VIRGINIA FISHERY RESOURCE GRANT PROGRAM

FRG 2023 Final Report

Title: Controlling Epibiont Pests

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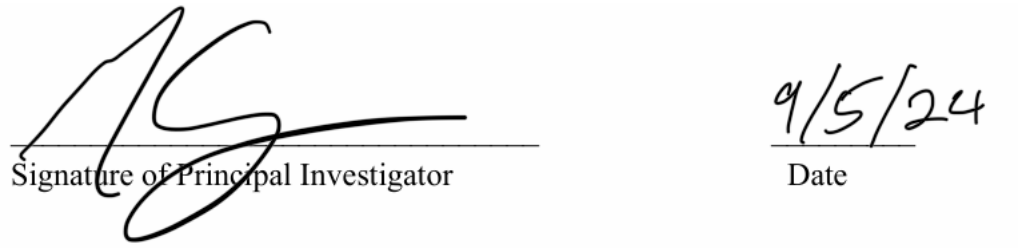


Signature of Principal Investigator

9/5/24

Date

Introduction

Stalked ciliates are a type of protozoa that have branched or unbranched stalks containing inverted bell-shaped bodies. The presence of cilia, or hair-like structures, on the oral region of the organism are their defining characteristic. These organisms primarily feed on small bacteria and debris, though some also consume other ciliates. Their life cycle includes both a free-swimming stage and a sedentary stage, and they are commonly found in freshwater, brackish, and marine environments with high bacterial populations. Stalked ciliates often colonize the surfaces of living organisms, a phenomenon known as epibiosis. Historically, epibiosis was considered a commensal relationship, but recent studies have shown that epibionts can sometimes cause significant harm to their hosts (Overstreet 1987; Shinn et al. 2015; Mahasri et al. 2021).

There are several privileges that epibiont pests enjoy while being attached to their host: ease of transport, protection from predators, availability of nutrients, and expansion of their range. They may occasionally provide benefits to their host by masking chemical scents from predators (Fernandez-Leborans & Gabilondo 2007). However, this relationship is not always commensal, if they share the same niche, both the epibiont and their host may end up competing for resources. In some instances, the epibiont may decrease growth and reproduction of the host, and impede normal physiological functions of the host. Solitary stalked ciliate epibionts may have little to no effect on their hosts, but colonial stalked ciliates could have a significant impact.

The detrimental effects of stalked ciliate infestations are documented for commercially important crustaceans (primarily shrimp and copepods), but not bivalves. In crustaceans, stalked ciliates primarily infest the gills and cause mechanical obstruction and physical damage, which deprives the host of oxygen. However, other adverse effects of epibiont relationships with crustaceans include decreased fecundity, interference with feeding and locomotion, and increased sensitivity to contaminants. In our oyster hatchery, we have experienced both light and severe infestations in bottle and nursery systems, resulting in slowed growth and increased mortality of oyster seed. Similar issues have been reported by other local hatcheries.

Infestations occur on the shell particularly around the growing edge or “bill” where feeding currents from the oyster are likely strongest. As oysters open and establish a feeding current to commence feeding, stalked ciliates are observed being advected into feeding current and subsequently contacting sensory tentacles of the mantles which cause the oyster to shut and discontinue feeding. This cycle will happen repeatedly with oysters attempting to begin feeding and quickly stopping, undoubtedly reducing consumption and resulting in sluggish growth in light infestations or significant mortality in heavy infestations.

While chemical control methods for stalked ciliates have been explored in crustacean aquaculture, there is limited published literature on control methods for bivalve aquaculture. One study (Shinn et al. 2014) located at a commercial horseshoe crab (*Limulus polyphemous*) hatchery with an infestation of *Zoothamnium duplicatum* found success in exposing the juveniles to 10 ppm chlorine in freshwater for one hour and changing the substrate to aquarium sand to eradicate the ciliates. In addition, an earlier study conducted by Texas A&M (Johnson et al. 1973) found 25 ppm formalin was effective in controlling *Zoothamnium* on penaeid shrimp in commercial ponds. For spiny lobster (*Panulirus homarus*) larvae, Vijayakkumaran & Radhakrishnan (2003) determined that a dip in 100 ppm of malachite green or a short bath treatment for 10 minutes in 10 ppm malachite green is the most effective in eradicating *Zoothamnium*. These findings suggest that different control methods for stalked ciliates may be more or less effective depending on the species and system.

In phase 1, we identified, with the help of Dr. Snyder, the most prevalent stalked ciliates that are found in our bottle and nursery systems: Suctorians, *Zoothamnium* spp., and *Vorticella* spp. The method of controlling these infestations at our hatchery consists of agitating the seed for one minute in a diluted vinegar solution (pH 6) and following with a thorough rinse of freshwater. This effectively knocks the zooids off of *Zoothamnium* and *Vorticella*, but the Suctorians remain unaffected. We know that this method does not drastically harm our production of oyster seed. However, in phase 2, we wanted to explore other methods of mitigation to determine if there is a treatment that is more effective in ridding these infestations and also to determine how oyster seed of varying sizes will respond to treatments. In 2023, there was only one documented infestation, so the project was extended into 2024 at no cost to provide additional time for testing treatments and conducting bioassays.

Objectives

This project had two main objectives:

1. Assess the tolerance of small (bottle-sized) and larger (nursery-sized) oyster seed to various treatment concentrations to avoid harm during treatment.
2. Test and compare treatments for seed in bottle and nursery systems to determine which specific treatment is most effective.

A third and fourth objective was created during the project extension to help gather information about these infestations in other regions of the United States and compare stalked ciliate coverage and types inside the hatchery versus ambient water outside:

3. Create a survey that could be sent out on an aquaculture listserv, posted on Aquaculture Information Exchange, and presented at conferences, where the aquaculture community

can weigh in on their issues with infestations and share their mitigation strategies anonymously.

4. Deploy sentinel slide racks, for the purpose of attracting epibionts, in ambient water adjacent to OSH and one slide rack inside one of the hatchery systems to compare the coverage and types of stalked ciliates in hatchery systems versus ambient water.

Methods

Experiments

A. Experiments 2023

During the 2023 hatchery season, there was only one outbreak of stalked ciliates which we identified as Suctorians. One experimental trial was conducted using different concentrations of the lab soap "Alconox" as the treatment (Table 1, Experiment # 1). Oyster seed were split into 12 cups with 75 mL of seed (approximately 1 mm, and numbering about 500k) in each cup. They were then assigned to one of three treatments (in triplicates): 5g Alconox in freshwater, 10g Alconox in freshwater, and 15g Alconox in freshwater. Three cups of seed were reserved as the control, in which no Alconox was added. Before the start of the experiment, 25 seed from each cup were haphazardly chosen and placed under the microscope to obtain an average count of stalked ciliates per individual. After counts were recorded, each cup of seed was individually emptied on a screen and dipped in the assigned concentration of Alconox for one minute, then rinsed with freshwater for one minute. Each replicate was then placed into miniature upweller bottle systems for four days. (These experimental bottle systems are part of OSH R&D infrastructure.) At the end of the 4-day cycle, 25 seed in each bottle were haphazardly examined again to record an average number of stalked ciliates per individual. Volume of seed in each bottle was measured immediately after treatment and just before assessment to determine, indirectly, growth and survival.

B. Experiments 2024

In the 2024 hatchery season there was one recorded infestation. Due to space limitations, experimental trials on the infested cohort were conducted in duplicate for each treatment in our R&D bottle system. The four experimental trials completed in 2024 were based on treatments we wanted to test in 2023. Each experiment tested a different treatment (Table 1, Experiment #2-4): Alconox (surfactant), diluted vinegar, and a hypersaline solution. The fourth experiment was testing diluted isopropyl alcohol as another treatment method. For each individual experiment,

seed were split up into equal volumes (75 mL, approximately 1 mm, and numbering about 50k per bottle) across 6 bottles (duplicates) and assigned to one of 3 treatments (treatment 1, treatment 2, control). At the end of the 4-day cycle, 25 seed in each bottle were haphazardly examined again to record an average number of stalked ciliates. Volume of seed in each bottle (X = 6) was also measured to determine growth and survival.

Table 1. Outline of experiments conducted in 2023 and 2024. "C" represents control.

Experiment #	Start Date	End Date	Treatments	# of Replicates
1	5/1/23	5/4/23	Alconox 5g, 10g, 15g, C	3 replicates/ treatment
2	3/15/24	3/19/24	Alconox 20g, 30g, C	2 replicates/ treatment
3	3/19/24	3/22/24	Brine (60 ppt) 3 min, 5 min, C	2 replicates/ treatment
4	3/22/24	3/25/24	Diluted vinegar (pH 4) 60 sec, 120 sec, C	2 replicates/ treatment
5	3/25/24	3/29/24	1-part Isopropyl alcohol & 3 parts freshwater. 60s, 90s, C	2 replicates/ treatment

Bioassays

A. Bioassays 2023

Bioassays in 2023 were used to determine the tolerance of seed to different treatment concentrations and exposure times (Table 2, Assays #1-10). There were two types of bioassay: bottle and upweller. Four bioassays were completed in the bottle system in 2023 (Table 2, Assay # 1-4). For bottle bioassays, seed approximately 1 mm in size were separated into 8 cups of equal volume (~ 75 mL) and assigned a treatment. They were dipped in their respective solutions for one minute, followed by a one-minute freshwater rinse and placed into the bottle system. After a 4-day period, they were removed from the bottles and their volume was recorded. The exact same steps in this assay were repeated for the last 3 assays to test different concentrations of surfactant, diluted vinegar, and salinity respectively (Table 2, Assays #2-4).

For upweller bioassays, seed (4 mm size) were divided into equal volumes (75 mL per treatment) across 12 experimental silos (11.4 cm wide) and those silos were individually assigned to a treatment. They were dipped in their respective treatment for one minute or more, depending on the specific assay, followed by a freshwater rinse for one minute. At the end of the 4-day cycle, the volume of seed was recorded to determine effects on growth and survival. There was a total of 6 bioassays completed for the outdoor upweller system (Table 2, Assay # 5-10). Bioassays #5 and 6 used conservative treatments. For #5, the effects of Alconox lab soap at three different concentrations on seed health when compared to the control (normal seawater).

