ABSTRACT

Title of Thesis:	DETERMINING FEEDING RATES IN EASTERN OYSTERS (*Crassostrea virginica*) USING NATURAL SESTON FLOW-THROUGH SYSTEM
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Bivalves are prized for the ecosystem services they provide. The removal of particles from the water column through filter feeding and resulting water quality benefits, known as the biofiltration services, of bivalves have been studied for over a century. This has created a wealth of knowledge around the mechanistic drivers of bivalve feeding activity. Recently, Chesapeake Bay ecosystem-wide models have begun incorporating Eastern Oyster (*Crassostrea virginica*) biofiltration. Acute feeding variability is critically important when estimating oyster biofiltration services at ecosystem scale. Typically, natural seston clearance rate studies last a limited timeframe, with a focus on specific environmental events such as an increase in temperature, drop in salinity, or a tidal cycle.

To capture the highly variable filter feeding rate of bivalves, such as the Eastern Oyster, studies have used highly controlled laboratory conditions, with single environmental variable modification. These studies often use indirect methods for estimating clearance rates that

commonly lack high-resolution capability. Furthermore, these studies are labor intensive and time consuming, and as a result, few studies have monitored bivalve feeding activities over long periods to understand variation in activity or how these rates may change with seasonal shifts in conditions. These limitations have led to a shortage of knowledge around how clearance rates of oysters vary in response to ambient conditions over both short-term (hourly) and long-term (seasonal) time scales.

This study leverages advances in semi-autonomous aquatic observing to track high-resolution, long-term feeding responses of bivalves to subtle variations in environmental conditions. Oyster *ex situ* clearance rates in the Choptank River (Maryland, USA) were estimated under flow-through conditions, and logged in real-time using fluorometers among replicate oysters over 5-day experiments for 9 months. The measured clearance rates from this system were compared to a mechanistic clearance rate model used by the Chesapeake Bay Program, which is used to estimate the role of oysters in controlling water quality in the Bay. Environmental data were evaluated to build a statistical and random forest model to predict how oyster clearance rates respond to prevailing environmental conditions. This monitoring system and resulting models enable a deeper understanding of feeding variability and how natural seston and environmental variability directly influence oyster physiology.

DETERMINING FEEDING RATES IN EASTERN OYSTERS
(*Crassostrea virginica*) USING NATURAL SESTON FLOW-THROUGH SYSTEM

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2023

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Acknowledgements

This thesis research would not have been possible without the knowledge and support of my advisor, Dr. Matthew Gray. His direction and ideas made this system come to life. I would like to thank my committee members: Dr. Greg Silsbe for his support and influence throughout this study, and Dr. Vyacheslav Lyubchich for his countless hours of coding and statistical training. Thank you to the Horn Point Shellfish Aquaculture Innovation Laboratory which built the framework of my bivalve knowledge, offered much needed support throughout this study, and continue to provide friendship.

This thesis work was primarily funded by an award from the Ratcliffe Foundation as well as a fellowship from Horn Point Laboratory. Additional funding was provided by the Isaac Walton League of America, and the National Science Foundation. I am very grateful for the ability to conduct my research and grow my knowledge based on this funding.

An integral part of this research was developing my personal knowledge of plumbing, electrical, and the use of power tools. A very big tank you to the Horn Point AREL maintenance department, Ralph, Bear, and Chris for sharing their knowledge and patience during the development of this system.

Finally, I would like to thank my friends and family who have supported me throughout my years at Horn Point. Friends have been an essential part of making Cambridge feel like home. My parents have always been my biggest cheerleaders and I would not be where I am today without their guidance and encouragement. My dog, Franklin, was the best field tech I could ask for. He was always ready to accompany me for weekend and late-night trips to the lab. Last, but not least, I would like to thank my amazing husband, Kevin, for helping me pursue my dreams and placing roots wherever he is planted.

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Chapter 1: Review of Bivalve Feeding Measurements

Introduction

Oysters are considered ecosystem engineers due their transformative effects on the local environment and includes their suspension-feeding activity, which removes particles from the water column and can improve water quality (Dame et al., 2001). As suspended particulates are removed from the water column by oysters, the nutrients absorbed from the particles are sequestered into oyster tissues and shell, buried as waste products, (Locher et al., 2020), or may stimulate denitrification (Kellogg et al., 2013). As a result, many State and Federal Agencies, as well as NGOs, have advocated for oyster restoration as a key nature-based tool to help improve water quality (e.g., NOAA, Oyster Recovery Partnership, Chesapeake Bay Foundation), bringing greater attention to the strategy of biofiltration as a eutrophication mitigation strategy. These agencies and many stakeholders within the aquaculture community have also argued that the water quality benefits of oysters are conserved under culture conditions (Humphries et al., 2016), touting oyster farms as another tools against eutrophication.

Previously reported estimates of particle removal by bivalves at ecosystem scale are often based on models that parametrize animal feeding rates from maximum clearance rates determined in laboratory settings either by Jordon (1987) or collective values reported in Newell & Langdon (1996) (Newell et al., 2005; Cerco & Noel, 2007, Fulford et al., 2007). These models scale the maximum clearance rate as a function of the environmental conditions to calculate instantaneous clearance rate. However, bivalves in nature rarely encounter environmental conditions that elicit the maximal feeding activity observed in laboratory settings. In addition, Crandford et al. (2011) found that cultured algae as a food source resulted in higher clearance rates than natural seston diets, which consists of detritus and less nutritious suspended particulate. Therefore, basing a model on a maximum clearance rate constructed under near ideal conditions with cultured diets may lead to overestimating the clearance rate of *in situ* bivalves. This becomes especially apparent when scaling up feeding activity to populations of oysters at ecosystem level, as will be explored in chapter 2 of this thesis.

A clearance rate model scaled to the ecosystem level based on natural seston feeding studies has been done by Gobler et al. (2022). In this thesis, an ecosystem-wide model was created based on clearance and growth rates from previous studies of *M. mercenaria* fed natural seston in the Shinnecock Bay of New York (Hibbert, 1977; Doering & Oviatt, 1986; Grizzle et al., 2001). Relationships between weight and clearance rate were determined based on these clearance rate studies. The modeled allometric rates were proven to closely match empirical measurements taken by a second study (Wall et al, 2008) that also relied on natural seston concentrations. This example of an ecosystem wide clearance rate estimation model for *M. mercenaria* demonstrates how modelers could use previously reported clearance rates to examine ecosystem-wide biofiltration services, however, to parameterize a model such as this, robust estimates for clearance rates under natural seston conditions were required for the specific study organism. These data are lacking for the eastern oyster (*Crassostrea virginica*, Gmelin 1791) and therefore cannot currently be modeled for the Chesapeake Bay.

The physiological process of filter feeding relies primary on the gills for ingestion and sorting. In oysters, cilia along the gills create currents to capture suspended particles in the water (Newell & Langdon, 1996). These currents also allow the gills to sort and transport particles either into the mouth or reject them into the mantle. Newell & Jordan (1983) determined that oysters are able to distinguish between organic and non-organic particles and preferentially select nitrogen rich particles. Oysters will also regulate particle ingestion when the amount of seston is too high, such as in turbid environments (Newell & Langdon, 1996). Rejected particles are ejected from the mantle as pseudofeces. Selected food particles are transported into the mouth where they make their way through the digestive tract, and are expelled as feces.

Oysters are known to moderate feeding and close their valves throughout the day (Loosanoff & Nomejko, 1946) and this mechanism leads to high variability throughout diurnal and tidal cycles even under stable laboratory conditions. Currently, estimated clearance rates generally assume a constant or seasonal rate, mostly grounded in stable temperature conditions (e.g., Fulford et al., 2007; Cerco & Noel, 2007). This is due to high amount of human input needed to create long-term clearance rate datasets. However, it has been indicated that oyster feeding and clearance rates are correlated to environmental conditions, such as temperature, salinity, organic content, and turbidity, all of which fluctuate on varying time scales (Gray & Langdon, 2018). Dynamic estuarine conditions that are heavily impacted by human activity, such as those in the Chesapeake Bay, where this study is located, are both highly variable and may be frequently suboptimal for oysters (Kirby & Miller, 2005). Therefore, previous estimates of oyster feeding activity that fail to account for fine scale variability may produce biofiltration estimates that are grossly inaccurate when summing over long periods of time (month, season, etc.) and at ecosystem scale.

In this chapter, traditional methods for collecting bivalve clearance rates will be discussed. This analysis creates a justification for improved clearance rate methods. Finally, the objective of this thesis is to outline and utilize an improved clearance rate method to collect data at a short temporal scale across a long period of time. It should be noted that clearance rate and filtration rate are often treated as synonyms in the literature; therefore, clearance rate will be used hereafter. Filtration rate is measured as the mass of particulate matter removed over time (mg/hr), and clearance rate is measured as the volume of water cleared of particles per hour (L/hr).

Traditional Methods

Acknowledging the importance of feeding for the production of animals and the ecosystem services that extend from this activity, researchers have been seeking to determine the feeding rates of oysters for over a century (e.g., Nelson, 1923). Many methods to quantify the filtration and clearance rates of oysters have been developed and implemented over time. Estimating oyster clearance rates are measured directly by measuring volumetric flow of water processed by the animal or indirectly by measuring or monitoring a proxy with a known volumetric concentration (i.e., decline of algae due to feeding) that can serve to determine the processing rate of the animal.

There are many indirect methods and examining a few are important to illustrate common drawbacks to their use that prevent detailed or long-term observations of bivalve feeding activity to be collected. The static method involves placing an organism or group of organisms in an aquarium with a known concentration of algae and measuring its decline over time while accounting for settlement. The mean algal removal rate, reported as algal concentration (mg/L) removed per hour, is measured using light microscopy with a counting cell or electronic particle counters (i.e., Coulter Counter). The removal rate is assumed to be proportionate to the clearance rate or volume of water processed by animals per unit time. This method produces the mean clearance rate for bivalves as opposed to an instantaneous rate and therefore does not capture the variability in feeding between sampling points that is known to occur in the clearance rates of eastern oysters. In order to better capture variability, one could take more frequent measurements, however the measurements would only be as resolved as the frequency of measurements. Even with the aid of a particle counter, the time involved in collecting and processing the water samples would be still impractical and laborious for any experiment longer than a few hours.

The biodeposition method, another indirect measure of bivalve feeding activity, can be used when the concentration of algae is too high to measure using the static method (Gray & Langdon, 2018). This method uses gravimetric measurements of inorganic matter in biodeposits of oysters to estimate clearance rate. This method is useful it allows for long-term, *ex situ* monitoring of oyster feeding activity under flow-through condition; however, biodeposits must be collected and the resultant estimates of feeding are averaged between sampling points. Some automation to the biodeposition method has occurred (see Cranford and Hargrave, 1993), but this was accompanied by course sampling rates and feeding rate estimates. In general, a major drawback to the biodeposition method is labor requirements for biodeposition collection and processing, which has prevented its use for fine-scale estimates of clearance rates.

Direct method often uses the feeding current generated by the organism to determine the pumping rate, which is considered equivalent to the clearance rate when the retention efficiency of particles is 100% (Riisgård, 2001a). One method uses a barrier, usually a rubber apron, that is placed between two volumes of water with a bivalve seated at the barrier. The bivalve pumps water from one side of the barrier to the other and the difference in the volume over time is used to calculate the pumping rate of the organism. This method can only be used with classes of bivalves with well-defined exhalent siphons (i.e., mussels and clams), of which the eastern oyster is not. Criticisms of this method suggest that feeding may become distorted due to the influence of increasing hydrostatic pressure on the exhalent-side of the apparatus (Riisgård, 2001a). Additionally, the apparatus must be custom built, is limited to a single size-class of animal once constructed, and requires copious amounts of time and practice to use well. This method also involves a heavy investment of time, similar to the indirect method, and runs into similar sampling volume constraints that prohibit long-term sampling. Particle image velocimetry represents a relatively new and powerful way to track bivalve clearance rates (e.g., Du Clos and Jiang, 2018; Wang et al. 2020); however, the optical requirements to track particles and sensitivity of the equipment require copious amounts of preparation prior to measurement and do not readily lend themselves long-term studies.

In addition to the limiting conditions stated above, both the indirect and biodeposition method have commonly been performed under laboratory conditions due to the sensitive nature of the methods. Historically, simulating natural variability was considered too difficult to pursue at the short time scales to mimic tidal effects and other short timescale phenomena. Reproducing water quality variability, especially in terms of pH, in laboratories has improved in recent years (e.g., Gimenez et al., 2019; Keppel et al, 2016) and there have been studies done to compare *in situ* clearance rate measurements with those collected in the laboratory (Grizzle et al., 2008). However, the division between laboratory and field studies may be overcome using flow-through conditions with natural water, such as those described by Agüera et al. (2023) and in chapter 2 of this thesis. The indirect and biodeposition method, as well as emerging technological methods, will be the focus of the remainder of this chapter.

Overtime, methods for determining the filtration and clearance rates of shellfish have become more complex in tandem with advances in technology. Riisgård (2001a) provides an overview of many of the popular methods in the literature, which has been summarized in Table 1. Starting from the direct and indirect method, scientists have diversified methods to include technology such as fluorometers and automated dosing pumps. However, there are many more techniques that have been tested (see Table 1). Some of these methods have allowed for longer trials with more frequent measurements; however, introducing alterations to existing methods or developing new approaches to data acquisition may impair comparisons between studies, as Riisgård (2001a) highlights.

Table 1. Desciption, example of studies that have used the method, and pros and cons of common feeding rate methods

	Method	Description	Example Source	Pros	Cons
Direct	Direct Method	Leveraging the morphology of some bivalves to measure the volume of water intake and output of the organism directly from the siphon	Famme et al. (1986)	Produces a reliable averaged clearance rate under a variety of conditions	Cannot be done for bivalves without a siphon, only estimates averaged rate
	Particle Image Velocimetry	High-resolution particle tracking under highly controlled condition to estimate volumetric flow in to and out of animals.	Du Clos & Houshuo (2018) Wang et al. (2020)	Very high resolution and detailed observations	Studies are brief and require expensive equipment and sophisticated analysis to obtain a sing clearance rate
Indirect	Biodeposition Method	Gravimetric measurement of particulate inorganic matter in biodeposits over time that act as a tracer determine total volume of water processed by animal	Cranford & Hill (1999) Iglesias et al. (1998) Mitchell (2006)	Produces reliable removal rates under a variety of conditions Can be used at high turbidity levels	Only as resolved as the sampling rate High human input
	Ex Situ Flow-through Method	Flow-through feeding chambers with fluorometry or coulter counters before and after oyster exposure to determine volume processed by oysters	Vajedsamiei et al. (2021) Filgueira et al. (2006) Steeves et al. (2022)	Produces a reliable instantaneous clearance rate if automated under a variety of conditions	Dependent on the flow-through method Expensive equipment costs
	In Situ with Fluorometry Method	Water is measured using a fluorometer upstream and downstream of reef; difference in fluorometric reading used to estimate volume processed by reef	Grizzle et al. (2008) Hanson et al. (2011)	Can estimate reef-scale clearance rate, including epibionts that add to filtration services	Must been done on short time scales when flow is laminar Cannot control for confounding variables Low signal:noise ratio

Table 1 continued. Desciption, example of studies that have used the method, and pros and cons of common feeding rate methods

Indirect Cont.	Static, Clearance Method	Measuring the algal concentration in feeding chambers over defined intervals until a preset percent removal has been reached	Coughlan (1969) Widdows (1985) Casas et al. (2018)	Simple and reliable; provides averaged clearance rate under a variety of conditions	Only estimates averaged rate Cannot be used at high algal concentrations
	Steady-state Method	Dosing pump is used to keep aquarium at a constant algal concentration, with the rate of removal estimated	Vismann (1990)	Produces a reliable averaged clearance rate under a variety of conditions	Can only use monoculture diets with known algal concentrations

In an attempt to resolve previous misunderstandings and improve future studies, Riisgård (2001a) makes recommendations for taking filtration and clearance rate measurements, focusing on the basic assumptions that need to be met for each method to improve and create reproducible measurements. However, Riisgård's conclusions as to the basic assumptions for methods did come under debate by a fellow researcher in a comment written by Widdows (2001). Widdows (2001) claims that many of the faults to the methods highlighted by Riisgård (2001a) focused on problems with studies performed 30-40 years prior, before methods, such as the flow-through method, were refined. Specifically, the importance of flow rate for the flow-through method was mentioned. The flow rate is very important due to the impact of recirculation within the system, which re-exposes the animal to food, overestimating the clearance rate. Widdows (2001) states that the flow rate has been refined for flow-through systems and that studies utilizing different systems justify differences in flow rates across many studies. Widdows (2001) also claims that Riisgård dismisses studies that do not report the maximum clearance rate of bivalves under ideal conditions. Many studies are performed specifically under non-ideal conditions in order to measure the influence of certain environmental parameters, and therefore would not have maximum clearance rates at ideal conditions to report.

