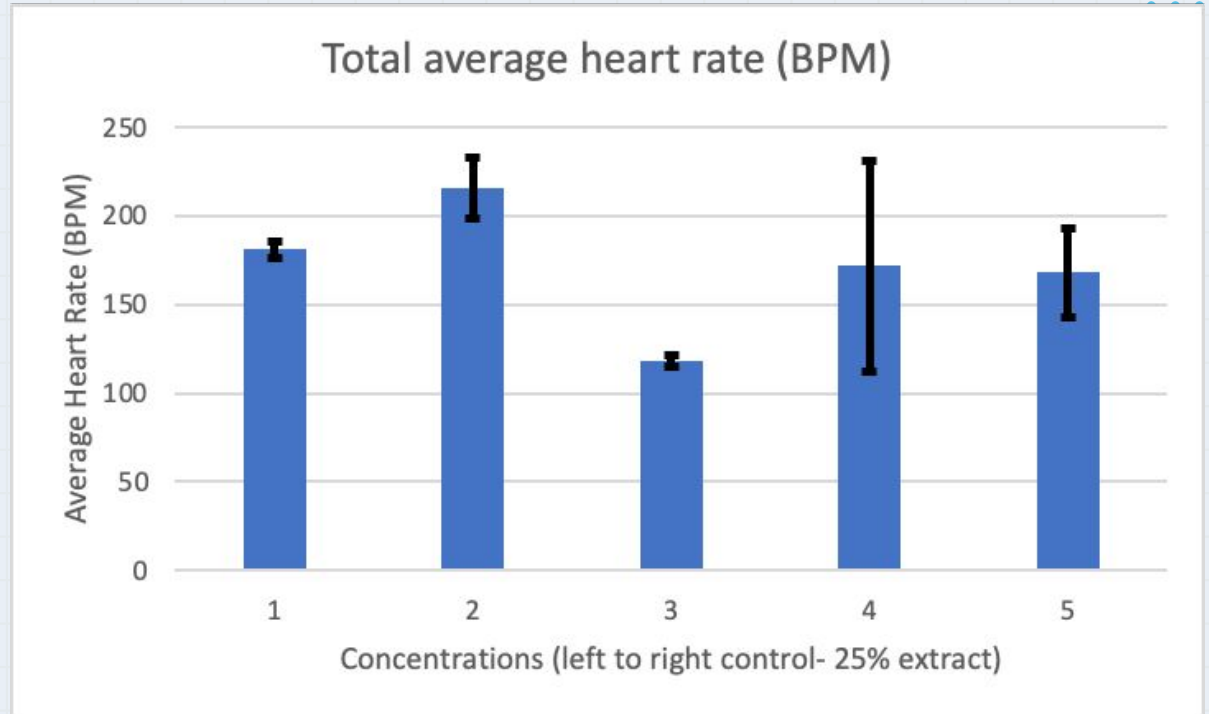
Daphnia

- Control (methanol- 0% plant extract)
 - 10% plant extract
 - 15% plant extract
 - 20% plant extract
 - 25% plant extract
- We used two Daphnia for each extract and control



Stimulatory Properties

- Based on results we found no correlation between accelerated heart rate to higher percentages of plant extract
 - Our results varied in effect causing both high and low heart rate.





Conclusions

Conclusion

In conclusion, Goldenrod doesn't seem to have any toxicity since most of the brine shrimp survived compared to the other substances. Goldenrod seems to have medicinal properties for antifungal use, help with inflammation in the kidneys and bladder, infections, and anti-inflammatory. Had some medicinal properties against *M. Fruticola* (brown rot) and *P. expansum* (blue mold) in stone fruits. It seems to be a stimulus since its heart rate increased slightly when exposed to the extract. In order for us to find out if it is truly medicinal, and its purpose we would have to conduct more experiments.



Future directions

- Prevention of fungal growth



Reflection

GoldenRod in our studies showed little signs of medicinal properties



References

- Rahimi, O., Asadi Louie, N., Salehi, A., & Faed Maleki, F. (2022). Hepatorenal Protective Effects of Hydroalcoholic Extract of *Solidago canadensis* L. against Paracetamol-Induced Toxicity in Mice. *Journal of toxicology*, 2022, 9091605. <https://doi.org/10.1155/2022/9091605>
- Thakore P, Liang TH. Urolithiasis. [Updated 2023 Jun 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK559101/>
- Elshafie, H. S., Grul'ová, D., Baranová, B., Caputo, L., De Martino, L., Sedlák, V., Camele, I., & De Feo, V. (2019). Antimicrobial Activity and Chemical Composition of Essential Oil Extracted from *Solidago canadensis* L. Growing Wild in Slovakia. *Molecules*, 24(7), 1206. <https://doi.org/10.3390/molecules24071206>
- Frey, F. M., & Meyers, R. (2010). Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. *BMC Complementary and Alternative Medicine*, 10(1). <https://doi.org/10.1186/1472-6882-10-64>
- Luciano-Rosario, D., Keller, N. P., & Jurick, W. M., II. (2020). *Penicillium expansum*: Biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. *Molecular Plant Pathology*, 21(11), 1391–1404. <https://doi.org/10.1111/mpp.12990>

References

Fursenco, C., Calalb, T., Uncu, L., Dinu, M., & Ancuceanu, R. (2020). *Solidago virgaurea* L.: A Review of Its Ethnomedicinal Uses, Phytochemistry, and Pharmacological Activities. *Biomolecules*, *10*(12), 1619.

<https://doi.org/10.3390/biom10121619>

Ahmed, M. A., Shafiei-Masouleh, S.-S., Mohsin, R. M., & Salih, Z. K. (2023). Foliar Application of Iron Oxide Nanoparticles Promotes Growth, Mineral Contents, and Medicinal Qualities of *Solidago virgaurea* L. *Journal of Soil Science and Plant Nutrition*, *23*(2), 2610–2624. <https://doi.org/10.1007/s42729-023-01218-2>

Vilanova, L., Valero-Jiménez, C. A., & van Kan, J. A. L. (2021). Deciphering the *Monilinia fructicola* Genome to Discover Effector Genes Possibly Involved in Virulence. *Genes*, *12*(4), 568. <https://doi.org/10.3390/genes12040568>

Luciano-Rosario, D., Keller, N. P., & Jurick, W. M., II. (2020). *Penicillium expansum*: Biology, omics, and management tools for a global postharvest pathogen causing blue mould of pome fruit. *Molecular Plant Pathology*, *21*(11), 1391–1404. <https://doi.org/10.1111/mpp.12990>

References

Nandi, M., Macdonald, J., Liu, P., Weselowski, B., & Yuan, Z. (2018). *Clavibacter michiganensis* ssp. *michiganensis*:

Bacterial canker of tomato, molecular interactions and disease management. *Molecular Plant Pathology*, 19(8), 2036–2050. <https://doi.org/10.1111/mpp.12678>

Scales, B. S., Dickson, R. P., LiPuma, J. J., & Huffnagle, G. B. (2014). Microbiology, Genomics, and Clinical Significance of the *Pseudomonas fluorescens* Species Complex, an Unappreciated Colonizer of Humans. *Clinical Microbiology Reviews*, 27(4), 927–948. <https://doi.org/10.1128/cmr.00044-14>

Thank you

Brine Shrimp, Daphnia, and
Seton Hill University for
making this research possible



Micropollutants & Waste Water

A research presentation by [REDACTED], [REDACTED],

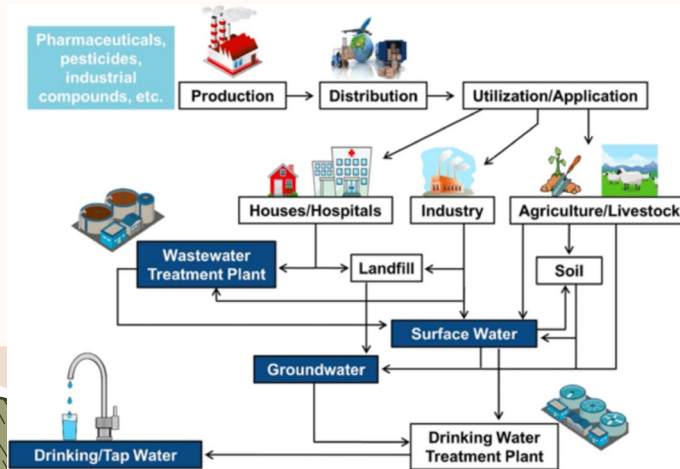
[REDACTED]

Team SEB

February 20, 2024

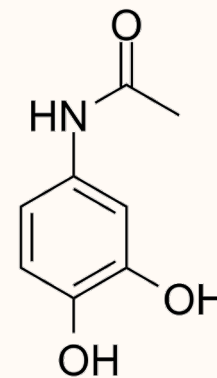
Introduction to Micropollutants

- Organic chemicals are the main concern
 - PPCPs, Pharmaceuticals, Pesticides
 - Low concentrations, but long-term exposure
- Researchers are determining which pollutants are of the highest concern



Introduction to Acetaminophen as a Micropollutant

- One of the most common OTC painkiller
- Excreted into water sources after consumption in native and metabolized forms
- Present in ppt/ppm, but, intermediates can be harmful
 - Depends on environment
 - Hydroquinone and benzoquinone = toxic
- Drinking water and food can allow for these toxic metabolites to reenter the body



Organic Structure
of Acetaminophen

Research Question



Big Picture

Target medicine as a pollutant in wastewater can pose issues to not only humans but other plants and animals in the environment



Goal

Determine the effects of the medicine on different organisms in order to study and determine what effects are positive vs negative, and how our research can work to combat micropollution



Importance

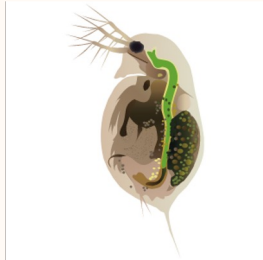
Previous research shows potential toxic effects, more research can contribute to determining these effects

Overall Research Approach

Week 3

Daphnia Exposure

Observe effects of
heart rate



Week 4

Yeast Exposure

Observe cell count
and cell survival



Week 5

Duckweed
Exposure

Observe qualitative
and quantitative data
such as color and
frond number



Week 9

Darkling Beetle
Exposure

Observe morphology
and survival data



01 Daphnia

Experiment & Results





Previously Researched Data

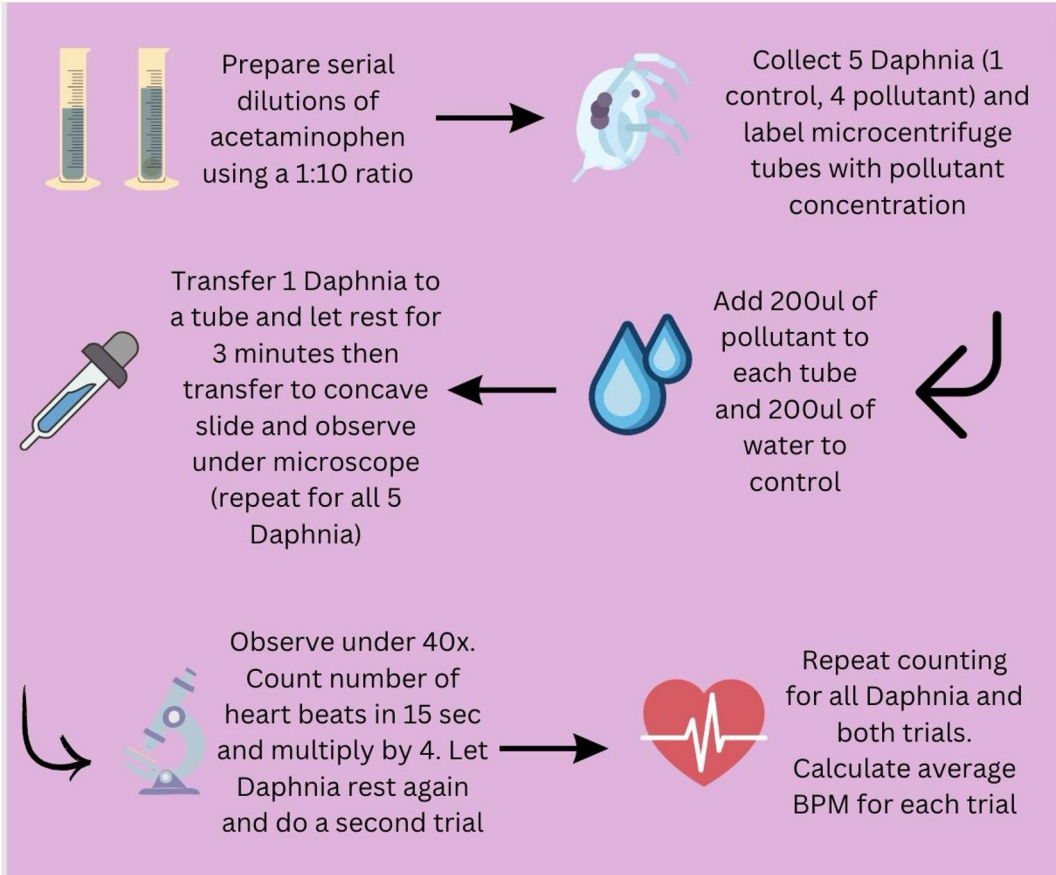
- Experiment tests many aspects of Daphnia
 - Not heart rate
- Daphnia growth is not adversely affected by ibuprofen
 - Body surface area increases, with a spike at 12-14 days and 80mg.
- Population growth rate is adversely affected
 - As ibuprofen concentration increases, growth rate decreased
- Daphnia reproduction rates were adversely affected.
 - The amount of juveniles present decreased significantly as time went on, until there were none
 - As ibuprofen concentration increases, reproduction rate decreases