It was determined that seed were more tolerant of the treatments than initially thought. Therefore, the rest of the 2023 upweller bioassays focused on longer treatment contact times to see if there was an effect on seed health (Table 2, Assay #7-10). Due to space limitations in upwellers, the first contact-time assay was conducted with duplicates. Seed were split into equal volumes (75 mL per treatment across 6 silos and assigned to one of three treatments: 2 minutes sitting in non-agitated, undiluted vinegar, 2 minutes in undiluted vinegar while being agitated, and 2 minutes in regular seawater as the control. Volume was measured after the 4-day cycle to determine effects on growth and survival. The last three upweller assays were completed using 2-4 mm seed in triplicates of equal volume (75 mL per silo) to determine effects of longer contact time with Alconox, brine, and vinegar (between 10 and 60 minutes) on seed health (Table 2, Assay # 8-10).

Table 2. Outline of bioassays conducted in 2023 and 2024. BOT = bottle assay; UP = upweller assay; "C" = control.

Assay #	Start Date	End Date	Treatments	# of Replicates
1 (BOT)	3/9/23	3/13/23	Diluted vinegar pH 5, pH 6, pH 7, C	2 replicates/ treatment
2 (BOT)	3/14/23	3/17/23	Brine 40 ppt, 50 ppt, 60 ppt, C	2 replicates/ treatment
3 (BOT)	3/20/23	3/24/23	Diluted vinegar pH 3, pH 4, pH 5, C	2 replicates/ treatment
4 (BOT)	4/11/23	4/15/23	Alconox 10g, 20g, 30g, C	2 replicates/ treatment
5 (UP)	7/5/23	7/8/23	Alconox 10g, 20g, 30g, C	3 replicates/ treatment
6 (UP)	7/11/23	7/14/23	Diluted vinegar pH 4, pH 5, pH 6, C	3 replicates/ treatment
7 (UP)	7/15/23	7/18/23	Vinegar 120 sec w/ no agitation, 120 sec w/ agitation	2 replicates/ treatment
8 (UP)	7/18/23	7/21/23	Brine (60 ppt) 10 min, 20 min, C	3 replicates/ treatment
9 (UP)	7/21/23	7/24/23	Alconox (70g) 10 min, 20 min, C	3 replicates/ treatment
10 (UP)	7/24/23	7/27/23	Vinegar 20 min, 60 min, C	3 replicates/ treatment
11 (UP)	4/1/24	4/1/24	Alconox 10g dip 60s, hard freshwater spray 60s, C	2 replicates/ treatment
12 (UP)	4/5/24	4/5/24	Copper sulfate (5 mL in 15 L of freshwater)	2 replicates/ treatment

B. Bioassays 2024

There were two bioassays completed in 2024 (Table 2, Assay # 11-12 to test the immediate effectiveness of certain treatments on removing stalked ciliates. The first assay (Table 2, Assay # 11) was to compare the effectiveness of a hard freshwater spray for 60 seconds versus continuously dipping the seed in freshwater containing 10g of Alconox lab soap for 60 seconds followed by a freshwater rinse. Seed were evenly split into 6 cups (30 mL of seed each, approx 4 mm) and the average number of stalked ciliates was calculated before and after treatment by haphazardly picking 25 seed and counting stalks attached to them. This same method was repeated to test the effectiveness of dipping seed in copper sulfate (Table 2, Assay # 12) for one-minute at the recommended concentration of 5 mL per 15 L of water.

Survey

The survey was designed to determine if other hatcheries or farms experienced stalked ciliate outbreaks, what time of year they occur, what systems infestations occur most often, and what control methods are used to mitigate infestation. This survey was created on the "jotform" website for easy distribution. It was posted on the Aquaculture Information Exchange website, sent through the East Coast Shellfish Grower's Association listserv, and presented at the Virginia Aquaculture Conference and North East Aquaculture Conference and Expo.

Slide Rack Observations

Dr. Richard Snyder from VIMS provided us with two slide racks to monitor stalked ciliates in situ. A method employed regularly by his lab. One slide rack with four slides was deployed in ambient water adjacent to the hatchery and one slide rack with four slides was deployed within a bottle system inside the hatchery for a period of 7 days. At the end of the 7-day period, both slide racks were removed from their respective locations and each of the 4 slides were examined under a microscope to identify and determine overall coverage of ciliates. These qualitative observations were used to determine if the species present and coverage of ciliates were similar between both locations.

Results and Discussion

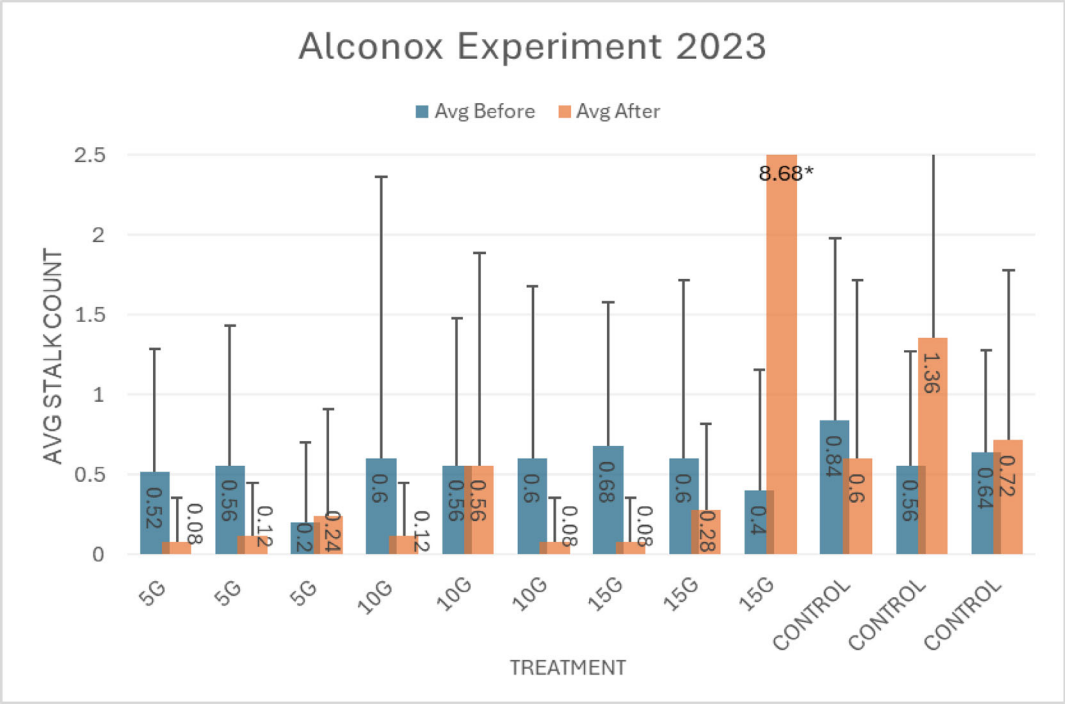
Experiments

A. Experiments 2023

Results of the Alconox experiment showed a decrease in stalk count in 7 of the 9 replicates while stalk count in the control increased (Fig. 1). The 5g concentration seemed to have been effective

for the first two replicates, where the average stalk count decreased at the end of the 4-day period, but the 3rd replicate had increased average stalk count. Similarly, the 10g and 15g treatments had the same results amongst their replicates. One of the control replicates showed a slight decrease in average stalk count, while the other two replicates had an increase in average stalk count. This experiment displays variance around the stalk count and variance around the replication which may limit the reliability of the results. The variability could have been impacted by environmental conditions of the water and the system at the time of the experiment and also by the health of the seed. For example, the third replicate for 15g of Alconox showed the highest average stalk count after the treatment, which indicates that the infestation proliferated in the conditions of that specific bottle whether it was related to environmental conditions or the health of the seed. In future experiments, it would be best to consider increasing the sample size of seed for counting stalks and managing environmental variability within the system. The exposure to the different concentrations of Alconox did not appear to affect the growth of the seed because the ending volumes were greater than the starting volumes (Appendix A, Fig. 1).

Figure 1. Alconox concentration experiment conducted in 2023 in the R&D bottle system. Error bars represent standard deviation.



B. Experiments 2024

The first experiment conducted during the outbreak of 2024 focused on examining the effect of Alconox in higher concentrations during the short SOP contact time (60 seconds). After the 4-day experimental period, the average stalk count doubled in the treatment replicates and tripled in the control replicates (Fig. 2). Observationally it seems that Alconox has the potential to knock off zooids initially, but the stalks remain intact and it does not help mitigate the infestation over the course of a grading cycle (4 days). The second experiment used diluted vinegar (pH 4) as a treatment with contact times of 60 seconds and 120 seconds compared with a control. This treatment is able to remove zooids of *Zoothamnium* and *Vorticella* effectively, but not Suctorians (observation). It also seems like this treatment has a greater effect of mitigation over a grading cycle period. The average number of stalks only increased in the control replicates (Fig. 3).

A hypersaline, or brine, solution of 60 ppt was examined in the third experiment (Fig. 4) with contact times of 3 minutes and 5 minutes with no agitation. In the first 3-minute replicate, stalk number increased slightly after the 4-day grading cycle. However, for the second 3-minute replicate and the 5-minute replicates, stalk count average did not increase, but the averages were only slightly less than the starting averages. This may show that the osmotic shock on stalked ciliates is not as effective as a chemical treatment would be over time. Lastly, the final experiment tested a new treatment of diluted isopropyl alcohol that was inspired by one of the answers given in the stalked ciliate survey combined with contact times of 60 and 90 seconds. Results of this experiment were inconclusive because in two of the treatment replicates, average stalk count increased while two treatment replicates saw a decrease in average stalk count (Fig. 5). One control replicate showed an increase in average stalk count while the other showed a decrease. Although some impact was seen on the average number of stalks during each experimental trial, the seed used were already experiencing stress, and not much growth occurred during the experiments (Appendix A, Figures 1-3). This may have been a combination of environmental factors, the infestation, and treatments combined.

Figure 2. Alconox concentration experiment conducted in 2024 in the R&D bottle system. Error bars represent standard deviation.