In a reply to Widdow's comment, Riisgård (2001b) responds to the lack of consistency in flow rates and suggests a study that refines the influence of chamber geometry and flow rate on feeding rates, which was ultimately performed by Filgueira et al. (2006). This study consisted of a systematic examination of chamber geometry and flow rates for *Mytilus galloprovincialis* to determine when bivalves hit each of the three stages related to clearance rate and flow: dependency phase, independency phase, and inhibition phase. These phases demonstrate that if the flow rate is too slow, clearance rate is dependent on the flow rate and therefore will not be preforming at its maximum. If flow rate is too fast, the system will be in the inhibition phase that causes the bivalve to not be able to capture particles and will once again not be able to perform at its maximum. However, if the system is able to keep a flow rate in the independency phase, the animal can clear water freely. This set the standard for flow-through studies to operate in the independency phase, and validates the wide range of flow rates that have been present in many flow-through studies as highlighted by Riisgård (2001a).

In relation to the lack of maximum clearance rates that was dismissed by Widdows (2001), Riisgard points to allometric models that have been used to determine feeding rates in the past. These equations require the maximum clearance rate to accurately scale clearance rates based on weight. However, with recent studies that aim to extrapolate from single organism clearance rates to ecosystem scale, the influence of environmental parameters on clearance rates becomes vital to the function of the model and therefore are no longer as dependent on the maximum clearance rates, as will be reinforced in chapter 2 of this thesis. With such a vast body of research at its foundation and debates on the validity of methods, choosing the most appropriate feeding rates to scale up during ecosystem model creation can become a daunting task, as outlined by Ehrich & Harris (2015).

Many studies that create oyster clearance rate models have the goal of predicting clearance rates based on previous experimentation (i.e., Cerco & Noel, 2007; Fulford et al., 2007; Powell et al., 1992). Laboratory studies are useful to tease out mechanistically how individual or multiple environmental factors influence feeding activity (Jordan, 1987; Baldwin, 1995; Gray & Langdon, 2018), but combining these effects into a mechanistic model has previously been shown to have poor predictive strength for describing how species feed under naturally varying conditions (Gray & Langdon, 2018). With the benefits and drawbacks to mechanistic models based on laboratory studies, many scientists in other fields have turned towards machine learning models that rely on larger conglomerations of data (Baker et al., 2018). Researchers interested in modeling feeding rates may find machine learning a useful tool for estimating clearance rates.

Machine learning is able to isolate important predictor variables from large datasets, whereas mechanistic modeling is based on the hypothesized effects of the parameters of interest (Baker et al., 2018). With the large amount of environmental data that has been collected in the Chesapeake Bay region, creating machine learning models that predict processes in the region is becoming a possibility (Maloney et al., 2022). Oyster biofiltration is one of the processes that could benefit from machine learning as oysters have a complex relationship between feeding rates and environmental conditions (Newell & Langdon, 1996).

Random forest models are considered a supervised machine learning method. In a random forest, many decision trees are combined to determine an output. These models alone are not able to determine causality, but they are able to capture more complex relationships than traditional linear models. In the past, large ecosystem models have used combined linear and non-linear relationships to represent ecosystem processes (Cerco & Noel, 2007; Elrich & Harris, 2015). Traditionally, machine learning cannot be used to determine causality, but using additional analysis and knowledge of the study system (Zhao & Hastie, 2019). Baker et al. (2018) suggests the pros and cons of mechanistic and machine learning models contrast each other and therefore may be able to be used symbiotically to model complex systems that include known and unknown effects. Baker et al. (2018) uses the example of clinical settings when researchers know the direct mechanistic relationship between medication and disease, but may not know how a patient's history may alter these effects. In the instance of oyster filtration services, the relationship between some environmental conditions may be easier to predict mechanistically, than others, such as the location of an organism.

Why we need improved methods

Despite a century of research and evolution of methods, more data are needed to better understand the variability of oyster feeding under natural conditions. Previous data from laboratory, field, and modeling studies have shown that oyster filter-feeding can influence water quality (Coen et al., 2007; Cerco & Noel, 2007; Kellogg et al., 2014), however the scope of this influence has been called into question due to varying estimates feeding rates (Gray & Langdon, 2018). Riisgård

(2001a) points to inconsistent and improper use of methods as a reason for contradicting rates. Even with varying rates under laboratory settings, clearance rate measurements from oysters functioning in the ecosystem, not under ideal conditions, are needed to continue research into the larger impacts of the biofiltration services of bivalves. Such as the case with ecosystem models.

Ehrich & Harris (2015) focused on refining the maximum filtration rate used in ecosystem-wide models based on previous filtration rate studies from a variety of methods, such as those listed in Table 1. The maximum rate they determined from a literature review was 0.17 (± 0.07) m^3/g Dry Tissue Weight (DW)/day or 7.08 L/hr/g DW. This maximum value is then scaled based on mechanistic equations generally determined under laboratory conditions. Laboratory experiments frequently put oysters in ideal conditions and fluctuate a single environmental to tease out the mechanistic response of organisms. Methods used in the laboratory, such as some indirect methods, are focused on calculating average clearance rates across the experimental period, due to the sampling rate of the method. An average rate could serve as the maximum clearance rate for a mechanistic model, but is not able to collect high-resolution clearance rates that capture variation for models that need many data points.

A specific organization that is interested in these models is the Chesapeake Bay Program (CBP). One of the goals of the CBP is to maintain and improve a modeling system that simulates and predicts biogeochemistry, oxygen concentration, water clarity, and chlorophyll *a* concentration in the Bay (Hood et al. 2021). The CBP uses the Cerco & Noel (2005) mechanistic model to determine the effects of oyster restoration on the bay. The specifics of this model will be highlighted later in this thesis, however the maximum clearance rate for the Cerco & Noel model is set to 22.9 L/hr/g DTW based on laboratory experiments done by Jordan (1987). Comparing this to those by Ehrich & Harris (2015) of 7.08 L/hr/g DTW, there is a 15.82 L/hr/g DTW difference. In general, any model trained on previously measured clearance data will predict a maximum clearance rate when scaling, however depending on how that maximum clearance rate was determined, in the laboratory or in the field, oysters experiencing ideal conditions will have extremely different maximum modeled rates. This difference in estimates becomes more problematic when scaling up models to predict volumes of water cleared by an entire reef or over long periods. This will be seen in chapter 2 of this thesis when the Cerco & Noel model is used to predict clearance rates. An improved consistent method that is able to capture the clearance rates of oysters at a high-resolution over long periods, would allow for biogeochemical models, such as the CBP ecosystem model to more accurately incorporate the biofiltration services of the eastern oyster or other important filter-feeding organisms.

In addition to the need for improved methods for modeling, there is also the existence of new technologies for sampling that can now be incorporated into feeding rate systems. One of the largest improvements in technology has been the shrinking size and cost of fluorometers and ease of using them via software that aids data logging and management. One of the first studies using fluorometers to determine clearance rates was Haure et al. (2003), with a single benchtop fluorometer taking clearance rate measurements every 16 minutes. Benchtop fluorometers tend to be expensive, and are limited in their sampling frequency due to the inherent limitations of the structure of the system for flow-through. This limited Haure et al. (2003) in the number of chambers, sampling frequency, and overall length of experiment. Vajedsamiei et al. (2021) overcomes these limitations by utilizing multiple hand-held fluorometers that collect data every second. Although still costly, advances in hand-held fluorometer technology have allowed for smaller designs that are able to sample at a much higher rate. Hand-held fluorometers are also able to connect directly to computers that can process data and streamline data analysis, making large datasets easier to manipulate. In addition, fluorometers have seen a general improvement in reliability over the decades since their inception (Zeng & Li, 2015).

To predict the impacts of filter-feeding bivalves on ecosystems, such as the economically and culturally important Eastern oyster in the Chesapeake Bay, scientists should be adapting and incorporating improved technology and measurements into data analyses. Feeding rate datasets are ripe for improving using new technology benefiting the larger bivalve community through increased capture of variability in bivalve feeding rates, which can then be incorporated into ecosystem-wide models.

How to Improve

To improve the accuracy of models that use experimentally defined clearance rate values in a range of naturally occurring environmental conditions, one must go back to the methods for collecting clearance rates. Recent technologies in ocean observing have opened up new avenues for semi-autonomous physiology measurements. Specifically, the use of fluorometry to determine the concentration of chlorophyll *a* in water has gone through innovation to take measurements on an increasingly finer scale, over longer periods of time, with smaller instruments (Zeng & Li, 2015).

Fluorometers measure concentration of the molecule chlorophyll *a*, which is involved in the process of photosynthesis, in order to estimate plant biomass in a sample. This method has been used to measure phytoplankton biomass in ocean ecosystems since 1966, but has gone through many innovations since the days of static sampling benchtop systems (Leeuw et al. 2013). Most importantly, *in vivo* fluorometers allow scientists to measure the concentration of chlorophyll *a* in a living cell without the use of preservatives. With this innovation, scientists could measure the *in vivo* concentration of chlorophyll *a* and infer the concentration of phytoplankton as frequently as the sensor is able to measure. This is particularly useful when measuring the instantaneous rate of removal of phytoplankton by filter-feeders.

Vajedsamiei et al. (2021) uses *in vivo* fluorometers for mussel clearance rate experiments, where individual feeding chambers are operated on flow-through with a single fluorometer assigned to each mussel. The fluorometers used in this experiment, Turner Instruments Cyclops 7F, measures chlorophyll *a* every second. Chlorophyll *a* concentration is used to calculate clearance rates using code provided by Vajedsamiei et al. (2021). A rapid and straightforward framework of processing and storing clearance rates across trials saves human input hours. Measuring clearance rates every second provides shellfish physiologist high temporal scale rates for experiments that can be run indefinitely.

Secondly, sondes that can measure the environmental parameters that are of interest to clearance rate models have become increasingly common. By employing sondes alongside experimental set-ups for feeding rates, the environmental data that is needed to parameterized clearance rate models can be easily collected. Accessible aquatic sondes make the environmental conditions of dockside *ex-situ* experiments that rely on raw seston concentrations easier to measure, and therefore allow environmental parameters more frequently included in studies.

Using available technology, such as *in vivo* handheld fluorometers and easily accessible sondes, feeding rate studies can be done at higher frequency over longer timeframes with less human input.

Thesis Objectives

The goal of this study was to monitor oyster feeding over long timescales (weeks to months) with high-resolution in order to 1) better understand the variability in feeding activity at various time scales and 2) understand the impacts of environmental conditions on oyster feeding rates. A semi-autonomous monitoring system was created so that causal relationships between oyster feeding responses and variations in biotic and abiotic conditions in an estuary may be teased out. This project aims to refine oyster clearance rate values under a variety of natural conditions as well as create and compare a statistical and machine learning model with a frequently used modeling method. Project data have several applications including offering a range of clearance rates under varying environmental conditions to update existing models of oyster filtration services and provide a more precise understanding about the ecological role of oysters in natural water bodies.

Chapter 2: Creation and Verification of FOFS

Introduction

Oysters are ecosystem engineers well recognized for the suite of services they provide, many of which have transformative effects over the local environment (Jones et al., 1994; Coen et al., 2007). Arguably, some of their most recognized services are those that extend from their filter-feeding activity, which includes biofiltration, biodeposition, and nutrient cycling (Dame et al., 1984; zu Ermgassen et al., 2013; Gray et al., 2019; Nelson et al., 2004). However, the filtering capacity of oysters has been decreasing largely due to loss of habitat (Beck et al., 2011). As result there have been numerous studies, such as those outlined in Chapter 1, that seek to measure clearance rates. These studies have also commonly sought to understand the environmental and endogenous factors that influence the processing rates of bivalves.

There are numerous approaches to measure the clearance rates of oysters and other bivalves that include the static clearance method and biodeposition method, as outlined in chapter 1. Perhaps the most common way to collect clearance information is using the static system clearance method described by Coughlan (1969). This static method typically involves measurements of water samples processed with a particle counter over a defined amount of time to quantify the depletion of algae in the suspension. The biodeposition method, which estimates the total volume of water processed by the animal by gravimetrically measuring that particulate inorganic material (PIM) found in the pseudofeces and feces and accounting for PIM in the water column during the study period. This is a powerful method that enables the calculation of other physiological rate processes, including ingestion, absorption, and egestion. Although these two methods are comprehensive, they both suffer from lack of fine temporal scale measurements due to the amount of human input required for fine-scale data collection. Due water or sample processing constraints, these methods also are easiest to employ under controlled laboratory conditions.

Laboratory feeding experiments with constant seawater conditions generally vary a single environmental parameter, such as temperature, salinity, or total suspended solids (TSS) (Loosanoff, 1953; Loosanoff, 1958; Suedel et al., 2015). This is appropriate when examining the mechanistic effects of environmental factors on clearance rate (Jordan, 1987). However, *in-situ* oysters can experience brief fluctuations in several environmental parameters simultaneously (Gray & Langdon, 2018), such as during a summer rain event which delivers in a cool and fresh water influx.

A common constraint to long-term or high-frequency monitoring of bivalve clearance experiments has been the dependence on manual labor to take samples and process data. For example, there are few examples of seasonal studies due to the need for laborious measurements, inhibiting understanding of how clearance rates under natural conditions vary overtime and at seasonal scales. Cranford & Hill (1999), represent one of the few studies that used the biodeposition method to monitor the daily feeding physiology of blue mussels (*Mytilus edulis*) and giant scallops (*Placopecten magellanicus*); however, their approach integrated and averaged feeding metrics over each day, providing no information about feeding variability within a given day. Indeed, most methods currently in use estimate an average clearance rate rather than providing instantaneous measurements, so highly resolved variability feeding information is lacking for many ecologically important species.

Previous studies that contain highly resolved data are frequently focused on specific environmental influences on clearance rates (e.g., Domnik et al., 2016; Vajedsamiei et al., 2021; Suedel et al., 2014; Casas et al. 2018). These studies are able to demonstrate that single environmental parameters, such as temperature, TSS, and salinity, affect feeding ability. Occasionally, two parameters are modified in one study (i.e., Hutchinson & Hawkins, 1992; Casas et al., 2018). However, these experiments generally rerun trials with one continuously modified parameter and one parameter modified in a step-wise fashion, such as with Moullac et al. (2007). This emphasizes the need to collect highly resolved data with multiple varied parameters, such as in an *ex-situ* environment.

Peterson et al. (2004) compares three methods, the indirect, biodeposition, and flow-through, with mussels finding that the biodeposition method tends to underestimate clearance rates compared to the indirect and flow-through method. Peterson et al. (2004), also found that mussels from different regions have different clearance rates, but was not able to find a direct cause for the differences in clearance rates. This study highlights the importance of choosing a method that reliably measures clearance rates.

In chapter 1 of this thesis, the need for highly-resolved, long-term studies in natural conditions were emphasized to increase knowledge of clearance rates under riverine conditions. To build on this knowledge, large-scale ecosystem models could benefit from the inclusion of the biofiltration services of oysters and other bivalves in their calculations, and determine the influence of filtration services. Of particular interest is the mechanistic oyster feeding model developed by Cerco and Noel (2005) for the Chesapeake Bay Program. This model combines a series of environmental response functions of oysters to water temperature, salinity, total suspended sediment, and dissolved oxygen and estimates oyster feeding over time. The Cerco & Noel model is used by the Chesapeake Bay Program to estimate the role of oyster restoration for improving water quality. However, the maximum feeding rates and the general strength of this approach have been called into question (Wheeler, 2020; Gray and Langdon, 2018) because they are inherently not able to capture complex relationships between predictor variables (Baker et al., 2018). The model was presented in a report in 2005 (Cerco & Noel, 2005), and then published in a peer reviewed journal in 2007 (Cerco & Noel, 2007). The 2007 publication will be referenced for the remained of this chapter.

Therefore, this study aimed to adapt a previously published Fluorometer and Oximeter equipped Flow-through Setup (FOFS) method as described by Vajedsamiei et al. (2021) that collects highly resolved, instantaneous clearance rate measurements of bivalves under flow-through, *in situ* conditions, and logs and stores the data autonomously. The system was applied to measure oyster feeding rates on the

Choptank River from August, 2022 to April, 2023. The goal of this research was to improve resolution of oyster feeding rates and determine environmental drivers of feeding activity in this system. Secondly, the observed rates of feeding were compared to estimates provided by Cerco and Noel (2007) to test the power of this model to predict oyster feeding activity in the Choptank River. Additionally, since this project employed a novel approach to monitoring oyster feeding activity, a tertiary but overarching was to understand and evaluate the efficacy of FOFS for long-period use in this system.