(Heckman 2007)

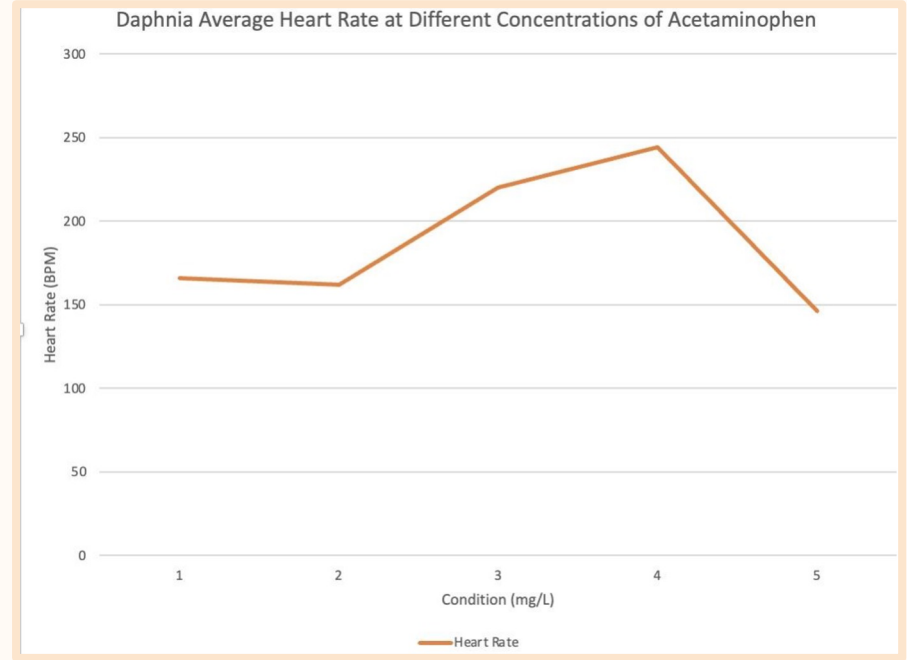


Purpose and Process

Purpose: to determine the stimulatory or sedative effects of acetaminophen on Daphnia heart rate



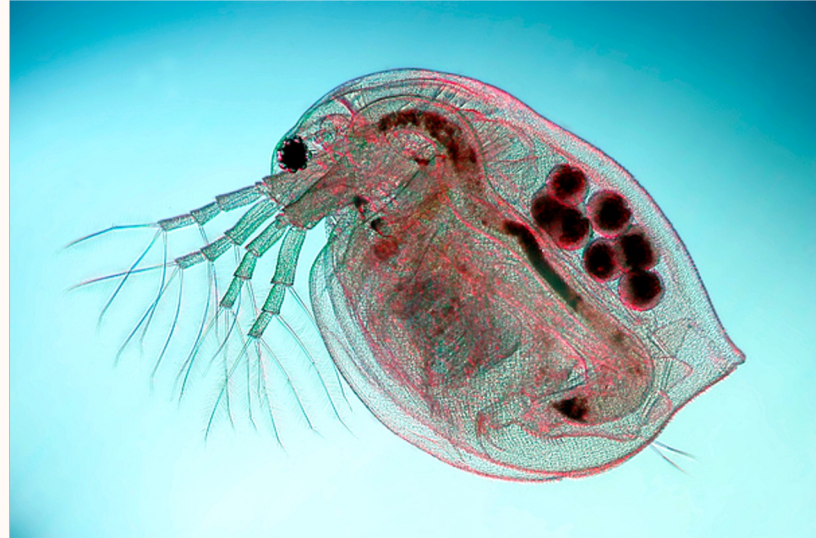
Condition (mg/L)	Heart Rate (bpm)
100	166
10	162
1	220
0.1	244
0	146



Data (Pics & Graphs)

Conclusions & Error

- Conclusion: as the concentration of acetaminophen increases, the heart rate decreases. The control was an outlier.
- Error: damaging of Daphnia in transfer could affect heart rate.



02 Yeast

Experiment & Results



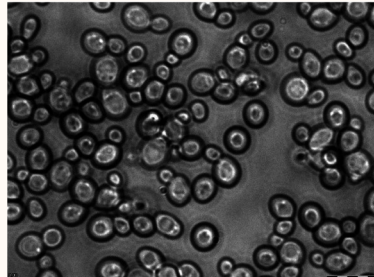
Previous Research/ Studies

There have been many different studies completed on yeast. Yeast is such a great source to study because it's easy to take care of, reproduces fast, and cheaper. Yeast research has been done on a variety of different topics/experiments.

One specific experiment was whether caffeine had an affect on cell concentration

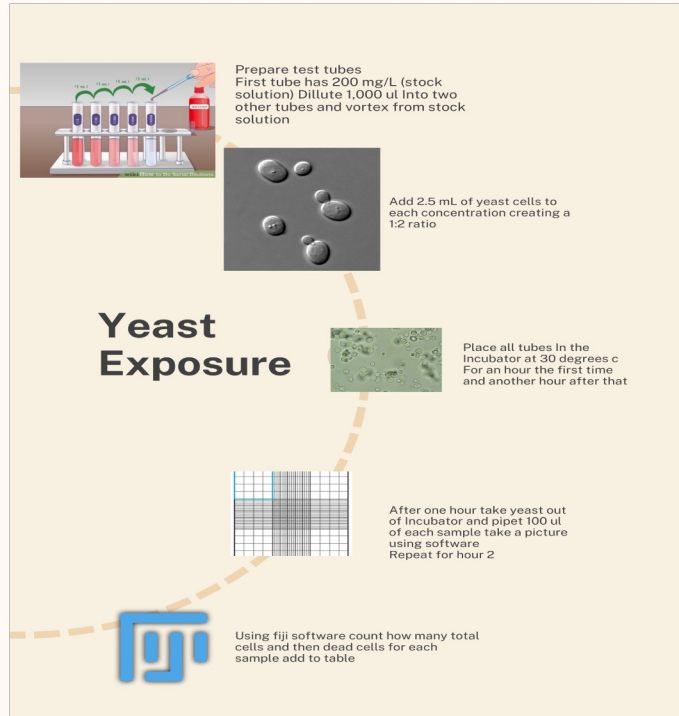
Results

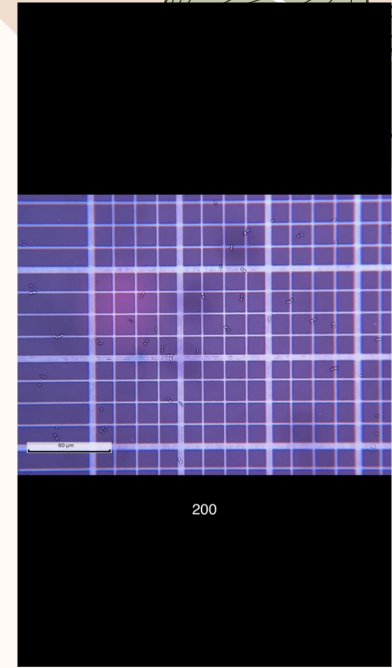
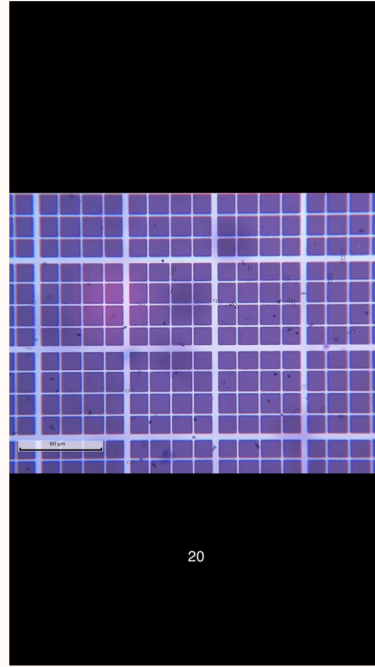
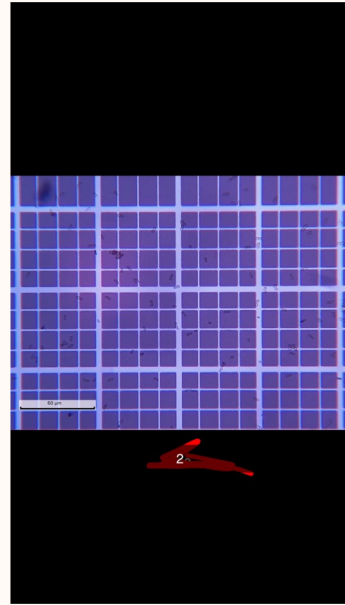
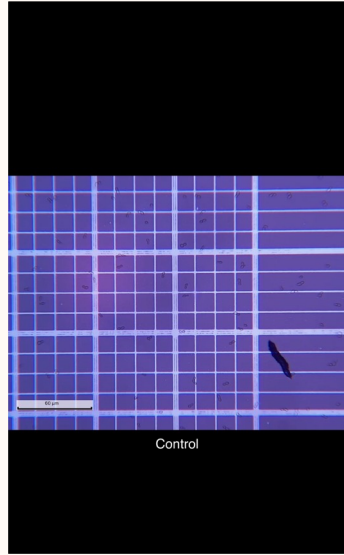
- The caffeine negatively affected cell concentration
- As more caffeine was added the less cells there were present (Hu 2017)



Purpose and Procedure

Conduct an experiment on yeast using acetaminophen observing and monitoring cell count and living cell count





Data

Table 1. Cell Concentration Over Time Following medicine/metabolite Exposure

Condition	Time point (h)	Total Cell Count 16-square set #1	Total Cell Count 16-square set #2	Average Total Cell Count	Cell Concentration (cells/mL)
200 mg/L medicine or metabolite (stock)	1	24	13	18.5	185,000
	2	21	14	17.5	175,000
20 mg/L medicine or metabolite	1	19	12	15.5	155,000
	2	12	30	21	210,000
2 mg/L medicine or metabolite	1	15	13	14	140,000
	2	25	9	17	170,000
Control	1	15	16	15.5	155,000
	2	22	12	17	170,000



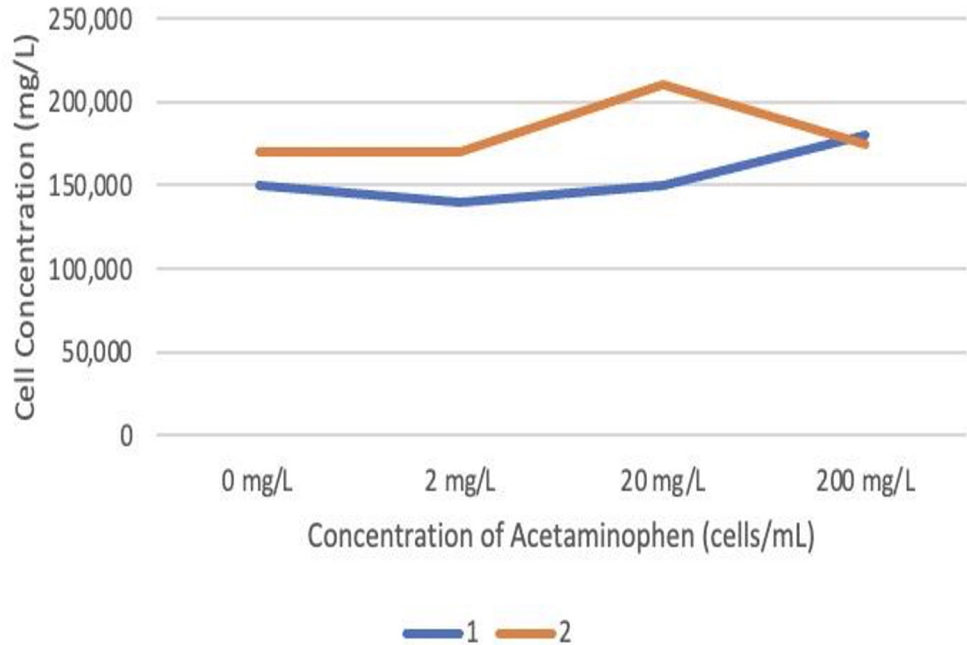
Data continued

Table 2. Proportion of living cells over time Following medicine/metabolite Exposure