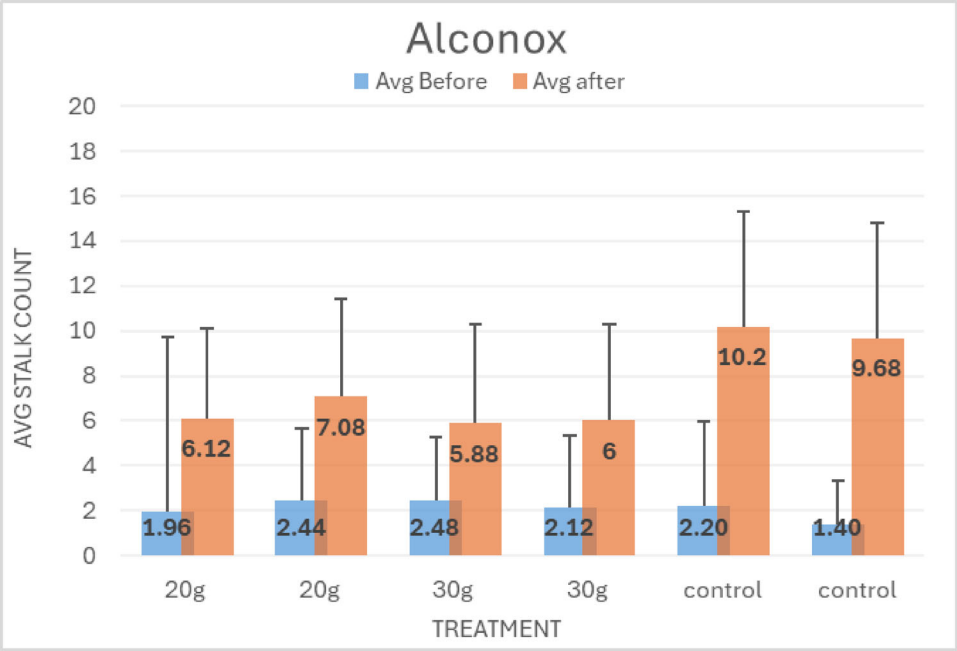


Figure 3. Vinegar concentration experiment conducted in 2024 in the R&D bottle system. Error bars represent standard deviation.

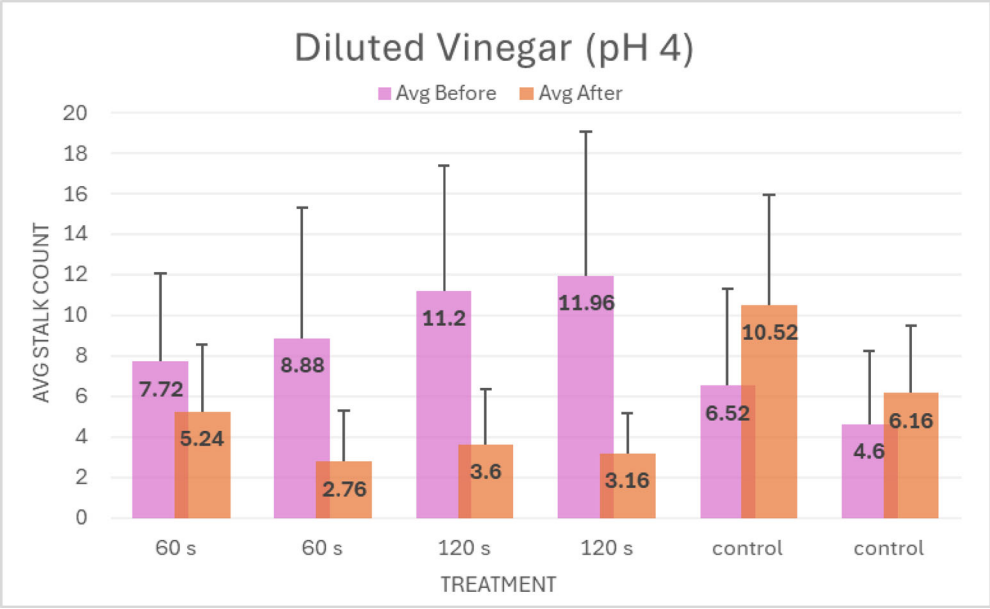


Figure 4. Hypersaline concentration experiment conducted in 2024 in the R&D bottle system. Error bars represent standard deviation.

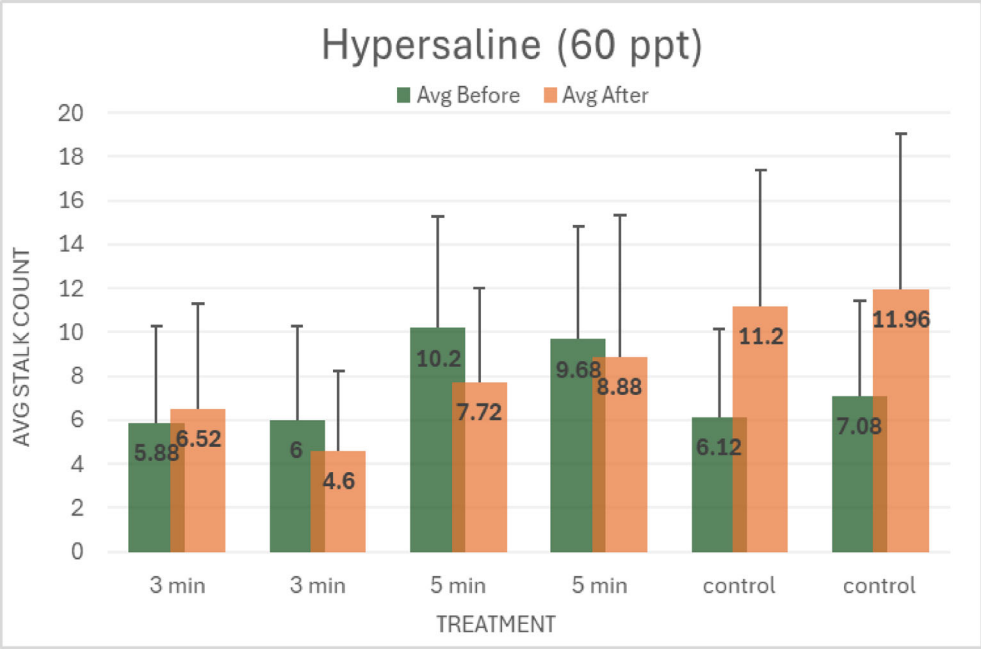
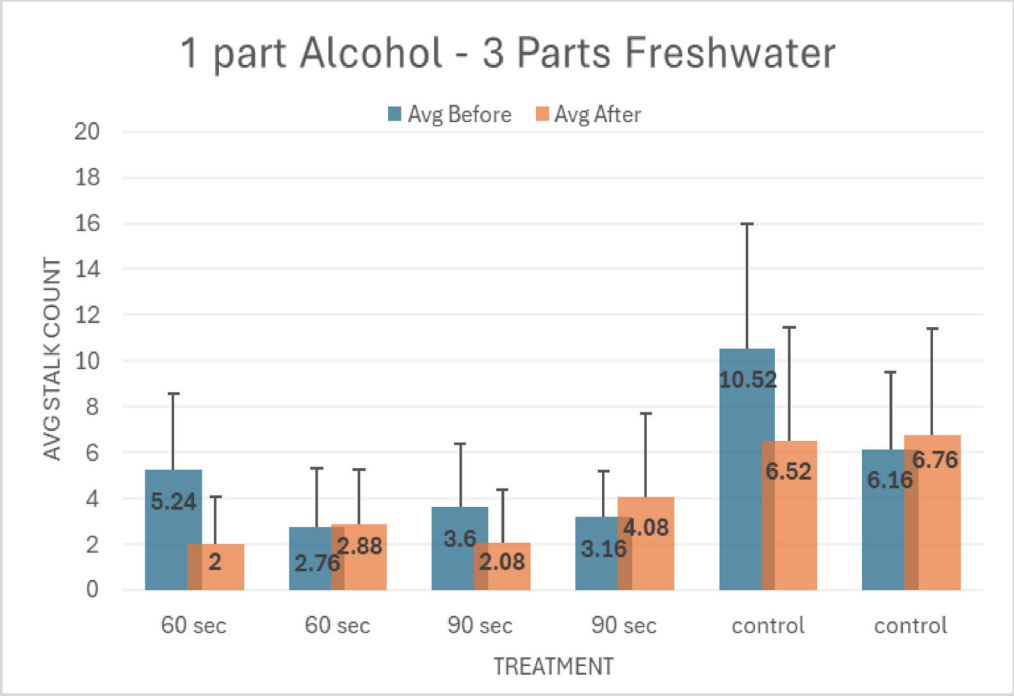


Figure 5. Diluted isopropyl alcohol concentration experiment conducted in 2024 in the R&D bottle system. Error bars represent standard deviation.



Bioassays

A. Bioassays 2023

Results of the bioassays conducted in 2023 show that the concentration of the treatments do not affect growth and survival of seed when the contact time is short (1 minute) and agitation is continuous, like in our standard operating procedure for using diluted vinegar (Appendix A, Figures 5-10). However, for certain, more potent treatments, like vinegar, the longer contact times without agitation do have an effect on seed growth and survival. The first undiluted vinegar trial with a longer contact time had similar results between agitation for 2 minutes and no agitation for 2 minutes (Appendix A, Fig. 11) where volume of seed still increased over the course of 4 days, mortality was very minimal if any, and this growth was similar to the growth seen in the control. The second longer-contact vinegar trial examined the contact time of 20 and 60 minutes unagitated (Appendix A, Fig. 14). This is the only bioassay where significant mortality occurred (observation) and while there was still some growth, it was not comparable to the growth of the control. In contrast, longer contact times with Alconox and brine did not seem to have a significant effect on growth and survival of seed (Appendix A, Fig. 12 & 13). No obvious mortality was observed and volumes increased across the board for all replicates.

B. Bioassays 2024

The assays conducted in 2024 concentrated on examining immediate effects of treatments instead of a 4-day cycle. The first assay was comparing the effectiveness of Alconox and a hard freshwater spray at knocking-off stalked ciliates from oyster seed (Fig. 6). In this particular assay, it seems that a freshwater spray with hard pressure for one minute has a greater immediate effect on stalked ciliates when compared to dipping seed in an Alconox and water solution for one minute. The Alconox dip had roughly a 40-50% decrease in average stalk count while the freshwater spray had roughly an 80% decrease.

Dr. Richard Snyder at VIMS suggested trying copper sulfate, commonly used in aquaculture and aquarium settings to control protozoan ciliates, as another possible treatment. Since using copper sulfate on a large scale could be tricky due to strict site regulations and environmental hazards, this assay focused on a small-scale immediate trial involving two cups containing 30 mL of seed each. Before and after average stalk counts show that the copper sulfate was able to reduce stalked ciliates by 40-50% (Fig. 7). While copper sulfate seems like it could be another treatment option, more trials need to be completed to see if there are similar results.

Figure 6. Comparison of diluted alconox versus freshwater spray on the number of stalks present. This was an assay looking at immediate effects. Error bars represent standard deviation.

