ABSTRACT

CAMPBELL, MATTHEW DWAIN. The Investigation of Biological and Engineering Parameters Intrinsic to Intensive Culture of Oysters, *Crassostrea virginica*, in Upweller Systems. (Under the direction of Dr. Steven G. Hall).

As oyster aquaculture continues to grow, there is an increased need to develop sustainable and profitable operations. The use of upweller culture units in oyster nurseries is widely practiced as a technique that enhances the ability to rear large quantities in a semi-controlled environment. Hydrodynamics within these systems are critical to operation because it affects oyster growth due to food supply, oxygen, and metabolic waste buildup. As the industry continues to mature, it is imperative that the influence of hydrodynamics on oyster aquaculture is thoroughly understood. A review of hydrodynamics effects on oysters revealed feeding and growth limiting velocities are reported that range from 1 to above 22 cm s\(^{-1}\). This is in contrast to thriving oyster reefs in a natural setting that exist and thrive above 15 cm s\(^{-1}\). Upweller systems have also reported a wide range of bulk velocities from 0.5 to 7.1 cm s\(^{-1}\). In practice, higher current velocities are desirable because they increase delivery of food to the oysters, improve water quality, and enhance dispersal of biodeposits. Guidance for flow rate operations have been primarily anecdotal and thus there is a need to develop a more mechanistic assessment of upweller systems.

A series of experiments were conducted to investigate the application of hydrodynamic-based engineering models to oyster upweller systems. The application of packed bed reactor theory was investigated, which has the potential to improve optimization of these culture methods. The Ergun equation and the axial diffusion model developed from packed bed reactor theory were evaluated using a set of controlled experiments to determine the hydrodynamic properties of a packed bed of oysters. The Ergun equation was able to accurately predict the
hydrodynamic equivalent diameter distribution of oyster shells (μ=3.18 mm, σ=0.74 mm). This oyster shell diameter and void ratio distribution gained through the Ergun equation was used in the relationship of axial diffusion and superficial velocity. The mean axial diffusion coefficient in the oyster bed was estimated $1.65 \times 10^4$ m$^2$/s at 0.01 m/s and $7.26 \times 10^4$ m$^2$/s at 0.08 m/s. The data gained from these experiments was used to develop mechanistic models calibrated through Bayesian inference. The use of Bayesian inference allows for greater understanding of the credibility of individual parameters (i.e., rates and physical attributes) within these mechanistic formulations. This work established a baseline methodology to systematically evaluate and optimize bivalve upweller culture systems.

The determination of oyster filtration magnitude and variability is also important in the development of mechanistic models for upweller system optimization. The filtration of oysters within an upweller system was explored though controlled experiments. Five upweller experimental units were constructed on-site at an oyster farm in North Carolina. The sedimentation and filtration within these upwellers were analyzed using various sampling and analysis methods. Individual particles were observed using a portable FlowCAM (Flow Cytometry And iMaging) system equipped with auto-imaging. This analysis revealed dynamics within the upweller oyster bed that were not possible to observe with total suspended solids testing alone. While there was no clear trend on removal of solids by TSS, when analyzed by particle and size class with FlowCAM, a mean of 35.5% (σ=8.6%) of small particles (5-200 micron) were removed on each pass through the system. The mean individual filtration rate of oysters was 3.44 L/hr/g (σ = 0.80 L/hr/g, n=4), which was similar to other studies.

By applying engineering principles to oyster upweller systems, there is an opportunity to identify and quantify critical parameters. This work establishes a framework to understand and
optimize these upweller systems to improve production yield and increase sustainability of the industry. The application of mechanistic models allows for the optimization in diverse situations and the ability to improve designs of culture systems.
The Investigation of Biological and Engineering Parameters Intrinsic to Intensive Culture of Oysters, *Crassostrea virginica*, in Upweller Systems

by
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DEDICATION

To my wife, Becky, and daughters, Kate and Nora.
I would like to thank the William White Endowment of the North Carolina Agriculture Foundation for generously providing funding for this research. I would like to especially thank my wife, Becky, daughters, Kate and Nora for all of their support and sacrifice during this time. I would like to thank my committee members for serving and giving me guidance and support along the way. My advisor, Steve Hall, is an amazing mentor and friend. My lab group was especially supportive during the study and were always willing to join in and help out when I needed a hand. Astrid Schnetzer and her lab taught me so much about microalgae and were generous with their time in helping me with the FlowCAM. The BAE shop staff were always very helpful and I learned a lot from them on machining various components of the experiments; thank you Neil, Joe, and Robbie! Ryan Kelly and Tommy McArthur from our MARC facility were very supportive and helpful in setting up experiments and helping me acquire oysters! Thanks to faculty at BAE for review of papers and experimental methodology. Cong Tu from the BAE EAL Lab for helping with lab analysis and letting me shadow him on some of the more advanced techniques. Chris Matteo from Chadwick Creek Oyster Farm was extremely helpful in giving me guidance on culture operations and providing the oysters for this study. And all this by the grace of God that I was even afforded the opportunity to endeavor such a challenging and rewarding accomplishment! Jesus, you sustained and encouraged me in the most challenging moments, thank you!
Matthew Campbell was born and raised in Louisiana. He has been fascinated with biological systems in the coastal environment his entire life. Growing up in Louisiana, he became keenly aware of the interaction of the coastal environment and the people who inhabit it. He graduated from the Louisiana State University Biological and Agricultural Engineering Department with a Bachelor’s degree in 2002 and a Master’s degree in 2004. His Master’s thesis was entitled, “Analysis and Evaluation of a Bioengineered Submerged Breakwater.”

After graduating from LSU, Mr. Campbell worked as a coastal engineer for the State of Louisiana and then in consulting as a senior engineer. He gained extensive experience with the integration of biology and engineering in the marine environment. His experience included multiple phases of project development including client management, feasibility evaluation, scope of work development, numerical modeling and analysis, preliminary design, final design, permitting, specification development, bidding process, and construction management. Mr. Campbell thrives in a multidisciplinary environment and has managed a wide variety of teams. Mr. Campbell has also founded two companies and successfully commercialized coastal technologies that he patented and developed. His 15 years of experience in the coastal engineering field has given him keen insight into coastal development and dynamics throughout the world.

Mr. Campbell joined North Carolina State University as a graduate student in 2017 and as a research associate at Marine Aquaculture Research Center with an emphasis on marine aquaculture engineering. Mr. Campbell leveraged his background in coastal engineering to investigate aquaculture techniques and technologies that will promote a more sustainable industry.
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CHAPTER 1: INTRODUCTION

Oyster aquaculture is a rapidly growing global industry that is expanding and diversifying. Oyster aquaculture has been touted as a potentially beneficial marine aquaculture practice that can enhance the surrounding environment and operate synergistically with other types of aquaculture, such as multi-trophic techniques (Soto, 2009; Granada et al., 2016). A thorough understanding of the physical and biological mechanisms within these culture systems is required to advance the practice. There has been a concerted multidisciplinary effort to investigate the physical, biological, ecological, and economic interactions within oyster aquaculture (Goslin, 2015).

An understanding of hydrodynamics (i.e. interactions of fluid motion and solid bodies) within oyster aquaculture practices is critical due to the impacts on feeding, growth, and survival (Goslin, 2015; Kennedy, Newell, and Eble, 1996; Wildish and Kristmanson, 1997). Upweller (i.e. upflow) systems have been used to culture juvenile oysters to reduce predation and increase yields at a vulnerable period in the oyster life cycle. Upweller systems are particularly influenced by the flow and the shape of the culture units. Flow through these systems regulates food, oxygen, and water quality within the oyster bed. Higher flows increase oyster growth, but could be limiting due to feeding inhibition, damage, and required energy inputs (Appleyard and Dealeris, 2002; Pfeiffer and Rusch, 2001; Rodhouse and O’Kelly, 1981; Ver and Wang, 1995).

This study addressed some of the knowledge gaps that are inhibiting the advancement of oyster aquaculture. This dissertation has been developed as a series of papers exploring the role of hydrodynamics in oyster aquaculture, engineering framework for modeling and analysis, and biological variability within culture systems. The first paper is a review of the hydrodynamic impacts on oyster aquaculture. The second paper develops the framework of applying packed
bed reactor theory to oyster upweller systems. The third paper explores the inherent variability of oyster filtration in oyster upweller systems. This body of work establishes a framework to understand and optimize these upweller systems to improve production yield and increase sustainability of the industry.
CHAPTER 2: HYDRODYNAMIC EFFECTS ON OYSTER AQUACULTURE SYSTEMS: A REVIEW

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Abstract
Hydrodynamics (i.e., interactions of fluid motion with solid bodies) affect oyster aquaculture within every phase of farming. Although it has many direct and indirect implications to the success of any particular aquaculture endeavour, hydrodynamics is the least understood of environmental factors affecting oyster growth. As the industry continues to mature, it is imperative that the influence of hydrodynamics on oyster aquaculture is thoroughly understood. Hydrodynamics also interacts with other environmental factors, such as salinity, temperature, turbidity, food supply and oxygen, which affect the health and growth of oysters through mixing and transport. Proper siting and management of aquaculture requires a comprehensive understanding of the hydrodynamics involved and its impact on the culture of oysters. Unfortunately, literature is inconsistent on oyster feeding and growth response to the influence of hydrodynamics. Feeding and growth limiting velocities are reported that range from 1 to above 22 cm s⁻¹. This is in contrast with thriving oyster reefs in a natural setting that exist and thrive above 15 cm s⁻¹. Upweller systems have reported bulk velocities that range from 0.5 to 7.1 cm s⁻¹. In practice, higher current velocities are desirable because they increase delivery of food to the oysters, improve water quality, and enhance dispersal of biodeposits. This paper summarizes the findings of those studies in regard to hydrodynamics and provides suggestions for future work.

Key words: aquaculture, flow, hydrodynamics, oyster, upweller, velocity.

Introduction
Marine aquaculture is a rapidly growing global industry, with oysters contributing a substantial proportion in many parts of the world. The demand for seafood has increased as the marine environment and the natural fisheries have been increasingly stressed. Global capture fisheries have reached a maximum threshold primarily due to the overexploitation of natural marine fish stocks. Aquaculture is becoming an increasingly greater source of global seafood supply compared with wild-caught. This is largely due to the stagnating wild-caught supply and increasing demand for healthy protein from fish. Recently, aquaculture production has surpassed capture fisheries; providing over 50% of the total global fish supply (FAO, 2016).

As the aquaculture industry continues to expand, there is an ever increasing need to develop sustainable methods that maximize yield and profitability while protecting, or even improving, the surrounding environment. Oyster aquaculture has been touted as a potentially beneficial marine aquaculture practice that can enhance the surrounding environment and operate synergistically with other types of aquaculture, such as multi-trophic techniques (Soto 2009; Granada et al. 2016). A thorough understanding of the physical and biological mechanisms within these culture systems is required to advance the practice. There has been a concerted multidisciplinary effort to investigate the physical, biological, ecological and economic interactions within oyster aquaculture.

Hydrodynamics (i.e., interactions of fluid motion with solid bodies) are important to oyster aquaculture because of the effects on feeding, growth and survival (Kennedy et al. 1996; Wildish & Kristmanson 1997; Goslin 2015). Excessive velocity can produce differential pressures around inhalant and exhalent regions resulting in feeding inhibition (Wildish & Kristmanson 1997). Hydrodynamics can also interact with the surrounding environment that have direct and indirect effects on oysters (Dame 2012). The
hydrodynamic environment from tides, waves and currents affect the locations where oyster aquaculture can be conducted (Mallet et al. 2003, 2013; Bishop & Hooper 2005; Comeau 2013; Theuerkauf et al. 2017). Previous attempts to understand the flow effects on oyster growth and filtration have been inconclusive and poorly understood (ZuERMass et al. 2013). The optimal stocking density, which is important with considering flow requirements through an oyster bed, has been empirically determined and is not fully understood (Manzi et al. 1986; Appleyard & Dealeris 2002). Regardless of the type of oyster aquaculture system, it is important to consider the hydrodynamic effects.

This paper attempts to synthesize the significant research that has been conducted on oysters since the 1970s relative to the hydrodynamic effects. In addition to literature that directly addresses this specific topic, studies related to other non-siphonate bivalve species, such as mussels and scallops, have been included. Although there are conceivable differences in response, incorporation of these diverse studies is thought to be useful in our understanding of relevant hydrodynamic interactions.

Oyster aquaculture methods

Oyster aquaculture is believed to exist for over 2000 years with the mention of oyster culture in Greece by Aristotle in 350 B.C. (Goslin 2015). The practice has developed and spread globally since that time. Although agriculture in general has experienced massive development through mechanization, the oyster aquaculture industry has not experienced the same (Matthiessen 1970). The oyster industry in North America has historically relied on primitive methods including tongs and dredges for harvesting oyster reefs. According to Matthiessen (1970), the primary species traditionally cultured in the US has been the Eastern Oyster, Crassostrea virginica, followed by the Pacific oyster, Crassostrea gigas, the Olympia oyster, Ostrea lurida and the European flat oyster, Ostrea edulis.

Modern oyster aquaculture practices rely on three distinct culture phases: spat or seed production, nursery and grow-out (Goslin 2015). Each of these phases is advancing through technology development, understanding of oyster biology and environmental interactions, and scientifically informed regulation. Additional advances in the area of genetic modification (i.e. ploidy) have the potential to increase growth rates and disease resistance (Walton et al. 2013).

Hatcheries are used within the oyster aquaculture industry to accommodate the abundance and reliability limits of wild-caught sources for spat (i.e. free swimming oyster larvae attached to a substrate). Hatchery methods have been explored since the 1920s through the US Bureau of Commercial Fisheries Laboratory in Milford, Connecticut (Matthiessen 1970). Many other regions throughout North America have increased hatchery research and technology development (Helm et al. 2004). Although there are many places throughout the world that continue to rely on wild-caught spat, many regions are developing hatcheries to accommodate the growing industry (Helm et al. 2004; Goslin 2015).

Nursery culture of spat and juvenile oysters is practiced to reduce predation and increase yields at a vulnerable period in the oyster life cycle. This is usually done in raceways or upweller (i.e. upflow) systems. Upweller systems are conducted on land-based or raft/barge systems. These types of systems typically have much higher density capacity than other methods, such as raceways (Goslin 2015). A conceptual floating upweller system for rearing juvenile oysters is shown in Figure 1. These types of systems are heavily influenced by flow rate through the culture silos.

The grow-out phase of aquaculture is the longest and possibly the riskiest period during the oyster culture process. Traditionally, this has occurred through ‘shell-on-bottom’ practices where the oysters are harvested with tongs or dredges from submerged reefs. Suspended culture techniques include off bottom racks, floating cages, string culture and long line cages that hold single or culchless oysters that are considered a higher value product (Matthiessen 1970; Goslin 2015).

Variations in the techniques described above have been conducted with positive results. Powell and Wheaton (1993, 1995) designed and constructed a method of floating platforms with suspended racks in the Chesapeake Bay for production of oysters. They observed over a 2 year study that their system produced higher density and quality than long line systems used elsewhere in the bay (Powell & Wheaton 1993, 1995). Semi-controlled systems have been implemented through the use of salt marsh ponds, such ‘claires’ in France, for fattening oysters or exposing to them specific food sources that give them the desirable blue gills (Goslin 2015).

Oyster growth and mortality in the natural environment

The natural environment influences the growth rate of oysters through direct and indirect interactions. Food supply is a major contributor to oyster growth and is affected by flow, temperature, population density, and turbidity. Other factors such as salinity, temperature, and oxygen are dynamic and affect oyster growth and survival. Site hydrodynamics have primary and secondary effects on oyster growth by directly inhibiting feeding through physical damage at high velocities and regulating feeding through supply of food. Hydrodynamics also have indirect effects
including turbulence induced turbidity, aerial exposure times from tides, dissolved oxygen concentration through mixing, and selection of predatory pressure types. Other environmental factors that can influence growth rates include pollutants, ocean acidification, sediment properties and light (Shumway 1996; Dame 2012; Goslin 2015).

Oyster growth is directly linked to feeding, which is influenced by the natural environment (Dame 2012). Oysters and other bivalves feed by removing suspended seston from the water column, which is composed of phytoplankton, detritus and inorganic particles. Non-siphonate bivalves, such as oysters, capture seston by filtering the water through their gills. The gills are lined with bands of cilia that catch and transport the suspended particles to the mouth (Newell & Langdon 1996). Physical phenomenon within the environment, such as hydrodynamics, can affect feeding mechanisms, and regulate the supply of food (Wildish & Kristmanson 1997).

Natural environmental factors have combined effects creating conditions that increase mortality and decrease the growth rate of oysters. For example, Rybovich et al. (2016) demonstrated the negative influence of the combined effects of low salinity and high temperature on the growth and mortality of oysters. Furthermore, the effects were more pronounced in larger market size oysters, which were more negatively affected by the higher temperatures than the juvenile oysters.

Environmental factors effect on filtration rate
Filtration rate (or clearance rate) has widely been used as a surrogate for oyster feeding and interactions with the surrounding environment. Filtration rate (L h⁻¹) is a metric for the amount of suspended particles removed from the water column by oysters. Clearance and filtration rates are synonymous, if one assumes that 100% of all suspended particles ‘cleared’ are retained by the oyster (i.e. no particle rejection).

Lab studies and field studies have attempted to determine oyster filtration under various conditions. These studies have yielded a multitude of results on reported filtration rates and suggestions how they should be applied. Grizzle et al. (2008) observed filtration rates in a field study (1.21 L h⁻¹) and compared them with previously conducted lab and field experiments relative to a standardized 1 g dry weight oyster biomass. Lab studies were suggested to have over predicted filtration rates compared with in-situ measurements because they did not properly account for various environmental factors. Flows and wind waves were thought to significantly affect in-situ measurements of filtration rates due to sediment resuspension further complicating studies (Grizzle et al. 2008).

Models have been developed to analyze the filtration rate under various environmental conditions. Erich and Harris (2015) reviewed three oyster filtration models that included temperature, total suspended solids (TSS) and salinity. The three models were compared with other filtration rates reported previously in the literature. Fulford et al. (2007) predicted higher filtration rates than the other models, while Powell et al. (1992) had relatively lower rates (3-4 times lower). In general, higher temperatures corresponded with increased filtration rates in all three models. The Cerco and Noel (2005) model predicted similar responses to TSS as Fulford et al. (2007). Salinity had a relatively small effect
on filtration rate than other factors in these models (Ehrlich & Harris 2015).

Recently, ZuErmgassen et al. (2013) conducted a review on environmental factors affecting oyster filtration rate. The information was synthesized in an attempt to quantify ecosystem service losses due to population decline of the eastern oyster, *Crassostrea virginia*. The study primarily based the assessment on water temperature and oyster size for estuary-wide scale estimates. Some variables were acknowledged to elicit ‘all or nothing’ responses in filtration rates, which include salinity and dissolved oxygen. Although flow rate and seston quantity effect oyster filtration and growth, they were not included in the analysis because incorporating factors that are spatially and temporally dynamic was not feasible for the scope of the study. ZuErmgassen et al. (2013) acknowledged that the effect of flow rate on oyster filtration rates is not well understood and previous studies have presented conflicting results.

**Oyster aquaculture modelling and site selection**

Modellers have incorporated aquaculture-environmental interactions that help to assess and manage proposed oyster aquaculture farms. One of these models developed by Ferreira et al. (2007) is termed the Farm Aquaculture Resource Management (FARM) model, which utilizes environmental variables (i.e. current velocity, temperature, and chlorophyll a concentrations) and farm layout (i.e. culture technique, species, and stocking density) as inputs into bivalve growth and economic models. This model allows for the evaluation and assessment of proposed site layout, environmental impact and profitability. Another model developed by Hawkins et al. (2013) termed ShellSIM uses a more generic approach that allows for analysis of multiple habitats and species. This model was incorporated into a geospatial model, ShellGIS, which incorporates local hydrodynamics. The hydrodynamics are determined from data collection or numerical models for refined evaluation of the site conditions and impacts to the surrounding environment (Newell et al. 2013).

All of the above mentioned models include the hydrodynamics (i.e. current speed) as an input variable. The hydrodynamics of a proposed aquaculture site can affect the structural integrity of the culture system, oyster growth, and the impact on the environment. Proper siting of aquaculture requires a good understanding of the hydrodynamics and its impact on the aquaculture emplacement. As Newell et al. (2013) discussed the use of their model for aquaculture siting and planning, they emphasized that higher current velocities are desirable because they deliver food to the oysters and increased dispersal of oyster biodeposits. Unfortunately, potentially inhibiting effects of the hydrodynamics are not incorporated into the existing models.

**Effects of grow-out culture method on oyster feeding and growth**

Oyster aquaculture has engaged in various culture methods depending on the phase of growth, regional traditions, environmental conditions and market demand. The various culture methods have shown to have varying success depending on physical environmental factors. Hydrodynamics conditions generated by currents and waves affect growth rates in floating cage culture, which have been attributed to movement and disturbance of the oysters (Mallet et al. 2003, 2013; Bishop & Peterson 2005). Additionally, oysters grown on bottom can be affected by low dissolved oxygen, which is correlated with the hydrodynamic environment (i.e. lack of flow and mixing).

Oysters grown near the bottom may be subject to low oxygen events and increased predatory pressure. Grabowski et al. (2004) noted increased growth of *Crassostrea ariakensis* but not *C. gigas* as rack height of culture units were raised off the bottom. Another example was reported by Walton et al. (2013) where they conducted an experimental field test to explore the effect of ploidy and gear on oyster survival, growth and disease prevalence. The field test used a combination of triploid and diploid oysters placed in four culture gear types: (i) low profile bottom cages; (ii) longline baskets; (iii) floating cages; and (iv) floating bags. The experiment was conducted in Grand Bay, Alabama from May to October. The ploidy did not seem to have an effect on survival, while gear type did demonstrate significant decreased survival in bottom cages. Bottom cages also had the poorest growth rates among the gear types.

Comeau (2013) compared filtration rates and growth of the oyster, *C. virginica*, in floating and bottom culture. The oyster shell height and dry tissue weight of each method was correlated to the observed filtration rates. The ratio of dry tissue weight to shell height was larger for the suspended than the bottom culture. The filtration rates to dry tissue weight were lower for suspended culture than the other methods. Although the growth rates for the floating culture were higher, the relative filtration rates (and impact on surrounding phytoplankton population) were relatively lower than the bottom culture (Comeau 2013).

Wave action can also affect oyster growth especially in floating cages where oyster motion can inhibit growth. Bishop and Peterson (2005) deployed floating and fixed oyster aquaculture racks at six locations across coastal North Carolina. The oyster, *C. ariakensis*, growth was lower in the floating racks than in the fixed racks suspended 15 cm above the bottom. Mallet et al. (2003) also noted...
negative impacts of oyster growth in floating cages in higher wave energy environments.

As noted above, the movement of oysters within cages or bags could have negative effects on oyster growth. Mallet et al. (2013) compared growth of oysters in floating cages and attached to floating horizontal ropes. The oysters attached to ropes had limited movement and significantly greater growth rates than the cage cultured oysters across experimental sites. This was partially attributed to the damage of shell edge (i.e. new shell growth) or feeding disturbances in floating cages, especially in high wave action areas within the cage culture systems (Mallet et al. 2013).

Another example of movement effects on growth was conducted by Mallet et al. (2009) in their study of biofouling impacts and handling strategies of floating oyster bag systems. It is currently standard practice to control biofouling on oyster culture gear in order to allow for maximum flow through cages or bags by turning them and exposing them to the air. In their study, the frequency of handling to reduce biofouling did not correspond to increased oyster growth rates. On the contrary, Mallet et al. (2009) suggested that the activity of bag turning could be detrimental to oyster growth.

In-situ hydrodynamics on oyster reefs

The hydrodynamics in the natural environment may give us insights into the conditions that oysters can thrive. Lenihan (1999) measured oyster growth and mortality on various three dimensional reef structures with low to high relief. Higher growth and lower mortality rates were observed on the crest of the reefs corresponding to the highest current velocities. The velocity explained 81% of the variation of growth and mortality where higher velocities corresponded with higher growth rates and lower mortality. The velocities on the crest were approximately 8 cm s⁻¹ on average and approximately 17 cm s⁻¹ during faster flow periods.

Higher precision velocity measurements are possible near the oyster reef surface with the implementation of Doppler technology in the marine environment. Styles (2015) measured tidal current velocities over an oyster reef in the North Inlet estuary located near Georgetown, South Carolina with a SonTek acoustic Doppler velocimeter (ADV). He recorded velocities 10 cm above the bed within a tidal creek at 30 cm s⁻¹ above an oyster reef and up to 40 cm s⁻¹ on the opposite bank to the reef.

Data analytics have been utilized on large-scale hydrodynamic spatial and temporal data sources to establish correlations to potential oyster habitat. Theuerkauf et al. (2017) correlated the presence of oysters from ground-truthed oyster maps with estimated wave exposure using the Wave Exposure Model developed by the United States National Oceanographic and Atmospheric Administration. The threshold for presence of oysters was estimated to be approximately 500 J m⁻² total representative wave energy in one wavelength per unit wave crest length.

These studies suggest that oysters can survive in higher energy environments under certain conditions. This information could be used to establish experimental bounds in future studies endeavouring to explore the upper limits of oyster growth and survival. These types of studies are useful in advancing our understanding of how and where we can implement oyster aquaculture.

Effects of flow on oysters

Previous attempts to understand the flow effects on oyster growth and filtration have been inconclusive and poorly understood (ZuErmgassen et al. 2013). Studies of other non-siphonate bivalves have revealed some insights into how flow and water currents affect feeding and growth. Kirby-Smith (1972) was the first to grow bay scallops, Argopecten irradians, in a controlled tube study investigating the effects of water currents on growth. The experimental apparatus consisted of eight opaque tubes of 7.6 cm diameter that were stacked within a box. The end of the pipes had rubber stoppers with an inserted glass tube to regulate flow. The inflow to the pipes was driven by a head differential in a calming chamber. This allowed Kirby-Smith (1972) to maintain a constant and consistent flow through the pipes. The 'bulk velocity' or superficial velocity was calculated by dividing the flow rate by the pipe area. The scallops were grown in a series of twelve segmented partitions in each of the tubes. The system was fed with unfiltered water from the Beaufort Channel, Beaufort, North Carolina. Two separate experiments were run in the summer and winter for 3 and 6 weeks, respectively. Shell length was measured at the end of the experiments with Vernier calipers to the nearest 0.1 mm.

The scallops had a growth response to the flow rate in which they were subjected. They had negligible growth at 12.5 cm s⁻¹ and maximum growth at 0.21 cm s⁻¹. The scallops also exhibited differential growth depending on the flow and their position in series along the tube. Scalars near the inflow under the 0.75 cm s⁻¹ exhibited slower growth than those near the exit. Conversely, scallops near the inflow under 0.21 cm s⁻¹ exhibited faster growth than those near the exit. This indicates a possible hydrodynamic limitation on the higher flow conditions and food limitation in the lower flow condition near the exit.

Controlled studies have also been conducted on oysters primarily in saltwater flumes, which attempt to approximate laminar flow conditions. Grizzle et al. (1992) investigated the growth response of Eastern oyster,
C. virginica, at various current speeds (1-8 cm s$^{-1}$) in two separate experiments over summers of 1990 and 1991. These experiments were conducted in multiple flow-through flumes. The 1990 summer experiment included three current speeds (2, 4, and 8 cm s$^{-1}$) that did not reveal significant differences in oyster shell growth. The 1991 summer experiment demonstrated significant differences ($P = 0.042$) in oyster shell growth between the four current speeds (0, 1, 2, and 4 cm s$^{-1}$). The optimum growth rate for the oysters occurred at 1 cm s$^{-1}$ and showed a decreasing growth response in higher (>1 cm s$^{-1}$) and lower current speeds (0 cm s$^{-1}$). Grizzle et al. (1992) hypothesized that this was due to the relationship of inhalant velocities and the optimum current speeds to feeding activity. Although the 1991 experiment showed a significant difference in growth rate at 1 cm s$^{-1}$, all of the velocities in the 1990 experiment had comparable growth rates to those exposed to the 1 cm s$^{-1}$ velocity in the 1990 experiment. Therefore, the overall results from these experiments are inconclusive relative to the effect of current speed on oyster growth rate.

Harsh and Luckenbach (1999) conducted a series of flume experiments investigating the effect of current speed on filtration activity of Eastern oysters, C. virginica. The experiments consisted of placing a bed of oysters (90 oysters) in a saltwater flume where they were subjected to eight current velocities (0.65--22.0 cm s$^{-1}$). The study did not demonstrate a significant correlation between current speed and filtration rate. On the other hand, the study demonstrated that feeding activity up to current speeds of 22 cm s$^{-1}$, which is much higher than those shown in other studies by Grizzle et al. (1992), which suggested feeding inhibition may occur at current speeds over 1 cm s$^{-1}$. Harsh and Luckenbach (1999) emphasized that the physical particle distribution and vertical mixing has a relatively large impact on the perceived filtration rates, which they warned could have a large impact on in situ measurements of filtration rate estimates.

