Temperature and Water Flow Effects on the Maturation of Freshwater Sponge Gemmules from *Ephydatia fluviatilis*

By

Sean Willis

A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirement for graduation from the University Honors College

Fall 2025

Thesis committee

Dr. Cole Easson, Thesis Director

Dr. Steven Howard, Thesis Committee Chair

Dr. Jessica Arbour, Second Reader

Temperature and Water Flow Effects on the Maturation of Freshwater Sponge Gemmules from *Ephydatia fluviatilis*

By

Sean Willis

APPROVED:

Dr. Cole Easson, Thesis Director
Associate Professor, Biology

Dr. Steve Howard, Thesis Committee Chair
Professor, Biology

Dr. Jessica Arbour, Thesis Second Reader
Professor, Biology

Acknowledgments

I owe my success in this research to the Middle Tennessee State University biology department, and to the many advisors and peers who have assisted me along the way. I am particularly grateful to Dr. Cole Easson for guiding my hand as a student researcher and managing my constant questions and concerns. I would also like to thank Gabriel Barton and Dr. Jessica Arbour for assisting me in the development of my experimental design and preliminary methods, on course with previous research.

Abstract

Freshwater sponges are benthic aquatic animals, often found fixed to hard substrates in many of Tennessee's waterways. They are multicellular, non-motile, and heterotrophic invertebrates, collecting nutrients via a process known as filter-feeding and in some cases, an algal symbiont. Sponges may produce sexually or asexually, and this study sought to investigate the cues for asexual reproduction by analyzing the growth of asexual structures called gemmules in the laboratory. Gemmules require specific environmental cues to induce hatching and subsequent growth. Prior to the onset of this study, aquaria were filled and left to incubate for one month in an effort to develop a microbiota and simulate *in situ* environmental conditions. The choice species for research was *Ephydatia fluviatilis*, collected from Stewart's Creek in Smyrna, TN, and stored as refrigerated gemmule samples in the Easson lab. This species was chosen based on previous successful hatching trials in the laboratory. Water circulation and varying temperatures were manipulated to test for their influence on gemmule maturation. These results provide the first experimental data to optimize husbandry conditions for Tennessee's sponges. Temperature and flow independently were statistically significant contributors to the maturation of *E. fluviatilis*. Fifteen degrees Celsius water temperature contributed to the greatest overall growth area of sponge tissue in the final three of five weeks spent in treatment. Flow also resulted in a greater area of growth at week two alone. Additionally, both temperature and flow had compounding effects on the growth of sponge tissue. This experiment was conducted to be used as a preliminary study to investigate freshwater sponges, and their biotechnical application for water quality monitoring and selective filtration.

Table of Contents

Acknowledgements......iii

Abstract......iv

List of Tables......vi

List of Figures......vii

CHAPTER I: INTRODUCTION......1

CHAPTER II: MATERIALS AND METHODS......5

A. Storage......5
B. Protocols......5
  Sterilization......5
  Incubation......5
  Strekal's Concentration Calculations......6
  Treatment......6
  Growth in Variable Groups......8
  Statistical Analysis......9

CHAPTER III: RESULTS......9

  Temperature......10
  Flow......11
  Area of Inhibition......11

CHAPTER IV: DISCUSSION AND CONCLUSIONS......21

CHAPTER V: REFERENCES......24

List of Tables

Table 1. Strekal's Ingredients..6

Table 2. Treatment Within Tanks...7

Table 3. Statistical Results for Tissue Growth and AOI...12

List of Figures

Figure 1. Sponge Morphology..2

Figure 2. Freshwater Sponge with Algal Symbiont...3

Figure 3. Experimental Design of Treatments..8

Figure 4. Tissue Growth...12

Figure 5. Area of Inhibition..13

Figure 6. Tissue Growth vs. Temperature...14

Figure 7. Tissue Growth vs. Temperature with Standard Deviation.................................15

Figure 8. Tissue Growth vs. Flow..16

Figure 9. Tissue Growth Given Flow and Temperature..17

Figure 10. Tissue Growth Given Flow and Temperature with Standard Deviation..........18

Figure 11. Mean Area of Inhibition...19

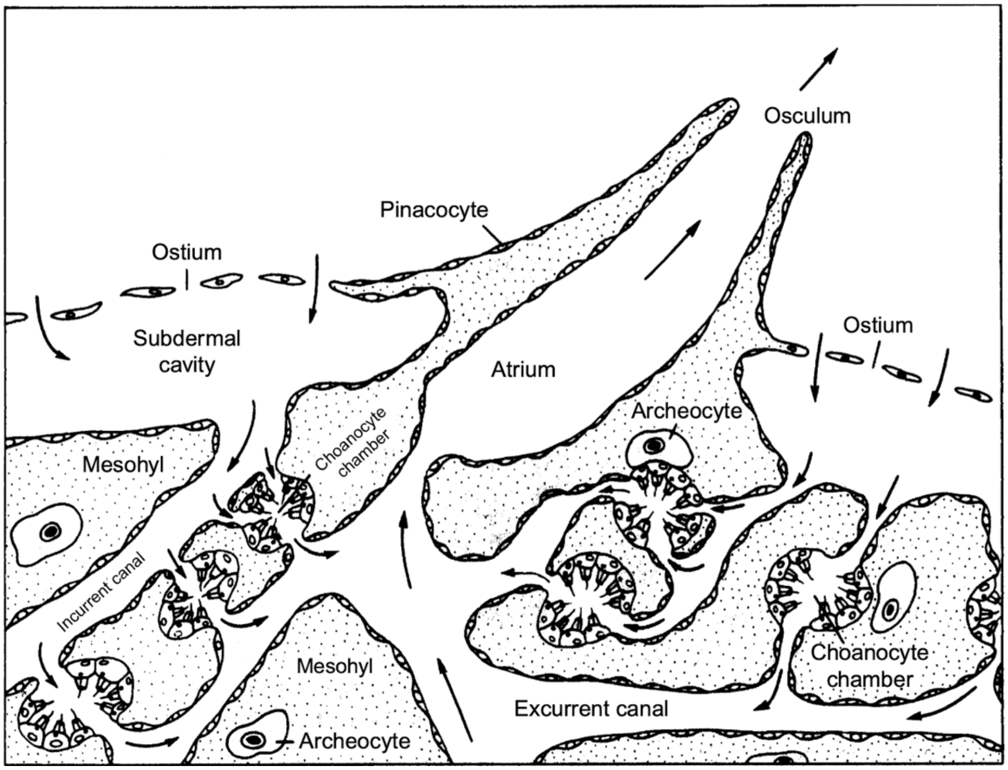
Figure 12. Representative Gemmules...20

INTRODUCTION

Phylum Porifera encompasses a range of sessile fresh and salt-water invertebrates commonly known as sponges (Cocchiglia et. al 2013). Porifera likely appeared in the Neoproterozoic era around 890 million years ago and rose as one of the earliest multicellular life forms (Tuner, 2021). Though they lack organs, they are nonetheless included in the kingdom Animalia and have wide ranging morphology, including encrusting, tubes, vases, and massive forms. Of the total 8,500 sponge species currently known to exist only 250 live in freshwater and all are in the order Spongillida (Copeland, 2020). Freshwater sponges are classified into 45 genera within 6 families and are found worldwide in aquatic habitats (Manconi & Pronzato, 2008).