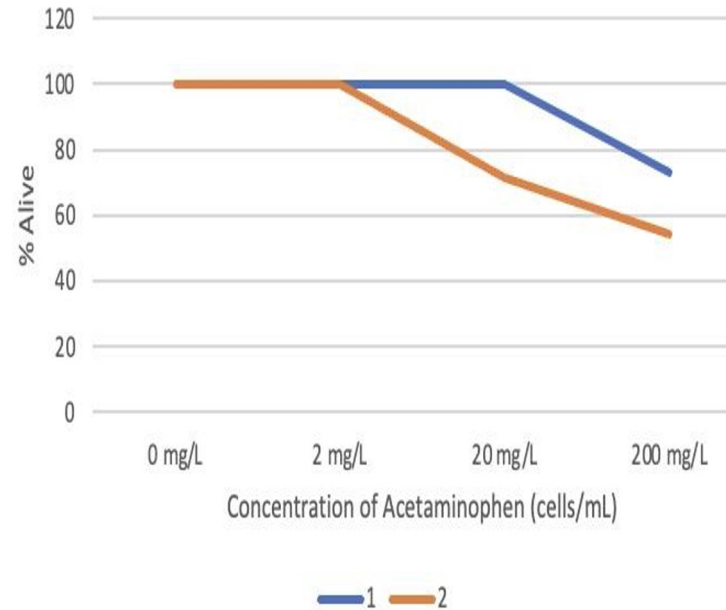
Condition	Time point (h)	Living Cell Count 16-square set #1	Living Cell Count 16-square set #2	Average Living Cell Count	Percentage Living Cells (divide average living cell count by the average cell count in Table 1)
200 mg/L medicine or metabolite (stock)	1	19	8	13.5	72.9%
	2	7	12	9.5	54.3%
20 mg/L medicine or metabolite	1	19	12	15.5	100%
	2	6	14	15	71.4%
2 mg/L medicine or metabolite	1	15	13	14	100%
	2	25	9	17	100%
Control	1	15	16	15.5	100%
	2	22	12	17	100%

Graphs

Total Cell Concentration vs Time



% Alive vs Time



Errors and Conclusion

ERRORS:

- Transferring Yeast
- Using Fiji software incorrectly
- Using unsterile lab equipment
- Not putting yeast back in the incubator immediately after using them



Conclusion: As the concentration of acetaminophen increases, less yeast cells are alive. Although there is not an obvious set pattern between cell concentration and acetaminophen concentration

Conclusions



Daphnia

As the concentration of acetaminophen increases, the average heart rate of Daphnia decreases



Yeast

As the concentration of acetaminophen increases, less yeast cells are alive. There is not an obvious pattern between cell concentration and acetaminophen concentration

Overall

Overall, it seems as if acetaminophen has adverse effects on the organisms that we have experimented with so far

Future Directions

There are many future steps for our research on acetaminophen

- Complete duckweed experiment
- Start and Complete Beetle experiment
- Our independent experiment/research
- Final poster
- Is acetaminophen a toxic micropollutant to the environment?



References

Phong Vo, H. N., Le, G. K., Hong Nguyen, T. M., Bui, X.-T., Nguyen, K. H., Rene, E. R., Vo, T. D., Thanh Cao, N.-D., & Mohan, R. (2019). Acetaminophen micropollutant: Historical and current occurrences, toxicity, removal strategies and transformation pathways in different environments. *Chemosphere*, 236, 124391.

<https://doi.org/10.1016/j.chemosphere.2019.124391>

Yang, Y., Zhang, X., Jiang, J., Han, J., Li, W., Li, X., Yee Leung, K. M., Snyder, S. A., & Alvarez, P. J. J. (2021). Which micropollutants in water environments deserve more attention globally? *Environmental Science & Technology*, 56(1), 13–29.

<https://doi.org/10.1021/acs.est.1c04250>

Hu, Lucy et al. "Effect of caffeine concentration and incubation time on the cell concentration of wild-type *Saccharomyces cerevisiae*." *The Expedition* 6 (2017): n. Pag.

<https://www.semanticscholar.org/paper/Effect-of-caffeine-concentration-and-incubation-on-Hu-Jeong/e8db0e6a66bdd7b74bcc3ddcdf0383a029cf28bf>

HECKMANN, L., CALLAGHAN, A., HOOPER, H., CONNON, R., HUTCHINSON, T., MAUND, S., & SIBLY, R. (2007). Chronic toxicity of ibuprofen to daphnia magna: Effects on life history traits and Population Dynamics. *Toxicology Letters* 172(3) 137–145. <https://doi.org/10.1016/j.toxlet.2007.06.001>



Any Questions?

Introduction

- Brine Shrimp are any of several small crustaceans, and they play important roles in saline aquatic and marine ecosystems.¹
- Brine shrimp are used in the lab due to their simplicity, cheapness, and convenience.¹
- Newly-hatched brine shrimp, are strongly attracted to white light or sunlight. Some experiments use light to move newly-hatched brine shrimp from the chambers they are hatched in.¹
- **Hypothesis: If the brine shrimp are exposed to IB then they will move more slowly to the light (even though they are attracted to light) because the IB will slow their movement.**



Discussion

- The results of this study reveal that ...
- Our hypothesis was correct and the effects of Ibuprofen on brine shrimp are negative.
- The effect Ibuprofen had on brine shrimp was slowing down their activity.
- Higher concentrations of IB slowed down the brine shrimp more than lower concentrations.
- Some brine shrimp showed no movement at all.
- Brine Shrimp movements being inhibited by IB will affect them getting a source of food.
- This shows how micropollutants, specifically painkillers affect biological systems in a negative way

Methods

Figure 1: Picture showing steps of brine shrimp procedure.

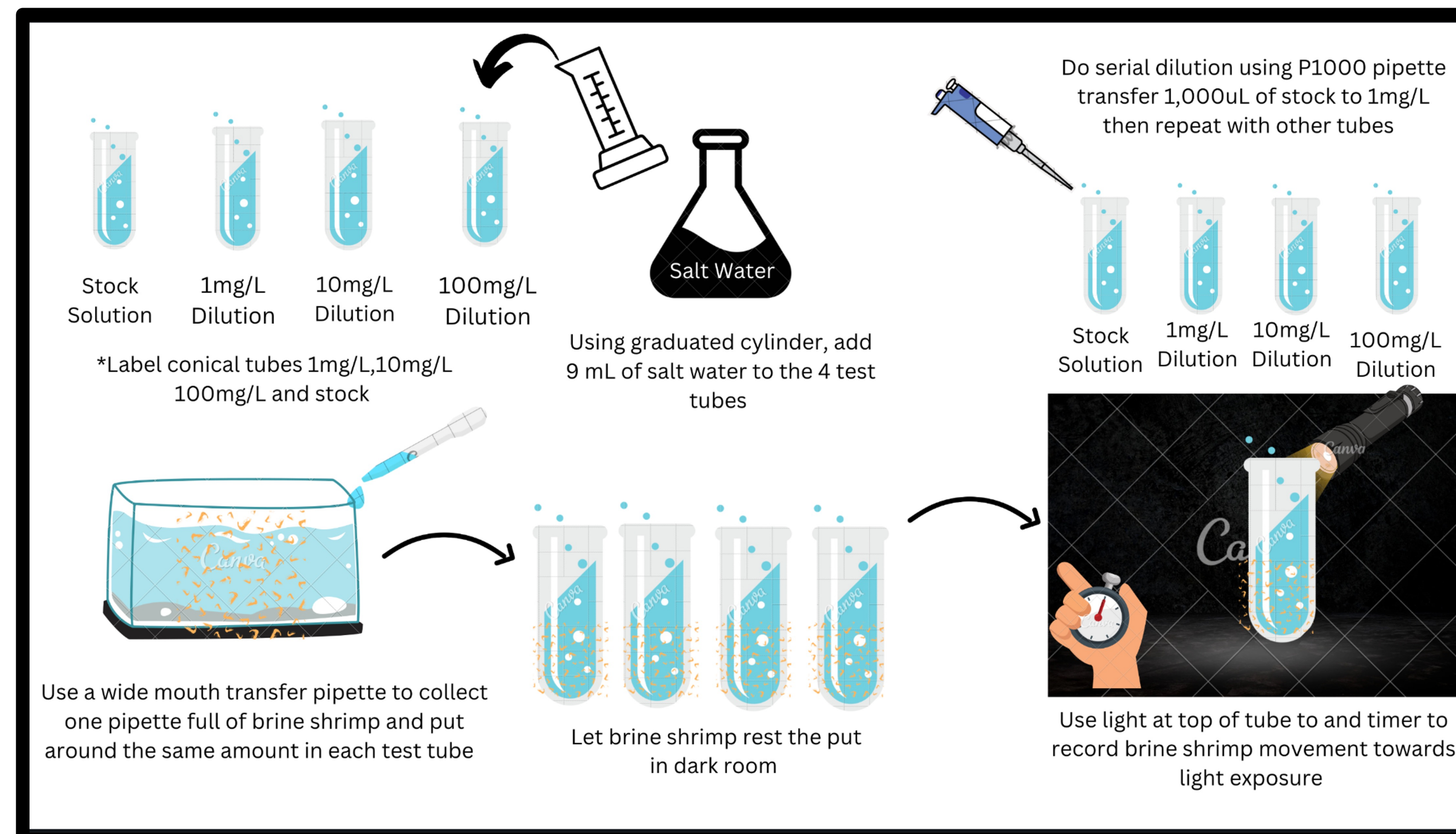
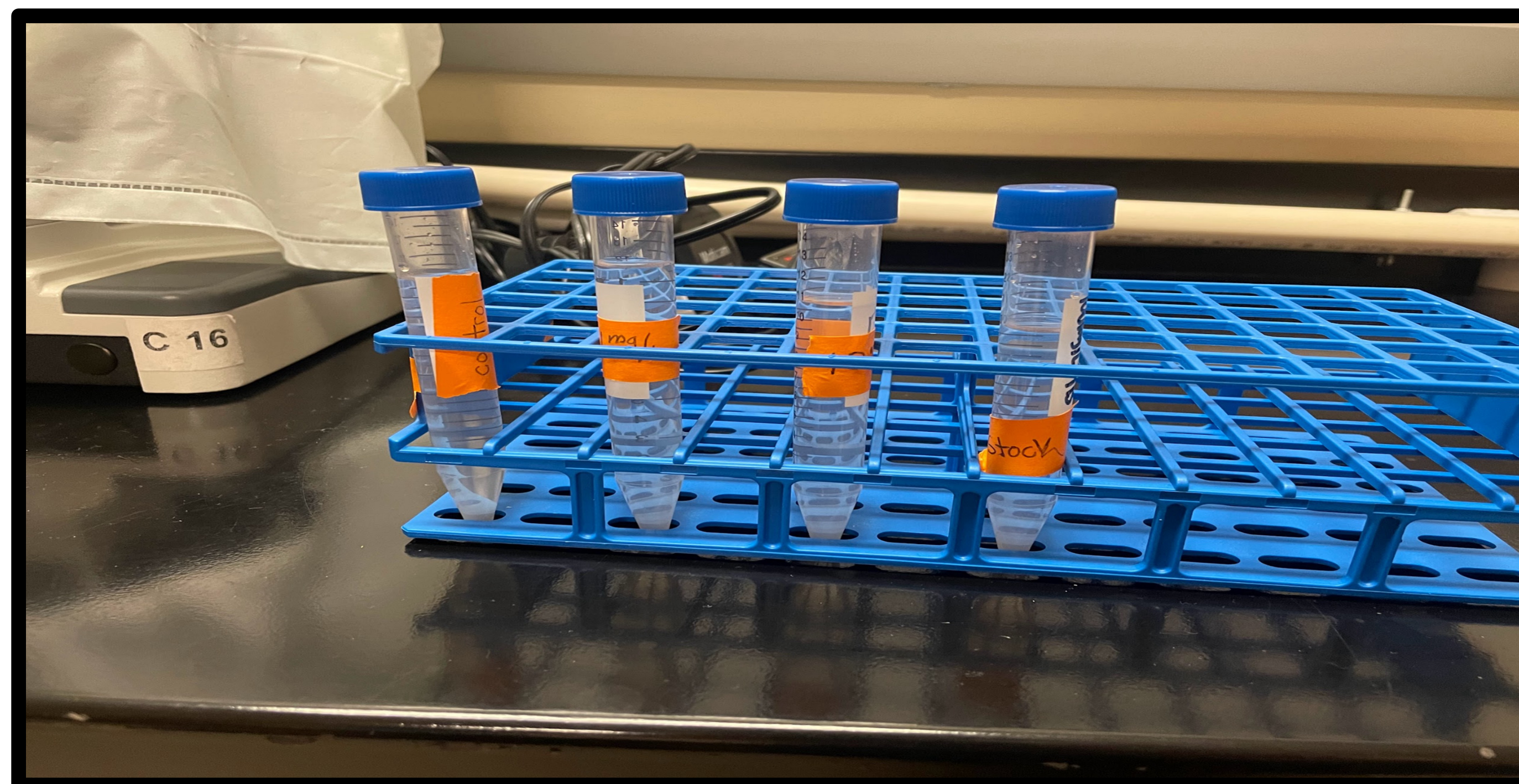