Another complicating factor in investigating influence of flow on oyster growth and filtration is seston flux (i.e. multiplicative interaction of flow and seston concentration). Experiments that have been conducted in situ and in controlled flume experiments experienced difficulty decoupling these factors. In order to avoid the confounding phenomena of vertical mixing and seston flux, Lenihan et al. (1996) investigated the effect of current speed on growth of juvenile oysters, C. virginica, cultured in a tube, similar to Kirby-Smith (1972), where they were subjected to five different flows (0, 0.5, 2.5, 4, and 7 cm s$^{-1}$). The water used in the flow through culture tube was taken from Bogue Sound in North Carolina, and filtered with an 80 μm mesh to create two seston concentrations (i.e. ambient and reduced concentrations). Although growth and seston flux were positively correlated, flow and seston concentration acted independently and in different ways. This could be related to the mechanisms and interactions of oyster feeding currents, partial pressures around the organisms, and the maximum feeding capability of the oyster. Furthermore, it is logical to assume that increasing velocity would at some point inhibit feeding as described by Wildish and Kristmanson (1997). Lenihan et al. (1996) stated, ‘...the apparent relationship between oyster growth and food flux is fundamentally misleading and coincidental,’ implying their understanding of limits to the previous logic.

On the other hand, seston flux has been suggested to affect populations of oysters more than individuals (Wildish & Kristmanson 1997). At the population scale of an oyster reef, the seston flux can affect the growth of oysters relative to their location (Bayne 2017). This is because individual oysters feed on the concentration of ambient seston, which is affected by surrounding oysters filtering activity and local hydrodynamics. Bayne (2017) provides a good discussion of how seston flux can affect a population of oysters and their spatial distribution. As the horizontal flux of seston is consumed by the oysters in the front of the reef, the oysters in the lee of the reef can become food limited. This can lead to competition and ‘self-thinning’ of the reef. The hydrodynamics around the reef can affect the concentration experienced by the oysters due to turbulent mixing, which is effected by the ambient flow and the reef structure. Lenihan (1999) conducted observations that demonstrated this correlation of oyster growth and hydrodynamics on natural and constructed reefs.

These studies highlight the complexity of measuring the impacts of flow on filtration and growth of oysters. The interconnected variables of flow, resuspension and seston flux have made it difficult to develop a mechanistic model for oyster filtration and growth under various flow environments. An important parameterization would be on the upper range of current speeds before the oyster’s filtration and feeding activity is inhibited. The maximum current speed investigated on the studies mentioned was 22 cm s$^{-1}$ (Harsh & Luckenbach 1999), which may not capture the maximum current speed that oysters can feed and grow.

The experimental velocities from studies mentioned above were compiled and tabulated in Table 1 for comparison. The growth responses to velocity are also reported. The negative responses reported by Grizzle et al. (1992) are not consistent with studies conducted by Lenihan et al. (1996) and Harsh and Luckenbach (1999). This could be due to a number of experimental factors inherent in the experimental apparatus, growth versus filtration measurement, the lack of statistical power to detect differences, and precision of measurements.
Table 1  Superficial velocities utilized in laboratory experiments exploring response of flow on growth (G) and filtration rate (FR) of the eastern oyster, *Crassostrea virginica*

<table>
<thead>
<tr>
<th>Source</th>
<th>Superficial velocities (cm s⁻¹)</th>
<th>G or FR trend (+, –)</th>
<th>Experimental apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harsh and Luckenbach (1999)</td>
<td>0.7, 1.0, 2.1, 4.2, 6.0, 10.4, 13.7, 22.0</td>
<td>+ Up to 22 cm s⁻¹ (FR)</td>
<td>Flume</td>
</tr>
<tr>
<td>Grizzle et al. (1992)</td>
<td>2.0, 4.0, 8.0</td>
<td>NS trend (G)</td>
<td>Flume</td>
</tr>
<tr>
<td>Grizzle et al. (1992)</td>
<td>0.0, 1.0, 2.0, 4.0</td>
<td>– Above 1 cm s⁻¹ (G)</td>
<td>Flume</td>
</tr>
<tr>
<td>Lenihan et al. (1996)</td>
<td>0.0, 0.5, 2.5, 4.0, 7.0</td>
<td>+ Up to 7 cm s⁻¹ (FR)</td>
<td>Tube</td>
</tr>
</tbody>
</table>

*No significant trend was found related to growth rate and flow rate.

### Effects of flow on upwelling culture systems

Flow rates in upwelling oyster culture systems are important because they regulate food supply and other factors related to growth. Studies were reviewed dealing with the effects of flow and velocity on upwelling bivalve aquaculture systems. Greater understanding of the hydrodynamic effect on upwelling systems allows for the management and optimization of factors such as food concentration, temperature, oyster size and stocking density.

Models have been developed to evaluate the relative importance of each of these variables within upwelling systems. Rodhouse and O’Kelly (1981) developed an empirical model for optimum flow rates in upwelling aquaculture systems for two species of oysters: *C. gigas* and *O. edulis*. They conducted five separate experiments investigating the effects of flow rate, food concentration, temperature, body size and culture column shape. The filtration rates of oysters were approximately equal to the flow rate at lower values (i.e. below 1 mL min⁻¹ g⁻¹) and levellled off at higher flow rates. The effect of food concentration and culture tube shape (i.e. wide or narrow) had no significant effect on the filtration rate. The optimum flow rate model was developed with respect to water temperature and live weight of oysters with the data obtained from the individual experiments. The 90% filtration rate was chosen to optimize the flow rate in order to maximize the nutritional needs of the oysters while minimizing ‘wastage of algae in an open system’ (Rodhouse & O’Kelly 1981).

The series of experiments conducted during this study were useful in explaining effects of temperature and weight individually. Due to the nature of the experimental setup (i.e. a series of individual factors), there can be no inference of interactions between the factors. For example, food concentrations were varied at a constant high flow rate. Results from this experiment cannot be used to infer filtration rates at lower flow rates (or higher flow rates) than what was used because the oysters may respond differently under those combined conditions. Additionally, Rodhouse and O’Kelly (1981) acknowledged that their model was sensitive to live weight, which could be affected by interactions of temperature and food availability.

This effect was observed in the experiments conducted by Spencer (1988) who used fertilized seawater to increase food supply to outdoor upwelling systems. The flow rate affects the growth differentially depending on the concentration of food. Equations were developed to determine the expected filtration rates due to water flow rate, temperature, algae concentration and oyster size. These equations were used to adjust the flow rate within the individual treatments to achieve five levels of filtration rates (15%, 25%, 35%, 45% and 55%). This study demonstrated the ability to control flow rate as a means of maximizing growth of oysters.

Flow rate is not only important in regulating food supply and growth, but may mitigate disease and other issues related to overstocking. Bishop and Hooper (2005) conducted experiments on the growth and mortality of oysters, diploid *C. virginica* and triploid *C. ariakensis*, grown in upweller systems under high and low flow rates at high and low stocking densities. The effect of disease, *Polyspora* spp., treatment was also investigated. The effect of stocking density was minimal in both species at the experimental flow rates. There was a differential response to flow rate between species, where *C. virginica* had a higher growth rate at high flows and *C. ariakensis* did not exhibit the same response. On the other hand, increasing flow rate had a significant effect on decreasing the presence of *Polyspora* spp. in both oyster species (Bishop & Hooper 2005).

The variability in environmental conditions in the field can affect oyster growth within upweller systems. Flow rate can mitigate detrimental environmental conditions, such as low dissolved oxygen levels. A number of studies have shown or suggested that flow rate is an important factor to consider in managing bivalve aquaculture systems in variable natural environments.

Manzi et al. (1986) was one of the first to publish results of upweller nursery systems in natural waters recognizing that natural variability could affect oyster growth rates. The study observed growth of northern quahog, *Mercenaria mercenaria*, in an upweller system in South Carolina. The system contained multiple stocking densities under a constant flow rate (2.5 mL min⁻¹). Growth increased as flow rate per kg of biomass increased (i.e. decreased stocking...
density). This relationship was also affected by season where temperature and phytoplankton abundance was variable. Maximum growth occurred in April and October. Time periods with high water temperatures, such as July, had lower growth especially in high stocking density cultures.

Appleyard and Dealteris (2002) conducted a series of field experiments to determine the effect of food supply (adjusted by flow rate) on growth of the northern quahog, *M. mercenaria*. The experiments were conducted in floating upweller systems located in the upper region of Point Judith Pond in Wakefield, Rhode Island. The effective flow rate was adjusted to low, medium, and high rates (i.e. 38, 58, 77 µg chl-a min⁻¹, respectively) by modifying the flow rate through the floating upwellers. The stocking density of biomass was modified to low (0.3 L cm⁻²) and high (0.6 L cm⁻²). The results were in general agreement with previous work, which concluded that higher food supply was positively correlated with increased growth rates. The low stocking densities grew significantly faster than high stocking densities. On the other hand, other factors were not controlled in the study since it was conducted in the field, which affected the results. The dissolved oxygen concentration significantly limited growth as morning dissolved oxygen was lower between experiments. Appleyard and Dealteris (2002) suggested that future research should investigate methods and technology for increasing dissolved oxygen in upweller systems.

Li et al. (2012) measured the filtration rates of a commercial oyster nursery utilizing a floating upweller system in the Peconic Estuary, New York. The flow rates used in the system were typical of commercial operations. The system was shown not to have a significant impact on the phytoplankton within the surrounding environment. On the other hand, environmental factors such as temperature and dissolved oxygen did affect the observed filtration rates.

The above mentioned studies suggest a positive relationship of growth rate and increased flow rate. If the flow rate is increased to the point of fluidization of the oyster bed, increases in the interstitial space between the individual oysters occurs. There are potential advantages of this state of flow including increased food availability to high stocking density cultures. Pilot studies and evaluation of these types of systems for oysters and other bivalves have been conducted. Ver and Wang (1995) designed and tested an oyster upweller nursery system that was fed with shrimp pond effluent. The study examined the fluidization of juvenile oysters, *C. virginica*, in an upweller aquaculture system. The oysters were cultured in a 22.8 cm thick bed. The flow was increased until fluidization of the oyster bed occurred, then the flow was decreased until fluidization ceased. This methodology was conducted to determine the velocity at beginning of fluidization, *vfl* and velocity at minimum fluidization, *vMF*. The *vMF* is a reproducible state achieved by decreasing flow once fluidization has occurred. The pressure and bed expansion was measured and correlated to flow rates. Ver and Wang (1995) correlated oyster weight and height to the *vMF* which they assumed was the optimal growing conditions.

Although this study contributes to our knowledge of fluidization of oyster beds in this particular upweller system, it is lacking in its ability to identify optimums and upper limits for culture in upweller systems. The *vMF* equation was determined by fitting the data (r² = 0.96), although there was no discussion of how it related to fluidized bed theory. Future studies should include a more thorough investigation of the range of velocities and their effect on the growth of the oysters. Additionally, comparisons to fixed and fluidized bed theory could make this technique more widely applicable.

The fluidized bed system has shown to have growth advantages when compared with other more traditional nursery aquaculture systems in some cases. Pfeiffer and Rusch (2001) conducted growth experiments of the northern quahog, *M. mercenaria*, under various culture systems related to flow type: stacked trays with downward flow, traditional upweller (i.e. low flow) and fluidized flow upweller (i.e. high flow). The clams were fed a constant food ratio (1% of dry algae biomass per day) for each of the treatments in order to isolate the effects of food supply. The fluidized flow conditions exhibited better growth than the downward flow and upward low flow rates. This study did not, however, identify whether the optimum flow rate was at fluidization velocity and further investigation is warranted.

The flow rates in the systems discussed above were normalized by calculating the superficial velocity. The tabulated superficial velocities are shown in Table 2 for comparison. The highest superficial velocities were in the system studies by Bishop and Hooper (2005) followed by fluidized flow systems explored by Ver and Wang (1995). The earlier work by Manzi et al. (1986) and Rodhouse and O’Kelly (1981) used the lowest superficial velocities within the group. The commercial operation studies by Li et al. (2012) operated at a more moderate superficial velocity when compared with the other systems. These systems operated in various environments and across multiple species, which make it difficult to directly compare performance. On the other hand, this analysis does give some insight into the range of velocities that oysters and other bivalves can be successfully cultured. Additional work is necessary to determine optimum flow rates and superficial velocities at given stocking densities and variable environmental conditions, such as low dissolved oxygen. One additional factor to consider is the balance between optimum growth of the oysters and energy consumption of the system.
Table 2  Superficial velocities within upflow bivalve nursery systems observed in previous research

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Superficial velocity (cm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bishop and Hooper (2005)</td>
<td>Crassostrea virginica</td>
<td>7.1</td>
</tr>
<tr>
<td>Bishop and Hooper (2005)</td>
<td>Crassostrea ariakensis</td>
<td>7.1</td>
</tr>
<tr>
<td>Ver and Wang (1995)</td>
<td>C. virginica</td>
<td>3</td>
</tr>
<tr>
<td>Bishop and Hooper (2005)</td>
<td>C. virginica</td>
<td>2.2</td>
</tr>
<tr>
<td>Bishop and Hooper (2005)</td>
<td>C. ariakensis</td>
<td>2.2</td>
</tr>
<tr>
<td>Spencer (1988)</td>
<td>Ostrea edulis</td>
<td>1.6</td>
</tr>
<tr>
<td>Spencer (1988)</td>
<td>Crassostrea gigas</td>
<td>1.6</td>
</tr>
<tr>
<td>Pfeiffer and Rusch (2001)</td>
<td>Mercenaria mercenaria</td>
<td>1.6</td>
</tr>
<tr>
<td>Li et al. (2012)</td>
<td>Mercenaria mercenaria</td>
<td>1.6</td>
</tr>
<tr>
<td>Rodhouse and O'Kelly (1981)</td>
<td>C. virginica</td>
<td>1.2</td>
</tr>
<tr>
<td>Appleyard and Dealeris (2002)</td>
<td>M. mercenaria</td>
<td>0.8</td>
</tr>
<tr>
<td>Rodhouse and O'Kelly (1981)</td>
<td>Ostrea edulis</td>
<td>0.6</td>
</tr>
<tr>
<td>Manzi et al. (1986)</td>
<td>M. mercenaria</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Discussion and future work

As the oyster aquaculture industry expands and diversifies, there is an increasing interest in optimizing aquaculture systems and evaluating the impacts on the surrounding ecosystem. The hydrodynamics of an aquaculture site can affect the structural integrity of the culture system, oyster growth and the impact on the environment. Proper siting of aquaculture requires a comprehensive understanding of the hydrodynamics and its impact on the culture of oysters. Typically, higher currents are desirable because they increase delivery of food to the oysters, improve water quality, and disperse biodeposits. This results in both higher growth rates and lower impact on the environment. On the other hand, extreme hydrodynamic energy can inhibit growth through physical damage. Unfortunately, information on oyster feeding activity and growth is lacking in regard to hydrodynamic effects. A greater understanding of how hydrodynamics affect oyster feeding activity and growth could have implications on culture techniques, oyster stocking densities, siting criteria and management.

Oysters are affected by direct and indirect factors in the natural and cultured environments. Hydrodynamics play a crucial role in determining the extent that these factors influence oyster growth. Salinity, temperature, turbidity, food supply and oxygen are highly variable in nature, but are also correlated with the hydrodynamics of the environment through mixing and transport. The hydrodynamic environment may also influence what types of predators are present at the aquaculture site (Kennedy et al. 1996; Dame 2012; Goslin 2013).

Typically oyster aquaculture consists of some type of bed configuration stocked to a specific density. This configuration has fluid-oyster, suspended/dissolved substance-fluid-oyster and oyster-oyster interactions that need to be considered. When a flow passes through a porous bed of oysters, they are both feeding and excreting metabolic wastes. This can have detrimental effects downstream to other oysters that either lack food or are inhibited through re-filtration of metabolic wastes (Kirby-Smith 1972; Lenihan et al. 1996). Increasing the flow through a bed of oysters could minimize these effects (Ver & Wang 1995; Pfeiffer & Rusch 2001; Appleyard & Dealeris 2002; Bishop & Hooper 2005).

Hydrodynamics also influences the type of culture method and locations where oyster aquaculture can be conducted. Culture methods are more effective in various types of hydrodynamic environments. Floating cage culture may not be as successful in areas of high wave energy as bottom culture (Mallet et al. 2003, 2013; Bishop & Hooper 2005). Conversely, bottom culture in areas with low flow and mixing may be susceptible to low dissolved oxygen (Grabowski et al. 2004; Walton et al. 2013).

Upwelling systems are primarily influenced by the flow and shape of the culture unit. Flow through these systems regulates food, oxygen and water quality within the bed of oysters. Flow should correspond with stocking density and seston concentration for proper management (Manzi et al. 1986; Spencer 1988; Bishop & Hooper 2005). Higher flows increase oyster growth, but could be limiting due to feeding inhibition, damage and required energy inputs (Rodhouse & O’Kelly 1981; Ver & Wang 1995; Pfeiffer & Rusch 2001; Appleyard & Dealeris 2002).

Future work should include controlled experiments that separate variability in environmental conditions from the hydrodynamic effects on oysters. As the hydrodynamics can influence other parameters that have an effect on oyster growth and feeding, controlled experiments are required to better understand and parameterize effects. Specifically, inhibiting mechanisms due to velocity and pressure differences need to be investigated further. The indirect effects of vertical mixing and transport of sediment and dissolved oxygen warrant investigation, especially in suspended aquaculture settings. The management of these types of culture methods could benefit from this knowledge.

Feeding is an important mechanism to the growth of oysters. Velocity and movement could both independently affect feeding during nursery and grow-out phases. These effects should be studied for both fixed and mobile (i.e. unattached) individual oysters. The velocity effects should be studied in the higher range in order to determine inhibiting velocities. Motion thresholds of feeding inhibition are also unknown and warrant further study.

The type of culture units (i.e. bag or cage configuration) used for grow-out of oysters can affect the current speeds experienced by the oysters and potentially their growth. This includes mesh size, shape and orientation of the culture units. Future work is needed to determine how these...
parameters affect the flow regimes within the culture units. Additionally, secondary impacts, such as biofouling and predation should be included as they will affect overall growth of the oysters.

Flow regimes and packing densities influence the provision of dissolved oxygen and removal of wastes including carbon dioxide, solids and nutrient rich waters within oyster culture systems. The optimization of design and management practices for these systems could be enhanced through knowledge gained from experiments exploring optimal flow with relation to supply of oxygen and removal of waste materials.

Future studies could have broader impacts by exploring interactions between physics and biology that have applications in many marine and aquaculture fields. For example, these interactions have applications to alternate culture systems such as artificial reefs and living shorelines. These systems are influenced by their hydrodynamic environment and the desired performance (i.e. ecological restoration or shoreline protection) is directly linked to the biophysical interaction.

Upwelling systems in particular should be studied further to understand the hydrodynamics through a bed of oysters. This has implications on stocking densities, flow, size sorting and other management strategies. Further investigation into the application of fixed bed reactor theory to this culture method could be applied and could generate further innovations in oyster culture and related fields.

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CHAPTER 3: APPLICATION OF PACKED BED REACTOR THEORY AND BAYESIAN INFERENCE TO UPWELLER CULTURE OF JUVENILE OYSTERS

Publication status

At the time of writing, this Chapter was submitted to the Aquaculture Engineering and under peer review.

Abstract

The use of upweller culture units in bivalve nurseries is widely practiced as a technique that enhances the ability to rear large quantities in a semi-controlled environment. However, guidance has varied for optimal flow rates, and thus there is a need to develop a more mechanistic assessment. The application of packed bed reactor theory, including axial diffusion models, would improve optimization of these culture methods. The following paper presents a series of controlled experiments to determine the hydrodynamic properties of a packed bed of oysters. The data gained from these experiments is used to develop mechanistic models calibrated through Bayesian inference. Specifically, the Ergun equation and the axial diffusion model are used to predict the experimental data. The Ergun equation was able to accurately predict the hydrodynamic equivalent diameter distribution of oyster shells ($\mu=3.18$ mm, $\sigma=0.74$ mm). This oyster shell diameter and void ratio distribution gained through the Ergun equation was used in the relationship of axial diffusion and superficial velocity. The mean axial diffusion coefficient in the oyster bed was estimated $1.65 \times 10^4$ m$^2$/s at 0.01 m/s and $7.26 \times 10^4$ m$^2$/s at 0.08 m/s. The use of Bayesian inference allows for greater understanding of the credibility of individual parameter distributions (i.e., rates and physical attributes) within these mechanistic formulations. This work establishes a baseline methodology to systematically evaluate and optimize bivalve upweller culture systems.
1. Introduction

Aquaculture is the fastest growing protein sector in the world with shellfish being a large portion of the industry (FAO, 2018). Additionally, shellfish such as oysters are filter feeders that can enhance water quality, and efforts are currently being undertaken to repopulate native species in estuaries (Goslin, 2015). Understanding is still limited on both what affects oyster feeding and how this contributes to growth and development. While genetics and environmental factors such as temperature and salinity are understood to be important (Rybovich et al., 2014), the interactions between water movement and biology (i.e. feeding and development) are not fully understood (Campbell and Hall, 2019).

Water movement through a bed of oysters can affect feeding, growth and survival due to the supply of food, oxygen, and removal of wastes (Goslin, 2015; Kennedy, Newell, and Eble, 1996; Wildish and Kristmanson, 1997). The water movement combined with ambient seston concentrations (i.e. oyster food source) affects the optimal stocking density used in the culture units (Appleyard and Dealteris, 2002; Manzi et al., 1986). These factors become critical in the design and operation of oyster culture systems, such as upwellers.

Upweller (i.e. upflow) aquaculture systems (Figure 1) have been used to culture juvenile oysters to reduce predation and increase yields at a vulnerable period in the oyster life cycle. These types of systems typically have much higher stocking density capacity than other methods (Goslin, 2015). Upweller aquaculture systems are particularly influenced by flow that regulates food, oxygen, and water quality within the bed of oysters being cultured. The superficial velocities reported in the literature range from 0.5 - 7.1 cm s⁻¹ (Campbell and Hall, 2019). In practice, higher current velocities are desirable because they increase delivery of food to the oysters, improve water quality, and enhance dispersal of biodeposits, but this must be balanced
with energy and pump cost (Appleyard and Dealeris, 2002; Pfeiffer and Rusch, 2001; Rodhouse and O’Kelly, 1981; Ver and Wang, 1995). The application of engineering design models would allow for the optimization of flow rates at given stocking densities and variable environmental conditions, as well as improved design of upweller culture units.

Figure 1. Typical cross section of an upweller culture unit for oyster aquaculture.

A typical upweller system (Figure 1) consists of a culture unit with a screened bottom that supports a bed of oysters, which is assumed to be analogous to porous media in a packed bed reactor (Green and Perry, 2007; Ranade, 2001). This assumption allows the application of packed bed reactor theory to upflow aquaculture systems. Packed bed reactors contain catalyst in the form of a packed bed to increase chemical conversion efficiency. Theory and models have been developed for these reactors within chemical engineering and related fields to understand the dynamics and optimize these reactors. Application of packed bed reactor models, such as the axial diffusion model discussed in Nauman (2008), could greatly enhance understanding of these systems and empower the ability to optimize culture systems and methodology. The axial diffusion model was chosen because it can be used to simulate the actual concentrations of food
or waste experienced by oysters at different layers within the packed bed. This information could be critical in the analysis of whether oysters at the top layer are starving or being exposed to degraded water quality from oysters in lower layers.

The axial diffusion model developed within packed bed reactor theory is widely used in chemical reactor design and could be applied to oyster upweller systems. The axial diffusion model has a long history in chemical engineering and has been shown to be valid in open boundary conditions (i.e. diffusion occurs at the boundaries). The model represents the change of concentration of a solution as it passes through a packed bed. This model accounts for reaction rates relative to superficial velocity, $v_s$, and axial diffusion through the packed bed. It is assumed that the reaction rates in this model are analogous to the filtration rates of the oysters, as shown in Section 2.1 below. The governing equation for the axial diffusion model is shown below in Equation 1 (Nauman, 2008).

\[
\frac{v_s}{dC}{dz} = D_L \left[ \frac{d^2C}{dz^2} \right] + k_c
\]

Where, $C$ is the concentration of the reactant (mg/l); $v_s$ is the superficial velocity or area averaged velocity ($v_s = \frac{Q}{A}$) (m/s); $D_L$ is the empirically derived axial diffusion coefficient (m$^2$/s); $z$ is the position from the start of the packed bed in the axial direction (m); and $k_c$ is the reaction rate (g/s).

The diffusion coefficient is a physical parameter that must be determined experimentally to apply the axial diffusion model, which is currently unknown for oysters. The diffusion coefficient is a function of the velocity, particle diameter, and void space as discussed in Section 2 below.
The objective of this study was to develop estimates for the axial diffusion coefficient with respect to superficial velocity and particle size for a packed bed of juvenile oysters. This was accomplished through physical lab experiments in combination with Bayesian parameter estimation techniques. The particle size was estimated by applying relationships between pressure loss and hydrodynamically equivalent particle size (i.e. Ergun equation). These representative particle sizes were used in conjunction with physical tracer studies to relate diffusion coefficients to velocity. It was hypothesized that the axial diffusion coefficient would increase with increasing water velocity moving through the packed oyster bed and this mechanistic relationship was applied within a Bayesian framework. The resulting mechanistic models and parameter estimates could be applied to the axial diffusion model for full scale field operations.

2. Theory

2.1. Derivation of Axial Dispersion Model for Oysters

A conceptual mass balance model for seston movement through a packed bed of oysters was developed for the upweller system to show the applicability to the axial diffusion model. The conceptual mass balance is shown in Figure 2 where the oysters are removing seston from the interstitial space (i.e. void space) in the oyster bed.
Figure 2. Mass balance of seston concentration, $C$, within the tube that is filtered out of the water by oysters. The oyster actively filters seston from the interstitial space immediately around it as water flows through the bed.

The formulated conceptual mass balance from Figure 2 is shown in Equation 2 below.

Equation 2

$$V_{int} \frac{dC}{dt} = QC_{in} - F_R C - QC$$

Where, $V_{int}$ (m$^3$) is the interstitial volume of fluid that is inside of the oyster bed ($V_{int} = V \varepsilon$); $\varepsilon = \frac{volume \ of \ void}{volume \ of \ solid}$ is the void ratio; $C$ (mg/l) is the concentration of seston within the oyster bed thickness and leaving; $C_{in}$ is the concentration of seston entering the oyster bed; $Q$ (m$^3$/s) is the flow rate in the upflow tube; $F_R$ (m$^3$/s) is the composite filtration rate of the bed of oysters and is a function of stocking density, individual oyster size, bed depth, and other environmental parameters (i.e. temperature, salinity, suspended solids, and dissolved oxygen).

If it is assumed that the system is in steady state, $\frac{dC}{dt} = 0$, then Equation 2 can be simplified to
This is similar to the formula used by Grizzle et al. (2008) and others when conducting field experiments to determine filtration rates of oysters. This formula was useful in its simplicity of gathering data in the field but disregards any other effects that could be attributed to turbulent diffusion. This could explain some of the previous discrepancies in hydrodynamic effects to oyster filtration noted by others (Grizzle et al., 2008; Grizzle et al., 1992; Harsh and Luckenbach, 1999; Lenihan et al., 1996). Forsyth (2014) noted in her numerical mass balance model that the inclusion of diffusion had a significant impact on the profile of seston concentration across an oyster reef. This result validated previous observations of particle gradients and food limitation across reefs (Lenihan, 1999).

If the mass balance (Equation 2) is reformulated by adding diffusion at the boundaries (denoted by subscripts 1 and 2), discretizing the packed bed length, substituting \( Q = v_s \pi r^2 \) then

\[
V \frac{dC}{dt} = v_s \pi r^2 (C_1 - C_2) - D_L \pi r^2 \left( \frac{dC_1}{dz} - \frac{dC_2}{dz} \right) - F_R C
\]

By substituting \( V = \pi r^2 \Delta z \) and assuming that the system is in steady state, \( \frac{dC}{dt} = 0 \), then the equation reduces to

\[
v_s \frac{dC}{dz} = D_L \left( \frac{d^2 C}{dz^2} \right) - \frac{F_R}{\pi r^2 \Delta z} C
\]

Where, \( r \) is the radius of the tube; and \( \Delta z \) is the discretized layer thickness in the tube that is representative of the composite filtration rate, \( F_R \). It can be seen that this model is very similar to

\[
F_R = \frac{(C_{in} - C)}{C} Q
\]
the axial diffusion model (Equation 1). Therefore, the reaction rate \( k_c \) can be approximated as

\[- \frac{F_R}{\pi r^2 \Delta z} C \text{ above.} \]

2.2. Relationship between Axial Diffusion and Velocity

The Peclet number is a dimensionless relationship that describes mixing within a reactor by relating the axial diffusion coefficient, \( D_L \), superficial velocity, and reactor mixing length, \( L \), as can be seen in Equation 6 (Nauman, 2008):

\[
\text{Equation 6} \quad Pe_L = \frac{v_s L}{D_L}
\]

The Peclet number, \( Pe_L \), can be used to solve for the axial diffusion coefficient, \( D_L \), experimentally by measuring tracer concentrations across the packed bed. The methods for determining \( Pe_L \) experimentally and solving for \( D_L \) are discussed in Section 3.4. The particle Peclet number, \( Pe_p \), (Equation 7) is used to relate the size of particles to the diffusion coefficient. It should be noted here the difference between the two Peclet numbers. While these two dimensionless numbers are expressed based upon length of bed (\( Pe_L \)) and diameter of particle (\( Pe_p \)), the diffusion coefficient is considered the same. This allows for the calculation of the diffusion coefficient experimentally through \( Pe_L \) and then applied to characteristic packed bed diameters through \( Pe_p \).

\[
\text{Equation 7} \quad Pe_p = \frac{v_s d_p}{D_L}
\]

The mechanistic relationship used in this study for the axial diffusion coefficient and the influence of velocity is explained by Delgado (2006). The effective axial diffusion measured within the tracer experiment is actually composed of axial diffusion due to turbulence and
molecular diffusion. As \( v_s \) decreases to 0, then \( D_L \rightarrow \frac{D_m}{\tau} \), where \( D_m \) is the molecular diffusion, and \( \tau \) is the tortuosity factor. As \( v_s \) increases to infinity, the effect of turbulence becomes dominant and molecular diffusion becomes negligible, which leads the Peclet number to approach an asymptote \( (P_{e\infty}) \). Equation 7 can be re-written with respect to \( D_L \) and combined with the effect from molecular diffusion to yield Equation 8 (Delgado, 2006).