During filter feeding, water enters the body through openings called ostia and flows through gradually widening canals into a choanocyte chamber (Figure 1). Choanocytes are special cells unique to phylum Porifera and are composed of a microvilli collar and a flagellum. The flagellum beat to increase the flow of water throughout the canals, and the microvilli act as a final filter, around 0.01 um in diameter (Reiswig et. al, 2010).

Sponges acquire nutrients via the filtration of water and/or from an algal symbiont (Figure 2). Many freshwater species with access to sunlight form symbiotic relationships with eukaryotic algae, resulting in a green coloration of their cells (Copeland, 2020). While the size of particles that a freshwater sponge is capable of consuming is known (Frost, 1980), data on their diets *in situ* are markedly difficult to obtain (Skelton and Strand, 2013). Sponges are suspension feeders utilizing choanocytes to pull water through their bodies actively. They also utilize ambient current to assist in filtration to reduce the energy cost of pumping. Leys et. al (2011) determined that some species strategically filtered up to two-thirds of their daily water volume during heavy flow periods.



Reiswig, H. M., Frost, T. M., & Ricciardi, A. (2010). Ecology and Classification of North American Freshwater Invertebrates. McGill. http://redpath-staff.mcgill.ca/ricciardi/Reiswig et al 2010 T&H sponges.pdf

FIGURE 1: SPONGE MORPHOLOGY
Illustration of a cross section of sponge tissue; a general layout of dermal and subdermal channels, cellular composition, and direction of water flow.

U.S. Department of the Interior. (2020, October 6). Freshwater sponges (U.S. National Park Service). National Parks Service. https://www.nps.gov/articles/freshwater-sponges.htm

FIGURE 2: FRESHWATER SPONGE WITH ALGAL SYMBIONT
Freshwater sponge growing on hard substrate in symbiosis with a resident green alga.

Freshwater sponge reproduction may occur sexually or asexually. Asexual reproduction can be undergone via fragmentation or by forming tiny reproductive spheres called gemmules. (USDI, 2020). Gemmules form when specialized spicules encase masses of totipotent cells within them. Sponges shield these gemmules in a specialized protective coating and produce them seasonally, usually before stressful environmental conditions. Each gemmule contains cells called thesocytes, which function as energy stores for development beyond germination. Studies on the gemmule lifecycle indicate that germination often requires an extended period of dormancy, particularly in exposure to cold temperatures (Reiswig et. al 2010). This process is known as vernalization. Pronzato et. al (1993) proposed that exogenous factors like temperature and desiccation dictate the length of phases within a sponge's life cycle. Other environmental variables like pH may also significantly impact gemmule hatchability (Benfrey & Reiswig, 1982).

Ephydatia fluviatilis, which was collected from the Stewart's Creek, Smyrna, TN, was the subject of this research. Recent trials in the Easson lab suggest this species is reliable in terms of hatching and growth in a laboratory setting. E. fluviatilis gemmules used in this study have undergone extended vernalization periods in refrigerated storage in the Easson lab at 4℃. Previously published protocols from the University of Alberta, Canada were initially followed for *in vitro* growth (Leys, 2019). However, a recent study in the Easson laboratory effectively modified protocols for these specific local species.

Following successful hatching, gemmules were transplanted into several 10-gallon tanks. Maturing sponges were subjected to a fully factorial experiment evaluating flow and temperature as environmental variables. Determining the optimum conditions for freshwater sponges to hatch and grow to maturity has mounting potential for bioengineering. Cartwright et. al (2024) suggests that mature freshwater sponges may be useful as a biomonitoring tool for qualitative analysis of bacteria in water sources. Alongside bacterial detection, sponges also have the potential for the selective filtration of gray water (Cartwright, 2017).

CHAPTER II: MATERIALS AND METHODS

A. Storage

The species of sponges used in this research was *E. fluviatilis*, collected from Stewart's Creek in Middle Tennessee in the months prior to experimentation. All gemmules were then refrigerated at 4°C for an extended period in the Easson lab.

B. Protocols

Sterilization

Gemmules were stored in a DI water solution, then pipetted into a 10 cm petri dish. The petri dish was flooded with the 1% hydrogen peroxide solution and left for 5 minutes. Following sterilization, gemmules seen floating on top of the solution were discarded and gemmules at the bottom were kept. The solution was slowly poured out, being careful to keep the viable gemmules in the petri dish. Each petri dish was flooded with DI water and poured out in the same manner 3 times.

Incubation

Viable gemmules were transplanted into a separate petri dish within a 2X concentration of Strekal's medium (Table 1). The solution was changed every 48 hours for 14 days. After 14 days, the petri dishes were moved into one of six 10-gallon tanks filled with freshwater.

Strekal's Concentration Calculations

TABLE 1: 10X STREKAL's INGREDIENTS FOR 500 mL WATER
Quantities of each individual constituent required to make a 10X solution of Strekal's medium

Compound	Amount (mg)
magnesium sulfate heptahydrate ($MgSO_4$ $7H_2O$)	108.33
calcium carbonate ($CaCO_3$)	5.04
sodium silicate nonahydrate ($NaSiO_3$ $9H_2O$)	28.42
potassium chlorate (KCl)	7.55

Calculations for 2X concentration
Volume of tank = 31,135.3 mL
31,135.3 / 500 = 62.2706 (multiplier for ingredients)

((108.33 mg) / 5) 62.2706 = 1.35 g $MgSO_4$ $7H_2O$
((5.04 mg) / 5) 62.2706 = 62.270 mg $CaCO_3$
((28.42 mg) / 5) 62.2706 = 353.95 mg $NaSiO_3$ $9H_2O$
((7.55 mg) / 5) 62.2706 = 94.686 mg KCl

Treatment

The water in the tanks contained a 2X concentration of Strekal's medium. Water in the tanks was continuously cycled, removing and replacing 10L of 2X Strekal water every 30 days. A submersible pump with PVC attached was used to simulate unidirectional flow within the tanks (Figure 3). Flow rate treatment was varied between medium and no flow for independent tanks. The temperature of each tank was monitored and graded at 15°, 18°, and 23° C. Tanks were kept in three separate incubators to manipulate temperature. The effect of both flow rate and temperature variation was surveyed simultaneously in a fully factorial design. Each tank was previously filled with water and left to stagnate for one month prior to the outset of the experiment. A complementary bacterial biome within the tank was grown during this time in order to provide excess or missing nutrients to the gemmules during development. 6 tanks in total were monitored, 1 treatment per tank, each containing 3-4 petri dishes. Gemmules were sorted into the following variable groups for each 10-gallon tank:

TABLE 2: TREATMENT WITHIN TANKS

Experimental setup of temperature and flow treatment within each individual tank.

