Integrated Aquaculture

University of New Hampshire

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I. Abstract

The development of efficient aquaculture systems is a topic of growing importance as extant wild fisheries become depleted. This project sought to create a hatchery system for use by an aquaculture enterprise that was culturing both green sea urchins (*Strongylocentrotus droebachiensis*) and European oysters oyster (*Ostrea edulis*), while also investigating the applicability of the sea squirt (*Ciona intestinalis*) as a bio-component (but not commercial product) of the hatchery system. The project investigated the most effective feeding strategies, and fabricate a prototype hatchery that was inexpensive and easily fabricated.

II. Introduction

The National Oceanic and Atmospheric Administration (NOAA) defines aquaculture as the “breeding, rearing, and harvesting of plants and animals in all types of water environments including ponds, rivers, lakes, and the ocean.” Recently, overfishing of wild stocks has increased the demand for an expansion of aquaculture capabilities, in turn driving an effort to create more efficient growth and harvesting systems. In the local Gulf of Maine region, aquaculture has expanded in scope from traditional fish and shellfish operations to include the culture of echinoderms, valued for their roe on the international market.

Many traditional aquaculture models involve the raising of a single species. This practice has the potential to be highly profitable, but comes with a set limitations which make monocultures undesirable for widespread adoption. When working with a single species, disease is often a significant problem, since all of the cultured individuals would be susceptible and can lead to significant die offs affecting one or many regional aquaculture installations. Along with this, pollution and nutrient loads are of particular concern when dealing with single-species aquaculture. To combat these problems, it is often useful to integrate multiple species into a system to diversify the culture. This allows for a more robust financial model, so that fiscal sustainability does not rely on the success or failure of a single crop. Also, with careful selection of species and how they are associated, nutrient inputs and outputs can be balanced in order to decrease the overall impact of the system on water quality.

Often, aquaculture enterprises will spawn their own stock using an initial selection of organisms. Each of these organisms typically requires its own unique hatchery system that is carefully designed to support only its intended species. If more than one species is desired for cultivation, multiple hatcheries must be built and maintained to support the different species. Furthermore, variations in spawn times during the year frequently means that only one spawn system would ever be in use at one time.

Our project sought to address this issue by designing, building, and testing a hatchery system that could spawn more than one aquaculture species, eliminating the need for multiple hatchery systems. The project also aimed to develop a system where multiple species could be cultured alongside each other, for periods when spawn cycles overlapped. This goal would require performing feeding trials to determine what feeds were optimal for the intended species, balancing the need for rapid growth against concerns for complexity, cost, and varying needs between species. Ideally, the system would be inexpensive, easily constructed and maintained, and easily expandable. The hatchery must accommodate the various needs of the cultured organisms at the initial stages of their development, ensuring the highest possible survival rates during a critical period of growth.

For the initial system, it was decided that the prototype hatchery be designed to spawn both the green sea urchin (*Strongylocentrotus droebachiensis*) and the European oyster (*Ostrea edulis*). These were selected both because of their availability and their potential economic value in a fully-realized aquaculture system. In addition, the team sought to integrate a third species, the sea squirt (*Ciona intestinalis*) as a biological component of the system. Typically

regarded as an invasive species, *Ciona* also has the potential to act as a hardy bio-filter in hatchery systems. As a tunicate, it is also a relatively rich source of protein that, when pelletized, might be able to be used as a viable artificial food source for other cultured species.

This report seeks to outline the overarching design choices of our system, including a background on the species used, and a description of how those biological constraints affected the design. The report then discusses the results of several feeding trials performed on the cultures species, and the early results of the prototype hatchery. The report concludes with a retrospective on the relative success of the various choices, and makes recommendations for future work.

III. Species Overview

Species 1: The Green Sea Urchin (*Strongylocentrotus droebachiensis*)

The green sea urchin can be found in the northern reaches of the world's oceans, ranging from the Atlantic to the Pacific. *S. droebachiensis* thrives in cold waters, inhabiting mostly polar waters, with extreme southern reaches of its range extending to Puget Sound in the Pacific to the coasts of the British Isles and New England in the Atlantic. Preferring rocky substrata, adult *S. droebachiensis* is found in many habitats ranging from the lower intertidal down to depths of 1,000 meters where it prefers to feed on macroalgae and other detritus. Prized for their roe, there is a well-established fishery and market for urchins, representing millions of pounds of harvest and millions of dollars in New England alone (Lawrence, 2007).

Green sea urchins reproduce by broadcast spawning, releasing their gametes directly into the water column where fertilization takes place. This process usually takes place in early to mid-Spring, between February and April. Once fertilized, the eggs develop into pelagic larvae that live suspended in the water column for a variable period of five months to two weeks, depending on food availability and temperature. During this period they survive feeding on phytoplankton and particulate matter. After this period, the motile larvae attach to suitable hard benthic substrates where they continue to grow into adulthood (Lawrence, 2007).

This larval stage is of particular importance in the aquaculture of urchins because it may have influence the overall size and health of the adult individuals, and thus their market values. A series of studies suggest that the diets of juvenile urchins can affect both the volume of roe

production in adults as well as overall growth rates and body sizes of adults (Harris, 2006). Another study found that the water temperature in which larval urchins were raised affected adult body size, finding that optimal growth occurred in temperatures around 15˚C (Harris, 2006). These findings demonstrate that proper care of juvenile urchins is critical to increasing the sale value of the adult product.

Species 2: The European oyster (*Ostrea edulis*)

The European, or Belon oyster is native to Europe, occurring along the western Atlantic coast from Morocco to Norway, throughout the British Isles, and parts of the Mediterranean. In the 20th century, it was introduced to North America, where it can now be found on the east coast from Rhode Island to Maine (Jaziri, 1990).

As a bivalve mollusk, *O. edulis* is a filter feeding invertebrate which possesses a shell of calcium carbonate. As adults, they are sessile, living attached to hard substrates of shallow coastal waters up 20 meters and filtering out particulate matter, phytoplankton and zooplankton. They prefer lower salinities near the mouths of estuaries, tolerating levels up to 23 parts per thousand (ppt) as adults (Barnabe 1994).

European oysters are protandric hermaphrodites, changing sexes up to two times during the spawning season, which generally occurs in summer. Males release sperm into the surrounding seawater, where they are taken up into the females. Females store eggs internally and after fertilization, they are incubated for a period of eight to ten days, after which they are released. Adult oysters can produce up to one million eggs during a single spawning season.

Once released, the oyster larvae, or spat, spend another eight to ten days suspended in the water column, dispersing and feeding on smaller planktonic organisms before swimming down and attaching to suitable substrate. Once attached, they reach sexual maturity after two to three years (Barnabe 1994).

Species 3: The Vase Tunicate (*Ciona intestinalis*)

Ciona intestinalis is an invasive tunicate originating from Northern Europe. It has been introduced to several continents including Africa, South America, Australia, and North America (Pagad, 2007). This organism has a larval stage and an adult sessile stage. It was most likely introduced through the larval stage where it was transported in vessels for aquaculture purposes, like shellfish. *C. intestinalis* can be found in shallow waters or up to 1000 feet deep in coastal waters. They place themselves on hard surfaces such as pilings, rocks, and man-made substances. *C. intestinalis* has two siphons, one inhalant siphon where they take in suspended particles and oxygen and they also have an exhalent siphon where they excrete wastes and excess water (Pleus, 2014). Their mass is made up of about 95% water weight but condensed, can have about 15% protein.

Previous studies done on the filtration rate and feeding preference of *C. intestinalis* have shown that they reduce the amount of phytoplankton in the surrounding waters. It was said that over time, these results may turn into a serious problem for a shift in the food conditions of other organisms as well as *C. intestinalis* itself (Riisgard, 1996). A separate study discussed the food quality of *C. intestinalis* for specific dynamic actions. Results of this study showed that different food qualities had an effect on the respiratory rates of *C. intestinalis.* The ingestion of

one species of plankton resulted in a 20% specific dynamic action coefficient (DAC) while the ingestion of another species resulted in an insignificant increase in respiratory rates (Sigsgaard, 2003).

IV. Design Criteria

While designing the hatchery, the team sought to follow several guiding principles that would define what the expectations of the hatchery ought to be.

Principle 1: The system should be simple and affordable

Ideally, the system should be easily purchased and fabricated, so that new aquaculture enterprises can begin without requiring a large investment of capital. All parts should preferably be available in a local hardware store, keeping construction and maintenance uncomplicated and straightforward.

Principle 2: The system should accommodate multiple species

Current hatchery systems are generally designed only for the spawning of a single species, which only occurs during part of the year. By creating a hatchery that can support multiple organisms that spawn at different times of the year, the system may be used by an aquaculture effort to diversify their cultured species, without extra infrastructure investment.

Principle 3: The system must maintain a constant, uniform flow

Uniform flow allows the cultured larvae to filter greater volumes of water, leading to faster growth. In addition, uniform flow prevents eddies and areas of low flow. This avoids having the developing organisms settle on the bottom, which would lead to their death. The hatchery must also take measures to avoid any areas of strong current within the tank, particularly near any meshes. Strong flow risks trapping the larvae against the mesh.

V. *Ciona intestinalis* Filtration

Overview:

Ciona intestinalis is an invasive tunicate that has been introduced to several continents, including the Gulf of Maine in the United States. *C. intestinalis* most likely originated from Northern Europe but was introduced to the United States in the 1940s. *C. intestinalis* can be found on pilings and other hard surfaces along the coast of the Gulf of Maine filtering out the suspended particles in the water. Studies have shown a direct correlation with *Ciona intestinalis* living and inhabiting man-made structures and aquaculture landings. The objective of this project was to test whether *C. intestinalis* would filter out phytoplankton or *Artemia* at a faster rate which would also indicate a feeding preference. It was hypothesized that phytoplankton would be preferred. This experiment was held for just over a month with samples taken daily. Results showed that indeed, phytoplankton was preferred over *Artemia* by a 93.78% ±0.0121 to an 86.28% ± 0.883% per every twenty-four hours. Size of the feeding source may have played a role in the preference of *C. intestinalis*.

Introduction:

Invasive species are species which were not previously inhabited in an area but have since been introduced into a new area where they did not previously live. Many consider an invasive species as a negative impact on a system but some invasive species do have no impact and can increase species diversity and overall either help or have no impact on the ecosystem (Simberloff, 2013). It is easy to introduce a new species to an ecosystem with the increased

transportation via ship. Many species can be introduced from the ballast water in ships. This is similar to how the tunicate, *Ciona intestinalis* was introduced (Pagad, 2007).

Ciona intestinalis is an invasive tunicate originating from Northern Europe. It has been introduced to several continents including Africa, South America, Australia, and North America. This organism has a larval stage and an adult sessile stage. It was most likely introduced through the larval stage where it was transported in vessels for aquaculture purposes, like shellfish (Pagad, 2007). *C. intestinalis* can be found in shallow waters or up to 1000 feet deep in coastal waters. They place themselves on hard surfaces such as pilings, rocks, and man-made substances. Previous studies have shown that *Ciona intestinalis* prefer to inhabit areas with artificial surfaces and aquaculture landings, such as oyster farms (Dumont *et al.*, 2011). *C. intestinalis* has two siphons, one inhalant siphon where they take in suspended particles and oxygen and they also have an exhalent siphon where they excrete wastes and excess water (Pleus, 2014). Their mass is made up of about 95% water weight but condensed, can have about 15% protein. *Ciona intestinalis* is a filter feeder that is considered a mucus feeder. An endostyle within the brachial sac of *C. intestinalis* consistently secretes a mucus which gets transported through the digestive pathway of the organism. Any suspended particles that *Ciona intestinalis* ingests will be trapped in this secretion and will remain in this mucus until excretion (Carver, 2006).