Equation 8

\[
D_L = \frac{v_s d_p}{\varepsilon Pe_{e\infty}} + \frac{D_m}{\tau}
\]

The characteristic packed bed diameter, \( d_p \), cannot be measured directly for oysters. The high variability of the shell shape and size adds to the challenge. Therefore, the hydrodynamic equivalent spherical diameter (ESD) must also be determined experimentally. The methodology for determining ESD is explained in Section 2.3 below.

2.3. Determining Hydrodynamic Equivalent Spherical Diameter

The equivalent spherical particle diameter, \( d_p \), is an important parameter in the relationship between \( D_L \) and \( v_s \) as shown in Equation 8 above for the particle Peclet number. The difficulty arises when determining which dimension has the most effect on the diffusion characteristics of the bed. Section 3.2 below discusses the highly variable and bimodal dimensions of oysters. Additionally, the shape affects how the particles interact with the fluid dynamics. The concept of equivalent spherical diameter can be used to determine the diameter of spheres that exhibit identical hydrodynamic properties to the irregularly shaped oysters. The equivalent spherical diameter can be estimated using conventional relationships, such as those established for pressure drop and packed bed particle diameter by Ergun (1952).

The Ergun equation was parameterized to represent the friction factor of a packed column as a function of a modified particle Reynolds number yielding Equation 9 (Ergun, 1952).
Equation 9 assumes that the viscous and kinetic energy losses are additive, which is valid for laminar to turbulent flows (McCabe, Smith, & Harriott, 2005).

\[
-\frac{\Delta p}{L} = 150 \frac{\mu v_s (1 - \epsilon)^2}{d_p^2 \epsilon^3} + 1.75 \frac{\rho v_s^2 (1 - \epsilon)}{d_p \epsilon^3}
\]

Where \(\Delta p\) (Pa) is pressure drop across the packed bed; \(d_p\) (m) is the equivalent spherical diameter; \(\rho\) (kg/m\(^3\)) is density of the fluid; and \(\mu\) (Pa-s) is dynamic viscosity of the fluid.

3. Materials and Methods

3.1. Physical Setup

Juvenile oyster shells and glass spheres were subjected to increasing superficial velocities to determine bulk parameters (i.e. \(d_p\) and \(D_L\)) for the packed bed. The physical experiments were conducted using an upflow tube constructed from 2-inch (50 mm) polycarbonate tubes packed with juvenile oyster shells (3 – 20 mm) and glass spheres (15 mm) that were subjected to 12 superficial velocities (0.9, 1.5, 2.5, 3.1, 4.0, 4.6, 5.6, 6.2, 7.1, 7.7, 8.6 and 9.3 cm s\(^{-1}\)). The packed bed materials were placed into the polycarbonate tubes held in place by stretchable plastic mesh (polypropylene with 7.5 mm opening) at each end of the packed bed zone (33 cm). The upflow tubes were designed to create a predictable and repeatable flow within a porous packed bed of solid material. The tubes had ports on each side of the porous bed zone which allowed for conductivity meter insertion and pressure measurement via pitot tubes. Figure 3 depicts the experimental setup with locations of the measurement and injection points relative to the packed bed.
Figure 3. Experimental setup of the upflow tube with packed bed section (Not to Scale). Measurement points and injection site are also shown.

The desired superficial velocities were generated using a King flow meter (0 - 3.5 GPM) to regulate the flow rate in the tubes. The flow meters were calibrated by measuring the time to fill a 4000 mL Nalgene graduated cylinder. The flow rates had standard deviation of +/- 0.03 GPM (n=7).

3.2. Packed Bed Materials

The juvenile oyster shells used in these experiments were sourced from a local oyster farmer, Chadwick Creek Oyster Company in Bayboro, NC. The oysters were of the “Lola” genetic line and originally sourced from Virginia Institute of Marine Science as spat (3 mm). Representative samples of oysters were taken directly from the FLUPSY (Floating Upweller System) and allowed to decompose in the open environment. These remaining shells were
cleaned; and a small amount of epoxy was applied to secure shells that were gapping or had disconnected valves.

It is recognized that any one set of geometric data will be influenced by genetics and culture techniques. However, it is still important to assess typical variation in geometric properties. Oyster geometry is largely dictated by shell development. Oysters create biogenic structures with their shells that are approximately 95% calcium carbonate (Kennedy et al., 1996). Measuring the physical properties of oysters was necessary to better understand their interaction with fluid flows. The width, length, and height of the oysters were measured with digital calipers. The dimensions and shape of the shells within a sample of juvenile oysters are highly stochastic (Kennedy et al., 1996). The width (mean=3.95 mm, sd=1.20, n=300), length (mean=10.93 mm, sd=2.56, n=300), and height (mean=11.53 mm, sd=2.87, n=300) were measured from representative samples taken from the juvenile oyster shells used in these experiments.

The void ratio of the spheres and oysters were also measured experimentally. The oysters and spheres were placed in a 300 mL beaker and filled to the 200 mL mark. Water was added with a graduated cylinder to the beaker until it reached the 200 mL mark. The amount of liquid added was used to calculate the void ratio, $\varepsilon = \frac{\text{volume of void}}{\text{volume of solid}}$ for oysters (mean=0.643, sd=0.018, n=3) and glass spheres (mean=0.407, sd=0.013, n=3).

Another physical attribute of the packed bed experiments that should be noted is the ratio of the tube diameter to the size of the packed bed particles ($D/d_p$). Delgado (2006) stated that ratios less than 12 would have effects from the walls that could influence the measurable hydrodynamics. The ratio of tube diameter to the particle size was 3.3 for the glass spheres and 12.9 for the oysters. This could affect the results for the glass spheres, but not likely for the
oysters as the ratio was above 12, which Delgado (2006) noted would have measurable effects due to the walls. Since the purpose of the glass spheres in these specific experiments were to validate the modeling approach within these experiments, the scaling limitation was deemed not an issue due to the limited application to this experimental setup. On the other hand, the diffusion results from the oysters could potentially be scaled up because the ratio was sufficiently above the threshold.

3.3. Pressure Drop Across Bed

Pressure drop, $\Delta p$, across the packed bed was measured by the water level differential in a pitot tube connected to ports on both sides of the packed bed. The pitot tubes consisted of 3.5 mm ID vinyl air tubes that were connected at ports 185 mm above and below the packed bed. A single pitot tube was routed from the port below the packed bed over a fastener above the upflow tube and down to the port above the packed bed. The line was purged before each run and enough air was allowed in the line to determine the pressure differential.

3.4. Tracer Study

Diffusion coefficients, $D_L$, were estimated using a tracer, similar to methods described in Nauman (2008). A known amount of conservative tracer (i.e. salt solution) was injected in a single pulse at the bottom of the upflow tube at the injection site depicted in Figure 3. The conservative tracer pulse consisted of 5 ml of salt solution 123 g/L (18.5 g Instant Ocean™ sea salt per 150 ml deionized water) with red food coloring (FD&C Red 40&3). The red food coloring was added to visualize salt plume moving through the packed bed.

Electrical conductivity meters were used to measure the salt content of the water passing by the measurement point on either side of the packed bed. The conductivity meters measured the voltage change as the salt plume passed. They were inserted into the upflow tubes at the port
locations 48 mm above and below the packed bed and adjusted to measure in the center of the tube. The conductivity meters used in the experiments were constructed using commercially available electronics and calibrated with the salt solution used in these experiments. The conductivity meter used a programmable circuit board, Arduino Uno (www.arduino.cc), for data acquisition and sensing. The voltage change for the expected salinity range was adjusted to give sufficient accuracy by the addition of a 10k ceramic resistor to each sensor. The unit was connected by a shielded wire to two 28AWG jumper wires with 2.5 mm contacts consisting of tinned beryllium-copper. The two contacts were encased in black plastic housings and separated by 3mm.

The conductivity meters were calibrated by measuring voltage at various salt concentrations; ten 150 mL beakers filled with deionized water and increasing amounts salt from 0 to 350 mg (n=10). The voltage changed from 2.5 to 3.5 volts in a linear relationship up to 3.3 volts (r²=0.93). Therefore, it was assumed that this voltage range could be used as a direct measurement of the salt concentration of the water passing by meters. The voltage pulses recorded by the conductivity meters were normalized using the impulse function so that the diffusion coefficient could be calculated.

The impulse function (Equation 10), as described in Nauman (2008), was calculated as:

\[
 f(t) = \frac{C(t)}{\int_0^\infty C(t) dt}
\]

where \(C(t)\) is the concentration (i.e. voltage) over time at each sensor.

The impulse function was used to calculate the moments of time, \(\mu_n\), which has a general form:

\[
 \mu_n = \int_0^\infty t^n f(t) dt
\]
where moments are calculated as:

\begin{equation}
\text{1st moment (mean), } \bar{t} = \mu_1 = \int_0^\infty t f(t) dt
\end{equation}

\begin{equation}
\text{2nd moment, } \mu_2 = \int_0^\infty t^2 f(t) dt
\end{equation}

The second moment about the mean, \((t-\mu)^2\), or the variance, \(\sigma_t^2\), is calculated as:

\begin{equation}
\sigma_t^2 = \mu_2 = E((t - \bar{t})^2) = \int_0^\infty (t - \bar{t})^2 f(t) dt = \mu_2 - \bar{t}^2
\end{equation}

The dimensionless variance, \(\sigma^2\), was calculated for each of the superficial velocities, \(v_s\):

\begin{equation}
\sigma^2 = \frac{\sigma_{t,2}^2 - \sigma_{t,1}^2}{t_2^2 - t_1^2}
\end{equation}

The dimensionless variance was used to calculate the \(Pe_L\) using a relationship developed by Levenspiel and Smith (1957), which is shown in Equation 16 below.

\begin{equation}
\sigma^2 = \frac{2}{Pe_L} - \frac{2}{Pe_L^2} [1 - e^{-Pe_L}]
\end{equation}

The mean residence time, \(\bar{t}\), (Equation 12) was used to find the average velocity of plume movement \(\bar{u} = \frac{L}{\bar{t}}\). The experimentally determined Peclet number, \(Pe_L = \frac{\bar{u}L}{D_L}\), was then used with the average velocity and length of packed bed to determine the axial diffusion coefficient, \(D_L\), as discussed in Nauman (2008).
4. Modeling Approach

A Bayesian inference calibration approach was selected to gain insights into the credibility of the parameter estimation. Bayesian inference relates to the method of statistical inference using Bayes’ rule to allocate credibility for parameters based upon prior knowledge and logical relationships within mathematical models (Kruschke, 2015). Prior knowledge about the parameters and their variability can be gained from established literature, experimentation, or other knowledge of the system. Improved predictions from a Bayesian model are achieved as more information becomes available, which would allow for future improvements to be gained systematically. The model was developed within PyStan, which is a Python based implementation of the Bayesian inference package, Stan, that uses Hamiltonian Monte Carlo sampling with No-U-Turn algorithms (Carpenter et al., 2017; Stan, 2018). The simulations were run for 5,000 iterations with a maximum tree length of 15 and adaptive delta of 0.99 to minimize divergences within estimations.

The modeling framework, which is used to infer (i.e., estimate) critical parameters and associated distributions in a stepwise manner, is illustrated in Figure 4. The first set of parameters (i.e. equivalent spherical diameter, \(d_p\), and void ratio, \(\varepsilon\)) were estimated through the Ergun equation (Equation 9) and then used in the Peclet based relationship to estimate superficial velocity, \(v_s\), and diffusion coefficient, \(D_L\). The diffusion coefficient can be used in the axial diffusion equation for various velocities and other geometric configurations. The diffusion coefficient is critical in this formula in transitional Reynolds numbers (10<Re<2000) as explained by Nauman (2008) and Delgado (2006).
Figure 4. Modeling framework of how Bayesian models will be applied to determine representative equivalent spherical diameter, $d_p$, void ratio, $\varepsilon$, and diffusion coefficients, $D_L$, with their associated distributions.

The Ergun equation (Equation 9) was embedded in the Bayesian framework and was validated using glass spheres since the parameters were more defined from measurements and in the literature. The methodology of using Ergun’s equation to determine hydrodynamic spherical equivalent size with the experimental setup was confirmed by first estimating the $d_p$ for glass spheres and comparing to the actual measurements. The parameterized model had the normal distribution, $\mathcal{N}$, form:
The prior distributions used in the model were gathered from literature and direct measurement. The void ratio was determined from a review and mathematical formulation developed by Delgado (2006), which had a normal distribution \( \varepsilon \sim \mathcal{N}(0.39, 0.03) \), which also aligns with direct measurements. The assumed particle diameter was developed considering measured data and had a distribution, \( d_p \sim \mathcal{N}(15.3, 0.5) \) mm. Coefficients established by Ergun (1952) were used as priors with estimates of standard deviation, \( C_1 \sim \mathcal{N}(150, 20) \) and \( C_2 \sim \mathcal{N}(1.75, 1) \). The standard deviation of the normally-distributed model error (\( \sigma \)) had wide uniform (i.e., uninformative) prior distribution, \( U(0:1000) \) m\(^2\)/s.

Once the methodology for estimating \( d_p \) was validated using spheres, the same approach was used with oysters. The coefficients (i.e. \( C_1 \) and \( C_2 \)) and model error, \( \sigma \), maintained the same prior distributions as the model for the spheres. The void ratio used a prior distribution developed from physical measurements conducted when measuring packed bed material \( \varepsilon \sim \mathcal{N}(0.64, 0.018) \). The characteristic diameter of the oysters was assumed to be close to the smallest dimension (i.e. width) measurements and had the prior distribution: \( d_p \sim \mathcal{N}(3.95,1.25) \) mm.

The model for axial diffusion, \( D_L \), was re-parameterized for the Bayesian framework from the particle Pelcet number (Equation 8), as shown in Equation 18:
The prior distributions for this model were provided from the results of the previous model estimates and literature. The estimates of $d_p$ and $\varepsilon$ from the previous Ergun model were used as prior distributions. Nauman (2008) suggested $Pe_\infty = 5$ as $v_s$ is increased to high Reynolds numbers ($Re>2000$) and Delgado (2006) suggest that $Pe_\infty = 2$ as $v_s$ is increased to high Reynolds numbers. This information was used to create the prior distribution $Pe_\infty \sim \mathcal{N}(2, 3)$. The molecular diffusion, $D_m$, (m$^2$/s) was bound by 0 with a uniform prior distribution. Prior distributions for tortuosity were gathered from Delgado (2006) and assumed to be $\tau \sim \mathcal{N}(1.41, 0.5)$ for spheres and $\tau \sim \mathcal{N}(1.93, 0.5)$ for oysters (as represented as solid cylinders). The error between observed and predicted axial diffusion coefficients was assumed to be normally distributed with model error, $\sigma$ (m$^2$/s), having an uninformative uniform prior distribution, $U(0:1000)$.

5. Results and Discussion

5.1. Measurements and Modeling of Pressure Drop

The Bayesian modeling estimates for the parameters from the Ergun equation with sphere data were compiled and analyzed. Table 1 lists the estimated parameter means, standard deviations (sd), credible intervals, effective sample size (n_eff), and $\hat{R}$. $\hat{R}$ values are the Gelman-Rubin statistic and should be below 1.1 for convergence on an estimated parameter to be achieved (Gelman et al., 2013).

The Bayesian model provided realistic parameter estimates that compared well with physical measurements and literature. The diameters estimated for glass spheres (mean=15.28
mm, sd=0.5) matched the physical measurements (mean=15.3 mm, sd=0.5). The estimated Ergun constants were in general agreement with the literature (i.e. C1=150 and C2=1.75), where the model estimated C1=154.5, and C2=0.62. The discrepancies in the constants could be due to the specific hydrodynamics of the experimental units compared to what Ergun (1952) used, see Section 3.2.

The posterior distributions of parameter estimates were approximately the same as the prior distributions. The exception was the Ergun constant C2, which was smaller than the prior distribution, $C_2 \sim \mathcal{N}(1.75, 1)$. The other exception was the model error, which was very small compared to the wide uniform prior distribution, $U(0:1000)$. This indicates that the data was only informative for the C2 and model error parameters for the glass spheres and that the prior distributions for $\varepsilon$ and $d_p$ were similar to reality.

Table 1. Parameter estimates for spheres from Ergun’s equation.

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>sd</th>
<th>2.50%</th>
<th>97.50%</th>
<th>n_eff</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$</td>
<td>0.39</td>
<td>0.03</td>
<td>0.34</td>
<td>0.44</td>
<td>7721</td>
<td>1.00</td>
</tr>
<tr>
<td>$d_p$ (mm)</td>
<td>15.28</td>
<td>0.5</td>
<td>14.29</td>
<td>16.25</td>
<td>13927</td>
<td>1.00</td>
</tr>
<tr>
<td>C1</td>
<td>154.49</td>
<td>19.34</td>
<td>116.71</td>
<td>192.21</td>
<td>14156</td>
<td>1.00</td>
</tr>
<tr>
<td>C2</td>
<td>0.62</td>
<td>0.17</td>
<td>0.36</td>
<td>1.03</td>
<td>7484</td>
<td>1.00</td>
</tr>
<tr>
<td>$\sigma$ (Pa)</td>
<td>26.93</td>
<td>7.13</td>
<td>17.07</td>
<td>44.44</td>
<td>9993</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The agreement with the Bayesian model parameter estimates for particle diameter of the glass spheres strongly suggested that this approach could also be used effectively on the oyster packed bed material. The estimated parameters for the packed bed of oysters were determined using all the data and with the highest point removed, which was suspected of being an outlier.
The highest point was suspected of being an outlier because of the extreme jump in pressure drop relative to change in velocity (Figure 5). Table 2 shows the parameter means, standard deviations (sd), and effective sample size (n_eff) for both cases. The $\hat{R}$ was approximately 1.00 for all parameters both cases.

The Ergun constants estimated for the oysters were in general agreement with the literature (i.e. $C_1=150$ and $C_2=1.75$). The estimate of $d_p$ for the oysters (mean=3.18, sd=0.74) was slightly smaller than the physical width measurements (mean=3.95 mm, sd=1.20). This could have been due to the irregularity in the bed and individual oyster nesting. The “cup” of the oysters allowed them to nest together creating decreased void spaces, which would mimic smaller particle sizes.

The differences between the “all data” and the “without high point” were also analyzed. The estimated equivalent spherical diameter, $d_p$, for the “all data” case (mean=3.18, sd=0.74) and the “without high point” case (mean=2.45, sd=0.45) were notably different. The model estimated $C_1=149.27$, sd=19.97 and $C_2=2.88$, sd=0.77 for the “all data” case; and $C_1=154.95$, $\sigma=19.3$ and $C_2=1.9$, sd=0.39 for the “without high-point” case. The “without high-point” case had estimates that were closer to the literature values. This relative predictive capacity of each case is revealed when the model error ($\sigma$) is observed, where “all data” was 343.66 and “without high point” was 44.7.
Table 2. Parameter estimates from Ergun model for all oyster pressure data and without extreme high point.

| Parameter | All Data | | | | Without High Point | | | |
|-----------|---------|------|------|------|---------|------|------|
|           | mean    | sd   | n_eff | mean | sd     | n_eff |
| Ε         | 0.63    | 0.02 | 13472 | 0.64 | 0.02   | 8472  |
| d_p (mm)  | 3.18    | 0.74 | 8525  | 2.45 | 0.45   | 4996  |
| C1        | 149.27  | 19.97| 15098 | 154.95| 19.3   | 10209 |
| C2        | 2.88    | 0.70 | 8972  | 1.9  | 0.39   | 5052  |
| σ (Pa)    | 343.66  | 87.86| 10011 | 44.7 | 14.02  | 5687  |

The posterior distribution of parameter estimates relative to the prior distribution were relatively different for the oysters and the glass spheres. The C1 and C2 Ergun constants were much closer to the literature for the oysters. The value for C2 was smaller and closer to the literature for the “without high point” case and had a smaller posterior distribution than the prior. The $\epsilon$ was approximately the same as the prior distribution, $\mathcal{N}(0.64, 0.018)$, for both the “all data” and “without high point” cases. The $d_p$ was closer to the prior distribution, as noted earlier for the “all data” case. This indicates that the data was informative for the $d_p$ and C2 estimates, where the high point had an impact on the estimate.

The $\epsilon$ and $d_p$ from the “all data” case were used in the model for diffusion even though the estimates for the “without high point” case appear to be better. This was because the oyster shells were noted to have shifted when the drag and buoyant forces were greater than the weight at the highest velocity. This could have caused the pressure to spike due to the oyster bed being compressed and modified diffusion within the tube. The distributions for the “all data” case were applied in the diffusion modeling analysis to maintain consistency.
The parameters estimated from the Bayesian model were used to calculate the predicted pressure drop with increasing velocities, where the estimated pressure is shown for glass spheres and oysters (“all data” case) along with observed data in Figure 5. The model fit for glass spheres had an $R^2=0.996$ The estimated values for the pressure drop for oysters were calculated using the parameters estimated in both “all data” and “without high point” cases. The “all data” case had $R^2=0.95$ and the “without high point” case had $R^2=0.998$. The predictive skill of these models was acceptable and could be used in future analysis.

![Figure 5. Estimated and observed data for pressure differential as superficial velocity increases for spheres and oysters (“with all data” case).](image)

5.2. Measurement and Modeling of Diffusion Coefficient

An example impulse function is shown in Figure 6, where the conservative tracer plume can be seen at both conductivity sensors for the oyster bed subjected to 4.6 cm s$^{-1}$ superficial velocity. This data was collected for each of the 12 velocities for both packed bed materials and used to calculate corresponding diffusion coefficients.
Figure 6. Impulse function, f(t), at sensors on each side of the packed bed as the salt plumes pass through a packed oyster bed subjected to superficial velocity of 4.6 cm s\(^{-1}\). The variance of the plume will be used to determine the axial dispersion coefficient for each velocity.

As described in Section 2.2 above, the Peclet number, \(Pe_L\), and diffusion coefficient, \(D_L\), were calculated from the plume data. Figure 7 shows the diffusion coefficient for each corresponding superficial velocity for both glass spheres and oysters. In general, as the superficial velocity increased, the diffusion coefficient also increased for both the glass spheres and the oysters.

Figure 7. Experimentally determined diffusion coefficients, \(D_L\), for glass spheres and oysters.
The posterior distributions from the previous Ergun models (i.e. $d_p$ and $\varepsilon$) were used as prior distributions in the Bayesian models that predict diffusion as a function of superficial velocity for both spheres and oysters. Other parameter prior distributions were selected based upon the literature as described in Section 3.4.

The glass sphere estimates of posterior distributions were estimated using Bayesian inference for the diffusion model. The estimated parameter means, standard deviations, credible intervals, effective sample size ($n_{\text{eff}}$), and $\hat{R}$, are listed in Table 3. The $Pe_\infty$, which is the stable Peclet number as velocity goes to infinity, was 4.42 and is close to the estimate of 5 by Nauman (2008) for homogeneous particles. The $d_p$ and $\varepsilon$ remained consistent with the measured values and the posterior distribution estimated by the Ergun model. The estimation of the tortuosity, $\tau$, also remained consistent with the literature for glass spheres (Delgado, 2006). The model error, $\sigma$, for glass spheres was 1.96.

The sphere diameter and void ratio inferred with the Bayesian diffusion model were approximately the same as those from the Ergun based model. The diffusion model using oysters as a packed bed inferred slightly different estimates than the Ergun based model. The estimate for diameter, $d_p$, for the diffusion model (mean =3.37, sd =0.73) was slightly larger than the Ergun based model (mean=3.18, sd=0.74). The estimate for void ratio, $\varepsilon$, for the diffusion model (mean =0.61, sd =0.1) was lower and had a smaller standard deviation than the Ergun based model (mean =0.63, sd =0.2).
Table 3. Parameter estimates from particle Peclet based model for axial diffusion for glass spheres.

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>sd</th>
<th>2.50%</th>
<th>97.50%</th>
<th>n_eff</th>
<th>( \bar{R} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d_p ) (mm)</td>
<td>15.27</td>
<td>0.5</td>
<td>14.3</td>
<td>16.26</td>
<td>7928</td>
<td>1.00</td>
</tr>
<tr>
<td>( T )</td>
<td>1.59</td>
<td>0.47</td>
<td>0.7</td>
<td>2.55</td>
<td>7536</td>
<td>1.00</td>
</tr>
<tr>
<td>( Pe_\infty )</td>
<td>4.42</td>
<td>1.01</td>
<td>2.96</td>
<td>6.92</td>
<td>5117</td>
<td>1.00</td>
</tr>
<tr>
<td>( E )</td>
<td>0.39</td>
<td>0.03</td>
<td>0.33</td>
<td>0.45</td>
<td>6435</td>
<td>1.00</td>
</tr>
<tr>
<td>( D_m ) (10^4 \text{m}^2\text{s}^{-1})</td>
<td>4.01</td>
<td>2.12</td>
<td>0.69</td>
<td>8.91</td>
<td>5322</td>
<td>1.00</td>
</tr>
<tr>
<td>( \sigma ) (10^4 \text{m}^2\text{s}^{-1})</td>
<td>1.96</td>
<td>0.5</td>
<td>1.24</td>
<td>3.16</td>
<td>5846</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The oyster estimates of posterior distributions for the diffusion model and are listed in Table 4. The estimated parameter means, standard deviations, credible intervals, effective sample size (n_eff), and \( \bar{R} \). The mean \( Pe_\infty \) was calculated as 0.69 with a standard deviation of 0.39, which is below the estimate of 2 by Delgado (2006) for homogeneous particles. The \( d_p \) and \( \varepsilon \) remained consistent with what was measured and inferred in the Ergun model, although \( d_p \) was slightly higher than Ergun estimates, and the \( \varepsilon \) was slightly lower than Ergun estimates. The tortuosity, \( \tau \), was slightly higher than the prior distribution for solid cylinders (mean=1.93, sd=0.5). This is intuitive because of the relative complexity of the irregular formed oyster shells. The model for oysters had a model error of 1.39, which was smaller than for the glass spheres.
The diffusion coefficients estimated from the Bayesian model were consistent with the literature for spheres within the range of Reynolds numbers included in this experiment (Nauman, 2008). The Peclet number based on the particle size was calculated and plotted versus the particle Reynolds number, which is shown in Figure 8 for the glass spheres. As the velocity increases to create fully turbulent conditions (Re>2000), the Peclet number approaches an asymptote (Delgado, 2006; Nauman, 2008).

Figure 8. Peclet number based on particle diameter at increasing particle Reynolds numbers for the glass spheres in packed beds.
The Bayesian models for diffusion through spheres and oysters had good prediction capabilities. The model for spheres had $\sigma=1.96$, and the model for oysters had $\sigma=1.39$. The coefficient of determination for the models were generally good for spheres ($R^2=0.71$) and oysters ($R^2=0.82$). The linear model form discussed in Delgado (2006) appears to be appropriate but could be further evaluated with more data. The higher values of diffusion at the higher velocities in the oyster experiments could be related to the bed shifting as noted in Section 5.1.

A Monte-Carlo simulation of predicted values using the posterior parameter distributions was conducted to analyze the prediction and credible intervals. The posterior distributions of the parameters were used to generate predictions of the diffusion coefficient for the glass spheres and the oysters. The predicted values for these 50,000 simulations were used to generate the 95% prediction and 95% credible intervals. The mean estimate of the linear representation (blue line) is shown for glass spheres (Figure 9) and oysters (Figure 10) along with the observed values of diffusion (blue dots) with increasing velocity. The 95% credible intervals (red lines) represent the uncertainty in the diffusion coefficient estimate. The 95% prediction interval (dotted black line) represents the residual error including experimental error. The prediction and credible intervals were smaller for the oysters than for the glass spheres.
Figure 9. Predictions of axial diffusion, \( D_l \), for glass spheres were calculated using Monte-Carlo simulation based upon posterior distributions and residual uncertainty, where the dashed black lines are the 95% prediction interval. The mean diffusion estimate (blue line) and the raw diffusion data (blue dots) were calculated from the posterior distributions. The solid red lines are the 95% credible intervals for the model (i.e. without residual uncertainty).
Figure 10. Predictions of axial diffusion, $D_L$, for oysters were calculated using Monte-Carlo simulation based upon posterior distributions and residual uncertainty, where the dashed black lines are the 95% prediction interval. The mean diffusion estimate (blue line) and the raw diffusion data (blue dots) were calculated from the posterior distributions. The solid red lines are the 95% credible intervals for the model (i.e. without residual uncertainty).