Figure 2: Picture showing labeled conical tubes with brine shrimp with the control group (0 mg/L IB), 1 mg/L of ibuprofen, 10 mg/L of ibuprofen, and the stock group (100 mg/L IB)



Results

More ibuprofen= the longer it will take the brine shrimp to move towards the light

Figure 3: The Average Time it Took for the Brine Shrimp to Move Towards the Light

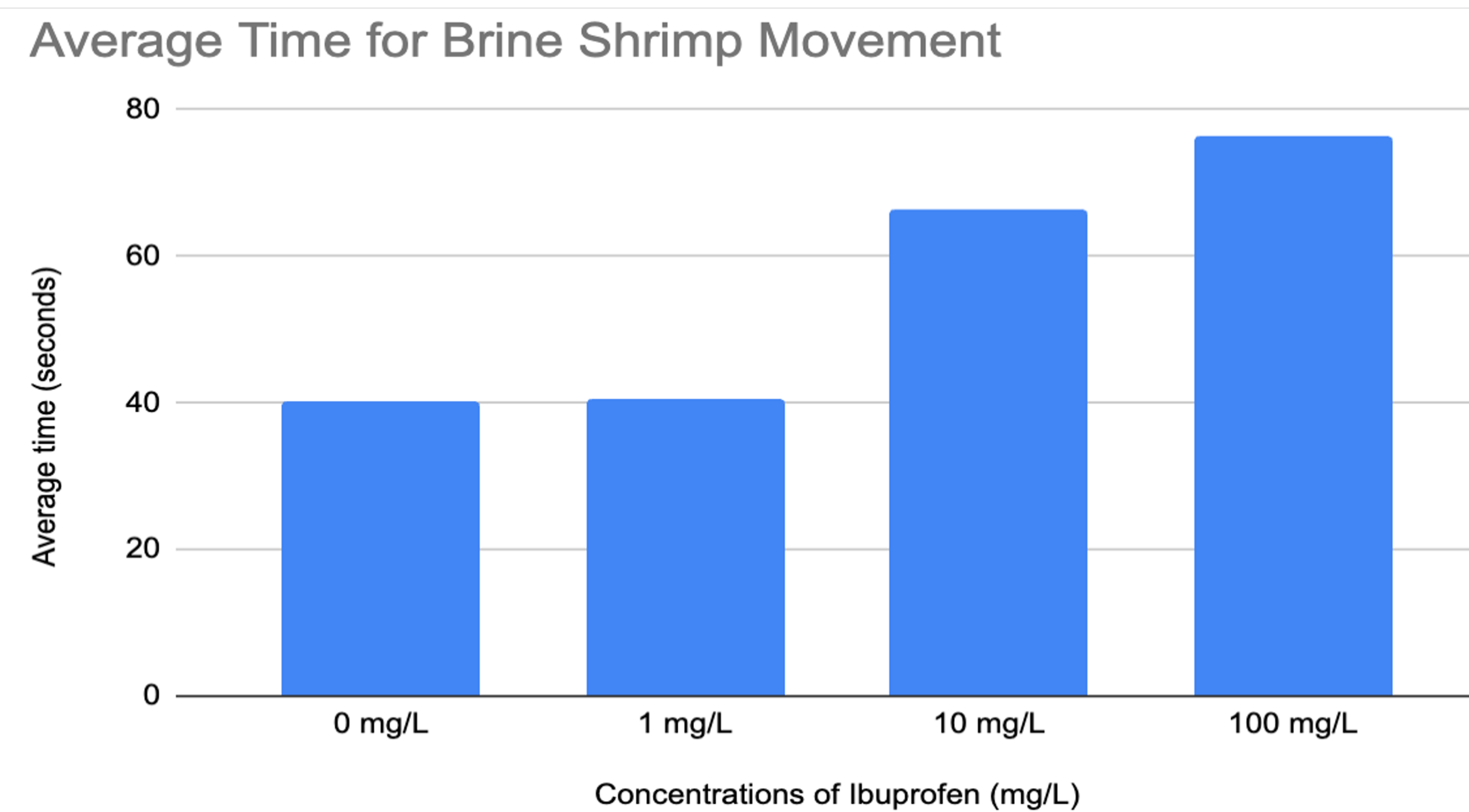
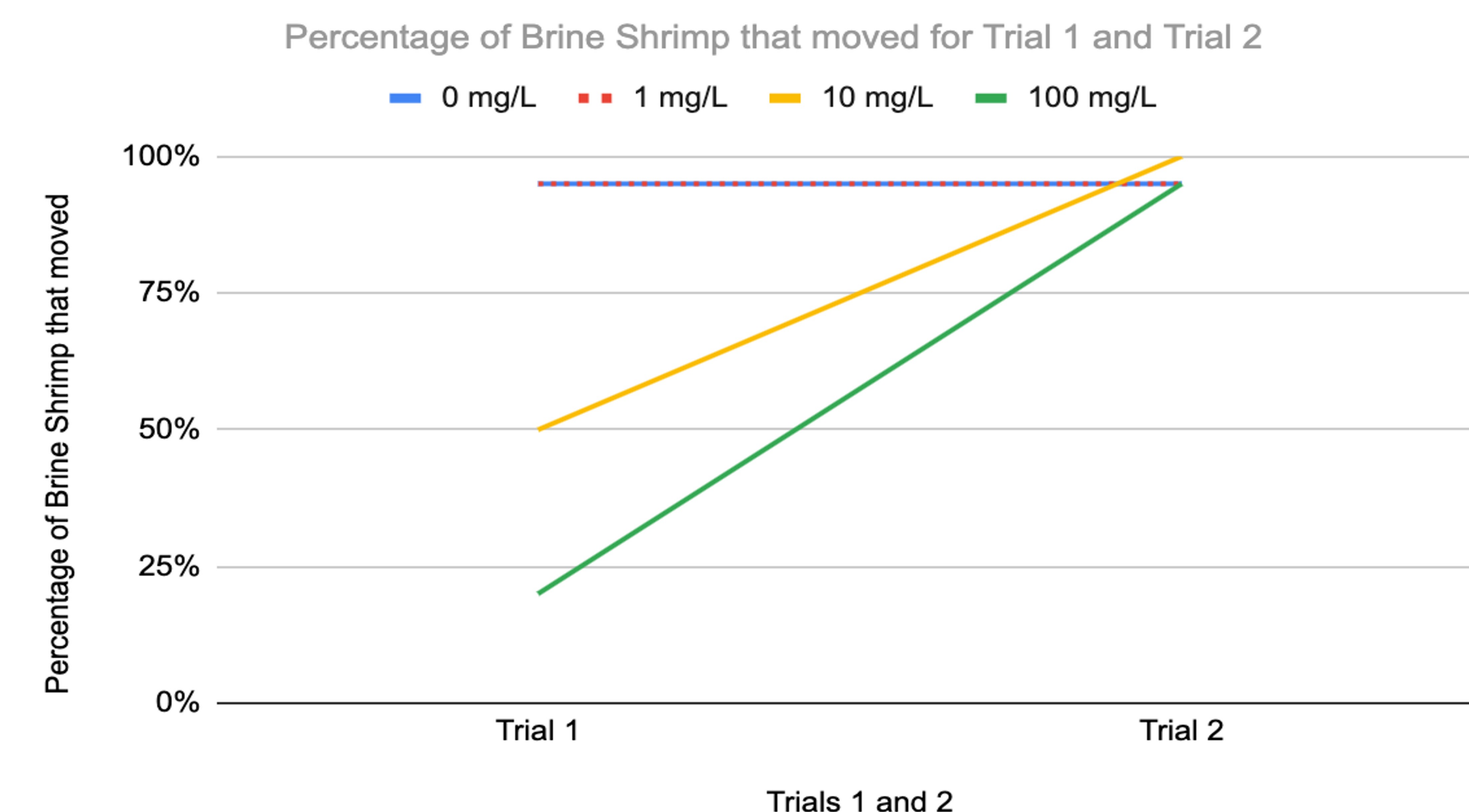


Figure 4: Percentage of Brine Shrimp that Moved



Future Directions

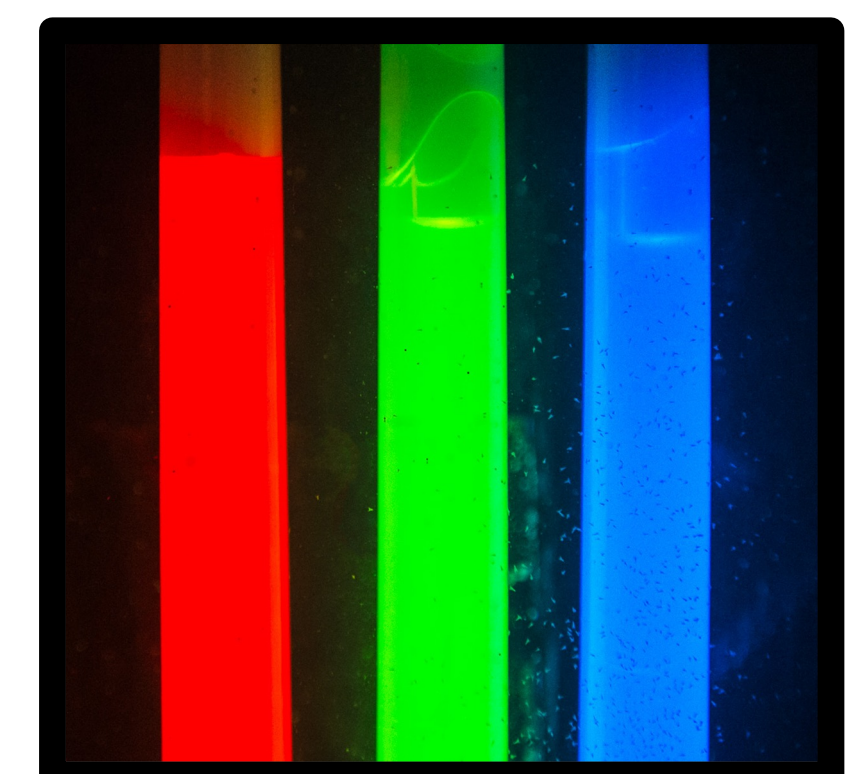
In an experiment brine shrimp were determined to be particularly useful animals for observations and experiments on feeding²

- It is easy to observe the brine shrimp feeding on algae
- Future experiment: Observe if their rate of feeding on algae is slowed when exposed to different concentrations of ibuprofen.



According to research brine shrimp were more strongly attracted to blue light, and less to other colors (red and green)³

- As sunlight travels through water, red and yellow light are absorbed, leaving only blue and green to be transmitted.
- Brine shrimp have evolved to be most sensitive to blue light, the color of light that's best transmitted in water.
- Future experiment: Determine whether brine shrimp exposed to different concentrations of ibuprofen favor different colors of light.



References:

1. Zhang, Y., Mu, J., Han, J., & Gu, X. (2012). An improved brine shrimp larvae lethality microwell test method. *Toxicology Mechanisms & Methods*, 22(1), 23-30. <https://doi-org.setonhill.idm.oclc.org/10.3109/15376516.2011.583297>
2. Dockery, M. (2000). Investigating the feeding behaviour of brine shrimps. *Journal of Biological Education (Society of Biology)*, 34(4), 211. <https://doi.org/10.1080/00219266.2000.9655720>
3. Plankton Rainbow: Biology & Perception Science Activity | Exploratorium Teacher Institute Project. "Plankton Rainbow Shine Some Light on Brine Shrimp Behavior.", Exploratorium, 2024. www.exploratorium.edu/snacks/plankton-rainbow.