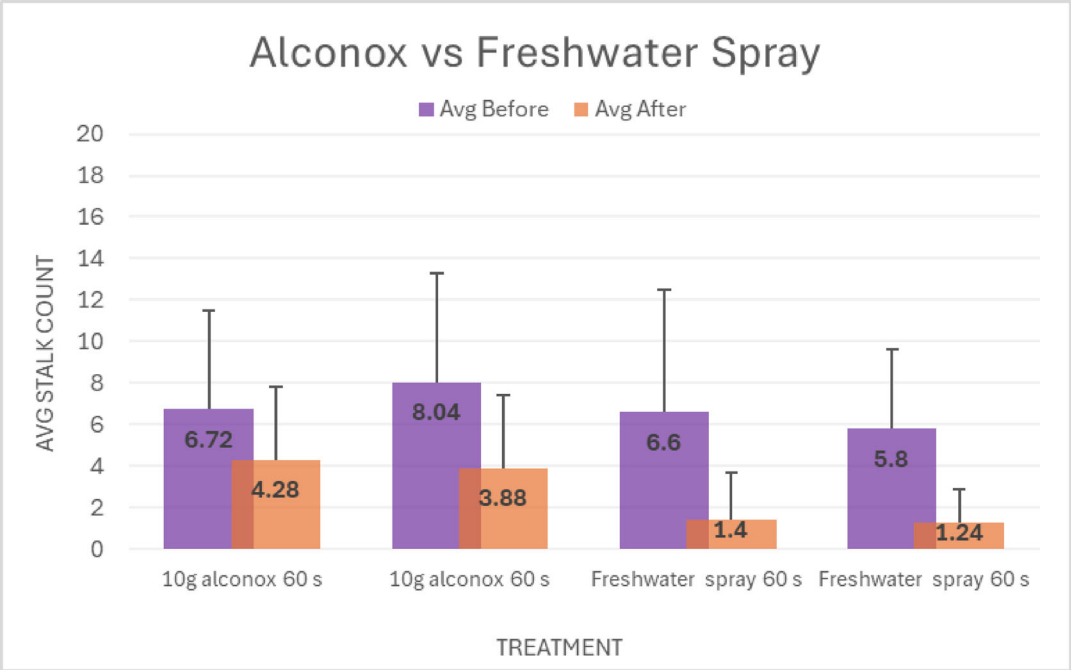
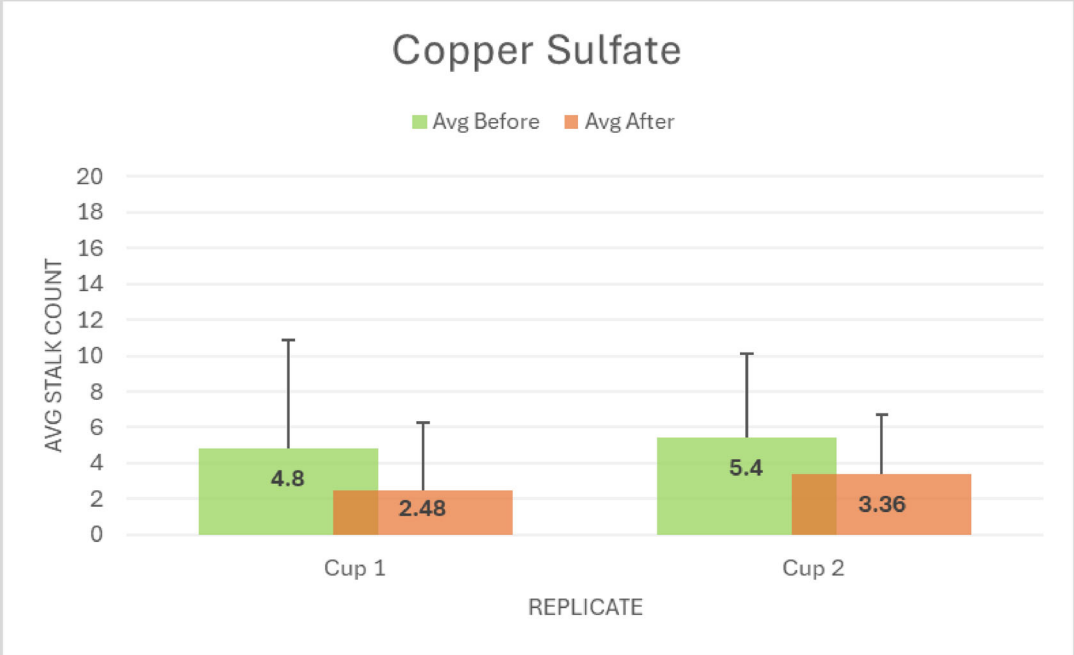


Figure 7. Examining the immediate effects of copper sulfate on the number of stalks present. Error bars represent standard deviation.



Survey

The stalked ciliate survey had a total of 21 participants from the Gulf and east coast ranging from Texas through Massachusetts and encompassing a wide range of salinities (Appendix B, Table 1). Out of the 21 participants, 19 of them said they do experience stalked ciliate infestations (Fig. 8). Most of the participants experience these infestations primarily in spring and summer (Fig. 9), which seems to line up with the timing of when our hatchery sees them. According to survey results, systems with the highest prevalence of stalked ciliate infestations are downweller and upweller systems (Fig. 10). Responses to treatment methods varied depending on what is feasible for their individual operations (Appendix B, Table 2). For example, freshwater dipping does not work for our hatchery because salinity is low, but it seems effective for hatcheries or farms located in higher salinity areas. Some other common suggestions from the survey included a hard freshwater spray, hard saltwater sprays, brine dips, desiccation, and more frequent or aggressive grading.

Figure 8. Pie chart showing the results from 21 participants about their encounter with stalked ciliate infestations.

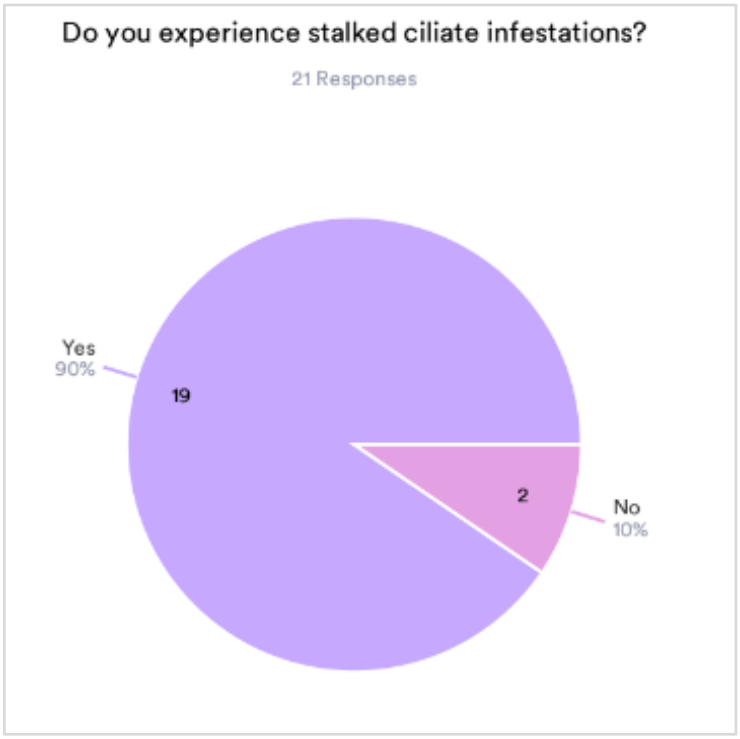


Figure 9. Pie chart showing the results for what time of year participants commonly see stalkedciliate infestations at their location.

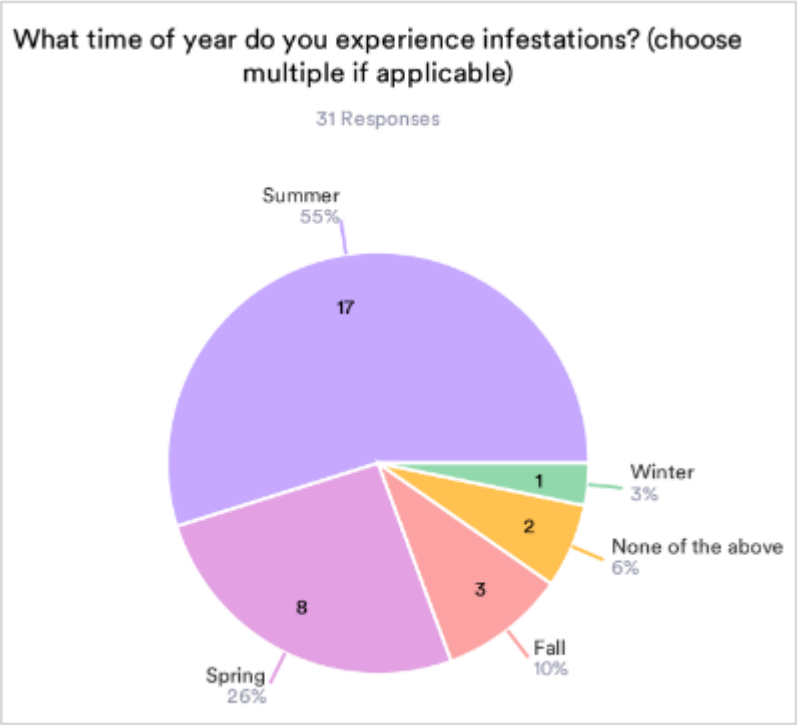
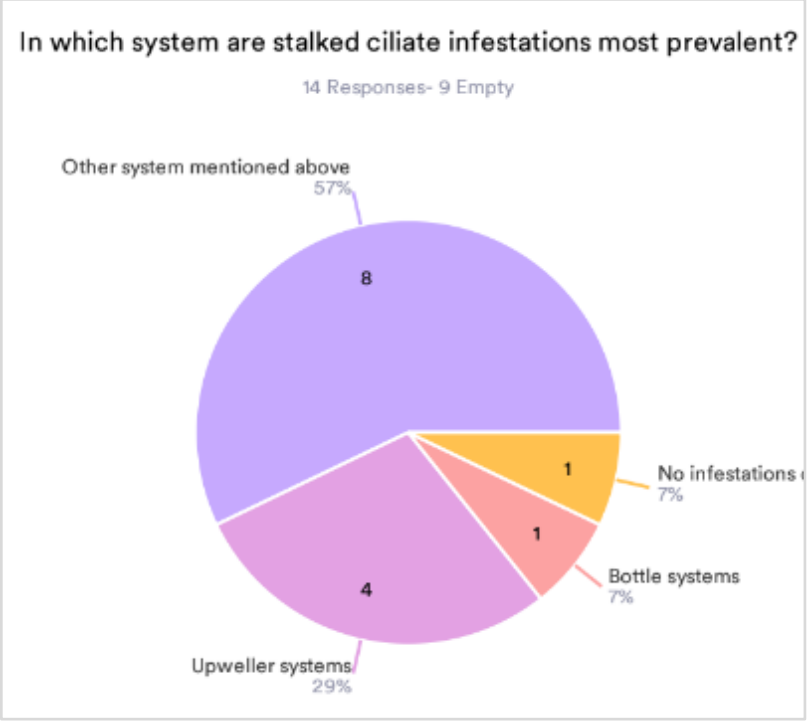


Figure 10. Pie chart showing the results for which systems experience the most infestations at our participant's locations.