6. Conclusions

This paper presented three unique approaches to analysis of hydrodynamics within an upweller aquaculture system. The application of the axial diffusion model allows for the quantification of the hydrodynamics within the packed bed. This greater understanding will allow for the optimization of these types of culture methods and could lead to innovations in their design. The addition of Bayesian inference allows for a robust analysis of all relevant parameters applied within the mechanistic models. The step-wise framework of modeling
pressure and then diffusion allowed parameters to be correlated by two independent tests giving them more confidence and allowing transfer to the field.

The Hamiltonian Monte Carlo sampling provided reasonable estimates for particle equivalent spherical diameter, void ratio, tortuosity, and axial diffusion coefficients. This suggests that this method is a viable approach to modeling bulk oyster bed parameters with stochastic characteristics, such as shape and size. The estimated equivalent spherical diameter yielded encouraging results when applied to the Peclet based Bayesian model for diffusion. The Monte-Carlo simulations of axial diffusion coefficients using the posterior parameter distributions were reasonable when compared to the observed data. The consistency between the estimated parameters from the two models and physical measurements demonstrates the validity of this approach.

Another benefit of applying Bayesian inference to this model is that the prior parameter distributions can be improved over time. This allows the model to improve as more data is gathered from future experiments, field data collection, or otherwise. At a pragmatic level, the ability to correlate pressure with diffusion coefficients could also yield useful outcomes to farmers that could easily measure pressure drop on each side of culture units. For example, pressure measurements could allow the farmer to optimize flow rates to produce sufficient food and water quality for oysters throughout the culture unit.

Future work should explore a range of variations in oyster culture scenarios. These should include different salinities, sizes of oysters, and sedimentation rates within culture units. The exploration of diffusion characteristics of algae and other seston should be conducted as they could behave differently than inert tracers (i.e. diatoms may settle and flagellates have motility). The influence on sedimentation within the bed would also affect the diffusion through the packed
bed. The validation of the model on actual culture units in the field could reveal the importance of these various parameters.

Diffusion is an important parameter for applying the axial diffusion model to oyster upweller systems. This study developed a model estimate of diffusion with increasing velocities that can be used to design and optimize upweller systems using the axial dispersion model. An analytical form of the model can be used to determine if the oysters are getting enough food at a specific flow rate, oyster size, and depth of oyster bed. Other parameters still need to be explored further, such as the oyster filtration rate and the factors affecting it (Campbell and Hall, 2019; zu Ernsgassen et al., 2013). Since oysters grow in size within these systems, both their short term (e.g. opening and thus enlarging the bed) and long term (growing, thus altering size and shape of oysters over time) should be explored. In addition, practical applications of these insights should be developed and transferred to oyster farmers and other situations with similar challenges. Simple local measurement of critical parameters such as pressure, salinity, temperature, oxygen and turbidity are each of interest in optimizing, managing and controlling these types of systems. The current work could serve as a baseline for further analysis of oyster filtration under a variety of scenarios.
7. References


CHAPTER 4: ANALYSIS OF FILTRATION THROUGH A CONTROLLED UPWELLER AQUACULTURE SYSTEM CONTAINING JUVENILE OYSTERS, *CRASSOSTREA VIRGINICA*

Abstract

As oyster aquaculture continues to grow, there is an increased need to develop sustainable and profitable operations. Intensive systems, such as upwellers, are critical components in modern oyster aquaculture that could be optimized using engineering principles. The oyster filtration is an important parameter in the development of mechanistic models for upweller system optimization. The filtration magnitude and variability of oysters within an upweller system was explored though controlled experiments. Five upweller experimental units were constructed on-site at an oyster farm in North Carolina. The sedimentation and filtration within these upwellers were analyzed using various sampling and analysis methods. Individual particles were observed using a portable FlowCAM (Flow Cytometry And iMaging) system equipped for auto-imaging. This analysis revealed dynamics within the upweller oyster bed that were not possible to observe with total suspended solids testing alone. The FlowCAM method revealed a shift in particle size to larger diameters from the incoming water to the water passing through the oyster bed. This method was able to measure a 35.5% filtration rate of algae by the oysters with a standard deviation of 8.6%. The mean individual filtration rate of oysters was 3.44 L/hr/g ($\sigma = 0.80$ L/hr/g, n=4) and similar to other studies. The results from this study will inform mechanistic models for system optimization and provide oyster farmers with guidance on maintenance due to sedimentation buildup in culture units.
1. Introduction

Marine bivalves, including oysters, have become a substantial portion (15.6 million tons in 2016) of seafood production globally where the vast majority are produced through aquaculture according to the recent FAO (2018) State of the World Fisheries report. Oyster aquaculture has experienced increased challenges due to declining natural stocks, water quality degradation, siting, and regulatory issues (Goslin, 2015). The natural stocks have become stressed due to degraded water quality and overexploitation by capture fisheries. Due to these pressures and a rising demand for farmed oysters, aquaculture has increasingly replaced capture fisheries. As the aquaculture industry continues to expand, there is an ever increasing need to develop sustainable methods that maximize yield and profitability (FAO, 2018).

Oyster and other bivalve aquaculture operations have utilized upweller systems to increase yield and profitability for the past 50 years. These systems are typically used during the early life cycles (i.e. juvenile stage) where oysters are vulnerable to predation and water quality issues. Higher stocking densities and smaller footprints are also achievable with these systems compared to other methods, such as ponds or tanks (Goslin, 2015). A typical upweller system consists of a silo with a mesh bottom that supports a bed of oysters. Water is pumped from the bottom of the silo through the bed of oysters to provide food, supply oxygen, and remove metabolic wastes (Spencer, 1988; Williams, 1981). The flow rate is important to supply these critical conditions to the oysters, but the operational cost must be accounted for in determining optimal rates (Appleyard and Dealeris, 2002; Bishop and Hooper, 2005; Pfeiffer and Rusch, 2001; Rodhouse and O’Kelly, 1981). The FAO Hatchery manual recommends 10 to 20 l/min per kg of spat to achieve sufficient growing conditions (Helm and Bourne, 2004). Although these
types of recommendations have been given as guidelines for culture, a thorough understanding of the physical and biological mechanisms within these culture systems has yet to be fully explored.

A large body of knowledge has accumulated on oyster filtration (i.e. what the oyster removes from the water column) within an estuary setting due to their ecological significance (Bayne, 2017). Grizzle (2006) conducted studies in an estuary on the ability of oysters and mussels to clear seston from the water column using fluorometry. Grizzle (2008) compared individual filtration rates from field observations (removal of Chl-a of -0.6 to 6.0 L h⁻¹) in the estuary to lab studies and concluded the need for these studies in the natural setting due to the large variability. Dame (2012) concluded similarly with his discussions on filtration in the natural environment. Zu Emergassen et al. (2013) conducted an extensive review of environmental factors affecting oyster filtration rate in an attempt to quantify ecosystem service losses due to population decline of the eastern oyster, *Crassostrea virginica*, in a variety of environmental conditions. Various filtration rate models that consider effects from temperature, total suspended solids (TSS), and salinity have been developed (Ehrich and Harris, 2015).

Although the filtration rate of oysters has been investigated extensively in the natural setting, these rates have not been applied to upweller culture systems to optimize their performance.

Studies that have observed oyster filtration rates in upweller systems have shown high temporal variability. Manzi et al. (1986) was one of the first to describe the effect that environmental conditions had on the filtration rate of shellfish in upweller systems, where a similar stocking density of 10 kg/m² had a filtration rate that ranged from 8.11% in the winter to 62% in the summer. Li et al. (2012) recorded fluorometry measurements within and downstream of a Floating Upweller System (FLUPSY) in Peconic Estuary, New York over several months. They observed high temporal variability in filtration by the upweller systems that were correlated
with temperature and dissolved oxygen changes in daily and seasonal conditions (Li et al. 2012). They recorded chlorophyll removal rates of -50% to 40% with a median of 6.45%. These studies note the temporal and environmental changes that affect filtration rate of oysters within an upweller system, but do not adequately describe the inherent variability in filtration oysters exhibit within the same set of environmental parameters. They also do not describe the other filtration mechanisms that could occur within the upweller systems (i.e. sedimentation).

A greater understanding of the dynamics within these systems must be achieved to optimize the upweller culture systems. The application of engineering principles to these systems could enhance the approaches to design and optimization of operations. Nobre et al. (2017) developed a mass balance approach based on “rules of thumb” in an effort to utilize information gathered from how oysters performed in estuaries to culture systems. While these types of tools are useful in sizing operations and stocking densities, they give little insight into how systems could be optimized through engineering design. Campbell et al. (in review) developed a promising mechanistic model based on packed bed reactor theory that could be used for engineering design and optimization. This mechanistic model also applied Bayesian inference to account for the stochastic nature of biological parameters, such as size, shape, and filtration rate. A better understanding of the importance of parameters and their variability must be achieved to effectively use these types of mechanistic models.

This study explored the sources and magnitude of filtration variability within an upweller culture system. The bulk filtration of an upweller oyster bed can be divided into oyster filtration, biodeposit conversion, and sedimentation, which is illustrated in Figure 1. Variability in each of these rates could affect the overall filtration observed external to the upweller system. This variability of different rates could explain some of the variation noted by other researchers (Li et
al. 2012; zu Emergassen et al., 2013). The objective of this study was to determine if there was a significant decrease in concentration of algae particles from the incoming water to the water after the oyster bed to determine the magnitude of filtration. The second objective was to determine if there was a significant difference in particle size filtration by the oyster bed. An expected outcome was to quantify the variability of oyster filtration within an upweller system within the same conditions. A secondary outcome was to determine other mechanisms of oyster bed filtration and their relative importance.

![Diagram](image.png)

**Figure 1.** Bulk filtration of algae through a bed of oysters can be divided into filtration by oysters, conversion to biodeposits, and sedimentation.

### 2. Methods and Materials

The experimental setup was developed to represent the hydrodynamics that occur within an upweller system. The experimental units were installed on-site at Chadwick Creek Oyster Company farm in Bayboro, NC. The source water for the experiment was pumped from mid-water column near the existing FLUPSY (Floating Upweller System) in Jones Bay, adjacent to Pamlico Sound. The source water parameters were measured at the start of the experiment. The
salinity was 22 ppt; the temperature was 19 °C; the dissolved oxygen was 7.1 mg/L; and the pH was 8.01.

The experimental units were designed to have a controlled and repeatable flow through the bed of oysters. There was a total of 5 experimental units that had a controlled flow rate of 0.5 GPM, which are shown in Figure 2. This flow rate was chosen because it was identified by Campbell and Hall (2019) that it represented a typical upweller system superficial velocity within the oyster bed (i.e. 1.5 cm/s). The flow was controlled using King flow meters (0 - 1 GPM), which were calibrated with a 4000 mL Nalgene graduated cylinder. The flow rates had standard deviation of +/- 0.03 GPM (n=7). The oysters were suspended as a 280 mm (+/- 20mm) bed within tubes constructed of 2-inch (50 mm) polycarbonate. The oysters were held in place by stretchable plastic mesh (polypropylene with 7.5 mm opening) on top and bottom of the bed. The polycarbonate tubes had ports on either side of the oyster bed to measure pressure differential and acquire water samples.

Figure 2. Upweller experimental units at Chadwick Creek Oyster Company farm in Bayboro, NC.
The oysters used in this experiment were taken from one of the silos in the on-site FLUPSY system. The oysters were of the “Lola” genetic line from Virginia Institute of Marine Science and had a mean length of 8.04 mm ($\sigma=0.81$ mm, $n=142$) and a mean height of 7.21 mm ($\sigma=1.63$ mm, $n=142$). Before the oysters were taken from the FLUPSY system, the silos were washed as per the daily maintenance routine. This removed a large amount of sedimentation within the bed and was representative of what the oysters would have experienced within a FLUPSY.

The bed height and pressure loss through the bed was measured over the duration of the experiment. Measurements were taken every 30 minutes for 4.5 hours. The pressure loss through the oyster bed was measured with a differential pitot tube connected to sampling ports above and below the bed. The pitot tubes consisted of 3.5 mm ID vinyl air tubes that were connected at ports 185 mm above and below the mesh holding the oyster bed in place. The bed height was measured before pressure was taken for each of the experimental units and care was taken to minimize disturbances. The bed height also served as an indication of feeding during the experiment due to bed expansion as oysters opened their shells.

Water samples were taken from the tubes above and below the oyster beds to analyze the filtration by measuring the particle concentration. Sampling ports allowed the collection of water at the center of the tubes through locations 48 mm above and below the plastic mesh holding the oysters in place. Oysters were allowed to adjust to the system water flow for 50 minutes before water sampling began. Samples were collected slowly into 500 ml Nalgene water sample bottles over a 45 min period. This slow sampling method had an approximate flow rate of 7 ml/min.

During the slow sampling, it was observed that larger particles were not entering the sampling port inlets. A quick sampling method was used to capture particles that had different
hydrodynamic properties. This quick sampling method opened ports completely allowing the flow rate to increase from 7 ml/min to approximately 800 ml/min. This quick sample represented more of a snapshot than the slow sampling method, which represented more of an average over 45 minutes. After samples were collected, they were placed within a cool dark ice chest until they reached the laboratory fridge the same day. An aliquot of 10 ml was taken from each and 0.5 ml of Lugol’s iodine was added to preserve the samples.

The sedimentation within the bed of oysters was analyzed by taking samples at the beginning and end of the experiment. A 235 ml sample of oysters was taken from the top of the oyster bed and placed within a glass container. This was combined with 470 ml of onsite fresh well water and mixed together. The oysters were gently stirred until particles within the bed were released from the oysters. The water and particles were poured into a 500 ml Nalgene bottle for storage and transport back to the lab for further analysis.

Total suspended solids (TSS) analysis was performed on the samples using EPA Method 160.2 in the Environmental Analysis Lab at North Carolina State University. The glass fiber filters were prepared by washing with deionized water and placing them in an oven at 105 °C for 24 hrs. The samples were vacuum filtered onto glass fiber filters and rinsed with deionized water to remove dissolved solids. The sample volume was recorded for each sample. The filter samples were placed in the oven at 105 °C for 24 hrs. The samples were weighed and subtracted from original filter weight. The TSS was calculated for each sample by dividing sample dry weight by volume filtered.

The particle concentrations of the water samples were analyzed using a portable FlowCAM (Flow Cytometry And iMaging) system equipped for auto-imaging, and detection of individual particles passing through a flow cell. The FlowCAM software captures images of each
particle and records information on the size, diameter, and color properties of each cell
(Spaulding, 2014). The 10mL aliquot preserved water samples were used in the FlowCAM
analysis. At least 5000 particles between 5 µm and 200 µm were counted for each sample.
Images produced by the FlowCAM were reviewed for issues such as lodged particles and repeats
of the same image before finalizing processing. The particles identified with the FlowCAM were
compiled along with individual identification numbers, properties, and corresponding images.

The FlowCAM software included photo-recognition algorithms that were used to identify
biodeposits within the water samples (Spaulding, 2014). An example of images captured by
FlowCAM are shown in Figure 3, where the larger amorphous particles are considered
biodeposits. The photo-recognition algorithm was trained using 50 images of biodeposits from
FlowCAM images. These biodeposit images and corresponding parameters were screened from
the database using the same training set for all samples.

Data gathered from the TSS, FlowCAM, pressure and bed height analysis were compiled
and analyzed with open source software. The data were compiled and analyzed using python
3.7.4 libraries including Scipy v0.15.1, Pandas v0.25.2, Numpy v1.15.5. The Scipy v0.15.1 stats
module was used to conduct t-test statistics, which have produced consistent results when
compared to other commercial software applications (Oliphant, 2007; Scipy (2015); Millman and
Aivazis, 2011; McKinney, 2010). The combination of hardware and software tools allowed us to
test the effects of the oyster bed on the concentration of algae particles.
3. Results and Discussion

3.1. Bed Height

As discussed in Section 2 – Materials and Methods, the bed height was measured throughout the experiment and was used as a metric for oyster feeding during the experiment. As the oysters feed, they open their shells and the bed expands. Figure 4 illustrates the bed height over the duration of the experiment for each tube. The slow sampling period (grey polygon) and quick sampling time (red dotted vertical line) are shown in the figure as a reference. The oysters were feeding for most of the sampling period, with a few exceptions in T2 and T5. These could have been due to temporary disturbances during measurement as the bed was expanded in the subsequent measurements. The variation of bed height was highest for tube 5 ($\mu=286$ mm, $\sigma=6.2$ mm) and lowest for tube 3 ($\mu=281$ mm, $\sigma=2.5$ mm).
Figure 4. Bed height measurements during the course of the experiment for each tube. The duration of the slow sampling (grey polygon) and quick sampling (vertical red dotted line) are shown.

3.2. Filtration of Total Suspended Solids

The total suspended solids (TSS) at the top and the bottom of the oyster bed were calculated for each of the tubes for both the slow and quick sampling methods. The TSS measurements were plotted for comparison, which are depicted in Figure 5. The mean bottom concentration for the slow sampling method was 11.94 mg/l (σ= 1.33, n=5) and the quick sampling method had a mean of 14.06 mg/l (σ=3.21, n=5). The mean top concentration for the slow sampling method was 10.95 mg/l (σ= 1.58, n=5) and the quick sampling method had a mean of 15.33 mg/l (σ= 2.85, n=5). A two sample t-test conducted for these sampling methods determined that the bottom concentrations for both sampling methods were consistent with means being the same (t=1.3680, p=0.2085, df=8), but yielded significant differences at the top (t=3.00, p=0.0171, df=8) of the oyster bed. Therefore, the incoming water had the same concentration for both methods, but different concentrations above the oyster bed.
The effects of the oyster bed acted differently depending on the sampling methods. Although the bottom concentrations between sampling methods were not significantly different, the top measurements had larger concentrations for the quick sample than for the slow sample. Among sampling methods the top and bottom concentrations had a similar standard deviation, $\sigma$, for each sampling method. Overall, the quick sampling method had a larger standard deviation than the slow sampling method.

![Figure 5. Strip plot of Total Suspended Solids (TSS) at the top and bottom of the packed bed of oysters for the slow and quick sampling methods for each tube. A vertical jitter algorithm was applied to the data to minimize overlapping points.](image)

The data was “paired” by taking the difference from the top and bottom TSS concentrations to determine the filtration of the oyster bed. The TSS differences between the top and bottom of each oyster bed tube were calculated and are listed in Table 1 for TSS and as a percent of the bottom concentrations. The mean, $\mu$, and standard deviation, $\sigma$, of the differences were calculated for both the slow and quick sample methods. The percent differences represented the filtration rate due to the oyster bed relative to the incoming bottom concentration.
The measurements had a large variation and did not demonstrate a significant difference from top to bottom. A two-sided single sample t-test was conducted to determine if the concentration differences between the top and bottom were significant for each sampling method. There were no significant differences in TSS from bottom to top for the oyster beds using the slow (t=0.8603, p=0.4381, df=4) or the quick (t=-0.6363, p=0.5591, df=4) sampling methods. The TSS method was not able to demonstrate filtration by the oysters due to the high variability in measurements.

**Table 1. TSS solids difference between the top and bottom of packed bed of oysters with corresponding percentages of bottom concentration for the slow and quick sampling methods for each tube.**

<table>
<thead>
<tr>
<th>Tube</th>
<th>Slow Sample</th>
<th>Quick Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSS Difference (mg/l)</td>
<td>Percent Difference</td>
</tr>
<tr>
<td>1</td>
<td>2.63</td>
<td>20.00%</td>
</tr>
<tr>
<td>2</td>
<td>3.94</td>
<td>29.50%</td>
</tr>
<tr>
<td>3</td>
<td>-2.75</td>
<td>-26.20%</td>
</tr>
<tr>
<td>4</td>
<td>0.94</td>
<td>8.80%</td>
</tr>
<tr>
<td>5</td>
<td>0.16</td>
<td>1.30%</td>
</tr>
<tr>
<td>μ</td>
<td>0.98</td>
<td>6.68%</td>
</tr>
<tr>
<td>σ</td>
<td>2.55</td>
<td>21.30%</td>
</tr>
</tbody>
</table>

3.3. Sediment Retention within the Oyster Bed

The amount of material retained within the bed was calculated from initial and final TSS measurements (mg/l) of the rinsed oysters. The tabulated measurements with calculations of difference, $TSS_d$, and retention rates, $r_R$, (mg/hr) are in Table 2. The $TSS_d$ was calculated by
subtracting the final, TSSₜ, from initial, TSSᵢ, measurements. The \( r_R \) was calculated from Equation 1 below:

\[
\frac{V_B(TSS_i + TSS_d) V_w}{V_o} \frac{V_w}{V_o} = \frac{\Delta t}{\Delta t}
\]

Where \( V_B \) (l) is the average oyster bed volume; \( V_w/V_o \) is the ratio of water to oyster sample volume; and \( \Delta t \) (hrs) is the duration between the initial and final samples.

The \( r_R \) was more consistent than the TSSₙ calculations, which had higher variability that appeared to be correlated to the TSSᵢ. The TSSₜ concentrations were more consistent than the TSSᵢ, which suggest there may have been a non-linear increase of material within the bed. It should be noted that the \( r_R \) was calculated from the addition of TSSᵢ and TSSₙ because sedimentation occurred in the space where previous material was removed during the initial rinse of the oyster bed. The \( r_R \) represents the net increase of material buildup within the bed over time.
Table 2. Sediment concentrations within each tube at the beginning, TSSI, and end, TSSf, of the experiment. The difference, TSSd, and rate of accumulation, rR, were calculated from these measurements.

<table>
<thead>
<tr>
<th>Tube</th>
<th>TSSf (mg/l)</th>
<th>TSSI (mg/l)</th>
<th>TSSd (mg/l)</th>
<th>rR (mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>482.6</td>
<td>475</td>
<td>7.6</td>
<td>104</td>
</tr>
<tr>
<td>2</td>
<td>418.2</td>
<td>277.3</td>
<td>140.9</td>
<td>87.7</td>
</tr>
<tr>
<td>3</td>
<td>459.1</td>
<td>188</td>
<td>271.1</td>
<td>99.6</td>
</tr>
<tr>
<td>4</td>
<td>492.5</td>
<td>348.8</td>
<td>143.6</td>
<td>101.6</td>
</tr>
<tr>
<td>5</td>
<td>407.4</td>
<td>323.8</td>
<td>83.6</td>
<td>90.1</td>
</tr>
</tbody>
</table>

The percent retained of the incoming bottom concentration was calculated and tabulated in Table 3. The quick sample bottom concentration, TSSb, was multiplied by the flow rate to get the load rate, rL, to the oyster bed. The percent retained was expressed as a ratio of retention rate to loading rate, rR : rL. The percent retained had a mean, µ, of 6.29% and a standard deviation, σ, of 1.47%.

The 6.29% of the material accumulating within the bed could be due to various processes. The material entering the bed could be algae, biodeposits, inorganic particles, and detritus. The material trapped within the bed could also be biodeposits (i.e. feces and pseudofeces) produced by the oysters as they clear particles from the water column, digest or reject, and excrete them. It was not possible to determine the composition from the TSS measurements, but visual inspection at the time of sampling confirmed that larger particles and biodeposits were a substantial portion of this material.
Table 3. Load rate of TSS was calculated from bottom concentration (quick sample method) and flow rate.

The retention rate in the oyster bed was represented as a percentage of loading rate.

<table>
<thead>
<tr>
<th>Tube</th>
<th>TSSb (mg/l)</th>
<th>rL (mg/hr)</th>
<th>rR (mg/hr)</th>
<th>Percent Retained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.1</td>
<td>2166.6</td>
<td>104</td>
<td>4.80%</td>
</tr>
<tr>
<td>2</td>
<td>13.9</td>
<td>1579.3</td>
<td>87.7</td>
<td>5.60%</td>
</tr>
<tr>
<td>3</td>
<td>10.7</td>
<td>1211.3</td>
<td>99.6</td>
<td>8.20%</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>1362.7</td>
<td>101.6</td>
<td>7.50%</td>
</tr>
<tr>
<td>5</td>
<td>14.7</td>
<td>1665.6</td>
<td>90.1</td>
<td>5.40%</td>
</tr>
<tr>
<td>μ</td>
<td>14.1</td>
<td>1597.1</td>
<td>96.6</td>
<td>6.29%</td>
</tr>
<tr>
<td>σ</td>
<td>3.2</td>
<td>365.1</td>
<td>7.2</td>
<td>1.47%</td>
</tr>
</tbody>
</table>

Pressure differential from the top and bottom of the bed was also measured over the duration of the project, which could give some insight into sedimentation over time. The pressure differential over bed height for each tube is shown in Figure 6 over the duration of the experiment. Bed pressure differential generally increased over time, where higher rates of increase occurred at the beginning of the experiment. This could be an indication of asymptotic behavior of sedimentation within the oyster bed. This could also be due to the rapid infilling of the rinsed bed volume immediately after initial rinse was performed. Future studies should be considered to analyze entire bed accumulation at various bed depths with corresponding pressure differentials.
3.4. Filtration of Total Particles

The total particle concentrations were calculated from FlowCAM measurements, compiled, and analyzed for each water sample. A strip plot (Figure 7) was created to visualize the samples from the top and bottom of the oyster bed for the slow and quick sampling methods. The mean and standard deviations for top and bottom concentrations between the sampling methods were compared. The mean bottom concentration for the slow sampling method was 52464 counts/ml (σ= 6153, n=5) and the quick sampling method had a mean of 40516 counts/ml (σ=2376, n=5). The mean top concentration for the slow sampling method was 36267 counts/ml (σ=4714, n=5) and the quick sampling method had a mean of 45449 counts/ml (σ=10385, n=5). A two-sample t-test determined that the concentrations were consistent with the two sampling methods being the same at the bottom (t=-1.4708, p=0.1796, df=8) and top (t=1.800, p=0.1095, df=8).
The variation of the two sampling methods was different at the top and the bottom of the oyster bed. Although the bottom concentration data was consistent with sampling methods being the same, the variation of the slow sampling was larger than the quick sampling method. On the other hand, the top concentration variation was larger for the quick sampling than the small sampling method. This suggests that the oyster bed had a larger effect on the variation of the quick sampling method than the slow sampling method.

![Figure 7. Strip plot of particle concentrations at the top and bottom of the packed bed of oysters for the slow and quick sampling methods for each tube.](image)

The filtration of the oyster bed was analyzed similarly to the TSS approach using paired differences in top and bottom concentrations. The difference values for sample methods are shown in Table 4 for the concentration and the percent of bottom concentration for each tube. The mean, \( \mu \), and standard deviation, \( \sigma \), is also shown for each method. The mean difference was higher for the slow sample than the quick sample. The variation was higher for the quick sample than the slow sample method. A two-sided single sample t-test was conducted to determine if the concentration differences between the top and bottom were significant for each sampling method. The slow sampling method had a significant difference \((t=5.6279, \ p=0.0049, \ df=4)\) in
top and bottom concentrations. The quick sampling method had no significant difference ($t=-0.5726, p=0.5976, \text{df}=4$) in top and bottom concentrations of the oyster beds. This means that the slow sampling method established a filtration rate of the oyster bed that the quick sampling method could not, which is possibly due to a difference in the hydrodynamics of the sampling methods.

The FlowCAM results were more direct measurements of what the oysters filtered than the TSS measurements. In other words, the oysters filter individual particles from the water column as opposed to bulk mass including larger non-algal particles. This could explain the clear difference in the slow sample for the FlowCAM measurements ($\mu=30.45\%, \sigma=9.47\%$), but no difference in the TSS measurements ($\mu=6.68\% \sigma=21.30\%$). The difference observed with the quick sample was not significant for both the TSS and the FlowCAM, but the mean filtration was higher for the FlowCAM than the TSS analysis.
Table 4. FlowCAM particle concentration differences between the top and bottom of packed bed of oysters with corresponding percentages of bottom concentration for the slow and quick sampling methods for each tube.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Slow Sample</th>
<th>Quick Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td>(Count/ml)</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>17364</td>
<td>31.75%</td>
</tr>
<tr>
<td>2</td>
<td>12379</td>
<td>26.61%</td>
</tr>
<tr>
<td>3</td>
<td>8780</td>
<td>16.80%</td>
</tr>
<tr>
<td>4</td>
<td>25923</td>
<td>42.07%</td>
</tr>
<tr>
<td>5</td>
<td>16540</td>
<td>35.01%</td>
</tr>
<tr>
<td>μ</td>
<td>16197</td>
<td>30.45%</td>
</tr>
<tr>
<td>σ</td>
<td>6436</td>
<td>9.47%</td>
</tr>
</tbody>
</table>

3.5. Size and Shape of Particles

The FlowCAM also captured the size and shape of particles from the high-speed camera and image recognition algorithms (Spaulding, 2014). This allows for the analysis of sizes from the bottom and top of the oyster bed, which suggested a preferential filtering by the oyster bed. The quick sampling method had a mean bottom length of 12.6 µm (σ=0.43 µm) and a mean top length of 14.4 µm (σ=0.59 µm). The slow sampling method had a mean bottom length of 12.5 µm (σ=0.17 µm) and a mean top length of 12.83 µm (σ=0.58 µm). The difference of the particle length between the top and bottom of the oyster bed was significant for the quick sample (t=5.6820, p=0.0004, df=8), but not for the slow sample (t=1.2950, p=0.2315, df=8). This difference between the sampling methods could be due to hydrodynamic differences of the particles, where larger particles, including biodeposits, were not making it into slow samples as frequent as quick samples. This was also observed through visual inspection during sampling.
A histogram of particle length was developed for each of the tubes, shown in Figure 8. The histograms reveal a pattern of smaller particles on the bottom (5-12 µm) being removed by the bed. At the same time, there was a pattern of increased larger particles on the top (greater than 12 µm). This pattern was consistent with literature, where oysters can selectively filter particles of different sizes and densities (Espinosa et al., 2016; Nielsen et al., 2017; Rahmana et al., 2020; Ward and Shumway, 2004). The data also suggest that oysters were filtering the smaller particles and releasing larger particles (i.e. biodeposits).