Previous studies done on the filtration rate and feeding preference of *C. intestinalis* have shown that they reduce the amount of phytoplankton in the surrounding waters. It was said that over time, these results may turn into a serious problem for a shift in the food

conditions of other organisms as well as *C. intestinalis* itself (Riisgard, 1996). A separate study discussed the food quality of *C. intestinalis* for specific dynamic actions. Results of this study showed that different food qualities had an effect on the respiratory rates of *C. intestinalis.* The ingestion of one species of plankton resulted in a 20% specific dynamic action coefficient (DAC) while the ingestion of another species resulted in an insignificant increase in respiratory rates (Sigsgaard, 2003).

This experiment was conducted to test the filtration rate of *Ciona intestinalis* with two feeding sources; *Artemia* and phytoplankton concentrations. The goal of this experiment was to test how efficient of a filter feeder *C. intestinalis* is. Results could later be compared to the efficiency of oysters and other filter feeders in the Gulf of Maine. It was be hypothesized that *Ciona intestinalis* would filter out more phytoplankton than *Artemia* within a twenty-four hour time frame.

Methods:

Source of species

Ciona intestinalis was collected from the Coastal Marine Lab in New Castle, New Hampshire alongside the dock where pilings of several species were gathered. The organisms were pulled from the site into a bucket and transported to The University of New Hampshire Spaulding Life Science Building. They were kept and maintained in a recirculating tank system until the experiment began. This was a temporary home for the organisms while they relaxed from the removal of their natural habitat.

Plankton growth

Artemia was grown in a one gallon jar under a fluorescent bulb with two aerators in it. A half of a spoonful of *Artemia* eggs were placed into the jar after it was filled with filtered sea water. *Artemia* was proposed to hatch within 24 hours and at hatchling size, the protein level would be the highest. Water with the eggs were replaced every 48 hours. Kent Marine Phytoplex Phytoplankton was used with concentrated *Nannochloropsis, Tetraselmis* and *Isochrysis sp. Tahitian* ranging in size from 2 to 15 microns for the phytoplankton samples

Set-up

Fifteen Tupperware containers were gathered of equal size, shape, and color and filled with filtered sea water along with an air stone for increased flow and oxygen into the container. Containers were set up in the 10C cold room originally but then transferred to a room temperature environment. *C. intestinalis* was placed into a container with a designated number on the side of the container which would be used for future referencing. *Ciona* placed in containers 1-5 were to contain a feeding source of both concentrated phytoplankton and concentrated *Artemia*. *Ciona* placed in containers 6-10 were designated to contain concentrated *Artemia*. *Ciona* placed in the remaining 11-15 containers were designated to contain concentrated phytoplankton. Day one was given 1000 µm of *Artemia* and phytoplankton for designated containers while days 2-6 were given a reduced concentration of both feeding sources of 500 µm. After day 6, containers 1-10 were given concentrated amounts of the feeding sources of 250 µm. Concentrated amounts (in µm) were taken using a calibrated pipette.

Figure 5.1: Set-up design of the *Ciona intestinalis* filtration experiment.

Phytoplankton counts

Daily water changes were done on containers 1-5 and 11-15. 3 milliliter samples were taken from the containers before water changes were done and placed into a small tube. Tubes were labeled with the date, container number, initials, and whether it was both or a single food source fed. Tube samples were later tested in a fluorimeter for chlorophyll concentrations. Results were recorded and later entered into an excel sheet for data analysis.

Artemia counts

Daily water changes were done on containers 1-10 and remaining *Artemia* concentrations were taken using a 4 in^2 piece of cut plankton net. The mesh plankton net piece was placed on top of a funnel and the funnel was put into a bucket. A container was dumped into the funnel and remaining *Artemia* was caught into the plankton mesh. 95% alcohol was then used to pour over the plankton mesh to get the remaining *Artemia* in a small jar about half full with alcohol. Jars were labeled with date, container number, initials, and whether it was both or a single food source fed. Counts for *Artemia* were later taken using a dissecting microscope. Jars were stirred for an even distribution of *Artemia* and then one milliliter was removed using a pipette and placed into a counting dish with a 1.0 centimeter grid across the bottom. Counts of *Artemia* and remaining egg counts were done and recorded. The 1 milliliter count was done five times each per jar. Data was later entered into an excel sheet for further analysis.

Results:

C. intestinalis in the fifteen containers were of varied sizes but all ranged in the same size range. Figures 1-4 display the percent change of weight (grams), weight (mL displaced), length (millimeters), and width (millimeters) for each tunicate according to their designated number. Bars in orange represent the new organisms for numbers 4 and 5 due to mortality. A high percentage of the organisms decreased in all aspects of measurement.

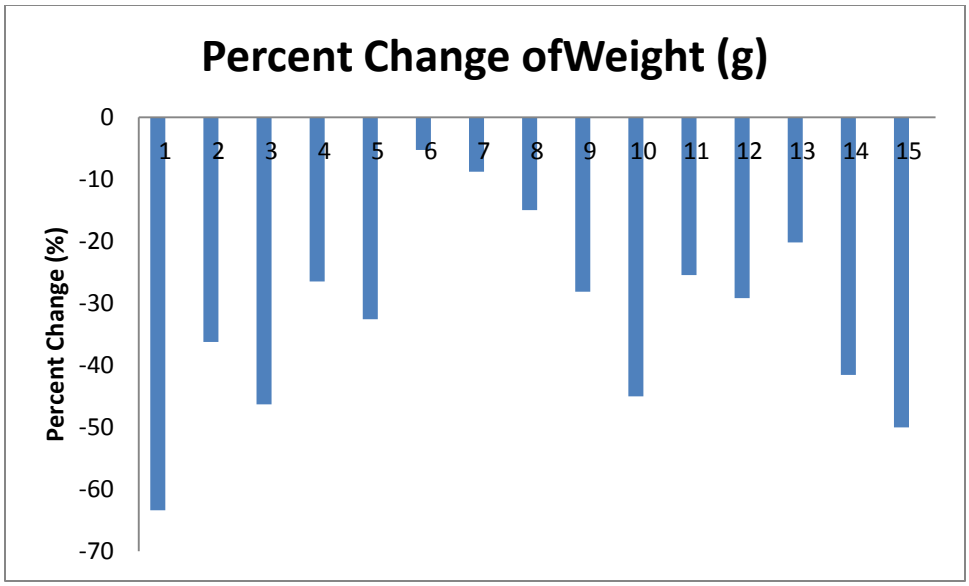


Figure 5.2: Percent change of weight (grams) for each organism, including replaced organisms after mortality (new organisms indicated in red). Organisms were classified by container number (1-5 being combined *Artemia* and phytoplankton, 6-10 being *Artemia*, and 11-15 being phytoplankton).

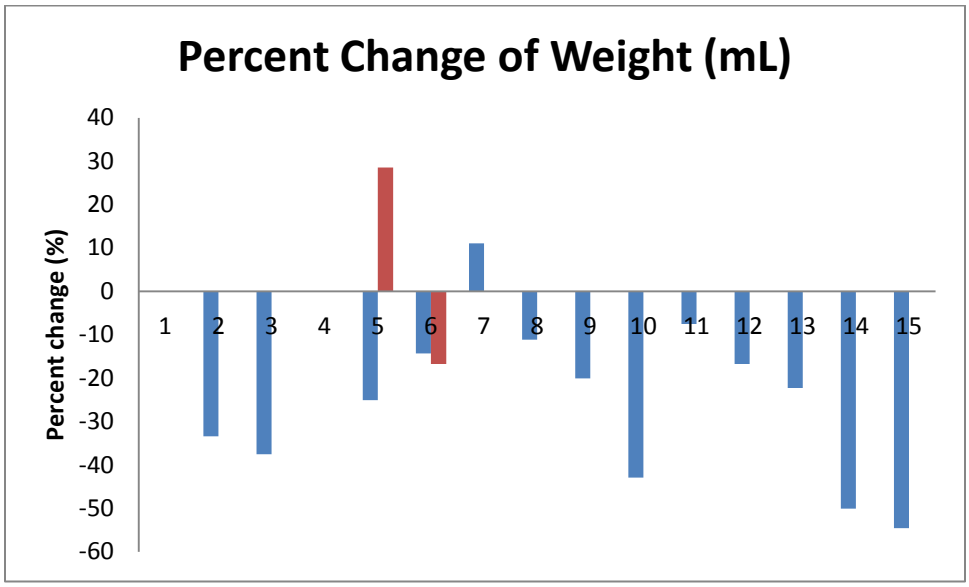


Figure 5.3: Percent change of weight (Milliliters displaced) for each organism, including replaced organisms after mortality (new organisms indicated in red). Organisms were classified by container number (1-5 being combined Artemia and phytoplankton, 6-10 being Artemia, and 11-15 being phytoplankton).

The weight was taken by grams and milliliters displaced. Overall, the weights, in grams, decreased from between 65% and 5%. In milliliters displaced, the weights decreased from an

average of 50% to 5%. Two organisms increased in weight at a percent change of 30% and 10%.

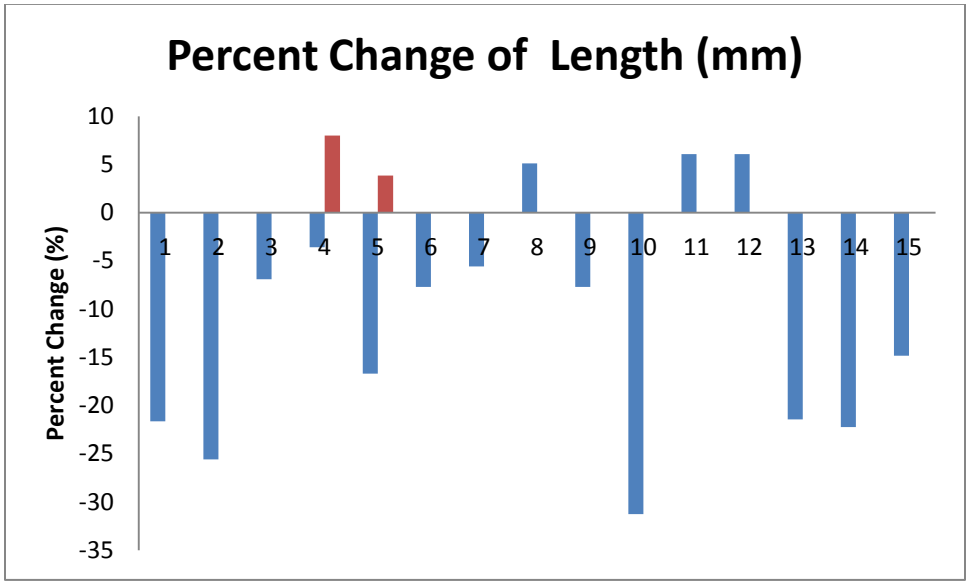


Figure5.4:: Percent change of length (millimeters) for each organism, including replaced organisms after mortality (new organisms indicated in red). Organisms were classified by container number (1-5 being combined *Artemia* and phytoplankton, 6-10 being *Artemia*, and 11-15 being phytoplankton).