Methods

The purpose of this study is to adapt the FOFS as described by Vajedsamiei et al. (2021) to make near-continuous measurements of oyster feeding activity over long time scales. Second, the FOFS was co-deployed alongside a suite of environmental sensors so that feeding activities may be statistically related to prevailing environmental conditions. The FOFS monitored three oyster feeding chambers and one chamber with an oyster shell (control) continuously during 5-day intervals spanning August, 2022 to April, 2023. At the end of each trial, oysters were sacrificed for allometric data and replaced with new oysters at the commencement of the next trial. FOFS data were analyzed by adapting code provided in Vajedsamiei et al. (2021) and used to create a model that estimates oyster clearance rate as a function of water quality parameters.

Site Description

The FOFS was assembled at the end of a 102-meter pier located at Horn Point Laboratory on the southern side of the Choptank River (Maryland, USA; Figure 1). The Choptank River is a mesohaline tributary on the eastern side of the Chesapeake Bay with an average salinity of 14.33 ppt during the experimental period. The NOAA National Data Buoy Center (NDBC) monitoring station, 44062, is located within 25 km of the study site.

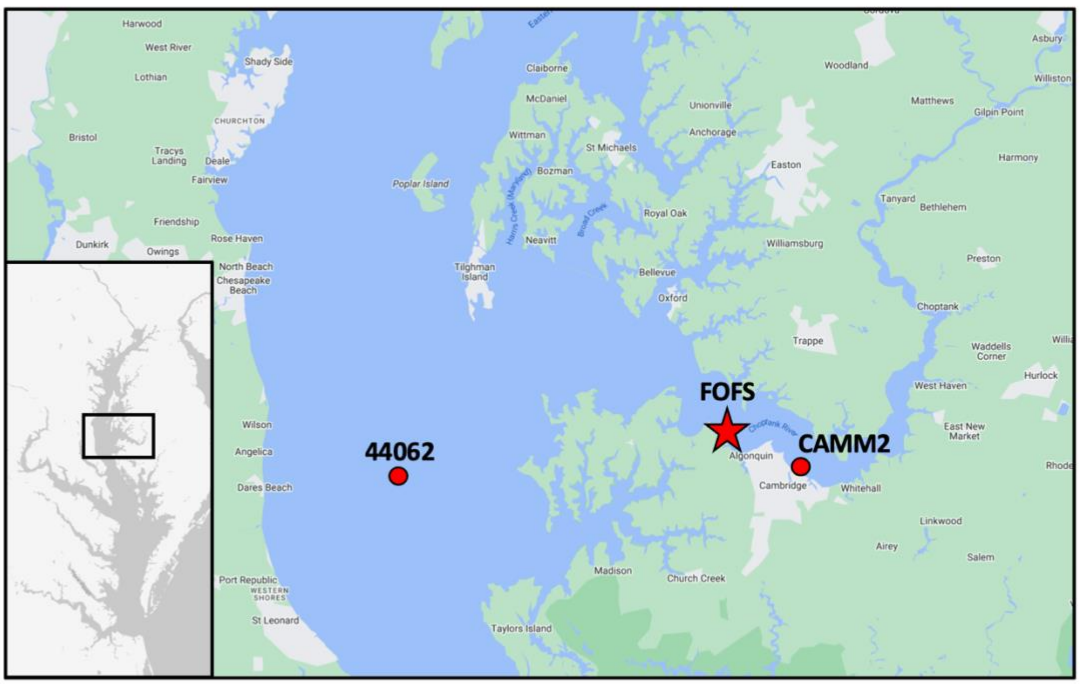


Figure 1. Map of the Choptank River and Chesepeke Bay (inset) with the location of the FOFS marked with a star and the locations of two NOAA NBDC stations marked with circles

Experimental Organisms

Diploid eastern oysters were acquired from The Choptank Oyster Company located in Cambridge, Maryland on May 11th, 2022. The 150 oysters ranged in length from 65 mm to 90 mm to meet the approximate range for market size oysters (i.e., 76 mm). The 150 oysters were split into two groups that became “indoor” and “outdoor” oysters (n indoor = 50; n outdoor = 100). Indoor oysters were held in the laboratory and exposed to raw river water under flow-through conditions at Horn Point Laboratory. These oysters were used primarily for preliminary laboratory based FOFS experiments (February 1st, 2022 - July 31st, 2022), but were used during the first three months of outdoor FOFS trials (August 1st, 2022 - November 1st. 2022). Outdoor oysters were spread across three 17 mm mesh bags and placed in the Horn Point Laboratory boat basin. On November 1st, 2022 the two populations were combined and placed in a single 17 mm mesh bag hanging from the Horn Point Laboratory setting pier.

Oysters were prepared for trials by gently washing the shells to remove sediment and epibionts (e.g., such as barnacles)., Oysters were then placed in a salt brine bath for 15 minutes, then allowed to air dry for at least one hour to remove *polydora* worms as described in Biofouling Control Strategies by Hood et al. (2020). During the one hour drying period oysters were marked with an identifying number using nail polish.

Length, width, height, dry tissue, and shell weight data were collected within one week of the end of each experiment for each oyster. Oysters were either removed immediately from the FOFS to be measured and shucked, or placed in a 1°C refrigerator for less than 7 days and measured and shucked at that time. Shucked oyster tissue and shell were separated with tissue placed on a metal weigh boat and dried in a 60 °C oven for at least 24 hours or up to 7 days. Shell was left in a dry environment for at least 24 hours or up to 7 days. The weight of the dry tissue and shell was collected at the end of the drying period by subtracting the known weight of an average metal weigh boat (2.22 g +/- 0.03). The condition index of the oysters was calculated using the dry tissue weight and dry shell weight equation described by Rainer & Mann (1992) (Equation 1):

$$Condition\ Index\ =\ Dry\ Soft\ Tissue\ Weight\ (g) *\ 1000/Dry\ Shell\ Weight\ (g)$$

(Equation 1)

FOFS Overview

The FOFS consists of organism feeding chambers with coupled oximetry and fluorometry sensors to measure the clearance and respiration rates of organisms. Turner Cyclops 7F Fluorometers located at the outflow of the feeding chambers measured the amount of chlorophyll a (chl a) being removed by the organism (

Figure 3). Using the flowthrough clearance rate method described by Riisgård (2001a), chl a concentrations were used as a metric for phytoplankton abundance. Clearance rate, or the volume of water oysters are able to clear of particles, was calculated using the difference between chl a in the control chamber and each experimental chamber multiplied by the flow rate of the system (Equation 2).

$$Clearance\ Rate\ \left(\frac{L}{hr}\right) = Flow\ Rate\left(\frac{L}{hr}\right) * (1 - \frac{Experi.Chamber\ Chl\ a\ \left(\frac{\mu g}{L}\right)}{Control\ Chamber\ Chl\ a\ \left(\frac{\mu g}{L}\right)})$$

(Equation 2)

Each trial ran for approximately five consecutive days. Trial length and frequency was dependent on biofouling conditions with an average trial length of 4.4 days. Each chamber was sealed with no air present at the initiation of the trial. The flow rate of the variable speed peristaltic pump (Golander Pump, BT100S-1 Basic Variable Speed Peristaltic Pump) was kept consistent at 7.9 L/hr in order to provide an adequate amount of food, but not so high as to inhibit filtration (Filgueira et al., 2006).

As mentioned in chapter 1 of this thesis, flow rate within a flow-through system is very important and can cause the method to over and under estimate the clearance rate. Previous studies for clearance rates of oysters in similar water body conditions to the Choptank River, estimate an average clearance rate of 9.03 L/hr/gram (g) at salinity of 15 ppt (Casas et al., 2018). The Choptank River's salinity is slightly lower than 15 ppt, at an average of 14.3 ppt across the study period, therefore the system flow rate was set as close to 8 L/hr as possible.

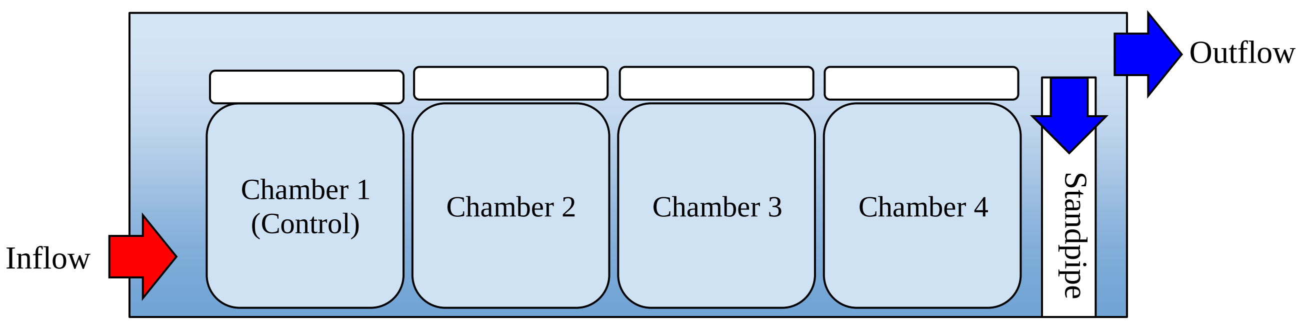
Water Bath

River water was pumped into a 33.2 L custom-built container at 400 ± 50 L/hr using a compact submersible pump (Little Giant 3E-34N), with copper mesh covering a PVC extension, used to increase surface area, on the inflow to avoid introduction of macro-organisms into the system (Figure 2). Water entered the bath at the lower portion and flowed out through a standpipe located in the downstream end of the water bath to ensure water moved across the chambers (

Figure 3). A second outflow was located in the upper right corner for to help prevent overflow.

Figure 2. Photo of inflow pump (blue) with PVC and copper mesh attachment

Figure 3. Diagram of FOFS water bath set-up with the inflow of water from river shown in red, and outflow of water from the bath shown in blue



In order to ensure the temperature of the water bath was similar to the river, Onset HOBO TidbiT MX Temperature 400' Data Loggers were placed separately in the river next to the pump and in the water bath. These sensors collected temperature data every 10 seconds for 24 hours as shown in Figure 4. A linear regression between the river and tank temperature show agreement between the two sensors was strong during the trial (r = 0.99, slope = 0.95, intercept = 0.92) confirming temperatures in the water bath reflected those in the Choptank River (Figure 5).

Figure 4. Comparion of the HOBO sensor temperature reading every 10 seconds for the Choptank River and the FOFS water bath over 24 hours

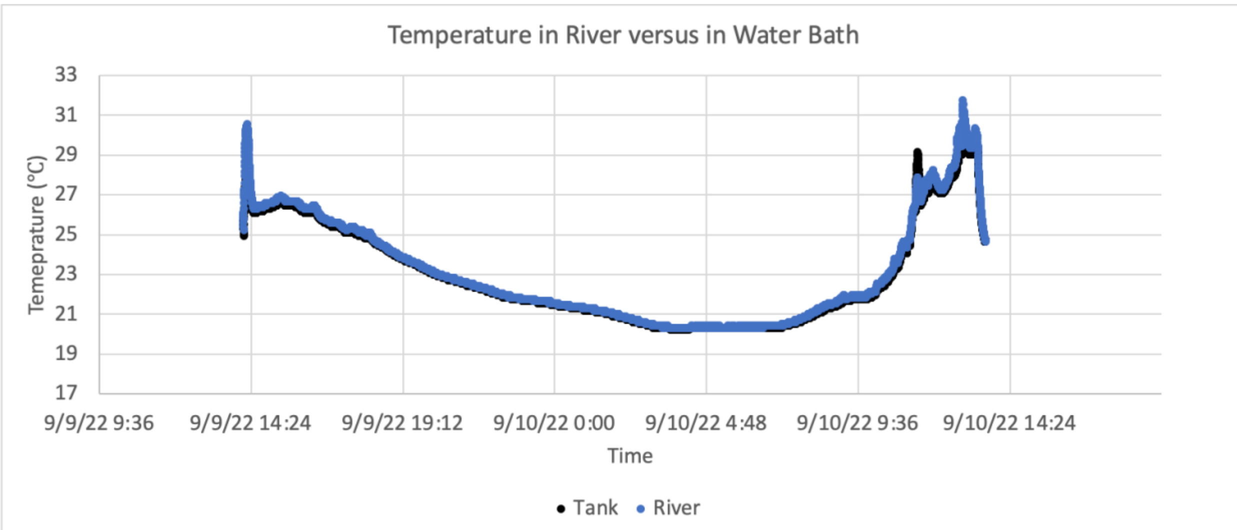
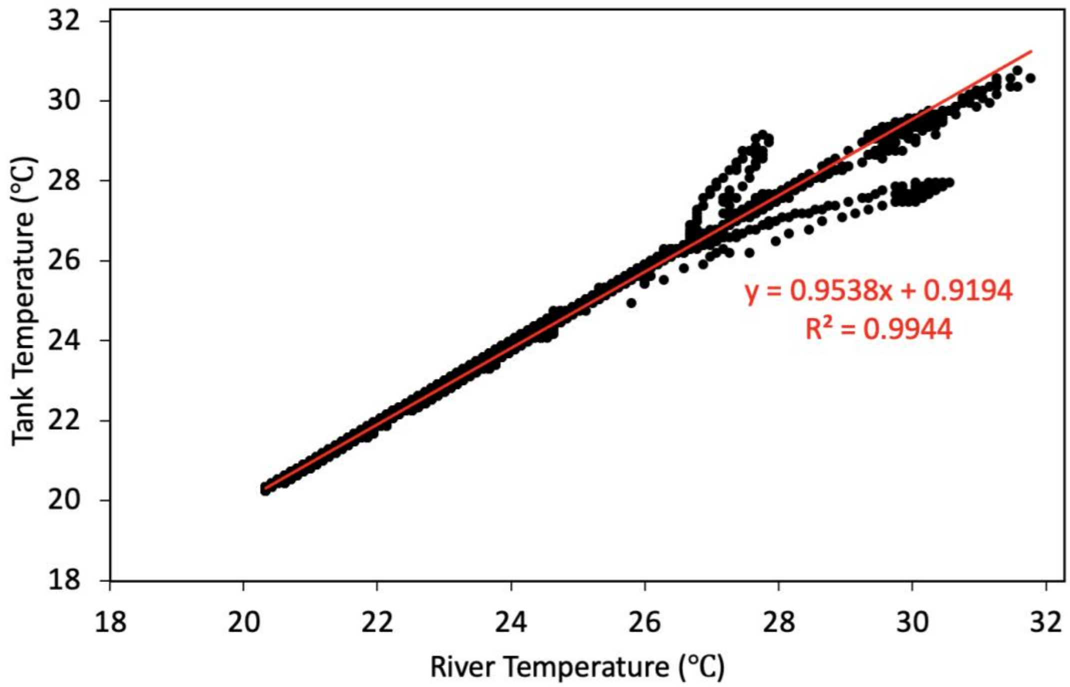


Figure 5. Relationship between Choptank River temperature and the water bath (Tank) temperature, with linear regression in red

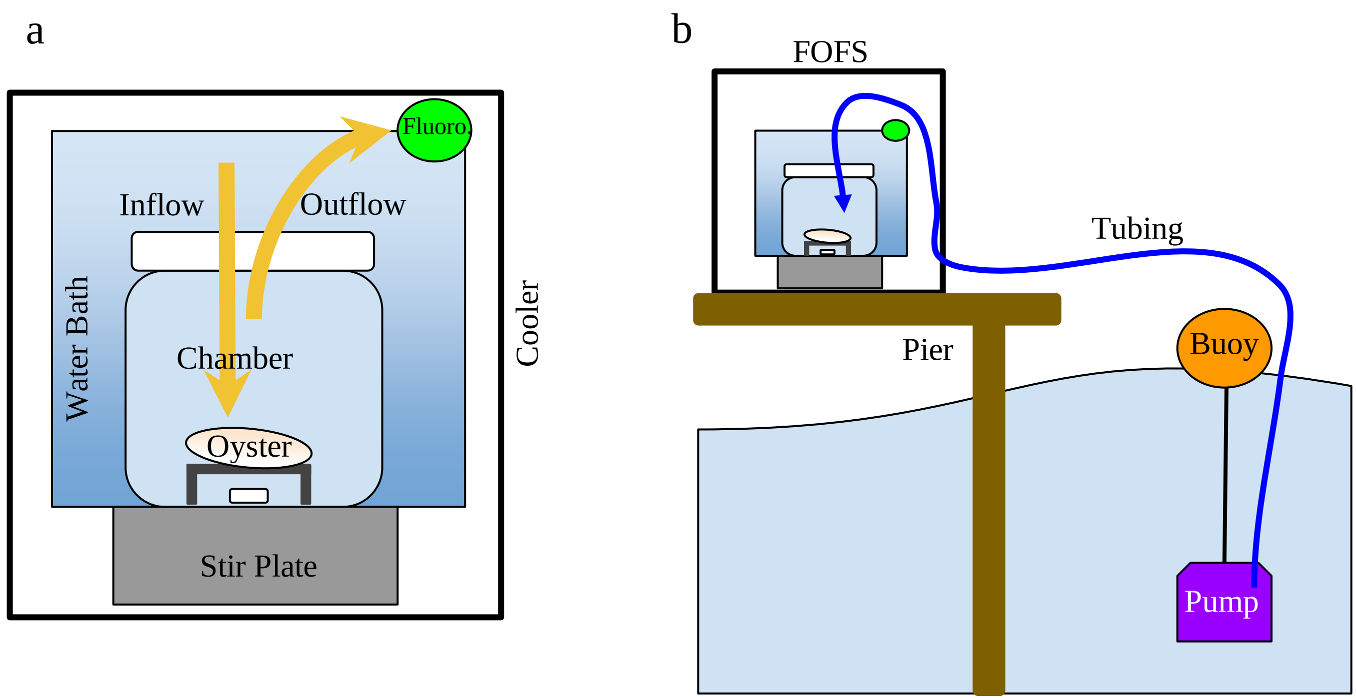


Chambers

Four 1.9 L glass wide mouth mason jars were used as mesocosm to simulate natural seston and oxygen conditions of the river. These chambers contained a stir bar and were placed in the water bath above magnetic stir plates (Hanna Instruments) to avoid settlement of particulate matter. Chambers contained a 17 mm plastic mesh "seat" for either the oyster or an empty oyster shell (control). An oyster shell was placed in control chambers to act as a substrate and account for bacteria and biofouling material that was not removed during the mitigation strategies outlined above or may accumulate on oysters during trials, which might influence feeding rate and respiration rate measurements. Oysters were placed with the right valve placed down to allow for filtration. Water was pumped into the system through 3.2 mm tygon tubing located through a hole in the plastic lid of the chamber. Inflow tubing extended to the bottom of the chamber to allow for new river water to be introduced to the oyster. Water exited through 3.2 mm tygon tubing located at the top of the chamber in order to allow for full circulation of water in the chamber. Outflow from each chamber was pumped through 30 cm of tubing to reach the fluorometer flowthrough cell.