Figure 8. Histogram of particle length at the top and bottom of the oyster bed using the quick sampling method.

A scatterplot of length versus width was developed to analyze the shape differences in the particles on the top and bottom of the oyster bed. An example plot of particle sizes for Tube 2 water sample from the quick sampling method is shown in Figure 9. The majority of the particles are smaller than 40 µm in length and are within a 2:1 ratio of length to width. The exception was a small number of bottom particles that were long and narrow (greater than 10:1), but did not
show up on the top. These could have been longer chain forming algae, diatoms, or detritus that are filtered by the oyster bed. The scatterplot also revealed larger particles on the top, which was consistent with the histograms above.

Figure 9. Scatter plot of measurements (width and length in µm) of particles from the top and bottom samples on Tube 2 using the quick sampling method.

3.6. Screening out Biodeposits

The biodeposits are primarily composed of feces and psuedofeces, which are fully digested or partially digested algal cells, discarded algal cells, and rejected particles that are encased in mucus. These cells were recognizable by the FlowCAM and were sorted from the other particles in the 12-200 µm size class.

The biodeposits increased in percentage from the bottom to top of the oyster bed for quick and slow samples. The slow sample had a mean increase of 6.0% (σ = 3.2%, n=5) and the quick sample had a mean increase of 11.0% (σ =2.4%, n=5). The differences in biodeposits were significant for both the quick (t=10.2128, p=0.00052, df=4) and slow (t=4.2396, p=0.0133, df=4) sampling methods. The quick sample had a slightly higher percentage increase in biodeposits. This was consistent with the histogram and size analysis, which once again suggested a difference in hydrodynamic properties of the biodeposits.
The concentrations of total particles were adjusted by removing the counts that the FlowCAM recognized as biodeposits. The difference calculations for sample methods are shown in Table 5 concentration and the percent of bottom concentration for each tube. The mean, \( \mu \), and standard deviation, \( \sigma \), is also shown for each method. The sampling methods were compared by conducting a two sample t-test on the bottom concentrations and determined that the methods did not have significant differences (\( t=-1.8472, p=0.1019, df=8 \)) and can be considered the same concentration. The percent difference (i.e. filtration) for the algal particles increased from the total particles due to removing biodeposits from the analysis.

Similar to the analysis of the TSS and total particles, a two-sided single sample t-test was conducted to determine if the concentration differences between the top and bottom were significant for each sampling method. The slow sampling method had a significant difference (\( t=6.8418, p=0.0024, df=4 \)) in top and bottom concentrations. The quick sampling method had no significant difference (\( t=-2.2850, p=0.0843, df=4 \)) in top and bottom concentrations of the oyster beds. Even though the quick sampling methods did not have significant differences from the top and bottom concentrations, the differences were more consistent than the total particles. This suggests that other particles were included in the sample that were not biodeposits (i.e. detritus) or were not recognized by the FlowCAM as biodeposits. Overall, the slow sample may serve as a better representation of the average filtration of algae by the oyster bed.
Table 5. FlowCAM algae concentration differences between the top and bottom of packed bed of oysters with corresponding percentages of bottom concentration for the slow and quick sampling methods for each tube.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Slow Sample</th>
<th>Quick Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td>(Count/ml)</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>15056</td>
<td>35.60%</td>
</tr>
<tr>
<td>2</td>
<td>13542</td>
<td>36.73%</td>
</tr>
<tr>
<td>3</td>
<td>9290</td>
<td>22.78%</td>
</tr>
<tr>
<td>4</td>
<td>22540</td>
<td>47.02%</td>
</tr>
<tr>
<td>5</td>
<td>13539</td>
<td>37.08%</td>
</tr>
<tr>
<td>μ</td>
<td>14794</td>
<td>35.84%</td>
</tr>
<tr>
<td>σ</td>
<td>4835</td>
<td>8.63%</td>
</tr>
</tbody>
</table>

4. Conclusions

The results of the experiment demonstrated different variabilities of filtration due to sampling techniques and analysis methods. Summary statistics of the concentration difference from bottom to top of oyster bed as a percent of the incoming bottom concentration (i.e. oyster bed filtration rates in percent) is shown in Table 6. The mean difference was higher for the slow sampling method versus the quick. The standard deviation was also higher for the quick sampling method. The TSS analysis had the highest variability and did not show a significant difference in the top and bottom concentrations for either sampling technique. The FlowCAM analysis had much better differentiation between top and bottom concentrations, although the quick sampling method did not show a significant difference.
Table 6. Summary statistics for the percent difference of concentrations at the top and bottom of the oyster bed.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>St Dev</th>
<th>t-value</th>
<th>p-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS_Slow</td>
<td>6.7</td>
<td>21.3</td>
<td>0.7008</td>
<td>0.5220</td>
<td>4</td>
</tr>
<tr>
<td>TSS_Quick</td>
<td>-13.1</td>
<td>29.1</td>
<td>-1.0056</td>
<td>0.3715</td>
<td>4</td>
</tr>
<tr>
<td>Total_Slow</td>
<td>30.4</td>
<td>9.5</td>
<td>7.1921</td>
<td>0.0020</td>
<td>4</td>
</tr>
<tr>
<td>Total_Quick</td>
<td>5.5</td>
<td>21.9</td>
<td>0.5568</td>
<td>0.6073</td>
<td>4</td>
</tr>
<tr>
<td>Algae_Slow</td>
<td>35.8</td>
<td>8.6</td>
<td>9.2834</td>
<td>0.0007</td>
<td>4</td>
</tr>
<tr>
<td>Algae_Quick</td>
<td>19.1</td>
<td>18.7</td>
<td>2.2814</td>
<td>0.0847</td>
<td>4</td>
</tr>
</tbody>
</table>

The knowledge of particle parameters was very informative in understanding the filtration dynamics within the oyster bed. The particle counts from the FlowCAM was more useful than bulk mass (i.e. TSS) for determining filtration rates. The TSS analysis did not account for the sizes of particles or the types of particles. This resulted in ignoring biodeposits and detritus, which likely contributed to the high variability in observed filtration through TSS analysis.

The FlowCAM allowed for counting particles and the observation of the particle size. The size shift from smaller particles on the bottom to larger particle on the top of the oyster bed suggested production of biodeposits was important to consider. Additionally, the larger shift in the quick sampling method suggested differences in the hydrodynamic properties of the biodeposits. The FlowCAM demonstrated a superior potential to measure oyster filtration by being able to differentiate between algal cells and biodeposits, which resulted in a more definite depiction of oyster filtration of algal particles.
An estimate of the filtration rate for an individual oyster per standardized 1 g dry weight tissue was calculated based upon the filtration percentages for each tube using the slow sampling method to compare with existing literature. The filtration rate, $FR_i$, was estimated using Equation 2 below:

$$FR_i = \frac{Q \times FR_{bulk}}{DTW \times \Phi \times A_b \times H_b \times 1000}$$

Where, $Q$ is the flow rate (L/hr); $FR_{bulk}$ is the filtration rate in percent; $DTW$ is the dry tissue weight (g); $\Phi$ is the number of oysters per liter; $A_b$ is the area of bed (cm$^2$); $H_b$ is the bed height (cm). The dry tissue weight (DTW), in grams, was calculated from the mean shell length, $L_{shell}$ (mm), using the equation developed by Ross and Lukenbach (2006) $DTW = 0.00008 \times L_{shell}^{2.175}$. The mean $FR_i$ was calculated as 3.44 L/hr/g ($\sigma = 0.80$ L/hr/g, n=4).

These rates were reasonable when compared to the literature. Grizzle et al. (2008) reported filtration rates of natural reefs in an estuary of 1.21 L/hr. When these values are converted to the standardized 1 g DWT, the filtration rate was 4.11 L/hr/g. Shumway (2003) reported filtration rates of 4.8 L/hr/g in 14-18 °C. Rissgard (1988) study reported 6.79 L/hr/g at optimum temperatures of 27 °C. When this value is corrected using Cerco and Noel (2005) temperature correction, the filtration rate becomes 3.26 L/hr/g at 20 °C. Li et al. (2012) measured fluorescence changes before and after FLUPSY system for 4 months and had a minimum filtration rate of 0.31 L/hr/g and a maximum filtration of 2.21 L/hr/g. The higher filtration rates observed in this study when compared to Li et al. (2012) were likely due to the experimental units having controlled flow, consistent sampling methods, and using particle counts (as opposed to fluorescence).
The results of this study will inform mechanistic models and provide guidance to oyster farmers. The magnitude and variability of the oyster filtration can be used in models such as the axial diffusion model for system optimization. The sedimentation rates measured would be useful in determining the cleaning schedule of culture units. These rates could be applied to guidance for farmers to determine how often oyster beds should be rinsed of biodeposits and other buildup.

Future work will be needed to further explore the dynamics of sedimentation and biodeposit production within the upweller systems. This study observed a mean of 96.6 mg/hr sedimentation occurring within the bed, or 6.29% of the bottom concentration loading. Progressive sedimentation was also observed through the increasing pressure differential over time, which indicates that accumulation was modifying the hydrodynamics within the bed. The composition of the sedimentation material was not differentiated but was likely largely due to biodeposits. Further investigation of these rates and composition is warranted spatially within the bed and temporally. Information from future experiments could be useful in the development of cleaning protocols implemented at the farm level.

Another topic of future work should focus on feeding patterns of oysters temporally and spatially within the upweller bed. During the study, it was observed that the oysters fed asynchronously within the bed, but could be explored more to determine timing and patterns within an upflow system. The feeding also changed the bed density, which could also affect the hydrodynamics (i.e. velocity experienced by the individual oysters and diffusion of particles). The velocity effects on filtration rates of oysters are still poorly understood and warrant further investigation within upweller systems (zu Emergassen et al., 2013; Campbell and Hall, 2019).
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CHAPTER 5: CONCLUSIONS AND FUTURE WORK

Hydrodynamics are Important in Oyster Aquaculture

Hydrodynamics are important in most aquaculture operations, but particularly important in oyster aquaculture. The flow of water and how it interacts with the oysters influences food supply and water quality. The growth and survival of oysters is largely dependent on the hydrodynamic environment in which they grow. Typically, higher currents are desirable because they increase delivery of food to the oysters, improve water quality, and disperse biodeposits. The hydrodynamic environment may also influence what types of predators are present at the aquaculture site (Dame, 2012; Goslin, 2015; Kennedy et al., 1996). On the other hand, extreme hydrodynamic energy could inhibit growth through physical damage. A greater understanding of how hydrodynamics affect oyster feeding activity and growth could have implications on culture techniques, oyster stocking densities, siting criteria, and management (Campbell and Hall, 2019).

Upweller culture systems are particularly influenced by hydrodynamics. Flow through these systems regulates food, oxygen, and water quality within the bed of oysters. Flow should correspond with stocking density and seston concentration for proper management (Bishop & Hooper, 2005; Manzi et al., 1986; Spencer, 1988). Higher flows increase oyster growth, but could be limiting due to feeding inhibition, damage, and required energy inputs (Appleyard & Dealeris, 2002; Pfeiffer & Rusch, 2001; Rodhouse & O’Kelly, 1981; Ver & Wang, 1995).

Guidance for upweller operational flow rates is highly variable in literature although upwellers have been used for the past 50 years (Campbell and Hall, 2019). There has been a lack of understanding in the dynamics occurring within these systems, which hinders the development of engineering design and optimization. There is a need to gain a deeper understanding and develop a framework for engineering design of these systems.
Mechanistic Model Application

A mechanistic model can establish a framework for the engineering design and optimization of upweller systems by identifying important parameters and their corresponding influence. A deeper understanding of the dynamics within the system can be gained by applying physics-based relationships utilized in engineering design. This work analyzed the hydrodynamics within an upweller system and applied them to the axial diffusion model. The dimensionless relationships for Reynolds and Peclet numbers were also established, which allows the hydrodynamics to scale to culture systems at the farm scale. The parameters established within the axial diffusion model can be used as a foundation for further understanding the biological and physical dynamics within upweller systems. For example, the filtration rate of the oyster bed can be investigated independently from diffusion. This greater understanding will allow for the optimization of these types of culture methods and could lead to innovations in their design.

The relationship between oyster size, shape, flow rate, and diffusion were explored in this work as parameters within the axial diffusion model. The pressure loss through the bed was also explored to validate the application of packed bed theory. The experiments validated that the engineering theory established for packed bed reactors could be applied to these upweller systems with irregular and heterogeneous juvenile oysters. The parameter analysis revealed comparable estimates of size and diffusion coefficients to measurements and literature values.

The stochastic nature of oyster parameters, such as size and shape, was accounted for by utilizing Bayesian inference in combination with the mechanistic model. The addition of Bayesian inference allows for a robust analysis of all relevant parameters applied within the mechanistic models. Hamiltonian Monte Carlo sampling provided reasonable estimates for
particle equivalent spherical diameter, void ratio, tortuosity, and axial diffusion coefficients. This suggests that this method is a viable approach to modeling bulk oyster bed parameters with stochastic characteristics, such as shape, size, and filtration in the future. The estimated equivalent spherical diameter yielded encouraging results when applied to the Peclet based Bayesian model for diffusion. The Monte-Carlo simulations of axial diffusion coefficients using the posterior parameter distributions were reasonable when compared to the observed data. The consistency between the estimated parameters from the two models and physical measurements demonstrates the validity of this approach.

Bayesian inference also allows for the improvement of parameter estimates and associated distributions over time. As more data is gathered from future experiments and field data, the model can be improved. At a pragmatic level, the ability to correlate pressure with diffusion coefficients could also yield useful outcomes to farmers that could easily measure pressure drop on each side of culture units. For example, pressure measurements could allow the farmer to optimize flow rates to produce sufficient food and water quality for oysters throughout the culture unit.

**Dynamics of Filtration within an Upweller System**

Filtration by the oysters is a critical biological parameter that warrants further investigation within upweller systems. Studies that have observed filtration rates of upweller systems have shown high temporal variability (Manzi, 1986; Li et al., 2012). They observed high temporal variability in filtration by the upweller systems that were correlated with temperature and dissolved oxygen changes in daily and seasonal conditions. These studies note the temporal and environmental changes that affect filtration rate of oysters within an upweller system, but do
not adequately describe the inherent variability in filtration oysters exhibit within the same set of environmental parameters.

Experiments were conducted to investigate the variability of filtration in an oyster upweller system. The results of the experiment demonstrated different variabilities of filtration due to sampling techniques and analysis methods. Additionally, the knowledge of particle parameters was very informative in understanding the filtration dynamics within the oyster bed. A portable FlowCAM (Flow Cytometry And iMaging) system equipped for auto-imaging, and detection of individual particles passing through a flow cell was used to measure the concentration before and after the bed of oysters. This method revealed a shift in particle size from the incoming water to the water passing through the oyster bed. This method was able to measure a 35.5% filtration rate of algae by the oysters with a standard deviation of 8.6%. The filtration rate of oysters was compared to field studies and was found to be similar.

**Future work**

While this work established a framework to understand the dynamics of flow and filtration to optimize the upweller systems, it also revealed areas where further work should be conducted. These areas of future work are related to the biological (i.e. filtration, biodeposit production) and physical (i.e. oyster shape, oyster size, and particle settling) parameters within the axial diffusion model. By application of Bayesian inference, the future analysis of the parameter distribution is also important.

The biological parameters, such as filtration, in the context of upweller systems should be further explored. Zu Emergassen et al. (2013) and Campbell and Hall (2019) highlighted the lack of understanding in the hydrodynamic constraints of oyster feeding. The limitation of oyster feeding due to high and low velocity rates requires more exploration within a packed bed setting.
Rybovich et al. (2014) demonstrated the combination of salinity and oxygen on mortality. These same combinations of limitation will need to be explored alongside the velocity effects. The interrelated dynamics of oxygen, food supply, and other water quality parameters to hydrodynamics must be understood in the context of the axial diffusion model.

The physical parameters must also be explored further as they apply to upweller systems. Specifically, the diffusion coefficient variability on live oysters during feeding of various sizes. As sedimentation occurs, the hydrodynamics within the system will also change. The sedimentation rate, including biodeposit production, and its correlation to other parameters needs to be understood more deeply.

Flow regimes and packing densities influence the provision of dissolved oxygen and removal of wastes including carbon dioxide, solids and nitrogenous wastes within oyster culture systems. The optimization of design and management practices for these systems could be enhanced through knowledge gained from experiments exploring optimal flow with relation to supply of oxygen and removal of waste materials.

Upweller systems in particular should be studied further to understand the hydrodynamics through a bed of oysters. This has implications on stocking densities, flow, size sorting, and other management strategies. Further investigation into the application of fixed bed reactor theory to this culture method could be applied and could generate further innovations in oyster culture and related fields.

Another topic of future work should focus the feeding patterns of oysters temporally and spatially within the upweller bed. During the study, it was observed that the oysters fed asynchronously within the bed. The feeding also changed the bed density, which could also affect the hydrodynamics (i.e. velocity experienced by the individual oysters and diffusion of
particles). The velocity effects on filtration rates of oysters are still poorly understood and warrant further investigation within upweller systems (zu Emergassen et al., 2013; Campbell and Hall, 2019).

Future studies could have broader impacts by exploring interactions between physics and biology that have applications in many marine and aquaculture fields. For example, these interactions have applications to alternate culture systems such as artificial reefs and living shorelines. These systems are influenced by their hydrodynamic environment and the desired performance (i.e. ecological restoration or shoreline protection) is directly linked to the biophysical interaction. For example, the growth of oysters on a living shoreline protection structure are influenced by the waves and currents impacting the structure surface and conversely, the biogenic structure created by oysters dissipate the waves and currents.

The application of the axial diffusion model to predict available food concentrations at variable velocities could also be applied to other culture methods and natural reefs. Additional parameters would need to be established to relate this theory to those situations, but much of the existing approach could be applied. The physical manifestation of diffusion within those systems could be explored to apply the same model to bag culture and even reef restoration within an estuary.
REFERENCES


Millman, J. K. and Aivazis, M. Python for Scientists and Engineers, Computing in Science & Engineering, 13, 9-12, DOI:10.1109/MCSE.2011.36


10-20, DOI:10.1109/MCSE.2007.58
with Lowered Salinities Differentially Impact Oyster Size Class Growth and Mortality.


Appendix A- Experimental Units

Design plans for tube experimental units used in the experiments.
PROJECT
Tube Construction

TITLE
Flang Plate OR

NC STATE UNIVERSITY
Bio&Ag ENGINEERING

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SCALE: 1:1
WEIGHT
SHEET 4/6
Appendix B- Conductivity Probe

Conductivity probe wiring schematic and script for Arduino.

Wiring Diagram

Arduino Script
int readPin = 0;
int conductivity0;
int conductivity1;
int conductivity2;
unsigned long time;
void setup() {
  Serial.begin(9600);
  //pinMode(3,OUTPUT);
}

void loop() {
  conductivity0 = analogRead(A0);
  float voltage0 = conductivity0*(5.0/1023.0);
  conductivity1 = analogRead(A1);
  float voltage1 = conductivity1*(5.0/1023.0);
  conductivity2 = analogRead(A2);
float voltage2 = conductivity2*(5.0/1023.0);
time=millis();
    Serial.print(time);// Send time
    Serial.print(",");
    //Serial.print("Sensor0 ");
    Serial.print(voltage0);
    Serial.print(",");
    //Serial.print("Sensor1 ");
    Serial.print(voltage1);
    Serial.print(",");
    //Serial.print("Sensor2 ");
    Serial.println(voltage2);
}
Appendix C- Plume Impulse Python Code

#Developed by Matt Campbell 1/29/20
### for re-use with appropriate citation given

# Import relevant libraries
import matplotlib
import matplotlib.pyplot as plt
import pandas as pd
import numpy as np
import pystan
import scipy
from sklearn import metrics
from scipy.interpolate import interp1d

----
result=[]
for f in range(1,27):
    filename = 'Run_{}.txt'.format(f)
    data = pd.read_csv(filename, names=['time (ms)', 'S1', 'S2', 'S3'])
    ## truncate to account for sensor reaching equilibrium
    data=data.loc[data['time (ms)']>=30000]
    ## Interpolate data to create regular interval and smooth
    I=500 #regular interval
    S2int=interp1d(data['time (ms)'],data['S2'], 'linear')
    S3int=interp1d(data['time (ms)'],data['S3'], 'linear')
    tt=np.arange(data['time (ms)'].min(),data['time (ms)'].max(),I) ## create array from min to max every I ms
    e2=S2int(tt)
    e3=S3int(tt)
    ## create new dataframe
    df=pd.DataFrame({'time': tt, 'S2': e2, 'S3': e3})
    ##Find leading edge of pulse
    peak2=df['S2'].max()-df['S2'].min()
    peak3=df['S3'].max()-df['S3'].min()
    xmin_2=df.loc[df['S2']>=peak2*0.2+df['S2'].min()]# set threshold for change in voltage
    xxm_2=xmin_2['time'].min() #move back 5 seconds from threshold
    df2=df.loc[df['time']>=xxm_2-5000] #move back 5 seconds from threshold
    #correct for drift in sensor
    nstart=df2.index[0]
    nend=df2.index[-1]
    v11=df2.loc[nstart,'S2']
    t11=df2.loc[nstart,'time']
    v12=df2.loc[nend,'S2']
t12 = df2.loc[nend, 'time']
rate1 = (v11 - v12) / (t12 - t11)
S2corr = df2['S2'] + rate1 * df2['time']
v21 = df2.loc[nstart, 'S3']
t21 = df2.loc[nstart, 'time']
v22 = df2.loc[nend, 'S3']
t22 = df2.loc[nend, 'time']
rate2 = (v21 - v22) / (t22 - t21)
S3corr = df2['S3'] + rate2 * df2['time']
df3 = pd.DataFrame({'time': df2['time'], 'S2': S2corr, 'S3': S3corr})

## Remove tails as function of peaks
peak2 = df3['S2'].max() - df3['S2'].min()
peak3 = df3['S3'].max() - df3['S3'].min()
xx = df3[df3['S2'] > df3.loc[df3.index[-1], 'S2'] + peak2 * 0.05]
xxx = df3[df3['S3'] > df3.loc[df3.index[-1], 'S3'] + peak3 * 0.05]

## Reduce curve to near zero
s2red = df4['S2'] - df4['S2'].min()
s3red = df4['S3'] - df4['S3'].min()
tt3 = np.arange(0, len(s2red) * 1, 1)
df4 = pd.DataFrame({'time': tt3, 'S2': s2red, 'S3': s3red})
df4 = df4.fillna(0)

## Calculate area under the curve
A2 = metrics.auc(df4['time'], df4['S2'])
A3 = metrics.auc(df4['time'], df4['S3'])
ReS2 = df4['S2'] / A2
ReS3 = df4['S3'] / A3

## Calculate 1st moment (mean) using trapezoidal rule

t2_r = []
t3_r = []
n = np.array(df4['time']).index  # find indexes

for i in range(n[0], n[-2]):
    t2_r.append(df4.loc[i, 'time'] * 0.5 * (ReS2[i + 1] + ReS2[i]) * t1)

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t3_r.append(df4.loc[i,'time']*0.5*(ReS3[i+1]+ReS3[i])*I)
t2_bar=sum(t2_r)
t3_bar=sum(t3_r)
t_bar=t3_bar-t2_bar

## calculate 2nd moment
mu2_r=[]
mu3_r=[]
n=np.array(df4['time'].index)#find indexes
for i in range(n[0],n[-2]):
    mu2_r.append(np.power(df4.loc[i,'time'],2)*0.5*(ReS2[i+1]+ReS2[i])*I)
    mu3_r.append(np.power(df4.loc[i,'time'],2)*0.5*(ReS3[i+1]+ReS3[i])*I)
mu_2=sum(mu2_r)
mu_3=sum(mu3_r)

## Calculate second moment about the mean, variance
mu2_rr=[]
mu3_rr=[]
for i in range(n[0],n[-2]):#find indexes for i in range(n[0],n[-2]):
n=np.array(df4['time'].index)
    mu2_rr.append(np.power(df4.loc[i,'time']-t2_bar,2)*0.5*(ReS2[i+1]+ReS2[i])*I)
    mu3_rr.append(np.power(df4.loc[i,'time']-t3_bar,2)*0.5*(ReS3[i+1]+ReS3[i])*I)
var2_t=sum(mu2_rr)
var3_t=sum(mu3_rr)

## Calculate the dimensionless variance
var=(var3_t-var2_t)/(np.power(t3_bar,2)-np.power(t2_bar,2))

Pe=1
while True:
    # formula for var and Pe
    var2 = 2/Pe-2*np.power(Pe,2)*(1-np.exp(-Pe))
    Pe += 0.001
    if var2 <= var:
        break
    # print('var', Pe)
    # print(var, Pe)
    print(f, var,t_bar)
    result.append([var,Pe,t_bar])

from pandas import DataFrame
dm = pd.DataFrame(result, columns=['Var', 'Pe', 't_bar'])
export_Pyresult = dm.to_excel ('Toexcelfile.xlsx', index = None, header=True) #Don't forget to add '.xlsx' at the end of the path

for f in range(1, 27):
    filename = 'Run_{:02d}.txt'.format(f)
    volt = pd.read_csv(filename, names=['time (ms)', 'S1', 'S2', 'S3'])
    plt.figure(2)
    plt.plot(volt['time (ms)'], volt['S2'])
    plt.plot(volt['time (ms)'], volt['S3'])
    print(filename)
    plt.pause(0.05)
    plt.show()
    
for f in [10]:
    filename = 'Run_{:02d}.txt'.format(f)
    data = pd.read_csv(filename, names=['time (ms)', 'S1', 'S2', 'S3'])
    plt.figure(2)
    plt.plot(data['time (ms)'], data['S2'])
    plt.plot(data['time (ms)'], data['S3'])
    print(filename)
    plt.pause(0.05)
    plt.show()

    S2int = interp1d(data['time (ms)'], data['S2'], 'linear')
    S3int = interp1d(data['time (ms)'], data['S3'], 'linear')
    tt = np.arange(data['time (ms)'].min(), data['time (ms)'].max(), 1)  
    df = pd.DataFrame({'time': tt, 'S2': S2int(tt), 'S3': S3int(tt)})

    peak2 = df['S2'].max() - df['S2'].min()
    peak3 = df['S3'].max() - df['S3'].min()
    xmin_2 = df.loc[df['S2'] >= peak2 * 0.2 + df['S2'].min()]  
    xxm_2 = xmin_2['time'].min()
    df2 = df.loc[df['time'] >= xxm_2 - 5000]  # move back 5 seconds from threshold

    nstart = df2.index[0]
    nend = df2.index[-1]

    # correct for drift in sensor
v11=df2.loc[nstart,'S2']
t11=df2.loc[nstart,'time']
v12=df2.loc[nend,'S2']
t12=df2.loc[nend,'time']
rate1=(v11-v12)/(t12-t11)
S2corr=df2['S2'] + rate1*df2['time']

v21=df2.loc[nstart,'S3']
t21=df2.loc[nstart,'time']
v22=df2.loc[nend,'S3']
t22=df2.loc[nend,'time']
rate2=(v21-v22)/(t22-t21)
S3corr=df2['S3'] + rate2*df2['time']

df3=pd.DataFrame({'time': df2['time'], 'S2': S2corr, 'S3': S3corr})

## Remove tails as function of peaks

peak2=df3['S2'].max()-df3['S2'].min()
peak3=df3['S3'].max()-df3['S3'].min()

xx=df3[df3['S2'] > df3.loc[df3.index[-1],'S2'] + peak2*0.05]
xxx=df3[df3['S3'] > df3.loc[df3.index[-1],'S3'] + peak3*0.05]

xxy=xx.drop(columns=['S3'])
xxz=xxx.drop(columns=['S2'])

df4=pd.merge(xxy, xxz, how='outer', on='time')

## Reduce curve to near zero

s2red=df4['S2']-df4['S2'].min()
s3red=df4['S3']-df4['S3'].min()

tt3=np.arange(0,len(s2red)*I,I)
df4=pd.DataFrame({'time': tt3, 'S2': s2red, 'S3': s3red})
df4=df4.fillna(0)

## calculate area under the curve

A2=metrics.auc(df4['time'], df4['S2'])
A3=metrics.auc(df4['time'], df4['S3'])

ReS2=df4['S2']/A2
ReS3=df4['S3']/A3

## Calculate 1st moment (mean) using trapezoidal rule

t2_r=[]
t3_r=[]
n=np.array(df4['time'].index)#find indexes
for i in range(n[0],n[-2]):
    t2_r.append(df4.loc[i,'time']*0.5*(ReS2[i+1]+ReS2[i])*I)
    t3_r.append(df4.loc[i,'time']*0.5*(ReS3[i+1]+ReS3[i])*I)