Length of each organism varied greatly. The majority of the organisms decreased in length while five organisms increased in overall length. Organisms 10 decreased by 32%, the largest decrease. The largest increase in length in millimeters was 7% for organism 4 (the replacement).

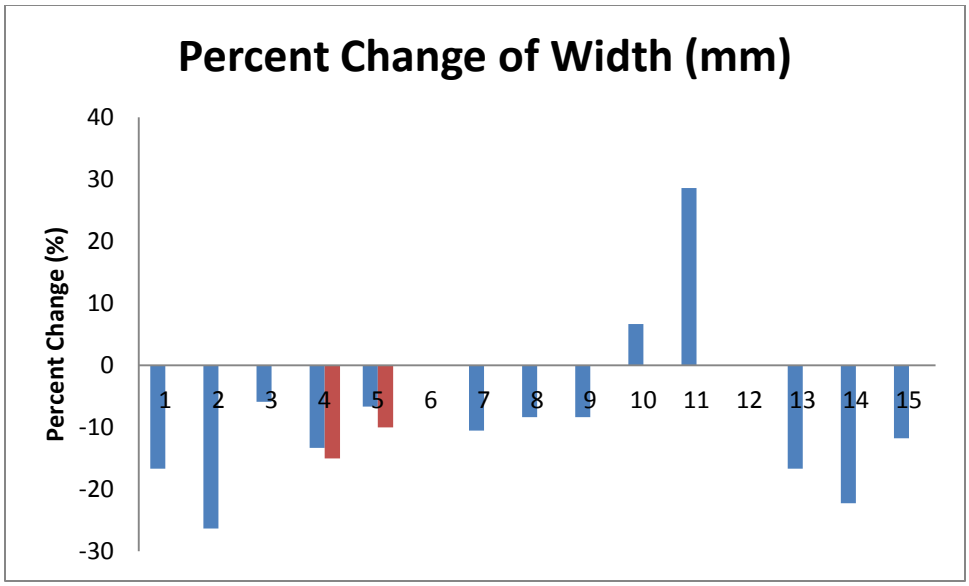


Figure5.5: Figure 1: Percent change of width (millimeters) for each organism, including replaced organisms after mortality (new organisms indicated in red). Organisms were classified by container number (1-5 being combined *Artemia* and phytoplankton, 6-10 being *Artemia*, and 11-15 being phytoplankton).

The width in millimeters of each organism also varied greatly. The largest decrease in an organism was organisms 2 at a 26% decrease. The largest increase in width of an organism was organism 11 at 28%.

Concentrations of both phytoplankton and *Artemia* were given in varying quantities over the course of the experiment. 1000 µm was given on the first day of the experiment (figure 5) while days 2-6 were given 500 µm of both phytoplankton and *Artemia* (figure 6) and the remainder of the experiment were given 250 µm to *Artemia* designated containers (6-10) and 250 µm of both *Artemmia* and phytoplankton to the combined containers (1-5) (figure 7). Phytoplankton designated containers (11-15) were still given 500 µm.

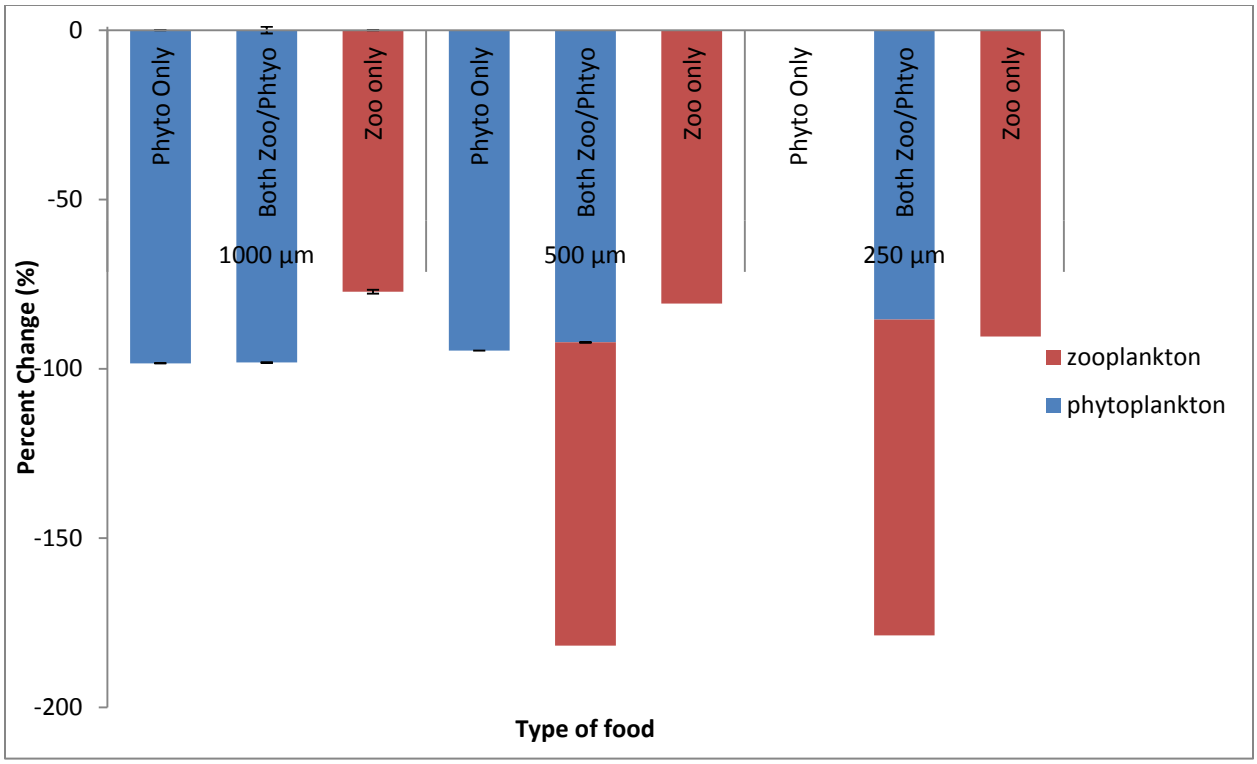


Figure 5.6: Total percent change of consumption of phytoplankton and *Artemia* at a concentration of 1000 µm. Phytoplankton was consumed at 98.39% ± 0.011% while phytoplankton while combined was consumed at 98.19% ± 0.011%, and *Artemia* was consumed at 77.25% ± 1.57%. Total percent change of consumption of phytoplankton and *Artemia* at a concentration of 500 µm. *Artemia* was consumed at 80.74% ± 0.31% while *Artemia* while combined was consumed at 89.53% ± 0.972% , phytoplankton while combined was consumed at 92.22% ± -7E-04 %, and phytoplankton while combined was consumed at 94.66% ± -7E-04%. Figure 7: Total percent change of consumption of phytoplankton and *Artemia* at a concentration of 250 µm. *Artemia* was consumed at 90.53% ± 0.58 % while *Artemia* while combined was consumed at 93.34% ± 1.012%, and phytoplankton while combined was consumed at 85.45% ± 0.04%.

Phytoplankton in a concentration of 1000 µm had a 98.39% consumption within 24 hours. Phytoplankton while combined with *Artemia* had a consumption of 98.19% within 24 hours. *Artemia* had a consumption of 77.25% within 24 hours . Data from *Artemia* while combined with phytoplankton was not taken. Phytoplankton in a concentration of 500 µm had a 94.66% consumption within 24 hours. Phytoplankton while combined with *Artemia* had a consumption of 92.22%. within 24 hours *Artemia* while combined with phytoplankton was consumed by 89.53% within 24 hours. *Artemia* had a consumption of 80.74% within 24 hours. Phytoplankton while combined with *Artemia* had a consumption of 85.45% within 24 hours.

Artemia in a concentration of 250 µm had 93.34% consumption within 24 hours. *Artemia* had a consumption of 90.53% within 24 hours. Data from phytoplankton while combined with phytoplankton was not taken.

Table 5.1: Total percent change of all phytoplankton samples and all *Artemia* samples.

	Artemia	Phytoplankton
Total Percent Change	93.7825	-86.2776

The overall consumption of all phytoplankton, including while combined with *Artemia* had 93.78% consumption rate in a 24 hour period. *Artemia* had an overall consumption rate of 86.27% within a 24 hour period, including *Artemia* combined with phytoplankton.

Discussion:

The results from the 1000 µm concentration showed that phytoplankton was filtered out by 98.39%, phytoplankton while combined with *Artemia* was filtered out by 98.19%, and *Artemia* was filtered out by 77.25%. the concentration was decreased by half due to a significant amount of food still in the containers, or so it looked but this could have been feces or the organisms may have rejected the plankton after they attempted to eat it. Once the food sources were decreased to 500 µm, phytoplankton was filtered out by 94.66%, phytoplankton while combined with *Artemia* was filtered out by 92.22%, *Artemia* while combined with phytoplankton was filtered out by 89.83%, and *Artemia* was filtered out by 80.74%. Concentrations for the combined phytoplankton and *Artemia* as well as just *Artemia* were decreased by 50% again down to 250 µm. At 250 µm, phytoplankton while combined with *Artemia* were filtered out by 85.45%, *Artemia* while combined with phytoplankton were filtered

out by 93.34%, and *Artemia* solely was filtered out by 90.53%. Overall for the three treatments combined, phytoplankton, combined and solely given, was filtered out by 93.78% per 24 hours while *Artemia* combined and solely given was only filtered out by 86.28% per 24 hours.

Riisgard's study from 1996 studied the filtration rates of *Ciona intestinalis* on phytoplankton. The results of this study determined that populations of phytoplankton significantly decreased with the presence of *C. intestinalis* (Riisgard, 1996). This study relates to the results of this study, both experiments had a decrease in phytoplankton populations from the feeding of *Ciona intestinalis*. There were limited studies done on the filtration rates of *Ciona intestinalis* feeding on *Artemia*.

It was hypothesized that the phytoplankton would be filtered out faster than the *Artemia* within a 24 hour time frame. The hypothesis was supported fully. Phytoplankton was filtered out overall with a 93.78% over 86.28% of *Artemia.* Both phytoplankton and *Artemia* were not filtered out as much as expected but there was still a high filtration rate of both food sources. It was thought that *C. intestinalis* would filter out the small amount of water in the container within just a few hours. This was not the case for these organisms but it also may be due to the decreased health status of all of the organisms. As it was seen above in fifures 1-4, almost all of the organisms had a decrease in weight, length, and width over the course of the experiment. There were four mortalities throughout the experiment. Organisms 4, 5, 9, and 15 all original organisms died throughout the experiment. Organism 15 died soon after the experiment started so data was only taken from the replacement. Organism 9 died in the last days of the experiment so there was no replacement for this organism. Organisms 4 and 5

were replaced after mortality halfway through the experiment so both the original and replacements were used in the data analysis.

Stress may have been a contributor to the mortalities of these organisms but may also have contributed to the decreased weight, length, and width of each organism. Temperature may have been too warm in the room temperature degree as well as a lack of flow of water. *C. intestinalis* lives in a habitat where water flow is relatively high due to the need to feed on suspended particles. *C. intestinalis* growth may have also been affected by the continuous removal from their container for measurements and water changes. This is highly stressful for the organisms and it may have been the largest contribution to mortality and decreased growth rate.