Incubator	Tank	Treatment
1	1	15° C, Flow
	2	15° C, No Flow
2	3	18° C, Flow
	4	18° C, No Flow
3	5	23° C, Flow
	6	23° C, No Flow

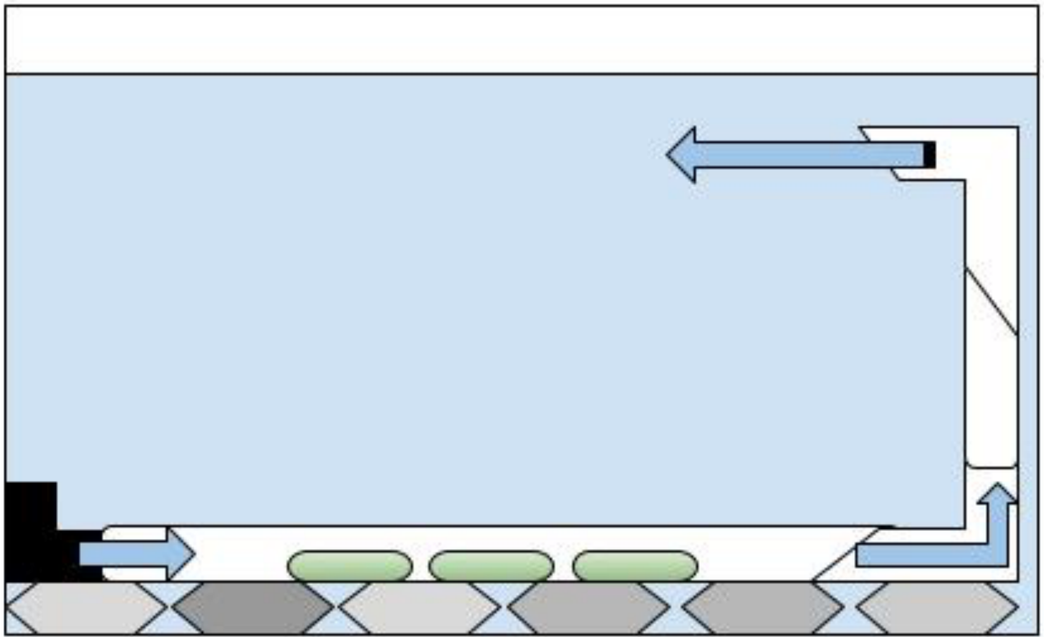


FIGURE 3: EXPERIMENTAL DESIGN OF TREATMENTS
Diagram of treatment using unidirectional flow in aim to simulate the *in situ* environment of *E. fluviatilis*.

Growth in Variable Groups

Following hatching, mature miniature sponges were continually cycled in the Strekal solution as a growth medium. Given favorable environmental conditions, early sponge skeletal growth may develop within 1 week (Höhr, 1977; Ilan, Dembo & Gasith, 1996; Funayama et al., 2005). Petri dishes with sponge growths were removed from the tanks, imaged every 7 days, and then placed back in the same location. Growth was measured using a dissecting microscope and the program ImageJ. Tissue area was quantified as a relation to the diameter of the gemmule of origin set at 1.

Statistical Analysis

The program R Studio was utilized for all statistical calculations. Normality was determined for all data (Shapiro-Wilk, $p < 0.05$). ANOVA was used for main effects and interaction between temperature, flow, time; Tukey's HSD was run for a post hoc test to discern pair-wise differences for variables. R studio was also used for visualization using ggplot2. A single 3-way ANOVA was run with the factors temp, flow, and time for interaction (Table 3).

CHAPTER III: RESULTS

Analysis of growth was performed on *E. fluviatilis* gemmules used in the experiment. Gemmules were expected to show significant variation in tissue size across temperature and flow treatments (Figure 12). Each dish and individual gemmule were treated identically, Sample size in each treatment varied between 9 and 14 depending on the relative success of hatching during incubation. Once the gemmules adhered to each dish, initial control measurements were taken after two weeks of incubation, denoted as "T0".

Initial gemmule size varied widely in diameter when measured using the ImageJ program. Because of this variation prior to T0, metric measurements would not be representative of the relative success of each treatment. As a result, tissue area was measured as a relation to the diameter of the gemmule of origin set as 1 unit. Some gemmules later adhered to the dishes after placement in the tanks and were therefore given an initial value of 1. Only significantly dense tissue and spicule growth were considered in each area calculation (Figure 4). Tissue growth varied significantly at key time points as a function of both temperature and flow individually, and no significant variation in tissue size was found before placement in tanks at T0 (Shapiro-Wilk, $p < 0.05$). A 3-way ANOVA did find that the three variables of temperature, flow, and time had statistically significant interactions with one another (Figures 9, 10). Temperature and flow ($p = 0.002$); flow and time ($p < 0.001$); and temperature and time ($p < 0.001$); all have an interaction (Table 3).

Temperature

The area of tissue growth between treatments saw statistically significant variation as a function of temperature (ANOVA, $p < 0.001$). Week 1 was the first time point to show variation, with the 15° C treatment showing a greater area of growth than the 18° C treatment (Tukey's HSD, $p < 0.05$) (Figures 6,7). At week 2, no differences among temperature treatments were observed (ANOVA, $p > 0.05$). Week 3 through 6 showed significant variation among temperature treatments, particularly at the lowest temperature. At week 3, the 15° C treatment saw a greater area than either 18° C or 23° C (Tukey's HSD, $p < 0.003$), with both 18° C and 23° C showing no variation from one another. In weeks 4 and 5 the same trend continued with increasing confidence, as all p-values fell below 0.001.

Flow

The area of tissue growth in relation to flow showed no variation at T0 (ANOVA, $p = 1$). The results produce a compelling trend when visualizing only the means of tissue area at each time point (Figure 8). However, after considering the variation around each mean, week 2 is the only phase at which there were statistically significant differences among flow treatments, with the mean area of tissue growth being significantly greater with flow than without (ANOVA, $p < 0.001$).

Area of Inhibition

In the weeks following T0, an area of inhibition (AOI) was observed surrounding the tissue growth of most gemmules. Due to the unexpected nature of this find and difficulty in observation within the image frame, a much smaller and much more variable sample size was used to calculate the means and standard deviation of the AOI in each treatment. AOI measurements were determined as apparent and significant displacement of surrounding biofilm around each hatched sponge (Figure 5). Those gemmules whose AOI was merging with one another were not measured along with those where the AOI was outside the bounds of the image.

AOI was significantly affected by variation in temperature (ANOVA, $p < 0.001$) and time in isolation (ANOVA, $p = 0.002$), but not by flow (ANOVA, $p = 0.09$). A 3-way ANOVA did find an interaction between temperature and time ($p < 0.001$). For temperature, mean AOI at 15° C was greater than the mean at 18° C (Tukey's HSD, $p < 0.02$) but not different than 23° C (Tukey's HSD, $p = 0.059$), and the mean at 18° C was less than that at 23° C (Tukey's HSD, $p < 0.05$). The total AOI for all samples over time showed a steady decline after week 2 (Figure 11). AOI at weeks 1, 2, and 3, varied from week 5 (Tukey's HSD $p < 0.05$), with week 4 showing no variation from any other week.

TABLE 3: STATISTICAL RESULTS FOR TISSUE GROWTH AND AOI
Analysis of confidence from ANOVA tests for tissue growth and AOI.