## calculate 2nd moment
mu2_r=[]
mu3_r=[]

n=np.array(df4['time'].index)#find indexes

for i in range(n[0],n[-2]):
    mu2_r.append(np.power(df4.loc[i,'time'],2)*0.5*(ReS2[i+1]+ReS2[i])*I)
    mu3_r.append(np.power(df4.loc[i,'time'],2)*0.5*(ReS3[i+1]+ReS3[i])*I)

mu_2=sum(mu2_r)
mu_3=sum(mu3_r)

## Calculate second moment about the mean, variance
mu2_rr=[]
mu3_rr=[]

for i in range(n[0],n[-2]):#find indexes for i in range(n[0],n[-2]):
    n=np.array(df4['time'].index)
    mu2_rr.append(np.power(df4.loc[i,'time']-t2_bar,2)*0.5*(ReS2[i+1]+ReS2[i])*I)
    mu3_rr.append(np.power(df4.loc[i,'time']-t3_bar,2)*0.5*(ReS3[i+1]+ReS3[i])*I)

var2_t=sum(mu2_rr)
var3_t=sum(mu3_rr)

## Calculate the dimensionless variance

var=(var3_t-var2_t)/(np.power(t3_bar,2)-np.power(t2_bar,2))

Pe=1
while True:
    # formula for var and Pe
    var2 = 2/Pe-2/np.power(Pe,2)*(1-np.exp(-Pe))
    Pe += 0.001
    if var2 <= var:
        break

    # print('var', Pe)
    # print(var, Pe)
    print(f, var,t_bar)
result.append([var, Pe, t_bar])

volt = df4
plt.figure(2)
plt.plot(volt['time'], ReS2)
plt.plot(volt['time'], ReS3)
---
## Appendix D- Bayesian Model Python Code

### Developed by Matt Campbell 1/29/20
### for re-use with appropriate citation given

### Import necessary packages
import pystan
import matplotlib
import matplotlib.pyplot as plt
import scipy
import pandas as pd
import numpy as np
import arviz as az
from sklearn.metrics import r2_score
from sklearn.metrics import mean_squared_error

### https://mc-stan.org/users/documentation/case-studies/pystan_workflow.html

# Ingest raw data from pressure and impulse analysis (Impulse.ipynb)
filename = 'Hydro_Results.csv'
data = pd.read_csv(filename)
data.head()

# Create arrays for velocity and pressure for glass spheres
vs_sp=data.loc[(data['Media'] == 'Glass sphere')][['Vs']]  
vs_oy=data.loc[(data['Media'] == 'Oyster')][['Vs']]  

### convert to m/s
vs_sp=vs_sp/100  
vs_oy=vs_oy/100

pr_sp=data.loc[(data['Media'] == 'Glass sphere')][['Pressure']]  
pr_oy=data.loc[(data['Media'] == 'Oyster')][['Pressure']]  

### convert pressure from mm h2o to Pa
rho=997.5
p_sp=pr_sp*rho*9.81/1000  
p_oy=pr_oy*rho*9.81/1000

plt.scatter(vs_sp,p_sp, color='red',marker='o',label='Glass Spheres')  
plt.scatter(vs_oy,p_oy,color='blue',marker='>',label='Oysters')  
plt.xlabel('superficial velocity (m/s)', fontsize=12)  
plt.ylabel('pressure (Pa)', fontsize=12)  
plt.legend(loc='upper left')  

fname='pressure_data.png'
**1) Glass sphere pressure analysis**

# Create arrays for velocity and pressure for glass spheres
vs=data.loc[(data['Media'] == 'Glass sphere')]['Vs']
## convert to m/s
vs=vs/100
pr=data.loc[(data['Media'] == 'Glass sphere')]['Pressure']
## convert pressure from mm h2o to Pa
rho=997.5
p=pr*rho*9.81/1000
plt.scatter(vs,p)
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('pressure (Pa)', fontsize=12)

###*_1a) Stan code for glass spheres relating Ergun’s equation_*

```stan
erguncode = """
  data {
    int<lower=0> N;
    vector[N] vs; //superficial velocity
    vector[N] p; //pressure differential across bed
    real rho;
    real mu;
    real L;
  }
  parameters {
    real eps;
    real<lower=0> sigma;
    real<lower=0> dp;
    real<lower=0> C1;
    real<lower=0> C2;
  }
  model {
    C1~normal(150,20);
  }
"""
```
C2~normal(1.75,1);
eps~normal(0.39,0.03);
\(dp\)~normal(15.3,0.5); \(//\) convert to mm (multiplied by 1000) to get more resolution
sigma~uniform(0,1000);
for (i in 1:N) {
p[i]~ normal(L*(C1*(mu*vs[i]*(1-\(\epsilon\))^2)/((dp/1000)^2*\(\epsilon\)^3)+C2*(rho*vs[i]^2*(1-\(\epsilon\)))/((dp/1000)*\(\epsilon\)^3)), sigma);
}

```

dat={'N': len(vs),
     'mu': 0.000919, # dynamic viscosity of water
     'rho': 997.5, # density of water
     'L': 0.33, # length of bed
     'vs': vs,
     'p': p}

sm = pystan.StanModel(model_code=erguncode)
fit = sm.sampling(data=dat, seed=194838, iter=10000, chains=4, control=dict(adapt_delta=0.9,
max_treedepth=12))

#summary of estimated parameters with credible intervals
print(fit)

#convert results into DataFrame
result_fit=fit.summary()
df_result=pd.DataFrame(result_fit['summary'],index=result_fit['summary_rownames'],
columns=result_fit['summary_colnames'])
df_result

#plot estimated pressure with parameters from Bayesian Inference
N= len(vs)
eps= df_result['mean'] ['\(\epsilon\)']
mu=0.000919 # dynamic viscosity of water
rho=997.5 # density of water
L=0.33 # length of bed
C1= df_result['mean']['C1']
C2= df_result['mean']['C2']
dp= df_result['mean']['\(dp\)']
pest_sp=[]
for i in range(N):
    pest_sp.append(L*(C1*(mu*vs[i]*np.power((1-eps),2))/np.power((dp/1000),2)*np.power(eps,3))+C2*(rho*np.power(vs[i],2)*(1-eps))/((dp/1000)*np.power(eps,3))))
# prest=np.array(pest)*1000/(rho*9.81) #convert pressure back to mm H2O
plt.plot(vs*100,pest_sp, label='est')
plt.scatter(vs*100,p, label='data')
plt.legend(loc='upper left')
plt.xlabel('superficial velocity (cm/s)', fontsize=12)
plt.ylabel('pressure (Pa)', fontsize=12)
fname='spheremodel.png'
plt.savefig(fname, dpi=300)

#Calculate RMSE and r2
print('RSME=',np.sqrt(mean_squared_error(p, pest_sp)))
print('rsquare=',r2_score(p, pest_sp))

#Plot posterior plots from MCMC sampling
azdata = az.from_pystan(posterior=fit,
                        observed_data=['p'],
                      )
zplot_pair(azdata, divergences=True);
plt.savefig('sphereAz_pair.png', dpi=300)

#Plot posterior distributions for the estimated parameters
az.plot_trace(azdata);
plt.savefig('sphereAz_trace.png', dpi=300)

**2) Oyster pressure analysis**
Use same procedure used for the glass spheres

# Create arrays for velocity and pressure for oysters
vs=data.loc[(data['Media'] == 'Oyster')]['Vs']
## convert to m/s
vs=vs/100
pr=data.loc[(data['Media'] == 'Oyster')]['Pressure']
## convert pressure from mm h2o to Pa
rho=997.5
p=pr*rho*9.81/1000

#remove last point and re-run analysis
# vs=vs.loc[12:22]
# p=p.loc[12:22]
# pr=pr.loc[12:22]

###*2a) Stan code for oysters relating Ergun’s equation*
erguncode = """'
data {
  int<lower=0> N;
  vector[N] vs; //superficial velocity
  vector[N] p;  //pressure differential across bed
  real rho;
  real mu;
  real L;
}

parameters {
  real<lower=0> eps;
  real<lower=0> sigma;
  real<lower=0> dp;
  real<lower=0> C1;
  real<lower=0> C2;
}

model {
  C1~normal(150,20);
  C2~normal(1.75,1);
  eps~normal(0.64,0.018); //void ratio for oysters
  dp~normal(3.95,1.25); // convert to mm (multiplied by 1000) to get more resolution, used
distribution of the smallest diameter
  for (i in 1:N) {
    p[i]~ normal(L*(C1*(mu*vs[i]*(1-eps)^2)/((dp/1000)^2*eps^3)+C2*(rho*vs[i]^2*(1-eps))/((dp/1000)*eps^3)), sigma);
  }
}"

dat={'N': len(vs),
     'mu': 0.000919, # dynamic viscosity of water
     'rho': 997.5, # density of water
     'L': 0.33, #length of bed
     'vs': vs,
     'p': p}

sm = pystan.StanModel(model_code=erguncode)
fit = sm.sampling(data=dat, iter=10000,seed=194838,chains=4, control=dict(adapt_delta=0.99, max_treedepth=15))

#summary of estimated parameters with credible intervals
print(fit)

#convert results into DataFrame
result_fit=fit.summary()
df_result=pd.DataFrame(result_fit['summary'], index=result_fit['summary_rownames'],
columns=result_fit['summary_colnames'])
df_result

#plot estimated pressure with parameters from Bayesian Inference
N= len(vs)
nstart=vs.index[0]
nend=vs.index[-1]
eps=df_result['mean']['eps']
mu=0.000919 # dynamic viscosity of water
rho=997.5 # density of water
L=0.33 # length of bed
C1=df_result['mean']['C1']
C2=df_result['mean']['C2']
dp=df_result['mean']['dp']
pest_oy=[]
for i in range(nstart,nend+1):
    pest_oy.append(L*(C1*(mu*vs[i]*np.power((1-eps),2)/(np.power((dp/1000),2)*np.power(eps,3)))+C2*(rho*np.power(vs[i],2)*(1-eps))/((dp/1000)*np.power(eps,3))))
# prest=np.array(pest)*1000/(rho*9.81)

plt.plot(vs*100,pest_oy, label='est')
plt.scatter(vs*100,p, label='data')
plt.legend(loc='upper left')
plt.xlabel('superficial velocity (cm/s)', fontsize=12)
plt.ylabel('pressure (Pa)', fontsize=12)
fname='oystermodel.png'
plt.savefig(fname, dpi=300)

#Calculate RMSE and r2
print('RSME=',np.sqrt(mean_squared_error(p, pest_oy)))
print('rsquare=',r2_score(p, pest_oy))

#Plot posterior plots from MCMC sampling
azdata = az.from_pystan(posterior=fit, observed_data=['p'],
                          )
azdata
az.plot_pair(azdata, divergences=True);
# fname='oyster_pair.png'
# plt.savefig(fname, dpi=300)
# Plot posterior distributions for the estimated parameters
az.plot_trace(azdata);
# fname='oyster_trace.png'
# plt.savefig(fname, dpi=300)

# Create arrays for velocity and pressure for glass spheres
vs_sp=data.loc[(data['Media'] == 'Glass sphere')]['Vs']
vs_oy=data.loc[(data['Media'] == 'Oyster')]['Vs']
## convert to m/s
vs_sp=vs_sp/100
vs_oy=vs_oy/100
pr_sp=data.loc[(data['Media'] == 'Glass sphere')]['Pressure']
pr_oy=data.loc[(data['Media'] == 'Oyster')]['Pressure']
## convert pressure from mm h2o to Pa
rho=997.5
p_sp=pr_sp*rho*9.81/1000
p_oy=pr_oy*rho*9.81/1000
plt.scatter(vs_sp,p_sp, color='red',marker='o',label='Glass Spheres')
plt.plot(vs,pest_sp, color='red')
plt.scatter(vs_oy,p_oy,color='blue',marker='>',label='Oysters')
plt.plot(vs,pest_oy,color='blue')
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('pressure (Pa)', fontsize=12)
plt.legend(loc='upper left')
fname='overall_pressure_data.png'
plt.savefig(fname, dpi=300)

# plot all diffusion data for comparison
data_r=data
L=0.33 #length of bed in m
data_r['Pe'].loc[18]=46.3 #from analysis in Impulse.ipynb
data_r['u_bar']=L/(data_r['t_bar']/1000)
data_r['D_L']=10000*data_r['u_bar']*L/data_r['Pe']
data_g=data_r.loc[data['Media']== 'Glass sphere']
data_o=data_r.loc[data['Media']== 'Oyster']
plt.scatter(data_g['Vs']/100, data_g['D_L'],color='red',label='Glass Sphere')
plt.scatter(data_o['Vs']/100, data_o['D_L'],color='blue',label='Oyster',marker='>')
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('Diffusion Coefficient $(10^4$ x $m^2/s)$', fontsize=12)
plt.legend(loc='upper left')
fname='Diffusion raw.png'
plt.savefig(fname, dpi=300)
# Create arrays for spheres

datasp=data.loc[data['Media'] == 'Glass sphere']
data3=datasp

L=0.33 # length of bed in m
vs=data3['Vs']/100 # velocity in m/s
PeL=data3['Pe']
u_bar=L/(data3['t_bar']/1000)

# calculate Diffusion coefficient for the bed

D_L=10000*u_bar*L/PeL

# Develop initial guess of line fit

dp=15.2 # from Ergun analysis
tau=1.41 # used value for spheres
Pel_inf=3 # initial guess
eps=0.4 # from Ergun analysis
Dm=0.05 # initial guess
DL_est=Dm/tau+10000*((vs/eps)*(dp/1000)/Pel_inf)

plt.scatter(vs, D_L)
# plt.plot(vs,DL_est)
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('Diffusion Coefficient (10^-4 x m^2/s)', fontsize=12)

## *3a) Stan code for glass spheres relating using Delgado (2006) relationship (DL=Dm'+ud/Pel_inf) *

erguncode = ""

data {  
  int<lower=0> N;
  vector[N] vs; // superficial velocity
  vector<lower=0>[N] DL; // axial diffusion coefficient
}

parameters {  
  real<lower=0> sigma;
  real<lower=0> dp;
  real<lower=0> tau;
  real<lower=0> Pel_inf;
  real<lower=0> eps;
  real<lower=0> Dm;
}
model {
    dp ~ normal(15.28, 0.5);
    tau ~ normal(1.41, 0.5); // used tortuosity value for spheres
    Pel_inf ~ normal(2, 3);
    eps ~ normal(0.39, 0.03);
    DL ~ normal(Dm/tau + 10000*(vs/eps*(dp/1000)/Pel_inf), sigma);
}

```python
#summary of estimated parameters with credible intervals
print(fit)
- #Plot posterior plots from MCMC sampling
azdata = az.from_pystan(posterior=fit)
azdata
az.plot_pair(azdata, divergences=True);
fname='diffusion_pair.png'
plt.savefig(fname, dpi=300)

#Plot posterior distributions for the estimated parameters
az.plot_trace(azdata);
fname='diffusion_trace.png'
plt.savefig(fname, dpi=300)
```

### *3b) Plot results of Stan estimates*

#extract the samples
stan_results = pd.DataFrame(fit.extract())

# produce the prediction for each sample that was drawn
# print credible intervals https://aidanrussell.com/2019/01/14/pystan-tutorial-1/
pred_df_stan = stan_results.copy()
summary_stan = pd.DataFrame(columns=['y_025', 'y_500', 'y_975'], index=vs)
for x in vs:
    pred_df_stan['y'] = x
# DL~normal(Dm/\tau+10000*(vs/\varepsilon(Dp/1000)/\text{d}_\text{L}),\sigma);

pred_df_stan["y_{x}"] = stan_results["Dm"]/stan_results["\tau"] + 10000*
pred_df_stan["x"] = pred_df_stan["y_{x} "]

summary_stan.loc[x, f"y_{025}"] = pred_df_stan[f"y_{x} "].quantile(q=0.025)
summary_stan.loc[x, f"y_{500}"] = pred_df_stan[f"y_{x} "].quantile(q=0.5)
summary_stan.loc[x, f"y_{975}"] = pred_df_stan[f"y_{x} "].quantile(q=0.975)

# # produce a chart in the style of the previous OLS confidence interval chart

summary_stan["ci_lower_diff"] = summary_stan["y_{025}"] - summary_stan["y_{500}"]
summary_stan["ci_upper_diff"] = summary_stan["y_{500}"] - summary_stan["y_{975}"]

fig=plt.figure()
plt.errorbar(
    summary_stan.index,
    summary_stan["y_{500}"],
yerr=summary_stan[ ["ci_lower_diff", "ci_upper_diff"] ].T.values,
    fmt="-",
capsize=4,
)
plt.scatter(vs, D_L, c="r", zorder=2)
plt.title("Stan Fit Including 95% Credible Interval")
# plt.ylim([-2.5, 12.5])
plt.ylabel("Diffusion Coefficient $(10^4 \times \text{m}^2/\text{s})$")
# plt.xlim([-0.5, 5.5])
plt.xlabel("Superficial Velocity (m/s)")
plt.show()
fname='sphere_95.png'
fig.savefig(fname, dpi=300)

summary_stan_sp=summary_stan

#convert results into DataFrame
result_fit=fit.summary()
df_result=pd.DataFrame(result_fit['summary'],index=result_fit['summary_rownames'],
columns=result_fit['summary_colnames'])
df_result

##*_3c) Use estimates to define relationship between \text{P}_\text{e}\text{P}_\text{e} and \text{Re}_*_*

mu=0.000919 # dynamic viscosity of water (kg*s/(m/s2))
rho=997.5 # density of water (kg/m3)
Pep_est=[]
Pep=[]
for x in vs:
    DLe=df_result['mean']['Dm']/df_result['mean']['tau']+10000*(x/df_result['mean']['eps'])*(df_result['mean']['dp']/1000)/df_result['mean']['Pel_inf']
    Pep_est.append(x/df_result['mean']['eps']*(df_result['mean']['dp']/1000)/(DLe/10000))
Pep=vs/df_result['mean']['eps']*(df_result['mean']['dp']/1000)/(D_L/10000)
Re=(vs)*rho*(df_result['mean']['dp']/1000)/(mu)
plt.xlabel('Particle Reynolds Number', fontsize=12)
plt.ylabel('Peclet Number Based on Particle Diameter', fontsize=12)
plt.scatter(Re,Pep)
plt.plot(Re,Pep_est)
plt.savefig(fname, dpi=300)

##*_3d) Generate Monte Carlo simulation from original Bayesian Model_*

erguncode = ""

data {
    int<lower=0> N;
    vector[N] vs; //superficial velocity
    vector<lower=0>[N] DL ; //axial diffusion coefficient
    int<lower=0> N_new;
    vector[N_new] vs_new;
}

parameters {
    real<lower=0> sigma;
    real<lower=0> dp;
    real<lower=0> tau;
    real<lower=0> Pel_inf;
    real<lower=0> eps;
    real<lower=0> Dm;
}

model {
    dp~normal(15.28,0.5);
    tau~normal(1.41,0.5); // used tortuosity value for spheres
    Pel_inf~normal(2,3);
eps~normal(0.39,0.05);
DL~normal(Dm/tau+10000*(vs/eps*(dp/1000)/Pel_inf),sigma);
}
generated quantities {
    vector[N_new] DL_new;
    for (n in 1:N_new)
        DL_new[n] = normal_rng(Dm/tau+10000*(vs_new[n]/eps*(dp/1000)/Pel_inf),sigma);
}

dat={'N': len(vs),
     'vs': vs,
     'DL': D_L,
     'N_new': len(vs_new),
     'vs_new': vs_new}
sm = pystan.StanModel(model_code=erguncode)
fitn = sm.sampling(data=dat, seed=194838, iter=5000, chains=4)
samples=fitn.extract()
sigma=samples['sigma']
dp=samples['dp']
tau=samples['tau']
Pep=samples['Pel_inf']
eps=samples['eps']
beta=samples['Dm']
DL_new=samples['DL_new']
DL_new2=np.where(DL_new<=0,np.nan, DL_new) #filter out negative numbers

##Create plot of Monte Carlo simulations for Diffusion and Pep_

####plot results for DL vs velocity
plt.figure(figsize=[8,6])
# for f in range(1,50):
#    plt.scatter(vs_new,DL_new2[f], alpha=0.1, color='red')
Dest=df_result['mean']['Dm']/df_result['mean']['tau']+10000*(vs/df_result['mean']['eps'])*df_result['mean']['dp']/1000/df_result['mean']['Pel_inf']
plt.scatter(vs,D_L, label='Raw Data')
plt.plot(vs,Dest, label='Mean Estimate')
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('Diffusion Coeff $(10^4 x m^2/s)$', fontsize=12)
plt.plot(vs,summary_stan['y_025'],color='red')
plt.plot(vs,summary_stan['y_975'],color='red',label='95% Credible Interval')
# create 95% predictive intervals
df_diff = pd.DataFrame([])
df_ci = pd.DataFrame({'025': []})
for f in range(0, 9999):
    df_diff[f's{f}'] = samples['DL_new'][f]

l_ci = df_diff.T.quantile(q=0.025)
u_ci = df_diff.T.quantile(q=0.975)

plt.plot(vs_new, l_ci, color='black', ls='--')
plt.plot(vs_new, u_ci, color='black', ls='--', label='95% Prediction Interval')
plt.legend(loc='upper left')
plt.ylim(0, 16)
fname = 'Diffusion_pred-gs.png'
plt.savefig(fname, dpi=300)

print('RSME=', np.sqrt(mean_squared_error(D_L, Dest)))
print('rsquare=', r2_score(D_L, Dest))

# Plot results for Pep and velocity
for f in range(100, 175):
    Re = (vs_new) * rho * (dp[f] / 1000) / (mu)
    plt.scatter(Re, Pep_new, alpha=0.1, color='red')
plt.ylim(0, 10)
plt.xlabel('Particle Reynolds Number', fontsize=12)
plt.ylabel('Peclet Number Based on Particle Diameter', fontsize=12)

Pep = []
for x in vs:
    DLe = df_result['mean']['Dm'] / df_result['mean']['tau'] + 10000 * (x / df_result['mean']['eps']) * (df_result['mean']['dp'] / 10000) / (DL_new['mean']['Pel_inf'])
    Pep.append(x / df_result['mean']['eps'] * (df_result['mean']['dp'] / 10000) / (DLe / 10000))

Rep = (vs) * rho * (df_result['mean']['dp'] / 1000) / (mu)
plt.plot(Rep, Pep)

### 4) Oyster Bayesian Inference for Diffusion**

# Create arrays for oysters
dataoy = data.loc[data['Media'] == 'Oyster']
data3 = dataoy

# replace run 10 data with updated analysis for specifics in data within Impulse.ipynb
# PeL.loc[18] = 46.3

L = 0.33  # length of bed in m
vs = data3['Vs'] / 100  # velocity in m/s
PeL = data3['Pe']

u_bar = L / (data3['t_bar'] / 1000)

# replace run 10 data with updated analysis for specifics in data within Impulse.ipynb
PeL.loc[18] = 46.3

# calculate Diffusion coefficient for the bed

D_L = 10000 * u_bar * L / PeL

# Develop initial guess of line fit

dp = 3.24  # from Ergun analysis
tau = 1.93  # used value for cylinders (Delgado, 2006)
Pel_inf = 0.5  # initial guess
eps = 0.64  # from Ergun analysis

Dm = 0.2  # initial guess

DL_est = Dm / tau + 10000 * (vs / eps) * (dp / 1000) / Pel_inf

plt.scatter(vs, D_L)
# plt.plot(vs, DL_est)
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('Diffusion Coefficient $(10^{-4} \times m^2/s)$', fontsize=12)

### 4a) Stan code for oysters relating using Delgado (2006) relationship

```
erguncode = ""
data {
  int<lower=0> N;
  vector[N] vs;  // superficial velocity
  vector<lower=0>[N] DL;  // axial diffusion coefficient
}
parameters {
  real<lower=0> sigma;
}
```
real<lower=0> dp;
real<lower=0> tau;
real<lower=0> Pel_inf;
real<lower=0> eps;
real<lower=0> Dm;

}

model {
dp~normal(3.18,0.74);
tau~normal(1.93,0.5); // used tortuosity value for solid cylinders (Delgado, 2006)
Pel_inf~normal(2,3);
eps~normal(0.63,0.1);
//Dm~uniform(0,10);
DL~normal(Dm/tau+10000*(vs/eps*(dp/1000)/Pel_inf),sigma);
}

""

dat={'N': len(vs),
     'vs': vs,
     'DL': D_L
}

sm = pystan.StanModel(model_code=erguncode)
fit = sm.sampling(data=dat, seed=194838, iter=5000, chains=4)

#Increase delta and max tree depth to decrease divergences, if necessary
# fit = sm.sampling(data=dat, iter=10000, seed=194838, chains=4,control=dict(adapt_delta=0.999, max_treedepth=20))

#summary of estimated parameters with credible intervals
print(fit)

#Plot posterior plots from MCMC sampling
azdata = az.from_pystan(posterior=fit)
azdata
az.plot_pair(azdata, divergences=True);
# fname='diffusion_pair.png'
# plt.savefig(fname, dpi=300)

#Plot posterior distributions for the estimated parameters
az.plot_trace(azdata);
fname='diffusion_trace-oys.png'
plt.savefig(fname, dpi=300)

##Plot results of Stan estimates_ *
#extract the samples
stan_results = pd.DataFrame(fit.extract())

# print credible intervals
https://aidanrussell.com/2019/01/14/pystan-tutorial-1/
predictions_stan = pd.DataFrame.copy()
summary_stan = pd.DataFrame(columns=["y_025", "y_500", "y_975"], index=vs)
for x in vs:
    predictions_stan["x"] = x
    # DL~normal(Dm/tau+10000*(vs/eps*(dp/1000)/Pel_inf),sigma);
    predictions_stan[f"y_{x}"] = stan_results["Dm"]/stan_results["tau"] + 10000*
    predictions_stan["x"]/stan_results["eps"]*(stan_results["dp"]/1000)/stan_results["Pel_inf"]
    summary_stan.loc[x, f"y_025"] = predictions_stan[f"y_{x}"].quantile(q=0.025)
    summary_stan.loc[x, f"y_500"] = predictions_stan[f"y_{x}"].quantile(q=0.5)
    summary_stan.loc[x, f"y_975"] = predictions_stan[f"y_{x}"].quantile(q=0.975)

# produce a chart in the style of the previous OLS confidence interval chart
summary_stan["ci_lower_diff"] = summary_stan["y_025"] - summary_stan["y_500"]
summary_stan["ci_upper_diff"] = summary_stan["y_500"] - summary_stan["y_975"]
fig=plt.figure()
plt.errorbar(
    summary_stan.index,
    summary_stan["y_500"],
    yerr=summary_stan["ci_lower_diff", "ci_upper_diff"].T.values,
    fmt="-",
    capsize=4,
)
plt.scatter(vs, D_L, c="r", zorder=2)
plt.title("Stan Fit Including 95% Credible Interval")
# plt.ylim([-2.5, 12.5])
plt.ylabel("Diffusion Coefficient $(10^4 x m^2/s)$")
# plt.xlim([-0.5, 5.5])
plt.xlabel("Superficial Velocity (m/s)"
plt.show()
fname="oyster_95.png"
fig.savefig(fname, dpi=300)

plt.scatter(vs,D_L)
plt.plot(vs,summary_stan["y_025"],color='black')
plt.plot(vs,summary_stan["y_500"],color='red')
plt.plot(vs,summary_stan["y_975"],color='black')
summary_stan_oy=summary_stan

# convert results into DataFrame
result_fit=fit.summary()
df_result=pd.DataFrame(result_fit['summary'], index=result_fit['summary_rownames'] , columns=result_fit['summary_colnames'])
df_result

###* _4c) Use estimates to define relationship between Pep and Re_*

mu=0.000919 # dynamic viscosity of water (kg*s/(m/s2))
rho=997.5 # density of water (kg/m3)
Pep=[]
Pep_est=[]
for x in vs:
    DLe=df_result['mean']['Dm']/df_result['mean']['tau']+10000*(x/df_result['mean']['eps'])*(df_result['mean']['dp']/1000)/df_result['mean']['Pel_inf']
    Pep_est.append(x/df_result['mean']['eps']*(df_result['mean']['dp']/1000)/(DLe/10000))
Pep=vs/df_result['mean']['eps']*(df_result['mean']['dp']/1000)/(D_L/10000)
Re=(vs)*rho*(df_result['mean']['dp']/1000)/(mu)
plt.xlabel('Particle Reynolds Number', fontsize=12)
plt.ylabel('Peclet Number Based on Particle Diameter', fontsize=12)
plt.scatter(Re,Pep)
plt.plot(Re,Pep_est)
fname='reynolds_oyster.png'
plt.savefig(fname, dpi=300)

###* _4d) Generate Monte Carlo simulation from original Bayesian Model_*

#generate new quantities
vs_new=np.arange(0.01,0.10,0.01)