Further experimentation would need to be conducted in a different environment to see what the true growth rates would be for *C. intestinalis*. If this experiment was to be conducted again, *C. intestinalis* would be placed in the 10C cold room, would be placed in a container where consistent removal was not mandatory, excluding weekly measurements. A constant flow of water would be used during the experiment to reduce stress from lack of oxygen and nutrients flowing across the siphons. It would be conducted in a recirculating tank with several species per tank and have an overall filtration rate from a group of organisms. There would be three separate systems, one with solely *Artemia,* one with solely phytoplankton, and one with both phytoplanktons and *Artemia*. The experiment would be conducted over a month time frame. A separate study could be conducted to test the survival and growth of *Ciona*

intestinalis at three separate temperatures, 10C, 15C, and 20C to reduce the daily measurements and stress rates of the organisms.

VI. *Ostrea edulis* Feeding Preference

Introduction:

Ostrea edulis is an invasive species that we have grown to enjoy quite a bit. They are a delicacy in some countries and are well liked as a food source in the United States. They were brought over from Europe to the United States for the purpose of farming. The demand for these oysters has increased and the need for a sustainable, cheap, and easy system to grow *O. edulis* is on the rise.

Ostrea edulis has been known to grow on firm bottoms with mud, shells, rocks, and sand. They prefer bases with shells to attach to also to make it easier for larvae to attach to. These oysters prefer to live in waters roughly 30 feet deep (ISSG, 2007). Oysters do well with other species in their environment. Several species benefit from *O. edulis* in the habitat, including the green sea urchin, *Strongylocentrotus droebachiensis*. These organisms live in similar environments together and do not interfere with one another. The green sea urchin can actual benefit from an oyster by consuming the algal substances on the shell of the oyster and can gain calcium from it as well (Chow and Kim, 2000). Urchins can also benefit the oysters by giving them a food source as well. As they feed on kelp they break down some of the material small enough for an oyster to siphon out of the water.

The purpose of this experiment was to test whether the European oysters would grow better with the help of an added food source from the green sea urchins food particulates. There would be a supplemental food source which can be bought from a local pet store of phytoplankton and zooplankton as a control. The oysters with the green sea urchin particulates would also be given this supplement. It

was hypothesized that the oysters with both food sources would benefit and grow more than the oysters with only the supplemental food source.

Methods/Materials:

Six tanks were used for the containment of the European oysters (*Ostrea edulis*) and green sea urchins (*Strongylocentrotus droebachiensis*). Three of the tanks were designated control tanks with four oysters per tank while the other three tanks were designated trial tanks also with four oysters per tank but included six urchins contained in a basket near the water surface in each tank. Each basket was constructed roughly 6" x6" with a plastic coated metal wire, safe for salt water environments. These baskets were suspended at the surface of the water, just below the water line to keep *S. droebachiensis* from escaping (Figure 1). Six urchins were placed in each basket with a single layer of sugar kelp (*Saccharina latissima*) (Figure 2). Oysters were kept in specific corners of the tank under the basket to have an organized way to tell which oyster was what. Oyster 1 was in the back left corner, oyster 2 in the back right corner, oyster 3 in the front left corner, and oyster 4 in the front right corner.

Over the course of a two month period the kelp was consistently kept present and the oysters were fed daily with a mixture of numerous substances. The combination consisted of: 1mL of Phytoplankton, 1mL of Instant Algae, 1 spoon of reef chili, and 98mL of Distilled Water. 15mL were placed into each tank after mixing the substances. Measurements were taken weekly, typically on Tuesdays or Fridays. The weight, length, and width of each oyster were measured weekly to determine if there was any growth. Black marks were put on the oyster shells to try and prevent any misunderstanding of where measurements would be taken.

Weight was taken using an electric scale and length and width were taken using a caliper. Data was entered into Microsoft Excel for further evaluation.

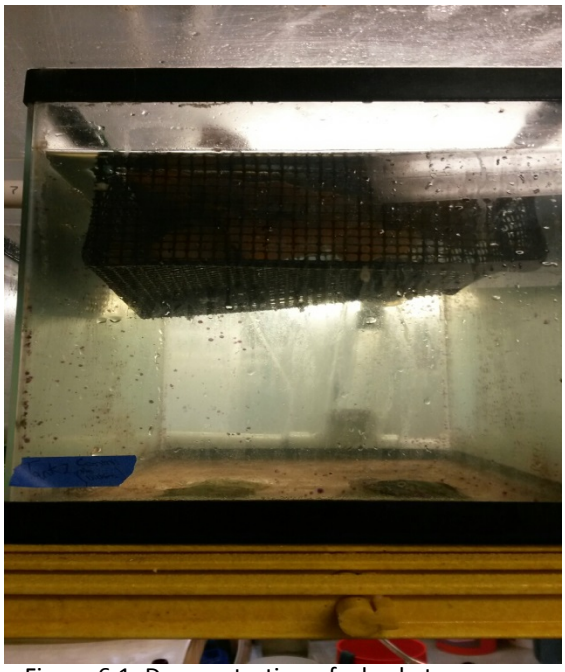


Figure 6.1: Demonstration of a basket suspended at the surface of the water above the oysters.

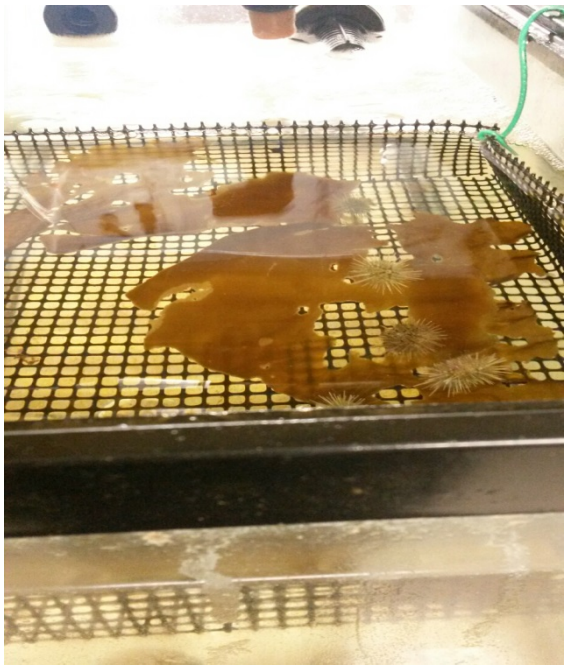


Figure 6.2: Demonstration of the setup of the urchin basket. Note that the kelp has already been consumed partially.

Results:

Over the two month period there was a slight increase in many of the oyster sizes, from all six tanks. Although there was no significant difference between the two, the oysters from the trial tanks with urchin particulates had an overall larger increase in growth. Tanks 1-3

(control) had an average increase of 1.1g from the beginning weight to the end weight while the length and the width only had an average increase of 0.1mm. The graph below shows the average weight, length, and width of each organism (Figure 3). Tanks 4-6 (trial) showed and average increase of 1.3g from beginning weight to ending weight. The length of these organisms had an average increase of about 0.3mm while the width had an average increase of 0.2mm over the two month period. The second graph shows the average weight, length, and width of each organism in tanks 4-6 (Figure 4).

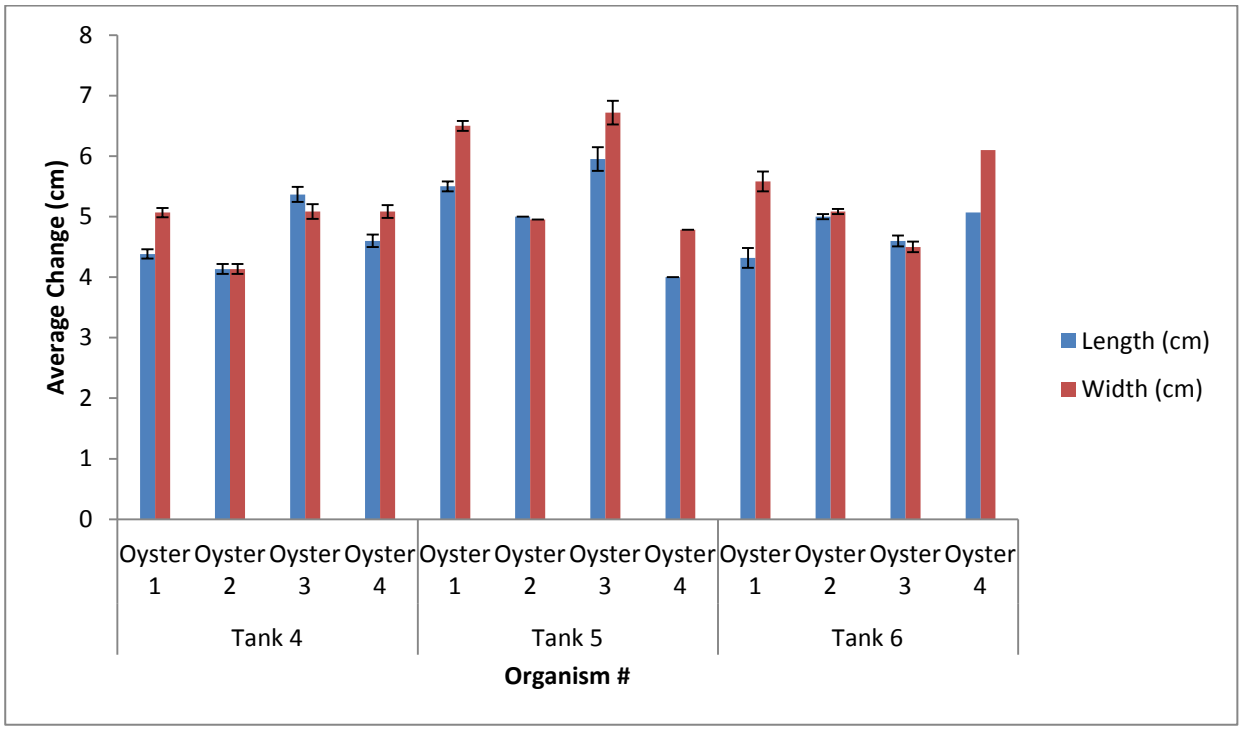


Figure 6.3: Three trial tanks where oysters were given phytoplankton and particulates as a food source.