Data set	Statistical Test	F	df	p	Fig.
Area of Tissue Growth	3-way ANOVA				
Time		34.84	5	< 0.001	
Temperature		68.24	2	< 0.001	
Flow		19.47	1	< 0.001	
Temperature x Flow		6.53	2	0.00163	9,10
Temperature x Time		9.72	10	< 0.001	6,7
Flow x Time		3.17	5	0.00804	8
Area of Inhibition	3-way ANOVA				
Time		4.33	4	0.00223	11

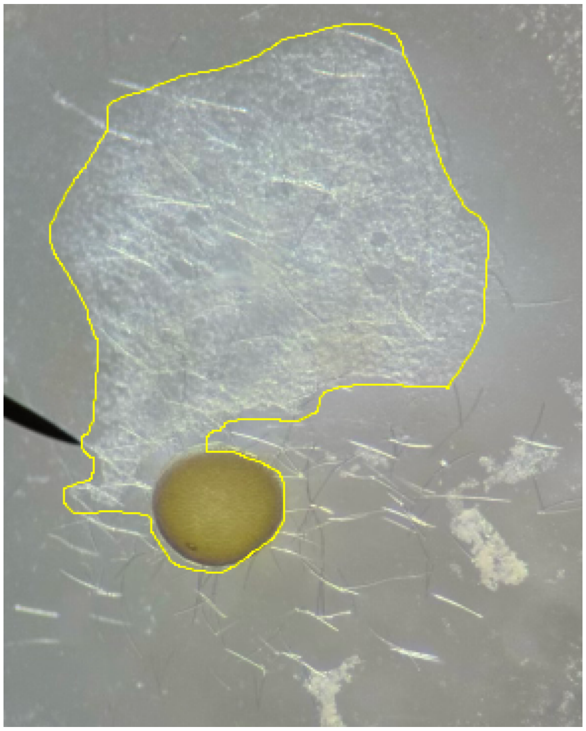


FIGURE 4: TISSUE GROWTH
Only significant tissue growth was considered when determining the area of growth for each individual gemmule, spicule growth alone was counted.

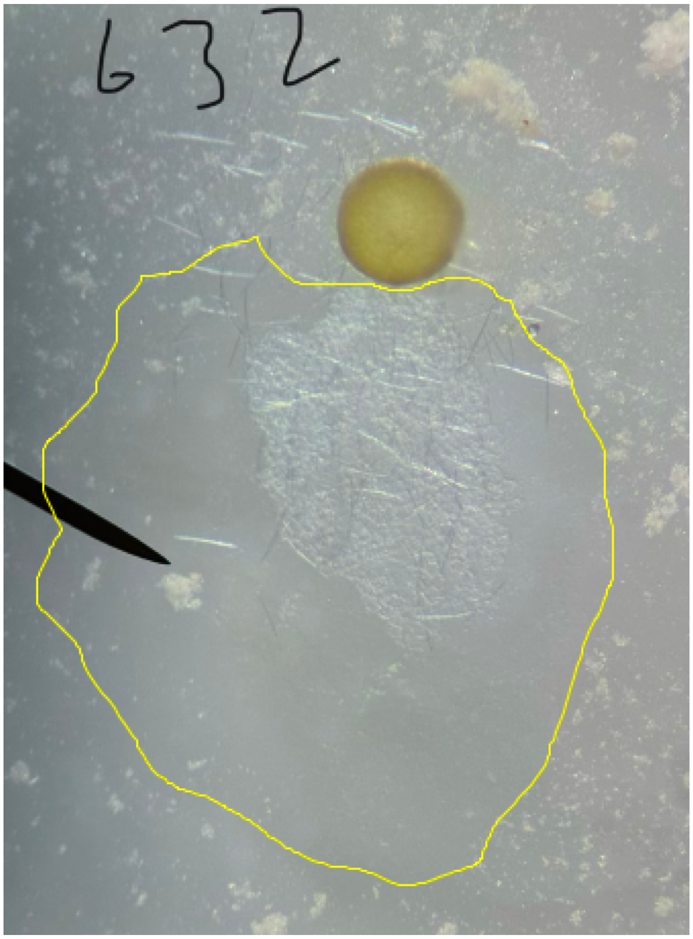


FIGURE 5: AREA OF INHIBITION
Only clear displacement of surrounding film was included in the measurements that determined mean and standard deviation data for the area of inhibition.

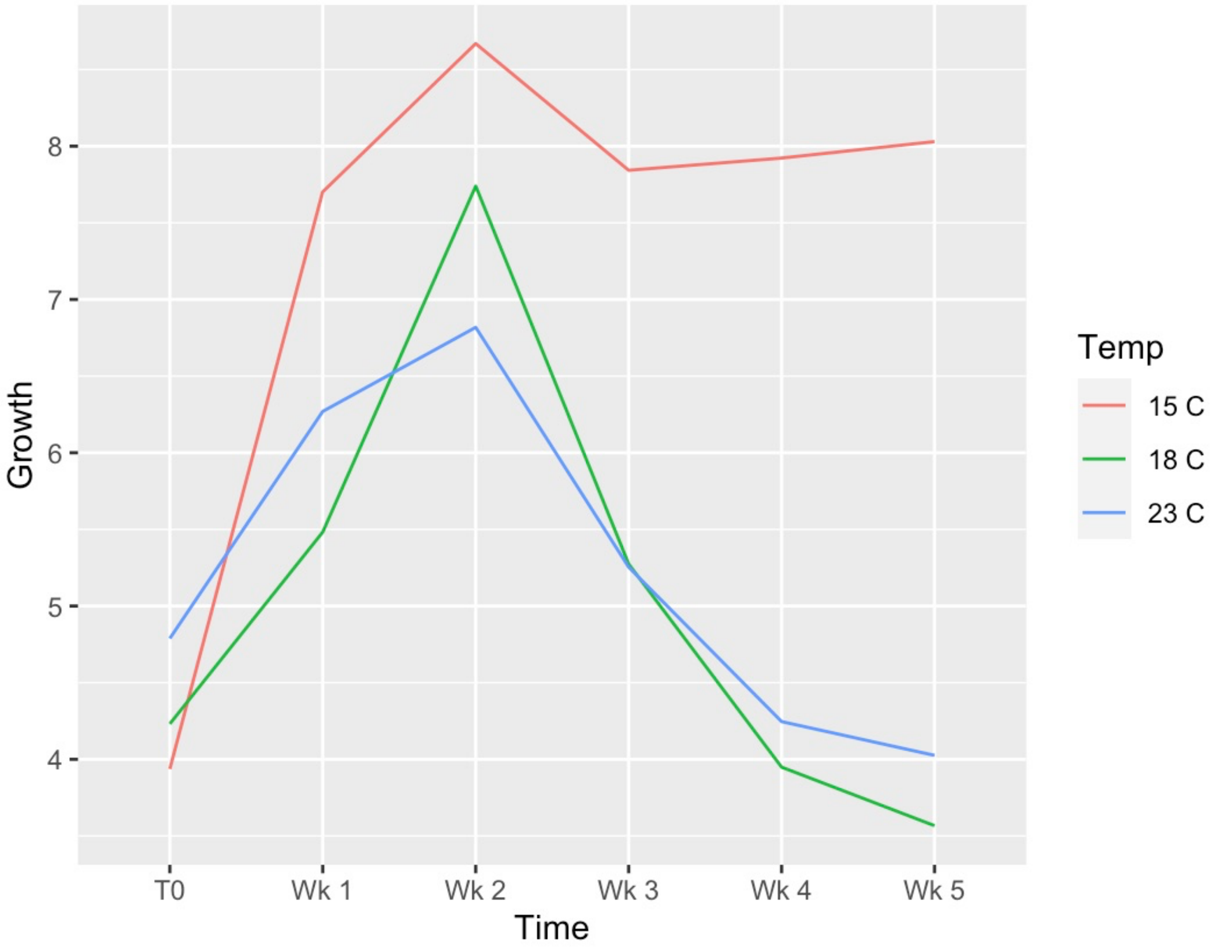


FIGURE 6: TISSUE GROWTH VS. TEMPERATURE
Means of tissue growth area within each incubator. Tissue growth was sustained considerably longer given the colder of the three temperature ranges.