```
erguncode = """
data {
    int<lower=0> N;
    vector[N] vs; //superficial velocity
    vector<lower=0>[N] DL ; //axial diffusion coefficient
    int<lower=0> N_new;
    vector[N_new] vs_new;
}
parameters {
    real<lower=0> sigma;
    real<lower=0> dp;
    real<lower=0> tau;
```
real<lower=0> Pel_inf;
real<lower=0> eps;
real<lower=0> Dm;
}

model {
  dp~normal(3.18,0.74);
  tau~normal(1.93,0.5); // used tortuosity value for solid cylinders (Delgado, 2006)
  Pel_inf~normal(2.3);
  eps~normal(0.63,0.1);
  //Dm~uniform(0,10);
  DL~normal(Dm/tau+10000*(vs/eps*(dp/1000)/Pel_inf),sigma);
}

generated quantities {
  vector[N_new] DL_new;
  for (n in 1:N_new)
    DL_new[n] = normal_rng(Dm/tau+10000*(vs_new[n]/eps*(dp/1000)/Pel_inf),sigma);
}

""

dat={'N': len(vs),
     'vs': vs,
     'DL': D_L,
     'N_new': len(vs_new),
     'vs_new': vs_new}
sm = pystan.StanModel(model_code=erguncode)
fitn = sm.sampling(data=dat, seed=194838, iter=5000, chains=4)

samples=fitn.extract()
sigma=samples['sigma']
dp=samples['dp']
tau=samples['tau']
Pep=samples['Pel_inf']
eps=samples['eps']
beta=samples['Dm']
DL_new=samples['DL_new']
DL_new2=np.where(DL_new<=0,np.nan, DL_new) #filter out negative numbers

#plot results for DL vs velocity
plt.figure(figsize=[8,6])
# for f in range(1,50):
#   plt.scatter(vs_new,DL_new2[f], alpha=0.1, color='red')
Dest=df_result['mean']['Dm']/df_result['mean']['tau']+10000*(vs/df_result['mean']['eps'])*(df_result['mean']['dp']/10000)/df_result['mean']['Pel_inf']
plt.scatter(vs,D_L, label='Raw Data')
plt.plot(vs,Dest, label='Mean Estimate')

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```python
plt.xlabel('superficial velocity (m/s)', fontsize=12)
plt.ylabel('Diffusion Coeff $(10^4 x m^2/s)$', fontsize=12)
plt.plot(vs,summary_stan['y_025'],color='red')
# plt.plot(vs,summary_stan['y_500'],color='brown')
plt.plot(vs,summary_stan['y_975'],color='red', label='95% Credible Interval')

# Create 95% prediction intervals
df_diff=pd.DataFrame([])
df_ci=pd.DataFrame({'025':[]})
for f in range(0,9999):
    df_diff[f's{f}']=samples['DL_new'][f]
    l_ci=df_diff.T.quantile(q=0.025)
    u_ci=df_diff.T.quantile(q=0.975)
    plt.plot(vs_new,l_ci,color='black',ls='--')
    plt.plot(vs_new,u_ci,color='black', ls='--',label='95% Prediction Interval')
plt.legend(loc='upper left')
plt.ylim(0,16)
fname='Diffusion_pred-oy.png'
plt.savefig(fname, dpi=300)

print('RSME=',np.sqrt(mean_squared_error(D_L, Dest)))
print('rsquare=',r2_score(D_L, Dest))
RSME= 1.2426781756461154
rsquare= 0.8155067658026198

# Create 95% credible intervals
df_diff=pd.DataFrame([])
df_ci=pd.DataFrame({'025':[]})
for f in range(0,9999):
    df_diff[f's{f}']=samples['DL_new'][f]
    l_ci=df_diff.T.quantile(q=0.025)
    u_ci=df_diff.T.quantile(q=0.975)
    plt.plot(vs_new,l_ci,color='black')
    plt.plot(vs_new,u_ci,color='black')

##### Plot results for Pep and velocity
for f in range(1,25):
    Re=(vs_new)*rho*(dp[f]/1000)/(mu)
```

Pep_new = vs_new/eps[f]*(dp[f]/1000)/(DL_new[f]/10000)
plt.scatter(Re, Pep_new, alpha=0.1, color='red')
plt.ylim(0, 2)
plt.xlabel('Particle Reynolds Number', fontsize=12)
plt.ylabel('Peclet Number Based on Particle Diameter', fontsize=12)

Pep = []
for x in vs:
    DLe = df_result['mean'][Dm]/df_result['mean'][tau]+1000*(x/df_result['mean'][eps]*)((df_result['mean'][dp]/1000)/df_result['mean'][Pel_inf])
    Pep.append(x/df_result['mean'][eps]*)((df_result['mean'][dp]/1000)/(DLe/10000))

Rep = (vs)*rho*(df_result['mean'][dp]/10000)/mu
plt.plot(Rep, Pep)
## Developed by Matt Campbell 1/29/20
### for re-use with appropriate citation given

### Import relevant libraries
import matplotlib
import matplotlib.pyplot as plt
import pandas as pd
import numpy as np
import scipy
import seaborn as sns
from scipy import stats

# Upload total suspended solids raw data
# values are in grams
filename = 'tss.csv'
df = pd.read_csv(filename)
df.head()

# Upload sample number and volume data that match for each sample
filename = 'Filter_Map.csv'
fmap = pd.read_csv(filename)
fmap.head()

# Combine the raw data with the sample map
df2 = pd.merge(df, fmap, how='left', on='Sample')
df2.head()

# Calculate the concentration for each sample
df2['Conc'] = (df2['Final'] - df2['Initial']) / df2['Volume']
df2['Conc'] = df2['Conc'] * 1000000  # convert from g/mL to mg/L#

# Set up definitions to extract difference between top and bottom
def des(dd, description):
    df_type = dd[dd['Description'] == description]
    return df_type;

def tube(dd, description, number):
    df_type = des(dd, description)
    exp_u = df_type[df_type['Tube'] == number]
    return exp_u;

def diff(dd, description, number):
    exp_u = tube(dd, description, number)
    top = exp_u[exp_u['Position'] == 'Top']
    bottom = exp_u[exp_u['Position'] == 'Bottom']
red = bottom.iloc[0].Conc - top.iloc[0].Conc
return red

def conc(dd, description, number, level):
    exp_u = tube(dd, description, number)
    level = exp_u[exp_u['Position'] == level]
    lvl = level.iloc[0].Conc
    return lvl

# set up definition to create table of whole and initial sample differences
# df_filt = pd.DataFrame({'Tube': [6, 7, 8, 9, 10]})

def chart(dd, Description):
    df_filt = pd.DataFrame({'Tube': [6, 7, 8, 9, 10]})

    df_filt['Top'] = [conc(dd, Description, 6, 'Top'), conc(dd, Description, 7, 'Top'), conc(dd, Description, 8, 'Top'), conc(dd, Description, 9, 'Top'), conc(dd, Description, 10, 'Top')]

    df_filt['Bottom'] = [conc(dd, Description, 6, 'Bottom'), conc(dd, Description, 7, 'Bottom'), conc(dd, Description, 8, 'Bottom'), conc(dd, Description, 9, 'Bottom'), conc(dd, Description, 10, 'Bottom')]

    df_filt['Diff'] = [diff(dd, Description, 6), diff(dd, Description, 7), diff(dd, Description, 8), diff(dd, Description, 9), diff(dd, Description, 10),]

    return df_filt

# Create table for bottom and top concentrations along with corresponding reductions
descrip = 'Initial Sample'
Islst = chart(df2, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom']

I_stats = pd.DataFrame({'Tube': ['Mean', 'St_Dev'], 'Top': [Islst['Top'].mean(), Islst['Top'].std()], 'Bottom': [Islst['Bottom'].mean(), Islst['Bottom'].std()], 'Diff': [Islst['Diff'].mean(), Islst['Diff'].std()]})

I_stats = pd.concat([Islst, I_stats], ignore_index=True)

print(descrip)
print(I_stats)

# print('Mean Top=', Islst['Top'].mean(), ' Standard Dev=', Islst['Top'].std())
# print('Mean Bottom=', Islst['Bottom'].mean(), ' Standard Dev=', Islst['Bottom'].std())
# print('Mean Diff=', Islst['Diff'].mean(), ' Standard Dev=', Islst['Diff'].std())

descrip = 'Whole Sample'\nwslst = chart(df2, descrip)
wslst['Top'] = wslst['Top']
wslst['Bottom'] = wslst['Bottom']
wslst['Diff'] = wslst['Diff'] / wslst['Bottom']

print(descrip)
print(wslst)

w_stats=pd.DataFrame({'Tube': ['Mean','St_Dev'], 'Top': [wslst['Top'].mean(),wslst['Top'].std()], 'Bottom': [wslst['Bottom'].mean(),wslst['Bottom'].std()], 'Diff': [wslst['Diff'].mean(),wslst['Diff'].std()]})

w_stats=pd.concat([wslst,w_stats],ignore_index=True)

print(descrip)
# print(wslst)
print(w_stats)

# print('Mean Top=',wslst['Top'].mean(), ' Standard Dev=', wslst['Top'].std())
# print('Mean Bottom=',wslst['Bottom'].mean(), ' Standard Dev=', wslst['Bottom'].std())
# print('Mean Diff=',wslst['Diff'].mean(), ' Standard Dev=', wslst['Diff'].std())

Initial Sample

<table>
<thead>
<tr>
<th>Tube</th>
<th>Top</th>
<th>Bottom</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>10.526316</td>
<td>13.157895</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>9.395973</td>
<td>13.333333</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>13.245033</td>
<td>10.493827</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>9.756098</td>
<td>10.691824</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>11.842105</td>
<td>12.000000</td>
</tr>
<tr>
<td>5</td>
<td>Mean</td>
<td>10.953105</td>
<td>11.935376</td>
</tr>
<tr>
<td>6</td>
<td>St Dev</td>
<td>1.587678</td>
<td>1.330178</td>
</tr>
</tbody>
</table>

Whole Sample

<table>
<thead>
<tr>
<th>Tube</th>
<th>Top</th>
<th>Bottom</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>13.815789</td>
<td>19.078947</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>20.238095</td>
<td>13.907285</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>13.333333</td>
<td>10.666667</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>15.333333</td>
<td>12.000000</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>13.907285</td>
<td>14.666667</td>
</tr>
<tr>
<td>5</td>
<td>Mean</td>
<td>15.325567</td>
<td>14.063913</td>
</tr>
<tr>
<td>6</td>
<td>St Dev</td>
<td>2.845740</td>
<td>3.214787</td>
</tr>
</tbody>
</table>

cat1=['Top']*5
cat2=['Bottom']*5
plt.figure(dpi=350)
df_tss1=pd.DataFrame({'Conc': Islst['Top'], 'Position': cat1, 'Sample': ['slow']*5})
df_tss2=pd.DataFrame({'Conc': Islst['Bottom'], 'Position': cat2, 'Sample': ['slow']*5})
df_tss3=pd.DataFrame({'Conc': wslst['Top'], 'Position': cat1, 'Sample': ['quick']*5})
df_tss4=pd.DataFrame({'Conc': wslst['Bottom'], 'Position': cat2, 'Sample': ['quick']*5})
df_tss=pd.concat([df_tss1,df_tss2,df_tss3,df_tss4],ignore_index=True)
# ax = sns.violinplot(x="Conc", y="Position", data=df_tss,inner=None, color=".8")
ax = sns.stripplot(x="Conc", y="Position", hue='Sample',data=df_tss, jitter=True, size=10, linewidth=1)
plt.xlabel('Conc. (mg/l)')
plt.legend()
plt.savefig('TSS_stripplot.png',bbox_inches="tight", pad_inches=0.1)
tv_dff = stats.ttest_ind(wslst['Top'], Islst['Top'])
print('Is there a difference between sampling methods?')
print('tvalue =', tv_dff[0], 'pvalue =', tv_dff[1])
tv_sl = stats.ttest_1samp(Islst['Diff'], 0)
print('Slow Sample')
print('tvalue =', tv_sl[0], 'pvalue =', tv_sl[1])

tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
print('Quick Sample')
print('tvalue =', tv_qu[0], 'pvalue =', tv_qu[1])

# create table for total particles
# slow sample
descrip = 'Initial Sample'
Islst = chart(df2, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom'] * 100
tv_sl = stats.ttest_1samp(Islst['Diff'], 0)
df_total_S = pd.DataFrame({'Group': ['TSS_S'], 'Mean': [Islst['Diff'].mean()], 'St_Dev': [Islst['Diff'].std()], 't-value': [tv_sl[0]], 'p-value': [tv_sl[1]], 'df': [4]})

# quick sample
descrip = 'Whole Sample'
wslst = chart(df2, descrip)
wslst['Top'] = wslst['Top']
wslst['Bottom'] = wslst['Bottom']
wslst['Diff'] = wslst['Diff'] / wslst['Bottom'] * 100
tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
df_total_Q = pd.DataFrame({'Group': ['TSS_Q'], 'Mean': [wslst['Diff'].mean()], 'St_Dev': [wslst['Diff'].std()], 't-value': [tv_qu[0]], 'p-value': [tv_qu[1]], 'df': [4]})

df_summary = pd.concat([df_total_S, df_total_Q], ignore_index=True)
print(df_summary)

# Create table of whole and initial samples
xs = chart(df2, 'Whole Sample')
xs = chart(df2, 'Initial Sample')
print(xs)

filename = 'pressure.csv'
df3 = pd.read_csv(filename)
df3

df_pr=pd.DataFrame([df3['6p'],df3['7p'],df3['8p'],df3['9p'],df3['10p']]).T
df_pr=df_pr.rename(columns={"6bh": "T1","7bh": "T2","8bh": "T3","9bh": "T4","10bh": "T5"})
df_bh=pd.DataFrame([df3['6bh'],df3['7bh'],df3['8bh'],df3['9bh'],df3['10bh']]).T
df_bh=df_bh.rename(columns={"6bh": "T1","7bh": "T2","8bh": "T3","9bh": "T4","10bh": "T5"})
df_time=pd.DataFrame({'Time': df3['Time']})

df_pr_tidy=pd.concat([df_time,df_pr],axis=1)
df_pr_tidy=pd.melt(df_pr_tidy,id_vars=['Time'],var_name='Tube',value_name='Pressure')
df_pr_tidy['Pressure']=df_pr_tidy['Pressure']*9.80665 # convert from mm H20 to Pa
df_bh_tidy=pd.concat([df_time,df_bh],axis=1)
df_bh_tidy=pd.melt(df_bh_tidy,id_vars=['Time'],var_name='Tube',value_name='Height')
df_prbh=df_pr_tidy

plt.figure(figsize=[8,4.8],dpi=350)

ax=sns.scatterplot("Time", y="Height", hue='Tube', alpha=0.75, data=df_prbh, style='Tube', s=75)

ax=sns.lineplot("Time", y="Height", hue='Tube', alpha=0.75, data=df_prbh, legend=False)

ax1=ax.axes

ax1.axvspan(50, 95, alpha=0.2, color='grey') #time period of slow sampling

ax1.axvline(x=105,c='red', ls='--')

plt.ylabel('Bed Height (mm)')
plt.xlabel('Time (min)')
plt.ylim(240,310)

plt.legend(loc='center left', bbox_to_anchor=(1, 0.5))
plt.savefig('BedHeight.png', bbox_inches="tight", pad_inches=0.1)

#Bed height statistics

def stat_height(df,tube):
    df_1=df.loc[df['Tube']==tube]['Height'].mean()
    df_2=df.loc[df['Tube']==tube]['Height'].std()
    return [df_1,df_2]
tubes=['T1','T2','T3','T4','T5']
sts=[]
for i in tubes:
    st1=stat_height(df_prbh,i)
sts.append(st1)
df_sts=pd.DataFrame('Tubes': tubes, sts)
df_sts=pd.DataFrame(sts,columns=['Mean','St Dev'],index=tubes)
df_sts=df_sts #Convert to mm
print(df_sts)

ax=sns.scatterplot("Time", y="Pressure", hue='Tube', alpha=0.75,data=df_prbh,
style='Tube',s=75)
plt.legend('Pressure Differential (Pa)')
plt.xlabel('Time (min)')
ax1=ax.axes
ax1.axvspan(50, 95, alpha=0.2, color='grey') #time period of slow sampling
ax1.axvline(x=105,c='red',ls='--')
ax.legend(loc='center left', bbox_to_anchor=(1, 0.5))

plt.figure(figsize=[8,4.8], dpi=350)
ax=sns.scatterplot("Time", y="Pr/BH", hue='Tube', alpha=0.75,data=df_prbh, style='Tube', s=75)
ax=sns.lineplot("Time", y="Pr/BH", hue='Tube', alpha=0.75,data=df_prbh, legend=False)
plt.legend('Pressure Differential (Pa)/ Bed Height (mm)')
plt.xlabel('Time (min)')
ax1=ax.axes
ax1.axvspan(50, 95, alpha=0.2, color='grey') #time period of slow sampling
ax1.axvline(x=105,c='red',ls='--')
ax.legend(loc='center left', bbox_to_anchor=(1, 0.5))
plt.savefig('Pressure.png',bbox_inches="tight", pad_inches=0.1)

# statistical analysis can be performed by:
-
# plt.plot(df3['Time'],df_pr['6p']/df_bh['6bh'],label='tube 6')
# plt.plot(df3['Time'],df_pr['7p']/df_bh['7bh'],label='tube 7')
# plt.plot(df3['Time'],df_pr['8p']/df_bh['8bh'],label='tube 8')
# plt.plot(df3['Time'],df_pr['9p']/df_bh['9bh'],label='tube 9')
# plt.plot(df3['Time'],df_pr['10p']/df_bh['10bh'],label='tube 10')
# plt.legend(loc=0)

#set up rinse difference definitions
def conc(dd,Description,number):
    c=tube(dd,Description,number)
    c2=c['Conc'].iloc[0]
    return c2
df_rns=pd.DataFrame({'Tube': [6,7,8,9,10]})
df_ac=pd.DataFrame({'Tube': [6,7,8,9,10]})
def rinse(dd,Description):
df_rns[Description]=[conc(dd,Description, 6),conc(dd,Description, 7),conc(dd,Description, 8),conc(dd,Description, 9),conc(dd,Description, 10),]
    return df_rns

## concentrations are in mg/l
xq=rinse(df2,'Final Rinse')
xq=rinse(df2,'Initial Rinse')
xq['Diff']=xq['Final Rinse']-xq['Initial Rinse']
print(xq)

## calculate the average bed hieght of the oysters (mm)
xq['Bed Hieght']=[df_bh['T1'].mean(),df_bh['T2'].mean(),df_bh['T3'].mean(),df_bh['T4'].mean(),df_bh['T5'].mean()]
print(xq)

# one cup of oysters (236.588 ml) and 2 cups (473.176 ml) of tap water were placed into
# measuring cup and mixed
# calculate how much sediment accumulated
xq['Bed Vol']=(xq['Bed Hieght']/10*np.pi*np.square(2.54))/1000 #calculate bed volume (l)
#Sedimentation occures to replace initial rinse up to final rinse (Initial Rinse + Diff)
# Assumes uniform sedimentation within bed. Would need to be investigated more in the future.
xq['Sed']=(xq['Initial Rinse']+xq['Diff'])*(473.176/236.588) # sediment accumulated in mg/L of
# oysters (473.16 ml=2 cups sample volume; 236.588 ml=1 cup oyster sample)
xq['Bed Sed']=xq['Bed Vol']*xq['Sed']# sedimentation in whole bed of oysters mg
xq['Rate']=xq['Bed Sed']/5.25 # sedimentation rate in mg/hr --5.25 hrs of accumulation

def sed_conc(dd,description,number):
    exp_u=tube(dd,description,number)
    top=exp_u[exp_u['Position']=='Top']
    bottom=exp_u[exp_u['Position']=='Bottom']

    df_rinses=...
```python
top_conc = top.iloc[0].Conc
bottom_conc = bottom.iloc[0].Conc
return bottom_conc

df_cc = pd.DataFrame({'Tube': [6, 7, 8, 9, 10]})

def sedcomp(dd, Description):
    df_cc = [sed_conc(dd, Description, 6), sed_conc(dd, Description, 7),
             sed_conc(dd, Description, 8), sed_conc(dd, Description, 9),
             sed_conc(dd, Description, 10),]
    return df_cc

ws_conc = sedcomp(df2, 'Whole Sample')

print(ws_conc)

df_coc = df_cc
df_coc['bottom'] = ws_conc

df_coc['sed_flow'] = df_coc['bottom'] * 0.5 * 227.12  # mg/hr at 0.5 gpm (227.12 L/h converts to 1 gal/min)
# df_coc['sed max'] = df_coc['sed flow']
df_coc['sed_rate'] = xq['Rate']  # mg/hr
df_coc['percent_ret'] = df_coc['sed_rate'] / df_coc['sed_flow']

df_coc_stats = pd.DataFrame({'Tube': ['mean', 'st_dev'], 'bottom': [df_coc['bottom'].mean(), df_coc['bottom'].std()]
                            , 'sed_flow': [df_coc['sed_flow'].mean(), df_coc['sed_flow'].std()]
                            , 'sed_rate': [df_coc['sed_rate'].mean(), df_coc['sed_rate'].std()]
                            , 'percent_ret': [df_coc['percent_ret'].mean(), df_coc['percent_ret'].std()]
                            })

df_coc = pd.concat([df_coc, df_coc_stats], ignore_index=True)

print(df_coc)

print('mean=', df_coc['percent_ret'].mean(), 'st dev=', df_coc['percent_ret'].std())
```

Appendix F- FlowCAM Python Code

##Developed by Matt Campbell 1/29/20
### for re-use with appropriate citation given

# Import relevant libraries
import matplotlib
import matplotlib.pyplot as plt
import pandas as pd
import numpy as np
import scipy
from scipy import stats
import seaborn as sns

filename='vial_number.csv'
vial=pd.read_csv(filename)
print(vial)

# init=vial.loc[(vial['Sample']=='Whole Sample') & (vial['Position'] == 'Top'),['Vial', 'Tube']]
# print(init)

z=[]
filename = 'data_export_vial24.csv'
data = pd.read_csv(filename)
count=data.shape[0]
z.append(count)
print(z)

# FlowCAM files can be screened by sizes
size_class=[12,250]#establish size class

###open each file and extract count
int_1=vial['Vial']
count=[]
for f in int_1:
    filename = 'data_export_vial{}.csv'.format(f)
data = pd.read_csv(filename)
    # data=data.loc[(data['Length']>size_class[0]) & (data['Length']<size_class[1])]
    c=data.shape[0]
    count.append(c)

vial['Count']=count

## Open summary file and export imaged volume
import re
stringToMatch = 'Fluid Volume Imaged'
matchedLine = 
V_image=[]
int_1=vial['Vial']

for f in int_1:
    filename='vial_{}_run_summary.txt'.format(f)
    with open(filename, 'r') as file:
        for line in file:
            if stringToMatch in line:
                matchedLine = line
                break
    ttx=re.findall("d+.d+", matchedLine)
    ttx=float(ttx[0])
    V_image.append(ttx)

vial['V_imaged']=V_image

# Calculate concentrations
vial['Volume']=vial['V_imaged'] *(1-0.5/10.5)   #Accounting for lugols addition (0.5 ml added to 10 ml water sample)
vial['Conc']=vial['Count']/vial['Volume']

###Refs for comparisons####
### algae count comparisons
##use mean wieght of cell to compare to counts from TSS
##https://mospace.umsystem.edu/xmlui/bitstream/handle/10355/46477/research.pdf

#Set up definitions to extract difference between top and bottom
def des(dd,description):
    df_type=dd[dd['Sample']==description]
    return df_type;
def tube(dd,description,number):
    df_type=des(dd,description)
    exp_u=df_type[df_type['Tube']==number]
    return exp_u;
def diff(dd,description,number):
    exp_u=tube(dd,description,number)
    top=exp_u[exp_u['Position']=='Top']
    bottom=exp_u[exp_u['Position']=='Bottom']
    red=bottom.iloc[0].Conc-top.iloc[0].Conc
    return red

def conc(dd,description,number,level):
exp_u=tube(dd,description,number)
level=exp_u[exp_u['Position']==level]
lvl=level.iloc[0].Conc
return lvl

# set up definition to create table of whole and initial sample differences
# df_filt=pd.DataFrame( {'Tube': [6,7,8,9,10]})

def chart(dd,Description):
    df_filt=pd.DataFrame( {'Tube': [6,7,8,9,10]})

df_filt['Top']=[conc(dd,Description,6,'Top'),conc(dd,Description,7,'Top'),conc(dd,Description,8,'Top'),conc(dd,Description,9,'Top'),conc(dd,Description,10,'Top')]

df_filt['Bottom']=[conc(dd,Description,6,'Bottom'),conc(dd,Description,7,'Bottom'),conc(dd,Description,8,'Bottom'),conc(dd,Description,9,'Bottom'),conc(dd,Description,10,'Bottom')]

df_filt['Diff']=[diff(dd,Description, 6),diff(dd,Description, 7),diff(dd,Description, 8),diff(dd,Description, 9),diff(dd,Description, 10),]
return df_filt

# # set up definition to create table of whole and initial sample differences
# df_filt=pd.DataFrame( {'Tube': [6,7,8,9,10]})

# def chart(dd,Description):
#     df_filt[Description]=[diff(dd,Description, 6),diff(dd,Description, 7),diff(dd,Description, 8),diff(dd,Description, 9),diff(dd,Description, 10),]
#     return df_filt

# Create table for bottom and top concentrations along with corresponding reductions
descrip='Initial Sample'
Islst=chart(vial,descrip)
Islst['Top']=Islst['Top']
Islst['Bottom']=Islst['Bottom']
Islst['Diff']=Islst['Diff']#/Islst['Bottom']
I_stats=pd.DataFrame( {'Tube': ['Mean','St_Dev'], 'Top': [Islst['Top'].mean(),Islst['Top'].std()],'Bottom': [Islst['Bottom'].mean(),Islst['Bottom'].std()],'Diff': [Islst['Diff'].mean(),Islst['Diff'].std()]})
I_stats=pd.concat([Islst,I_stats],ignore_index=True)
print(descrip)
print(I_stats)
# print('Mean Top=',Islst['Top'].mean(), ' Standard Dev=', Islst['Top'].std())
# print('Mean Bottom=',Islst['Bottom'].mean(), ' Standard Dev=', Islst['Bottom'].std())
# print('Mean Diff=',Islst['Diff'].mean(), ' Standard Dev=', Islst['Diff'].std())

descrip='Whole Sample'
wslst=chart(vial,descrip)
wslst['Top']=wslst['Top']
```python
wslst['Bottom']=wslst['Bottom']
wslst['Diff']=wslst['Diff']#/wslst['Bottom']

w_stats=pd.DataFrame({'Tube': ['Mean', 'St Dev'], 'Top': [wslst['Top'].mean(), wslst['Top'].std()], 'Bottom': [wslst['Bottom'].mean(), wslst['Bottom'].std()], 'Diff': [wslst['Diff'].mean(), wslst['Diff'].std()]})
w_stats=pd.concat([wslst, w_stats], ignore_index=True)

print(w_stats)

# print('Mean Top=', wslst['Top'].mean(), ' Standard Dev=', wslst['Top'].std())
# print('Mean Bottom=', wslst['Bottom'].mean(), ' Standard Dev=', wslst['Bottom'].std())
# print('Mean Diff=', wslst['Diff'].mean(), ' Standard Dev=', wslst['Diff'].std())

plt.scatter(Islst['Top'], cat1, s=200, c='#ff7f0e', marker='D', edgecolor='k', linewidths=1, label='Slow')
plt.scatter(Islst['Bottom'], cat2, s=200, c='#ff7f0e', marker='D', edgecolor='k', linewidths=1)
plt.scatter(wslst['Top'], cat1, s=100, c='#1f77b4', edgecolor='k', linewidths=1, label='Quick')
plt.scatter(wslst['Bottom'], cat2, s=100, c='#1f77b4', edgecolor='k', linewidths=1)
plt.ylim(2, -1)
plt.xlabel('Conc. (mg/l)')
plt.legend()
plt.savefig('FlowCAM_stripplot.png')

# plt.scatter(Islst['Top'], cat1, s=200, c='ff7f0e', marker='D', edgecolor='k', linewidths=1, label='Slow')
# plt.scatter(Islst['Bottom'], cat2, s=200, c='ff7f0e', marker='D', edgecolor='k', linewidths=1)
# plt.scatter(wslst['Top'], cat1, s=100, c='1f77b4', edgecolor='k', linewidths=1, label='Quick')
# plt.scatter(wslst['Bottom'], cat2, s=100, c='1f77b4', edgecolor='k', linewidths=1)
# plt.ylim(2, -1)
# plt.xlabel('Conc. (mg/l)')
# plt.legend()
# plt.savefig('FlowCAM_stripplot.png')

df_ps1=pd.DataFrame({'Conc': Islst['Top'], 'Position': cat1, 'Sample': ['slow']*5})
df_ps2=pd.DataFrame({'Conc': Islst['Bottom'], 'Position': cat2, 'Sample': ['slow']*5})
df_ps3=pd.DataFrame({'Conc': wslst['Top'], 'Position': cat1, 'Sample': ['quick']*5})
df_ps4=pd.DataFrame({'Conc': wslst['Bottom'], 'Position': cat2, 'Sample': ['quick']*5})
df_ps=pd.concat([df_ps1, df_ps2, df_ps3, df_ps4], ignore_index=True)

ax = sns.stripplot(x='Conc', y='Position', hue='Sample', data=df_ps, jitter=True, size=10, linewidth=1)
plt.xlabel('Conc. (count/ml)')
plt.legend()
plt.savefig('FlowCAM_stripplot.png', bbox_inches="tight", pad_inches=0)

# calculate statistics of the differences for total particles
tv_sample=stats.ttest_ind(wslst['Top'], Islst['Top'])
print('Is there a difference btw sample method?')
print('tvalue=', tv_sample[0], ' pvalue=', tv_sample[1])

print('Is there a difference btw bottom and top?')
tv_sl=stats.ttest_1samp(Islst['Diff'], 0)
print('Slow Sample')
```
print('tvalue =', tv_sl[0], 'pvalue=', tv_sl[1])

tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
print('Quick Sample')
print('tvalue =', tv_qu[0], 'pvalue=', tv_qu[1])