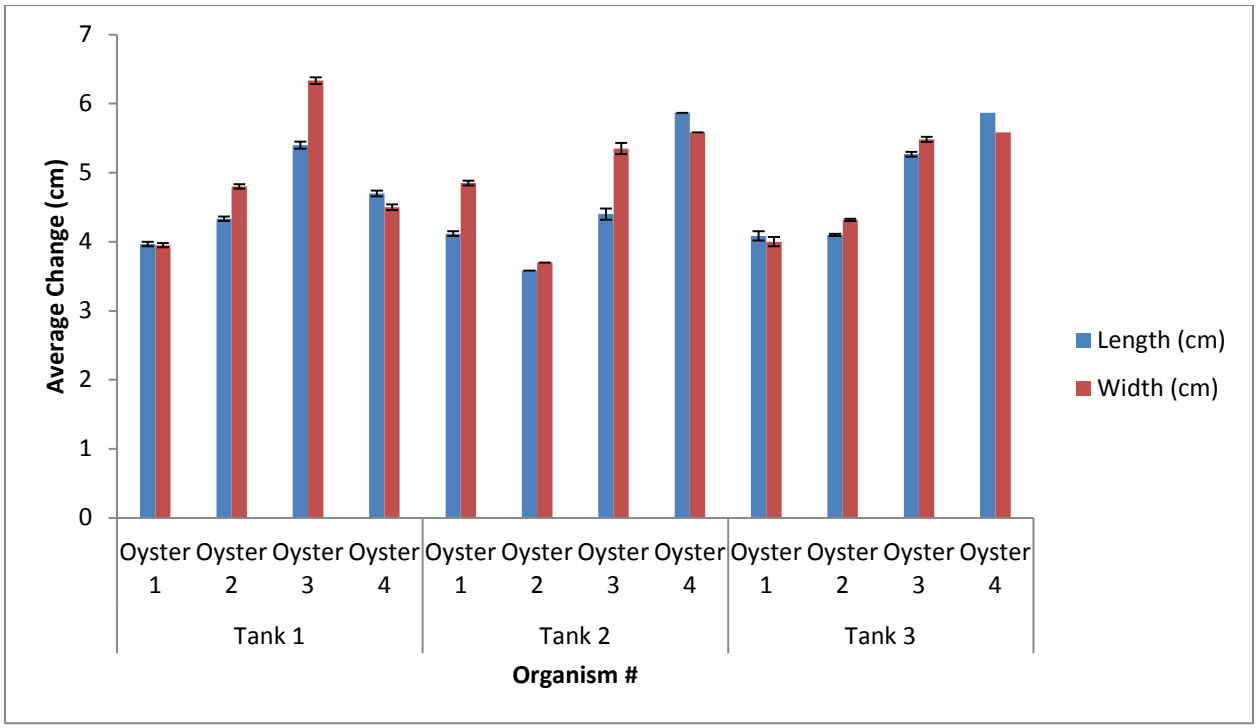


Figure 6.4: Three control tanks where oysters were given only phytoplankton as a food source.

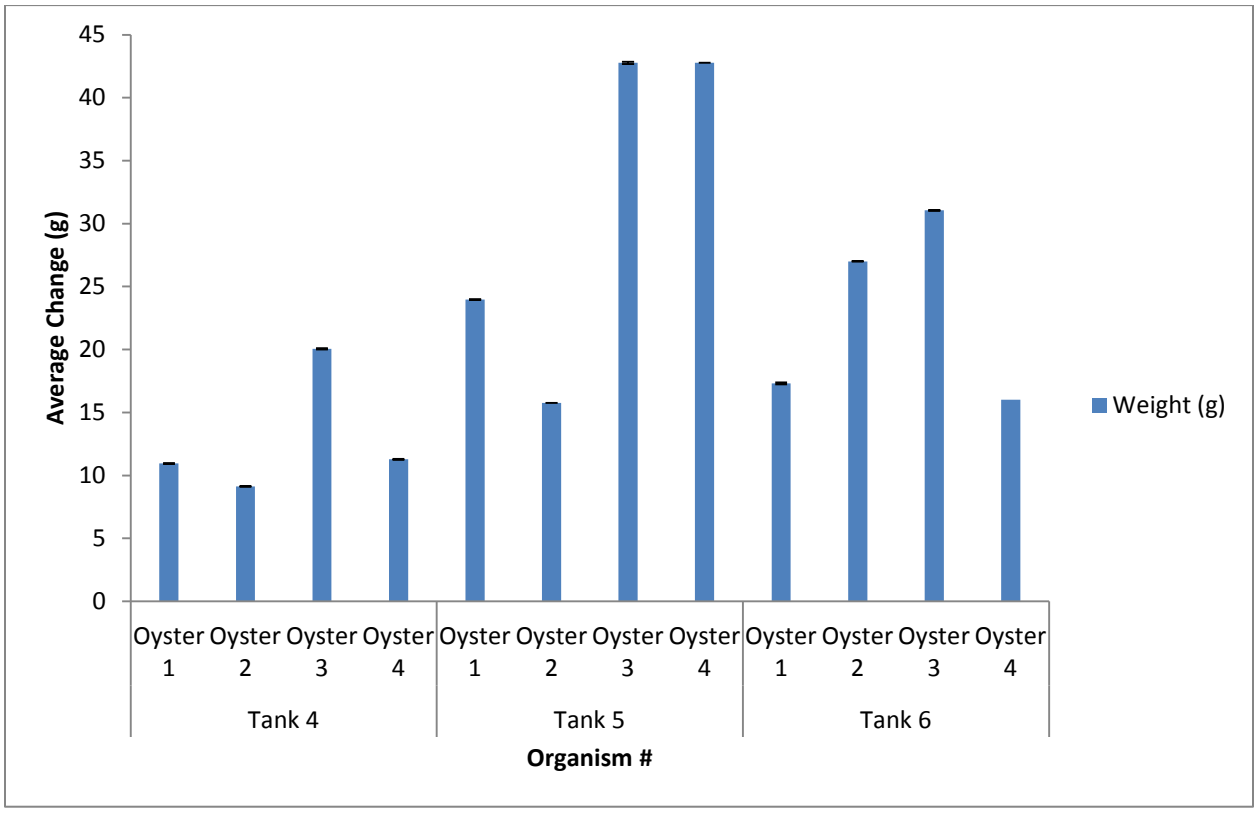


Figure 6.5: The average weight of the oysters in tanks 1-3.

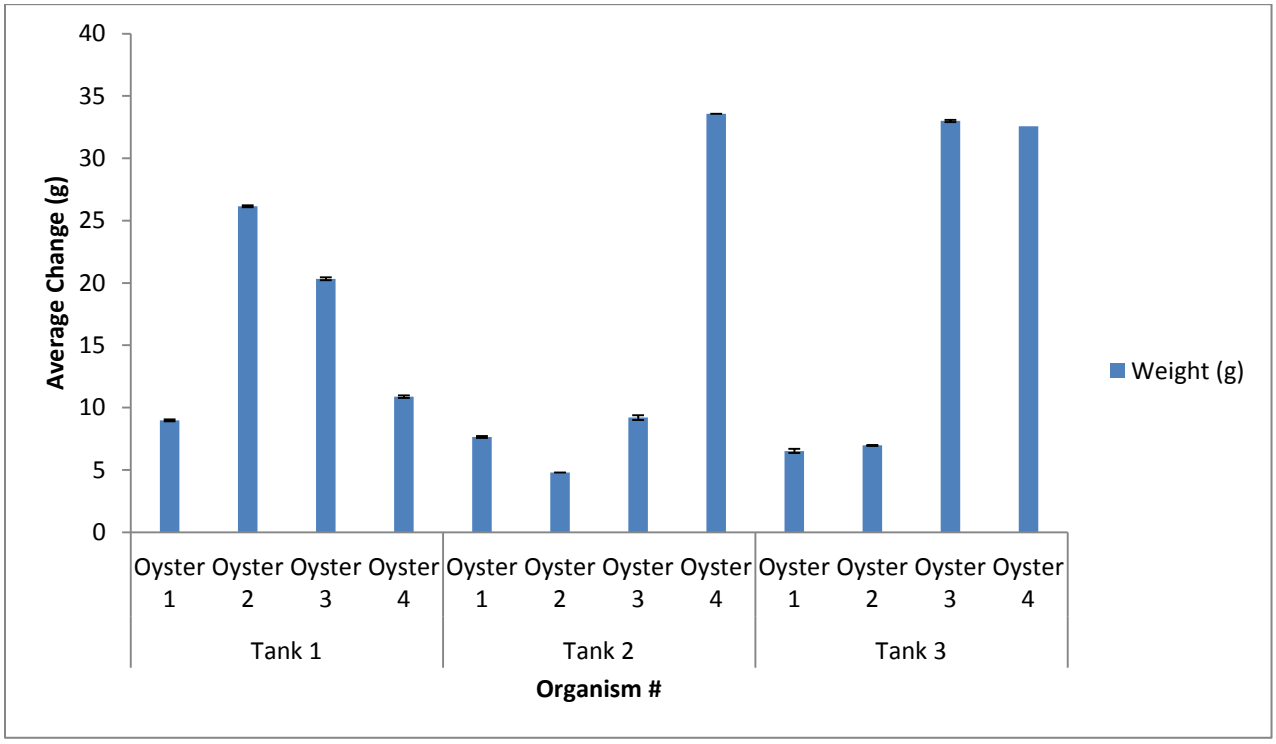


Figure 6.6: The average weight of the oysters in tanks 4-6.

Discussion:

The European oysters that were placed in the control tanks 1-3 had the available supplement mixture for food and showed an overall slight increase in size over the two month period. The increase of 1.1g as compared to the 1.3g increase from the trial tanks 4-6, where they had the same supplement plus the particulates from the green sea urchins feeding, showed that there was a benefit to having the option of oysters siphoning out the particulates as well as the supplement. A previous study published in 2009 showed that *Ostrea edulis* benefited more from having the option of feeding on natural substances, such as the algae

Tetraselmis suecica, rather than only having the option of having a supplemental food source from a local pet store. The spawning of *O. edulis* showed that more broods were produced when adult organisms were given the natural food sources (Helm, Holland, and Stephenson, 2009).

The growth of *Ostrea edulis* is rather slow. It takes roughly 2-3 years for an oyster to get to legal size. This would help explain why there was no significant difference in the data because of the short time frame. Two months is not a long time frame for a slow growing organism. Although there was a limited time frame for this experiment, it did show that there was a slight difference in the two systems. The control organisms grew but the organisms in the trial with both food sources grew a bit better. It was hypothesized that the oysters would grow better with both food sources of the supplement and the articulate matter over the oysters with just the supplement. The hypothesis was supported.

Further experimentation could include using the same basic idea to integrate it into the larval stage of each organism's life stage. Both organisms have a larval stage and supplemental food sources may not be the only way to feed them. Having natural food sources may also have benefits to the organisms similar to how it did in this experiment.

VII. Prototype Design

The schematic of a chosen hatchery design can be found in Figure 1 below. The prototype hatchery consists of a 5 gallon water bottle with its top cut off and a pipe standing vertically in its center. Two rectangular inlet slots are cut near the bottom of the pipe and four circular outlets are drilled near the top of it. The slots are covered in a mesh to keep the organisms from being pulled into the pipe. An air stone is placed inside the pipe between the inlet and outlet holes. Compressed air is pumped out of the air stone and rises to the top of the tank. The rising air creates a slow flow of water as seen in Figure 7.1.

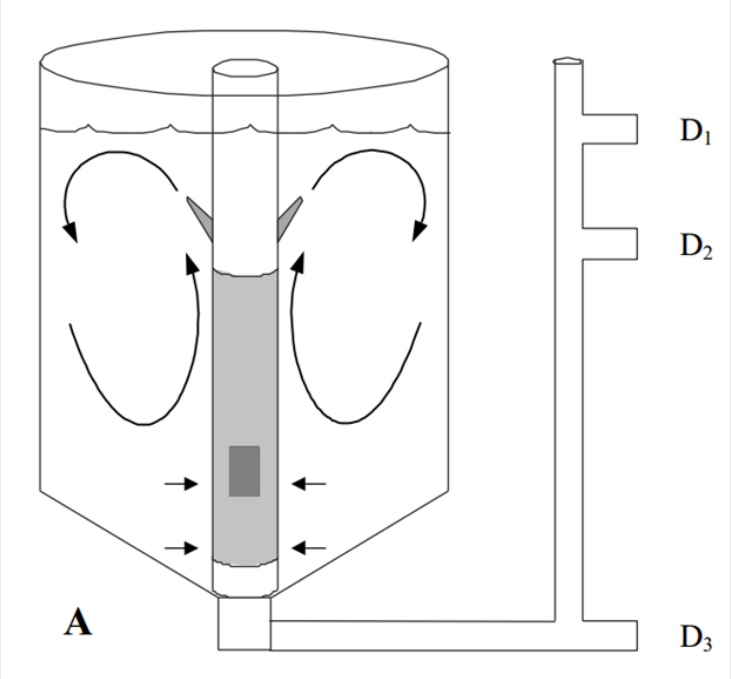


Figure 7.1- Larval tank schematic by Michael P. Russell

The flow inside the tank is designed to replicate the flow that the organisms would experience in the wild. It must not be so fast that the organisms cannot settle, but it cannot be so slow

that the organisms do not receive enough nutrients to grow properly. There should be flow throughout the tank so that the water is fully circulating and does not stay in one area for too long.