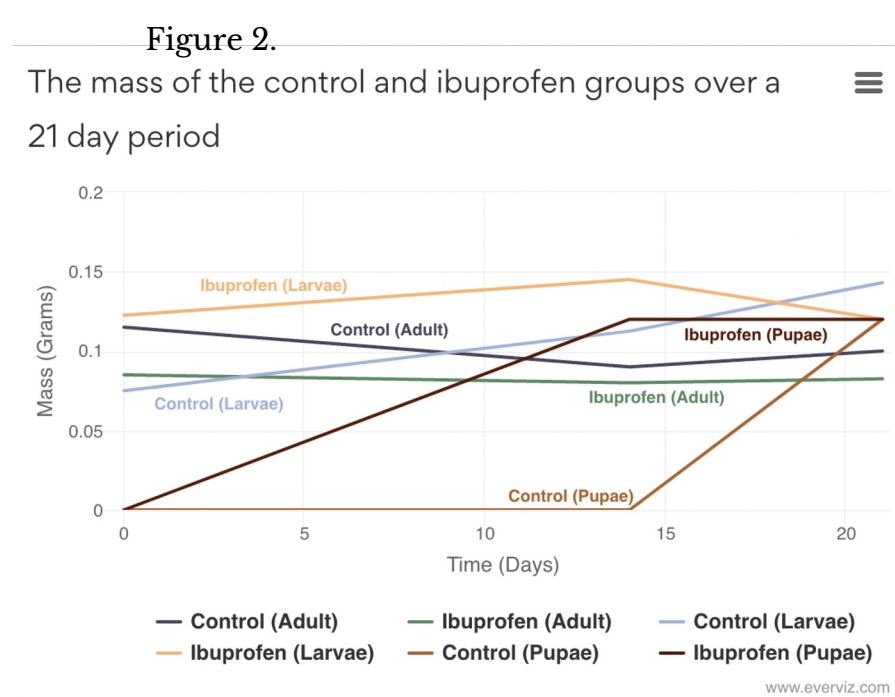
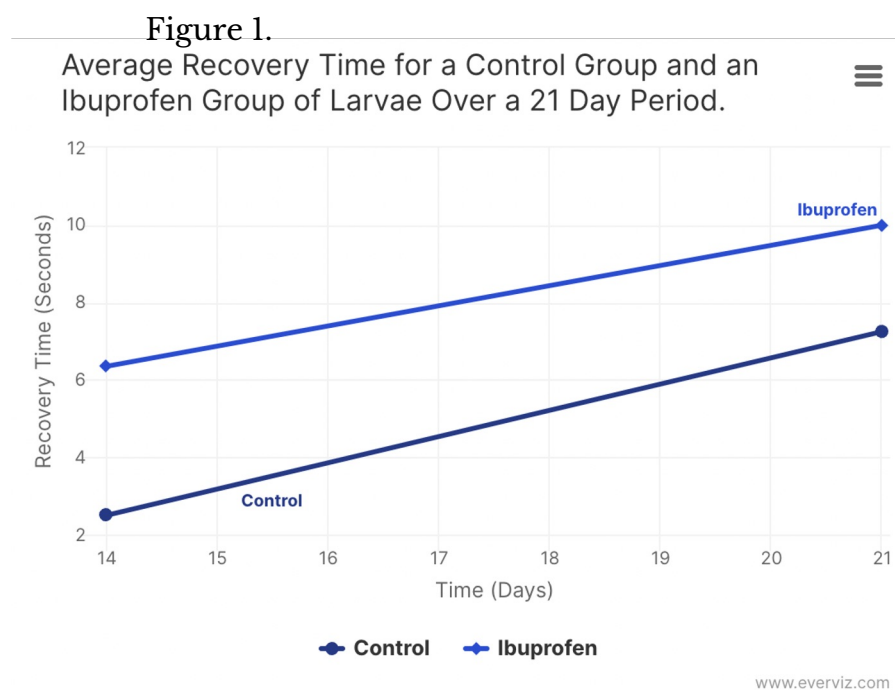
Acknowledgments:

- Thanks to lab partners
- Thanks to lab professors who got all of the materials for us

Introduction

Results

- The purpose of this experiment is to determine if the darkling beetles (*Tenebrio molitor*) (Phylogenomics of Darkling Beetles (Coleoptera: Tenebrionidae) From the Atacama Desert, 2023) prefer the ibuprofen water crystals or the control (uncontaminated) water crystals. Ibuprofen is nonsteroidal anti-inflammatory drug that you can find at any drug store (Ibuprofen, 2023). Ibuprofen is a micro pollutant that can get into soil and water ways. Furthermore, is ibuprofen beneficial or toxic to the darkling beetles when in there food source and living environment?
- The objective is to measure the mass of both the ibuprofen and control water crystals to determine which one the darkling beetles are drinking more. In finding what the darkling beetles drank more of the date will also be used to determine if it slowed down the natural predator response of the beetles and if it would show a decrease in the beetles development.
- This helps us infer how ibuprofen can affect other terrestrial organisms and ecosystems over time.
- Our previous experiment on Darkling beetles has led us to conclude that the ibuprofen increased the recovery time of the larvae shown in figure 1 and did not have a significant effect on the mass in all stages of life. The control masses were slightly higher for both the larvae and the adult beetles on day 21 shown in figure 2.
- Our hypothesis created from our previous research and studies is: **The darkling beetles will prefer the control water crystals over the ibuprofen water crystals.**



In order to account for evaporation, the data of each group was run through the following equation for both the Week 1 data set and the Week 2 Data set:

$$1 - \left(\frac{\text{Initial Mass} - \text{Final Mass}}{\text{Initial Mass}} \right) = \text{Estimated amount consumed by beetles}$$

Ibuprofen Consumed and Control Consumed vs. Average Change in Larvae Mass

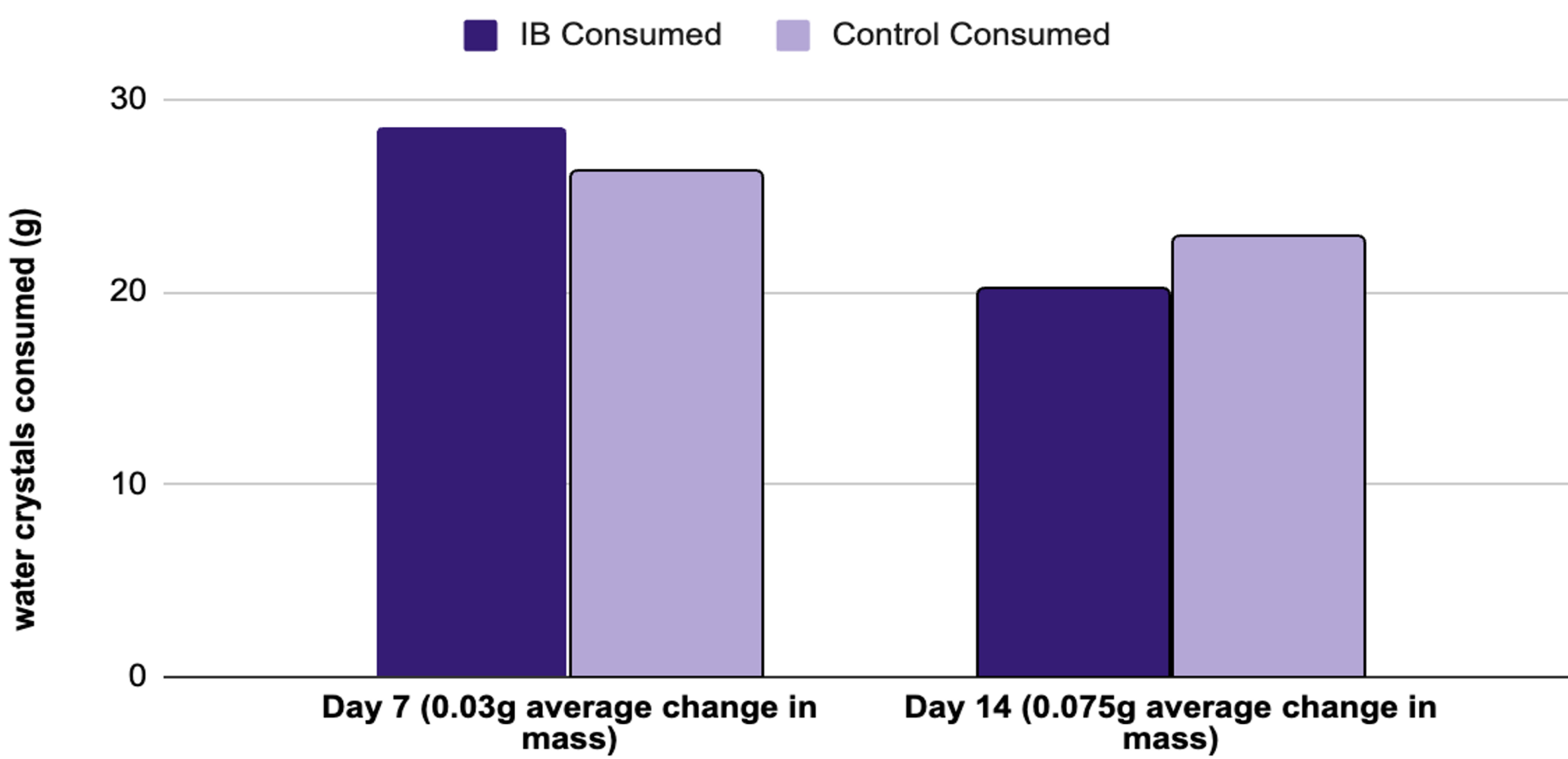
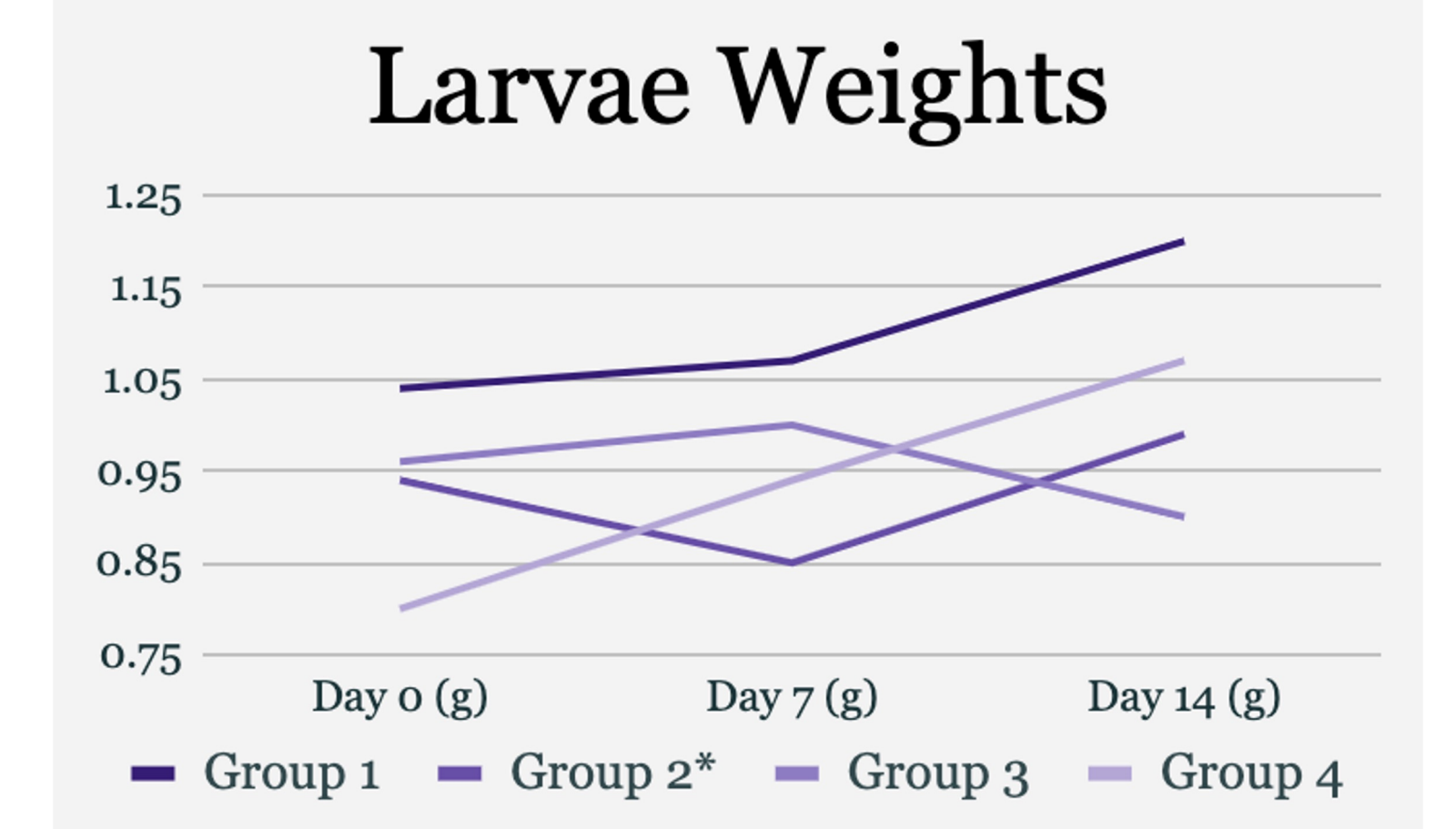
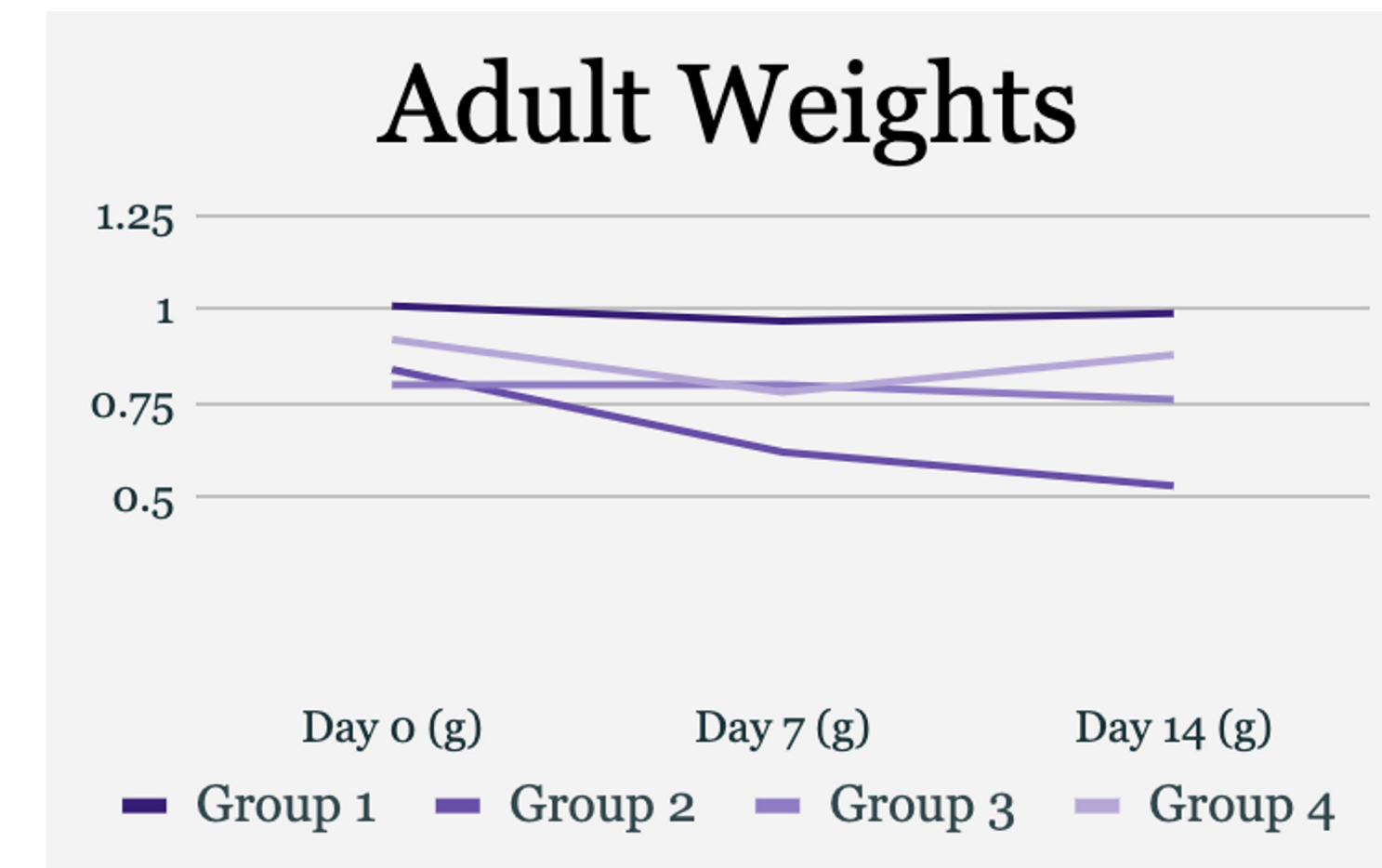