WT = vial.loc[(vial['Sample'] == 'Whole Sample') & (vial['Position'] == 'Top')]
WB = vial.loc[(vial['Sample'] == 'Whole Sample') & (vial['Position'] == 'Bottom')]
IT = vial.loc[(vial['Sample'] == 'Initial Sample') & (vial['Position'] == 'Top')]
IB = vial.loc[(vial['Sample'] == 'Initial Sample') & (vial['Position'] == 'Bottom')]

print('Quick Top =', WT['Conc'].mean(), '(mean)', WT['Conc'].std(), '(std)')
print('Quick Bottom =', WB['Conc'].mean(), '(mean)', WB['Conc'].std(), '(std)')
print('Slow Top =', IT['Conc'].mean(), '(mean)', IT['Conc'].std(), '(std)')
print('Slow Bottom =', IB['Conc'].mean(), '(mean)', IB['Conc'].std(), '(std)')
print('Quick =', (1 - WT['Conc'].mean() / WB['Conc'].mean()) * 100, '% removal')
print('Slow =', (1 - IT['Conc'].mean() / IB['Conc'].mean()) * 100, '% removal')

### Pseudofeces counts from image recognition

## particle size was screened for size class based upon shift in top to bottom sizes

int_1 = vial['Vial']
count = []
for f in int_1:
    filename = 'Psuedofeces/vial_{0}_data.csv'.format(f)
data = pd.read_csv(filename)
data = data.loc[(data['Length'] > size_class[0]) & (data['Length'] < size_class[1])]  # screen for size class
    c = data.shape[0]
count.append(c)

vial['PsuedoCount'] = count
vial['PsuedoConc'] = vial['PsuedoCount'] / vial['Volume']

WT = vial.loc[(vial['Sample'] == 'Whole Sample') & (vial['Position'] == 'Top')]
WB = vial.loc[(vial['Sample'] == 'Whole Sample') & (vial['Position'] == 'Bottom')]
IT = vial.loc[(vial['Sample'] == 'Initial Sample') & (vial['Position'] == 'Top')]
IB = vial.loc[(vial['Sample'] == 'Initial Sample') & (vial['Position'] == 'Bottom')]

print('Whole Top =', WT['PsuedoConc'].mean(), '(mean)', WT['PsuedoConc'].std(), '(std)')
print('Whole Bottom =', WB['PsuedoConc'].mean(), '(mean)', WB['PsuedoConc'].std(), '(std)')
print('Initial Top =', IT['PsuedoConc'].mean(), '(mean)', IT['PsuedoConc'].std(), '(std)')
print('Initial Bottom =', IB['PsuedoConc'].mean(), '(mean)', IB['PsuedoConc'].std(), '(std)')
def ps_diff(dd, description, number):
    exp_u = tube(dd, description, number)
    top = exp_u[exp_u['Position'] == 'Top']
    bottom = exp_u[exp_u['Position'] == 'Bottom']
    red = bottom.iloc[0].PsuedoConc - top.iloc[0].PsuedoConc
    return red

def ps_conc(dd, description, number, level):
    exp_u = tube(dd, description, number)
    level = exp_u[exp_u['Position'] == level]
    lvl = level.iloc[0].PsuedoConc
    return lvl

# set up definition to create table of whole and initial sample differences

def ps_chart(dd, Description):
    df_filt = pd.DataFrame({'Tube': [6, 7, 8, 9, 10]})
    df_filt['Top'] = [ps_conc(dd, Description, 6, 'Top'), ps_conc(dd, Description, 7, 'Top'), ps_conc(dd, Description, 8, 'Top'), ps_conc(dd, Description, 9, 'Top'), ps_conc(dd, Description, 10, 'Top')]
    df_filt['Bottom'] = [ps_conc(dd, Description, 6, 'Bottom'), ps_conc(dd, Description, 7, 'Bottom'), ps_conc(dd, Description, 8, 'Bottom'), ps_conc(dd, Description, 9, 'Bottom'), ps_conc(dd, Description, 10, 'Bottom')]
    df_filt['Diff'] = [ps_diff(dd, Description, 6), ps_diff(dd, Description, 7), ps_diff(dd, Description, 8), ps_diff(dd, Description, 9), ps_diff(dd, Description, 10),]
    return df_filt

# Create table for bottom and top psuedofeces concentrations along with corresponding reductions

descrip='Initial Sample'
Islst = ps_chart(vial, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom']
print(descrip)
print(Islst)
print('Mean Top=', Islst['Top'].mean(), ' Standard Dev=', Islst['Top'].std())
print('Mean Bottom=', Islst['Bottom'].mean(), ' Standard Dev=', Islst['Bottom'].std())
print('Mean Diff=', Islst['Diff'].mean(), ' Standard Dev=', Islst['Diff'].std())

descrip='Whole Sample'/wsllst = ps_chart(vial, descrip)/wsllst['Top'] = wsllst['Top']
wslst['Bottom']=wslst['Bottom']
wslst['Diff']=wslst['Diff']#/wslst['Bottom']
print(descrp)
print(wslst)
print('Mean Top=',wslst['Top'].mean(), ' Standard Dev=', wslst['Top'].std())
print('Mean Bottom=',wslst['Bottom'].mean(), ' Standard Dev=', wslst['Bottom'].std())
print('Mean Diff=',wslst['Diff'].mean(), ' Standard Dev=', wslst['Diff'].std())

#Strip plot of the psuedofeces concentrations

cat1=['Top']*5
cat2=['Bottom']*5
plt.figure(dpi=350)
df_ps1=pd.DataFrame({'Conc': Islst['Top'], 'Position': cat1, 'Sample': ['slow']*5})
df_ps2=pd.DataFrame({'Conc': Islst['Bottom'], 'Position': cat2, 'Sample': ['slow']*5})
df_ps3=pd.DataFrame({'Conc': wslst['Top'], 'Position': cat1, 'Sample': ['quick']*5})
df_ps4=pd.DataFrame({'Conc': wslst['Bottom'], 'Position': cat2, 'Sample': ['quick']*5})
df_ps=pd.concat([df_ps1,df_ps2,df_ps3,df_ps4],ignore_index=True)
# ax = sns.violinplot(x="Conc", y="Position", data=df_ps,inner=None, color=".8")
ax = sns.stripplot(x="Conc", y="Position", hue='Sample',data=df_ps, jitter=True,
size=10,linewidth=1)
plt.xlabel('Conc. (count/ml)')
plt.legend()
# df_ps
plt.savefig('FlowCAM_ps_stripplot.png')

# # test of the % psuedofeces

tv_sample=stats.ttest_ind(wslst['Bottom'],Islst['Bottom'])
print('Is there a difference btw sample method?')
print('tvalue =',tv_sample[0], 'pvalue=',tv_sample[1])

print('Is there a difference btw bottom and top?')
tv_sl=stats.ttest_1samp(Islst['Diff'],0)
print('Slow Sample')
print('tvalue =',tv_sl[0], 'pvalue=',tv_sl[1])

tv_qu=stats.ttest_1samp(wslst['Diff'],0)
print('Quick Sample')
print('tvalue =',tv_qu[0], 'pvalue=',tv_qu[1])

## table of % psuedofeces

descrip='Initial Sample'
Is_ps=ps_chart(vial,descrip)
Is_tot=chart(vial,descrip)
Is_percent=Is_ps/Is_tot
Is_percent['Diff'] = Is_percent['Top'] - Is_percent['Bottom']

descrip = 'Whole Sample'
ws_ps = ps_chart(vial, descrip)
ws_tot = chart(vial, descrip)
ws_percent = ws_ps / ws_tot
ws_percent['Diff'] = ws_percent['Top'] - ws_percent['Bottom']

print('slow sample')
print(Is_percent)

print('Mean Top =', Is_percent['Top'].mean(), ' Standard Dev =', Is_percent['Top'].std())
print('Mean Bottom =', Is_percent['Bottom'].mean(), ' Standard Dev =', Is_percent['Bottom'].std())
print('Mean Diff =', Is_percent['Diff'].mean(), ' Standard Dev =', Is_percent['Diff'].std())

print('quick sample')
print(ws_percent)

print('Mean Top =', ws_percent['Top'].mean(), ' Standard Dev =', ws_percent['Top'].std())
print('Mean Bottom =', ws_percent['Bottom'].mean(), ' Standard Dev =', ws_percent['Bottom'].std())
print('Mean Diff =', ws_percent['Diff'].mean(), ' Standard Dev =', ws_percent['Diff'].std())

print('Is there a difference btw bottom and top?')
tv_sl = stats.ttest_1samp(Is_percent['Diff'], 0)
print('Slow Sample')
print('tvalue =', tv_sl[0], 'pvalue =', tv_sl[1])

tv_qu = stats.ttest_1samp(ws_percent['Diff'], 0)
print('Quick Sample')
print('tvalue =', tv_qu[0], 'pvalue =', tv_qu[1])
df_pseudo_diff = pd.DataFrame({'Group': ['Psuedo_S', 'Psuedo_Q'], 'Mean': [Is_percent['Diff'].mean(), ws_percent['Diff'].mean()], 'St_Dev': [Is_percent['Diff'].std(), ws_percent['Diff'].std()], 't-value': [tv_sl[0], tv_qu[0]], 'p-value': [tv_sl[1], tv_qu[1]], 'df': [4, 4]})
print('summary')
print(df_pseudo_diff)

print('There is a', df_pseudo_diff['Mean'][0]*100, 'percent conversion to pseudofoeces with slow sampling')
print('There is a', df_pseudo_diff['Mean'][1]*100, 'percent conversion to pseudofoeces with quick sampling')

### Particle size

### All particles

avg = []
for f in int_1:
    filename = 'data_export_vial{}.csv'.format(f)
data = pd.read_csv(filename)
ag=data['Length'].mean()
avg.append(ag)

vial['SizeAvg']=avg

WT=vial.loc[(vial['Sample']=='Whole Sample') & (vial['Position']=='Top')]
WB=vial.loc[(vial['Sample']=='Whole Sample') & (vial['Position']=='Bottom')]
IT=vial.loc[(vial['Sample']=='Initial Sample') & (vial['Position']=='Top')]
IB=vial.loc[(vial['Sample']=='Initial Sample') & (vial['Position']=='Bottom')]

print('Whole Top =',WT['SizeAvg'].mean(),'(mean)',WT['SizeAvg'].std(),'(std)')
print('Whole Bottom =',WB['SizeAvg'].mean(),'(mean)',WB['SizeAvg'].std(),'(std)')
print('Initial Top =',IT['SizeAvg'].mean(),'(mean)',IT['SizeAvg'].std(),'(std)')
print('Initial Bottom =',IB['SizeAvg'].mean(),'(mean)',IB['SizeAvg'].std(),'(std)')

tv_sample=stats.ttest_ind(WT['SizeAvg'],WB['SizeAvg'])
print('Is there a difference btw bottom and top size?')
print('Quick Sample')
print('tvalue =',tv_sample[0], 'pvalue=',tv_sample[1])

tv_sample=stats.ttest_ind(IT['SizeAvg'],IB['SizeAvg'])
print('Is there a difference btw bottom and top size?')
print('Slow Sample')
print('tvalue =',tv_sample[0], 'pvalue=',tv_sample[1])

### The histogram for the whole (quick) sample according to size
l=[0,1,2,3,4] #position 0-4
tubText=[6,7,8,9,10]
fig=plt.figure(figsize=[10,6],dpi=350)
for f in l:
    tt=WT['Vial'].iloc[f]
    filename1 = 'data_export_vial{}.csv'.format(tt)
dx_T = pd.read_csv(filename1)
    bb=WB['Vial'].iloc[f]
    filename2 = 'data_export_vial{}.csv'.format(bb)
dx_B = pd.read_csv(filename2)
plt.subplot(2,3,f+1)
bins = np.linspace(0, 50, 50)
column = 'Length'
tubText=['Tube 1','Tube 2','Tube 3','Tube 4','Tube 5']
plt.hist([dx_B[column],dx_T[column]], bins, alpha=0.5, label=['bottom','top'], density=False)
plt.text(40, 700,tubText[f] ,fontsize=12, ha='center')
plt.xlim(0,50)
plt.ylim(0,1000)
# plt.set_ylabel('Frequency')
# plt.set_xlabel(column)
plt.legend(loc='center left', bbox_to_anchor=(1, 0.5))
plt.gcf().text(0.05, 0.5, 'Frequency', fontsize=12, rotation=90)
plt.gcf().text(0.33, 0.05, 'Length (microns)', fontsize=12)
## plt.show()
fig.savefig('Length-Hist-qs.png', bbox_inches="tight", pad_inches=0)

### The scatterplot for the particle sample according to size vs circularity
l=1 #position

# for f in l:
    tt=WT['Vial'].iloc[l]
filename1 = 'data_export_vial{}.csv'.format(tt)
df_T = pd.read_csv(filename1)
    bb=WB['Vial'].iloc[l]
filename2 = 'data_export_vial{}.csv'.format(bb)
df_B = pd.read_csv(filename2)
fig, ax = plt.subplots(dpi=350)
column1 = 'Length'
column2 = 'Width'

plt.scatter(df_T[column1],df_T[column2], c='#ff7f0e',marker='o',alpha=0.5, s=3,label='top')
plt.scatter(df_B[column1],df_B[column2], c='#1f77b4',marker='o',alpha=0.5,
s=2,label='bottom')
# cycler('color', ['#1f77b4', '#ff7f0e', '#2ca02c', '#d62728', '#9467bd', '#8c564b', '#e377c2',
' #7f7f7f', '#bcbd22', '#17becf']).
plt.ylim(0,100)
plt.xlim(0,150)
ax.set_ylabel(column2)
ax.set_xlabel(column1)
ax.legend(loc='upper left')
plt.show()
fig.savefig('Shape-scatter.png', bbox_inches="tight", pad_inches=0.1)

#### Pseudofeces only
avg=[]
for f in int_1:
    filename = 'Psuedofeces/vial_{}\_data.csv'.format(f)
data = pd.read_csv(filename)
    ag=data['Length'].mean()
    avg.append(ag)

### Pseudofeces only
avg=[]
for f in int_1:
    filename = 'Psuedofeces/vial_{}\_data.csv'.format(f)
data = pd.read_csv(filename)
    ag=data['Length'].mean()
    avg.append(ag)
vial['PsuedoSizeAvg'] = avg

WT = vial.loc[(vial['Sample'] == 'Whole Sample') & (vial['Position'] == 'Top')]
WB = vial.loc[(vial['Sample'] == 'Whole Sample') & (vial['Position'] == 'Bottom')]
IT = vial.loc[(vial['Sample'] == 'Initial Sample') & (vial['Position'] == 'Top')]
IB = vial.loc[(vial['Sample'] == 'Initial Sample') & (vial['Position'] == 'Bottom')]

print('Whole Top =', WT['PsuedoSizeAvg'].mean(), '(mean)', WT['PsuedoSizeAvg'].std(), '(std)')
print('Whole Bottom =', WB['PsuedoSizeAvg'].mean(), '(mean)',
    WB['PsuedoSizeAvg'].std(), '(std)')
print('Initial Top =', IT['PsuedoSizeAvg'].mean(), '(mean)', IT['PsuedoSizeAvg'].std(), '(std)')
print('Initial Bottom =', IB['PsuedoSizeAvg'].mean(), '(mean)', IB['PsuedoSizeAvg'].std(), '(std)')

tv_sample = stats.ttest_ind(WT['PsuedoSizeAvg'], WB['PsuedoSizeAvg'])
print('Is there a difference btw bottom and top size?')
print('Quick Sample')
print('tvalue =', tv_sample[0], 'pvalue =', tv_sample[1])

tv_sample = stats.ttest_ind(IT['PsuedoSizeAvg'], IB['PsuedoSizeAvg'])
print('Is there a difference btw bottom and top size?')
print('Slow Sample')
print('tvalue =', tv_sample[0], 'pvalue =', tv_sample[1])

vial['NonPs_Count'] = vial['Count'] - vial['PsuedoCount']

vial['NonPs_Conc'] = vial['NonPs_Count'] / vial['Volume']

vial['NonPs_Conc'].loc[(vial['NonPs_Conc'] < 0)] = 0

def tot_diff(dd, description, number):
    exp_u = tube(dd, description, number)
    top = exp_u[exp_u['Position'] == 'Top']
    bottom = exp_u[exp_u['Position'] == 'Bottom']
    red = bottom.iloc[0].NonPs_Conc - top.iloc[0].NonPs_Conc
    return red

def tot_conc(dd, description, number, level):
    exp_u = tube(dd, description, number)
    level = exp_u[exp_u['Position'] == level]
    lvl = level.iloc[0].NonPs_Conc
    return lvl

# set up definition to create table of whole and initial sample differences

def tot_chart(dd, Description):
    df_filt = pd.DataFrame({'Tube': [6, 7, 8, 9, 10]})
df_filt['Top']=[tot_conc(dd,Description,6,'Top'),tot_conc(dd,Description,7,'Top'),tot_conc(dd,Description,8,'Top'),tot_conc(dd,Description,9,'Top'),tot_conc(dd,Description,10,'Top')]

df_filt['Bottom']=[tot_conc(dd,Description,6,'Bottom'),tot_conc(dd,Description,7,'Bottom'),tot_conc(dd,Description,8,'Bottom'),tot_conc(dd,Description,9,'Bottom'),tot_conc(dd,Description,10,'Bottom')]

df_filt['Diff']=[tot_diff(dd,Description, 6),tot_diff(dd,Description, 7),tot_diff(dd,Description, 8),tot_diff(dd,Description, 9),tot_diff(dd,Description, 10),]

return df_filt

# Create table for bottom and top concentrations along with corresponding reductions
descrip='Initial Sample'
Islst=tot_chart(vial,descrip)
Islst['Top']=[Islst['Top']]
Islst['Bottom']=[Islst['Bottom']]
Islst['Diff']=Islst['Diff']/Islst['Bottom']
I_stats=pd.DataFrame({'Tube': ['Mean','St_Dev'],'Top': [Islst['Top']].mean(),Islst['Top'].std()],'Bottom': [Islst['Bottom']].mean(),Islst['Bottom'].std()],'Diff': [Islst['Diff']].mean(),Islst['Diff'].std()])
I_stats=pd.concat([Islst,I_stats],ignore_index=True)
print(descrip)
print(I_stats)

descrip='Whole Sample'
wslst=tot_chart(vial,descrip)
wslst['Top']=[wslst['Top']]
wslst['Bottom']=[wslst['Bottom']]
wslst['Diff']=wslst['Diff']/wslst['Bottom']
w_stats=pd.DataFrame({'Tube': ['Mean','St_Dev','Top': [wslst['Top']].mean(),wslst['Top'].std()],'Bottom': [wslst['Bottom']].mean(),wslst['Bottom'].std()],'Diff': [wslst['Diff']].mean(),wslst['Diff'].std()})
w_stats=pd.concat([wslst,w_stats],ignore_index=True)
print(descrip)
print(w_stats)

tv_sample=stats.ttest_ind(wslst['Bottom'],Islst['Bottom'])
print('Is there a difference btw sample method?')
print('tvalue =',tv_sample[0], 'pvalue=',tv_sample[1])

print('Is there a difference btw bottom and top?')
tv_sl=stats.ttest_1sampIslst['Diff'],0
print('Slow Sample')
print('tvalue =',tv_sl[0], 'pvalue=',tv_sl[1])

tv_qu=stats.ttest_1samp(wslst['Diff'],0)
print('Quick Sample')
print('tvalue =', tv_qu[0], 'pvalue=', tv_qu[1])

cat1=['Top']*5
cat2=['Bottom']*5
plt.figure(dpi=350)
df_ps1=pd.DataFrame({'Conc': Islst['Top'], 'Position': cat1, 'Sample': ['slow']*5})
df_ps2=pd.DataFrame({'Conc': Islst['Bottom'], 'Position': cat2, 'Sample': ['slow']*5})
df_ps3=pd.DataFrame({'Conc': wslst['Top'], 'Position': cat1, 'Sample': ['quick']*5})
df_ps4=pd.DataFrame({'Conc': wslst['Bottom'], 'Position': cat2, 'Sample': ['quick']*5})
df_ps=pd.concat([df_ps1, df_ps2, df_ps3, df_ps4], ignore_index=True)
# ax = sns.violinplot(x="Conc", y="Position", data=df_ps,inner=None, color=.8")
ax = sns.stripplot(x="Conc", y="Position", hue='Sample', data=df_ps, jitter=True, size=10, linewidth=1)
plt.xlabel('Conc. (count/ml)')
plt.legend()
# df_ps
plt.savefig('FlowCAM_algae_stripplot.png', bbox_inches="tight", pad_inches=0.1)

### Create Chart showing statistics of differences

# base dataframe

df_summary=pd.DataFrame({'Group': [], 'Mean': [], 'St_Dev': [], 't-value': [], 'p-value': [], 'df': []})

# create table for total particles
# slow sample
descrip='Initial Sample'
Islst=chart(vial, descrip)
Islst['Top']=Islst['Top']
Islst['Bottom']=Islst['Bottom']
Islst['Diff']=Islst['Diff']#/Islst['Bottom']
tv_sl=stats.ttest_1samp(Islst['Diff'], 0)
df_total_S=pd.DataFrame({'Group': ['Total_S'], 'Mean': [Islst['Diff'].mean()], 'St_Dev': [Islst['Diff'].std()], 't-value': [tv_sl[0]], 'p-value': [tv_sl[1]], 'df': [4]})

# quick sample
descrip='Whole Sample'

wslst=chart(vial, descrip)
wslst['Top']=wslst['Top']
wslst['Bottom']=wslst['Bottom']
wslst['Diff']=wslst['Diff']#/wslst['Bottom']
tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
df_total_Q = pd.DataFrame({'Group': ['Total_Q'], 'Mean': [wslst['Diff'].mean()], 'St_Dev': [wslst['Diff'].std()], 't-value': [tv_qu[0]], 'p-value': [tv_qu[1]], 'df': [4])

# create table for pseudofeces
# slow sample
descrip = 'Initial Sample'
Islst = ps_chart(vial, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom']
tv_sl = stats.ttest_1samp(Islst['Diff'], 0)
df_pseudos_S = pd.DataFrame({'Group': ['Psuedo_S'], 'Mean': [Islst['Diff'].mean()], 'St_Dev': [Islst['Diff'].std()], 't-value': [tv_sl[0]], 'p-value': [tv_sl[1]], 'df': [4])

# quick sample
descrip = 'Whole Sample'
wslst = ps_chart(vial, descrip)
wslst['Top'] = wslst['Top']
wslst['Bottom'] = wslst['Bottom']
wslst['Diff'] = wslst['Diff'] / wslst['Bottom']
tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
df_pseudos_Q = pd.DataFrame({'Group': ['Psuedo_Q'], 'Mean': [wslst['Diff'].mean()], 'St_Dev': [wslst['Diff'].std()], 't-value': [tv_qu[0]], 'p-value': [tv_qu[1]], 'df': [4])

# create table for algae (total - pseudofeces)
# slow sample
descrip = 'Initial Sample'
Islst = tot_chart(vial, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom']
tv_sl = stats.ttest_1samp(Islst['Diff'], 0)
df_algae_S = pd.DataFrame({'Group': ['Algae_S'], 'Mean': [Islst['Diff'].mean()], 'St_Dev': [Islst['Diff'].std()], 't-value': [tv_sl[0]], 'p-value': [tv_sl[1]], 'df': [4])

# quick sample
descrip = 'Whole Sample'
ws lst=tot_ch art(vial, descrip)
ws lst['Top']=ws lst['Top']
ws lst['Bottom']=ws lst['Bottom']
ws lst['Diff']=ws lst['Diff']#/ws lst['Bottom']
t v_qu=stats.t test_1 samp(ws lst['Diff'],0)
df_algae_Q=pd.DataFrame({'Group': ['Algae_Q'], 'Mean':[ws lst['Diff'].mean()], 'St_Dev':[ws lst['Diff'].std()], 't-value':[tv_qu[0]], 'p-value':[tv_qu[1]], 'df':[4]})

df_summary=pd.concat([df_total_S,df total_Q,df psuedo_S,df psuedo_Q,df algae_S,df algae_Q],ignore_index=True)

print(df_summary)

# Summary table in percentages

# create table for total particles
# slow sample
descrip='Initial Sample'
Islst=chart(vial, descrip)
Islst['Top']=Islst['Top']
Islst['Bottom']=Islst['Bottom']
Islst['Diff']=Islst['Diff']/Islst['Bottom']*100
tv_sl=stats.t test_1 samp(I slst['Diff'],0)
df_total_S=pd.DataFrame({'Group': ['Total_S'], 'Mean':[Islst['Diff'].mean()], 'St_Dev':[Islst['Diff'].std()], 't-value':[tv_sl[0]], 'p-value':[tv_sl[1]], 'df':[4]})

# quick sample
descrip='Whole Sample'
wst=chart(vial, descrip)
wst['Top']=wst['Top']
wst['Bottom']=wst['Bottom']
wst['Diff']=wst['Diff']/wst['Bottom']*100
tv_qu=stats.t test_1 samp(wst['Diff'],0)
df_total_Q=pd.DataFrame({'Group': ['Total_Q'], 'Mean':[wst['Diff'].mean()], 'St_Dev':[wst['Diff'].std()], 't-value':[tv_qu[0]], 'p-value':[tv_qu[1]], 'df':[4]})

# create table for pseudofeces
# slow sample
descrip='Initial Sample'
Islst=ps_chart(vial, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom'] * 100
tv_sl = stats.ttest_1samp(Islst['Diff'], 0)
df_pseudo_S = pd.DataFrame({'Group': ['Psuedo_S'], 'Mean': [Islst['Diff'].mean()], 'St_Dev': [Islst['Diff'].std()], 't-value': [tv_sl[0]], 'p-value': [tv_sl[1]], 'df': [4]})

# quick sample
descrip = 'Whole Sample'
wslst = ps_chart(vial, descrip)
wslst['Top'] = wslst['Top']
wslst['Bottom'] = wslst['Bottom']
wslst['Diff'] = wslst['Diff'] / wslst['Bottom'] * 100
tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
df_pseudo_Q = pd.DataFrame({'Group': ['Psuedo_Q'], 'Mean': [wslst['Diff'].mean()], 'St_Dev': [wslst['Diff'].std()], 't-value': [tv_qu[0]], 'p-value': [tv_qu[1]], 'df': [4]})

# create table for algae (total - psuedofeces)
# slow sample
descrip = 'Initial Sample'
Islst = tot_chart(vial, descrip)
Islst['Top'] = Islst['Top']
Islst['Bottom'] = Islst['Bottom']
Islst['Diff'] = Islst['Diff'] / Islst['Bottom'] * 100
tv_sl = stats.ttest_1samp(Islst['Diff'], 0)
df_algae_S = pd.DataFrame({'Group': ['Algae_S'], 'Mean': [Islst['Diff'].mean()], 'St_Dev': [Islst['Diff'].std()], 't-value': [tv_sl[0]], 'p-value': [tv_sl[1]], 'df': [4]})

# quick sample
descrip = 'Whole Sample'
wslst = tot_chart(vial, descrip)
wslst['Top'] = wslst['Top']
wslst['Bottom'] = wslst['Bottom']
wslst['Diff'] = wslst['Diff'] / wslst['Bottom'] * 100
tv_qu = stats.ttest_1samp(wslst['Diff'], 0)
df_algae_Q = pd.DataFrame({'Group': ['Algae_Q'], 'Mean': [wslst['Diff'].mean()], 'St_Dev': [wslst['Diff'].std()], 't-value': [tv_qu[0]], 'p-value': [tv_qu[1]], 'df': [4]})
df_summary=pd.concat([df_total_S,df_total_Q,df_psuedo_S,df_psuedo_Q,df_algae_S,df_algae_Q],ignore_index=True)

print(df_summary)

##from oystersize.ipynb
oys_density=2840.0 # number of oysters per liter
DTW=0.007443297435782392 # dry tissue weight (g) from lenght (mm)
#Establish bed hieghts from means, and std dev from Oyster_Hydro.ipynb
df_bh=pd.DataFrame({'T6': [279.142857, 4.947342],
                    'T7': [271.714286, 3.302236],
                    'T8': [280.857143, 2.544836],
                    'T9': [267.166667, 5.492419],
                    'T10': [286.333333, 6.186006]})
#Calculate oyster volume of each tube
vol_convert=np.pi*np.square(25.4)/1000000 #convert to liters
df_vol=df_bh*vol_convert
df_dens=df_vol*oys_density # number of oysters per tube
df_DTW=df_dens*DTW #g DWT/tube
df_DTW

#Flow rate within tubes from source water
rate_flow=60*3.7854118*0.5 # conversion from 0.5 gpm to L/hr
#apply to filtration rate
dff=Islst['Diff'].to_numpy()
df_flow=pd.DataFrame([dff],columns=['T6','T7','T8','T9','T10'])
df_flow=df_flow/100*rate_flow #total filtration rate of bed
df_clearance=df_flow/df_DTW
cNc=df_clearance.iloc[0,:].to_numpy()

print('mean clearance =',cNc.mean(), 'L/hr-g')
print('st dev =', cNc.std(), 'L/hr-g')
Appendix G- Sample FlowCAM Images

Example images from FlowCAM for Tube 7 using the quick sampling method.