The system was designed to be easily constructed using materials that could be inexpensively purchased at a local hardware store. A set of three tanks with one set of outlet valves cost about $150 and could be built by three experienced people in about three hours. Some materials and tools used in construction, such as mesh netting, drills, and saws, were already available and were not accounted for in the cost of production. The process of purchasing supplies and constructing the tanks could be made more efficient, which would lower costs. Purchases could be made in bulk and construction could be streamlined to produce less waste. Taking these into account, the total cost is expected to about the same.

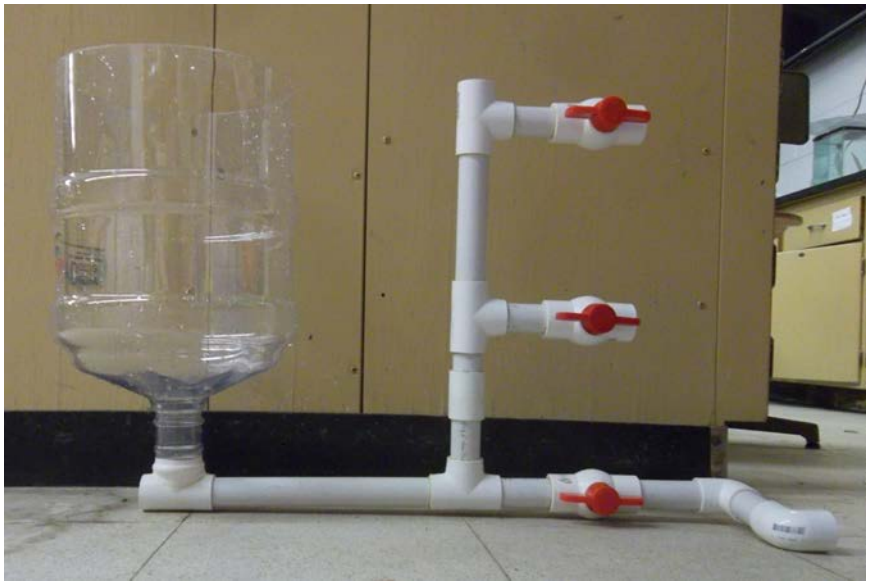


Figure 7.2 - One tank connect to outlet pipes

The outlet pipes, shown in Figure 2, were constructed using PVC pipe, tee joints, and ball valves. When the parts were confirmed to be cut to the proper lengths, they were primed

and cemented together. The top outlet valve can be used to lower the water level to the proper height if too much water is poured into the tanks. The middle valve lowers the water to a level that facilitates removing the organisms. The bottom valve completely drains the tanks.

The tops of the first set of bottles were cut off using a band saw, but the second set's were cut just as easily using a hand saw. The bottom necks of the bottles needed to be attached to PVC tee's that would connect to the rest of the tanks. The tank on the end could be attached to an elbow to end the set or it can be attached to a tee and capped to allow for more tanks to be added. Figure 3 shows three tanks set up so that more tanks could be added. Some bottles fit snuggly around the PVC tee while others were too large. The best method found for creating a watertight seal was to wrap the tee in electrical tape until it fit tightly in the bottle's neck. Additional tape can be wrapped around the connection to waterproof it further. Alternate waterproofing methods were also attempted. Silicone caulk and epoxy were used to seal gaps in the attachments, but there were problems having either material dry properly.

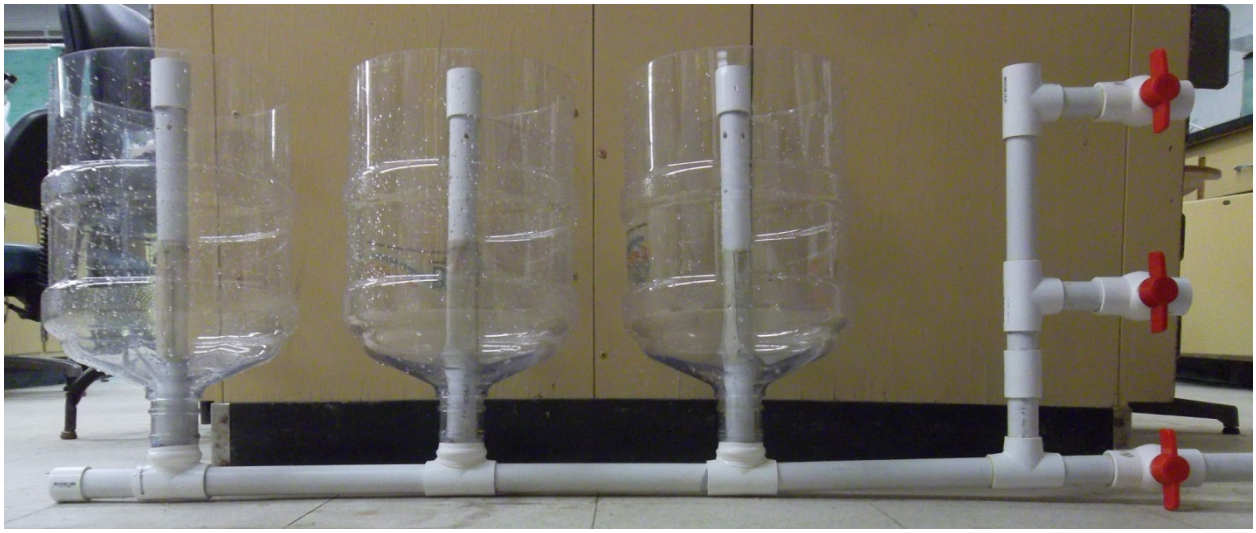


Figure 7.3 - A three tank set connected to outlet valves

VIII. Fluid Flow Analysis

Overview

The hatchery was designed to have perfectly cylindrical walls, but the first iteration was constructed using a 5-gallon water bottle with a handle that protruded into the tank. The team wanted to know if the handle would affect the flow throughout the tank in a way that would be detrimental to the growth of the organisms cultured inside. A secondary objective was to understand how the size of inlet slots in the central pipe of the hatchery affected flow.

The flow was analyzed through experimentation with the physical hatchery and using SolidWorks flow simulation. The hatchery was filled with water and allowed to reach a steady state of internal flow. Dye was released into an area of the tank so that the flow could be observed. This experiment was performed on a single-handled tank setup for multiple dye placement areas. The SolidWorks simulations were performed on models of a handled and handle-less tank with short, medium, and tall inlet slots. Velocity vector diagrams were obtained for certain planes within the hatchery during steady-state flow.

The experimentation and simulation both showed that flow velocity increased through the handle and decreased below the handle when compared to a handle-less tank. The changes in velocity are not significant to the growth of the organisms though. The simulations resulted in the decision that a taller inlet slot is more beneficial to circulation and the safety of the organisms. The handled tanks can be used for hatcheries with no ill effects on the organisms. It may be good to also test handle-less tanks as a comparison to the handled tanks.

Introduction and Background

The hatchery design called for a cylindrical tank, but the first water bottles purchased by the group had handles that protrude into the tank. The team was concerned that the handle would affect the flow in the tank with adverse effects on the growth of organisms. Figure 8.1 shows model of the handled bottle. It was thought that there may be increased flow through the handle that may suck in organisms or push them away from the area beneath it. The protrusion of the handle could also block flow on that side and create an area of no flow beneath it.

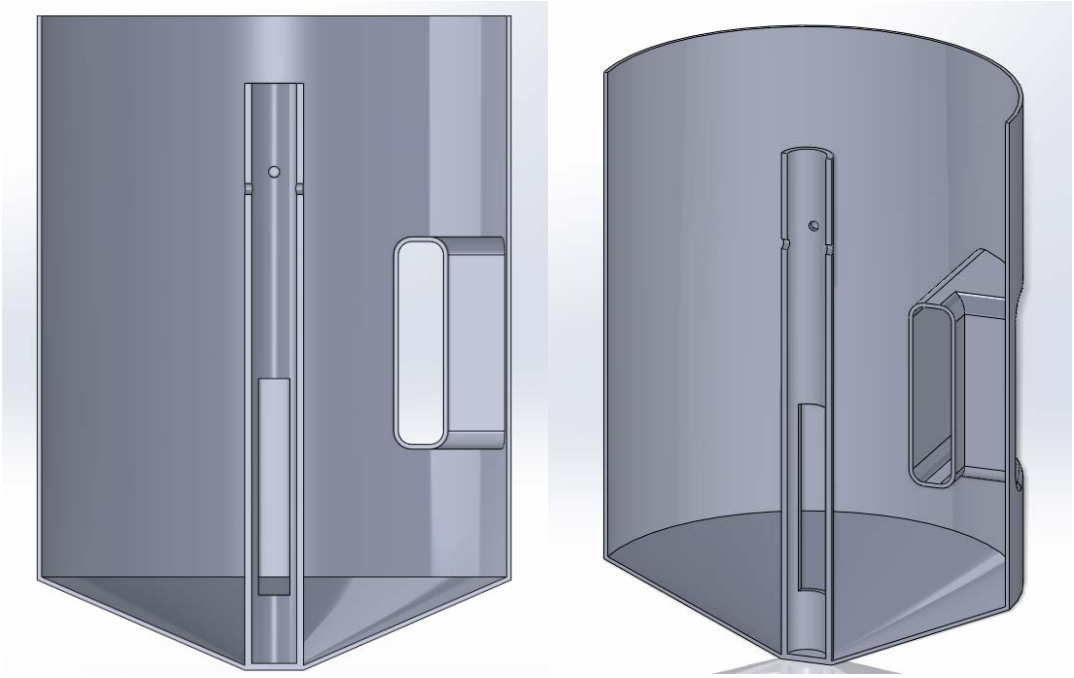


Figure 8.1 – Section view of the model of the handled bottle

The team also wanted to know how the height of the pipe inlet slots affected the flow throughout the tank. Smaller slots would cause faster flow into them compared to larger ones. The flow around the inlet should not be so fast to draw in the organisms, but it should pull in water from far enough away to maintain proper circulation.

Experimentation

Dye was used in a prototype hatchery to visualize the flow in certain areas of it. A tank was built using a handled water bottle and PVC pipe. The tank's drain was plugged with the bottle's cap and the tank was filled with water. The air stone was placed down the pipe through its top and the tank was left alone for some time to allow the flow to reach a steady state. Dye was then released into a chosen area of the tank and allowed to move around for about a minute. Each test was recorded with a video camera to be reviewed later.

Dye was placed above the handle to observe how it flowed through the handle. Several frames of this test can be seen in Figure 8.2. Some of the dye quickly moved through the handle and to the bottom of the tank. The dye took over 30 seconds to reach the area between the handle and the central pipe.