The use of a control chamber informs the initial chl *a* available in the river, but also accounts for loss of chl *a* due to settlement of suspended particles in the system. Stir bar speed was set to be approximately 500 rpm and kept consistent across all chambers to mitigate settlement of particles.

Figure 6. (a) Side view of the FOFS feeding chamber in water bath with the flow of water in and out of the chamber (b) FOFS inflow pump into the water bath on the pier



Clearance Rate Measurements

Turner Instruments Cyclops 7F fluorometers were used in conjunction with the Turner Flowthrough cap to collect chl *a* concentration data for each second. These four fluorometers were located immediately downstream of the experimental chambers as shown in Figure 6a. Each fluorometer measures the visible spectra of fluorescence within the flowthrough cell every second as millivolts (mV). These measurements are communicated to a computer using Turner Instruments Explorers and output as .csv files using the Explorer software. To determine chl *a* concentration, calibrations were performed every three months using the RWT 400 ppb direct concentration calibration method outlined in the Turner Instruments Cyclops 7F user manual. The correlation coefficients were incorporated into R code that processed the raw .csv files to convert mV to chl *a* (mg/L) at each second.

During warm weather trials, fluorometer sensor faces were cleaned every other day to remove biofouling. This involved removing the fluorometer flow-through cap, using a Kimwipe on the sensor face and in the flow-through cap, then replacing the cap. The need for this cleaning procedure was reduced to every two days during cold trials due to reduced biofouling pressure. The timing of this transition was based on known biofouling trends, generally coinciding with the colder water temperatures in the fall, and the first barnacle set in the spring. In between trials, the FOFS was rinsed with 5% bleach solution to avoid biofouling growth on equipment surfaces. Tubing was replaced every four trials, or when visual biofouling became present.

Outliers in fluorometer readings were removed, including when the fluorometer sensor faces were cleaned throughout the experiment. Any calculated clearance rate greater than 20 L/hr/g or less than -0.5 L/hr/g were removed as well as 60 seconds before and after the outlier point. The lower threshold of -0.5 L/hr/g was determined by plotting a relative frequency histogram to find where 50% of all negative clearance rates (Figure 7). All negative values between the threshold of -0.5 and 0 were adjusted to 0. Negative rates were not excluded completely from the dataset based on previous experiments that determined that feeding chambers do have some inherent differences in chl *a* concentration, even when empty (Figure 8). Although no statistical significance was present between empty chambers, the average standard deviation in chl *a* among the four chambers across this trial was 0.92. The process of correcting for negative clearance rates removed 45.3% of data points. The average clearance rate of all three chambers at each second was calculated and is hereafter referred to as the “chamber average”.

Figure 7. Relative frequency of negative clearance rates measured based on the average of the chambers

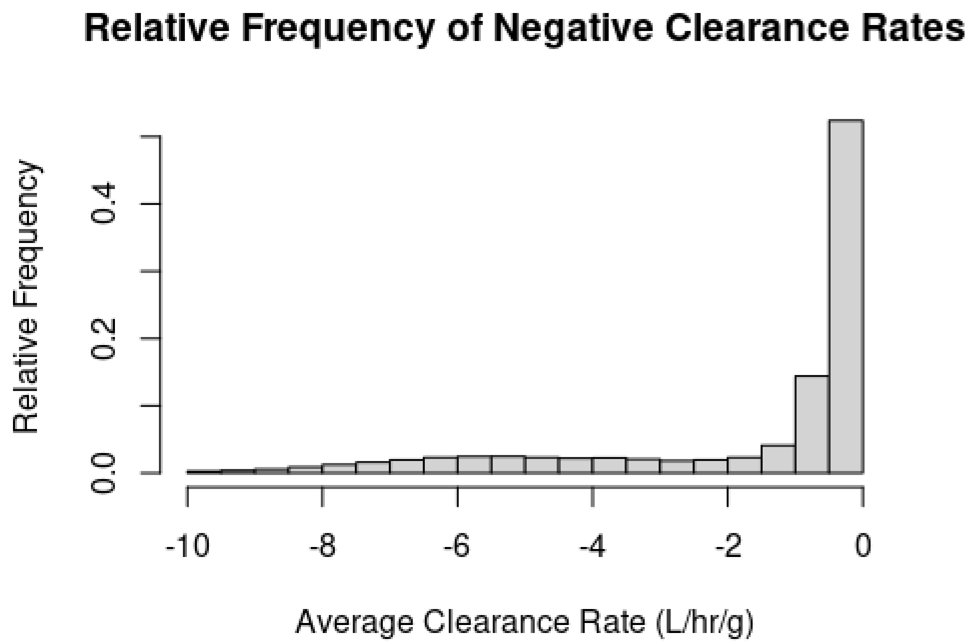
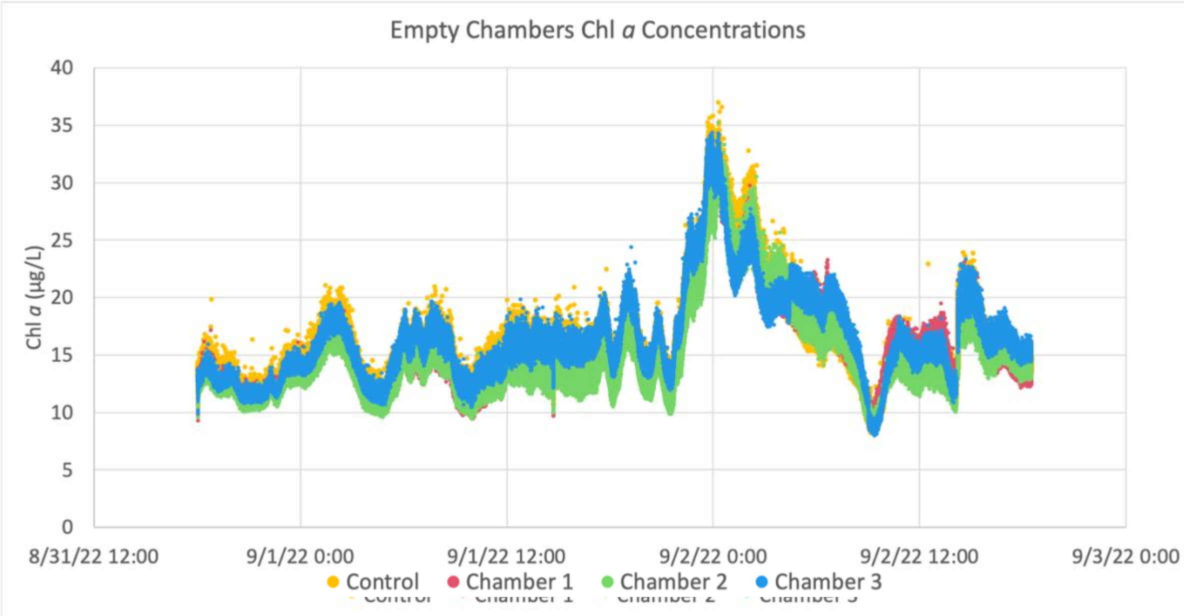


Figure 8. Chl *a* concentrations every second in three empty feeding chambers chambers for 24 hours



Labor requirements of the system generally consisted of three hours at the start of each trial to prepare the study organism and preparation of the FOFS. In-person checks were conducted daily around 10:00 to confirm system was functioning. Daily checks took approximately 10 minutes and included cleaning of sensors, removal of air bubbles in tubing and in the flow-through cap, and any water quality sampling. Takedown at the end of the trial required approximately two hours and consisted of cleaning the FOFS system, collecting oyster measurements, and uploading data into the system database. Resulting in approximately six man-hours per 5-day trial. This does not include time for troubleshooting, which occurred periodically and was driven largely by equipment failure.

Troubleshooting scenarios included, inflow pump failure as a result of water entering the electrical lines, and overloading of GFI outlets. Troubleshooting also involved determining the timeframe for changing out replaceable parts within the system, such as the silicone peristaltic tubing and stir plates. The FOFS was transitioned from an enclosed outdoor structure, housing additional sensor instruments, into the large insulated cooler that was used throughout the experimental period. This involved time for troubleshooting the new pumping within the system and determined to be a better fit for the system.

Environmental Monitoring

To measure environmental parameters of the study site, multiple environmental sensors were used in conjunction. Initially, the parameters collected were based on the environmental parameters that were used by Cerco & Noel (2007) (i.e., temperature, salinity, dissolved oxygen, TSS) to ensure that clearance rates observed as well as any resultant model that was developed in this study could be compared to their estimates. Additionally, we collected chl *a* data as it is commonly used as a proxy for food in many bivalve feeding studies. NOAA National Data Buoy Center (NDBC) station data, when available, was used to confirm accuracy and provide a historic context. Units were calibrated to comparable units using laboratory methods when possible, such as the case with turbidity and chl *a*.

Historic Data

NOAA NDBC Station 44062 is 25 km downstream of the Horn Point Lab setting pier and located in the mainstem of the Chesapeake Bay. This station has been collecting meteorological data since 2010 and oceanographic data since 2012. The oceanographic data includes water temperature (℃), salinity (ppt), dissolved oxygen (ppm), turbidity (FTU), and chl *a* (μg/L). Water quality at Station 44062 was not expected to be similar to water at Horn Point, which was monitored with a multiparameter water quality sonde (AquaTROLL, In-Situ® Colorado, USA); however, Station 44062 data were used to analyze historical data in order to determine how the experimental period compared to previous years. Oceanographic data from the NBCD website from January 2012 to March 2023 were analyzed using R code to determine the 10-year average and standard deviation (January 2012-March 2022), which also included data that overlapped the experimental period (i.e., August 2022-March 2023). Months not included in the experimental period, April-July, were excluded from the 10-year average to accurately compare the 10-year averages to the experimental period.

As shown in Table 2, the mean for each parameter within the experimental time frame were within one standard deviation of the 10-year mean for all parameters. These data suggest environmental conditions, except dissolved oxygen, throughout the experimental period were not unusual and similar to those in the Choptank River for the past decade.

Table 2. Ten-year average environmental parameter data from NOAA NBDC station 44062 from 2012 to 2022; * denotes when the experimental period mean was within one standard deviation of the 10-year mean

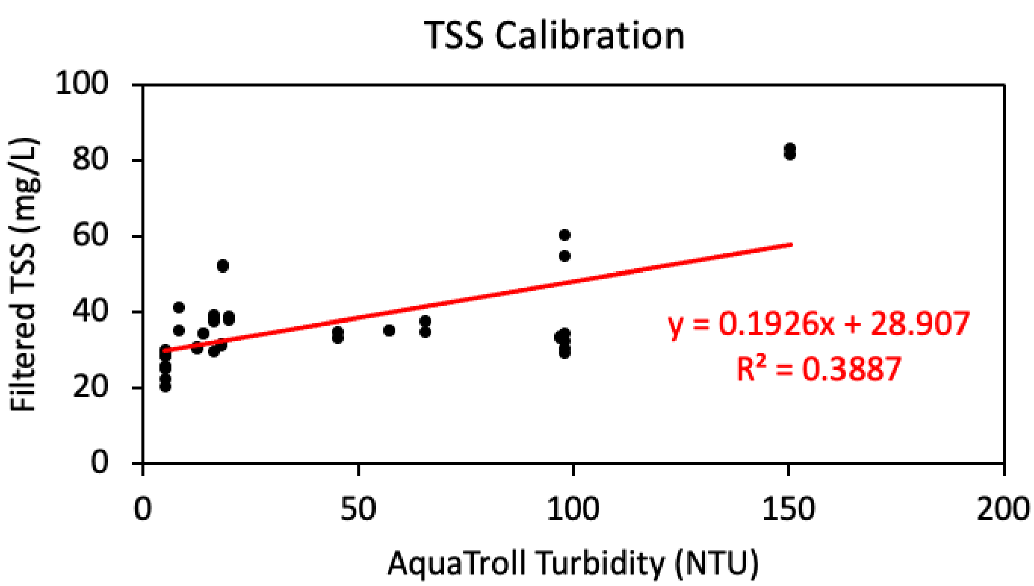
Parameter	10 Year Mean ± SD	Experimental Period Mean
Temperature (℃)	15.8 ± 8.9	12.52 ± 8.01*
Salinity (ppt)	13.6 ± 2.7	13.89 ± 0.81*
Dissolved Oxygen (ppm)	9.6 ± 2.2	11.69 ± 2.44*
Turbidity (FTU)	40.0 ± 44.4	26.38 ± 26.17*
Chl *a* (µg/L)	6.0 ± 6.5	11.95 ± 4.75*

Insitu AquaTROLL

An AquaTROLL 600 Multiparameter Sonde (In-Situ Inc.; Fort Collins CO, USA) was deployed beside the river inflow pump to collect environmental parameters alongside the FOFS. The AquaTROLL sensor collected temperature (℃), salinity (ppt), dissolved oxygen (mg/L), turbidity (NTU), and chl *a* (RFU) at one-minute intervals. These data were downloaded from the device at the end of each trial and compiled for the experimental period using R code. Measurements that required calibration to correlate to the units used by Cerco & Noel (2007), such as turbidity and chl *a*, were calibrated following methods from the manufacturer.