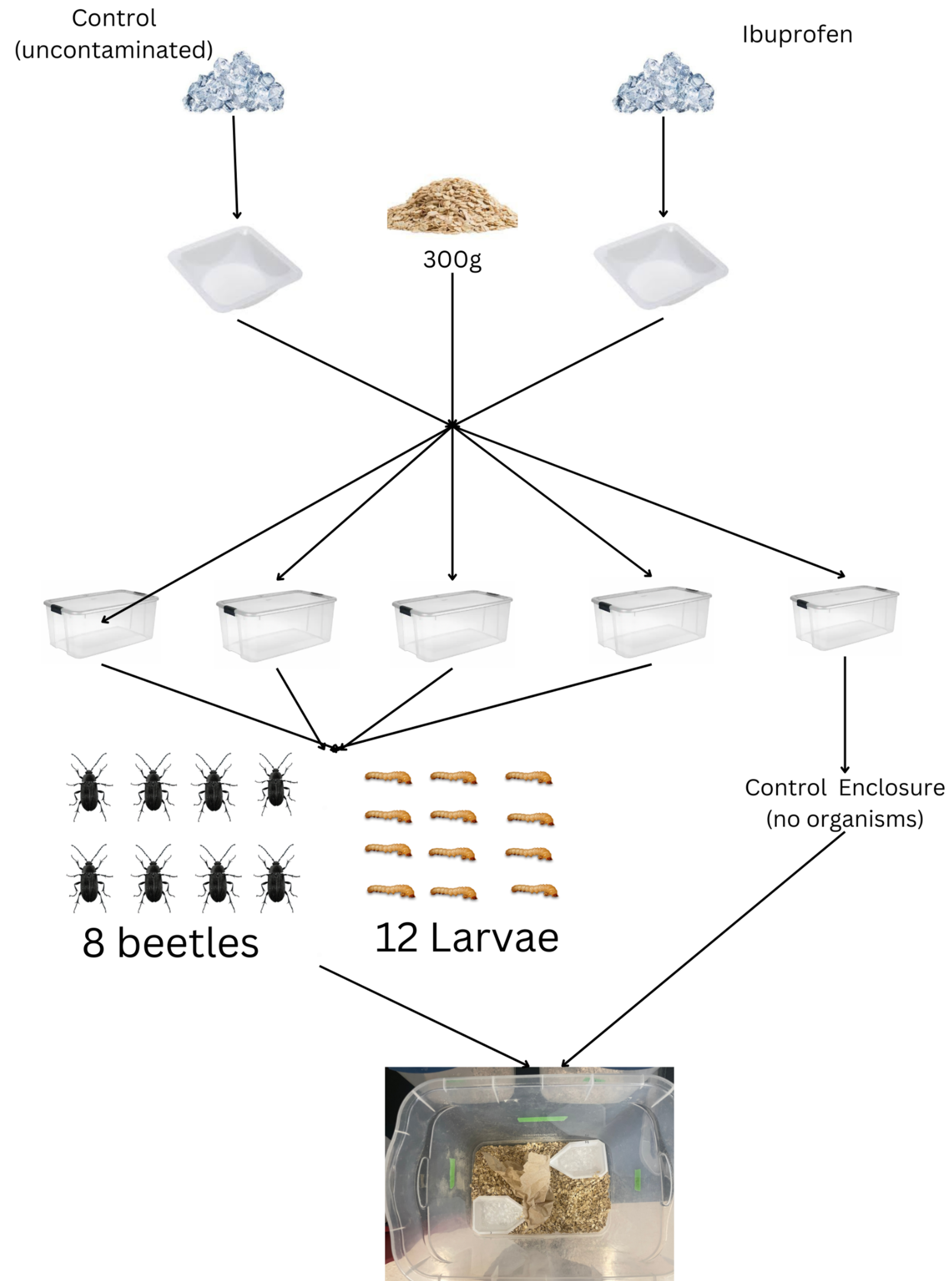
Figure 4: Average amounts of ibuprofen water consumed vs the average amount of control water consumed on day 0, day 7, and day 14 for Larvae.

Figure 7: Average Recovery Time for larvae measured on day 0, day 7, and day 14.



Methods

Figure 3. Flowchart of The Construction of The Enclosures



Ibuprofen Consumed and Control Consumed vs. Average Change in Adult Beetle Mass

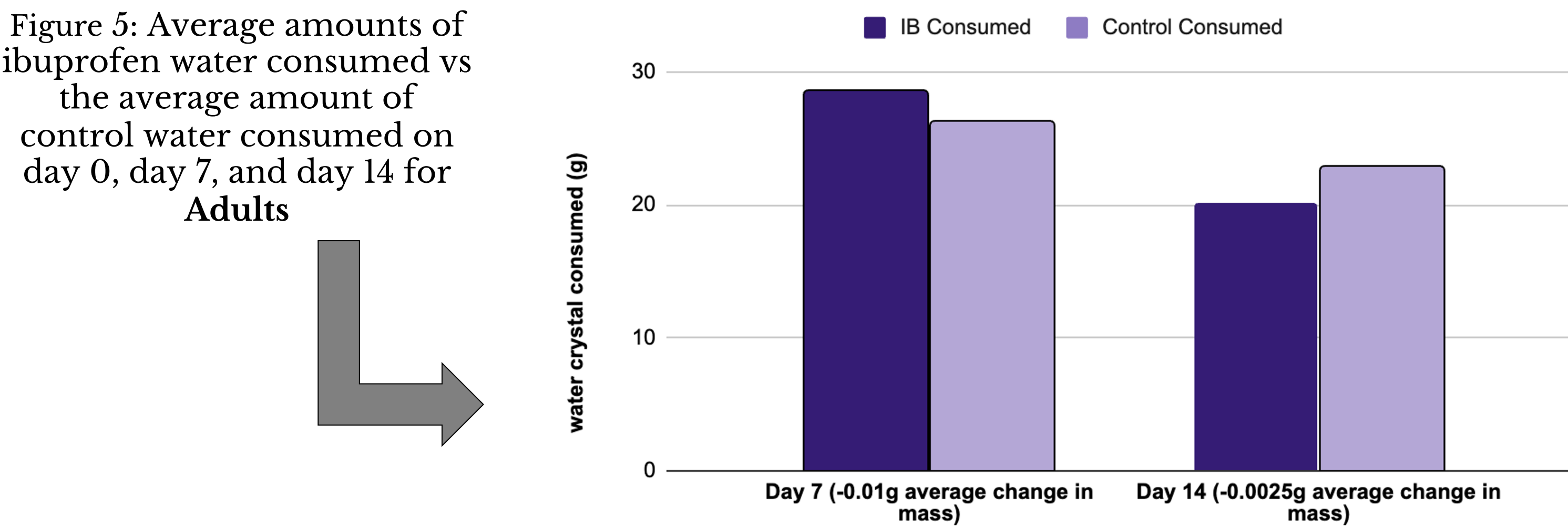
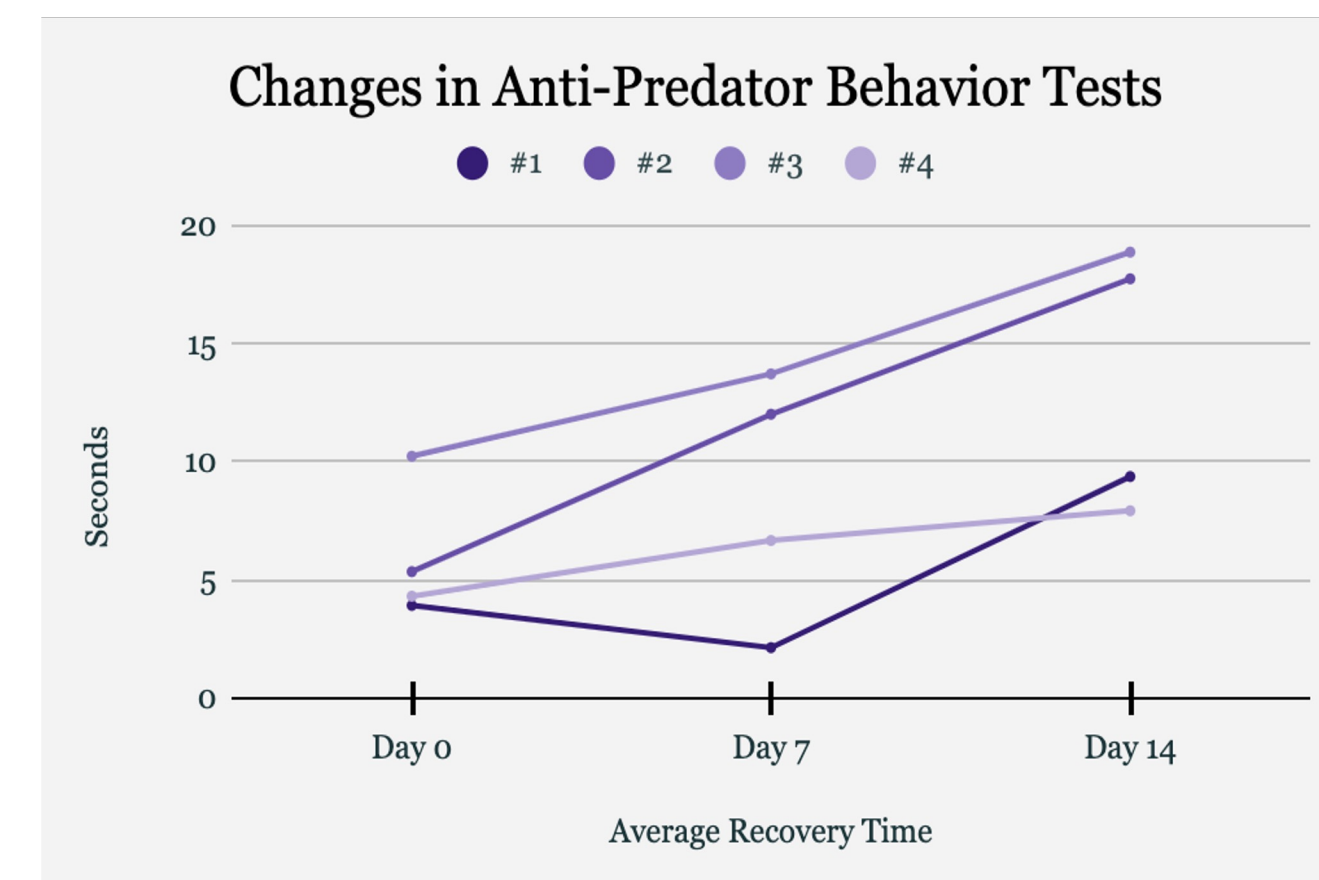
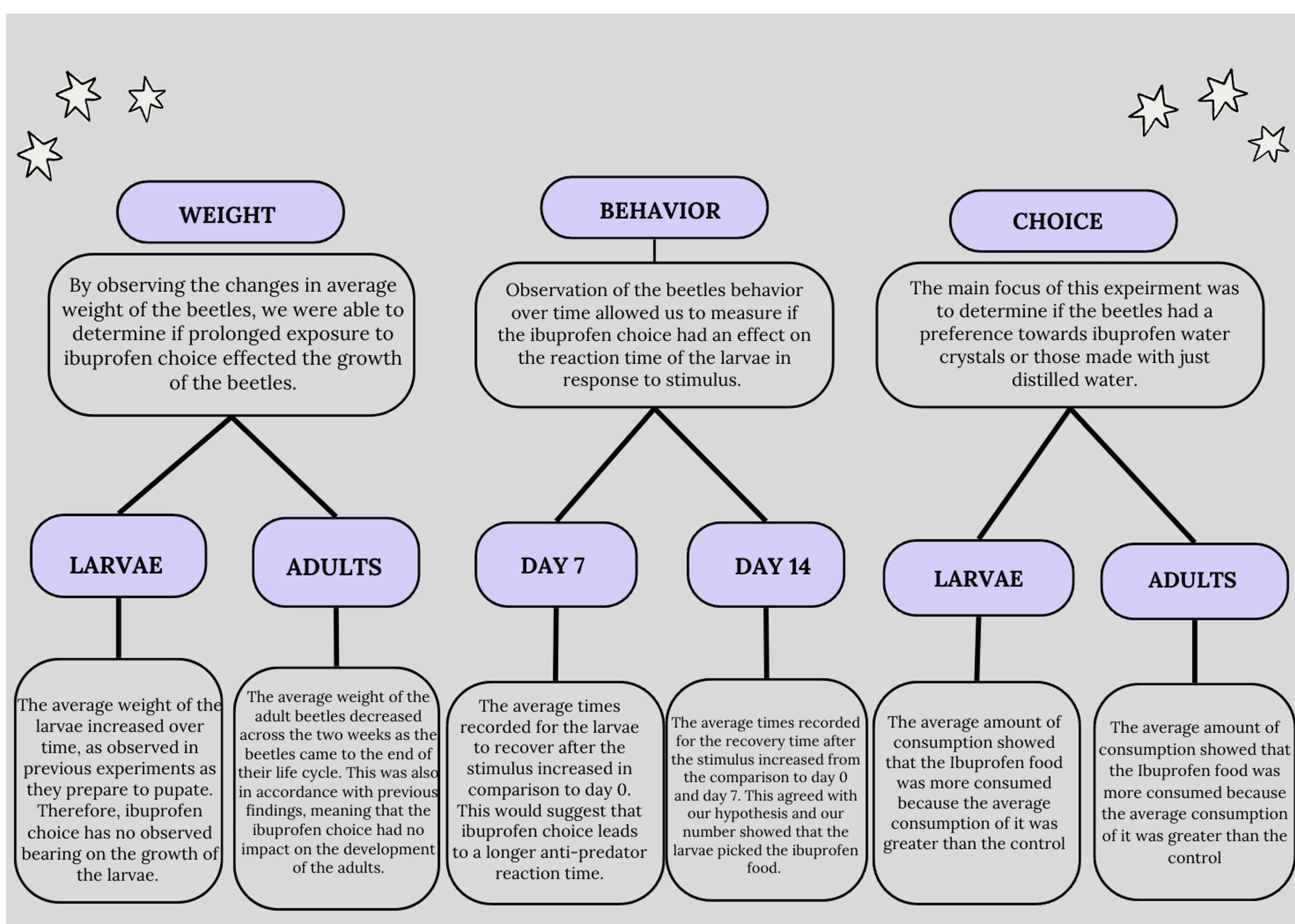


