ABSTRACT

Title of Document: DESIGN AND SUSTAINABILITY ANALYSIS OF OYSTER AQUACULTURE IN MARYLAND

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As the oyster aquaculture industry begins to develop in Chesapeake Bay, the design of sustainable operations becomes paramount. The design of a novel salty indoor recirculating aquaculture system was explored by testing the efficacy of algal turf scrubbers, sphagnum moss, and ultraviolet radiation in reducing nitrogen and coliform indicator organisms, while maintaining superior taste. Additionally, the environmental sustainability of outdoor oyster aquaculture operations was evaluated using emergy synthesis.

DESIGN AND SUSTAINABILITY ANALYSIS OF OYSTER AQUACULTURE IN MARYLAND

By

Timothy Robert Williamson

Thesis submitted to the Faculty of the Graduate School of the
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Dedication

This thesis is dedicated to my wife, Lucia, and my daughter, Liliana, who motivated me during graduate school and who served as a constant reminder of the bigger picture. I would also like to dedicate this thesis to my advisor, David Tilley, who often supported me financially and emotionally though his unwavering confidence in me.

Acknowledgements

I would like to thank my graduate committee members who guided me intellectually and motivated me by their example. Special gratitude is due to my colleagues Ashley Belle, Brandon Winfrey, Elliot Campbell, Scott Tjaden, and Freddy Witarsa for their advice, assistance and friendship. I would also like to thank Johnny Shockley, Ricky Fitzhugh and Chris Weir of Hooper's Island Oyster Aquaculture for the advice, their knowhow, oysters for our experiments and access to their facility for our emergy analysis. Finally, this thesis would not have been possible without the financial support of the Mtech: Maryland Industrial Partnerships.

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CHAPTER 1: Valve Activity in Cultured Oysters Exposed to Sudden Increases in Salinity

Abstract

The submergence of live cultured *Crassostrea virginica* in high salinity seawater is a practice the seafood industry may consider for enhancing taste and marketability. We investigated the response of triploid *C. virginica,* cultured in mesohaline areas of Chesapeake Bay, to sudden exposure to elevated salinity in artificial seawater. Valve activity and duration of valve closure was recorded for three levels of salinity—14 ppt, 22 ppt, and 28 ppt—in aerated 17-liter aquariums for 72 hours. We recored valve activity in the first 12 hours of exposure and monitored mortality for 72 hours. Valve activity was influenced by salinity and length of exposure. Higher salinity waters had less valve activity initially but all treatments exhibited strong valve activity within 72 h. The mean time to valve opening upon submergence was not statistically significant. We observed no mortality after the 72-hour period. The research showed that *C. virginica* resumes valve activity within a couple of hours of being submerged in artificial seawater with a range of 14 ppt to 28 ppt salinity. Aquaculturalists seeking to enhance taste through the use of short-term saltwater baths may not need an acclimation step before exposing *C. virginica* to aerated artificial seawater.

1. Introduction

Aquaculture production of eastern oysters, *Crassostrea virginica* (Gmelin 1791), in the Chesapeake Bay (herein referred to as the Bay) has shown considerable growth in recent years. There are 12 current water column leases for farms in

operation, and over 300 in review (MD DNR, 2013). Due to an increase in local production of oysters, growers in Maryland are seeking new ways to distinguish their product from competitors.

One method that has been proposed is to place live oysters in a saltwater bath for 24 hours to enhance taste prior to marketing. Growers currently improve taste by harvesting market-sized oysters from 5-15 ppt saline water of the Chesapeake Bay and transporting and submerging them in Chincoteague Bay in Virginia where salinities range from 23 ppt to 36 ppt (MD DNR, 2013). The re-submergence of harvested shellfish to new locations is known in the industry as relaying. In this case, it is used to increase the salt content of the meat but in other regions, the process is used to purge shellfish of harmful contaminants and pathogens (Oliveira et al, 2011). Oyster farmers claim that mortality rates in cultured Bay oysters are negligible during the short-term relaying process (Johnny Shockley, co-owner of oyster farm, pers. com., 2012).

Salinity and temperature are influential environmental factors on the life cycle, physiology, and growth rates and feeding in *C. virginica* (Kennedy et al, 1996), yet few studies have investigated valve activity after exposure to new salinity regimes. Glastnoff (1964) stated that reduced salinity resulted in partial or complete valve closure and a decrease in water flow through gills. Similarly, Loosanoff (1953) observed that valve closure lasted approximately 6 hours when oysters were exposed to lower salinities. However, there is little literature on valve closure during sudden increases of salinity.

Some studies have documented mortality induced by osmotic stress, especially when exacerbated by *Perkinsus marinus* infection, a protozoan parasite enzootic in Bay oyster populations (Peirce et al., 1992; Paynter et al., 1995). An earlier study found that sudden reductions in salinity induced valve closure that lasted for prolonged periods (19.3 ± 1.2 h) in *C. virginica* from the Gulf Coast, USA (Hand & Stickle, 1977). However, few studies are available regarding how sudden increases in salinity affects valve closure.

In this study, we investigated the resumption of pumping in cultured *C. virginica* from a meseohaline region of the Bay after being placed in artificial seawater (ASW) baths at 14 ppt, 22 ppt, and 28 ppt for 12 hours by recording valve movement and duration of closure. We then recorded mortality after 3 days of exposure to ASW.

2. Materials and Methods

2.1 Oysters

Specimens of cultured *C. virginica* were provided from a bottom-cage oyster farm in the mesohaline zone of the Chesapeake Bay (38°18'N, 76°13' W) in December 2012. During the harvest, oysters were removed from cages and placed on a conveyor belt that moved them through a tumbler machine and power-wash. Farm operators provided 135 randomly selected individual oysters for our

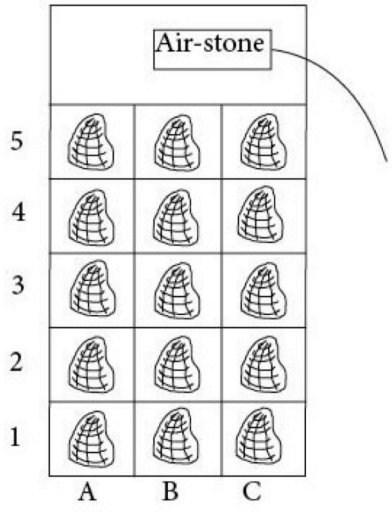

Figure 1.1. Each aquarium was arranged as above so that each oyster was individually labeled for observations during the study.

experiment. Upon delivery, oysters were stored overnight at 5 °C before being placed in artificial seawater aquariums for the experiment. Salinity at harvest was 15.5 ppt (MDDNR, 2012).

Figure 1.2. Oysters within the aquarium arranged in a grid pattern in order to monitor individuals.

2.2 Artificial Seawater

We made artificial seawater from de-chlorinated municipal drinking water and reef salt (Crystal Sea Marinemix^{TM}, Marine Enterprises International, Baltimore, MD, USA). We prepared three levels of salinities (14, 22 and 28 ppt) for 9 aerated aquariums so there were three replicates of each salinity. Each aquarium was given 17 l of artificial seawater at a stable temperature (Table 1.1). We then randomly arranged the aquariums on a lab bench in a lab set to room temperature.

We placed 15 oysters on the bottom of the aquariums arranged on a grid that was labeled on two axes—numerically and alphabetically—in order to mark individual oysters during the experiment (Fig. 1.1).

We recorded whether each individual had open valves each hour from 9:00 am until 9:00 pm for 12 h of exposure. Valves were checked by sight.

Table 1.1. Mean Water temperature of the three treatments.

Treatment	Mean Temp. (°C)
14 ppt ASW	18.1±0.14
22 ppt ASW	18.1±0.23
28 ppt ASW	18.0±0.29

2.3 Mortality

We held the oysters in the aquariums for an additional three days (72 h) to determine whether exposure to artificial seawater would cause mortality. At 72 hours, we recorded the amount of oyster deaths. Oysters were considered dead if gaping shells did not close upon touch.

2.4 Statistics

We analyzed the number of open valves per aquarium in a one-way ANOVA for hour 1, hour 6 and hour 12 to determine the effect of salinity and time held in aquariums using SAS 9.2 software. We determined the mean duration of valve closure after initial exposure and tested for significance in a one-way ANOVA.

3. Results and Discussion

3.1 Frequency of open valves

Figure 1.2 shows the percent of oysters with visibly open valves during each hour of exposure. Salinity and duration of exposure had a significant effect of the number of valves open during the experiment ($p<0.001$). We observed more oysters with open valves in the 14 ppt salinity treatment ($p=0.024$). The number of oysters

with open valves was not significantly different between the 22 ppt and 28 ppt seawater treatment. Duration of exposure also had an effect; more open valves were observed as more time passed while the oysters were exposed to the artificial seawater ($p<0.0001$).

Our results showed that 70% of cultivated oysters were able to open their valves within the first eight hours of exposure to a sudden change in salinity, within the range of 14 ppt to 28 ppt at 18º C. It may be that the quick resumption of valve activity was related to aerobic respiration after a period of stress in which oysters were harvested, cleaned and transported by refrigerated truck to the laboratory.

In their natural habitat, oysters experience sudden increases in salinity when the tides bring in cooler, oligotrophic water from the seas. These waters are typically low in food resources for oysters (Rheault & Rice, 1996), so a resumption in valve activity may not be related to a response to an environmental cue that signals increased food resources. In our experiment oyster were not fed and so any resumption of valve activity was not triggered by the presence of food, by rather the need for respiration.

We noted that only 2 of the 135 individuals were never observed with open valves. Both of these individuals were held in the 22 ppt artificial seawater. It is possible that these individual had open valves during periods that we did not observe, such as at night or between observations. Besides these exceptions, there appeared to be no adverse effects of placing cultured Bay oysters at salinities ranging from 14 ppt to 28 ppt at this temperature.

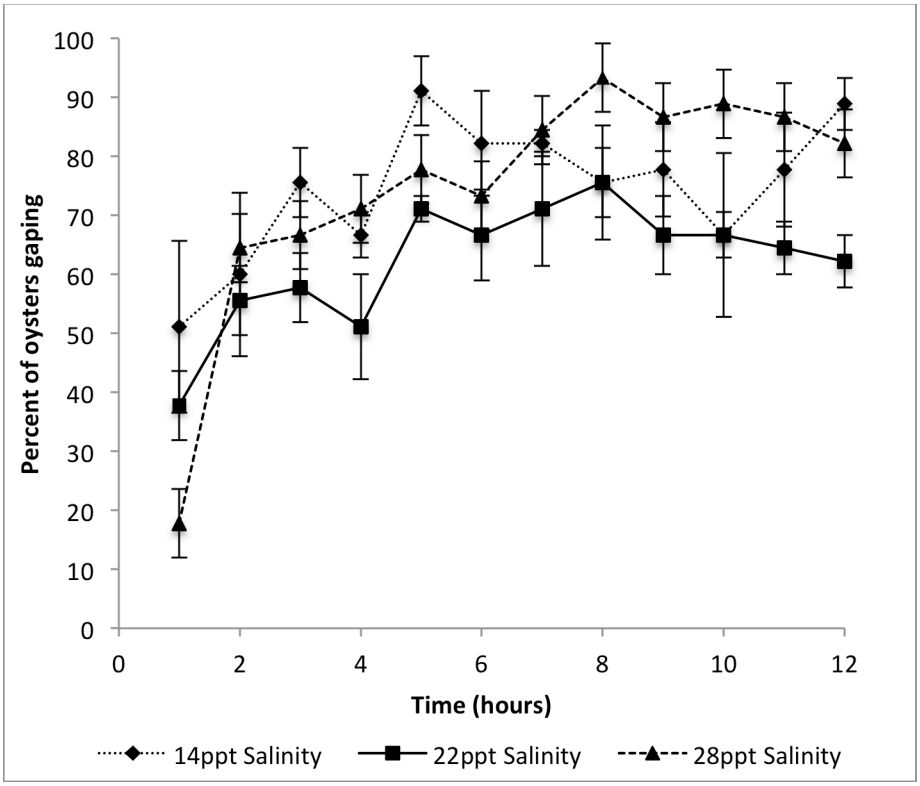

Figure 1.2. Percentage of oysters visibly gaping over the course of first 12 hours. No difference was detected in resumption of filtration between oysters in differing salinity regimes.

3.2 Time to Valve Opening

The mean time to valve opening upon submergence into a new salinity regime was 2.47 ± 0.16 hrs with no significant difference between the salinity treatments. Therefore, salinity levels did not affect duration of valve closure once oysters were exposed to a new salinity regime.

Our results contrast with Hand & Stickle (1977) in that we observed markedly lower durations of valve closure. The discrepancy could be related to the fact that, in their study, salinity rapidly dropped from 20 ppt to 10 ppt instead of being marginally decreased as in the 14 ppt treatment and raised as in the 22 ppt and 28 ppt treatments in our study. A significant decline in salinity may be more detrimental to the oyster physiology than significant increases. Fresh water flooding during period of higher than average water temperatures has often resulted in mass mortality of oysters in the wild (Shumway, 1996).

3.3 Mortality

At the end of the 3-day study (72 h), we did not observe any mortality of oysters in any of the salinity regimes. Therefore the temporary storage of live oysters in ASW to enhance taste may not constitute a shelf-life concern or a loss of product for marketing. However, we did not measure overall of condition of the oysters that may have been affected by the changes in salinity regime. This study could have been improved by the use of the condition index after Lawrence and Scott (1982).

3.4 Experimental Design

The experiment would have benefited from continuous monitoring equipment rather than visual assessments of valve activity. With continuous monitoring, a more accurate assessment of valve activity could have been made. Additionally, salinity treatments used in this experiment were determined in consultation with aquaculturalists seeking to enhance taste, rather than to learn about oyster physiology. In order to learn more, a further study could expose oysters to extreme salinities that

are outside of the organisms' optimal range of 14 ppt to 28 ppt (Shumway, 1996) and by examining condition of oysters after exposure as mentioned above.

For aquaculturalists that seek to enhance saltiness of cultured oysters, we conclude that an acclimation step is not needed to prevent mortality, when given ample oxygen supply, and salinity and temperature are maintained within the ranges of this experiment (14 to 28 ppt and 18º C). Finally, it is possible that cultured oysters could be held in salt water baths for as little as 8 hours since the majority (>50%) of oysters resume filtration within the first 3 hours of exposure.

4. Acknowledgements

We would like to the Department of Environmental Science and Technology at the University of Maryland, Ken Paynter for consultation and guidance, Sina Mirmozaffri for assistance, and Hooper Island Oyster Aquaculture for donating live oysters for the experiment. Funding was provided by the Maryland Industrial Partnerships program (#5019) with David Tilley as principal investigator.

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CHAPTER 2: Natural Water Treatment Systems in a Recirculating Aquaculture System for Storing Live Oysters

Abstract

Oyster aquaculture is a growing industry in the Chesapeake Bay. With 12 aquaculture water column leases in operation and over 300 leases currently in review, growers seek to distinguish their product from competitors through marketing and growth methods. One such method is the enhancement of taste by placing live oysters in artificial seawater in indoor recirculating aquaculture systems prior to marketing. This study tested the ability of green technologies, namely algal turf scrubbers^{TM} (ATS) and dried sphagnum moss leaves, and Ultraviolet (UV-C) disinfection to maintain water quality in a recirculating aquaculture system.

We constructed eight 216-liter recirculating aquaculture systems in a 2^3 factorial experimental design. ATS systems significantly reduced ammonium-nitrogen and nitrate-nitrogen concentrations, reduced temperatures, and increased DO concentrations. The ATS system were able to remove 1.7 ± 0.5 g-N m^{-2} day^{-1} but, due to under-sizing, were not able to maintain concentrations of nitrogenous wastes below tolerance levels for oysters. Our results indicate ATS systems could be used for filtration in aquaculture if appropriately sized. The sphagnum moss application had no effect on any parameter in this study and we could not make conclusions about its use in salt water systems with heavy biomass loading. In our experimental systems, unevenness of water temperature made analysis difficult; however, UV-C disinfection

suggested a trend of total coliform bacteria (MPN / 100 ml) reduction but was not statistically significant.

1. Introduction

In an effort to increase the marketability of eastern oysters (*Crassostrea virginica*) cultured in captivity in mesohaline waters, like tributaries of the Chesapeake Bay, the aquaculture industry is searching for methods to increase their salty flavor. One option is to physically move cultured oysters from mesohaline to saline waters three to five days before taking them to market. Depending on the distances travelled, this can be an energetically intense and financially costly undertaking. Another potential method is to move cultured oysters from their mesohaline habitat into a controlled, indoor wet storage system 24-h before taking them to market. Such methods are used in the shellfish industries in the United Kingdom, France, and Australia to reduce public health risks from oysters contaminated with harmful pathogens (Lee et al., 2008). To conserve water, energy and money, wet storage systems often rely on recirculation of the water. Due to the presence of a live organism, treatment of the recirculated water is necessary to maintain adequate water quality. Wet storage with recirculation and treatment is known as a recirculating aquaculture system (RAS).

In a RAS that holds live *C. virginica* in artificial seawater for raw human consumption, public health and product safety are chief concerns. Properly designed RASs can provide depuration, flavor enhancement and water purification. Depuration is a cleansing process that allows bivalves to naturally expel pathogens into a body of water that is disinfected by ozonation or ultraviolet light irradiation. Water

purification is the process of maintaining desirable chemical and physical properties of water for a given use.

Morales-Alamo and Haven (1979) tested a RAS for adding salt to the meat of *C. virginica*, that consisted of a culture basin which drained to a large reservoir, from which water was pumped through a UV-disinfection unit, and recirculated to the culture basin. Their study did not focus on the design of the recirculating aquaculture system; it focused on taste enhancement and the condition of cultivated oysters after a short period of submergence in the salting system. They submerged *C. virginica* for 24 hours and found that the process did not negatively affect the condition of the animal and sufficiently salted the meat, but the recirculating system was never intended for repeated use. While it had a UV-C irradiation unit to prevent bacterial growth during the salting period, it had no method for managing water quality beyond disinfection.

In order to reduce public health risks, disinfection is employed to prevent the development of harmful microorganisms in water that could be ingested by consumers of shellfish. UV irradiation, temperature control and ozonation are the most common methods of disinfection in the aquaculture industry and their effectiveness at preventing disease is enhanced when the three methods are used together (Summerfelt et al., 2009).

Temperature is widely regarded as a major environmental factor that aids in disinfection, especially in regards to pathogens within *C. virginica* (Kasper et al., 1993; Motes et al., 1998). Commonly associated with *C. virginica, Vibrio vulnificus* is capable of causing gastroenteritis and septicemia when infectious doses are

consumed along with the oyster, but nearly all cases of *V. vulnificus* infection have occurred from raw shellfish from the Gulf of Mexico in the United States (Martinez-Urtaza et al., 2010). The pathogen has a minimal growth temperature of approximately 13°C and an optimal growth temperature of 37°C (Kasper et al., 1993; Motes et al., 1998). Therefore water temperature ≤13°C is favorable for managing public health risks from consuming raw oysters.

Ozone disinfects water by destroying pathogens with oxidation. Ozonation can also convert harmful nitrite to nitrate, oxidize organic wastes, remove the yellowish color from humic substances (Summerfelt, 2003), flocculate solids (Davidson et al., 2011), and enhance the ability of other treatment systems. Ozonation can work well in combination with foam fractionation (Park et al., 2011).

Dissolved ozone is generally toxic to aquatic and marine life at low concentrations (Coman et al., 2005; Reiser et al., 2011) and, more importantly, ozone is toxic to humans. Because the risks associated with ozone toxicity are exacerbated in salt water systems containing bromide ions, ozone systems are considered impractical and economically unviable for most aquaculture operations (Schroeder et al., 2011).

UV irradiation, in contrast, constitutes minimal risk to cultured organisms and operators, but only provides disinfection. Specific wavelengths of UV radiation destroy the DNA of microorganisms, causing them to cease functioning or die. The most effective wavelength against all waterborne pathogens is 254 nm (Wheaton, 1993), but its efficacy is influenced by both water quality and characteristics of the target microorganisms (Hijnen et al., 2006). Certain organisms have greater UV

sensitivity than others.

The presence of suspended particles can inhibit light penetration and provide refuge for particle-associated bacteria (LeChevallier et al., 1988; Walters et al., 2013). Similarly, turbidity and coloration inhibit the ability of UV radiation to inactivate microorganisms by reducing light intensity. Gullian et al. (2012) discovered that the killing effect functions best at turbidities below 11.2 nephelometric turbidity units (NTUs) in aquaculture operations. Additionally, because water passes along a tubular section of pipe that houses the UV bulb, only water is disinfected. Attached microorganisms and biofilms in other regions of the aquaculture system are unaffected.

The use of dried sphagnum moss leaves has been used to prevent and remove biofilms in recreational swimming pools (Desai et al., unpublished report) but has not been tested in aquaculture. Sphagnum could potentially reduce the build-up of biofilms in an RAS, which could help maintain clearer water with less refugia for bacteria and viruses. Creative Water Solutions, LCC has marketed a patented device (patent # US20120152828 A1) that contains dried, autoclaved Sphagnum moss in order to reduce the need for backwash cycles in slow sand filters and chlorine additions in recreational swimming pools (Knighton & Fiegel, 2012).

Presumably, the sphagnum assists with disinfection by preventing biofilm growth, reducing chemical scaling on pool surfaces and inhibiting the growth of suspended bacteria in solution. However, independent testing to confirm these claims is lacking. The sphagnum moss has been used in the 50-m swimming pool at the University of Maryland's Eppley Recreation Center since 2010. Since its adoption,

backwash cycles to flush bio-films in slow-sand filters have significantly decreased, operators have used 50% less calcium hypochlorite (chlorination), and 93% less pH-buffering agents such as sodium bicarbonate (Desai et al., unpublished report).