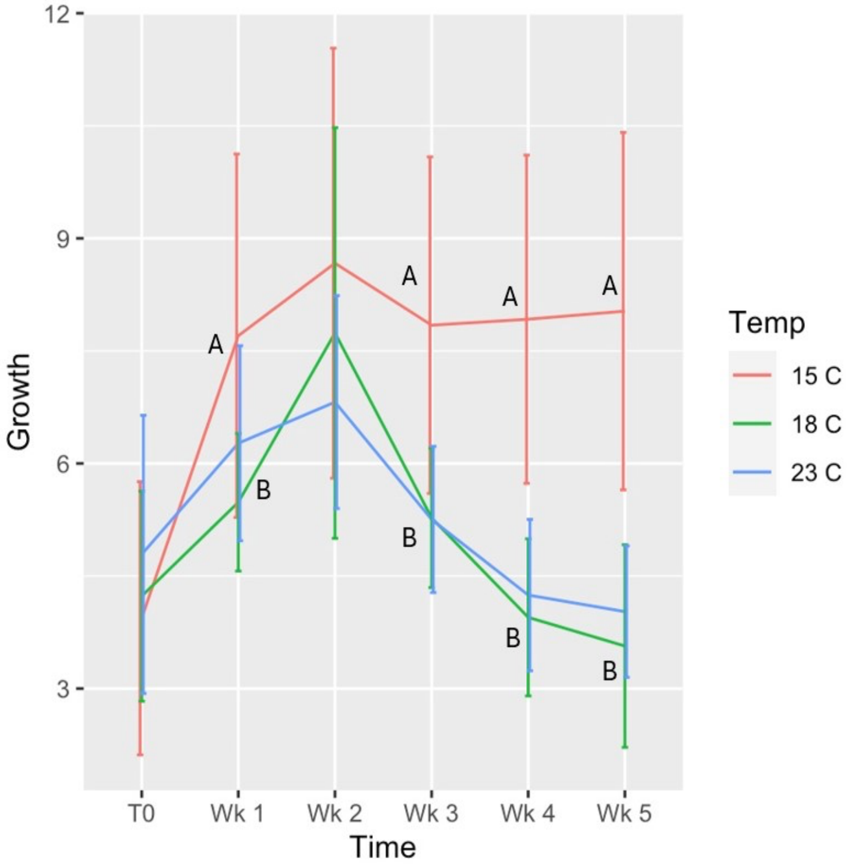


FIGURE 7: TISSUE GROWTH VS. TEMPERATURE WITH STANDARD DEVIATION Deviation around each mean displayed by horizontal bars at each time point. Due to the considerably variate nature of each mean, and small sample size, few conclusions of statistical significance can be drawn from the data from T0 to Wk 2 (Tukey's HSD, $p > 0.05$). In the following weeks however, the difference in growth does become significant, indicated by labels A and B.

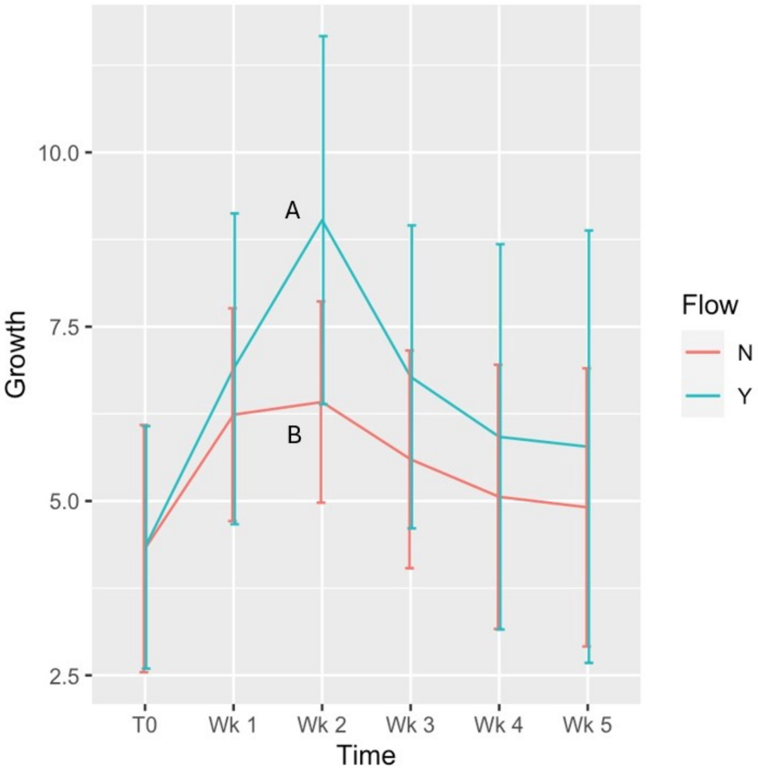


FIGURE 8: TISSUE GROWTH VS. FLOW WITH STANDARD DEVIATION
Mean tissue growth among tanks with and without regular water flow at each time point. Due to the variation around each mean, the only conclusion of statistical significance is that tissue area with flow at week 2 was greater than tissue area without flow at the same time point (Tukey's HSD, $p < 0.001$), differentiated in the figure as A and B.