Figure 5: Average amounts of ibuprofen water consumed vs the average amount of control water consumed on day 0, day 7, and day 14 for Adults

Figure 6: Average weights of both larvae and adults measured on day 0, day 7, and day 14.



Discussion



Future Directions

Future Directions/ Directions

- Perform another lab on choice of food with different concentrations of the ibuprofen to identify what amount of concentration changes the beetles choice in food and predator survival reaction.
- The data collected from the experiment showed a slight increase in the mass of the larvae and the beetle. Reaction time to a predator was increased and took longer than what we originally expected and this can be affected by the choice of ibuprofen being picked over control food. The data collected showed that the beetles and larvae drank and chose the water crystals with ibuprofen in it over the control group of water crystals. Evaporation was calculated and this showed that the beetles picked the ibuprofen over the control. The data can be concluded that ibuprofen is a micropollutant and can affect the development and predator survival instincts of the beetles if found in their food and habitat.
- Make sure in lab when weighing the masses of the boats that each weigh boat has the same amount of weight of food in each boat.

References:

Ibuprofen. (2023, September 15). MedlinePlus. Retrieved April 22, 2024, from <https://medlineplus.gov/druginfo/meds/a682139.html>
 Phylogenomics of darkling beetles (Coleoptera: Tenebrionidae) from the Atacama Desert. (2023, February 23). PubMed. Retrieved April 22, 2024, from <https://pubmed.ncbi.nlm.nih.gov/36855434/>

Acknowledgments:

We as a group, You're Cute, Genes, would like to thank all the beetles that were apart of this experiment and we hope the ones who have passed away that your life lives on in beetle heaven. We'd like to thank Dr. Rosier for all her help in this experiment and her guidance that we will take into our future labs here at Seton Hill.

Student Example: Weekly Experimental Review #2 – Daphnia

Student responses to questions are shown in **purple font**. This is the first time students are evaluating their results and making conclusions in the General Biology I course sequence. The example Weekly Experimental Review #4 is from the same student to show learning progression. These assignments are delivered in the quiz format in Canvas as an alternative to traditional lab reports.

Question 1

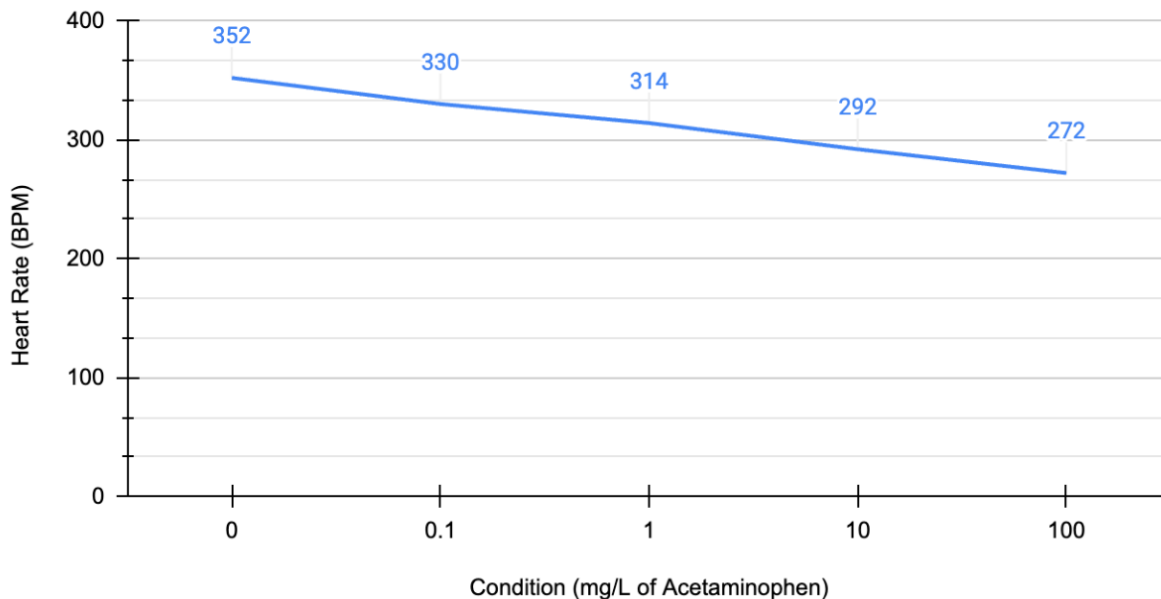
Summarize and describe the results of your Daphnia experiment. Be detailed. Comment on your positive and/or negative controls, and compare your exposed samples to these controls. Write your answer in paragraph form with complete sentences.

We determined that our results supported our hypothesis that the heart rate of Daphnia magna will decrease with increasing concentration of Acetaminophen. We saw a strong correlation between all of the Daphnia Magna in the various concentrations. The negative control was the concentration that was given 0 mg/L Acetaminophen. This gathered the heart rate of the Daphnia magna when it was in normal living water conditions. The other concentrations were 0.1, 1, 10, 100 mg/L. The 100 mg/L had the lowest heart rate.

Question 2

Upload an image of your graph that describe your results from this experiment. Make sure your graph has a title, axis titles, and shows the AVERAGE data from each sample (controls and exposed).

Daphnia Magna Heart Rate (BPM) vs. Condition (mg/L of Acetaminophen) - The Twins



Question 3

Explain the conclusions you can draw from the results described above. Write your answer in paragraph form with complete sentences.

Keep in mind the following:

- Conclusions should be supported by an explanation of what was observed in the results.
 - Ex. "We can conclude that a variety of bacteria was isolated from our basil leaves due to the diverse bacteria colony morphology observed on the stamp plates."
- Make sure your purpose and conclusions correspond to each other
 - Ex. If the purpose is to determine toxicity, then the conclusion of your experiment should include something about toxicity.
- Make sure your conclusions are set up like the following:
 - [Based on ____ results, we can conclude ____.]

Based on the heart rate results, we can conclude that as the concentration (mg/L) of Acetaminophen increased, the heart rate decreased based on examining the hearts of the Daphnia magnas through the microscopes. The purpose of this experiment was to examine the relationship between heart rate to concentration of Acetaminophen, which

was successfully performed because we saw a strong correlation between the two variables.

Question 4

List and explain any sources of error in your experiment. Write your answer in paragraph form with complete sentences.

Think beyond human error (ex. counting errors). Think about your experimental set up, how data was collected, how data was analyzed, your controls, etc & how they could lead to errors in your experimental results and the conclusions drawn from them.

A source of error in this experiment is adding in the acetaminophen solutions. There is uncertainty with the micropipette. For example, when 1000 microliters were added to the original test tube, there was an uncertainty of ± 1 , which would create uncertainty within the measurements for the concentration of the solution along with the dilutions that were made from the stock solution leading to unreliable concentrations. Only two trials were performed, to create more evidence, more trials should be performed along with testing more than 5 Daphnia's to reduce unreliability.

Question 5

Describe the future directions (aka "Next Steps") of your experiment. Write your answer in paragraph form with complete sentences.

What other experiments could be done or how could this experiment be changed to further investigate the experimental question? Do not simply state that the same experiment will be repeated.

To future research this experiment, we could examine how different organisms react to increasing Acetaminophen concentrations. Also, experimenting with other drugs, such as aspirin or melatonin, would be beneficial to see which drugs or medications affect the heart rates of species.

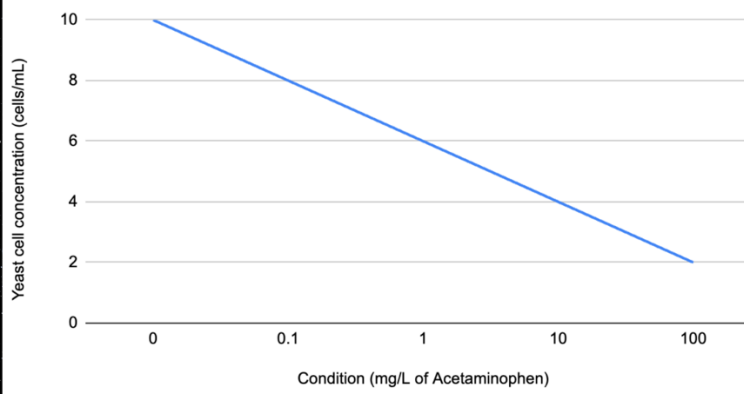
Question 6

Create a hypothesis diagram for the NEXT experiment we are doing in lab. Review the protocol first so you know what we are doing. The hypothesis diagrams should include information about the experiments being done and YOUR hypotheses of the results. See flowchart examples and recommendations [here](#).