Sphagnum moss is a group of hydrophytic bryophytes that are found in nutrient-poor bogs and fens in temperate climates around the globe (Andrus, 1986). A notable feature of sphagnum is the associated low pH and high water retention that can direct ecological succession in bogs. The acidifying process is likely the result of the sphagnum cell wall exchanging H^+ for dissolved cations, such as Ca^+, Mg^+, Fe^+, NH_4^+ and for its organic acid production (Clymo, 1964; Andrus, 1986). It is assumed that the high cation exchange capacity (CEC) is an adaptation that allows sphagnum to absorb nutrients from rain-fed water, which is low in nutrients (Hajek & Adamec, 2009). Studies have confirmed this by testing the sphagnum's ability to sequester heavy metals through cation exchange and found that the CEC capacity is linked to organic acids (Breuer & Metzer, 1990; Champagne & Li, 2009).

Stalheim et al (2009) tested the anti-septic ability of *sphagnan*, an acid derived from sphagnum moss, as an anti-septic and found that the acid was comparable in effect to hydrochloric acid (HCl) in low-buffering mediums, concluding that the reduction in pH is important to sphagnan's anti-septic qualities; however, sphagnan is baceteriostatic in that it halts growth of bacteria and was not found to reduce concentrations of bacteria. Mellegård et al. (2009) investigated phenol compounds derived from Sphagnum species and found little to no antiseptic effect, further isolating organic acids as the main culprit of its anti-septic properties. No known published studies have evaluated these anti-septic properties of sphagnum

in highly-buffered saline solution, such as artificial seawater.

C. virginica tolerates a range of water quality without adverse affects to growth rates and condition (Table 2.1); however, removal of solids and nitrogenous compounds, such as ammonium and urea, are critical to reduce risks to the cultured organisms and to the public's health. Biofiltration (also referred to as bacteriological filtration) is the most common method of waste reduction in RAS (Van Rijn, 2013).

Table 2.1. Oyster tolerance levels for critical water quality parameters.

Parameter	***Tolerance Levels***
Temperature (°C)	15-25[1]
Ammonium (mg/l)	0.0-5.5[1]
Nitrate (mg/l)	0.0-460.0[1]
pH	6.5-8.5[2,3]
Dissolved Oxygen (mg/l)	>3.5[1]

[1]Epifanio *et al,* 1976.
[2]Knutzen, 1981.
[3]Buchanan *et al*, 1998.

Biofiltration is the use of beneficial bacteria to break down harmful wastes into benign materials. The removal of nitrogenous wastes, such as urea, ammonia and organic nitrogen from solution is driven by microbial processes; decay of organic matter, nitrification of ammonium to nitrate in aerobic zones, and denitrification of nitrate (NO_3) to di-nitrogen gas (N_2) in anaerobic zones of the system (Van Rijn, 2013).

Biofilters are effective and economical as evidenced by their ubiquity in indoor aquaculture systems throughout the world, but they have drawbacks. Their

effluents are depleted of oxygen and high in carbon dioxide concentration (Adey and Loveland, 1998). In addition, the microbial processes eliminate useful forms of nitrogen from the system, as opposed to utilizing it as a resource for primary production, as seen in plant-based systems.

Another method of handling nitrogenous wastes is through phytoremediation systems that use photosynthesis and associated microbial communities to process wastes. In our study, we chose to use phytoremediation in the form of an Algal Turf Scrubber™ (ATS) to process wastes.

Algal Turf Scrubbers™, invented by Walter Adey of the Smithsonian Institute, were modeled after the algal communities in reef ecosystems, where significant wave action, nutrient inputs and light penetration provide for a highly productive algal community (Adey and Loveland, 1998). The system consists of a mesh substrate harboring a periphyton community in a shallow raceway trough. Untreated water is delivered to a tipping bucket that spills over when full to create a wave effect across the algal turf. The wave allows sufficient gas exchange for photosynthesis (Adey et al., 2011) and creates turbulence that prevents self-shading and increases mixing, reducing the diffusive layer around algal filaments and making nitrogen more available (Blersch et al, 2013). Algal biomass is periodically harvested to stimulate production. The biomass can then be used as a compost fertilizer (Mulbry et al., 2006), as a biofuel feedstock (Adey et al., 2011), or other natural product.

The primary role of the ATS is to reduce inorganic nutrients, but they also raise pH by removing carbon dioxide (CO_2), increase dissolved oxygen (DO), and reduce total suspended solids (TSS) (Craggs et al., 1996; Mulbry et al., 2010; Adey et

al., 2013). Cahill et al. (2010) built and tested a system similar to an ATS that used macroalgae, instead of microalgae, to treat water in RAS for the culture of abalone, *Haliotis iris*. At ammonia excretion rates of 0.015 g-NH_4^+ day^{-1}, seaweed filters maintained lower concentrations of NH_4^+ and NO_3^- than alternatives, which included biofilms in the uptake of nitrogenous wastes. Macroalgae growth rates (4.79 g m^{-2} day^{-1}) were considerably lower than reported rates by algal turf scrubbers that reach production rates of 30 g m^{-2} day^{-1} (Adey et al., 2011). The difference in rates could be attributed to lower nitrogen loading rates.

Objective

The purpose of this study was to quantify the extent to which algal turf scrubbers™, sphagnum moss, and UV-C disinfection affected the water quality (i.e., pH, dissolved oxygen, temperature, ammonium, nitrate and total coliform) of a saline recirculating aquaculture system that temporarily (< 24 h) stores daily batches of live *C. virginica* over a period of 5 days. Eight laboratory-scale recirculating aquaculture systems were designed, built and tested in the Ecosystem Engineering Design Laboratory, University of Maryland, College Park, USA in a 2^3 full factorial experimental design to quantify changes in key water quality parameters due to each of the three technologies: algal turf scrubbers™, sphagnum moss, and UV-C irradiation.

2. Methods

2.1 Description of Systems

Figure 2.1 shows the conceptual layout and water flow of the experimental RAS. The experimental RASs were comprised of open-topped 160-liter, polyester resin-coated culture basins (Hooper Island Oyster Aquaculture, Fishing Creek, MD) for holding *C. virginica* specimens; 57-liter, conical-bottomed, polyethylene settling basins (DenHartog Industries, Hospers, IA); 0.5 horsepower (375 W, 36 l min^{-1}) centrifugal pumps (Hayward Industries, Elizabeth, NJ); and polyvinyl chloride (PVC) plumbing. The total volume for each system was 217 liters.

Each RAS was randomly assigned water treatment components as per a 2^3 full factorial design (Table 2.2).

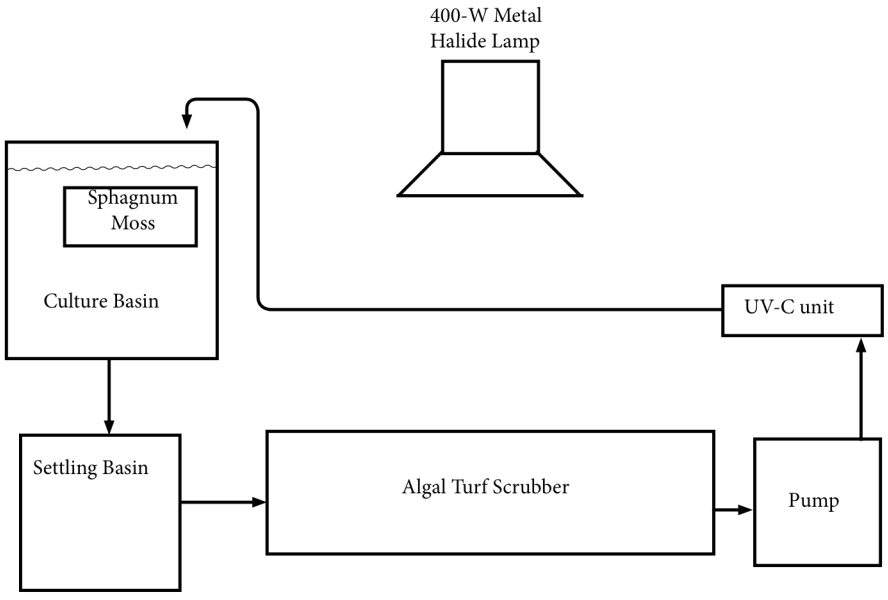

Figure 2.1 Schematic of the experimental recirculating aquaculture system.

2.2 Experimental Treatment Systems

2.2.1 Algal Turf Scrubbers™

Four RAS included a 104 cm x 106 cm Algal Turf Scrubber ™ (Living Ecosystems, Trappe, MD) positioned to receive water from the settling basin to a trapizoidal acryllic tipping bucket that produced the wave action needed for proper function. Untreated water flowed from the tipping bucket across an algal turf community on a 100 cm x 100 cm substrate of black polyethylene mesh with 3 x 4 mm openings (Industrial Netting, Minneapolis, MN) after Blersch et al (2013) (Figure 2.2). Water then drained via two circular openings (3.81cm ; 1.5in diameter) located in each corner of the end of the raceway to the centrifugal pumps. A pump then lifted water up to each oyster culture basin.

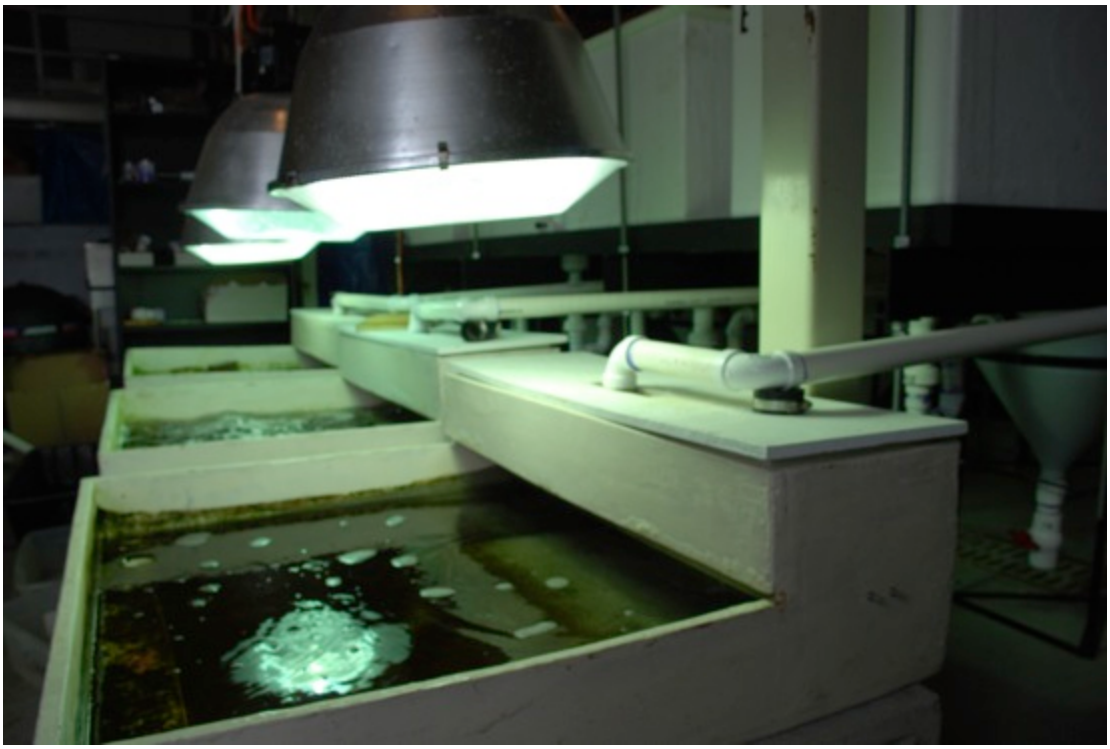

Figure 2.2. Photo of the ATS units, displaying the wide raceway trough and the 400-W metal halide lights used during the experiment.

Prior to the experiment, we seeded the ATS units by attaching a section of algal turf taken from an existing ATS in use at the Baltimore Harbor in the northern reaches of Chesapeake Bay. The ATS units were supplied with brackish water collected from an inlet on the Chesapeake Bay at Sandy Point State Park from February 2013 to April 2013 and were placed on a 12-hour diel cycle with a 400 W metal halide bulb (Valutek, Albany, NY). This ensured that the algal community had sufficient growth and diversity of algal species prior to the experiment. We harvested algal biomass from the ATS prior to the experiment in order to maximize growth and nutrient uptake.

2.2.2 UV Disinfection Units

UV disinfection consisted of a 57-watt UV-C bulb placed within a section of PVC piping (Aqua Ultraviolet, Temecula, CA) located so that it treated water immediately prior to returning to the culture basin.

2.2.3 Sphagnum Moss Application

We placed 5 3-gram packets of sphagnum moss (Creative Water Solutions, Plymouth, MN) directly in the culture basin in four of the units that received the sphagnum moss treatment. This is analogous to how the sphagnum moss is applied at the 50-m swimming pool at the University of Maryland's Eppley Recreation Center in College Park, Maryland, where it is applied in the sump reservoir.

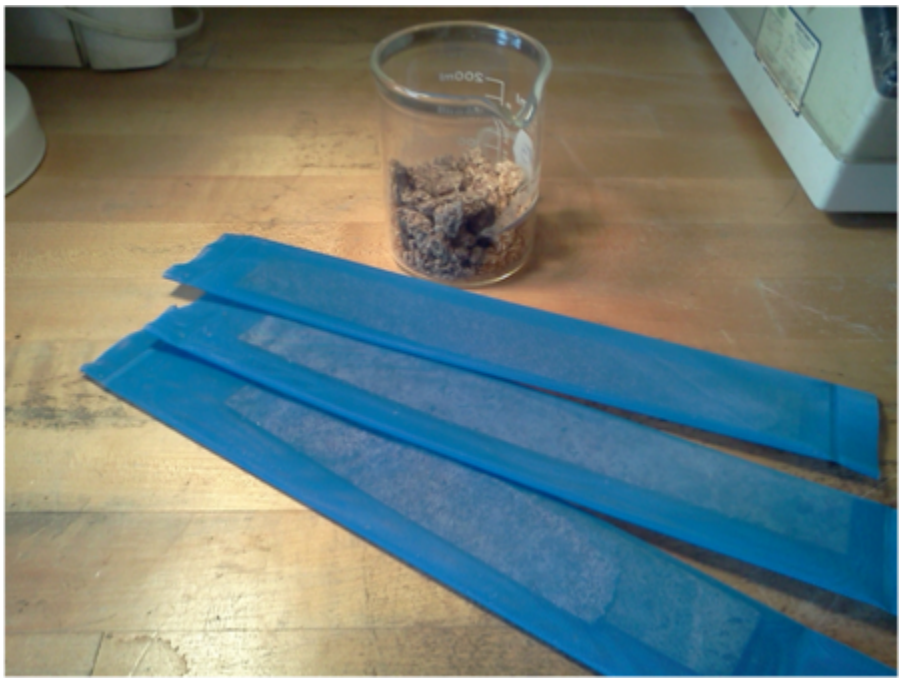

Figure 2.3. Blue mesh packets containing the dried sphagnum moss leaves for insertion into the water column.

2.2.4 Artificial Seawater

The day before we started the trials, we filled the entire RAS with 217 liters of de-chlorinated water produced from municipal drinking water that had been treated in a carbon filter to remove chlorine. We dissolved 6500 grams of Crystal Sea Marinemix (Marine Enterprises International, Baltimore, MD) into the 217 L of de-chlorinated water to create artificial seawater that had a salinity of 25 ppt. The Marinemix was not dried before we weighed it for additions, which accounts for the discrepancy between the mass added and the resulting salinity.

2.3 Experimental Design

C. virginica (51.6 ± 10.02 g, 7.4 ± 0.75 cm; n=60) were removed from on-bottom aquaculture cages in the Chesapeake Bay (38°18'N, 76°13' W). The oysters were rinsed in a tumbler (Hooper Island Oyster Aquaculture, Fishing Creek, MD, USA) and packed into polymer mesh bags holding 100 individuals each. Forty-eight (48) such bags were transported to the laboratory in a refrigerated truck and were stored in a walk-in refrigerator at 4°C until they were placed in the culture tank for the experiment.

Each day, we placed three mesh bags of 100 oysters (300 individuals total; 14.04 ± 0.99 kg; n=32) into culture tanks for 24 hours to replace the previously used three bags. The ratio of biomass to artificial seawater volume was 69.4 g-oyster liter^{-1}. At the start of each day, the oysters from the previous day were removed, and three bags were placed in the culture tanks for another 24 hours, which mimicked plans proposed by the industry.

Each experimental run was carried out for five days. A second replicate was performed fifty one (51) days following the first replicate. Between replicated experimental runs, each RAS system was cleaned with non-toxic all-purpose cleaner (Sunshine Makers, Inc, Huntington Beach, California) and all plumbing was cleaned with large pipe cleaners. Some sections of the plumbing could not be reached by the pipe cleaners and instead were left to soak in a soap-water mixture, then rinsed with running potable water.

Water treatment by the ATS, sphagnum moss application, and UV disinfection was tested in a 2^3 full factorial experimental design. Treatments were

applied randomly initially (Table 2.2), but for practical reasons, were not re-randomized between replications.

Table 2.2. Experimental design showing the eight treatments applied to the RAS units in the study. + and – denoting presence or absence of each technology used in the RASs.

Unit	UV-C Disinfection	Algal Turf Scrubber	Sphagnum Moss
1	-	-	-
2	+	-	-
3	-	+	-
4	+	+	-
5	-	-	+
6	+	-	+
7	-	+	+
8	+	+	+

2.4 Water Quality Analysis

Water samples were collected three times daily from the culture tanks to determine ammonia, nitrate, pH, dissolved oxygen, conductivity (a proxy for salinity), temperature, and total coliform counts (Table 2.3). Nitrate (NO_3^-- N) and NH_4-N concentrations were measured only in the second replication of the experiment due to an equipment failure during the first replication

Table 2.3. Water Quality Analytical Methods used in this study.

Parameter	Analytical Method	Equipment	Reference
Dissolved Oxygen	Luminescence Method	Hach Multiparameter Meter HQ40d	
Salinity	Conductivity	Hach Multiparameter Meter HQ40d	
Ammonium	Ammonia-Salicylate Method, Spectrophotometry	Hach DR 5000	Hach Method 8155
Nitrate	Cadium Reduction Method, Spectrophotometry	Hach DR 5000	Standard Methods 4500-NO_3
pH	Ion selective probe	pH probe (Accumet, Hudson, MA).	
Total Coliform	Multiple Tube Fermentation	Incubator	Standard Methods 9221

2.5 Statistical Analysis

Response variables were the change in dissolved oxygen concentration (mg/l), the change in pH, the change in NH_4^+ (mg/l), and the change in NO_3 (mg/l). Changes were calculated by subtracting the initial measurement by the intermediate final (96-hr) measurement after 96 hours of the experiment. In some cases, where negative changes occur (i.e. the concentration is reduced), we added 5 to all of the results for that parameter before data analysis in order to analyze in the statistical model. For the change in total coliform bacteria concentrations (Log MPN/100 ml) we subjected initial conditions from measurements taken after 48 hours and after 96 hours. Effects of treatments were analysed using one-way analysis of variance (ANOVA) using the PROC MIXED procedure in Statistical Analysis Software (SAS version 9.2, Cary,

NC). We used Pearson's correlation (PROC CORR) to determine relationships between parameter measures within treatments. Alpha was set at 0.05.

2.6 Oyster Nitrogen Excretion Rates

We estimated the oyster excretion rate of dissolved nitrogenous wastes (mg-N g-oyster^{-1} d^{-1}) based on the change in nitrogenous wastes concentrations for the control RAS systems using Eq. 1:

$$N_e = \frac{\frac{[N_b - N_a]}{[M]} * V}{dT} \qquad (1)$$

where N_b is the ending nitrogenous wastes (mg-N/l) at 24 hours and N_a is the initial nitrogenous wastes concentration (mg-N/l), M is the mass of oyster that were stocked (g), V is the volume of water in the system (l) and dT is the timestep (days). This was calculated for each day. From this we calculated the mean nitrogen excretion per day (mg-N g wwt oyster^{-1} day^{-1}).

We calculated the daily nitrogen loading rate by Eq 2.

$$N_D = N_e \; x \; M \qquad (2)$$

where N_D is the daily nitrogen load, N_e is the excretion rate from equation 1, and M is the mass of the oysters stocked per day.

2.7 Nutrient Uptake by Algal Turf Scrubber™

We estimated uptake of nitrogenous wastes by the Algal Turf Scrubber™ (mg-N m^{-2} d^{-1}) by calculating the difference in mass of nitrogenous wastes between

the control RAS units and those with ATS systems and divided by the time step (days) as in equation 3:

$$N_Q = \frac{[N_0 - N_{ATS}] * V}{dT} \quad (3)$$

where N_0 is the average ending concentration of nitrogenous wastes in non-ATS systems (mg-N l^{-1}) and N_{ATS} is the average ending concentration of nitrogenous wastes in ATS systems (mg-N l^{-1}) , V is the total water volume (l) of the aquaculture systems and dT is the timestep (days). We calculated the nitrogen uptake rate for each day and determined the mean uptake by the ATS systems.

3. Results

Table 2.5 shows the mean change in water parameters during the experiment.

Table 2.5. Water quality results for the two (2) trials. The table shows the mean and standard error of the change in concentration or value for each parameter from the initial condition of the water to the end of the experiment. Asterisk (*) denotes significant difference ($p<0.05$) in the change of the parameter.