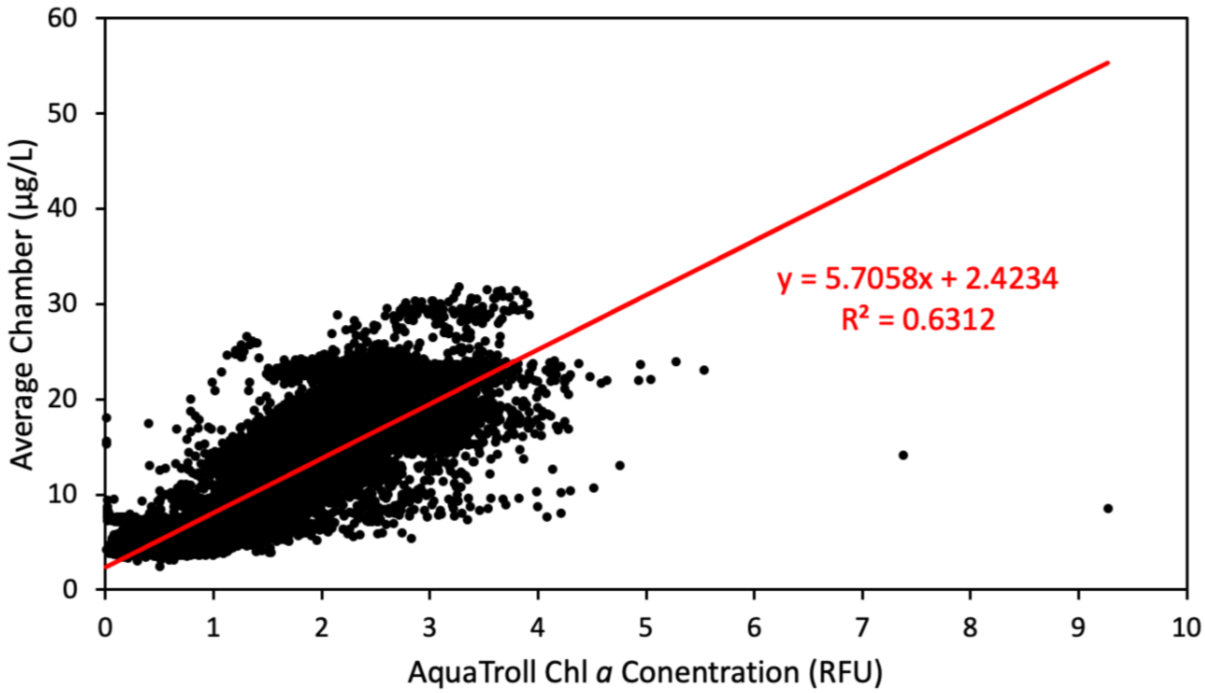
From turbidity, measured in NTU, total suspended sediment (TSS) was determined gravimetrically with water from the outflow of the water bath chamber was filtered onto pre-weighed microfiber filters (GE Whatman RTU) 3 days a week from August to September, and once a week from October to December. These filters were placed in a 60 °C drying oven for at least 24 hours, then weighed to determine the TSS concentration of the river. These TSS data were used to create a calibration curve between the AquaTROLL sensor and TSS from grab samples. The calibration equation obtained using a linear regression of the AquaTROLL turbidity measurements and the filtered TSS measurements was used to calculate the TSS throughout the experimental period (Figure 9).

Figure 9. Calibration curve for AquaTROLL turbidity reading (NTU) to Filtered TSS (mg/L)



Chl *a* is commonly collected in raw fluorescence units (RFU), however these units are relative, so calibration to units that represent total concentration of chl *a* is needed. A linear regression was used for the chl *a* (µg/L) concentration in the control chamber and the chl *a* (RFU) measurement from the AquaTROLL from August 3rd to October 14th, 2022 (Figure 10). This linear regression equation was used to convert the AquaTROLL chl *a* measurement in RFU to µg/L. As The calibrated chl *a* concentration in µg/L will be used for the remainder of this thesis.

Figure 10. Calibration curve for AquaTROLL Chl a Concentraiton (RFU) to Chl *a* concentration (µg/L)

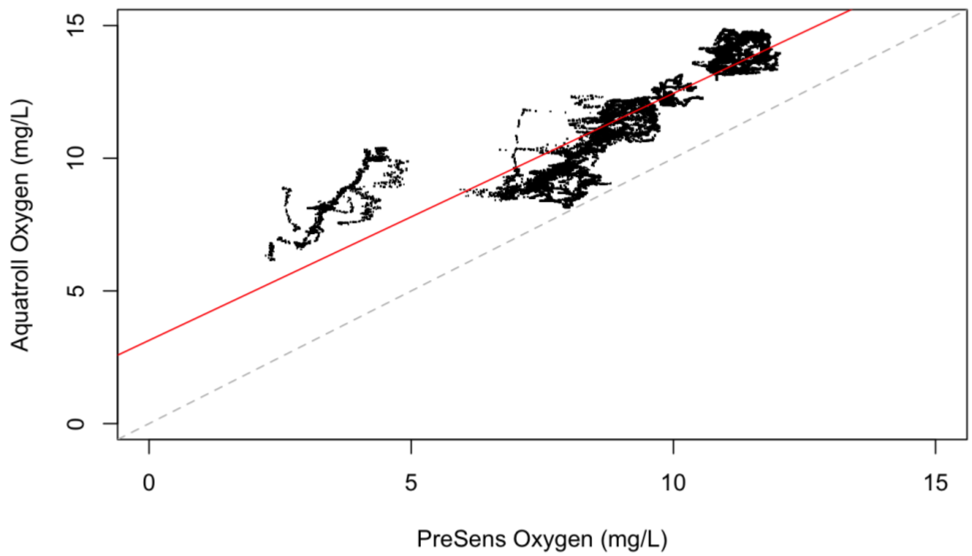


PreSens Oxygen Sensors

In each feeding chamber, a PreSens Oxygen Sensor Spot was mounted to estimate the respiration rate of oysters by subtracting the oxygen concentration of each experimental chamber from the control chamber and multiplying by the flow rate. By using the control as the baseline, the effects of microbial respiration can be accounted for. However, these data will not be further discussed in this thesis.

Dissolved oxygen measurements from these sensors were collected every 3 seconds through fiber optic cables and compiled via PreSens Measurement Studio. These data were compared to the dissolved oxygen data collected by the AquaTROLL sensor. All AquaTROLL DO measurements of zero were removed from the dataset (n=2160) and a linear regression was preformed between the PreSens control chamber and the AquaTROLL in the river (Figure 11). The slope of the regression (0.93) shows that there is a close to 1:1 relationship between the two sensors, and the intercept (3.1) shows that the AquaTROLL measures the DO 3 mg/L higher than the PreSens sensor. The PreSens DO measurement will be used for future measurements as this is what the oysters would be experiencing in the chambers.

Figure 11. Relationship between AquaTROLL oxygen measurements and the PreSens oxygen sensor dots. Linear regression line in red, 1:1 relationship shown in gray



Statistical Analysis

Cerco & Noel 2007 Model

To determine the effectiveness of this experiment and clearance rates being measured, the environmental conditions collected during the experiment were input into the clearance rate estimate model created by Cerco & Noel (2007). As described in chapter 1 of this thesis, the Cerco & Noel model is a mechanistic model, meaning it calculates the response variable based on the influence of scaled predictor variables. These models can be useful when calculating the individual influence of a predictor variable, such as the direct influence of increased temperature on oyster clearance rates and can help researchers to understand the mechanism behind the predictor variable. However, mechanistic models do not predict response variables well because they are inherently not able to capture complex relationships between predictor variables (Baker et al., 2018).

The Cerco & Noel model uses past experiments that determined the relationship between the specific predictor variables of temperature, TSS, salinity, and dissolved oxygen, and the response variable, clearance rate, to create the mechanistic model resulting in (Equation 3.) A total max clearance rate of 22.9 L/hr/g, calculated using the estimated filtration rate of 0.55 m^3/g/day determined by Jordan (1987) using water from the Choptank River at Horn Point Laboratory, is modified by the environmental conditions, which are scaled to be between 0 to 1. A value of 1 meaning the environmental condition results in the maximum clearance rate, and a value of 0 meaning the environmental condition results in no clearance.

$$Fr = f(T) * f(TSS) * f(S) * f(DO) * Fr_{max}$$

(Equation 3)

Temperature effects are calculated from experiments by Jordan (1987) at Horn Point Laboratory who placed oysters in flume chambers using raw river water, but at varying temperatures. A constant to control the effect of temperature on filtration was reported of 0.015. The temperature effect equation utilizes Ktg (0.015 $°C^{-2}$) as the constant effect of temperature on filtration (Jordan, 1987) and T_{opt} is the optimum temperature for filtration which is 27°C (Jordan, 1987) (Equation 4).

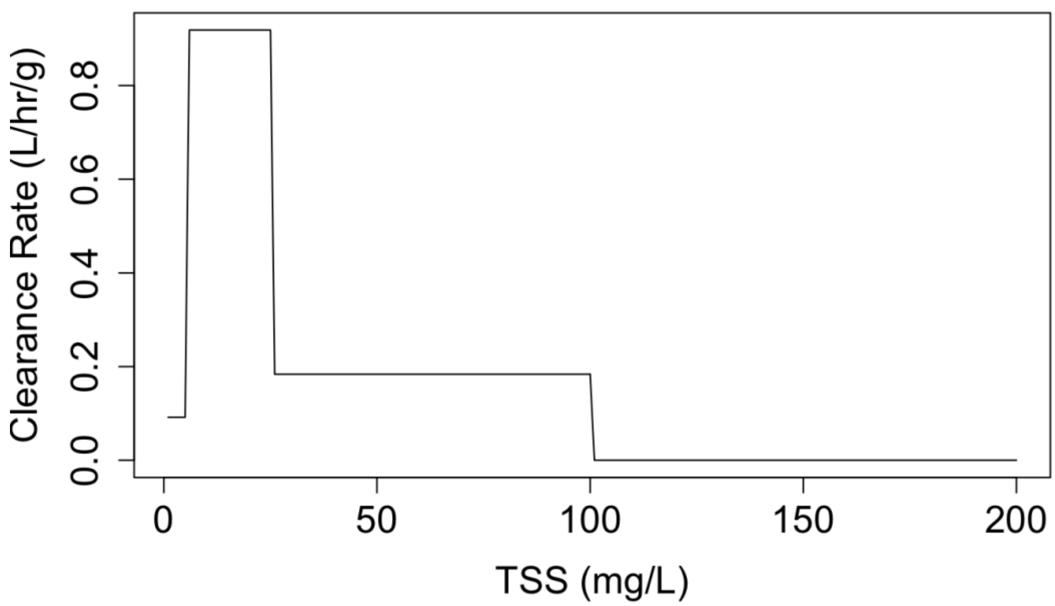
$$f(T) = e^{-Ktg*(T-T_{opt})^2}$$

(Equation 4)

TSS effects are calculated based on a stepwise function based on the results from Jordan (1987) shown in

Figure 12. This function results in the capacity for clearance rate to be highest between 5 and 25 mg/L of TSS and no water being cleared above 100 mg/L.

Figure 12. Cerco and Noel function for influence of TSS



Salinity is a strong driver for stress in oysters, specifically at low salinities (Casas et al., 2018; McCarty et al., 2020), and results in lower physiological performance when sub-optimal. The salinity effects are based on low-salinity experiments for oysters by Loosanoff (1953) to determine at what salinity clearance rate is halved (KHsoy), which was estimated to be 7.5 ppt (Equation 5).

$$f(S) = 0.5(1 + \tanh\,(S - KHsoy))$$

(Equation 5)

Finally, the effects of dissolved oxygen are based on a logarithmic curve. Do_{qx}, or when the clearance rate is one fourth of the maximum, is set at 0.7 mg/L, and Do_{hx}, or when the clearance rate is one half of the maximum is set to 1 mg/L (Equation 6). These values are based the combined effects of hypoxia on clearance and mortality for the general benthos. The Do_{hx} is the point at which the sigmoid curve for increased mortality overtakes the sigmoid curve for clearance in terms of fraction of maximum across hypoxic conditions.

$$f(DO) = \frac{1}{1 + \exp\left(1.1 * \frac{DO_{hx} - DO}{DO_{hx} - DO_{qx}}\right)}$$

(Equation 6)

With all the modifying equations combined into Equation 3, the model is able to scale the maximum clearance rate of 22.9 L/hr/g to estimate the instantaneous clearance rate. This model was used throughout this thesis as a comparison between the measured instantaneous rates and the models created using the data collected.

Multiple Linear Regression Model

A multiple linear regression model (MLR) was parameterized using the environmental conditions collected throughout this experiment as well as the measured clearances collected with the FOFS. This model was built using the stats R package (v4.1.1; R Core Team 2021). As stated previously, five environmental parameters were collected throughout the experimental period: water temperature (°C), salinity (ppt), dissolved oxygen (ppm), turbidity (mg/L), and chl *a* (μg/L). These parameters mirrored those used by the Cerco & Noel model, but with the addition of chl *a*. The dataset was split into a training and testing sets with the first 80% of data in the training set and the last 20% of data in the testing set for model validation. The varIMP function from the caret R package (v6.0.94; Kuhn 2008) was used to represent the importance of each environmental parameter in the multiple linear regression model.

Random Forest Model

A random forest model was built from a series of decision trees using the R package randomForest (v4.7; Liaw 2022). The goal of this model was to determine clearance rate of oysters under a variety of conditions, without relying on linear relationships. A training and testing dataset was used with 80% of data used for training and 20% used for testing, similar to the MLR model. A total of 500 trees were used. The variable importance for the model was retrieved to compare the importance of variables from the MLR and random forest model. The node purity describes how well each decision tree preforms using the residual sum of squares Purity in this context is a measure of how well a predictor decreases variance. However, percent increase in MSE can be used for machine learning models other than decision trees (reagro.org), so this metric was the focus of this analysis. In this random forest model, node purity and MSE present different orders of importance, therefore MSE was used as the predictor importance for the Random Forest model.

Results

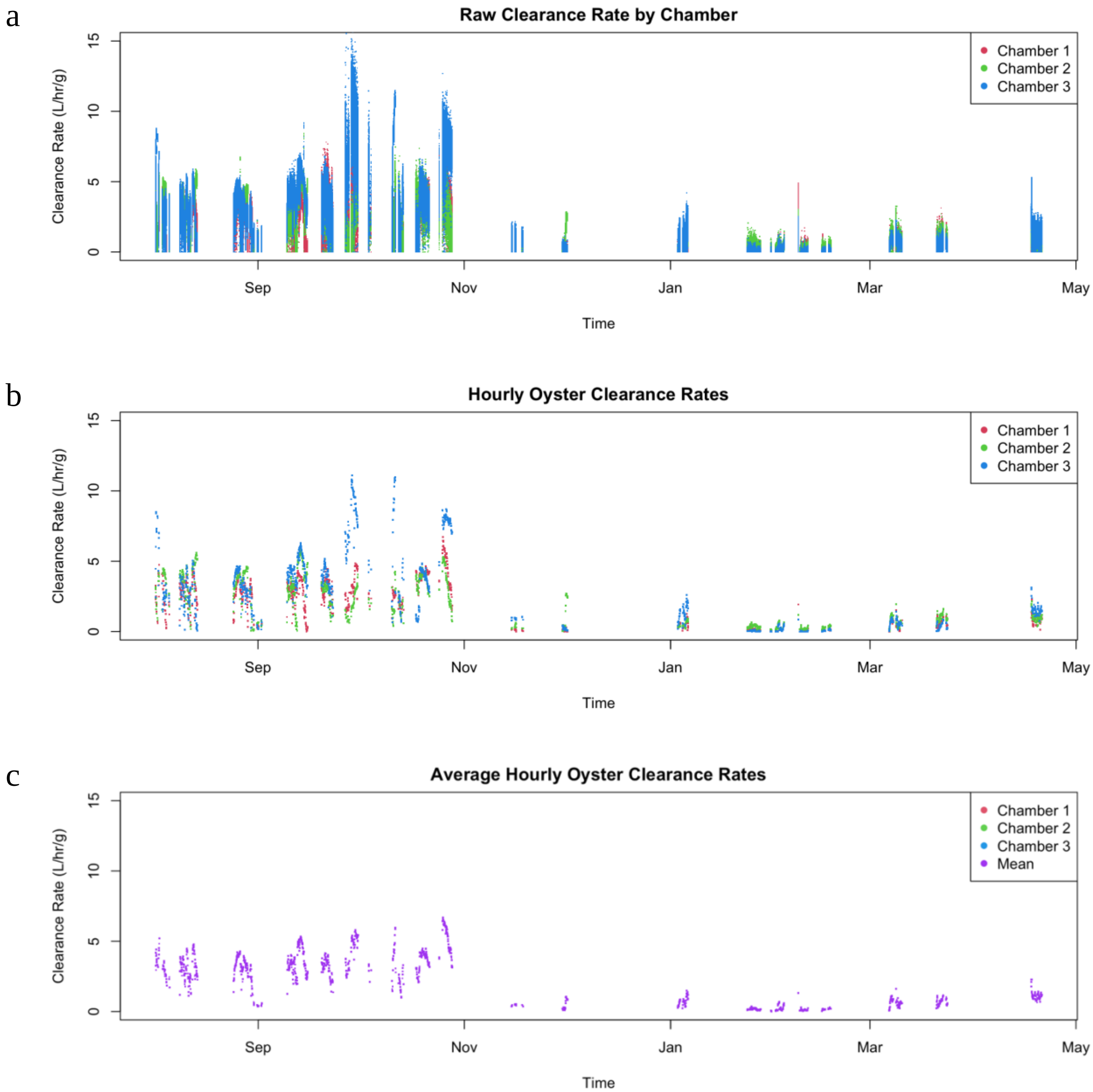
The clearance rate for each oyster in each chamber was calculated. The maximum clearance rate measured throughout the experimental period was 16.03 L/hr/g. The minimum clearance rate measured was -0.5 L/hr/g, however these negative values were adjusted to 0 L/hr/g. The summary statistics for each chamber and the three chambers combined, are listed in Table 3. There are no statistical differences observed among the three chambers. The monthly average of all chambers ranged from 0.21 L/hr/g in February to 4.18 L/hr/g in October, as shown in Table 5. The largest variation in feeding occurred in October (SD: ± 1.26 L/hr/g). The smallest variation in feeding occurred in December (SD: ± 0.09 L/hr/g).