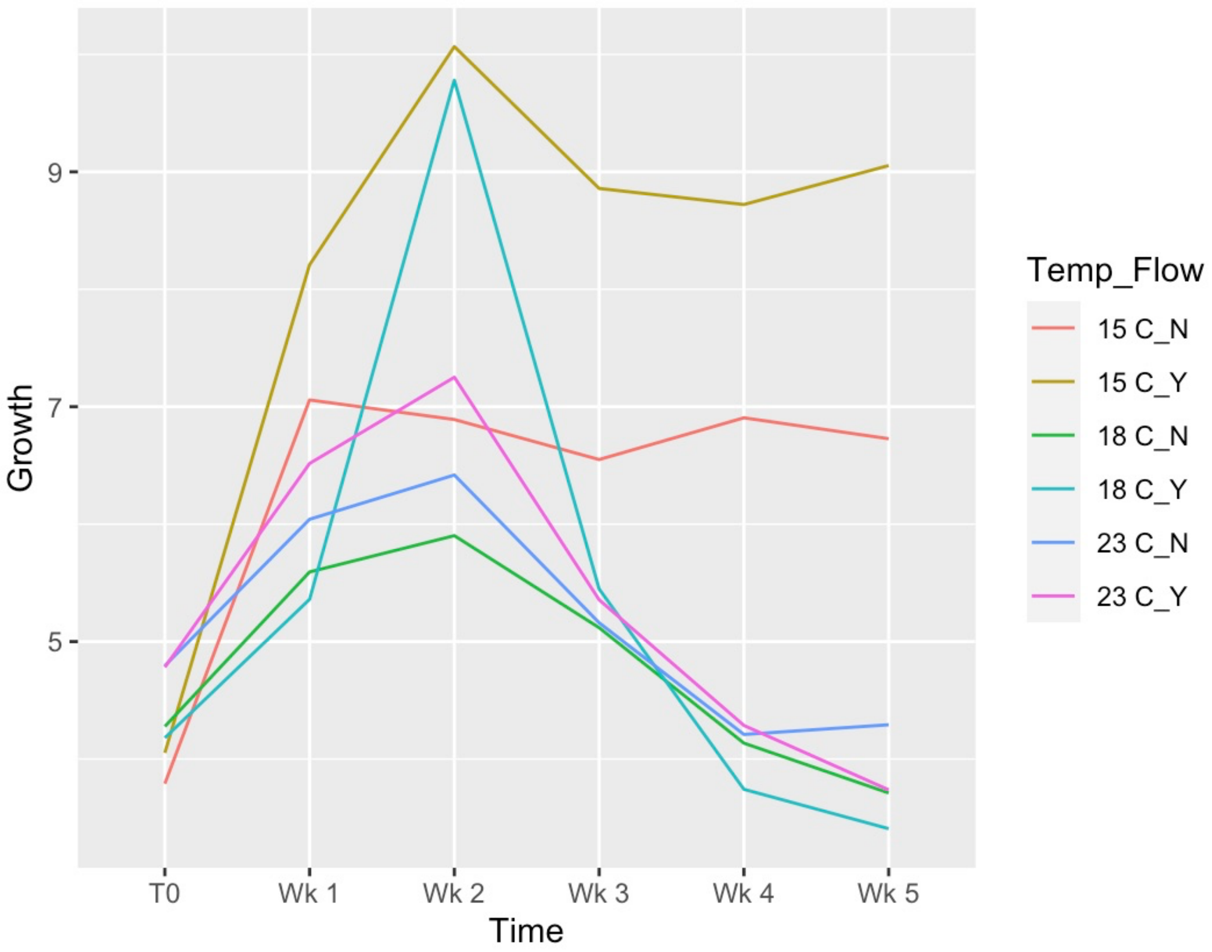


FIGURE 9: TISSUE GROWTH GIVEN FLOW AND TEMPERATURE
Mean tissue growth from each individual tank. Sample size within each treatment ranged from nine to 14 individuals.

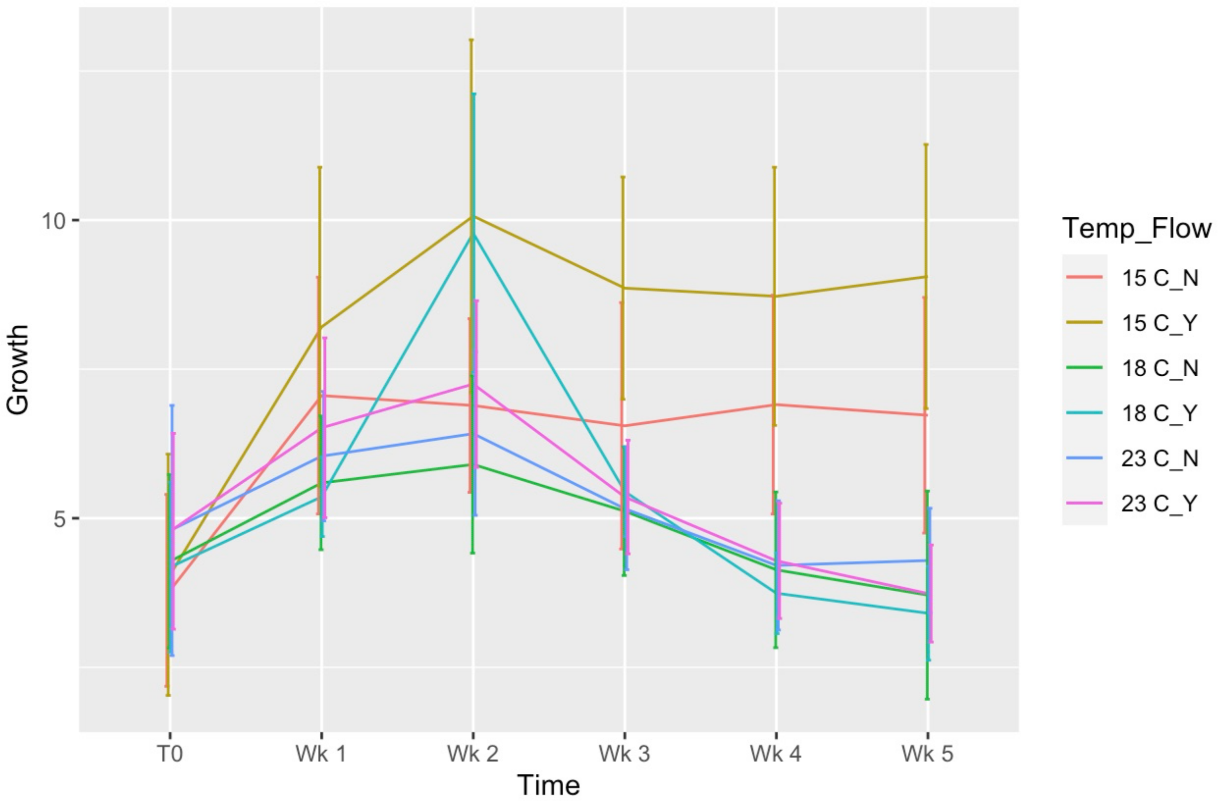


FIGURE 10: TISSUE GROWTH GIVEN FLOW AND TEMPERATURE WITH STANDARD DEVIATION

Deviation around each mean displayed by horizontal bars at each time point. Temperature, flow, and time had statistically significant interactions with one another, but not in conjunction.