Unit	Temperature (C)	DO (mg/l)	pH	Ammonium-N (mg/l)	Nitrate-N (mg/l)	Coliform (MPN/100ml)
Control	2.2 ± 1.2	-0.51 ± 0.24	-0.81 ± 0.18	52.6	3.9	ND
UV	3.8 ± 1.3	-0.33 ± 0.04	-0.90 ± 0.09	51.6	1.6	-0.22 ± 0.11
ATS	**1.1 ± 0.4**	**0.14 ± 0.16**	-0.57 ± 0.18	**17.4**	**5.6**	ND
UV * ATS	**0.6 ± 0.2**	**0.07 ± 0.08**	-0.82 ± 0.38	**16.8**	**4.5**	0 ± 0
Moss	2.8 ± 0.6	-0.29 ± 0.02	-0.63 ± 0.14	30.7	4.0	ND
UV * Moss	4.3 ± 0.2	-0.42 ± 0.13	-0.51 ± 0.01	44.1	2.4	-0.96 ± 0
ATS * Moss	**0.9 ± 0.9**	**0.14 ± 0.03**	-0.80 ± 0.35	**16.1**	**4.7**	88.2 ± 29.2
UV * ATS * Moss	**0.6 ± 0.0**	**-0.16 ± 0.13**	-0.64 ± 0.19	**16.1**	**21.7**	1.9 ± 0.5

3.1 Dissolved Oxygen

Daytime dissolved oxygen concentrations during the 96 h study were maintained above the minimum threshold for oysters in all of the experimental units (>3.5 mg/l; Table 2.1). Systems with ATS had significantly lower changes in dissolved oxygen concentrations during the daytime throughout the study than units without ATS (Table 2.5).

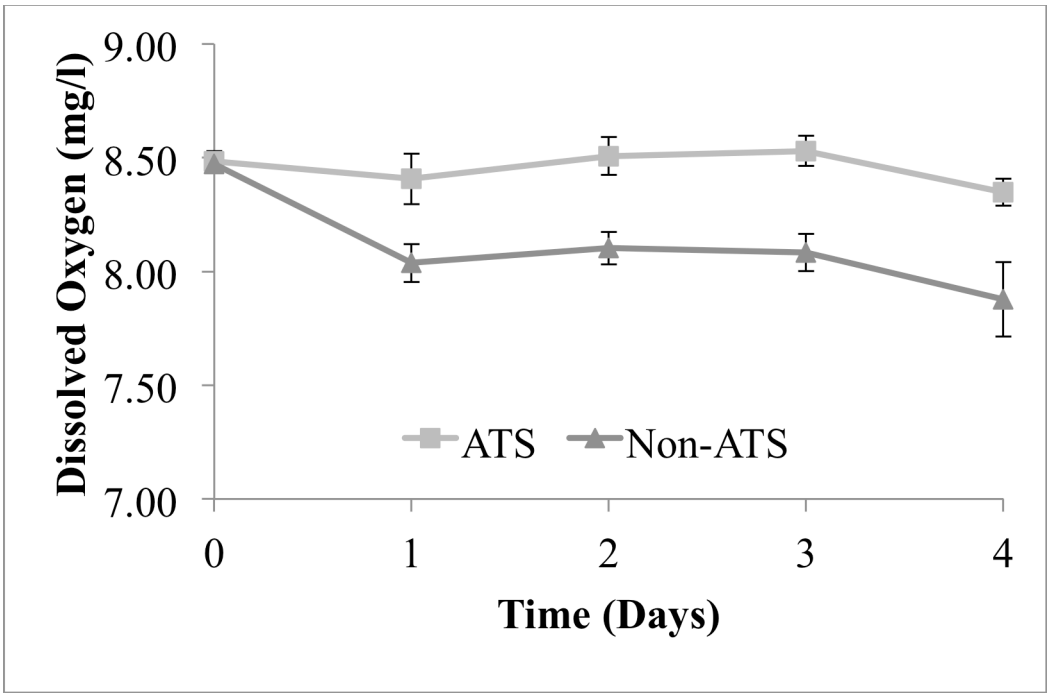

Figure 2.4. Mean dissolved oxygen concentrations (mg/l) during the two 96-hr trials. Units fitted with an ATS system had significantly lower changes in dissolved oxygen. Error bars are standard error.

Figure 2.4 displays the temporal pattern of dissolved oxygen between the units with and without ATS. Concentrations of dissolved oxygen began at 8.48 ± 0.04 mg/l (mean and SE) for ATS units and ended at 8.35 ± 0.06 mg/l, while units without ATS had a drop in oxygen from 8.47 ± 0.04 mg/l to 8.04 ± 0.08 mg/l in the first day and ended the study at 8.08 ± 0.16 mg/l. Dissolved oxygen was moderately or strongly

correlated to temperature in all of the aquaculture systems during both trials of the study (Table 2.10).

3.2 Temperature

ATS units had significantly lower changes in water temperature (Table 2.5; p=0.001). Water temperature in systems without ATS increased 2.4 ± 0.15° C more than those with ATS by the end of the 96-hr study (Figure 2.5). Difference in water temperatures were greatest during the second trial, when the UV + Moss system reached 31° C after 72 hours, while water temperature in the system with all three treatment systems (UV, Moss, and ATS) never reached above 26.9° C.

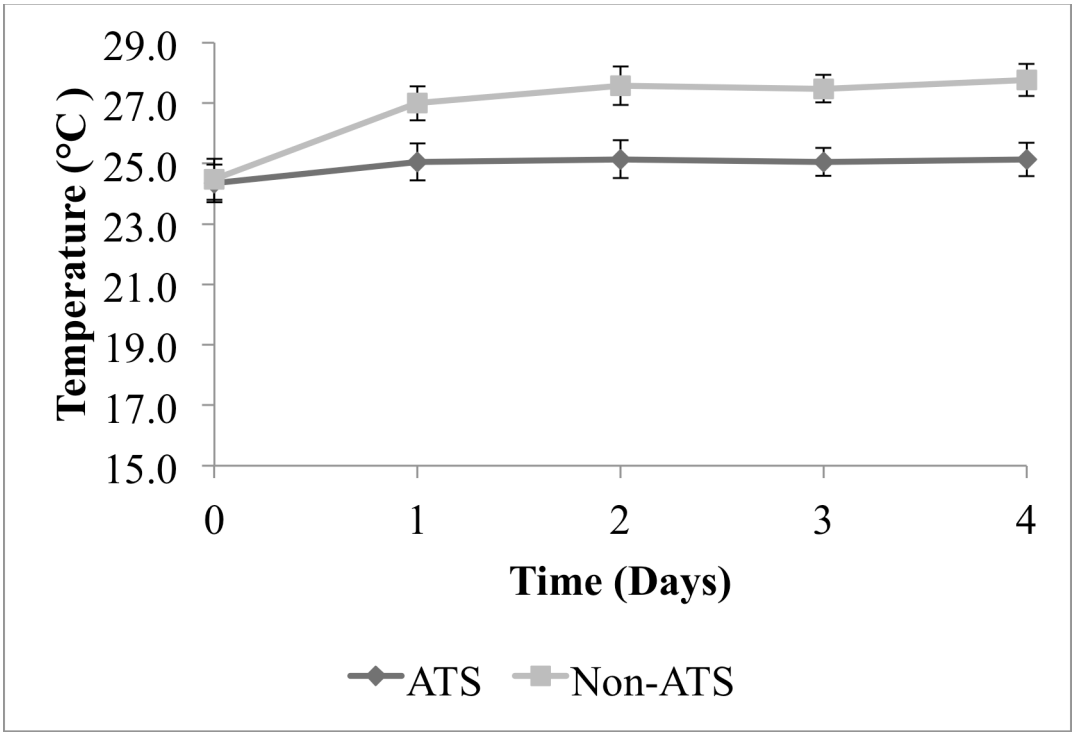

Figure 2.5. Mean water temperature in systems with ATSs and without ATSs during the two 96-hr trials. Error bars are standard error.

3.3 Dissolved Nitrogenous Wastes

Ammonium-N and nitrate-N were measured in the second trial of the experiment. In order to gain more degrees of freedom in the ANOVA statistical test, we removed the interaction effects from the model. ATS units significantly lowered nitrogenous wastes concentrations, but were not able to uptake the amount needed to maintain ammonium-N below tolerance levels for oysters. Ammonium-N concentrations were higher than the tolerance level for *C. virginica* (<5.5 mg/l; Table 2.1) during the second trial.

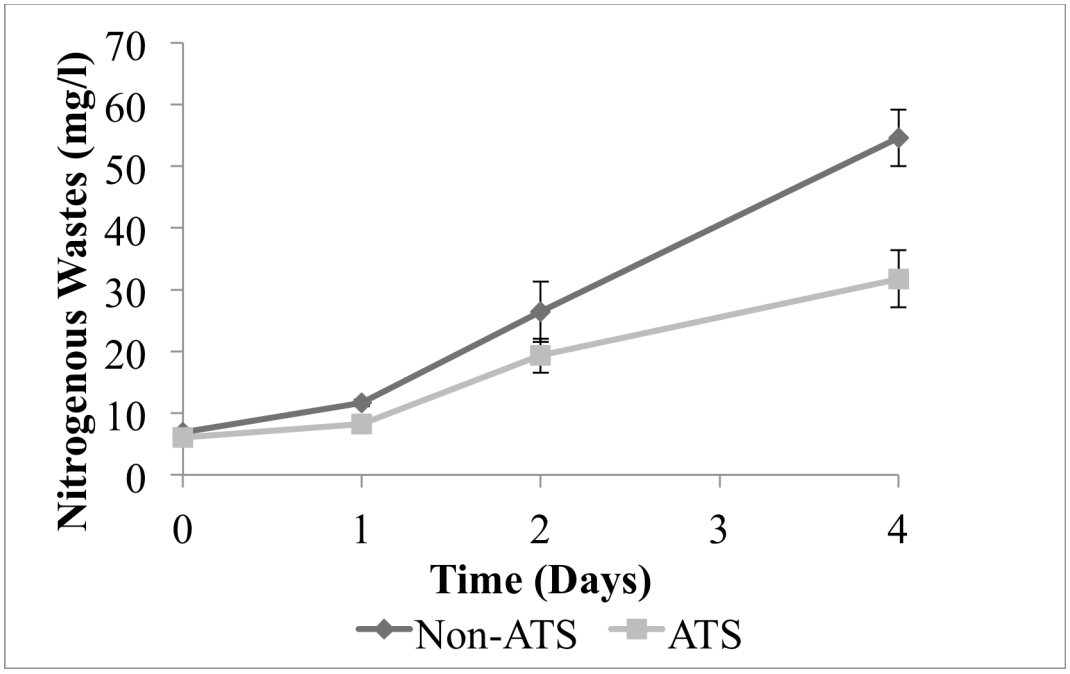

Figure 2.6. Mean dissolved nitrogenous waste concentration (ammonium-N, nitrate-N) in systems with and without ATSs during the second 96-hr trials.

The aquaculture system without treatment (the control) had the highest ammonium-N concentrations (53.2 mg/L NH_3-N) after 5 days of being stocked with oysters from the Bay, while units with ATS systems had the lowest concentrations (31.47 ± 4.63 mg NH_4) but was not statistically significant. Nitrate-N concentrations

were within the tolerance level for C. virginica (0.0-460.0 mg/l; Table 2.1) and were not significantly different in our trials.

3.4 pH

All systems showed a decline in pH from the initial conditions and no treatment had any effect on the pH levels. Overall pH showed a negative trend during the experiment (Figure 2.7).

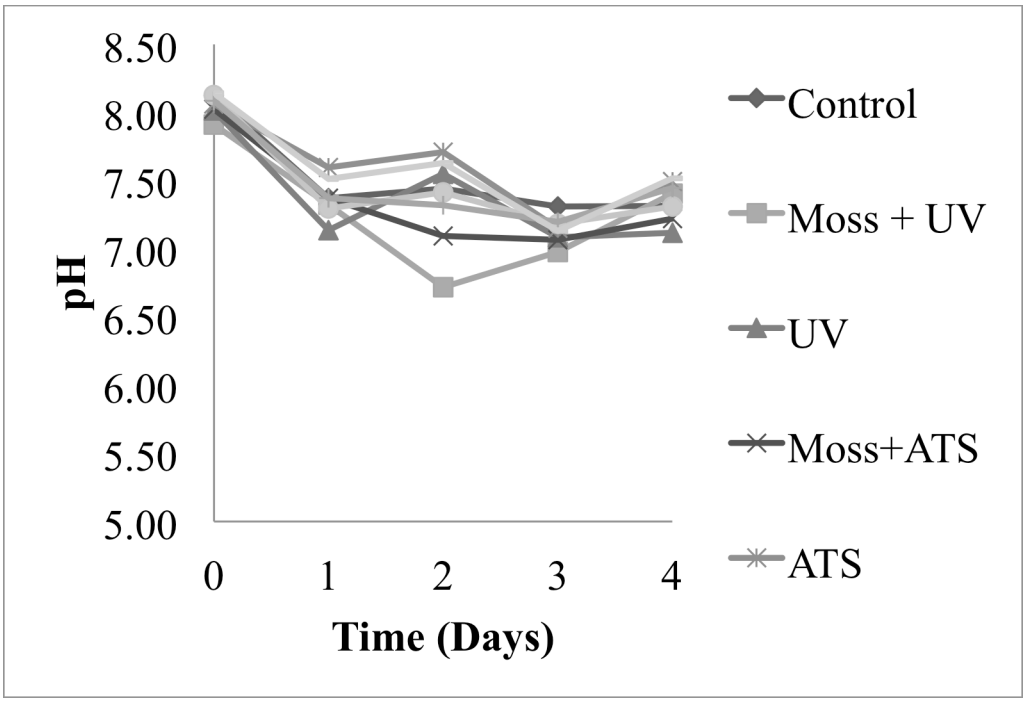

Figure 2.7. Mean pH in RAS systems during the two 96-hr trials. pH did not exhibit significant differences between treatments, but showed a overall negative trend during the experiment.

3.5 Coliform Bacteria

Figure 2.8 shows the coliform concentrations during the study. Some of the tests measured results that were higher than the maximum range of our test and were not included in the data set; however these indicated a much higher loading of bacteria. This occurred in the unit with sphagnum moss on its own during the first

trial at 48 hours, and during the second trial at 48 hours and 96 hours, in the unit without treatment (control) during the second trial at 96 hours, and in the unit with ATS on its own during the second trial. Therefore we analyzed the results of the change in Total Coliform after 48 hours in our ANOVA statistical test (Table 2.6).

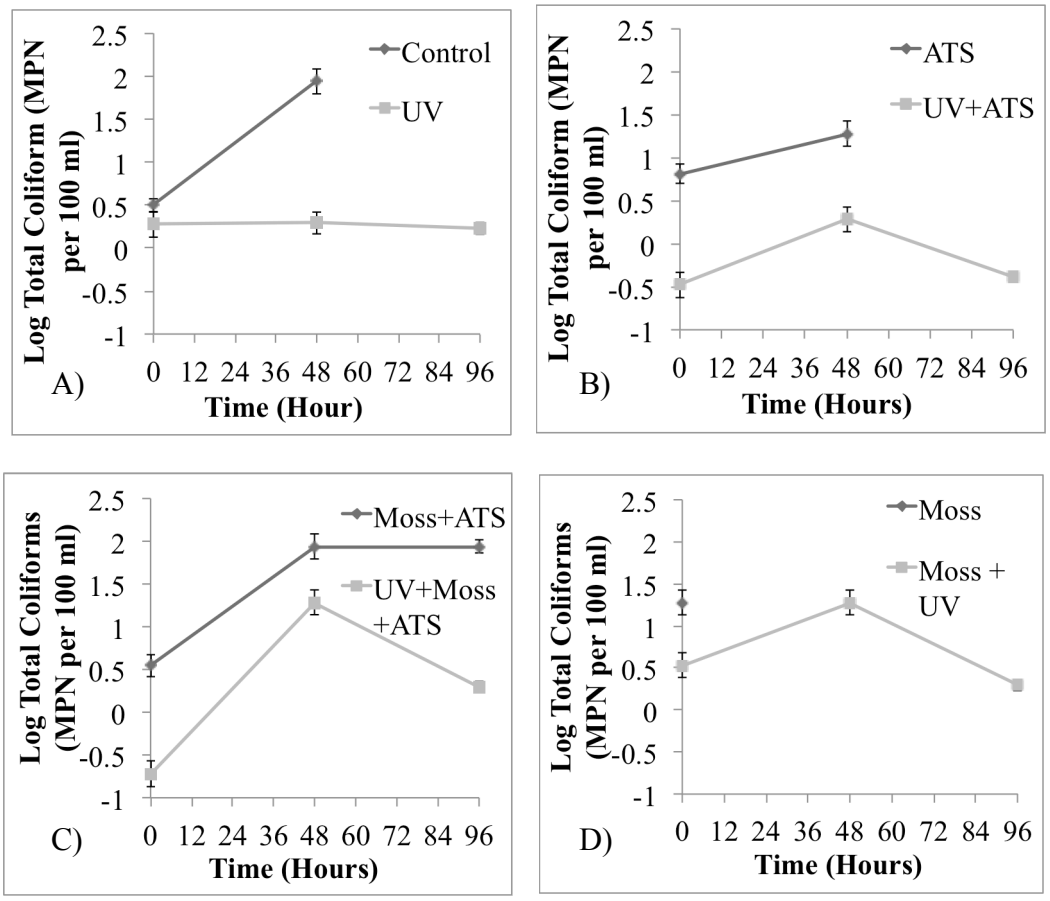

Figure 2.8. Mean total coliform bacteria concentrations (n=2) for A) control unit without treatment and unit with UV-C alone, B) unit with ATS and unit with ATS and UV-C, C) unit with moss and ATS and unity with UV-C, moss and ATS, D) Unit with moss alone and unit with moss and ATS. Test results that were above the range of detection are not included in the data set; therefore we did not have a large enough sample to make conclusions about the effect of sphagnum on coliform bacteria.

Systems fitted with UV-C disinfection units had significantly lower increases in total coliforms after 48 hours ($p=0.0065$) (Table 2.9). The control unit and the

moss + ATS unit showed a trend of higher bacterial load, but was only significant when α=0.10 (Table 2.6).

Table 2.6. ANOVA table for the mean change in total coliform bacterial counts (Log MPN/100 ml) from initial conditions to the end of the two trials.

Source of Variation	Sum of Squares	Degrees of Freedom	Means Squared	F-Statistic	P-value
UV	0.30858328	1	0.30858328	13.297274	0.0065
ATS	2.82676418	1	2.82676418	121.809121	0.1312
Moss	0.09593975	1	0.09593975	4.13417461	0.7647
UV x ATS	0.14309461	1	0.14309461	6.16614176	0.7151
UV x Moss	2.97092414	1	2.97092414	128.02117	0.1231
ATS x Moss	3.70388527	1	3.70388527	159.605464	0.0905
UV x ATS x Moss	1.94074112	1	1.94074112	83.6291798	0.2011
Error	0.18565205	8	0.02320651		

3.6 Excretion Rates and Nutrient Uptake by ATS

Oysters execreted NH_4-N and NO_3-N at a rate of 0.05 ± 0.03 mg-N g-oyster^{-1}(wwt) day^{-1}. Our total daily nitrogen load was 3.05 g-N day^{-1}. In comparison, the 1-m^2 algal turf scrubber^{TM} removal rate of nitrogenous wastes was 1.7 ± 0.5 g-N day^{-1}, which was 56% of the nitrogen-loading rate.

The rates of nitrogen uptake climbed initially (Fig. 2.9), reaching a maximum of 2.6 ± 0.3 g-N day^{-1}, and dropped to 1.7± 0.2 g-N day^{-1} on the final day of the experiment.

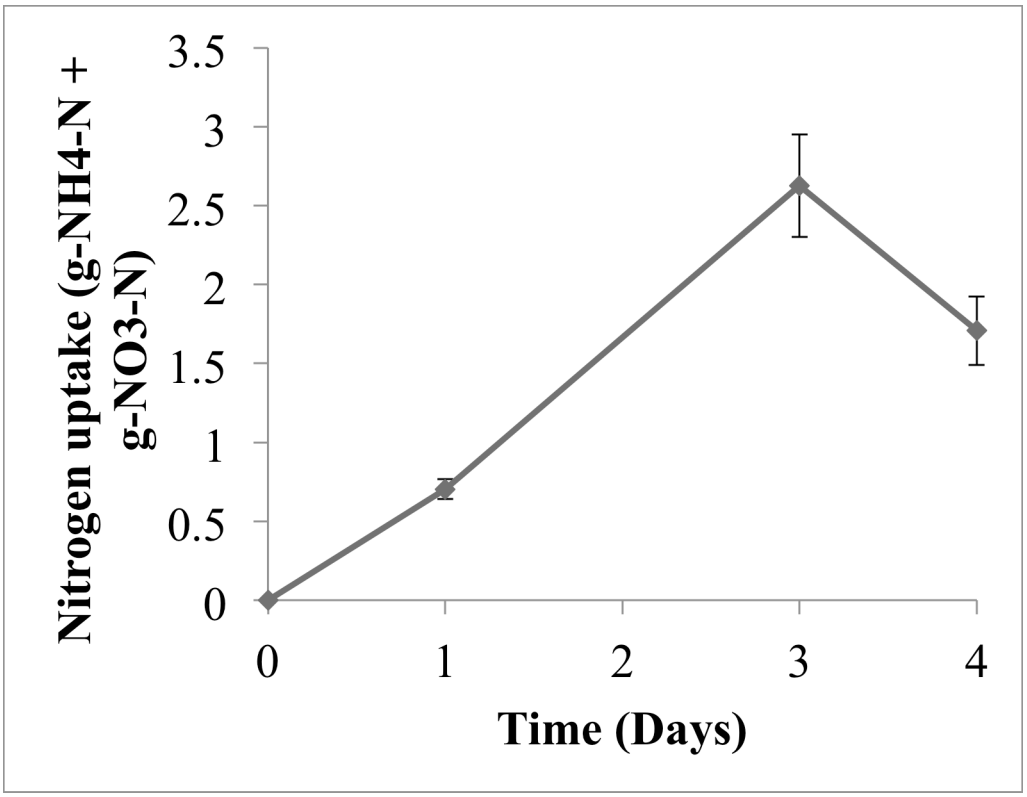

Figure 2.9. The mean nitrogen (NH_4-N and NO_3-N) take up bu the ATS units during the course of the experiment. Uptake rates climbed initially but dropped on the fourth day.