Table 3. Overall clearance rate (L/h/g) averages from each experimental chamber across the experimental period

	Mean ± SD	Median	Min	Max	n
Chamber 1	2.13 ± 1.67	2.31	0	10.44	3,916,839
Chamber 2	2.32 ± 1.70	2.41	0	9.10	3,916,839
Chamber 3	2.91 ± 2.67	2.72	0	16.03	3,916,839
Chamber Average	2.45 ± 1.81	2.73	0	10.69	3,916,839

Clearance rates measured at the per second rate captures the fine scale variation in feeding behavior of oysters in the Choptank River. Figure 13a illustrates the fine scale trends in clearance rate and differences between the three experimental chambers. However, due to limitations in computing power, to examine the daily trends across the 9-month experimental period, the average hourly clearance rate was calculated for each chamber (Figure 13b). This allows for hourly variation to be incorporated into analyses, but removes fine-scale differences in clearance rates. The impact of this approach is explored further in the discussion of this chapter. Additionally, the average clearance rate of the three chambers across every hour was calculated to acquire the average clearance rate across all hours (Figure 13c). The chambers were averaged to calculate the aggregate clearance rate at a specific time to better capture the clearance rate of an average oyster. The average clearance rate across all chambers will be used for all future analysis.

Figure 13. Oyster clearance rates over time (a) raw clearance rates among three feeding chambers (b) hourly average clearance rate among chambers (c) average hourly clearances of feeding chambers combined

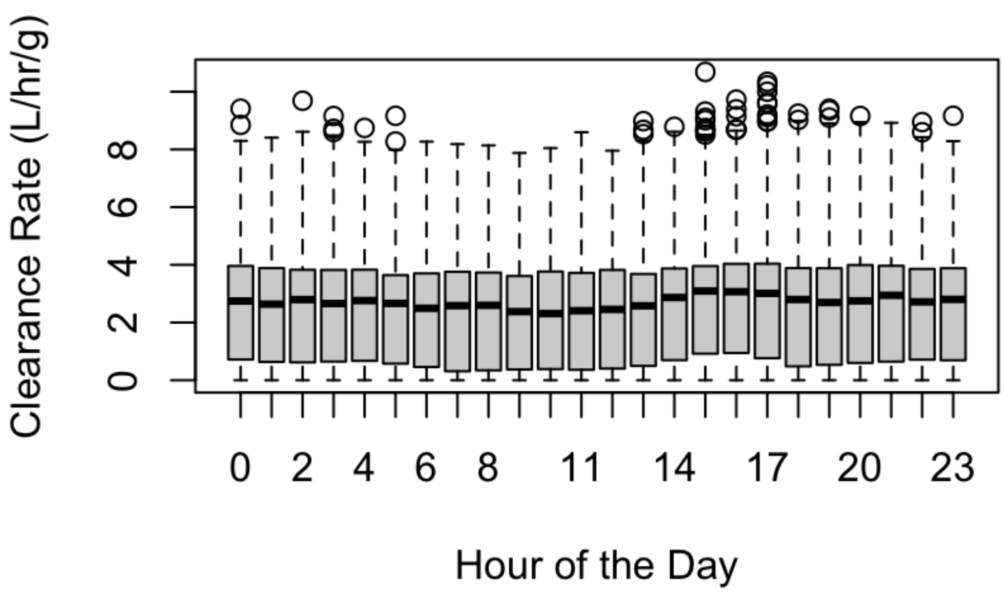


Daily Trends

Early studies into the feeding activity of oysters have shown no relationship between tidal or light cycles on feeding rates (Looseanoff & Nomejko, 1946); however, an analysis was warranted to confirm that the FOFS did not capture any diurnal relationships. The average clearance rates across all the chambers were grouped by hour of the day across all trials shown in

Figure 14. There was no significant difference in average clearance rate across all hours of the day.

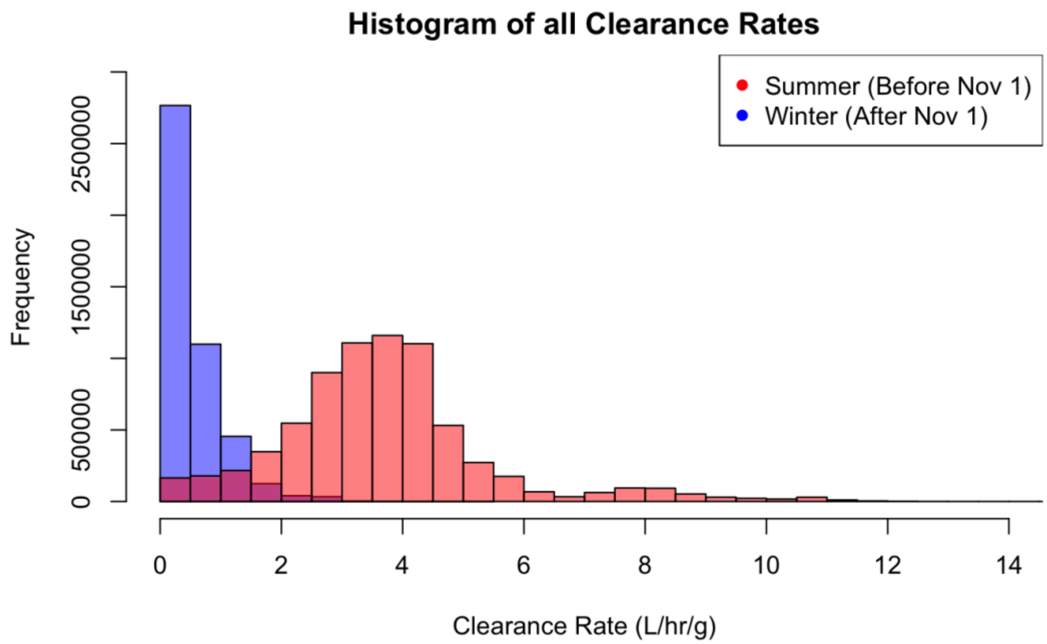
Figure 14. Average chamber clearance rates across experimental period grouped by hour of the day (24 hr)



Seasonality

Oysters are assumed to alter their clearance rate based on the season, not just based on environmental conditions (Cranford & Hill, 1999). A bimodal distribution of clearance rates is presented in the data. Separating data into seasons, Summer (data collected prior to Nov 1) and Winter (data collected after Nov 1), may explain the two modes present in the dataset (Figure 15). The delineation between Summer and Winter seasons was done based on monthly average water temperature being above or below 15°C. Long-term monitoring also revealed a reduced variation of the clearance rate based on the month. For example, when the average temperature of the water was greater than 15℃, the standard deviation of the clearance rate was above 0.5℃, but when the average temperature was below 15℃ the standard deviation of the clearance rate was below 0.5℃. This coincided with a lower overall clearance rate in the months where the average temperature was below 15℃. The seasonal effects on average clearance rate and variation can be seen in Figure 15, where Winter months, November and April, had a lower overall clearance rate and were clustered closely to 0 L/hr/g. The Summer months produced a higher overall clearance rate as well as a larger spread of activity.

Figure 15. Frequency of clearance rates grouped by season. Summer representing August 1st, 2022 - October 31st, 2022 (n summer= 2,408,858). Winter representing November 1st, 2022 – May 1st, 2023 (n winter= 1,507,981).



Intraspecies Variation

Dry tissue weight, shell weights, and shell dimensions were used to estimate an allometric relationship. Over time, there was positive linear relationship between the date of the trial and the condition index of the oysters (slope= 0.01, r^2=0.25, p-value= <0.05) from August to April. The mean condition index for the experimental period was 3.39 ± 1.53. There was also a positive linear relationship between the length and dry tissue weight of the oysters (slope = 0.06, r^2 = 0.43, p-value= <0.05). The intercept and coefficient from the linear regression derived from the relationship between dry tissue weight and oyster shell lengths found in this study were collated into Equation 7. This equation was specific to diploid oysters in the Choptank river over this experimental period.

$$Dry\ Tissue\ Weight\ (g)\ =\ 0.06 * Shell\ Length\ (cm) - 3.5$$

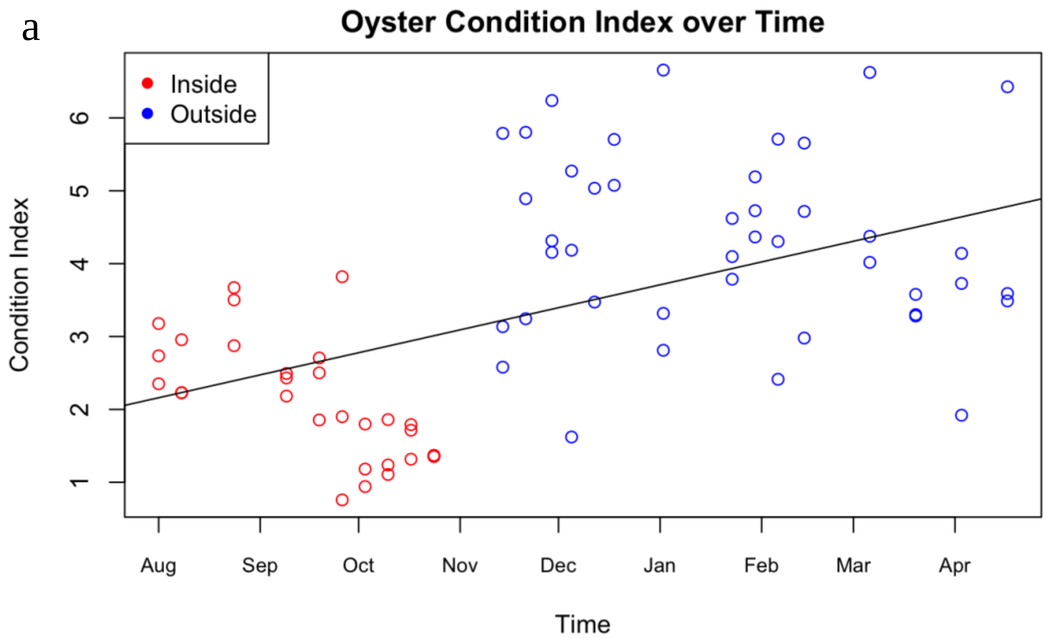
(Equation 7)

Table 4. Regression summary statistics for allometric model

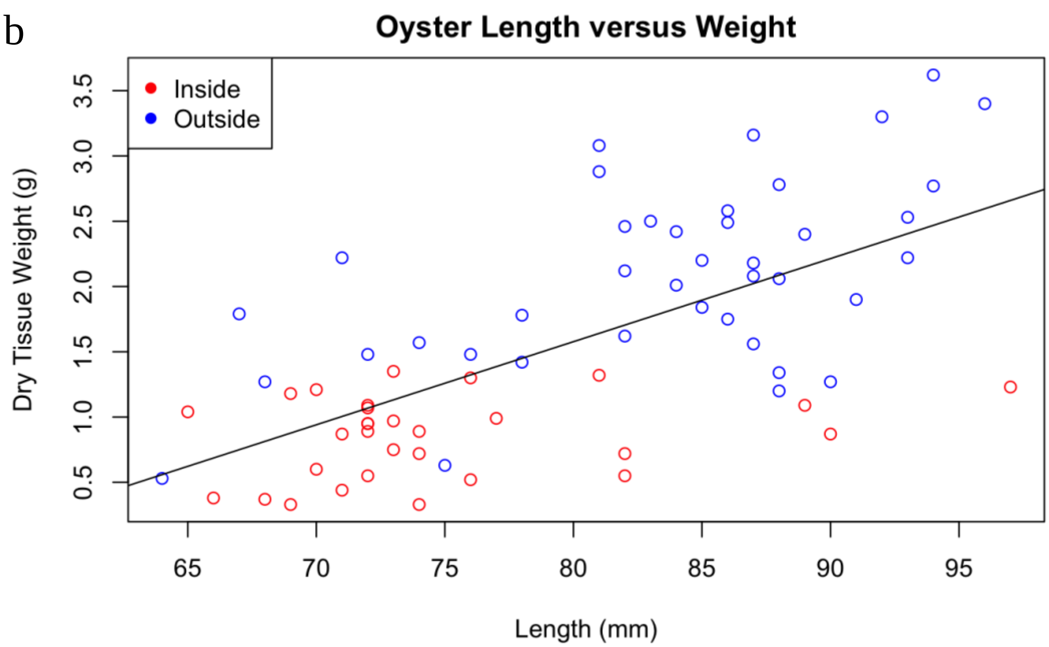
	Estimate	**Std. Error**	**T value**	**P-value**
Intercept	-3.515722	0.717073	-4.903	6.16 e-06
Shell Length	0.063666	0.008937	7.124	8.48 e-10

Figure 16. Oyster allometric relationships (a) Condition index over time (b) shell length versus dry tissue weight

a



b



Environmental Conditions

The data collected by the AquaTROLL overlapped with 0.5% of the total raw clearance rate data set collected at the per second rate. The lack of coverage by the AquaTROLL was due to mismatches in sampling rate between the AquaTROLL (per min) and the FOFS (per second). Routine maintenance and failure of the AquaTROLL did occur, creating weeklong gaps in dataset; however, general seasonal trends were clearly visible across the 9-month experimental period (

Figure 17).

Sample sizes for environmental data collected were notably small during two months (October n= 8, March n= 0). This was due to differences in sampling rate between the FOFS and AquaTROLL, as well as the sonde failure. However, data were collected are reported in Table 5.

January was the coldest month with an average water temperature of 5.50 ℃. August was the warmest month with an average water temperature of 30.21 ℃. The greatest temperature variation occurred in September (SD: ± 3.51). The highest salinity occurred in November (15.44 ± 0.20 ppt). The lowest salinity occurred in August (12.10 ± 0.70 ppt). The variation for salinity was generally low (SD: ± 0.81 ppt). The lowest DO occurred in August (6.68 mg/L), which aligns with the warmest month. February had the highest DO at 14.18 mg/L. Warmer months had higher DO variation, and colder months had lower DO variation. TSS was highest in September, with extremely high variation (47.96 ± 36.03 mg/L). TSS was lowest in April, again with extremely high variation (17.66 ± 22.83 mg/L). However, the coldest months, December, January, and February, had low TSS standard deviations (0.87 mg/L, 0.85 mg/L, 2.11 mg/L respectively). Finally, the chl *a* concentration was highest in October (17.64 μg/L). The chl *a* concentration was lowest in April at 3.03 μg/L. Overall, the average chamber chl *a* concentration was 11.95 ± 4.75 μg/L.