Slide Rack Observations

Slide racks were deployed inside and outside the hatchery a total of 5 times from early April through the end of May 2024. There were similarities between the stalked ciliate species observed inside and outside as well (Table 3), these are the same genuses that we generally observe in our systems. However, the coverage of stalked ciliates was greater on the slides deployed inside versus outside. The biodiversity is greater outside because the water has not been subject to filtration, therefore, competition for space and food is limited for stalked ciliates and they are exposed to more predators compared to systems inside the hatchery.

Table 3. Table outlining location of the slide racks, date of observation, and observations made.

Date	**Location**	**Observations**
4/11/24	Inside	Coverage is primarily *Zoothamnium spp.* and *Vorticella spp*, about 90% coverage on slides. Some free-swimming ciliates and tintinnids are present.
4/11/24	Outside	Mostly filamentous algae coverage, about 10-20% coverage of *Zoothamnium spp.* and Suctorians. Free-swimming ciliates, tintinnids, and barnacles present as well.
5/1/24	Inside	*Vorticella* spp., *Zoothamnium* spp., tintinnids, free-swimming ciliate *Apidisca*. About 80-90% coverage on slides.
5/1/24	Outside	Filamentous cyanobacteria, pennate diatoms, chain diatoms, nematode worms, *Vorticella* spp. (10-20% coverage)
5/7/24	Inside	Stichotrichs, scutio ciliates, *Vorticella* spp. and *Zoothamnium* spp. (80% coverage), copepods
5/7/24	Outside	Filamentous cyanobacteria, *Apidisca,* copepods, pennate diatoms, chain diatoms, nematode worms, Suctorian swarmers, stichotrichs, suctorians (10% coverage), *Vorticella* spp. (5% coverage)
5/24/24	Inside	*Vorticella* spp. (20%), nematode, copepod, free-swimming ciliates, pennate diatoms
5/24/24	Outside	Filamentous cyanobacteria, diatoms, *Vorticella* spp. (10% coverage), Suctorians (10% coverage)
5/30/24	Inside	*Vorticella* spp. 10% coverage on slides
5/30/24	Outside	95% coverage of *Zoothamnium* spp., nematodes, hydrozoans, pennate diatoms, filamentous cyanobacteria, chain diatoms, free-swimming ciliates

Conclusion

While it is evident that we cannot completely eradicate stalked ciliate infestations from our systems, our focus should shift to effective mitigation strategies and regular monitoring to catch infestations early. We hypothesize that these infestations could be a secondary issue of a stressed environment within the bottle system which highlights the importance of monitoring and adapting protocols accordingly. In 2024, only one infestation occurred in the bottle systems and we believe increasing the flow in those systems in this year may have contributed to the reduced observation of infestations by decreasing the amount of waste remaining in the system, and therefore, decreasing bacterial levels. There a couple reasons why increased water flow balances bacterial load in a system. First, higher water flow helps dilute the concentration of bacteria by spreading them out over a larger volume. Second, oxygen levels are enhanced by higher water flow. Many bacteria that contribute to disease thrive in low-oxygen environments, and better dissolved oxygen levels can make conditions less favorable for harmful bacteria and promote beneficial bacterial growth. Overall, slow-moving water allows bacteria to accumulate and form biofilms on surfaces, which is not ideal for healthy seed culture in a bottle system.

Furthermore, it's crucial to recognize that recirculating water, a standard practice during holding, can potentially worsen the issue by allowing waste, bacteria, and swimming ciliate zooids or cysts to accumulate and increase in density within the system. To address this, we implement strict sterilization procedures for our oyster seed, including cleaning the bottles twice a week and circulating bleach through them for 30 minutes to eliminate any microbes on the surfaces. However, oyster seed removed for grading might still have stalked ciliates attached to their shells and could introduce them back into the system. Given the high density of these systems and the significant waste produced from seed feeding, bacterial and ciliate densities can quickly rebound within days. Bacteria seem to be a bottom-up control for stalked ciliates since they are the primary food source and increasing flow rate across the board for seed holding systems could help decrease bacterial density and slow infestations.

The suggestion of continuous dosing of copper sulfate in our systems, while offering an alternative mode for addressing stalked ciliates, is impractical in continuous large-scale systems, due to the potential toxicity of copper sulfate to the environment. Furthermore, the continuous use of copper sulfate in aquaculture is expensive and logistically challenging and comes with strict site regulations that require careful monitoring and management. Given these issues, we concluded a continuous dosing method would not be feasible for our hatchery.

Going forward, it is important to build a more comprehensive understanding of the relationship between environmental parameters and onset of infestations. Setting up a regular monitoring plan that records water parameters and includes slide rack deployment to capture

stalked ciliate emergence will assist our team with defining these relationships. Additionally, from the results described in this project, it appears that a diluted vinegar dip is still the most efficient immediate treatment method for our seed, but it would be beneficial to further explore increased contact times with diluted vinegar or even desiccation measures. Reducing the need for recirculating water in bottle systems, while simultaneously increasing flow rates, could be the best strategy for decreasing the likelihood of severe outbreaks. Overall, future efforts should focus on refining treatment methods, monitoring strategies, and system management to mitigate the impact of stalked ciliates on oyster seed growth and survival.

Literature Cited

Shinn, A.P., Mühlhölzl, A.P., Coates, C.J., Freeman, M.A., Metochis, C. (2014) *Zoothamnium duplicatum* infestation of cultured horseshoe crabs (*Limulus polyphemus*), Journal of Invertebrate Pathology. doi: http://dx.doi.org/10.1016/j.jip.2014.12.002

Vijayakkumaran, M., Radhakrishnan, E.V. (2003) Control of epibionts with chemical disinfectants in the phyllosoma larvae of the spiny lobster, *Panulirus homarus* (Linneaus), In: *Singh et al. (Eds.)*, Aquaculture Medicine: 69-72.

Appendix A

A. Experiment Growth Figures

Figure 1. Volume before and after the 2023 Alconox concentration experiment.

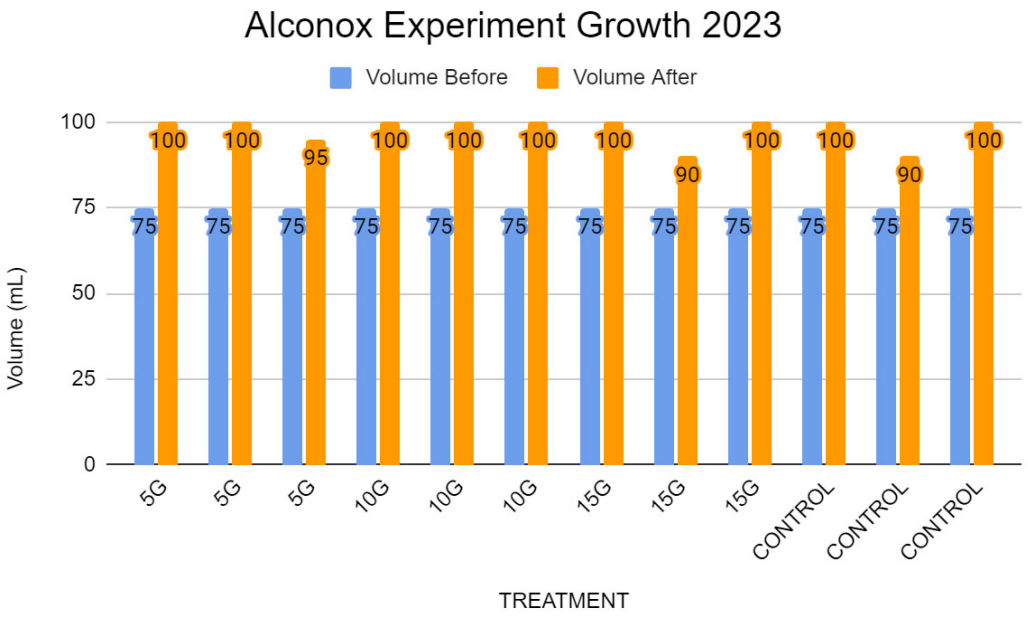


Figure 2. Volume before and after the 2024 Alconox concentration experiment.

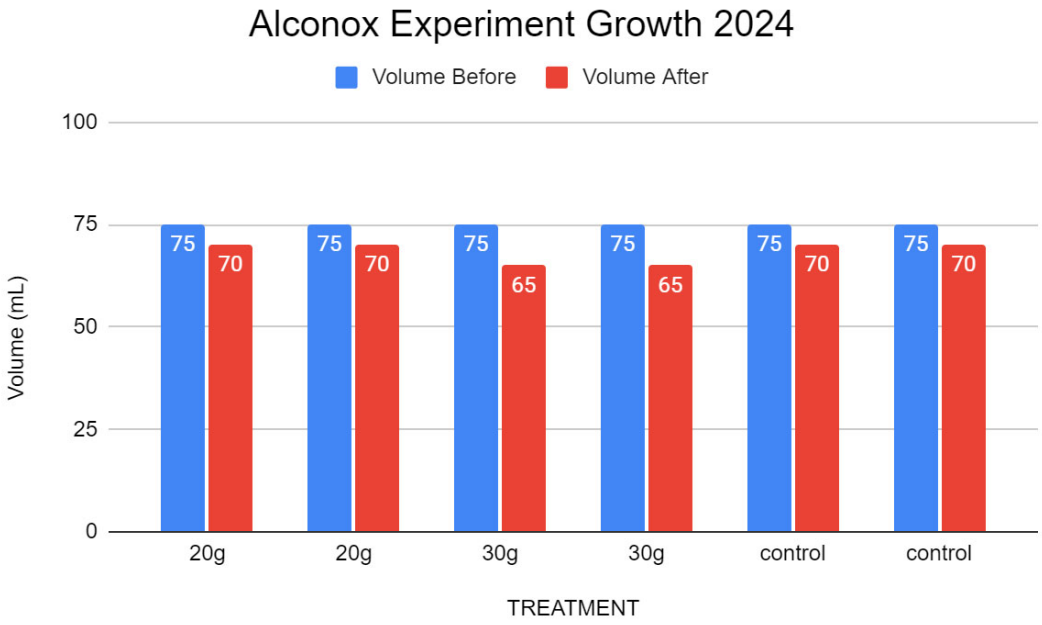


Figure 3. Volume before and after the 2024 hypersaline experiment.