3.7 Correlations in Water Quality

When water quality parameters were correlated in a Pearson's correlation, we found a moderate to strong inverse correlation between DO and temperature in all of the units (Table 2.10). Water pH and temperature were moderately inversely correlated in the control unit, ATS-only unit, the moss-only unit, and the UV x moss unit. Temperature and coliforms counts were only correlated in the UV-only unit, the ATS-only unit and the UV x moss unit. DO and water pH were often correlated, and water pH was often negatively correlated to total coliform counts.

Table 2.10 Pearson's Correlation Table for Water Parameters.

Parameters	Control	UV	ATS	UV x ATS	Moss	UV x Moss	ATS x Moss	UV x ATS x Moss
Temp x DO	-0.77874 **<0.0001** 20	-0.53265 **0.0061** 25	-0.812 **<0.0001** 19	-0.60697 **0.0045** 20	-0.51085 **0.0213** 20	-0.73833 **0.0003** 19	-0.59857 **0.0053** 20	-0.87017 **<0.001** 20
Temp x pH	-0.73138 **0.0013** 16	-0.39218 0.0787 21	-0.641 **0.0074** 16	-0.70885 **0.0021** 16	-0.49964 **0.0488** 16	-0.69102 **0.0102** 14	0.11645 0.6563 17	-0.37241 0.1555 16
Temp x Coliform	0.65893 0.1075 7	-0.51163 **0.0428** 16	0.7607 **0.0471** 7	0.70952 **0.0487** 8	-0.07252 0.9077 5	0.57721 0.1341 8	0.74379 0.0553 7	0.71293 0.0721 7
Temp x DIN	0.1915 0.8085 4	0.06824 0.8724 8	0.557 0.443 4	-0.45593 0.5441 4	-0.55901 0.441 4	-0.45498 0.545 4	-0.16724 0.8328 4	-0.88461 0.12 4
DO x pH	0.91421 **<0.0001** 14	-0.89768 **<0.0001** 20	0.6852 **0.0097** 13	0.80699 **0.0005** 14	0.23987 0.4088 14	0.65981 **0.0102** 14	0.18929 0.5169 14	0.45973 **0.0338** 15
DO x Coliform	-0.99745 **0.0026** 4	-0.79649 **0.0002** 16	-0.962 **0.0379** 4	-0.64299 0.2517 5	- - 2	-0.64947 0.1628 6	-0.32152 0.5343 6	-0.20947 0.7353 5
DO x DIN	-0.74855 0.4615 3	0.72516 **0.0418** 8	0.5643 0.4357 4	0.14733 0.4487 4	0.58312 0.4169 4	0.69647 0.3035 4	0.18563 0.8144 4	-0.26493 0.74 4
pH x Coliform	-0.81273 **0.0493** 6	0.75445 **0.0018** 14	-0.569 0.2383 6	-0.70144 0.079 7	0.7576 0.2424 4	-0.95368 **0.0009** 7	-0.88318 **0.0084** 7	-0.85366 **0.0306** 6
pH x DIN	-0.45552 0.5445 4	-0.97394 **0.001** 6	-0.179 0.8208 4	0.55133 0.4487 4	0.15764 0.8424 4	0.53752 0.4625 4	-0.06758 0.9324 4	0.64854 0.3515 4
Coliform x DIN	1 - 2	-0.78109 **0.0381** 7	- - 1	-1 - 2	- - 0	-1 - 2	0.55469 0.6257 3	-1 - 2

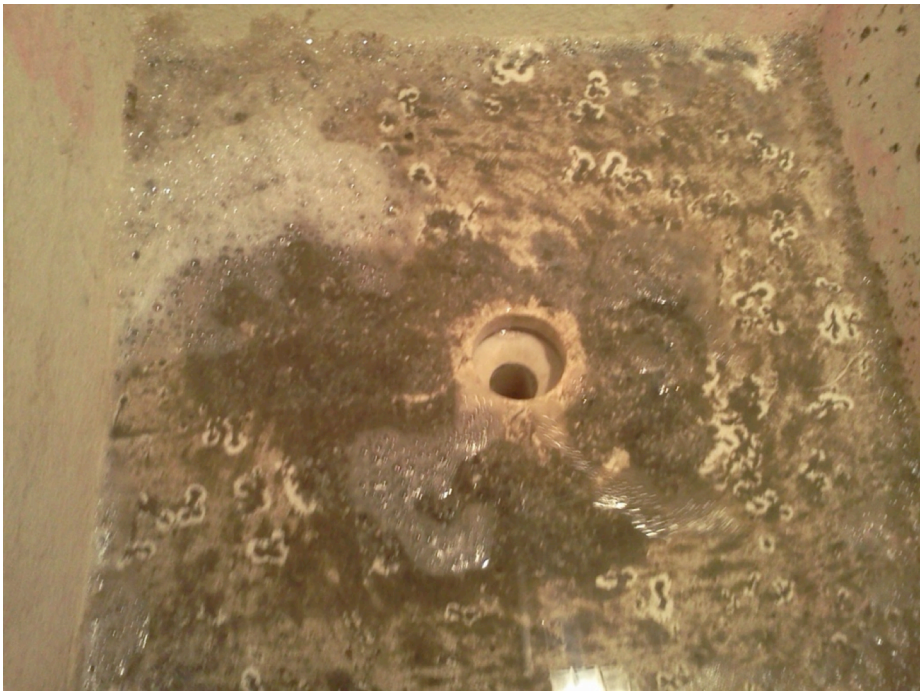

Figure 2.10 Oyster waste, shell particles and annelid worms collected at the bottom of the culture basin.

3.8 Qualitative Observations

In both trials, we observed excessive foaming in the aquaculture systems (Figure 2.10). Although foaming was not measured, the observations are important for aquaculturalists who wish to build salting systems like those in our study. There was no visible difference between the experiemental units, suggesting that none of the treatment technologies affected the amount of foam.

Additionally, we found large numbers of small, annelid worms (approximately 2 cm long) at the bottom of the cutlure basins, in the algal turf scrubbers^{TM}, and the settling basins after draining the system. We found similar worms attached to oyster shells while measuring oyster weights.

4. Discussion

4.1 Dissolved Oxygen, pH and Temperature

ATS systems increased DO concentration and decreased temperature but did not affect pH, as initially expected. The marginal increase in DO (<1 mg/l) was not critical from a practical sense since levels of DO in all systems were above the (>3.5 mg/l threshold for oysters. The difference in DO could be attributed to the photosynthesis of the algal community in the ATS. Considering the diel shifts from photosynthesis to respiration, DO was likely lower in the dark hours when the lights were switched off. However, we did not measure DO concentrations during the dark hours, which may have been important in determining the overall DO effect of the ATS systems. Many observers have documented significantly high DO concentrations during the day and substantial decreases during the dark hours in systems with substantial algal communities (Nimick et al, 2010).

The absence of DO production during dark hours may have also been marginal because it is likely that DO concentrations were more influenced by water movement and temperature. The aquaculture systems had a flow rate of 30 l min^{-1}, which means that the culture basin, where DO was sampled *in situ*, experienced a complete water exchange 11.2 times per hour (or once every 5.3 minutes). Ample circulation allows for greater contact time with the atmosphere for oxygen diffusion. Additionally, our Pearson's correlation test showed a significant strong inverse correlation between temperature and dissolved oxygen, and a correlation between water pH and DO. Therefore it is possible that the differences in temperature explain the differences in DO concentration. Water pH is generally not linked directly to DO

and the significant correlation is more likely the cause of two parameters decreasing during the same time period, due to respiration by oysters, rather than by directly affecting each other.

The uneven temperature across experimental units could have been attributed to differences in equipment (e.g. UV lights, different pump ages), the position of experimental units in the laboratory, or differential evaporative cooling from the ATS systems.

The UV lights and the pumps are electrical equipment that could have added heat to the water, as both were in direct contact with it. If the additional heat originated from the UV lights, then we would have seen a significant effect on temperature from the UV treatment, which we did not (see results; Table 2.6).

The centrifugal pumps in our experiment use contact water to cool the motors during operation. Coincidentally, the four (4) pumps used with the non-ATS treatments were older and had been used previously, while the other four (4) pumps used with the ATS treatments were newly purchased and unused. It is possible that the older pumps generated more heat due to their age and previous use. It is also likely that both factors --pumps and evaporative cooling in the ATS system-- contributed to water temperature differences. Regardless, we cannot conclude that the ATS systems alone had an affect on water temperature.

Water pH was unaffected by treatment factors and decreased over the course of the experiment. This is surprising since algal communities are known to raise pH and *sphagnum* spp. are known to lower it (Clymo, 1964; Andrus, 1986) but neither had an effect. In other studies, algal turf scrubbers^{TM} were shown to significantly raise

pH by the removal of carbon dioxide from water by algae, decreasing carbonic acid (H_2CO_3) and by extension the available hydrogen ions, increasing the pH. In our system we instead observed declining pH caused by the addition of respiration in oysters that out stripped photosynthesis and contributed CO_2 and decreased pH.

This point illustrates the need to carefully consider the ratio of respiration to photosynthesis when designing a constructed ecosystem to manage water quality. If respiration exceeds photosynthesis, CO_2, the primary waste product of respiration, builds up and affects the quality of the system. Therefore the ATS systems may have been too small to absorb enough CO_2 from respiration to prevent drops in pH.

4.2 Dissolved Nitrogenous wastes Management

Just as waste CO_2 from respiration accumulated in the systems, dissolved nitrogenous compounds also accumulated. Ammonium concentrations were over the recommended tolerance level for oysters (Table 2.1; Epifanio et al*,* 1976) and dissolved nitrogenous wastes reached concentrations considered hypertrophic for natural waters (Smith et al, 1999); however we did not observe any mortality during the course of the experiment. The 1-m2 algal turf scrubber^{TM} was able to reduce nitrogenous wastes concentrations to some extent, but were under sized. A larger surface area for the ATS periphyton community would be needed to maintain N concentrations below levels of concern.

Others have found that algae-based filtration systems were more effective when waters were more dilute than our systems and had less animal biomass (Cahill et al, 2010). This meant that we needed either less animal biomass, and therefore

more dilute wastes, or we needed a larger algal turf scrubber, to maintain acceptable water quality conditions.

This does not mean that ATS systems are not suited for aquaculture; but it highlights the need for proper sizing in the design process. Design of such systems would require accurate nutrient loadings to appropriately size algal turf scrubbers.

Our calculated rate of nitrogen uptake by the ATS systems was 1.7 ±0.5 g-N day^{-1} compared to the calculated excretion of 3.0 g-N day^{-1}, meaning that our ATS systems would need an algal turf area of least 1.8 m^2 to effectively manage water quality under similar conditions. There is also the possibilty that the ATS was limited by the available light from the single 400-W metal halide bulb on a 12-hour photoperiod on each system. The lamps produced between 68 and 72 W m^{-2} when measured with a pyranometer, which is considerably lower than global average of 340 w m^{-2} (NASA, 2012) however, our nitrogen removal was similar to the average annual nutrient removal rates in an ATS system used treat wastewater in Craggs et al (1996; 1.11 ± 0.48 g-N m^{-2} d^{-1}) but differed from removal rates in ATS systems used on tributaries of the Chesapeake Bay under diluted nutrient regimes as in Mulbry et al (2010; 0.25 g-TN m^{-2} day^{-1}).

The foam we observed in the aquaculture systems is an indicator of poor water quality caused by dissolved organic material that can break down into ammonia by microbial action (Wheaton, 1993). The foam may have contributed to the high amounts of ammonia observed in the study. These dissolved organics are mucus that oysters leak, perhaps to stimulate phytoplankton regeneration as hypothesized by Cognie & Barille (1998). In our system, this mucus may have

decomposed to ammonium and nitrate that could explain the higher concentrations of nitrogen that we observed.

4.3 Disinfection

Our aquaculture systems were not able to completely disinfect the water in the culture basins, but the UV treatment was able to reduce concentrations of coliform bacteria after the first 48 hours. However due to an error in the technique, measurements that were over range were excluded from our analysis, which makes drawing conclusions difficult from the data. Because of this, we could only statistically analyse data from the first sampling (initial conditions) and the second sampling (after 48 hours of the experiment).

Conditions in the aquaculture system were condusive to bacterial growth. Concentrations of coliform bacteria, are influenced by its source, exposure to solar and UV radiation, nutrient availability, predation, suspended particulate matter and turbidity (Campos et al., 2013). Therefore bacterial growth in our systems was likely fed by excess nutrients as evidenced by excessive foaming, oyster feces and psuedo-feces, dissolved nitrogenous wastes, and visible particulate matter (see Figure 2.8) that accumulated over time.

Suspended organic matter and particulates that accompanied the oyster into the system could have provided substrate for bacterial attachment. Such attachment by microbial communities has been observed in many studies (Characklis et al., 2005; Fries et al., 2006; Walters et al., 2013) and allows bacteria to avoid disinfection by chlorination and avoid UV-C light (LeChevallier et al., 1988; Walters et al., 2013).

Trapped air bubbles and particulate matter in suspension could have degraded the killing affect of UV light. Because the effluent from the settling basin was not pressurized, there was head-space in the pipes. The pumps then mixed this air with the water as it was delivered it to the UV lights. Suspended air bubbles could have prevented complete penetration of UV through the water, protecting a portion of the bacteria from being eliminated. Moreover, suspended matter could have provided refuges for bacteria as they passed through the UV disinfection unit. Therefore suspended solids are important measures to consider for aquaculture in additional to careful engineering.

However, it is clear whether suspended particulate matter and water clarity affected the anti-septic properties of the sphagnum moss application. Stalheim et al (2009) noted the anti-septic capacity of sphagnum mosses is linked to the reduction in pH by the sphagnum leaves; however we did not see a considerable change in pH. Reef salts used to produce artificial seawater have a pH buffering capacity by the inclusion of calcium (Ca^{+}), and this may have inadvertendly reduced the anti-septic effect of sphagnum moss. It is likely that sphagnum mosses may only be effective as an anti-septic in solutions without a notable buffering capacity, such as rain water or de-ionized water.

Temperature of the water also has an important control on microbial growth. A few degrees increase above 15C can promote rapid microbial growth. Temperatures close to 4C, will slow growth to a minimum. A comprehensive study of drinking water storages and facilities by LeChavellier et al. (1996) found that densities of coliform bacteria increased 18-fold from 0-5° C to above 20° C in

free-chlorinated water systems. LeChavellier et al. (1996) also found that water systems with low velocity storage tanks had the highest counts of bacteria. In our systems temperatures ranged from 24° C to 29° C and included low velocity settling basins. These two factors could have elevated coliform bacterial concentrations.

Vibrio vulnificus and *Vibrio parahaemolyticus* are the principle bacteria of concern for oyster aquaculture. These pathogens are naturally associated with oysters and can cause gastrointestinal illness in humans when consumed in high concentrations. The harmful occurrence of *Vibrios* is correlated with temperature where oysters are harvested, stored or processed (Duan & Su, 2005). Higher temperatures cause them to multiply to infectious dosages. Tamplin and Capers (1992) found that depuration with UV-C light in artificial seawater at 15 ppt reduces *v. vulnificus* concentrations only when water temperature is less than 15° C. This suggest that water temperatures in an indoor salting system should be maintained at 14°C or less.

In contrast, water temperature and total coliform counts was only significantly correlated in four (4) of the our experimental units --the UV-only unit, the ATS-only unit, the UVxATS-unit, and the UVxATSxMoss unit-- and these correlations were moderate. It may be that total coliform counts were more influenced by biomass loading than temperature in our case.

Another factor for the growth of *Vibrio* is salinity (Motes et al., 1998; Su et al., 2010). Salinity higher than 25 ppt can help reduce *Vibrio* concentrations in the oyster tissue (Motes et al., 1998; Su et al., 2010). Therefore systems for depuration

and salting oysters could improve disinfection by maintaining low temperatures and a high salinity.

4.4 Future Research

Future research on this aquaculture system could be improved by re-configuring the experimental design to include a larger sample size and number of replicates. Space requirements for aquaculture studies should be carefully considered as ATS systems require considerable space to function properly. Temperature control is also an important component of an experiment in water quality. Other measurements that should be included in further research into these systems include turbidity, regular TSS measurements, and characterization of settled solids to appropriate size solids fitration.

5. Conclusions

The goal of the experiment was to test the ability of three technologies to treat water quality in a recirculating aquaculture system used to salt large numbers of oysters during brief periods of time. Our results showed that the ATS system was the most effective treatment system for managing water quality in our systems. UV is likely to have been significantly effective for disinfection but our data was not able to support this conclusion, due to missing data points. There were not interaction effects uncovered from our experimental design, so we cannot conclude that the operation of the three technologies in concert had any effects on one another. They neither hampered nor enhanced the functioning of one another.

Overall, We determined that:

- UV-C reduced the growth of suspended total coliform bacteria.
- ATS systems reduced the accumulation of nitrogenous wastes and promoted higher oxygen levels during well-lit hours in the aquaculture system.
- Sphagnum moss did not affect any water quality parameter in our experiment but we cannot conclude that sphagnum was ineffective at disinfection because of missing data points.
- The factorial experiment design did not detect any interactions effects that either enhanced or hampered the function of the three technologies.
- Disinfection is important in a system that stores live oysters for human consumption because of the high amounts of coliform bacteria present in our systems.
- Nitrogenous waste management is needed in such a system due to the high rates of excretion and accumulation of ammonium and nitrate observed in our study.

Algal turf scrubbers showed the most promising results, and we were able to determine the nutrient uptake rates and relate them to excretion rates by oysters such that future designs can be sized to meet biomass-loading rates for oysters and other organism cultivated in recirculating systems.

Inland recirculating aquaculture relies on effective water treatment to reduce water consumption (Verdegem et al 2006), reduce disease, produce a quality product, and to reduce the potential for eutrophication when water is discharged to the

environment (Wilfart et al 2013). An effective recirculating aquaculture system could not be achieved by our treatment systems in this study, because hypertrophic conditions (high concentrations of NH_4+ and NO_3) and total coliform levels were higher than required by the National Shellfish Sanitation Program (US FDA, 2013). This is due to poor design of the aquaculture systems that allowed air bubbles to degrade the UV killing effect, and incorrect sizing of the ATS systems, which needed to be larger to treat the nutrient loads we witnessed.

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CHAPTER 3: Emergy Analysis of Oyster Aquaculture

Abstract

Cultivated oysters are often preferred by consumers and promoted by policy-makers, scientists and industry leaders because of an increasing awareness that wild fisheries are being depleted. Changes in governmental policy has made the process of leasing faster and simpler such that oyster aquaculture is a growing industry in Maryland, reflecting overall global growth of aquaculture in recent years. Despite this growth, scientific information on the impact of shellfish aquaculture is currently scarce and unclear. Emergy evaluation provides a scientific method of evaluating the sustainability and environmental impact of oyster aquaculture at a local and global scale. The objective of this study was to compare two oyster aquaculture farms that use differing methods of cultivation –floating raft aquaculture and bottom cage aquaculture– through an emergy analysis to quantify sustainability and environmental impact through emergy indicators. Emergy accounting was used to integrate the contributions of nature and the human economy to determine the sustainability of the two farms. The results showed that both farms are intensive systems, driven primarily by anthropogenic inputs. The emergy analysis did not favor either method, but the floating cage aquaculture had a lower environmental impact and a higher use of renewable emergy.

1. Introduction

Over-harvesting, habitat destruction and diseases have decimated the once abundant native population of Eastern oysters, *Crassostrea virginica*. Current oyster biomass estimates are thought to be 0.03% of the historical abundance in the early 1800s (Wilberg, et al, 2011). Dredging for oysters has destroyed 70% of suitable habitat from 1980 to 2009 (Wilberg, et al, 2011). The risk of extirpation is so high that scientists have recommended a moratorium on harvesting wild oysters and have promoted oyster aquaculture to alleviate stress on wild populations while maintaining a commercially important fishery (Mann and Powell, 2007; Wilberg et al, 2011).

The state of Maryland holds its portion of the Chesapeake Bay in the public trust and issues water-column leases to private oyster aquaculture businesses. There are currently 46 water-column leases, totaling 175 acres of Bay water column area for cage aquaculture and 30 additional water-column leases under review that would add approximately 115 acres of oyster aquaculture operations in the Chesapeake Bay (Maryland Department of Natural Resources, 2013).

Cultivation of the native Eastern oyster (*Crassotrea virginica*) has only recently been developed in Maryland along the Chesapeake Bay. Changes in fisheries management policy have made bottom leases more accessible to aquaculturalists (MD Department of Legislative Services, 2013) and the development of large-scale hatcheries in the region have made triploid stock more accessible, both for restoration efforts and aquaculture. A significant development in the Chesapeake Bay has been the success of the Horn Point Hatchery at the University of Maryland's Center for Environmental Science on the Eastern shore and the hatchery at the Virginia Institute of Marine Science in Norfolk, Virginia. These hatcheries now

produce selected genetic stocks of disease resistant, triploid oysters that are available for aquaculturalists to purchase at the eyed-larvae stage, clutched spat or attached to shell (spat-on-shell) for cultivation in open-systems.