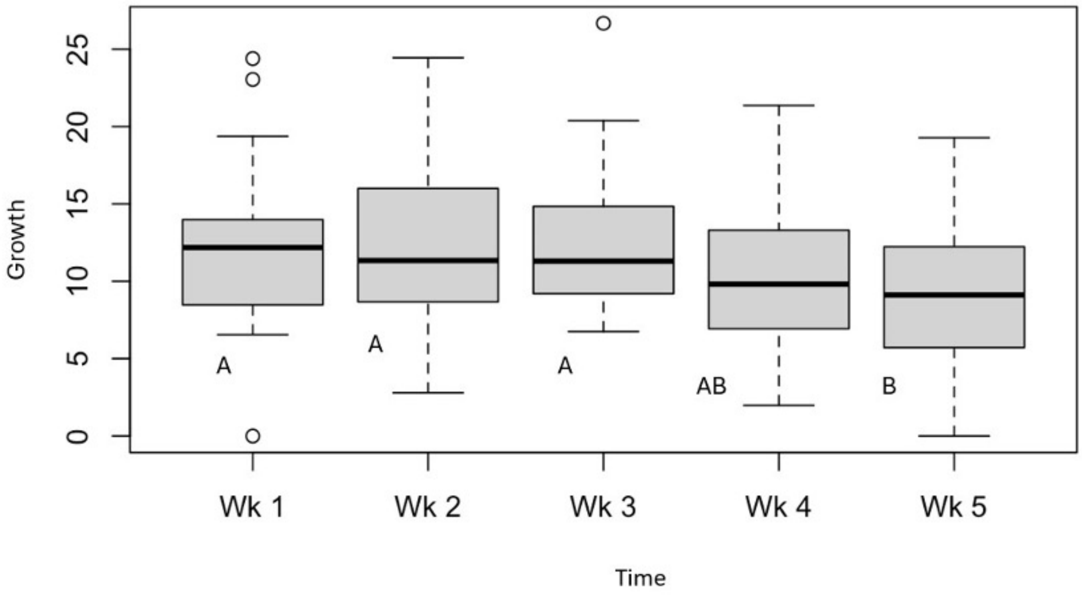


FIGURE 11: MEAN AREA OF INHIBITION
AOI showed a decline across all individuals between week 1 and week 5 (Tukey's HSD, $p < 0.001$). While the deviation around the mean is significant, the sample size for this metric was appropriately large to accept this interpretation (ANOVA. $p = 0.002$).

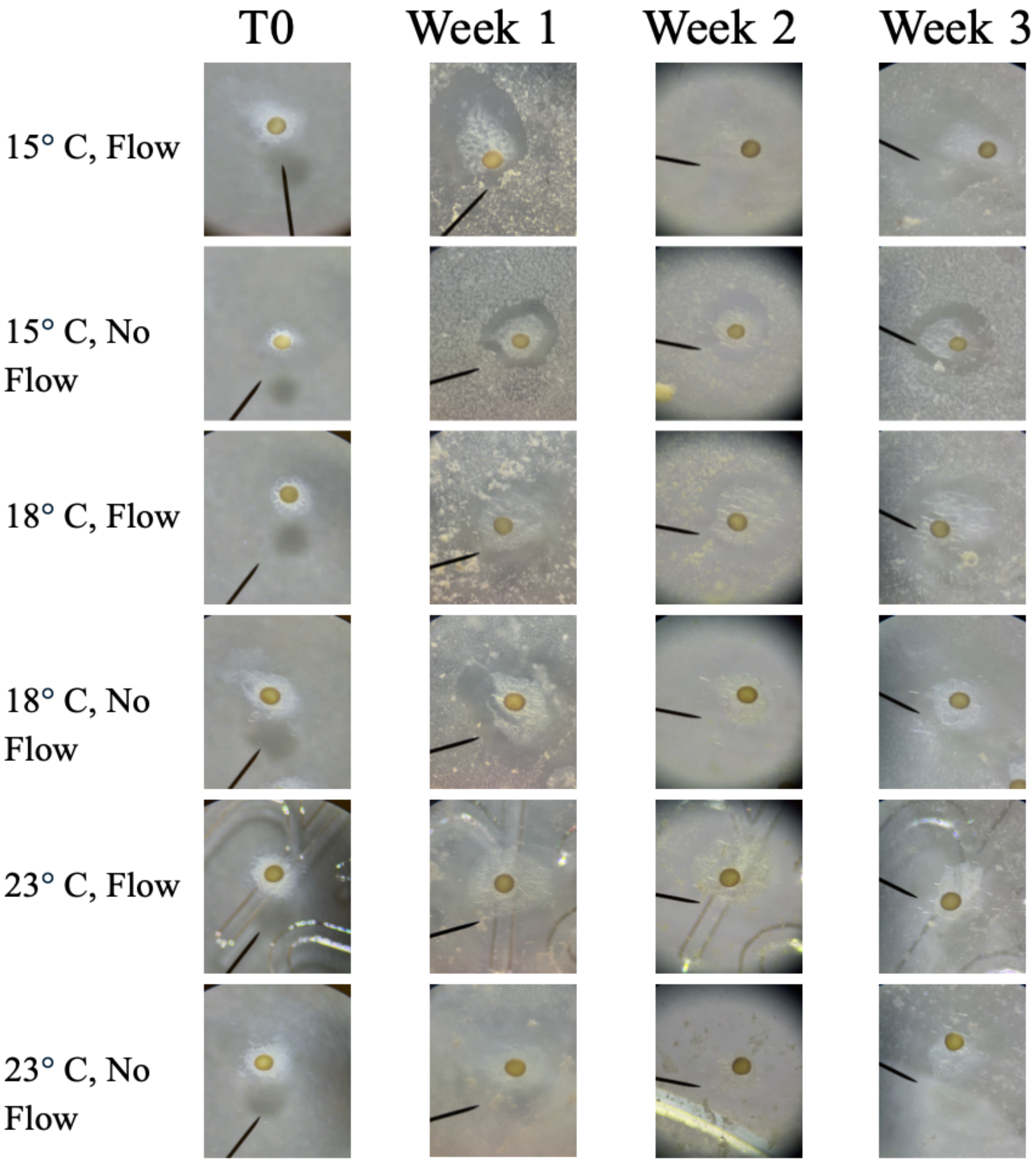


FIGURE 12: REPRESENTATIVE GEMMULES
Images displaying individual gemmules indicative of the overarching trends within each treatment from T0 through week 3.

DISCUSSION AND CONCLUSIONS

In the current study, variation in temperature and flow were related to differences in the growth of sponge tissue. The coldest temperature treatment of 15° C produced a notably greater average growth at key stages throughout the five weeks spent in tanks. The presence of flow was also a contributing factor for greater growth, but this was largely limited to earlier in the experiment. The original aim of this experiment was to find the optimum conditions by which sponges may grow to maturity. This was unfortunately not successful, as the gemmules with the greatest area of growth still plateaued following a peak at week two. The results did show, however, a much greater success with flow and a colder temperature range, than previous growth trials at room temperature with still water (Barton, 2023). While the sponges subjected to the coldest of the three treatments did plateau in growth, their mean area of growth was considerably larger and remained relatively fixed following week two. Conversely, both warmer treatments resulted in a marked decline in tissue area. These results suggest that temperatures within the colder ranges of the Stone's River in early spring, and the presence of flow, will facilitate the greatest possible tissue growth of sponge gemmules. Additionally, some gemmules whose tissue fused with one another saw a greater area of tissue growth than their expected sum. Since reliable estimates of individual growth could not be estimated, these gemmules were not included in the overall mean calculations for isolated individuals.

Given a larger sample size, it is possible that many more conclusions of statistical significance could be drawn at different time points, especially in regard to flow data, and the interaction between time, flow, and temperature in conjunction. The original experimental design had included only four treatments with high and low temperatures and flow/no flow, with two replicates of each treatment. However, in the early stages of the experiment, following the introduction of sponges into the tanks, it became apparent that two of four incubators had malfunctioned. One entire temperature treatment containing two tanks was unrecoverable as the incubator shut off entirely. The other incubator had a faulty temperature gauge and the water temperature of the two tanks within was in fact 18° C in contrast to the expected 15° C. A drastic reduction in sample size was a direct result of these unexpected complications.