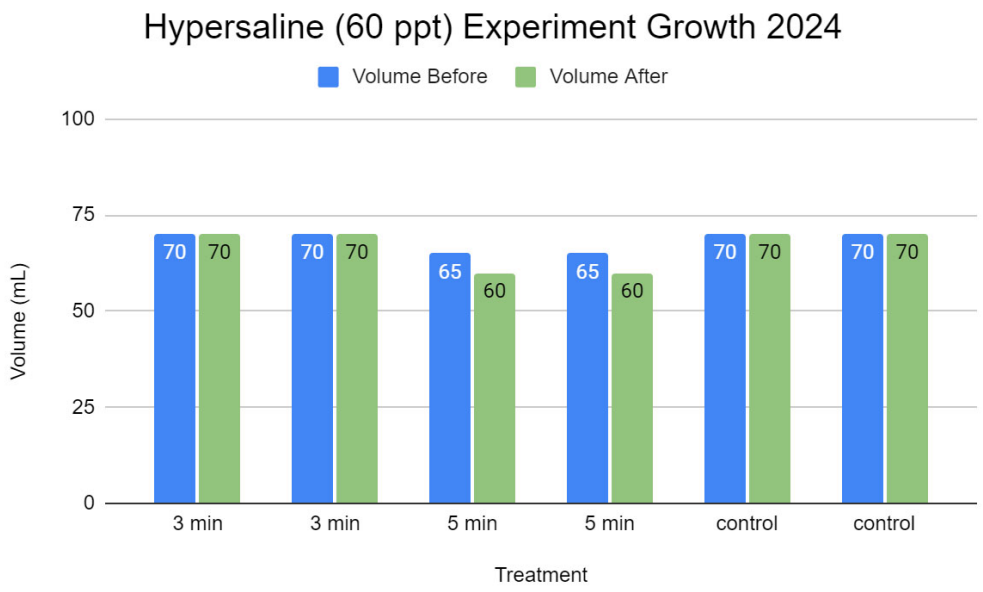
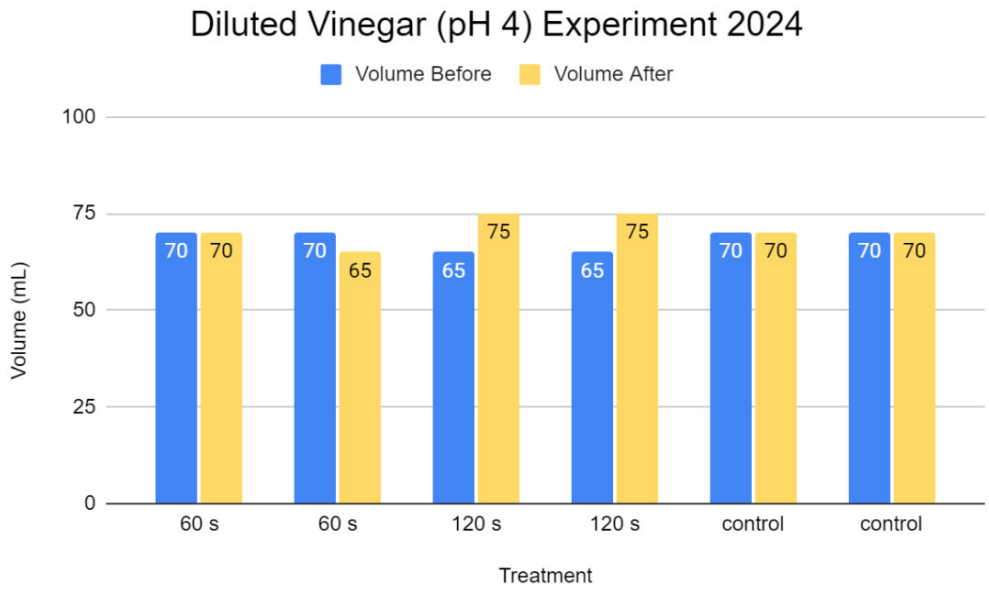


Figure 4. Volume before and after the 2024 diluted vinegar experiment.



B. Bioassay Growth Figures

Figure 5. Volume change for the bottle assay examining the effect of diluted vinegar concentrations on seed health.

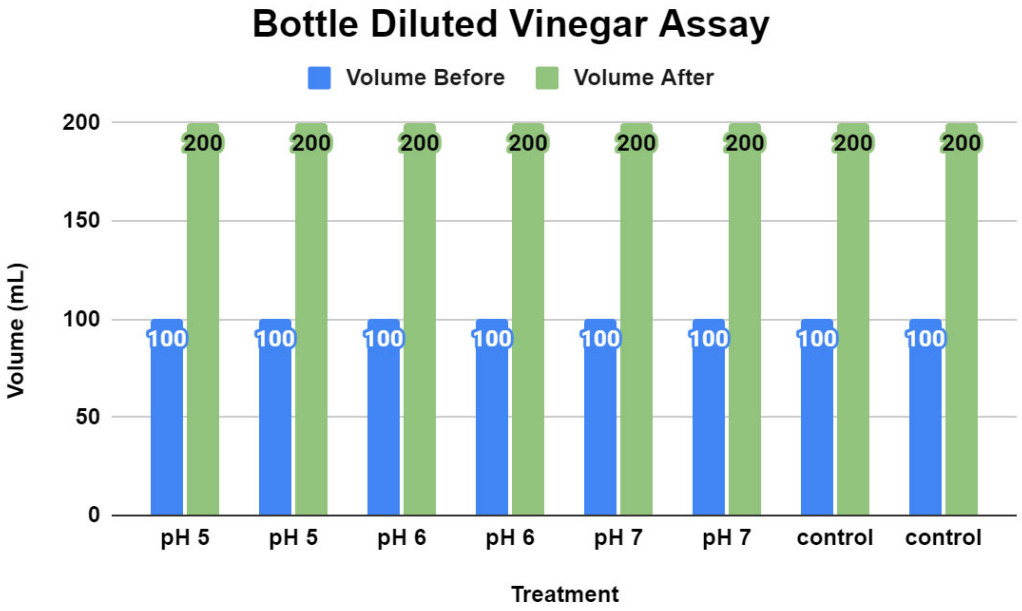


Figure 6. Volume change for the second bottle assay examining the effect of diluted vinegar concentrations on seed health. This trial was done with lower pH levels.

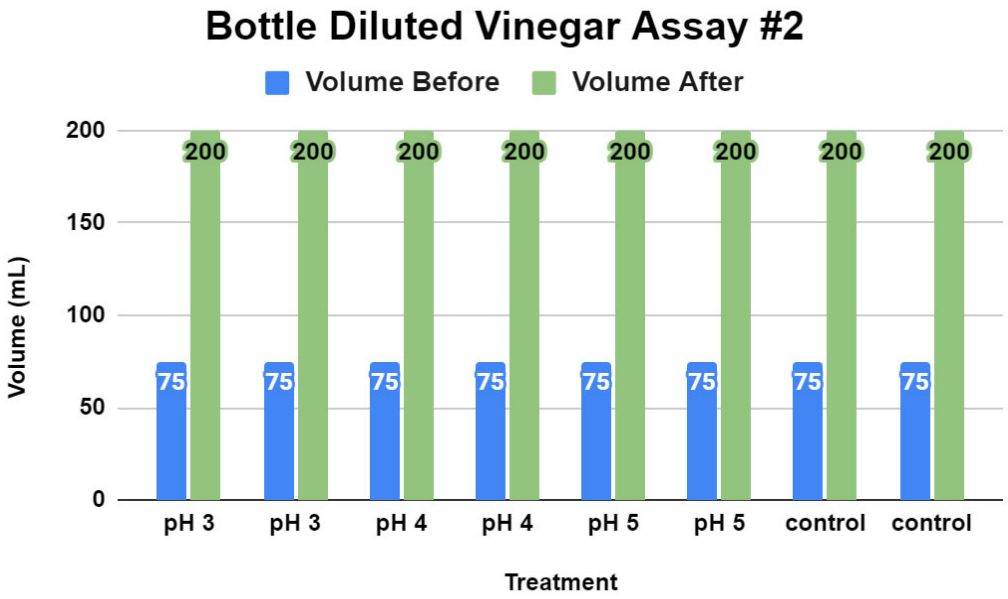


Figure 7. Volume change for the bottle assay examining the effect of hypersaline (brine) concentrations on seed health.

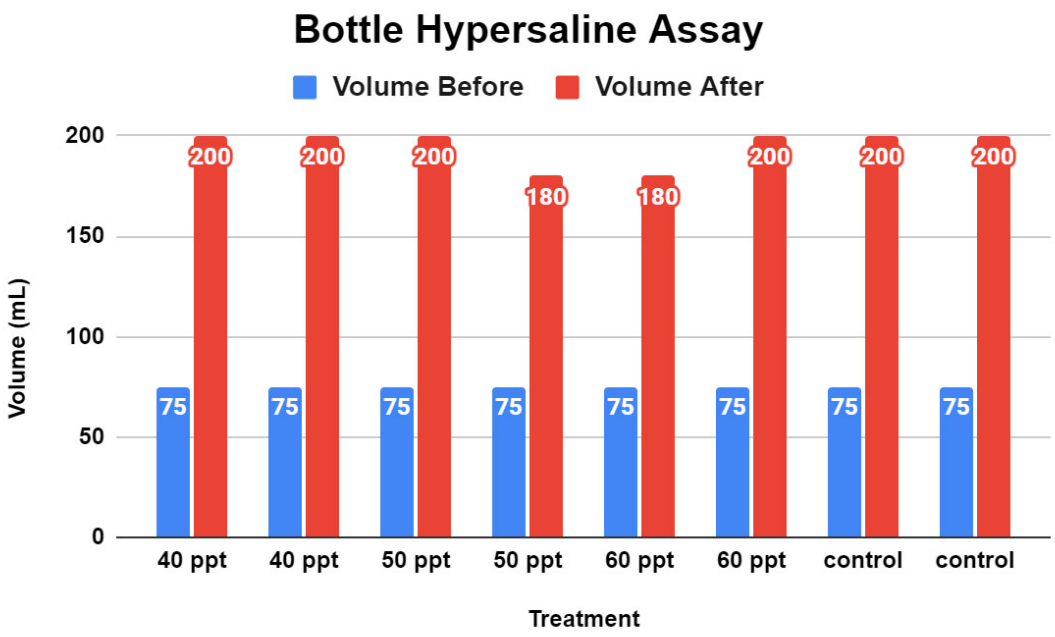


Figure 8. Volume change for the bottle assay examining the effect of Alconox lab soap concentrations on seed health.