The choice of larvae over clutched spat matters because eyed-larvae must first be allowed set to shell and grow to certain length before they can be deployed in the open estuary. First, the animals are set to tiny bits of shell in an on-shore aquaculture system. This process is referred to as "remote setting." Once they have set to shell, the oysters are then placed in protected nursery systems before they are deployed in cage or rafts in the estuary.

Cultched spat is larvae that have already attached to shell and can be immediately deployed into nursery systems or directly to the bay, depending on its size. In this case the setting process is done at the hatchery and oysters are grow to a certain size before being sold to aquaculturalists.

In 2011, an oyster aquaculture enterprise was established in an area on the Chesapeake Bay known as Tar Bay. The growers began producing large quantities of oysters for restaurants and wholesalers in the half-shell market; a high-end portion of the market. Over the course of 3 years, the farm became one of the largest producers of oysters in the state.

The farm developed new technologies for cultivating oysters including a customized boat for harvesting and maintaining cages, and a floating and on-shore nursery system. After oysters have grown to a certain size in the nursery system, they are placed in cages on muddy bottom in the estuary. These cages require regular maintenance and must be removed by power winch due to their weight. The farm

built a customized 36-ft boat that houses process equipment such as a power-winch, tumbler and pressure washer such that the boat functions as a mobile processing unit. I refer to this farm as the bottom cage aquaculture site in this paper.

An older farm (referred herein as the floating raft aquaculture site) established in 1999 along the Choptank River sub-estuary serves as a contrast to the bottom cage aquaculture site because of it's lower level of technology. The farm uses a different method of grow-out using floating rafts that hold oysters. Cultched spat (> 5mm in shell length) are purchased from a hatchery and deployed in plastic mesh bags on floats made of polyvinyl chloride (PVC) piping. These floats are placed along the Choptank river, close to shore where they can be managed by hand in shallow water. Because the oysters are held afloat in rafts, there is little need for machinery to remove and maintain the rafts.

The environmental impact of such oyster aquaculture systems is presently unclear. Nationally, disputes have arisen over aquaculture and the appropriate use of estuaries that have led to lawsuits in California (Drakes Bay Oyster Company V. Salazar, 2013) and Maine (Bernstein, 2007). Critics argue that aquaculture operations disrupt the local estuary environment and tarnish natural beauty. Proponents point to water quality improvements through filter-feeding and to sustainability as reasons why oyster aquaculture should be permitted in bays and estuaries.

Either position is difficult to argue given the complexity of the estuary environment. Regarding positive impacts, nutrient-remval via filter-feeding by cultivated oysters is unlikely to have a significant affect on water quality. Higgins et al (2011) assessed the potential of oyster aquaculture to remove nutrients (C, N, P)

from the estuarine environment through the process of bioassimiliation. They found that a harvest of 10^6 market-sized oysters (76 mm in shell length) removed 132 kg TN yr^{-1}, 19 kg TP yr^{-1}, and 3823 kg TC yr^{-1} through bioassimilation into shell. Flesh is not counted as a method of nutrient removal because it is reminieralized and returned to the system through consumption and decomposition.

Considering the rate of nutrient loadings into the Bay ($8.4x10^7$ kg-TN yr^{-1}; $4.03x10^6$ kg-TP yr^{-1}; USGS, 2000), the removal of nutrients through bioassimilation is insignificant. Therefore filter-feeding by bivalves cannot be relied upon to make a significant impact when nutrient loadings are several orders of magnitude higher than can be removed.

Another possible benefit is the provision of artificial reef structures in the form of oyster cages as habitat for estuarine species. Researchers have found commercially important finfish in and around aquaculture gear in greater abundance than muddy bottom areas of estuaries and bays (Tallman and Forrester, 2007; Erband and Ozbay, 2008; Marenghi and Ozbay, 2010). On the contrary, some consider that oyster aquaculture operations could compete for space with submerged aquatic vegetation (SAV) zones that also provides critically important habitat for fish, but research has not been conclusive in this area (Forrest et al, 2009).

However, considering that the primary water quality concern is the addition of nutrients to the Bay, oyster aquaculture certainly adds none. There is no artificial feed added to the system and the entire food budget of cultivated oysters is taken from the local environment. In that regard, oyster aquaculture may have a minimal impact on

water quality; neither degrading it significantly nor improving it in any meaningful way.

Beyond local ecological assessments, few scientific studies have provided quantitative information regarding the sustainability of oyster aquaculture and its impact on a global scale. We know of only one report that has attempted to quantify the sustainability of oyster aquaculture through carbon footprint accounting in the United Kingdom (Scottish Fisheries Research Forum, 2012). They found that oysters cultivated in the UK produced 1,281 kg CO_2-eq per MT of oysters produced at farm gate. This meant that from the hatchery stage to packaging, each kg of oyster produced 1.281 kg CO_2. More than half of the CO_2 is emitted during the management of the grow-out period, when oyster bags are constantly cleared of fouling organisms, sorted and re-bagged by size to allow for faster growth.

Carbon footprint accounting gives us an estimate of the amount of electricity and fuels that are consumed during the lifetime of a product, but it does not give us an estimate of the underlying energy basis for a system. It does not provide an objective measure of sustainability; instead we assume that processes or products with considerable carbon footprints are unsustainable because of their reliance on fossil fuels, which by nature are finite in abundance.

Ulgiati and Brown (1998) define sustainability by investigating two essential aspects of systems or processes. They argue that to be sustainable every process within a system must be environmentally sound, i.e. that it does not have negative environmental impacts that would hamper the systems productivity, and that every

system must provide a net benefit, or yield, to society. The system does not consume more resources than it produces.

If we accept this definition of sustainability, then carbon footprint accounting only gives us a measure of the first component –an estimate of the negative impact of the process or system. It does not provide us with an estimate of the net benefit to society or the system that contains it. This is an important factor in sustainability, because systems are nested within larger systems upon which they rely. If a system cannot provide a net benefit to the system that contains it, then the larger system becomes less productive and therefore less able to sustain internal sub-systems. By this manner, systems that do not provide a net benefit to society ultimately degrade their own ability to last. Therefore in order to evaluate the sustainability of a system or product, one must obtain an objective measure of sustainability that incorporates both determining factors –impact and yield.

Emergy analysis provides a systematic approach to evaluating impact and yield, by accounting for all inputs and outputs of a system within a framework that can integrate contributions from nature and the human economy on the same unit of measure. Impact can then be evaluated by the ratio of inputs brought in from the economy to the inputs from local free environmental inputs. Yield can be evaluated by a similar ratio that compares the amount of free renewable and non-renewable inputs from the environment to the amount from the human economy. Presumably, systems that utilize more free renewable inputs from the environment will have higher net yields and lower impact on the environment and are therefore more sustainable than those that do not.

Odum (1996) established a methodology for emergy analysis that we use in this study. Odum (1996; pp 7) defines emergy as "the available energy of one kind previously used up directly or indirectly that was used to produce a service or product." Emergy analysis then quantifies all direct and indirect inflows and outflows of emergy of a product or service with the same unit of measure, allowing for comparisons of different types of resources and energy. In this manner, the work of the environment and the human economy can be compared in a single analysis. In this paper, we quantify the inflows and outflows of emergy through two oyster farms in the Chesapeake Bay in order to evaluate the net emergy yield and environmental impact to make an objective statement about the sustainability of oyster farming.

Objective

The objective of this study was to quantify the sustainability of two oyster farms in the Chesapeake Bay with different local environments and grow-out methods. We determine the transformity of cultivated oysters, the percent of renewable emergy, the emergy yield ratio (EYR) and the environmental loading ratio (ELR) in order to quantify the sustainability of the two oyster aquaculture sites.

2. Methods

2.1 Site description

2.2 Overview

This study was performed using two 5-acre oyster aquaculture sites in the middle Chesapeake Bay. The Chesapeake Bay is a wind-dominated, micro-tidal estuary that receives a mean river discharge of 7.13×10^{10} m^3 yr^{-1} in its middle to

upper region from four major rivers; the Susquehanna, the Potomac, the Patuxent and the Choptank rivers (DNR, 2009).

The present study was carried out at two oyster aquaculture sites that use different methods of growing live oysters in the Chesapeake Bay estuary (Maryland, USA). The reared oysters in both sites are hatchery-produced disease-resistant triploid strains of Easter oysters (*Crassostrea virginica)*.

The aquaculture sites are located in designated Aquaculture Enterprise Zones (AEZs) that are certified by the state for oyster aquaculture based on water quality and location (i.e. not located in protected SAV zones, buffer zones, or oyster sanctuaries).

Table 3.1
Site Characteristics of the two Aquaculture Enterprise Zones

Characteristics	Bottom Cage Site	Floating Raft Site
Location	Tar Bay	Choptank River
Coordinates[a]	38°18'N, 76°13' W	38°37'N, 76°10' W
Area[a]	23,216 m^2	9,811.8 m^2
Bottom[a]	Muddy	Sandy
Depth[a]	1.45 m	0.91 m
Stocking Density[b]	194 oysters/m^2	153 oysters/m^2
Annual Yield[b]	4,500,000 oysters	1,500,000 oysters
Seed-Stock[b]	triploid eyed-larvae	5 mm triploid clutched spat

[a]Source: Maryland Department of Natural Resources.
[b]Source: Interview with farmers and direct observation.

Table 3.1 shows the different site characteristics between the bottom-cage and the floating-raft farms with regards to location, water characteristics, yield and stocking densities. Both farms bring oysters to market that are roughly the same size (>76 mm in length) and similar mass of oyster meat produced per unit (1.58 g-meat oyster^{-1}).

2.3 Bottom Cage Aquaculture Site

The bottom-cage facility purchases eyed-larvae from a hatchery and uses a process known as remote-setting to attach larvae to small bits of oyster shell in a land-based recirculating aquaculture tank. Once the larvae have set to shell, they are transferred to a flow-through aquaculture tank where they are fed on microalgae, particulate organic matter and dissolved organic matter from natural estuarine water (Figure 3.1). Once they reach a certain size, the young oysters are again transferred to a floating flow-through aquaculture system for further growth (Figure 3.2). Once large enough to be safely deployed in cages, the oysters are then transferred by boat to the AEZ where they remain in cages on bottom until they reach market size (76 mm). The bottom cage farm has 2000 such cages that sit on the bottom each holding 1500 to 3,000 oysters, depending on their growth stage.

The bottom cages require regular maintenance that must be done by boat. Cages are frequently removed from the bottom and the oysters are sent through a customized washing and tumbler system that cleans and sorts the oysters by size. The cages are then power-washed and cleared of fouling organisms and sediment to allow for unrestricted water flow. The sorted oysters are then restocked in cages. The

sorting is important to prevent over-crowding in cages as the oysters grow. Re-grading in this manner allows the oysters more room to grow within the cages.

Because the bottom cage AEZ is located 2 miles from the facility, the aquaculturalists use a 36-ft (~10 m) length boat for transport and on-deck tumbling. The boat runs on gasoline and has a diesel fuel generator for the power winch, pressure washer and tumbler grading machine.

Figure 3.1. The floating upweller nursery system in the foreground and the on-shore nursery in the background. Systems like these allow growers to purchase eyed-larvae at a lower cost and grow them to deployment size within 2 months.

Figure 3.2. A grower displays the containment system in the floating upweller for the young oysters.

Figure 3.3. The customized aluminum tumbler for cleaning and grading oysters. This system was located on a customized boat that functions as a mobile processing unit for oyster farm maintenance.

Figure 3.5. An oyster cage is being deployed into the bay after the re-grading process. At this stage, the cage is clear of fouling organisms and sediment.

Figure 3.6. This photo displays the sediment load and biofouling that must be removed from aquaculture cages to prevent water flow from being restricted to the oysters. Cages are typically cleaned once per month.

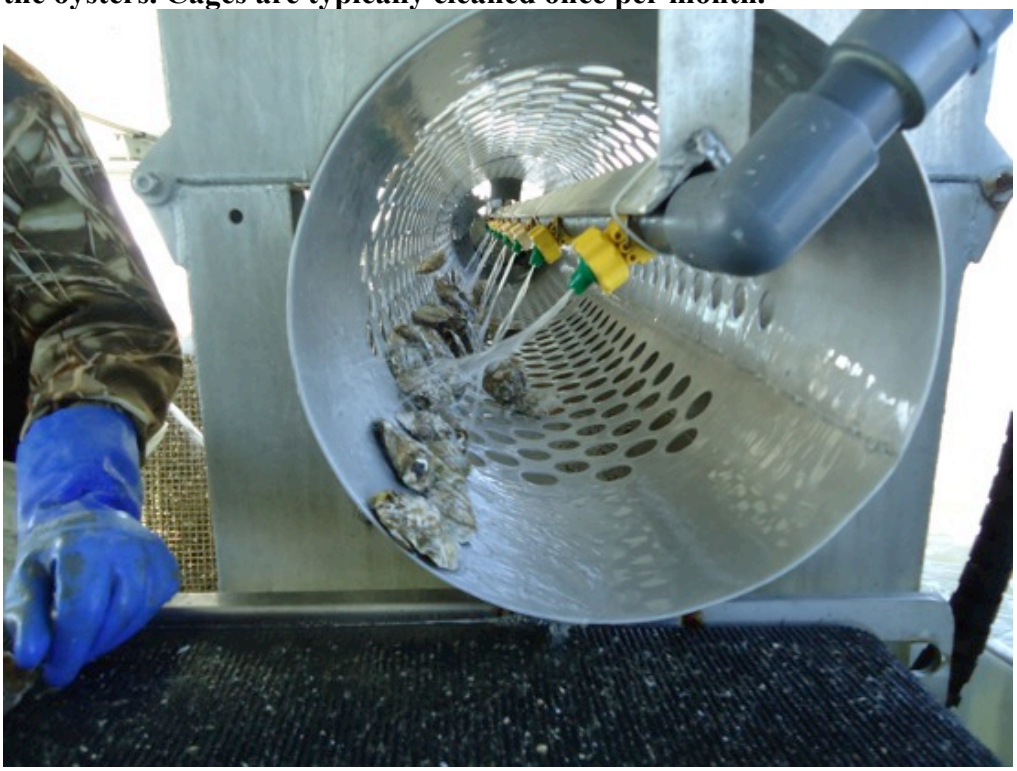

Figure 3.7. The tumbler unit cleaning and sorting live oysters. Oysters too large to pass through the perforated cylinder are harvested and sent to market.

2.4 Floating Cage Aquaculture Site

The floating raft aquaculture site forgoes the need for remote setting and a nursery system by purchasing young oysters (< 5mm in length), referred to as clutched seed oysters. Clutched seed has already been set to shell bits and is large enough to be deployed in floating rafts along the Choptank River, close to shore. The floating raft farm consists of roughly 2380 floating rafts made from PVC pipe and plastic mesh. The enclosed PVC pipe allows for buoyancy that suspends a mesh bag filled with live oysters at the water's surface.

The AEZ is located on a riverbank that is shallow enough to allow for a small boat or worker in chest waders to deal with biofouling. Periodically, the floats are flipped such that fouling organisms are exposed to the atmosphere and desiccated. When desiccation is not sufficient for removing biofouling, the rafts are brought to shore by hand and cleaned by a gasoline-powered pressure washer. Workers at the floating-raft aquaculture do not use machinery to tumble or sort oysters and all of the grading work is done by hand.

Figure 3.8. The floating raft aquaculture site featuring the lines of grow out rafts at the AEZ. Photo courtesy of Nicholas Ray.

Figure 3.9. The floating raft aquaculture site in winter. Notice the formation of ice in the lower flow areas around the rafts. Photo courtesy of Nicholas Ray.

Figure 3.10. A worker removes biofouling organisms with a gasoline powered pressure-washer. Photo courtesy of Nicholas Ray.

2.5 Emergy Analysis

We used an emergy analysis based on the methodology set forth by Odum (1996). Emergy analysis is the process of accounting for the work done by inputs from the environment, such as sunlight, wind, water movement, and the work done by inputs from the human economy, such as human labor, work by machinery and fossil fuels. This method of accounting is appropriate for our study, because oyster aquaculture is an open-system aquaculture system that operates at an interface between the estuary environment and the human economy. Natural contributions are matched with human labor, energy and materials such as cage structures to produce shellfish.

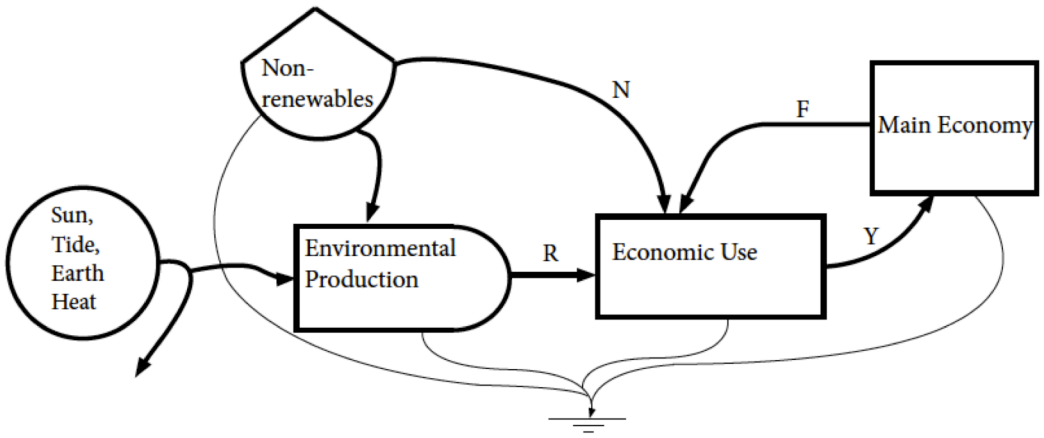

Figure 3.11. Basic system diagram of an economic production system displaying the flows of emergy categorized into by R) renewable emergy, N) non-renewable reserves, F) feedback emergy from the economy and Y) the emergy yield of the system.

Emergy is defined as the amount of solar energy equivalents used directly or indirectly in a system to produce a product or service. Emergy is measured in solar emjoules (sej). In this manner, an emergy analysis is able to include both the work of

nature and humans within a single analysis and therefore provides a quantitative assessment of what is required from humans and nature to produce a product or service.

Towards this goal, an energy systems diagram is first made in order to understand the flows of emergy within a system. Diagramming is done with the energy systems language as in Odum (1996). A basic emergy diagram is shown in Figure 3.11. Here the contributions of nature and of the main human economy are included in the conceptual diagram. Flows of emergy from different sources are categorized into free renewable emergy (R), free non-renewable emergy (N), feedback emergy from purchased from the economy (F), and the emergy yield of economic production (Y).

Once an energy systems diagram is completed, an emergy table is developed to account for all of the flows of materials and energy in the diagram. Data on material (g) and energy flows (j) are entered into the table and multiplied by a corresponding emergy transformity (sej/j or sej/g) to determine the emergy flow. In this manner, all inputs and outputs of the system are quantified using the same unit (sej).

In our case, two diagrams were made to represent the production systems of the two oyster aquaculture farms. Flows of emergy were then quantified by collecting data and using the appropriate transformities to accurately measure the proportional contribution of each flow. For consistency of transformities, we used the solar emergy global baseline of 15.83×10^{25} sej suggested by Campbell et al (2005).

2.6 Emergy Indicators

Once an emergy table is completed, emergy indicators (as shown in Table 3.2) are calculated to understand the functioning of the system in regards to emergy. These

Table 3.2 Emergy Indicators

Indicator	Equation	Significance
Renewable Emergy	R	The amount of emergy that is contributed by nature and considered free.
Non-renewable Emergy	N	The amount of emergy contributed by nature that is replenished at a lowe rate than it is used.
Feedback Emergy	F	The amount of emergy that is contributed from society as feedbacks from the economy.
Yield	R+ N+F = Y	The total renewable and feedback emergy.
Solar Transformity (Tr)	Y/E	The ratio of the total emergy inputs to the energy of the yield.
Renewability (%R)	100 x R/(R+N+F)	The percentage of the emergy inputs that are supplied by local renewable sources.
Emergy Yield Ratio (EYR)	Y/F	The ratio of emergy yield from the system to the emergy that is fed from outside the system.
Emergy Loading Ratio (ELR)	(F+N)/R	The ratio of purchased emergy and local non-renewable emergy to the local free renewable emergy.
Emergy Sustainability Index	EYR/ELR	The ratio of emergy yield to the emergy loading to the system.

Source: Odum, 1996; Ulgiati and Brown, 1998.

indicators are based on quantitative data of the various flows of emergy within the system analyzed.

2.6.1 Solar Transformity

In emergy evaluations cannot evaluate the efficiencies of energy transformations and processes because it would be lengthy and impractical to measure. Such an undertaking would require instrumentation, time and precise measurements. Instead emergy evaluations account for the total emergy inputs and outputs of systems. Then the solar transformity is calculated as in Equation 1 (also as shown in Table 3.2).

$$Solar\ Transformity = \frac{\sum emergy\ inputs}{energy\ output} \qquad 1.$$

Where the solar transformity is measured in solar emjoules per joule (sej/j), emergy inputs is measured in solar emjoules (sej) and energy output is measured in joules (j).

The solar transformity is inversely related to process efficiency because it is equal to the total emergy inputs to the systems, divided by the energy content of the product (Odum, 1996). The transformity is an indicator of efficiency because it includes the energy used to create a product or service. Items with a low transformity use less energy to be produced than those with higher transformities.

Tranformities may change over time as system develops efficiencies. The Lotka-Odum principle of maximum power states that competitive systems self-organize energy pathways and feedback loops that maximize the work done by the system (Odum, 1996; pp 16). In other words, under competitive forces, systems that

survive are those that tend to approach maximum efficiencies that are thermodynamically possible.