The plateau of tissue growth may have been a result of a nutrient insufficiency within the previously formulated Strekal's medium (Nichols, 2023). Each tank was left to sit in the incubators for a month prior to T0 in hopes of developing a biotic environment. The tanks, however, were exposed to minimal light throughout the five-week trial, and the sponges received no particulate biotic nutrient supplementation. Future studies will likely explore the optimum feed and lighting conditions, under a similar temperature range as were most successful in this experiment. Variation in the intensity of flow, and not just its presence, alongside a comparison of isolated gemmules—vs. those grown in conjunction—may also show significant results for tissue growth.

An area of inhibition (AOI) around each growing sponge was a gripping and unexpected discovery after the first week of experimentation. This find may be in part explained by the pumping mechanism implemented by sponge tissue using choanocytes (Reiswig et. al, 2010). If this be the case, the AOI is simply a physical feature surrounding sponge tissue, caused by the displacement and possible selective filtration of the surrounding sediment. Alternatively, previous studies have proposed that E. fluviatilis in particular may implement some strategy for bacterial suppression, as a means of preventing infection (Cartwright et. al, 2024). Tests commonly performed to measure the antimicrobial strength of certain fungi, like the Kirby-Bauer test, aim to analyze a zone of inhibition around fungal colonies (Bauer and Kirby, 1966).

These well documented techniques show strikingly similar results to the AOI observed in this experiment.

In synopsis, *E. fluviatilis* gemmules are most likely to grow to the current experimental maximum, and sustain that growth for a longer period of time when subjected to both colder water temperatures and flow. Future experimentation will seek to highlight weak points in this experimental design, and test a range of other possible variables, including feed, light, and flow rate.

References

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966 Apr;45(4):493-6. PMID: 5325707.

Barton, G. (2023). Growing Local Freshwater Sponge Gemmules to Adulthood for Preliminary Utilization as Gray Water Filters (undergraduate dissertation, Middle Tennessee State University).

Benfrey, T. J., & Reiswig, H. M. (1982, May 20). Temperature, pH, and photoperiod effects upon gemmule hatching in the freshwater sponge, Ephydatia mülleri (Porifera, Spongillidae). Wiley Online Library. https://onlinelibrary.wiley.com/doi/abs/10.1002/jez.1402210104

Cartwright A, Dooley JSG, McGonigle CD, Arnscheidt J. How suitable is freshwater sponge Ephydatia fluviatilis (Linnaeus, 1759) for time-integrated biomonitoring of microbial water quality? Access Microbiol. 2024 Apr 19;6(4):000691.v4. doi: 10.1099/acmi.0.000691.v4. PMID: 38737804; PMCID: PMC11083428. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11083428/

Cartwright, A. (2018, May). Freshwater sponges and their interaction with bacteria through filtration, retention and antimicrobial properties. Ulster University. https://pure.ulster.ac.uk/en/studentTheses/freshwater-sponges-and-their-interaction-with-bacteria-through-fi

Cocchiglia, L., Kelly-Quinn, M., & Lucey, J. (2013). Classification of Freshwater Sponge Collection at EPA Kilkenny. EPA. https://www.epa.ie/publications/research/biodiversity/Classification-of-freshwater-sponges.pdf

Copeland, John E. (2020) "Checklist of the Freshwater Sponges (Porifera: Spongillida) of Tennessee," Cumberland Mountain Naturalist: Vol. 1: Iss. 1, Article 1. https://digitalcommons.lmunet.edu/cumberland_mountain_naturalist/vol1/iss1/1/

Frost, T. M., 1980. Clearance rate determinations for the fresh- water sponge Spongilla lacustris: effects of temperature, particle type and concentration, and sponge size. Archiv Fur Hydrobiologie 90: 330–356. https://nmu.edu/biology/sites/biology/files/2021-01/Skelton_and_Strand_2013.pdf

Funayama et al. (2005) Funayama N, Nakatsukasa M, Hayashi T, Agata K. Isolation of the choanocyte in the freshwater sponge, Ephydatia fluviatilis and its lineage marker, Ef annexin. Development, Growth and Differentiation. 2005;47(4):243–253. doi: 10.1111/j.1440-169x.2005.00800.x.

Höhr (1977) Höhr D. Differenzierungsvorgänge in der keimenden Gemmula von Ephydatia fluviatilis. Wilhelm Roux's Archives of Developmental Biology. 1977;182(4):329–346. doi: 10.1007/bf00848384.

Ilan, Dembo & Gasith (1996) Ilan M, Dembo G, Gasith A. Gemmules of sponges from a warm lake. Freshwater Biology. 1996;35(1):165–172. doi: 10.1046/j.1365-2427.1996.00486.x.

P Leys, S., Grombacher, L., & Hill, A. (2019). Hatching and freezing gemmules from the freshwater sponge Ephydatia Muelleri v1. Protocols.Io.

Leys SP, Yahel G, Reidenbach MA, Tunnicliffe V, Shavit U, Reiswig HM. The sponge pump: the role of current induced flow in the design of the sponge body plan. PLoS One. 2011;6(12):e27787. doi: 10.1371/journal.pone.0027787. Epub 2011 Dec 13. PMID: 22180779; PMCID: PMC3236749. https://pubmed.ncbi.nlm.nih.gov/22180779/

Manconi, R., & Pronzato, R. (2008, January 1). Global diversity of sponges (Porifera: Spongillina) in freshwater. SpringerLink. https://link.springer.com/chapter/10.1007/978-1-4020-8259-7_3

Nichols, S. (2023). Growing Freshwater Sponges from Gemmules in the Laboratory V1. dx.doi.org/10.17504/protocols.io.x54v9dkj4g3e/v1

Pronzato, R.; Manconi, R. & Corriero, G. 1993. Biorhythm and environmental control in the life history of Ephydatia fluviatilis (Demospongiae, Spongillidae). Italian Journal of Zoology 60(1):63-67 https://www.tandfonline.com/doi/abs/10.1080/11250009309355792

Reiswig, H. M., Frost, T. M., & Ricciardi, A. (2010). Ecology and Classification of North American Freshwater Invertebrates. McGill. http://redpath-staff.mcgill.ca/ricciardi/Reiswig et al 2010 T&H sponges.pdf

Skelton, J., & Strand, M. (2013, January). Trophic ecology of a freshwater sponge (Spongilla lacustris) revealed by stable isotope analysis. NMU. https://nmu.edu/biology/sites/biology/files/2021-01/Skelton_and_Strand_2013.pdf

Stones River at US hwy 70 near Donelson, TN. USGS Water Data for the Nation. (2023, May). https://waterdata.usgs.gov/monitoring-location/03430200/#parameterCode=00010&showMedian=false&startDT=2023-05-01&endDT=2023-05-31

Turner, E.C. Possible poriferan body fossils in early Neoproterozoic microbial reefs. Nature 596, 87–91 (2021). https://doi.org/10.1038/s41586-021-03773-z

U.S. Department of the Interior. (2020, October 6). Freshwater sponges (U.S. National Park Service). National Parks Service. https://www.nps.gov/articles/freshwater-sponges.html