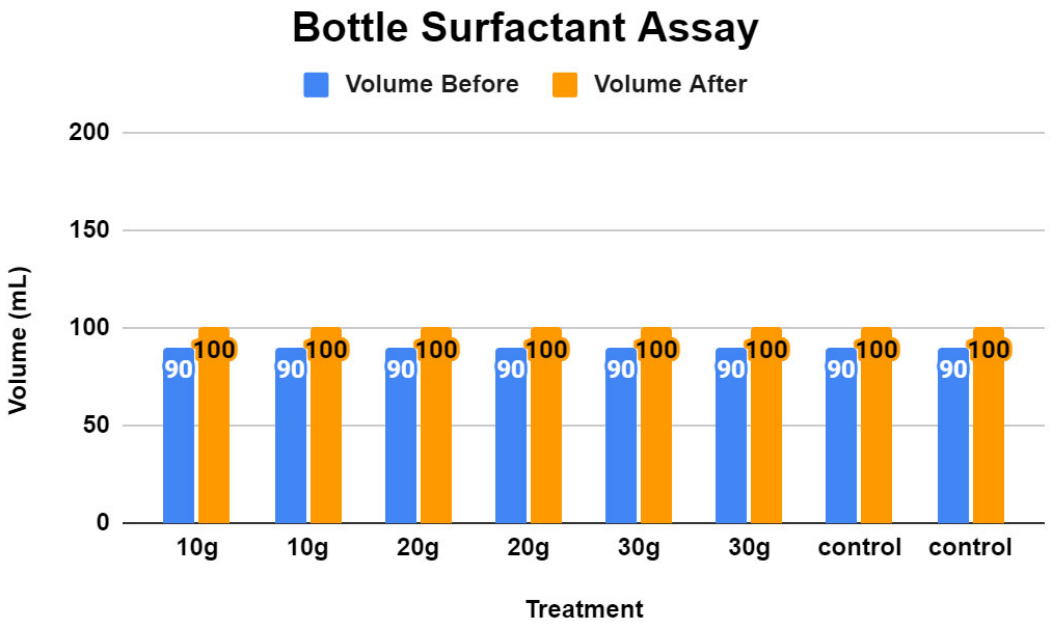


Figure 9. Volume change for the upweller assay examining the effect of Alconox lab soap concentrations on seed health.

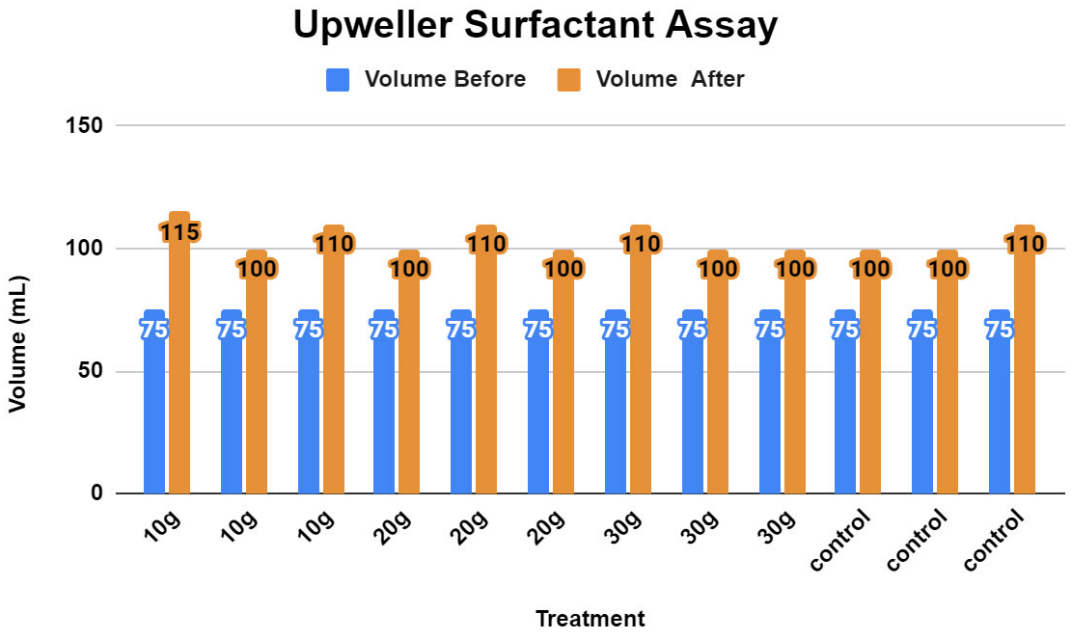


Figure 10. Volume change for the upweller assay examining the effect of diluted vinegar concentrations on seed health.

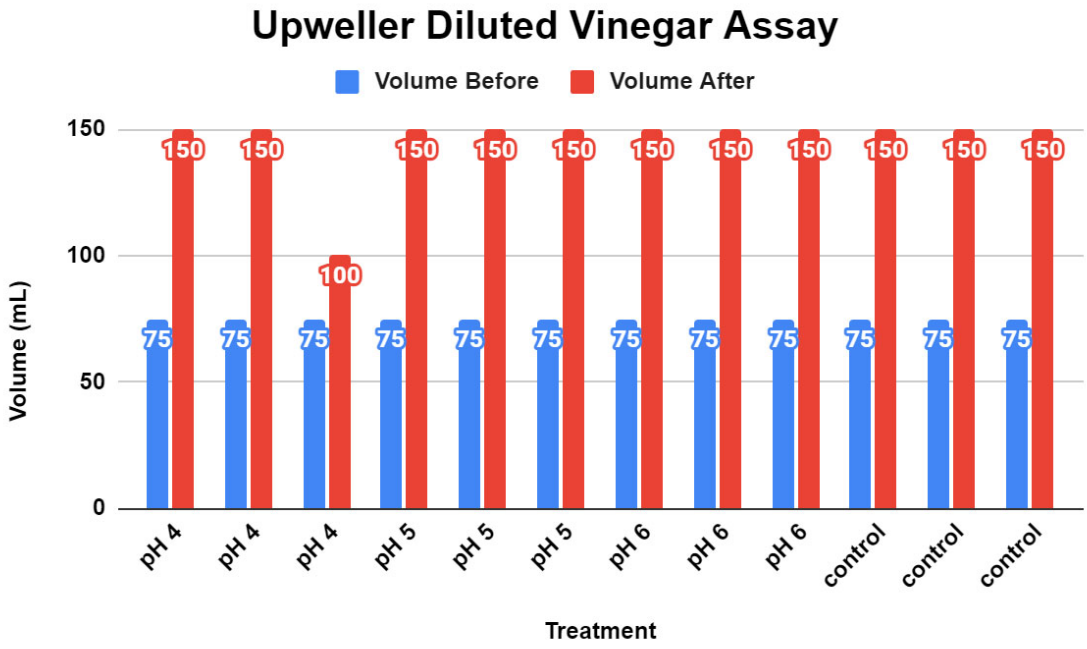


Figure 11. Volume change for the second upweller assay examining the effect of undiluted vinegar and greater contact time on seed health.

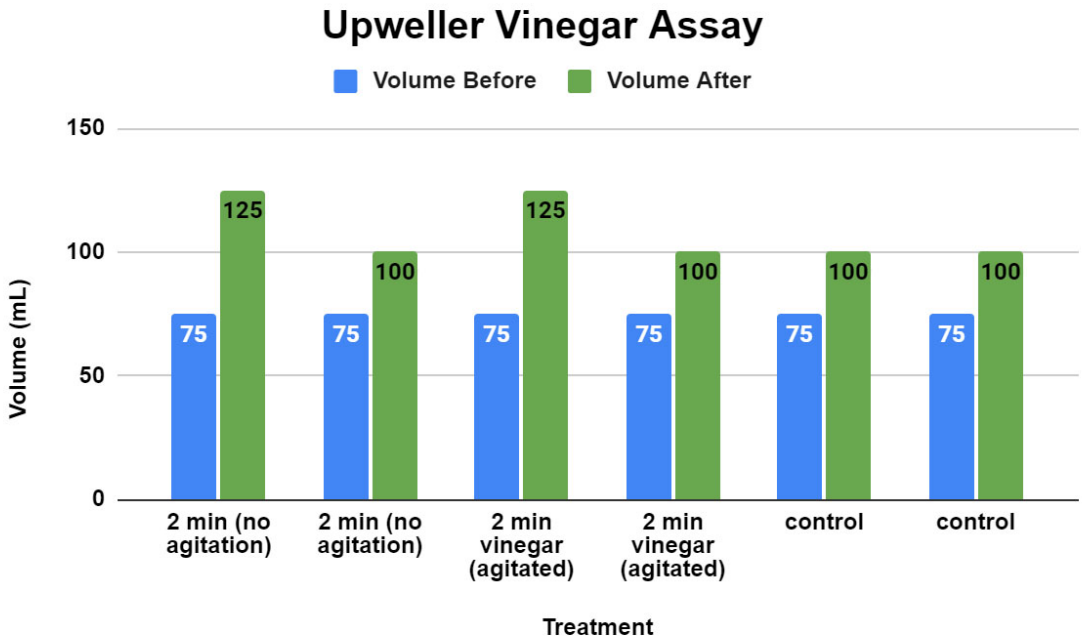


Figure 12. Volume change for the upweller assay examining the effect of hypersaline concentrations and greater contact time on seed health.