Mature ecosystems are assumed to have reached or are approaching optimum efficiencies because they have been in ecological competition for a long time (Odum, 1996; pp 18). Therefore in our study we compared the transformities of cultured oysters to the calculated transformity of 1.89×10^5 sej/j for intertidal wild oysters in a South Carolina oyster reef (Odum and Collins, 2001). A transformity similar to those found in nature would indicate that the aquaculture system is approaching maximum efficiency thermodynamically possible.

2.6.2 Percent Renewable (%R)

We determined the percentage of the total emergy inputs that were renewable as an indicator of sustainability. That is, systems that rely more on local renewable sources are assumed to last longer and be more sustainable unless some calamity or disturbance reduces or eliminates these sources.

As filter-feeding bivalves, oysters feed on phytoplankton, particulate organic matter, and dissolved organic material that they filter from estuary water. Stable isotope analysis has indicated that most of the assimilated carbon in oysters tissues is derived from phytoplankton and not from other sources (Langdon and Newell, 1996). Therefore we assumed that the oysters at these sites utilized only phytoplankton as a food source.

Research on the biology and ecology of Eastern oysters has identified a positive relationship of flow velocity and growth rate. This relationship is complex

and poorly understood (Newell and Langdon, 1996). Generally speaking, water circulation serves to deliver food sources and oxygen, while removing waste (feces, pseudo-feces, and oxygen depleted water) from the oyster reef. Grizzle et al (1992; as cited by Newell and Langdon, 1996) showed that growth was highest when current velocity was 1cm s^{-1} and that feeding ceased under conditions of no current velocity.

Research on the carrying capacities of various estuaries for shellfish aquaculture, including models simulations for carrying capacity and expected growth rates controlled by environmental parameters, show that water flow and concentration of chlorophyll-a are free environmental forcing functions of shellfish production (Pouvreau et al, 2006; Ferreira et al, 2007). Therefore we have included estuarine circulation emergy as an input to the system that contributes to oyster biomass.

Temperature is often regarded as a signal for oysters, influencing rates of filtration, gamete production and hibernation (Shumway, 1996; pp 467-503). However, accounting for solar emergy of temperature changes would be double-counting since temperature is a product of solar energy, which also produces phytoplankton and organics for oyster consumption. Therefore we ignored temperature as an emergy input in our analysis.

The percent renewable is calculated as in Equation 2.

$$\%\, R = \frac{R}{R + N + F}\, x\, 100 \qquad 2.$$

2.6.3 Emergy Yield Ratio

The Emergy Yield ratio (EYR) is the ratio of total emergy output (Y) of a system divided by the imported feedback emergy from the economy (F), as expressed in Equation 3.

$$EYR = \frac{R + N + F}{F} = \frac{Y}{F} \qquad 3.$$

Where Y is the emergy yield of the system (sej) equal to the total emergy inputs from renewable (R), non-renewable (N) and feedback from the economy (F). Therefore systems which have a higher emergy input from R and N than F will have a higher emergy yield ratio. This indicator provides an indication of the quantity of local free renewable emergy and free non-renewable emergy used for production. A high EYR indicates a greater utilization of R and N, while a low EYR (close to 1) indicates a greater dependence on purchased imported feedback emergy from the economy. It is important to note that systems that extract natural non-renewable resources as well as those with a high emergy input from R will have a EYR.

2.6.4 Environmental Loading Ratio

The Environmental Loading Ratio (ELR) is the ratio of feedback (F) and local non-renewable emergy (N) to the local renewable emergy (R) utilized in the system, expressed in Equation 4.

$$ELR = \frac{(F + N)}{R} \qquad 4.$$

The ELR is an indicator of the stress of a process on the local environment, as resources are drawn from other locations and local non-renewable resources are

redirected to the production activity (Ulgiati and Brown, 1998). By this equation, we can see that systems which are extractive, like mining, or that transform purchased inputs from the economy into new products, are likely to have a high ELR and by these measures are less sustainable.

2.7 Data Collection

We collected data by touring the facilities in June 2014, estimating resource use and measuring mass of aquaculture gear. We interviewed aquaculturalists about fuel use, and energy consumption (electrical costs). We recorded pump, boat and nursery basin sizing and recorded the types and amounts of materials used in the system. At the floating raft farm, we counted the amount of rafts at the site and measured the amount of polyvinyl chloride (PVC) piping was used in construction. The replacement periods for various equipment and materials was taken from Wieland (2007). Yearly emergy use of materials and equipment was calculated as in Equation 5.

$$Em = \frac{m}{P_r}\ x\ Tr \qquad 5.$$

Where Em is the emergy use per year (sej/yr), m is the mass of the material or equipment used at the aquaculture site, P_r is the replacement period of the material or equipment, and Tr is the transformity of the material or equipment.

Aquaculturalists provided production yield estimates (individual market-sized oysters for 2013 and we used this as annual yield of the systems. We assumed that both sites produced regular market-sized oysters (>76 mm in length) detailed in

Higgins et al (2011) as there was no evidence or claims from either farm that their oysters were different from market size.

Capital costs were determined using the VIMS Crop Budget Tool (VIMS, 2013) by entering the various parameters such as numbers of employees, annual production, equipment on site, and method (cage or raft culture). The annual capital cost was then entered into the emergy analysis as services from the economy and is included as a feedback emergy from the economy. The emergy of services was then determined by multiplying it by the emergy per dollar ratio (emdollars) as found on the National Emergy Accounting Database (NEAD, 2012).

Inputs from the natural environment were determined from site characteristics for water circulation from river geo-potential energy, wind and tidal energy. We calculated this from the change in water velocity before and after the aquaculture site using the Manning's equation and roughness coefficients for oyster reefs along the Gulf Coast as determined by Freeman (2010).

To calculate energy flow from particular organic matter, we calculated the average caloric intake from oyster metabolism in an intertidal reef ecosystem (Dame et al, 1992). Particular organic matter was assumed to embody emergy from sunlight and nutrients such as nitrogen and phosphorous and therefore these were not included as separate emergy inputs.

3. Results and Discussion

Figure 3.11 and 3.12 are the emergy diagrams of the raft and cage aquaculture systems. These diagrams show pathways of emergy into, within and out of the

aquaculture systems. The emergy diagrams correspond to the emergy tables (Table 3.3 and 3.4).

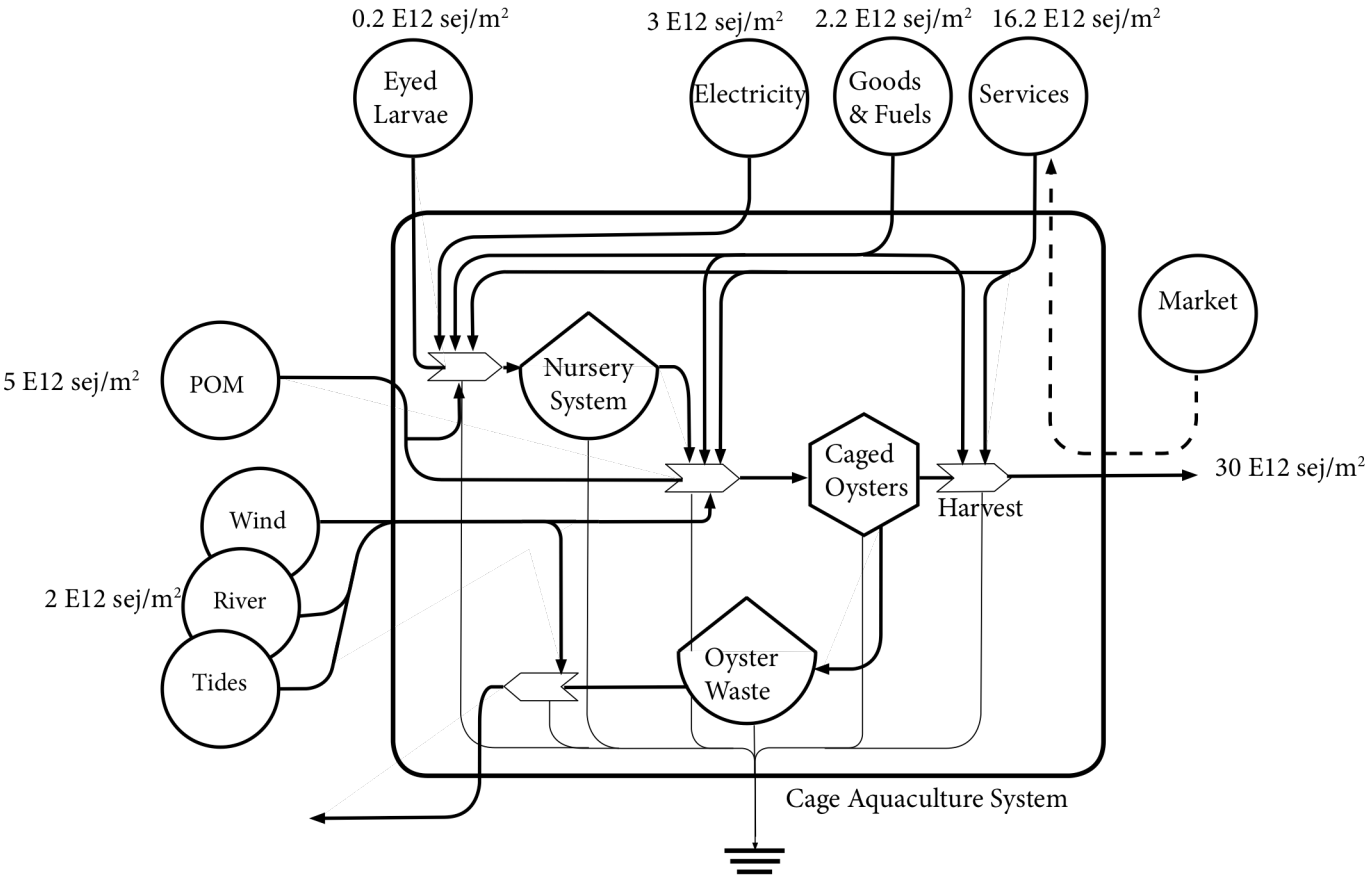

Figure 3.12. Energy Systems Diagram for Cage Oyster Aquaculture.

Table 3.3 Emergy Table for the Cage Culture Oyster Farm per unit area (m^2).

	Item	Data	Unit	Solar Transformity (sej/unit)	Emergy Flow (E12 sej/yr)	References for Transformity	Percent of Total Emergy
Renewable Resources (R)							
1	Sunlight	5.19E+09	j	1.00E+00	-	Odum, 2001	
2	Tides	7.29E+05	j	4.94E+04	-	Campbell, 2004	
3	River, Geopotential	6.70E+07	j	3.18E+04	2	Martin, 2002	7.22
4	POM (Microalgae)	9.72E+07	j	5.00E+04	5	Odum and Collins, 2001	16.46
Purchased Units (F)							
5	Eyed Larvae	8.89E-02	$	2.70E+12	0.2	NEAD, 2008	0.81
6	PVC	2.74E+00	g	5.85E+09	0.02	Brown and Buranakarn, 2003	0.05
7	Electricity	1.66E+07	j	2.08E+05	3	Dolan and Brown, 2009	11.71
8	Pressure-	1.88E+01	g	3.50E+09	0.1	Brown &	0.22
9	Steel	2.61E+02	g	4.30E+09	1	Brown & Buranakarn, 2003	3.80
10	Aluminum	5.16E-01	g	1.25E+10	0.01	Brown & Buranakarn, 2003	0.02
11	Copper	7.25E-03	g	9.80E+10	0.001	Cohen et al, 2007	0.00
12	Fiberglass	1.02E+01	g	3.00E+09	0.03	Ulgiati and Brown, 2002	0.10
13	Fuels, Gasoline	3.67E+07	j	3.86E+04	1	Bastianoni et al, 2009	4.80
Services							
14	Services	$ 1.90	$	2.70E+12	5	NEAD, 2008	17.34
15	Labor	1.64E+06	j	6.74E+06	11	Ingwersen, 2010	37.46
16	Renewable Emergy				7		
17	Feedback Emergy				23		
18	Total Emergy Flow				30		
Yield (Y)							
18	Market-sized Oysters	2.25E+06	j	1.31E+07	30		

See appendix for table footnotes.

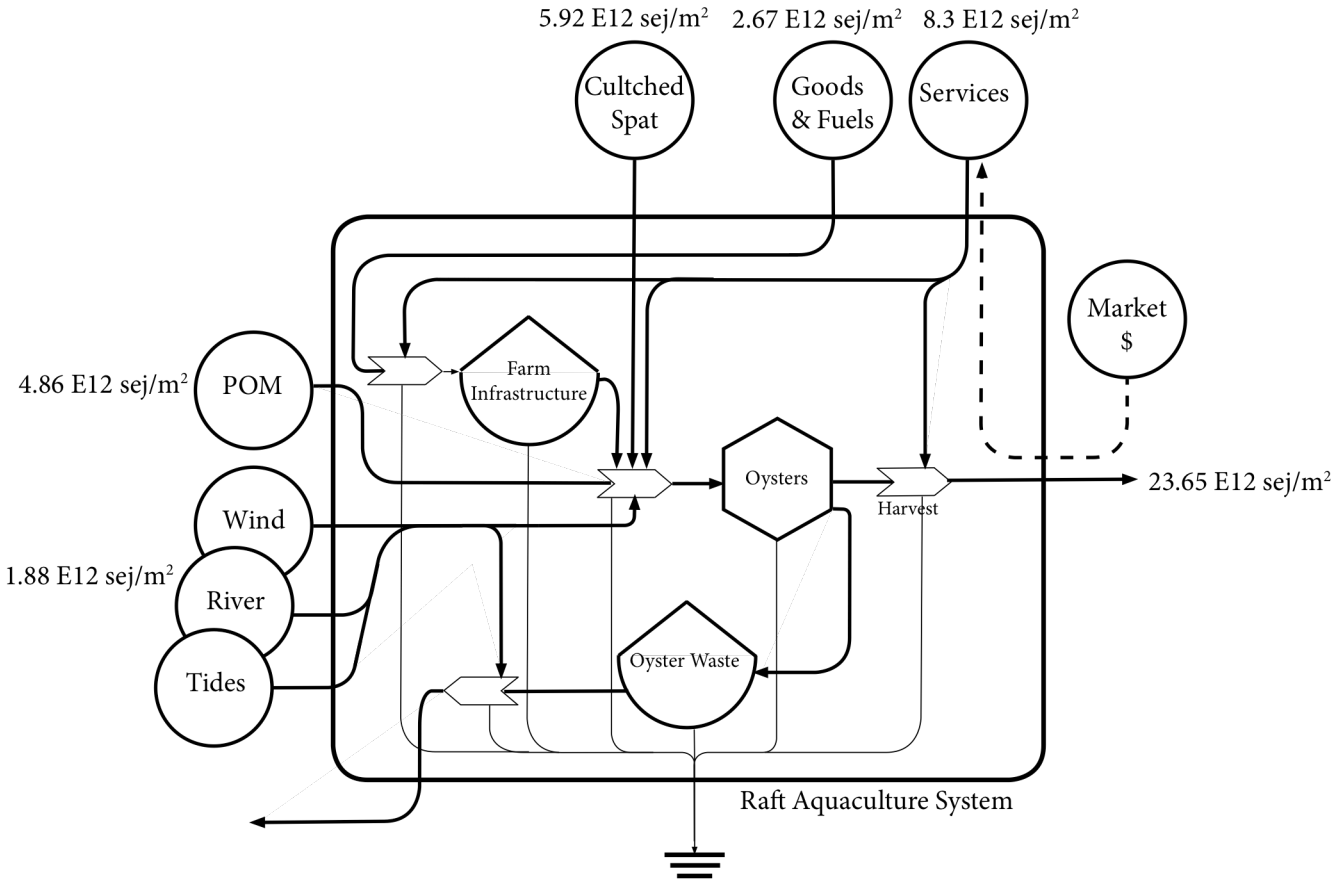

Figure 3.13 Energy systems diagram of a raft oyster aquaculture system.

Table 3.4 Emergy Table for the Raft Culture Oyster Farm per unit area (m^2).

	Item	Data	Unit	Solar Transformity (sej/unit)	Emergy Flow (E12 sej/yr)	References for Transformity	Percent of Total Emergy
Renewable Resources (R)							
1	Sunlight	5.19E+09	j	1.00E+00		Odum, 2001	
2	Tides	7.29E+05	j	4.94E+04		Campbell, 2004	
3	River, Geopotential	5.93E+07	j	3.18E+04	1.88	Martin, 2002	7.97
4	POM (Microalgae)	9.72E+07	j	5.00E+04	4.86	Odum and Collins, 2001	20.54
Purchased Units (F)							
5	Cultched Spat	2.19E+00	US$	2.70E+12	5.92	NEAD, 2008	25.01
6	PVC	1.65E+02	g	5.85E+09	0.97	Brown and Buranakarn, 2003	4.09
7	Machinery	8.32E-01	g	1.47E+10	0.01	Odum et. al.,1987	0.05
8	Electricity	7.86E+06	j	2.08E+05	1.64	Dolan and Brown, 2009	6.91
9	Fuels, Gasoline	1.39E+06	j	3.86E+04	0.05	Bastianoni et al, 2009	0.23
	Services						
10	Services	3.14E-01	US$	2.70E+12	0.85	NEAD, 2008	3.58
11	Labor	1.11E+06	j	6.74E+06	7.48	Ingwersen, 2010	31.61
12	Renewable Emergy				6.74		
13	Feedback Emergy				16.91		
14	Total Emergy Flow				23.65		
Yield (Y)							
15	Market-sized Oysters	5.33E+06	j	4.44E+06	23.65		

See appendix for footnotes.

The diagrams in Figure 3.12 and 3.13 show that cultivating oysters is the process of taking advantage of local renewable resources for feed (POM and water flow) and matching those with inputs from the economy, such as fuels, materials, services and labor. We determined that renewable emergy inputs account for 23.7 % and 28.5% of the emergy used by the cage and raft aquaculture sites, respectively.

The remainder of the emergy inputs is brought in from the greater system that contains the aquaculture farms, human society.

It is possible that we underestimated renewable emergy inputs. Our estimation of the oysters' primary food source, particulate organic matter (POM), was based on the metabolism of an intertidal oyster reef in South Carolina (Dame et al, 1992). Oxygen consumption was given on an energy-per-unit area measurement (6.5 kg-O_2 m^{-2} yr^{-1}), but did not include a reliable measurement of biomass or number of individuals present. We assumed that our aquaculture farms had the same biomass density as an intertidal oyster reef.

It is feasible that sub-tidal Chesapeake oysters consume greater amounts of energy per day due to being submerged, rather than periodically exposed at low tide, as has been witnessed in studies (Kingsley-Smith et al, 2009). Additionally, it may be that the selectively bred oyster strain used by both farms feeds at a different rate than a wild oyster. However, we could not find literature to support this other than increased growth rates in triploid oysters (Stanley et al, 1984), which may be explained by foregoing reproduction rather than greater energy intake.

Feedback emergy inputs (F) were different between the two farms in their composition and intensity. Figure 3.14 shows the different use of feedback emergy between the two farms. The bottom cage culture farm had a more diverse array of material inputs than the floating raft farm that used a small amount of material to produce oysters. This may be because that the raft does not have or use a boat, nursery system or customized tumbler like the bottom cage farm. In short, this is an indication of differing levels of technology at the two farms.

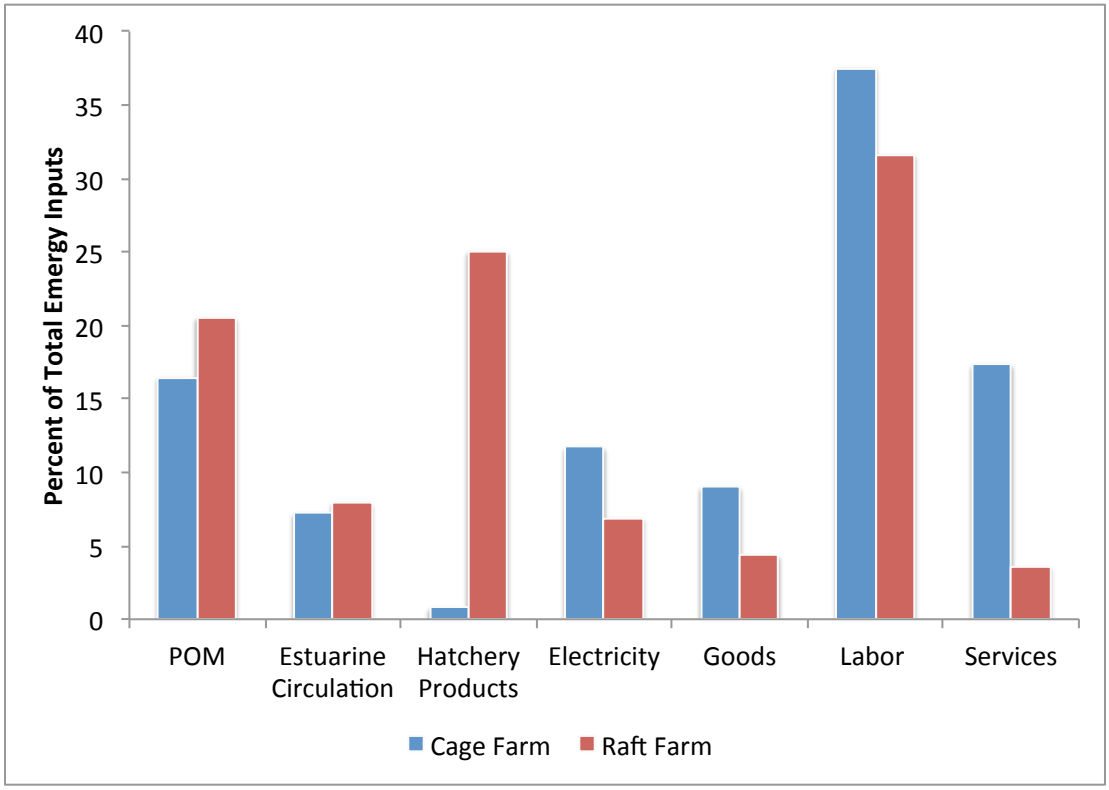

Figure 3.14. The relative differences in feedback emergy use by the two oyster aquaculture farms.