Yeast Cells

Exposure to Acetaminophen

Yeast cell concentration (cells/mL) vs. Condition (mg/L of Acetaminophen)



Hypothesis: As Acetaminophen concentration increases, the Yeast cell concentration (cells/mL) will decrease.

Student Example: Weekly Experimental Review #4 – Duckweed

Student responses to questions are shown in purple font.

Question 1

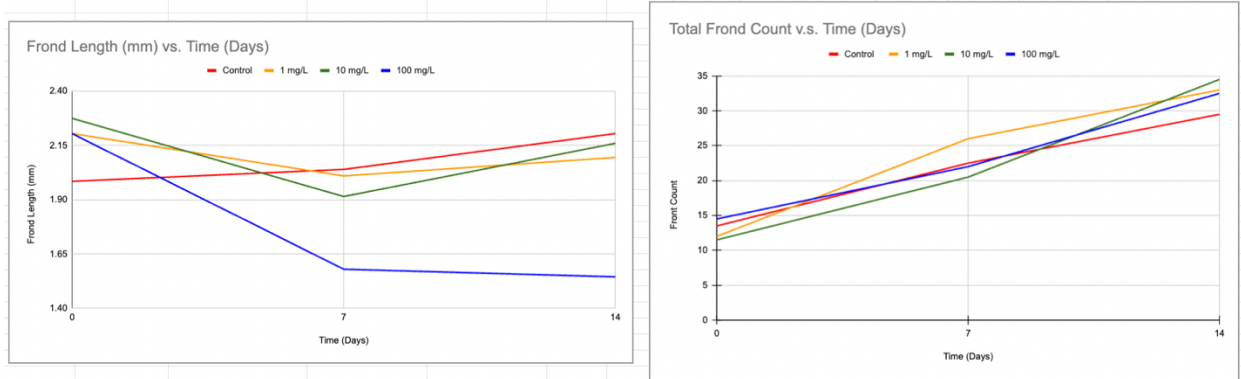
Summarize and describe the results of your Duckweed experiment. Be detailed. Comment on your positive and/or negative controls, and compare your exposed samples to these controls. Write your answer in paragraph form with complete sentences.

After 7 days of duckweed exposure to acetaminophen, the size of the frond length decreased in the 1 mg/L, 10 mg/L, and 100 mg/L solution of duckweed exposure to acetaminophen. The various concentrations of acetaminophen solutions decreased the frond length of the duckweed while the frond length of the negative control group increased. As shown in the graph, the higher the concentration of the acetaminophen, the more the frond length decreased. This shows that in the highest concentration, 100mg/L of acetaminophen, there is a clear decrease in the frond length of the duckweed. Additionally, the number of total fronds increased after 7 days for the 3 concentrations. Compared to the control, the 100mg/L solution increased the frond count at a greater rate. Furthermore, the solution in the 100mg/L concentration turned a dark brown color.

After 14 days, the size of the frond length increased. This differs from day 7 analysis because from day zero to seven, the frond length decreased. There is a clear effect of acetaminophen on the duckweed because the various concentrations of acetaminophen solutions decreased the frond length of the duckweed after 7 days but increased the length after 14 days. As shown in the graph, the higher the concentration of the acetaminophen from day 7-14, the more the frond length increased except for the 100 mg/L solution. This shows that with higher concentrations of acetaminophen, there is a clear correlation that it increases the frond length after 7 days of exposure at a greater rate except for 100 mg/L. Additionally, the number of total fronds increased after 14 days for the 3 concentrations. Compared to the control, the 100mg/L solution increased the frond count at a greater rate.

Question 2

Upload an image of your graph that describe your results from this experiment. Make sure your graph has a title, axis titles, and shows the AVERAGE data from each sample (controls and exposed).



Question 3

Explain the conclusions you can draw from the results described above. Write your answer in paragraph form with complete sentences.

Keep in mind the following:

- Conclusions should be supported by an explanation of what was observed in the results.
 - Ex. "We can conclude that a variety of bacteria was isolated from our basil leaves due to the diverse bacteria colony morphology observed on the stamp plates."
- Make sure your purpose and conclusions correspond to each other
 - Ex. If the purpose is to determine toxicity, then the conclusion of your experiment should include something about toxicity.
- Make sure your conclusions are set up like the following:
 - [Based on ____ results, we can conclude ____.]

Based on the 14-day duckweed exposure to acetaminophen decrease in frond length and increase in frond count in the four concentrations, we can conclude that the toxicity of acetaminophen positively affects the growth and negative survival of duckweed. There is a clear toxic effect of acetaminophen on the duckweed because the various concentrations of acetaminophen solutions decreased the frond length of the duckweed when compared to the control group. As shown in the graph, the higher the concentration of the acetaminophen, the more the frond length decreased

from day 0 to 14. Also shown in the graph, the higher the concentration of the acetaminophen from day 7-14, the frond length increased except for the 100 mg/L solution. Furthermore, the solution in the 100mg/L concentration turned a dark brown color.

This shows that with lower concentrations of acetaminophen present after day 7, there is a clear correlation that it increases the frond length but still decreases the frond length from day 0 to 14. The frond count stayed consistent in regards to the overall increase of the frond count when compared to the control group.

These results indicate that acetaminophen can affect the growth and survival of other aquatic plants that live in similar environments as duckweed. A consequence of acetaminophen exposure can cause environmental changes in duckweed where the natural selection of the duckweed that can survive in the presence of acetaminophen can lead to new species. Another consequence could be if the species could not evolve to the environmental change, then there would be no food for organisms that survive off of duckweed. This could cause a huge affect on organisms in the ecosystem because it could cause unexpected changes in the amount of duckweed present.

Question 4

List and explain any sources of error in your experiment. What caused them? Do these errors render your results meaningless? Write your answer in paragraph form with complete sentences.

Think beyond human error (ex. counting errors). Think about your experimental set up, how data was collected, how data was analyzed, your controls, etc & how they could lead to errors in your experimental results and the conclusions drawn from them.

A source of error in the experiment includes not sterilizing the tweezers to obtain the duckweed each time. A contaminant can come from the surface of the table where the tweezers were placed or if a group member's fingers touched the tweezers and then obtained the duckweed without sterilization. It could have introduced unexpected variables into the experiment. This could have caused the higher concentration of duckweed to turn the solution brown because there was a contaminant present. A way to reduce this source of error is to have a sanitizing station where the tweezers to obtain the duckweed stay sterile and new gloves must be worn by every individual who obtains the duckweed each time. Acetaminophen can degrade over time, especially in light so it may be a source of error in the experiment and could have caused unexplained changes in the frond survival and reproduction. Another source of error could have been sampling bias where randomly there were more healthy duckweed present in a sample than in another sample. This could lead to errors in the experimental results and cause the survival and reproduction to increase at a greater rate than a sample that happened to have slightly weaker or smaller duckweed.

A time-related source of error may be present if the experiment was conducted for too long or too little. This may have affected the results because there was a clear decrease in frond length after 7 days of exposure but after 14 days, there was an increase in frond length from 7-14 days. Even though there was an overall decrease in frond length, there was an increase from day 7 to day 14. This may have been due to a time-related error. Another source of error was the small sample size. This may have skewed the results because the whole experiment was based on the control groups. If the control groups contained duckweed that was either healthier than the other groups, then the data would have been different and different from other groups because there would have been results that looked like the acetaminophen affected the duckweed, when it was the duckweed itself. To fix this error, you would need a much larger sample size to reduce uncertainty to be able to draw accurate conclusions.

Question 5

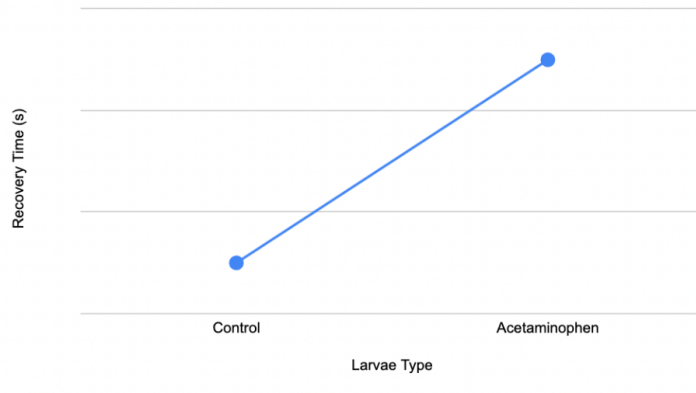
Create a hypothesis diagram for the NEXT experiment we are doing in lab, the beetle exposure study. Review the protocol first so you know what we are doing. The hypothesis diagrams should include information about the experiments being done and YOUR hypotheses of the results. See flowchart examples and recommendations [here](#).

Behavior Test of Darkling Beetles

Antipredator Behavior Test - single Larvae



Recovery Time (s) vs. Larvae Type

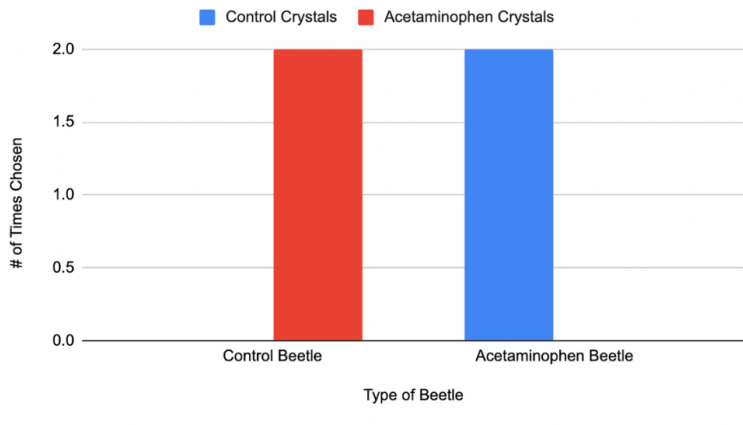


Hypothesis: The acetaminophen larvae will have a longer recovery time than the control larvae.

Choice Test - Adult Beetle (1)



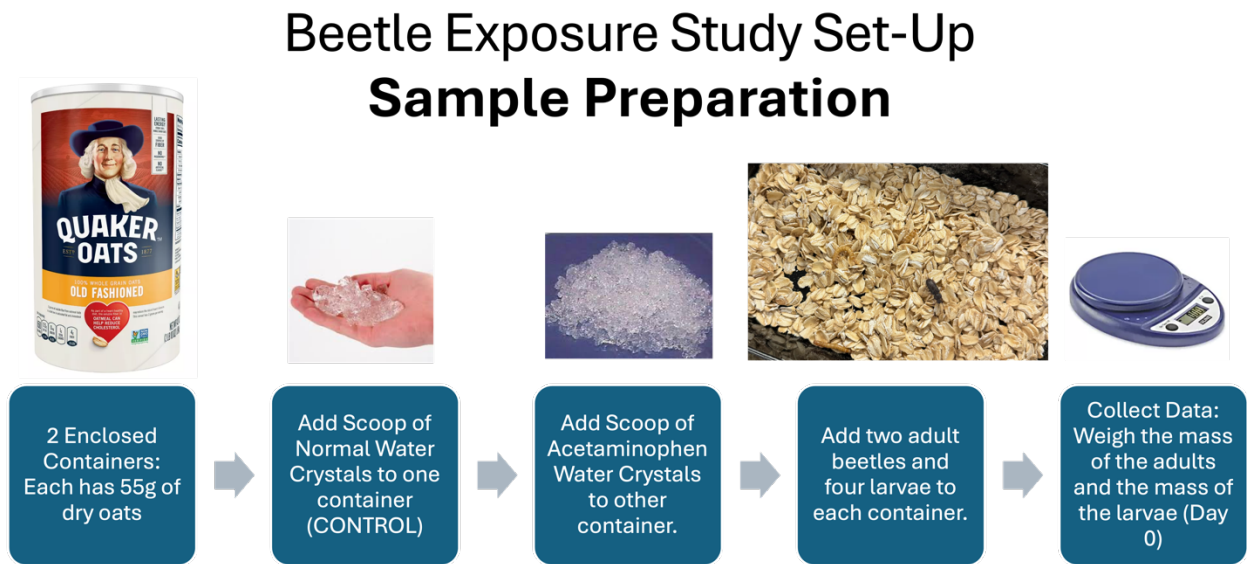
Choice Test



Hypothesis: The beetle exposed to the medicine will choose the control water crystals every time. The beetle exposed to control will go towards the acetaminophen water crystals every time.

Question 6

Create a diagram illustrating the methods for the beetle exposure study set-up completed during the lab before spring break. See flowchart examples and recommendations [here](#).



Question 7

After the beetle exposure study, you will be designing and carrying out an independent, follow-up experiment as a group. In a sentence or two, describe an idea for this experiment. Be sure to include which model organism you would like to study. The options are:

- Daphnia
- Yeast
- Duckweed
- Darkling Beetles
- Brine Shrimp

-Plants (lettuce, beans, or peas)

Our independent, follow-up experiment is determining how increasing the exposure time from 3 mins to 10 mins of acetaminophen affect the heart rate of *Daphnia magna*.