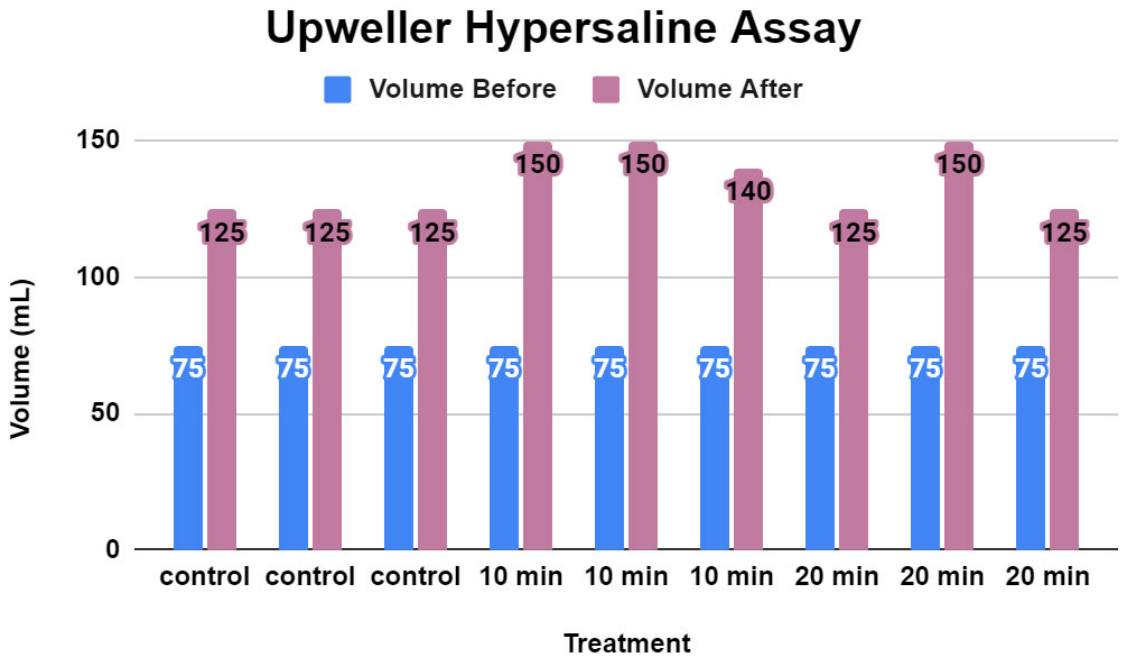


Figure 13. Volume change for the upweller assay examining the effect of surfactant concentrations and greater contact time on seed health.

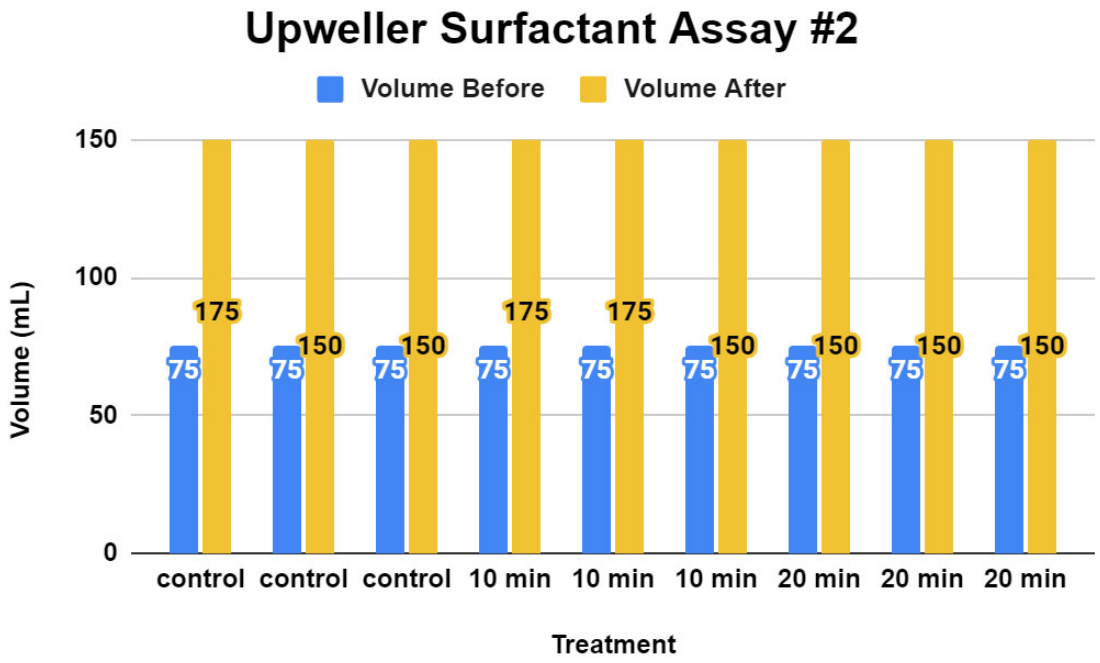
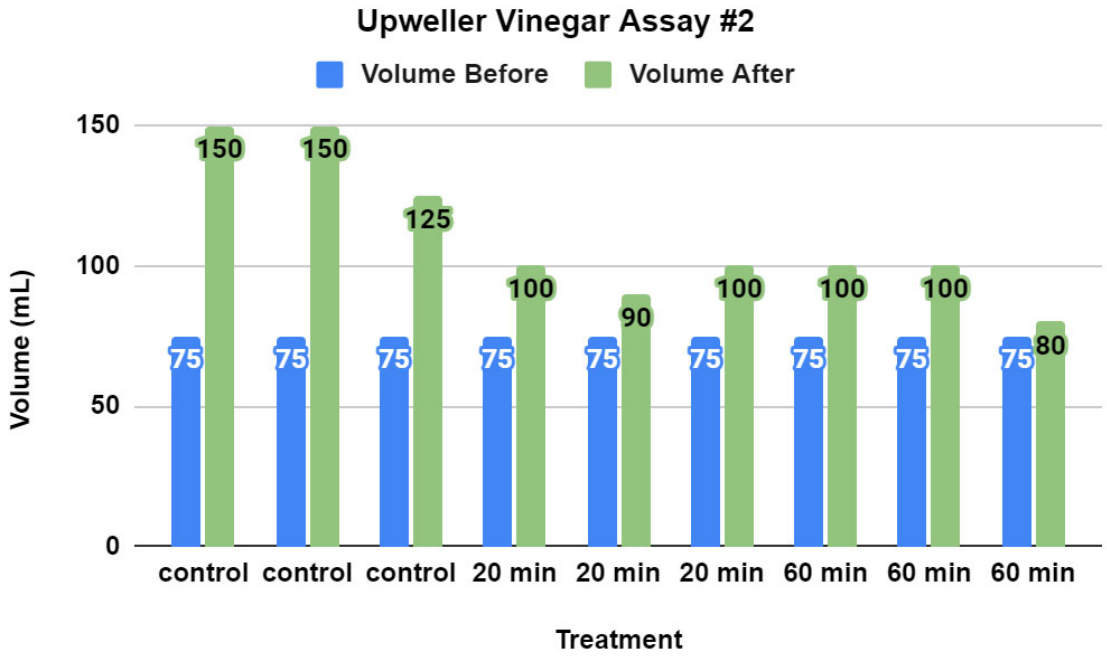


Figure 14. Volume change for the second upweller assay examining the effect of undiluted vinegar and greater contact time on seed health.



Appendix B.

Stalked ciliate survey data

Table 1. Table of stalked ciliate data recorded from the survey.

Date	Do you experience stalked ciliate infestations?	What time of year do you experience infestations?	Average salinity?	Do you have bottle systems, upweller systems, or both? If you have a DIFFERENT system please list the name.	In which system are stalked ciliate infestations most prevalent?
1/25/24	Yes	Spring	30 ppt	Downweller system, floating trays with air lifts	Other system mentioned above
1/12/24	Yes	Summer	26 ppt	Upweller	Upweller systems
1/12/24	Yes	Summer	30	Larval tanks, downwellers	Other system mentioned above
12/21/23	Yes	Spring - Summer	13-18%	upweller tanks, floating upweller system	Upweller systems - Other system mentioned above
12/12/23	Yes	Summer	28	upweller	Upweller systems
12/11/23	Yes	Summer	normally 15-20 ppt	both, ciliates first appeared in hatching tanks	Other system mentioned above
12/5/23	Yes	Spring - Winter	31	Upwellers, downwellers	Other system mentioned above
12/5/23	Yes	Summer	27	upwellers and downwellers	Other system mentioned above
12/5/23	Yes	Spring - Summer - Fall	17	both- and a hatchery- stalked ciliate noted in all systems	Bottle systems - Upweller systems
12/5/23	Yes	Summer	30	downweller	Other system mentioned above
12/5/23	No	None of the above	15	Upwellers	No infestations observed
12/5/23	Yes	Summer	13	upweller	Other system mentioned above
11/11/23	Yes	Spring - Summer			
11/11/23	Yes	Summer			
11/11/23	Yes	Summer			

Date	Do you experience stalked ciliate infestations?	What time of year do you experience infestations?	Average salinity?	Do you have bottle systems, upweller systems, or both? If you have a DIFFERENT system please list the name.	In which system are stalked ciliate infestations most prevalent?
11/11/23	Yes	Summer			
11/11/23	Yes	Spring - Summer			
11/11/23	Yes	Summer - Fall			
11/11/23	Yes	Spring - Summer			
11/11/23	Yes	Spring - Summer - Fall			
11/11/23	No	None of the above			

Table 2. Results for mitigation strategies from the participants of the stalked ciliate survey.

What mitigation strategies have reduced or eliminated infestations?
rinse trays and post set clams with fresh water every day. Keep trays clean.
Freshwater rinsing, Air dry
N/a
Excessive saltwater cleaning of seed. Have tried salt dips without success.
Not super sure but more cleaning seems to help.
We currently don't have any mitigation strategies. We are currently trying to figure out a method. They infested our pre-set larvae early in development killing broods before set.
Mostly we find vorticella on early post set juveniles, daily seawater rinsing and light rubbing of the animals keeps them under control. Very occasionally we will gently freshwater rinse. Have not had a problem with them in upwellers yet
grading/sieving. sometimes we rinse with freshwater but not sure if that actually helps.
Best mitigation for us has been to either grade the ciliates off using a series of sieves- success depends on size obviously and keeping systems clean from excessive fouling.
We were holding post-set razor clams in a downweller and had recurring ciliate infestations through the two summers that we attempted to hatchery culture the razors. Because we couldn't expose the small seed to any noxious treatments (i.e. freshwater), we relied on frequent sieving and flushing to control the ciliates. However, this did not prove to be very effective. This was quite a while ago that we ran these experiments - probably 20+ years ago.
None
We mostly see stalked ciliates in the larval stage inside the hatchery. Therefore, I have used a mixture of isopropyl alcohol and deionized water to kill the stalked ciliates in larval cultures.
Bottles - grade every other day with a good rinse. Once they hit 500 microns rinse every day with no grading.
Increased density in our bins to smother it out.
Fresh water? Mixed success. Brine dip for larger seed. Be sure to try on small batches first!
Smothering them
Drying, potential brine dipping
Wash constantly
Hard freshwater spray.
N/A
None tried.