The bottom cage farm used more emergy from electricity, pressure-treated wood, machinery (containing steel, aluminum, copper and other materials) fuels, fiberglass, and services from the economy and labor. In contrast, the floating raft culture site had more inputs of emergy from hatchery products and PVC for the construction of grow-out rafts.

However; feedback emergy from hatchery products, material for the grow-out system, electricity and fuels accounted for 36.3% of the emergy for the raft aquaculture system and only 21.5% at the cage aquaculture system. Instead, labor was the largest portion of feedback emergy from the economy, representing 31.6% and 37.5% of total emergy fro the raft and cage culture sites respectively.

Hatchery products were a significant input of emery for the raft culture site, constituting 25.1% of the total emergy inputs. Controversy, the cage culture site only relies on 0.8% of its emergy basis from hatchery products and instead uses more emergy from electricity and materials for nursing smaller eyed-larvae to a size that allows deployment in the estuary. Eyed-larvae are purchased and then raised in customized on-shore and near-shore aquaculture systems driven by pumps that run on electricity purchased from the energy grid. The feedback emergy from larvae is small; 0.2 E12 sej/m^2. In contrast, the raft culture site purchases larger cultched spat that can be deployed directly to floats in the estuary. The emergy input from cultched spat was higher at 5.92 E12 sej/m^2, encapsulating the work done at the hatchery to grow the spat to a larger size before purchase.

Services of the human economy represented a significant contribution of emergy (17.3% of total emergy flow) for the cage culture site, while the raft culture site relied on less emergy from services (3.6% of total emergy). Emergy from human labor was lower at the floating raft aquaculture site; 7 E12 sej/m^2 compared to 11 E12 sej/m^2 at the bottom cage aquaculture site. This may be due to the fact that the floating raft does not manage a nursery system as the bottom cage culture site or that the maintenance of floating rafts is less intense or that floating rafts receive less fouling and sediment that impedes water flow.

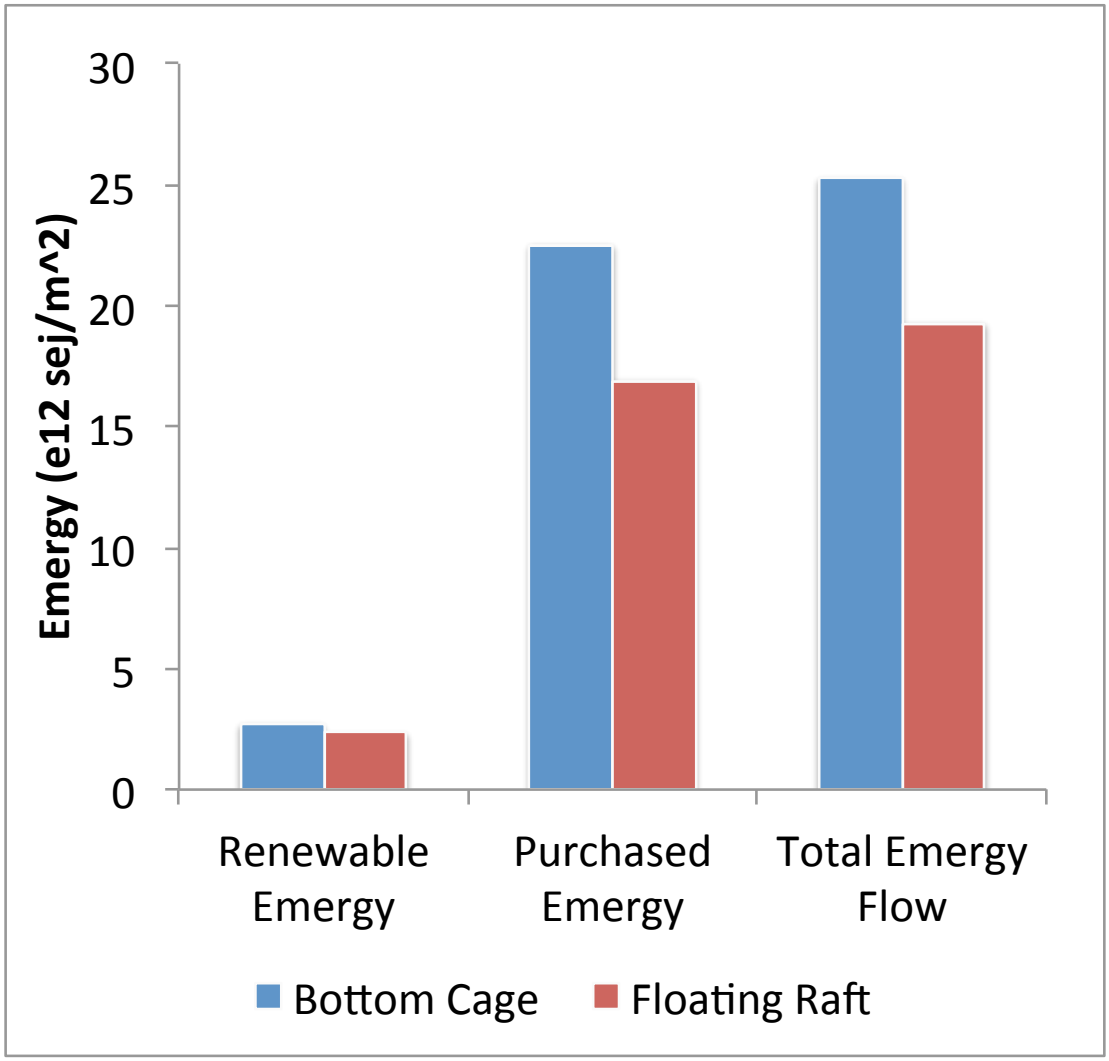

Figure 3.15. Comparison of the renewable emergy and feedback emergy flow at the two oyster aquaculture farms.

Neither site relied on free local non-renewable inputs. Therefore according to our analysis, there is no depletion of local non-renewable reserves such as soil, water, minerals or the like. Instead, all of the free local environmental inputs are renewable.

Overall, the cage culture uses more emergy from the human economy and more renewable emergy for production (Figure 3.15). The higher amount of feedback emergy is indicative of a greater reliance on technology than the raft culture site.

One possible flaw in our analysis is the use of the VIMS Crop Budget Tool for estimating similar capital costs for each farm per unit rather than obtaining operating costs from the farms. The tool could be over-estimating the costs of producing oysters. We calculated the cost of producing a single unit –100 oysters packaged in a box for the end-user. According to the VIMS Crop Budget Tool, the cage culture site spends $13.51 to produce a single package of oysters, and the raft culture site spends $9.40 to produce a single package. Packages are generally sold to the end-user for $47.00 to $50.00, which means that profits are acceptable for the enterprise. It follows then that the VIMS Crop Budget Tool is on the whole accurate at estimating capital costs of oyster aquaculture. Therefore we concluded that our estimations of emergy from services is accurate and that it is not overly faulty and clouding the analysis.

Table 3.5. Emergy Indicators for two oyster aquaculture farms.

Product	Transformity (sej/j)	EYR	ELR	Renewable Emergy (%)
Raft Oysters	4.44E+06	1.40	2.51	28.51
Cage Oysters	1.31E+07	1.31	3.224	23.68

Table 3.5 shows emergy indicators for the two oyster farms. The solar transformities of the two sites varied by an order of magnitude. The raft culture had the lower transformity of 4.44×10^6 sej/j and the cage culture site's oysters had a transformity of 1.31×10^7 sej/j. The difference may be attributed to the fact that the cage aquaculture site is located 3 km from shore and the use of a boat is necessary for maintenance of the cages and for harvest. Therefore much of the emergy from fuels is used in transport between the processing and packaging facility on-shore and the

grow-out location in the estuary. In contrast, the raft aquaculture site is located near shore in shallow water that allows for maintenance without the use of a boat.

Both farms had a transformity that was greater than the transformity of wild intertidal oysters; 1.89×10^5 sej/j (Odum and Collins, 2002). The higher transformity indicates that man-made aquaculture systems have not yet reached the efficiencies of natural oyster production in intertidal reefs.

The differences in transformities and corresponding production efficiency are likely due to the differences in emergy inputs of services and labor. If these two items are removed from our emergy analysis, the transformities of both farms drops to a number much closer to the 1.89×10^5 sej/J (Odum and Collins, 2002) transformity of wild oysters (1.97×10^6 for cage culture oysters and 2.88×10^6 for raft culture oysters). This means that the human economy and human labor are less efficient than natural systems.

Alternatively, transformity also corresponds to energy hierarchies and quality (Odum, 1996). Therefore another explanation for the difference in transformities between the cultured oysters and wild oysters could be that the products are different in quality. Higgins et al (2011) found that wild oysters had shells that were five (5) times greater in mass than the shell of cultured oysters.

It may be that the protection provided by aquaculture gear allows the oysters to divert energy toward the growth of tissue rather than shell resulting in a product of higher quality for human consumption (Don Merritt, pers comm.). In this way, the additional work of maintaining aquaculture gear and periodic cleaning of oysters to prevent excessive mortality and promote a lower shell-to-meat ratio results in a higher

transformity and higher energy quality. However, in this case, quality may be subjective and therefore we cannot fully explain the difference in transformities by discussions of quality.

In terms of renewable flows, the raft culture site only made marginally more use of local renewable emergy (R) than the cage culture site; 28.51 % compared to 23.7 %. The values are indicative of a process that is mainly driven by human effort and feedback from the larger system.

However, the natural contribution is not insignificant and represents approximately a quarter of the entire emergy flow of the production system. The Chesapeake Bay ecosystem provides the entire food budget for oyster growth and the necessary water movement for transport of food resources and waste.

The relatively low use of renewable emergy is also evident in the emergy yield ratios (EYR) for the oyster farms. The cage culture site had a ratio of 1.31 and the raft culture site had a ratio of 1.4. This indicates that resources from outside the Bay system are exploited to a greater extent than Bay resources. In other words, goods and services from the economy interact with a small contribution of free environmental emergy for production. Therefore a large portion of emergy is diverted from society to produce a small relative yield of free emergy from the local environment.

As with the EYR, the environmental loading ratios (ELR) are similar as well. The cage culture site had a slightly higher environmental loading ratio than the raft culture site (3.2 compared to 2.5). This may signify a difference in the amount of

development or technology used at the two farms. In other words, the cage culture farm has a more intensely developed operation than the raft culture farm.

In order to understand the indicators more completely, we compared our results with those of other food products as shown in Table 3.6. Cultured oysters are closer in their transformity to chicken eggs and have a similar EYR. In comparison to other aquaculture products such as shrimp and finfish, oysters are a higher transformity product, but have a much lower ELR. The evaluation from Vassallo et al (2007) showed that finfish (*S. Aurata*) are a lower transformity food with a lower of renewable emergy inputs and higher ELR. Similarly, even the organic shrimp production in Brazil was considerably more impactful on the environment than oyster

Table 3.6 Emergy indicators for agricultural and aquaculture food products

Product	Location	Transformity (E05 sej/j)	EYR	ELR	% R	Reference
Beef	USA	8.60	1.51	1.9	34.1	Brandt-Williams, 2002
S. aurata fish	Italy	13.20	1.2	5.00	16.69	Vassallo et al, 2007
Intensive Shrimp	Brazil	25.30	2.13	58.58	1.7	Lima et al, 2012
Organic Shrimp	Brazil	31.90	4.31	51.64	1.7	Lima et al, 2012
Eggs	USA	44.00	1.08	54.42	1.8	Brandt-Williams, 2002
Raft Oysters	USA	44.37	1.40	2.51	28.51	This Study
Cage Oysters	USA	131.05	1.31	3.22	23.68	This Study

aquaculture. This indicates that in relation to other forms of aquaculture, oyster aquaculture is a low impact practice. According to our study, this is due to the higher reliance on renewable emergy in the form of POM and natural water circulation.

One point of inconsistency with this conclusion is the fact that the shrimp farming system evaluated in Lima et al (2012) had considerably higher EYRs. This can be explained by differences in methodology. Lima et al (2012) included water resources as a free local non-renewable (N) source of emergy, which greatly

influences the EYR. In our analysis, water inputs were not included because estuary water is not consumed in the process and instead flows through the system without being lost or used up. Inclusion of estuary water as a free local non-renewable emergy input would have increased our EYR but would not have been accurate because water is not extracted and included as part of the emergy yield.

Beef is considerably different in terms of emergy, drawing more renewable emergy and representing a lower transformity. This may be due to the fact that cattle-ranching in the USA is an older practice than oyster aquaculture and represents a more mature industry than aquaculture. According to the maximum empower principle (Odum, 1996), the more mature a system, the greater the emergy efficiency of production. Moreover, beef is a primary source of protein for humans in the United States, while oysters are considered a luxury product, valued for taste and experience rather than for bulk nutrition.

In the broader context, our emergy analysis reveals that the methods of raft and cage culture are similar in terms of the amount of local renewable resources exploited and only differed by 4.8%. However, they differed in their environmental loading ratios (ELRs) and emergy density due to the different amount of equipment, maintenance methods and technologies employed.

Our emergy indicators did not show that either site took considerable advantage of local renewable emergies to produce a substantial emergy yield. Advances in technique to utilize more renewable emergy flows could improve the sustainability of either farm. Such advances could include recruitment of wild oyster spat from the Chesapeake ecosystem in place of purchased hatchery products or the

utilization of natural water flow for nursery systems. Other improvements might include reducing or eliminating the need to re-grade and remove biofouling from aquaculture gear. However, our analysis shows that the location of the farm in the estuary that necessitates the use of boat and fuel resources has an influence on the sustainability of the product and therefore the siting of the AEZ should be considered to reduce the amount of fuels and boats needed to manage the grow-out system (cages or floats). By siting aquaculture operations closer to the processing facility or base of operations, the system have a lower amount of feedback emergy required and therefore will have a higher sustainability

4. Conclusions

From the results of our emergy analysis, we can conclude the following:

- The process of cultivating oysters in the Chesapeake Bay is a labor-intensive process, requiring large amounts of feedback emergy. Feedback emergy (F) is dominated by labor for the aquaculture operation and the purchase of goods needed for production.
- The emergy yield ratio reveals that oyster farming has a low net emergy yield because the farms require large inputs from society and do not exploit significant free renewable resources for larvae production or maintenance of aquaculture sites.
- The transformity of oysters is higher than other sources of proteins, indicating that it is a luxury product rather than a human staple that supports the population.

- In comparison with other aquaculture products, oysters have a low environmental impact and higher use of renewable sources of emergy.
- Emergy indicators show that oyster cultivation has a higher sustainability than other aquaculture products, but lower sustainability than staple proteins such as beef, due to the intense work from the human economy needed to produce the product and lower percentage of renewable emergy used in the production process.

Appendix 1. Footnotes to Table 3.3

	Source	Calculation	Units	References
1	**Sunlight**			
	Fisheries Area =	23216.0	m^2	
	Insolation =	5584500000.0	J/m^2/yr	National Renewable Energy Laboratory, 2012. Taken from www.nrel.gov/gis/images/eere_csp/national_concentrating_solar_2012-01.jpg
	Albedo (Seawater) =	0.1		Payne, 1972.
	Energy per year (J/yr) = (fisheries area) x (insolation) x (1-albedo)	(23216 m^2) x (5.58e+09 J/m^2/yr) x (1-0.06)	j/yr	
	=	120574269360000.0	J/yr	
	Energy / Unit area = Joules/yr / area (23216 m^2)	5193585000.0	j/m^2/yr	
2	**Tides**			
	Fisheries Area=	23216.0	m^2	
	Tidal range=	0.5	m	Maryland DNR, 2013.
	Water density at salinity 15 ppt =	1005.9	kg/m^3	
	Gravity=	9.8	m/s^2	
	Tides per year=	730.0	tides/yr	
	Tidal Energy Absorbed per year = (area elevated)(0.	(23216 m^2) x (0.5) x (730 tides yr^-1) x (0.45m tide^-1)^2 x (1005.858 kg	j/yr	

	5, center of gravity)(tides y^-1)(height^2)(density)(gravity)	*m^-3) x (9.8 m* s^-2)		
	Tidal Energy Absorbed per year =	16914845573.2	j/yr	
	Energy / Unit area = Joules/yr / area (23216 m^2)	728585.7	j/m^2/yr	
3	**River, Geopotential**			
	Fisheries Area =	23216.0	m^2	
	Fisheries Width =	175.5	m	
	Cross Sectional area =	266.8	m^2	
	Water depth before aquaculture site =	1.2	m	
	Average Velocity before aquaculture site= Conversion factor (k)/ Gauckler-Manning's coefficient x Hydraulic Radius^2/3 x Slope^1/2	V=(k/n) Rh^2/3*S^1/2	m/s	Manning's Equation.
	k=	conversion factor	1 m^(1/3)/ s	

n=	Gauckler-Mannings Coefficient	unitless	
n (muddy bottom)=	0.0	unitless	Freeman, 2010.
Rh=	Hydraulic Radius	m	
Rh=	Cross-sectional area / wetted parameter	m	
Rh=	1.5		
S=	0.0	m/m	NOAA Nautical Chart 12230.
Water Depth after aquaculture site =	1.5	m	
Average velocity before aquaculture site =	(1/0.03) * 1.52^(2/3) * 0.00028^(1/2)	m/s	
Average velocity before aquaculture site =	0.7	m/s	
Flow rate before aquacultlure site = velocity x cross sectional area	(0.736) * (266.76)	m^3/s	
Flow rate before aquacultlure site=	196.4	m^3/s	
Flow rate before aquaculture site (yearly) = flow rate (m^3/s) x 3.15569e7	196.37 x 3.15569e7	m^3/yr	

s/yr			
=	6196967828.2	m^3/yr	
Average Velocity afer aquaculture site=			
Conversion factor (k)/ Gauckler-Manning's coefficient x Hydraulic Radius^2/3 x Slope^1/2	V=(k/n) Rh^2/3*S^1/2	m/s	
n (oyster reef)=	0.1	unitless	Freeman, 2010.
Average Velocity after aquaculture site =	(1/0.07) * 1.52^(2/3) * 0.00028^(1/2)	m/s	
=	0.3	m/s	
Flow rate after aquaculture site = velocity * cross sectional area	(0.315) * (266.76)	m^3/s	
	84.2	m^3/s	
Flow rate after aquaculture site (yearly)	flow rate (m^3/s) x 3.15569e7		
=	s/yr	m^3/yr	
=	2655843354.9	m^3/yr	
Mass of water per year before aquaculture	(6196967828 m^3/yr) * (1005.858 kg/m^3)	kg/yr	

site = flow rate * density of brackish water		
=	62332696656 97.8	kg/yr
Kinetic Energy before aquaculture site = 1/2 (mass) x velocity^2	1/2 * (6.2E+12 kg /yr) x (0.736 m/s)^2	Joules/yr
=	16889403775 37.3	Joules/yr
Mass of water per year after aquaculture site = flow rate * density of brackish water	(2655843355 m^3/yr) * (1005.858 kg/m^3)	kg/yr
=	26714012852 99.1	kg/yr
Kinetic Energy after aquaculture site = 1/2 (mass) x velocity^2	1/2 * (2.7E+12 kg/yr) * (0.315 m/s)^2	Joules/yr
=	13294865945 6.3	Joules/yr
Energy absorbed by aquaculture site = Energy of flow entering site - energy of flow leaving	1.69E+12 - 1.33E+11	joules/yr

	the site			
	=	15559917180 81.0	Joules/yr	
	Energy / Unit area = Joules/yr / area (23216 m^2)	67022386.2	Joules/ m^2/yr	
4	**Particulate Organic Matter (Microalgae)** Metabolism of intertidal oyster reef = Oxygen consumptoin rate (kg O2/m^2/yr)	6.5	kg O2/m^2 oyster reef/yr	Dame et al, 1992
	Free energy change (kcal) per mole of glucose decomposed =	686.0	kcal	
	ratio of glucose to oxygen consumed =	0.2	mol C6H12O6/ mol O2	
	Oxygen consumed per unit area = g-O2/m^2	6500.0	g O2/m^2 oyster reef	
	Moles to grams conversion = 1 mol O2 / 32 g-O2	0.03	mol/g	

	Kcal consumed per year = free energy change (kcal) per mole of glucose consumed x ratio of glucose ot oxygen consumed x oxygen csonumed per unit area x moles to grams conversion.	23224.0	kcal/m^2 oyster reef/yr
	Kcal ot joules converion = kcal/yr * 4184 joules	97169041.7	j/m^2 oyster reef /yr
5	**Eyed Larvae** cost /1,000,000 <5 um-culchless spat =	295.0	$
	# Spat Purchased /year=	7000000.0	spats
	Total Cost= Cost/1,000,000 spat * 7,000,000 spat=	2065.0	$/yr
	Cost / Unit area = cost/yr / area (23216 m^2)	0.1	$/m^2/yr