Table 5. Average clearance rate for each month for all chambers; average and standard deviation of environmental data taken from AquaTROLL for each month. Overall summary statistics for both clearance rates and environmental data

	CR ± SD	**CR n**	**Temp (°C)**	**Salinity (ppt)**	**DO (mg/L)**	**TSS (mg/L)**	**Chl *a* (µg/L)**	**AquaTROLL n**
August	3.21 ± 0.85	904,385	30.21 ± 2.38	12.10 ± 0.70	6.68 ± 1.27	41.01 ± 54.35	10.72 ± 4.24	508
September	3.80 ± 1.10	845,525	21.86 ± 3.51	13.95 ± 0.93	9.41 ± 1.31	47.96 ± 36.03	12.35 ± 3.80	4,293
October	4.18 ± 1.26	658,948	17.38 ± 2.16	15.07 ± 0.12	10.54 ± 0.44	36.71 ± 11.95	17.64 ± 1.10	8
November	0.26 ± 0.20	68,781	9.28 ± 0.88	15.44 ± 0.20	12.31 ± 0.30	19.78 ± 14.55	14.42 ± 2.52	1,150
December	0.96 ± 0.09	28,084	8.12 ± 0.37	15.31 ± 0.51	12.26 ± 0.07	18.75 ± 0.87	12.99 ± 1.26	497
January	0.35 ± 0.37	448,117	5.50 ± 0.60	13.88 ± 0.31	13.70 ± 0.42	17.73 ± 0.85	13.37 ± 2.79	5,276
February	0.21 ± 0.20	421,955	5.65 ± 0.23	13.72 ± 0.30	14.18 ± 0.35	18.00 ± 2.11	15.12 ± 3.06	2,755
March	0.70 ± 0.28	401,721	NA	NA	NA	NA	NA	0
April	1.20 ± 0.37	139,323	17.96 ± 1.16	13.35 ± 0.08	9.07 ± 0.41	17.66 ± 22.83	3.03 ± 0.73	2,315
Overall Mean	**2.45 ± 1.81**	**3,916,839**	**12.52 ± 8.01**	**13.89 ± 0.81**	**11.69 ± 2.44**	**26.38 ± 26.17**	**11.95 ± 4.75**	**16,772**
Overall Median	2.73		8.51	13.83	12.46	17.81	12.63	
Overall Min	0		4.63	9.86	3.22	15.81	2.57	
Overall Max	10.69		38.18	15.68	14.86	934.43	55.73	

Water temperature ranged from the maximum of 38.18°C in August to 4.68°C in January (

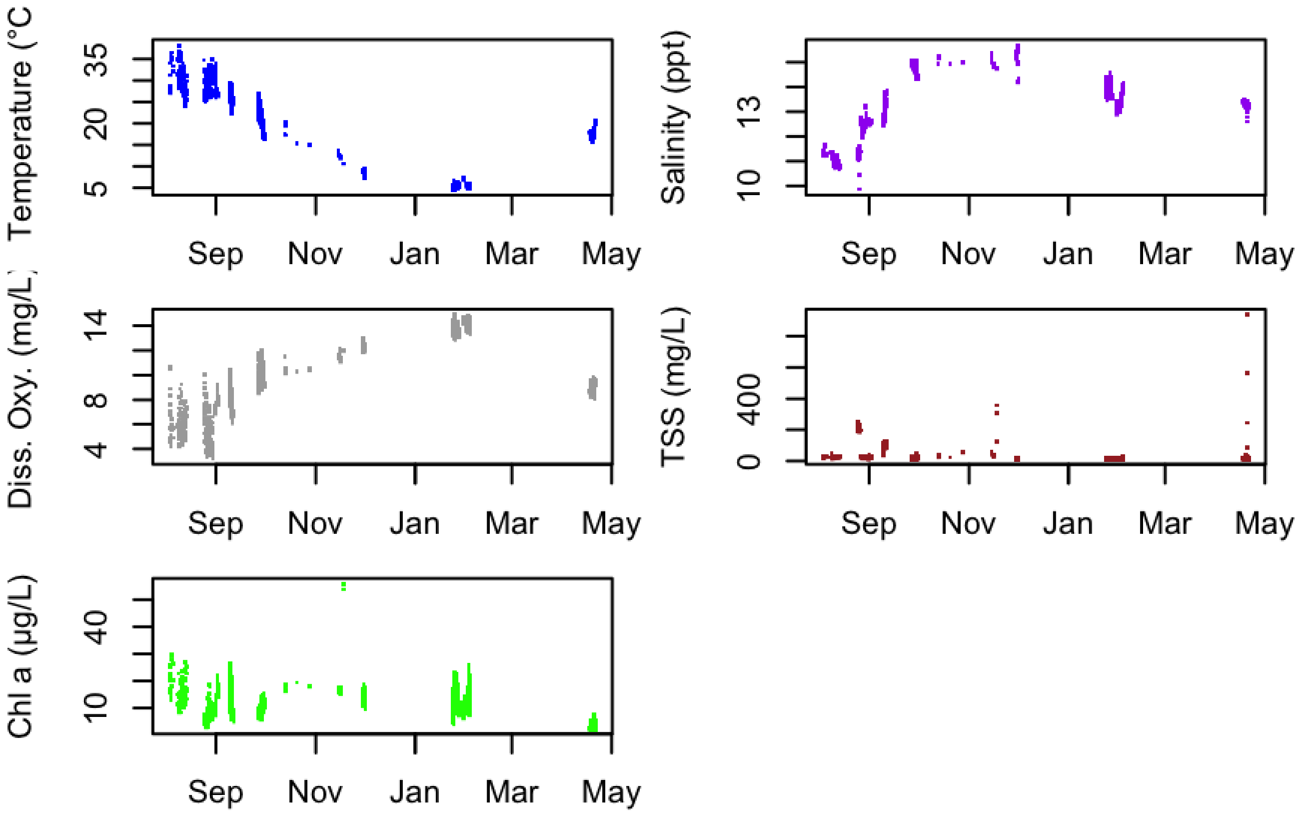
Figure 17a). The temperature increased at the end of the experimetnal period in April, indicative of spring conditions. Salinity followed an inverse trend to temperature with an increase in salinty to 15.69 ppt in October to January (

Figure 17b). However, the lowest overall salinity (9.86 ppt) occured in August, which was still well above the tolerance range for oysters (Casas et al., 2018; McCarty et al., 2020).

Dissolved oxygen followed the expected seasonal trend, which is a result of the dissolution of gases in warmer liquids (

Figure 17c). The highest avergage DO occurred in Feburary (14.18 mg/L) and the lowest average DO occurred in August (6.68 mg/L). The highest standard deviation for DO occurred in Septmeber (± 1.31 mg/L) and the lowest occurred in December (± 0.7 mg/L). TSS ranged from 17.66 mg/L in April to 47.96 mg/L in Septmeber. Although on average TSS across the experimental period was 26.38 mg/L, the standard deviation was high (± 26.17 mg/L), likely in due to rain events and algal blooms (

Figure 17d). Finally, the highest average clearance rate was 17.64 μg/L in October, and the lowest highest average clearance rate occurred in April (3.05 μg/L). The overall mean and standard deviation was 11.95 ± 4.75 μg/L. Chl *a* has a large spike in November, however, this is most likely due to sensor fouling that occurred



during the fall bloom.

Figure 17. AquaTROLL environmental conditions measured over time

Statistical Model

The outputs of the training and testing dataset for the MLR model are illustrated in Figure 18. The mean squared error (MSE) for the testing set of the model was 1.24. The normality of the residuals for the MLR model was determined using a Q-Q plot (Figure 19). The tails of the Q-Q plot deviate from the range of a normal distribution, suggesting that the residuals of the MLR model are not normally distributed.

The predicted values in the testing set were regressed with the measured clearance rates during the testing set period (

Figure 20). The slope of the regression was 1.008 ± 0.013 and the intercept was -0.007 ± 0.027. The range of the slope with error (0.995, 1.021) includes a slope of 1, and the intercept with error (-0.034, 0.02) includes an intercept of zero. These confidence intervals give strong evidence of an intercept of zero and a slope of one, meaning that the predicted values and modeled values show evidence of a 1:1 relationship. The variable importance was calculated using the absolute value of the T statistic for each predictor variable (Figure 21). This calculation determined that all predictor variables were significant (

Table 6). The order of importance was as follows: temperature, salinity, TSS, DO, and chl *a*.

Figure 18. Measured and modeled clearance rate using the multiple linear regression model with training prediction shown in blue and the testing prediciton shown in red.

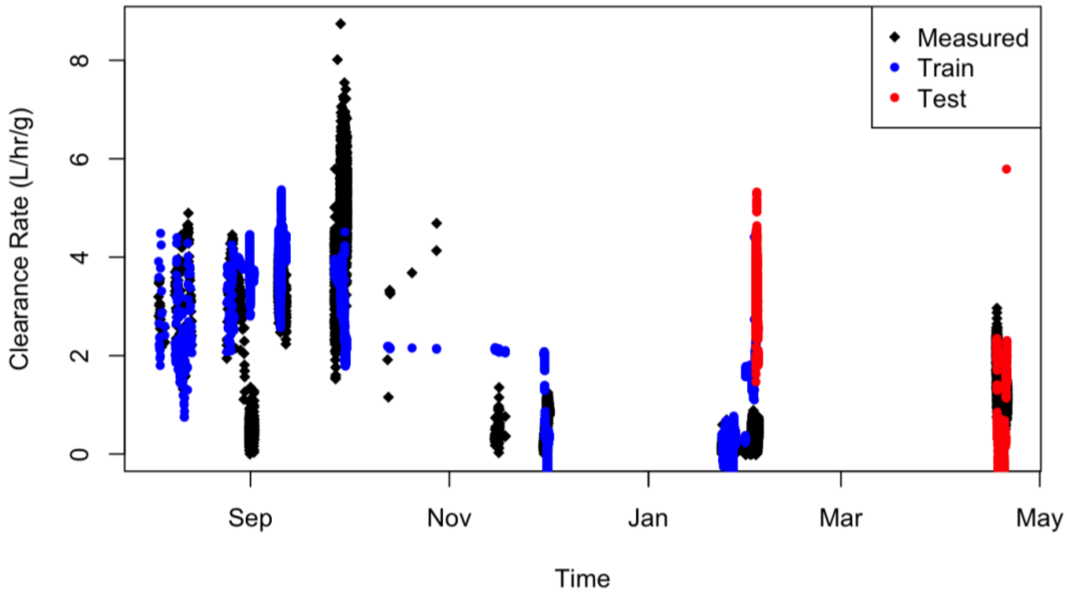


Table 6. Summary statistics for multiple linear regression model; Adjusted r^2 value: 0.629, Median Residual: -0.034

	Coefficient	Std. Error	T Value	P-value
(Intercept)	-14.674268	0.220805	-66.46	<2e-16
Temperature (°C)	0.219922	0.002804	78.43	<2e-16
Salinity (ppt)	0.835579	0.011929	70.05	<2e-16
TSS (mg/L)	0.005312	0.000449	11.83	<2e-16
Chl *a* (µg/L)	0.021708	0.002423	8.96	<2e-16
DO (mg/L)	0.117573	0.010105	11.63	<2e-16

Figure 19. Nomal Q-Q plot of residuals for multiple linear regression model

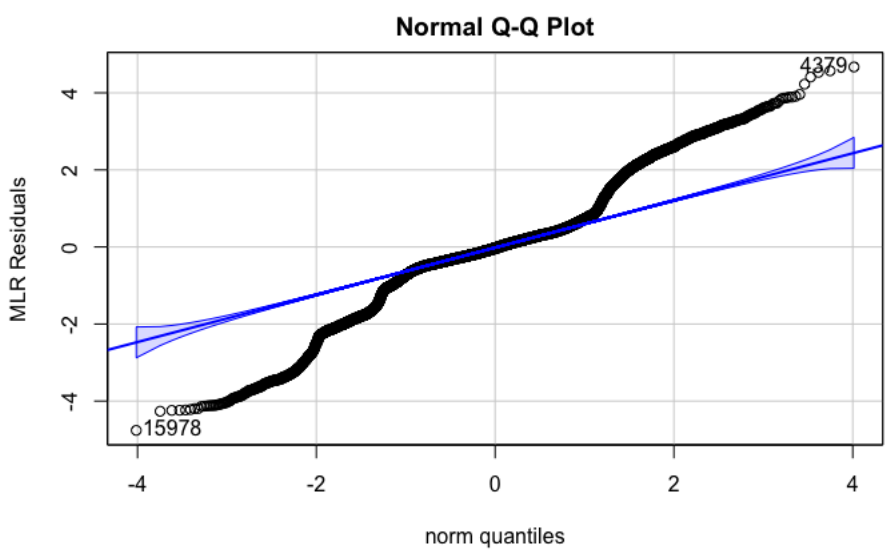


Figure 20. Measured clearance rates versus predicted clearance rates for the multiple linear regression model with 1:1 dashed gray line and linear regression line shown in red

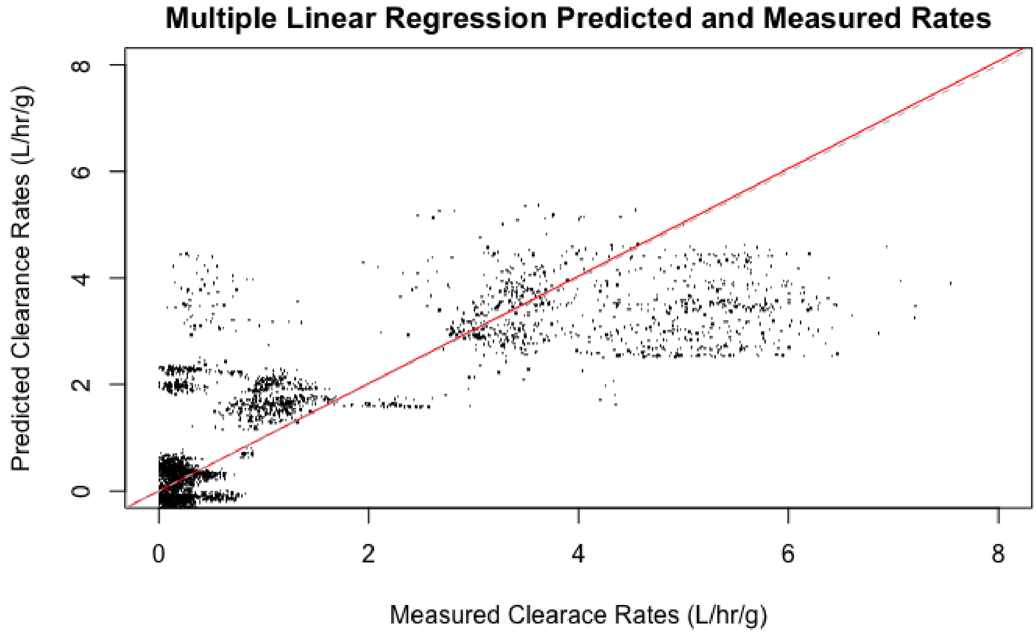
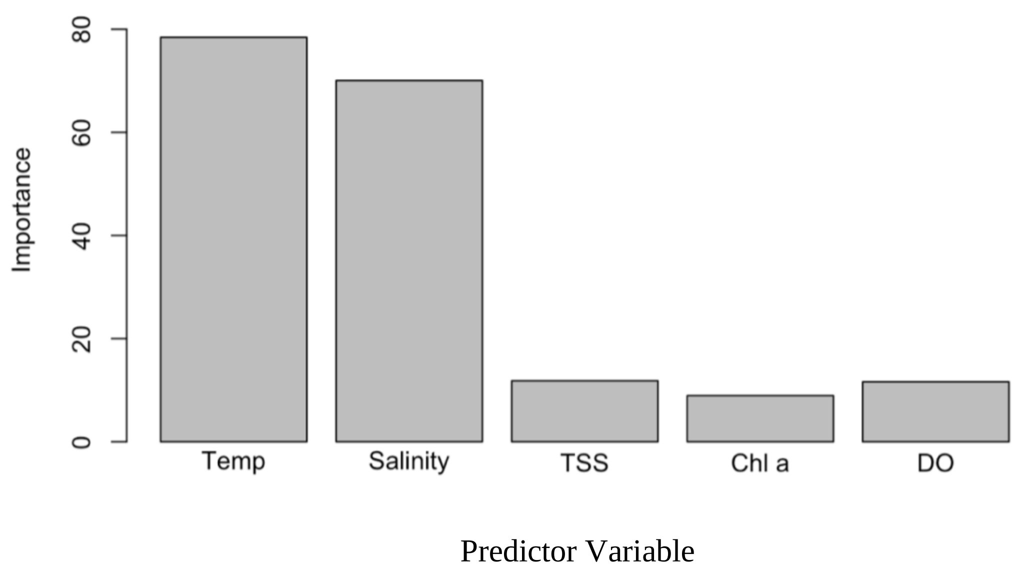


Figure 21. Multiple linear regression predictive variable importance (absolute value of the T statisic)



Random Forest Model

A random forest machine learning model was built using the same five predictors that were used for the multiple linear regression and Cerco & Noel model.

Figure 22 presents a timeseries plot of the measured clearances rates for the system and predicted data set based on the model. The model was able to explain 97.65% of the variance within the dataset and the mean square of the residuals was 0.08. The percent increase in mean square error (MSE) if each variable were to be removed from the model was highest in TSS and salinity (Table 7). This metric was used to represent the importance of each variable and the impact of the predictor variable on the decision of the model. Based on the node purity (Table 7), temperature was the best predictor of clearance rate (12,339.44) with DO as the second most important predictor variable (11,770.94).