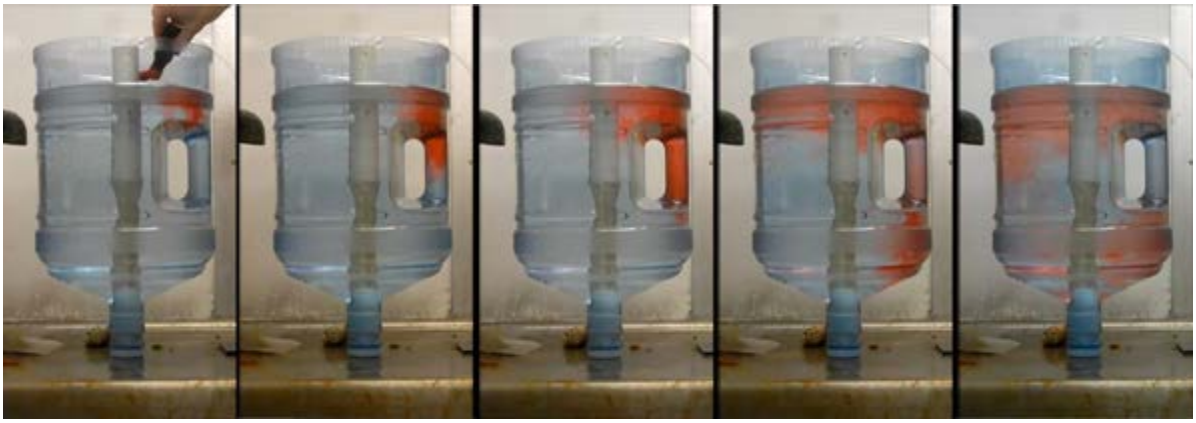


Figure 8.2 - Dye flowing through the prototype hatchery's handle over 30 seconds

In a similar test, dye was placed above the handle and on the side opposite the handle. As was observed in the first test, the dye flowed quickly through the handle in about 8 seconds. On the opposite side, which could simulate a cylindrical tank, the dye took about 24 seconds to reach the same level as the bottom of the handle.

In a third test, dye was placed into the top of the central pipe so that it would be released equally through the four outlet holes at the top of the pipe. Figure 8.3 shows several screenshots from this test. The dye flowed quickest through the handle in about 8 seconds, though it only took about 12 seconds for dye on the opposite side of the tank to reach the same level. Dye reached all areas of the tank over the course of a minute.

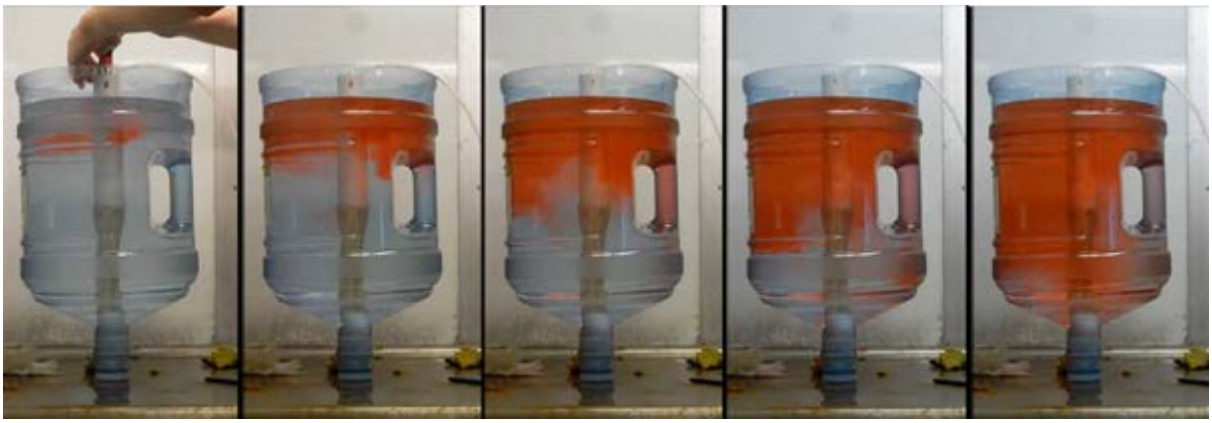


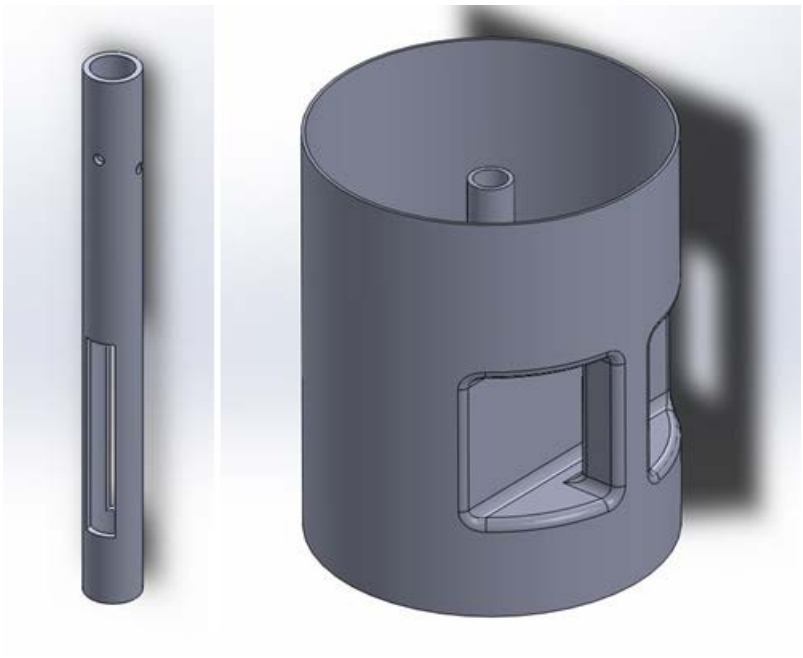
Figure 8.3 - Dye flowing through the central pipe's outlet holes

Dye was released in the top of the tank on the side facing the camera to see how the flow interacted with the protrusion of the handle. The flow resembled the flow on the left side of the tank where there was no handle. The water appeared to circulate normally in the front of the tank.

Other tests were performed where dye was released in the bottom of the tank. The results of these tests were affected by the hand that released the dye being removed from the tank. In all these tests, removing the hand altered the flow from its steady state that would be expected in normal operation of the tank. The results therefore did not reflect the true flow in the tank.

Simulation

SolidWorks models were created for the handled and non-handled water bottles. Three models of the central pipe were made to test short (2 in.), medium (4.8 in.), and tall (6 in.) inlet slots. Two models can be seen in Figure 8.4.



(a) (b)

Figure 8.4 – SolidWorks models of (a) the inner pipe with a medium inlet slot and (b) the handled tank

SolidWorks Flow Simulations were performed for a quantitative comparison of how different inlet sizes and having a handle would affect the flow in the tank. A total of 6 tank models were used by varying tanks with and without handles and by varying small, medium and large inlet sizes. Each Simulation was run with the same boundary conditions, shown in figure 6. The fluid flow inlet and outlet were set at a flow rate of 17.6 in^3/s so the entire 4.5 gallons that the tank held would circulate once per minute. Atmospheric pressure was set at the opening of the tank and gravity was taken into account.

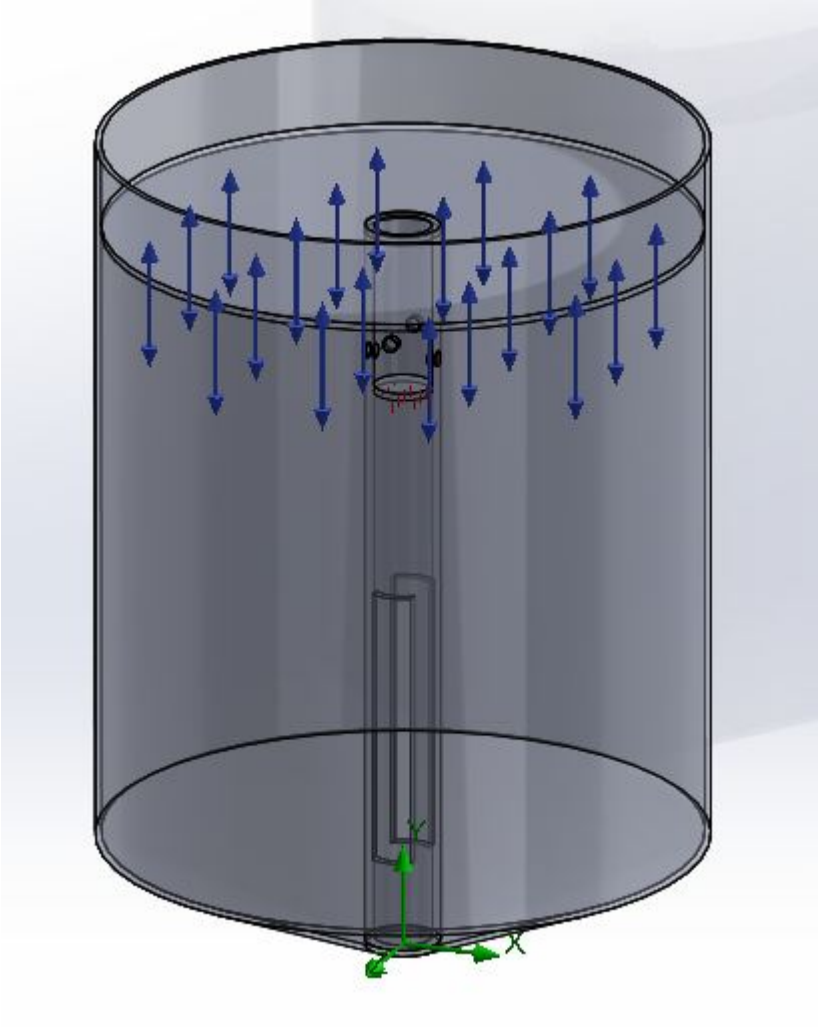


Figure 8.5 - Common boundary conditions for all of our flow simulations

Cut plots were created for each simulation with velocity vectors to show the flow distribution throughout the tank. Figures 8.6 to 8.8 show the cut plots for the model with no handle for the three different inlet sizes. In each case, the greatest velocity occurs at the outlets of the central pipe. The next greatest velocity is at the top of the inlet. The small inlet has the greatest velocity going in to the tube in order to maintain the mass flow rate. That large velocity is near the bottom of the tank where organisms may settle. This increased velocity near the bottom of the tank is undesirable because it increases the chances of an organism getting sucked against the mesh covering the inlet. The tall inlet creates a more distributed flow within the tank and keeps the increased velocity away from the bottom and sides of the tank where organisms will be growing.