6	**PVC Use**		
	Handling Trays		
	Total Mass per tray	1480.0	g
	Trays in use	30.0	trays
	total mass of trays = mass of tray x 30 trays	1480 x 30	g plastic
	=	1510.0	g plastic
	Nursery System		
	On-shore Aquaculture Basins =	440.0	lbs
	Conversion to grams = weight lbs * 453.592g	199580.5	g
	Replacement period	8.0	yr
	Yearly use = mass / replacement period	99790 g / 8 yr	
	=	24947.6	g/yr
	PVC piping(1.5 in) =	8.0	ft
	Lbs/ft =	0.5	lbs
	Mass = ft*lbs/ft	4.1	lbs
	mass (g) = lbs*453.592	1850.7	g
	Replacement Period	8.0	yr
	g/yr =	231.3	g/yr
	Floating Upweller Basin		
	Mass of upweller	20.0	lbs

basins = Number of upweller basins	30.0	basins	
Total basins mass pvc = weight (lbs) x number of basins (24) x 453.592g/lbs	272155.2	g	
Replacement Period	8.0	yr	Weiland, 2007.
Yearly use = mass / replacement period	34019.4	g/yr	
Floating Upweller Nylon Screens	Nylon		
Weight	0.5 lbs/screen		
Mass = weight (lbs) * Conversion (453.592g/lb)	226.8	g	
total screen use = = 30 * 226.796g/screen	6803.9	g	
Replacement period	8.0	yr	Weiland, 2007.
Yearly use = Mass / Replacement period	850.5	g/yr	
½ hp 1725 rpm 115V 20 amp electric motor pump	38.0	lbs	

=			
Total Mass = weight (lbs) * conversion (453.592 g/lb)	17236.5	g/pump	
Plastic content = 19% of pump mass	17,236 g/pump * 0.19	g/pump	
	3274.9	g pvc /pump	
Replacement Period	8.0	years	Wieland, 2007.
Yearly use = Mass (g/pump) / Replacement period yr)	409.4	g pvc/yr	
Total yearly use = yearly use * 5 pumps	2046.8	g pvc/yr	
Tumbler			
Total Mass	181440.0	g	http://www.fukuina.com/shellfish/regular_duty_oyster_tumbler_grader.htm
Plastic content = 0.5% * Mass	907.2	g	
Replacement period = Yearly use = Mass / Replacement period = Yearly use =	15.0 (907 g plastic) / 15 years 60.5	years g/yr	
Total PVC use / year=	63666.1	g/yr	

	PVC use / Unit area = g/yr / area (23216 m^2)	2.7	g/m^2/yr
7	**Electricity**		
	Electrical Utility Costs/Month=	1000.0	$/month
	Average Cost of Electricity=	0.1	$/kwh
	Monthly Electricity Use=	8928.6	kwh/month
	Kilowatthours to Joules=	32142857142.9	j/month
	Annual Electrical Use=	385714285714.3	j/year
	Electricity use / Unit area = j/yr / area (23216 m^2)	16614157.7	j/m^2/yr
8	**Pressure-treated wood**		
	Floating Upwellers		
	Dimensions =	2.4 m x 6.09 x 0.25 m	
	Volume =	2.2	m^3
	Density =	663.4	kg/m^3
	Mass of wood used in each upweller =	1454.4	kg
	Total mass for 3 upweller systems =	4363314.5	g

	Replacement Period =	10.0	yr	
	Yearly use =	436331.4	g/yr	
	Pressure-treated wood use / Unit area = g/yr / area (23216 m^2)	18.8	g/m^2/yr	
9	**Stainless Steel** *Aquaculture Cages*	*18143.7*	*g/cage*	EIA (Comercial Rate for Southern Mid-Atlantic)
	Cages in use yearly	2000.0	cages	
	mass of steel in cages =	36287400.0	g	
	Replacement period=	6.0	yr	Wieland, 2007.
	Yearly Use=	6047900.0	g/yr	
	5 Pumps ½ hp 1725 rpm 115V 20 amp electric motor pump =	38.0	lbs	
	Mass conversion to g= lbs*453.592 g=	17236.5	g	
	Total pump mass =	17236.5	g/pump	
	Steel content = 80% of mass =	17,236 g/pump x 0.80 = 13789.2	g/pump	

	total steel in pumps = steel content (g/pump) x 5 pump = s	13,789 g/pump x 5 pumps		
	=	86182.5	g steel	
	Replacement Period for pumps =	8.0	years	
	Yearly use = total steel mass / replacement period =	86,182 g steel / 8 years		
	=	10772.8	g steel/yr	
	Total yearly steel use = total g steel used in cages/yr + total g steel used in pumps/yr	6058672.8	g steel/yr	
	Steel use / Unit area = g/yr / area (23216 m^2)	261.0	g steel/m^2/yr	
10	**Aluminum Use**			
	Tumbler			
	Total Mass of tumbler (g) =	181440.0	g	http://www.fukuina.com/shellfish/regular_duty_oyster_tumbler_grader.htm
	aluminum content = 99% of mass	181440 g x 0.99	g	estimated as 99%
	=	179625.6	g	
	Replacement Period =	15.0	*yr*	

	Aluminum use / year = total mass of aluminum content / replacement period	179,626 g / 15 yr	
	=	11975.0	g/yr
	Aluminum use / Unit area = g/yr / area (23216 m^2)	0.5	g aluminum/m^2/yr
11	**Copper Use** *5 Pumps ½ hp 1725 rpm 115V 20 amp electric motor pump*	1138.0	*g/yr*
	=	38.0	lbs
	Mass conversion to g= lbs*453.592		
	g=	17236.5	g
	Total pump mass =	17236.5	g/pump
	Copper content = 1% of mass	17,236 g/pump x 0.01	
	=	172.4	g/pump
	total steel in pumps = steel content (g/pump) x 5 pump = s	172 g/pump x 5 pumps	
	=	861.8	g copper
	Replacement Period for pumps =	8.0	years

Yearly use = total steel mass / replacement period =	862 g copper / 8 years		
=	107.7	g copper/yr	
Tumbler			
Total Mass of tumbler system =	181440.0	g	http://www.fukuina.com/shellfish/regular_duty_oyster_tumbler_grader.htm
Copper content of tumbler electronics = 0.5% of total mass	907.2	g copper	
Replacement period of tumbler =	15.0	yr	Wieland, 2007.
Yearly copper use from tumbler = mass of copper in tumbler / replacement period =	60.5	g copper/yr	
Total yearly copper use = yearly copper use in pumps + yearly copper use in tumbler system	168.2	g copper /yr	
Copper use / Unit area = g/yr / area (23216 m^2)	0.0	g copper/ m^2/yr	

12	**Fiberglass use** *36-ft Fiberglass boat* Weight of boat = Conversion to mass = Weight (lbs) x 453.592 (g) Replacement Period = Yearly use = mass / replacement period = Fiberglass use / unit area = g/yr / 23216 m^2	7800.0 3538017.6 15.0 235867.8 10.2	lbs g yr g fiberglass/yr g fiberglass/m^2/yr	Wieland, 2007.
13	**Fuel, Gasoline** Energy Density = Total BTUs annually = BTUs to Joules = Yearly Use= Fuel use / Unit area = j/yr / area (23216 m^2)	6500.0 124340.0 808210000.0 1055.1 852706688528.5 36729268.1	gallons/year Btu/gal Btu/year Joules/BTU joules/yr j/m^2/yr	
14	**Services** *VIMS Crop Budget Expenses Tool Inputs*			

Boat Fuel =	6500.0	gallons/ year
Cost of Boat Fuel =	22750.0	$/year
Boat Maintenance =	500.0	$/year
Boat Insurance =	884.0	$/year
Business Liability Insurance =	400.0	$/year
LLC Registration fee =	50.0	$/year
Tax Accounting fees =	300.0	$/year
Property txes =	400.0	$/year
Depreciation Expense (boat) =	2806.0	$/year
Depreciation Expense (Hoist/powerwinch) =	343.0	$/year
Depreciation Expense (Cold room) =	1352.0	$/year
Depreciation Expense (Floating upweller) =	6075.0	$/year
Depreciation Expense (Sorter) =	1429.0	$/year
Rental Cost (water column lease) =	15.0	$/year
Commercial Fisherman Registration License =	190.0	$/year

	Oyster Aquaculture Product Owner's Permit =	10.0	$/year
	Oyster Aquaculture Harvester's Permit =	5.0	$/year
	Total operating Costs	44009.0	$
	Services / Unit area =$/yr / area (23216 m^2)	1.9	$/m^2/yr
15	**Labor**		
	Total employee-days applied=14 employees* 5 days/week* 52 weeks/year	3640.0	Working days/year
	Conversion = 2500 kcal/person/day x 4186 j/kcal	10465000.0	J/person/day
	Total energy = total employee-days* j / person/day	38092600000.0	J/yr
	Labor / Unit area =$/yr / area (23216 m^2)	1640790.8	j/m^2/yr
16	**Renewable**		

	Emergy			
	Renewable Emergy	= Sum of emergy inputs from item 3 -4		
	Feedback			
17	**Emergy**			
	Feedback Emergy =	Sum of items 5-15		
	Total			
	Emergy			
18	**Flow**			
	Total Emergy Flow =	Sum of items 1-15		
	Market			
	Sized			
19	**Oysters**			
	Yearly production of market sized oysters =	1500000.0	oysters/ yr	
	Energy content of 6 market sized osyters =	50.0	kcal/ 6 oysters	USDA. Taken from http://ndb.nal.usda.gov/ndb/foods/show/4696?qlookup=15245&max=25&man=&lfacet=&new=1
	Energy content of a market sized osyters =	50 kcal / 6 oysters		
		8.3	kcal/oysters	
	Annual energy yeild = energy content of market sized oyster * number produced (#	8.3 kcal * 4,500,000 oysters/yr		

ind)		
	12500000.0	kcal/yr
Conversion to joules = kcal * 4184 j	37500000 lcal * 4184 j	
=	52300000000.0	j/yr
Oyster energy produced / unit area = j/yr / area (23216 m^2)	2252756.7	

Appendix 2. Footnotes to Table 3.4

	Source	Calculation	Units	References
1	**Sunlight**			
	Fisheries Area =	9811.8	m^2	This Study
	Insolation =	5.58E+09	J/m^2/yr	National Renewable Energy Laboratory, 2012. Taken from www.nrel.gov/gis/images/eere_csp/national_concentrating_solar_2012-01.jpg
	Albedo (Seawater) =	0.07		Payne, 1972
	Energy per year (J/yr) = (fisheries area) x (insolation) x (1-albedo) =	(23216 m^2) x (5.58e+09 J/m^2/yr) x (1-0.06)	j/yr	
		5.10E+13	J/yr	
	Energy / Unit area = Joules/yr / area (9811.8 m^2)	5.19E+09	j/m^2/yr	
2	**Tides**			
	Fisheries Area=	9811.8	m^2	
	Tidal range=	0.45	m	Maryland DNR, 2013
	Water density at salinity 15 ppt =	1005.858	kg/m^3	
	Gravity=	9.8	m/s^2	
	Tides per year=	730	tides/yr	
	Tidal Energy Absorbed per year = (area elevated)(0.5, center of gravity)(tides y^-1)(height^2)(density)(grav	(23216 m^2) x (0.5) x (730 tides yr^-1) x (0.45m tide^-1)^2 x (1005.858 kg *m^-3) x (9.8 m* s^-	j/yr	

	ity)	2)		
	Tidal Energy Absorbed per year =	7.15E+09	j/yr	
	Energy / Unit area = Joules/yr / area (9811.8 m^2)	7.29E+05	j/m^2/yr	
3	**River Channel Flow**			
	Fisheries Area =	9811.8	m^2	
	Water depth before =	1.22	m	
	Water Depth at end of site =	0.9	m	
	Fisheries Width =	100	m	
	Cross Sectional area =	90	m^2	
	Average Velocity before aquaculture site= Conversion factor (k)/ Gauckler-Manning's coefficient x Hydraulic Radius^2/3 x Slope^1/2	V=(k/n) Rh^2/3*S^1/2	m/s	Manning's Equation
	k=	conversion factor	1 m^(1/3)/s	
	n=	Gauckler-Mannings Coefficient	unitless	

n (muddy bottom)=	0.03	unitless	Freeman, 2010
Rh=	Hydraulic Radius	m	
Rh=	Cross-sectional area / wetted parameter	m	
Rh=	0.9		
S=	0.0006	m/m	NOAA Nautical Chart 12230
Water Depth after aquaculture site =	0.9	m	
Average velocity before aquaculture site =	(1/0.03) * 0.9^(2/3) * 0.0006^(1/2)	m/s	
Average velocity before aquaculture site =	0.761	m/s	
Flow rate before aquacultlure site = velocity x cross sectional area	(0.761) * (90)	m^3/s	
Flow rate before aquacultlure site=	68.50	m^3/s	
Flow rate before aquaculture site (yearly) = flow rate (m^3/s) x 3.15569e7 s/yr =	68.5 x 3.15569e7	m^3/yr	
	2161654194	m^3/yr	

Average Velocity after aquaculture site=			
Conversion factor (k)/ Gauckler-Manning's coefficient x Hydraulic Radius^2/3 x Slope^1/2	V=(k/n) Rh^2/3*S^1/2	m/s	
n (oyster reef)=	0.07	unitless	Freeman, 2010
Average Velocity after aquaculture site =	(1/0.07) * 0.9^(2/3) * 0.0006^(1/2)	m/s	
=	0.326	m/s	
Flow rate after aquaculture site = velocity * cross sectional area	(0.326) * (90)	m^3/s	
	32.62	m^3/s	
Flow rate after aquaculture site (yearly)	flow rate (m^3/s) x 3.15569e7 s/yr	m^3/yr	
=	1029359140	m^3/yr	
Mass of water per year before aquaculture site = flow rate * density of brackish water	(2161654194 m^3/yr) * (1005.858 kg/m^3)	kg/yr	
=	2.2E+12	kg/yr	
Kinetic Energy before	1/2 * (2.1E+10 kg/yr) x (0.761	Joules/yr	

aquaculture site = 1/2 (mass) x velocity^2	m/s)^2	
=	6.30E+11	Joules/ yr
Mass of water per year after aquaculture site = flow rate * density of brackish water	(102935914 0 m^3/yr) * (1005.858 kg/m^3)	kg/yr
=	1.04E+12	kg/yr
Kinetic Energy after aquaculture site = 1/2 (mass) x velocity^2	1/2 * (1.01E+10 kg/yr) * (0.326 m/s)^2	Joules/ yr
=	5.51E+10	Joules/ yr
Energy absorbed by aquaculture site = Energy of flow entering site - energy of flow leaving the site	6.14E+9 - 5.37E+08	joules/ yr
	5.7E+11	Joules/ yr
Average Velocity at entrance of channel (mannings equation)	V=(k/n) Rh^2/3*S^1 /2	m/s
k=	1 conversion factor	m^(1/3)/s

n=	Gauckler-Mannings Coefficient	unitless	
n (oyster reef)=	0.07	unitless	Freeman, 2010
n (muddy bottom)=	0.03	unitless	Freeman, 2010
Rh=	Hydraulic Radius	m	
Rh=	Cross-sectional area / wetted parameter	m	
Rh=	0.9		
S=	0.0006	m/m	NOAA Nautical Chart 12230
Average Velocity over muddy bottom at entrance of channel =	0.761113415	m/s	
Flow rate at entrance of channel =	velocity x cross ectional area	m^3/s	
=	68.50020737	m^3/s	
Flow rate at entrance (yearly) =	flow rate (m^3/s) x 3.15569e7 s/yr	m^3/yr	
=	2161654194	m^3/yr	
Average Velocity over reef at aquaculture site=	0.326191464	m/s	
Flow rate at aquaculture site =	velocity x cross ectional area	m^3/s	
=	29.35723173	m^3/s	
Flow rate at aquaculture site (yearly) =	flow rate (m^3/s) x 3.15569e7 s/yr	m^3/yr	
=	926423225.	m^3/yr	

		9		
	Mass of water per year =	flow rate * density of brackish water	kg	
	Kinetic Energy entering site =	1/2 (mass) x velocity^2	Joules/ yr	
	=	6.31203E+11	Joules/ yr	
	Kinetic Energy exiting site =	1/2 (mass) x velocity^2	Joules/ yr	
	=	49686519488	Joules/ yr	
	Energy absorbed by aquaculture site =	Energy of flow entering site - energy of flow leaving the site	joules/ yr	
	=	5.81516E+11	Joules/ yr	
	Energy / Unit area = Joules/yr / area (9811.8 m^2)	59267035.83	j/m^2/ yr	
4	**Phytoplankton**			
	Metabolism of intertidal oyster reef =		kg O2/m^2 oyster reef/yr	
	Oxygen consumptoin rate (kg O2/m^2/yr)	6.5		Dame et al, 1992
	Free energy change (kcal) per mole of glucose decomposed =	686.0	kcal	

	ratio of glucose to oxygen consumed =	0.2	mol C6H12O6/ mol O2	
	Oxygen consumed per unit area = g-O2/m^2	6500.0	g O2/m^2 oyster reef	
	Moles to grams conversion = 1 mol O2 / 32 g-O2	0.03	mol/g	
	Kcal consumed per year = free energy change (kcal) per mole of glucose consumed x ratio of glucose ot oxygen consumed x oxygen csonumed per unit area x moles to grams conversion.	23224.0	kcal/m^2 oyster reef/yr	
	Kcal ot joules converion = kcal/yr * 4184 joules	97169041.7	j/m^2 oyster reef /yr	
5	**Cultched Spat**			
	cost / 1,000 5 mm-seed oysters =	10.75	$/1000 cultched spat	Horn Point Hatchery
	Number of Spat Purchased	2.00E+06	spat/yr	Schockley

	per year=			
	Total Cost=			
	Cost/1,000 seed * 2,000,000 spat=	2.15E+04	$/yr	
	$ / Unit area =$/yr / area (9811.8 m^2)	2.19E+00	j/m^2/ yr	
6	**PVC**			
	Floating rafts			
	Length of 4-inch piping for each raft=	577.85	cm	Four inch PVC = 0.632 lbs/ft.
	Mass per cm of PVC piping=	9.405	g/cm	
	Mass of PVC per float= length of pvc piping needed * mass per cm pvc piping	5434.7	g/float	286.67038 lbs/ 30.48 cm
	Floats in use annually=	2389	floats	Observed
	Total PVC use in floats = Number of floats * mass of pvc/float	5434.7 g pvc/float * 2389 floats		
	Total pvc in floats =	12983448.73	g pvc	
	Replacement period =	8	yr	
	Total use per year= Mass / Replacement Period	1622931	g/yr	
	PVC / Unit area =g pvc/yr / area (9811.8 m^2)	165	g pvc/m ^2/yr	

7	**Machinery**			
	Power washer mass=	40823	g	
	Replacement period=	5	yr	
	Yearly use = Mass / Replacement Period	40823 g machinery / 5		
	Yearly use =	8164.66	g/yr	
	Machinery / Unit area =g machinery/yr / area (9811.8 m^2)	0.832126623	g machinery/m^2/yr	
8	**Electricity**			
	Electrical Utility Costs/Month =	200	$/month	
	Average Cost of Electricity=	0.112	$ / kwh	EIA (Comercial Rate for Southern Mid-Atlantic)
	Monthly Electricity Use=	1786	kwh/month	
	Kilowatthours to Joules= kwh * 3,600,000 joules	6428571429	j/month	
		1786 kwh * 3600000 joules		
	Yearly Electrical Use=	7.71E+10	j/year	
	Energy / Unit area = Joules/yr / area (9811.8 m^2)	7.86E+06	j/m^2/yr	
9	**Fuel,**	104	gallons	

	Gasoline =		/year	
	Energy Density =	124,340	Btu/gal	
	Total BTUs annually =	12931360	Btu/year	http://www.afdc.energy.gov/fuels/fuel_comparison_chart.pdf
	BTUs to Joules =	1055.05585	Joules/BTU	
	Yearly Use=	13643307016	joules/yr	
	Energy / Unit area = Joules/yr / area (9811.8 m^2)	1390499.91	j/m^2/yr	
10	**Services =**			
	Boat Fuel =	1.04E+02	gallons/year	
	Boat Fuel (used in power washer) =	3.64E+02	$/year	
	Business Liability Insurance =	400.0	$/year	
	LLC Registration fee =	50.0	$/year	
	Tax Accounting fees =	300.0	$/year	
	Property txes =	400.0	$/year	
	Depreciation Expense (Cold room) =	1352.0	$/year	
	Rental Cost (water column lease) =	7.5	$/year	
	Commercial Fisherman Registration License =	190.0	$/year	
	Oyster	10.0	$/year	

	Aquaculture Product Owner's Permit = Oyster Aquaculture Harvester's Permit =	5.0	$/year
	VIMS Crop Budget Services	3078.50	$/yr
	Services / Unit area = $/yr / area (9811.8 m^2)	0.31	$/m^2/yr
11	**Labor**		
	Total man-days applied = 4 employees*5 days/week*52 weeks/year	1040	Working days/year
	Conversion= 2500 kcal/person/day x4186 j/kcal	10465000	J/person/day
	total energy = total man-days*j/person/day	10883600000	J/yr
	Labor / Unit area = j/yr / area (9811.8 m^2)	1109235.818	j/m^2/yr
12	**Renewable Emergy**		
	Renewable Emergy =	Sum of items 3-4	
13	**Feedback Emergy**		
	Feedback Emergy =	Sum of items 5-11	

14	**Total Emergy Flow**				
	Total Emergy Flow =	Sum of items 3-11			
15	**Market Sized Oysters**				
	Yearly production of market sized oysters =	1,500,000	oysters /yr		
	Energy content of 6 market sized oysters =	50	kcal/ 6 oysters	USDA. Taken from http://ndb.nal.usda.gov/ndb/foods/show/4696?qlookup=15245&max=25&man=&lfacet=&new=1	
	Energy content of a market sized oysters =	50 kcal / 6 oysters			
		8.3	kcal/oysters		
	Annual energy yeild = energy content of market sized oyster * number produced (# ind)	8.3 kcal * 4,500,000 oysters/yr			
		12500000	kcal/yr		
	Conversion to joules = kcal * 4184 j	37500000 lcal * 4184 j			
	=	52300000000	j/yr		
	Yield per unit area = j/yr / 9811.8 m^2	5330316.558	j/m^2/yr		

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