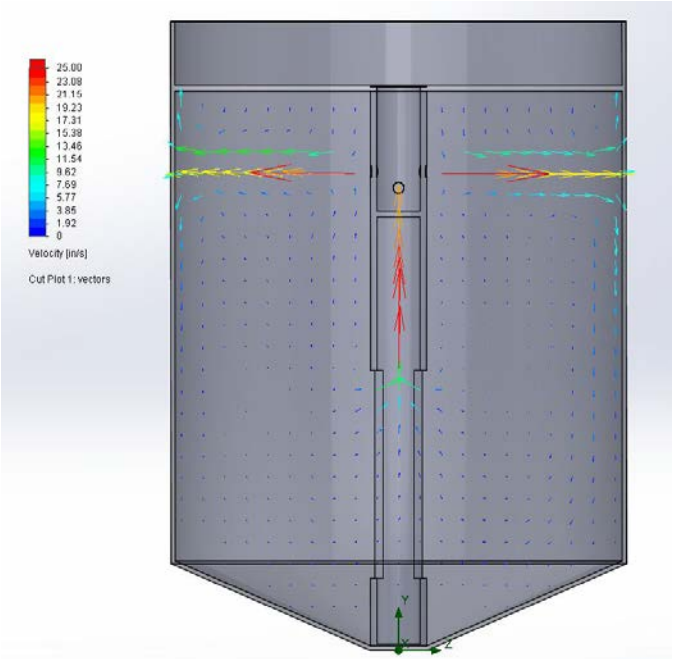


Figure 8.6 - Cut plot of velocity vectors for handle-less tank with small inlet

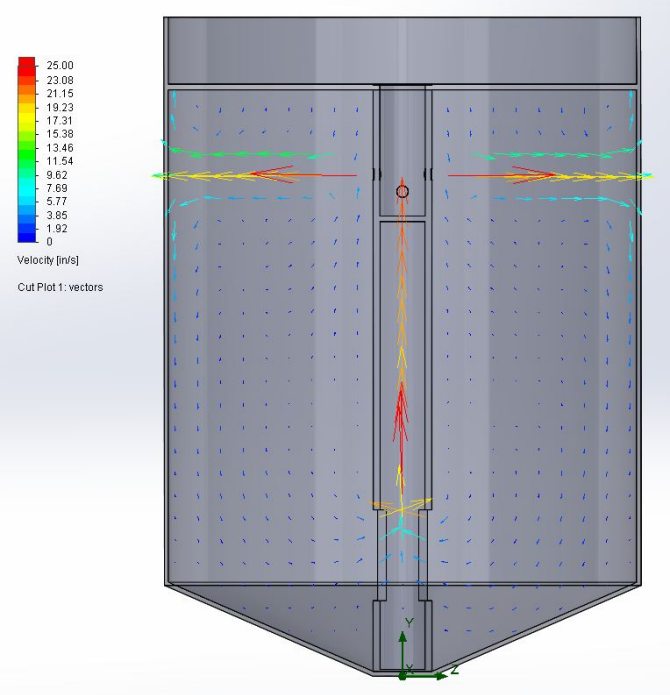


Figure 8.7 - Cut plot of velocity vectors for handle-less tank with medium inlet

The simulation results for a handled tank are similar to those from the dye experiment. Figure 8.9 shows an increase in velocity going down the handle compared to the opposite side of the tank. This increased flow is reduced towards the bottom of the handle and dissipates quickly after exiting the handle. It should not be a particular danger to the organisms. There is a small area of stagnation below and to the left of the handle. Flow seems to be more evenly distributed on the left side of the tank. This should not be a concern since these areas are away from the bottom and sides of the tank where the organisms will be growing.

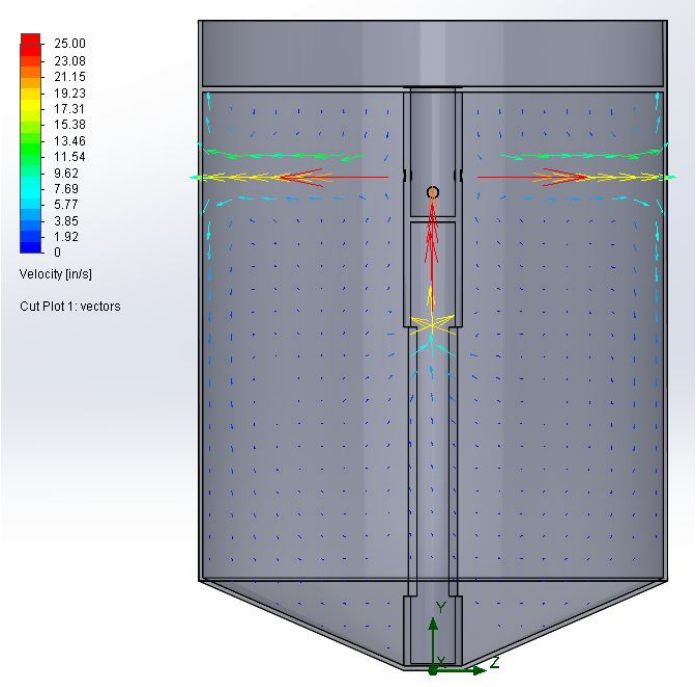


Figure 8.8 - Cut plot of velocity vectors for handle-less tank with tall inlet

Results

The dye experiment confirmed the concern that flow would be faster through the handle of the water bottle. The flow speed does not appear to be dangerous though. The flow quickly slows down after exiting the handle, so it is not dangerous for the creatures below it. Dye circulated throughout the entire tank demonstrating that nutrients would do the same when the tanks are used for aquaculture. For quantitative results from our SolidWorks flow simulations, velocity magnitudes were recorded at certain coordinates during each simulation. Figure 8.10 shows these coordinates inside the model of the handled tank.

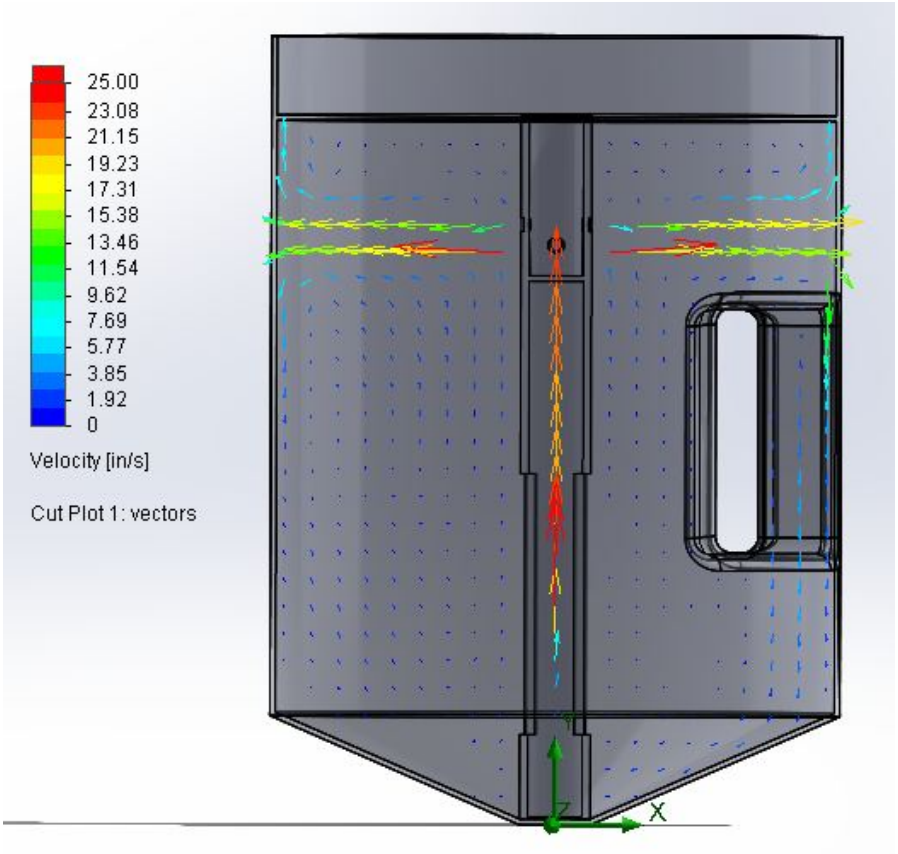


Figure 8.9 - Cut plot of velocity vectors for handled tank with medium inlet

Table 8.1 compares the recorded velocities for the three different inlet sizes for the handle-less model and the medium sized inlet for handled model. One large difference can be found between the tube inlet velocities of the small, medium, and tall inlet models. The velocity is about 8 times greater with the small inlet, which as discussed before could be a hazard for organisms growing at the bottom of the tank. Also, comparing velocities at the top of the handle between handled and handle-less models the velocity is about twice as large with a handle, although at the bottom of the handle the velocity does decrease significantly in the handled model. These quantitative results support the qualitative observations that a handled tank is appropriate for a hatchery.

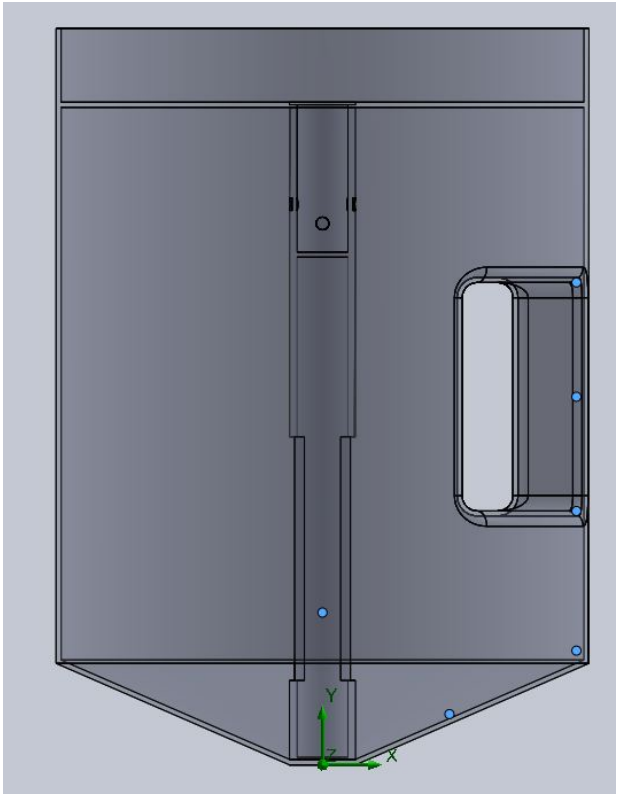


Figure 8.10 - Schematic of coordinates where velocity was recorded during each simulation.

	no handle			
location	small	medium	tall	handle
tube inlet	8.061141	1.031453	1.195624	8.183431
bottom	1.357278	0.267317	0.666647	1.881789
corner	0.233099	0.246356	0.257363	0.232267
handle bottom	1.558997	2.24935	1.621351	3.199414
handle mid	3.112073	3.921015	3.218595	4.552556
Handle top	5.947464	6.719234	6.111321	11.49734

Table 8.1- Velocity magnitudes (in/s) at specified coordinates

Conclusion

The physical experiment and the SolidWorks simulations yielded similar results as each other. There was an increased flow through the bottle's handle and decreased flow below part of the handle compared to a bottle with no handle. Neither change in flow rate would be detrimental to the growth of organisms in the hatchery. The protrusion of the handle removes a negligible amount of vertical space for organisms to grow. The overhang of the protrusion may be beneficial to the growth of the vase tunicate.

When choosing a pipe inlet size, a tall inlet would be best for circulation. Taller inlets pull in water from a larger area of the tank to circulate past the air stone. They also draw in water at a slower velocity than shorter inlets. The largest velocity caused by the inlet is located at the inlet's top, so a taller inlet would keep that higher in the tank and away from any organisms.

The handled water bottles can be used for aquaculture testing without any significant differences to handle-less bottles. They may even benefit the growth of certain organisms. Handle-less bottles could be purchased and used for comparison. When cutting inlet slots in the central pipe, taller slots are safest for the organisms and cause better circulation.

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