Figure 22. Measured and modeled clearance rate using the random forest model with training prediction shown in yellow and the testing prediciton shown in red

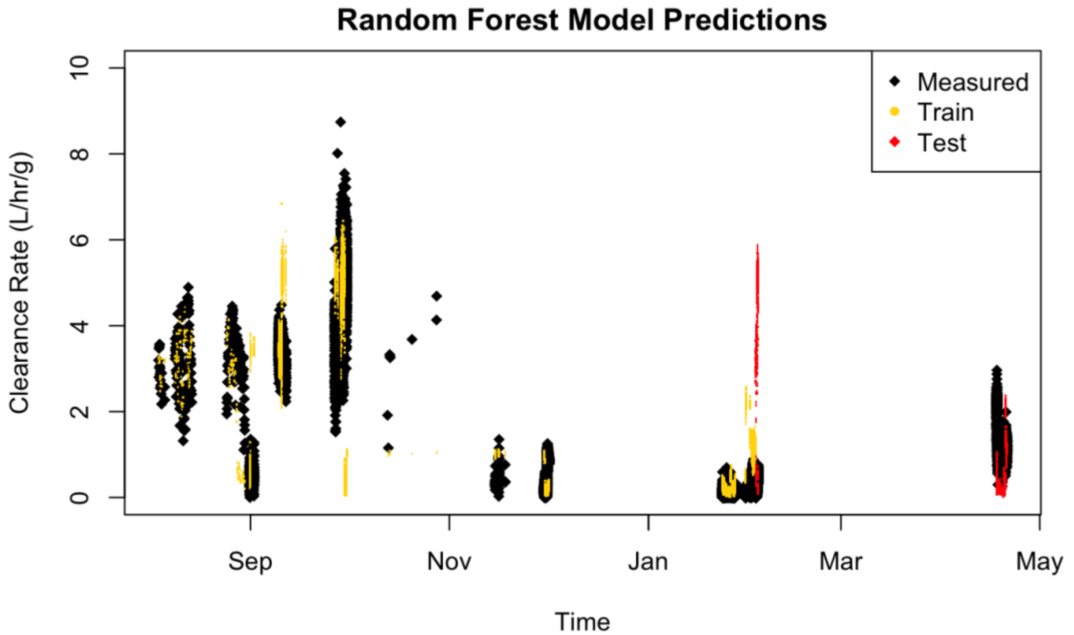


Figure 23. Measured clearance rates versus predicted clearance rates for the random forest model with 1:1 dashed gray line and linear regression line shown in red

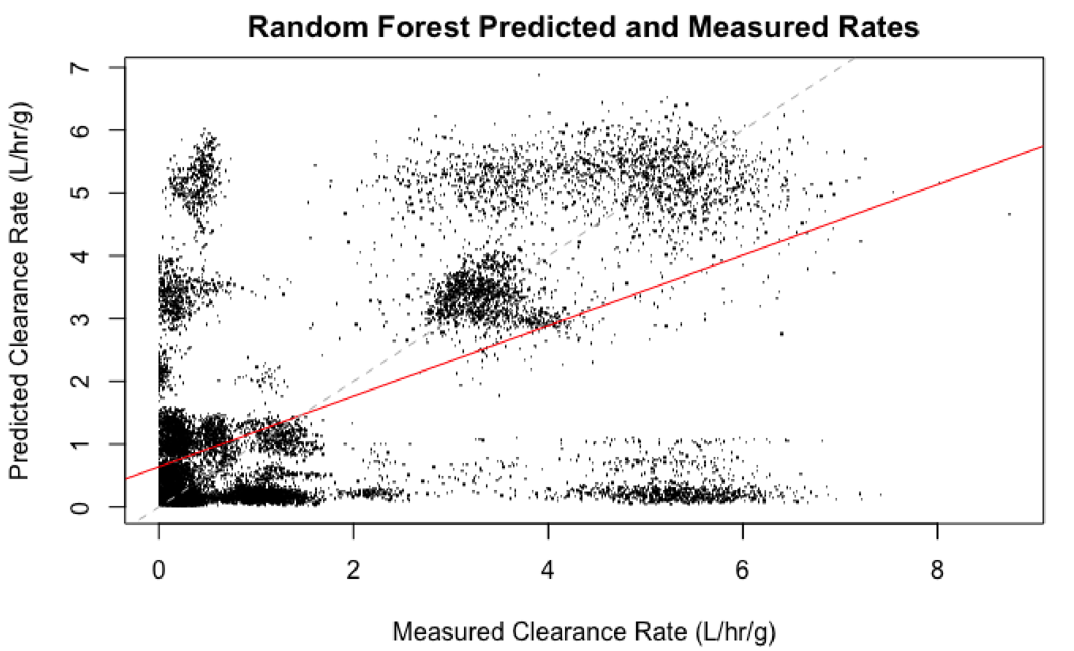


Table 7. Variable importance for the random forest model as percent increase in mean square error and increased node purity

Variable	% Inc MSE	Inc Node Purity
TSS (mg/L)	37.58	8466.78
Salinity (ppt)	37.33	8758.80
Temperature (°C)	29.93	12339.44
DO (mg/L)	29.21	11770.94
Chl *a* (µg/L)	26.90	4182.14

Comparing Models

The summary of clearance rates hindcasted from environmental conditions by the mechanistic Cerco & Noel model, MLR, and random forest model are shown in Table 8. The model with the highest mean clearance rate across the entire experimental period was the Cerco & Noel model (3.21 L/hr/g). The Cerco & Noel model also had the largest standard deviation (± 5.15 L/hr/g). The mean clearance rate for both the MLR and random forest model were very similar (1.46 L/hr/g), but the standard deviation for the random forest model was slightly higher for the random forest model (± 1.82 L/hr/g) than for the MLR model (± 1.46 L/hr/g). The mean squared error for the entire experimental dataset was highest using the Cerco & Noel mechanistic model (MSE= 22.72). The mean squared error was lowest for the MLR model (MSE= 1.26), suggesting that the MLR has the lowest mean squared error between the residuals for the entire dataset.

Figure 24a plots time on the x-axis to show the spread of measurements throughout the sampling period.

Figure 24b presents the clearance rates without gaps in time. This does depict clearance rates as consecutive values, even when jumps in time are present. However, a benefit to this visualization method is to represent how short-term variation is captured by each model, as well as the points at which the modeled rates deviate from the measured values. All models and measured clearance rates are illustrated in

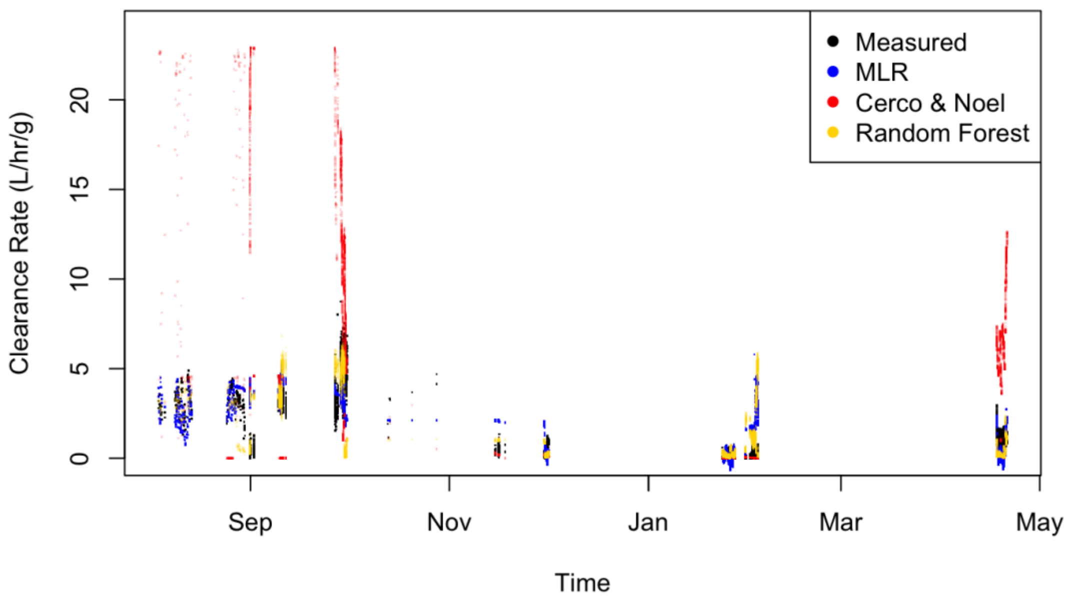
Figure 24b.

Table 8. Summary statistics and mean squared error of the full dataset of clearance rates for Cerco & Noel model, multiple linear regression model, random forest model, and measured avgerage chamber clearance rates; n= 28,128

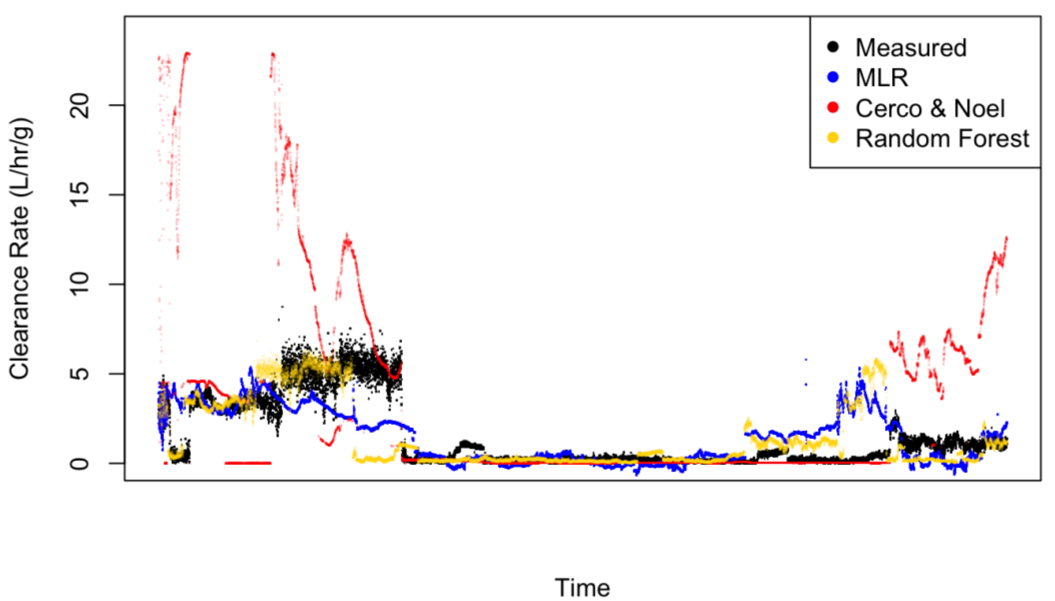
	Mean	Median	Min	Max	MSE
Cerco & Noel (2007)	3.21 ± 5.15	0.04	0	22.91	22.72
Multiple Regression Model	1.46 ± 1.46	1.37	-0.66	5.79	1.26
Random Forest Model	1.46 ± 1.82	0.46	0.02	6.05	2.92
Measured Average	2.45 ± 1.81	2.73	0	10.69	N/A

Figure 24. (a) Clearance rates for measured average chamber, Cerco & Noel model, multiple linear regression model, and random forest model (b) clearance rates for measured and modeled rates with index number on x-axis

a



b



Discussion

The FOFS system, components of which were developed for mussel feeding studies under laboratory conditions for hours of use (Vajedsamiei et al., 2021), was adapted and run over days to collect *ex-situ* oyster feeding rates. The system was able to collect clearance rate data over nine months with 5-day trials separated by 2 to 14 days depending on the time of year on a second-to-second timescale for a total of 60.7 days of observation. These high-resolution data were used to create and compare a multiple linear regression model and random forest model, as well as fed into a mechanistic model to estimate clearance rates of oysters over time. The data collected in this study add clarity about oyster feeding intensity and variation at several time scales, which has numerous ecological implications.

Measured Values

The clearance rates of oysters on a second-by-second timescale within the FOFS system revealed variation in clearance rates throughout daily and long-term feeding. The measured clearance rates in this study for summer months align well with previous estimates both in laboratory and field studies for warm months. The measured rates for October (4.18 L/hr/g; Table 5) were similar those estimated by Newell (1988; 5 L/hr/g) for summer months in the Chesapeake Bay. However, the short-term differences in these rates are not normally represented within feeding experiments due to the limitations in methods, such as the indirect method which determines an average clearance across the whole experimental period.

In the warmer months (August-October) the standard deviation in clearance rates were between ± 1.26 and ± 0.85 L/hr/g. This difference in rates is consistent with the variation between individual oysters at different time points, as well as the inconsistency of oysters in clearance rates in relation to being selective in their feeding (Palmer, 1980; Weissberger & Glibert, 2021). In the colder months (November-April), the standard deviation drops to between ± 0.37 and ± 0.09 L/hr/g. The decrease in the spread of clearance rates in colder months is consistent with a lower overall clearance rate and temperature during these months. However, water was being cleared even when oysters at moderate rates during the winter (e.g., ~1 L/hr/g in December; Figure 15) when others have assumed very low or no feeding occurring (Casas et al., 2018).

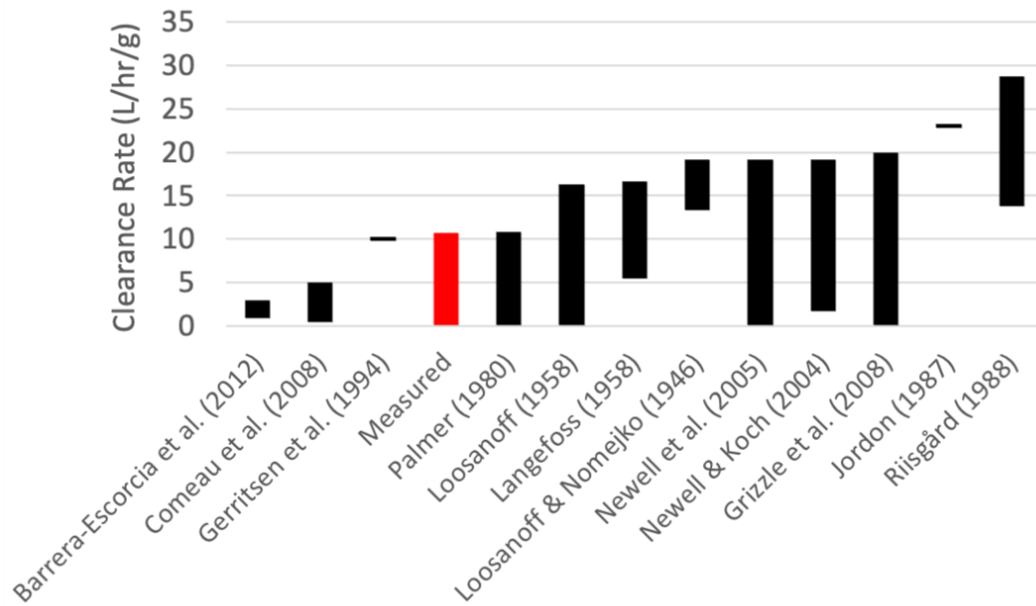
Continuing to look at the colder months, the average chamber mean clearance rate remained low (0.21-1.20 L/hr/g) during this time (Table 5). The mean clearance rates only began to increase again in April (mean CR= 1.20 ± 0.37) when the temperature rose to over 15 °C (April mean temp. = 17.96 ± 1.16 °C). This suggests that clearance rates for oysters in the Choptank River will be close to 0 L/hr/g, but with occasional feeding, in colder months, and will start to increase feeding rate again in April when the water temperature rises above 15 °C. Examining additional environmental variables, the salinity was higher in colder months, which should lead to higher clearance rates (Casas et al., 2018), but a decrease in clearance rates was observed at this time driven by reduced temperatures. Furthermore, the DO was higher and TSS was lower in cooler months, which should be more ideal for oysters as outlined previously in this chapter, however, this could also signal less food available, therefore the clearance rates were still low. This indicated that when temperature was low, it becomes the driving force for determining the clearance rates of oysters. This is consistent with current knowledge on valve gape (Mayrand et al., 2017; Tonk et al., 2023) which found a negative correlation between temperature and valve gap, suggesting that in colder months oysters will stay closed for longer, slowing feeding.

As poikilotherms, oyster metabolism is largely driven by environmental temperature. When the body temperature is low, metabolism becomes lower, and both feeding and respiration rates become lower (Tinsman & Maurer, 1974; Comeau et al., 2008). This decrease in metabolism could explain the decreased clearance rates of oysters in colder months. In addition, water viscosity is affected by temperature and has been found to impact the feeding conditions of oysters (see Podolsky, 1994).

The daily cycling of clearance rates did not show a statistically significant difference based on the hour of the day, however there was a non-significant increase in clearance rates between 14:00 and 18:00. This increase possibly corresponds to fluorescence quenching from increased solar intensity during this period (Rousso et al., 2021), decreasing the fluorescence readings in all chambers, but resulting in higher clearance rate readings in the feeding chambers. However, due to the lack of significant difference in clearance rates across the hours of the day, it is determined that there no clear daily cycling. In the future an analysis based on relative clearance rates may better capture variation between the hours of the day.

Considering the range of the measured clearance rates for this experiment and comparing them to previous studies, such as those compiled by Ehrich & Harris (2015), the measured range for this experiment was on the lower end of the clearance rate range spectrum (Figure 25). The rates were measured using a variety of methods under *in-situ* and laboratory conditions and by varying environmental parameters. These studies represent a large difference in maximum clearance rates with the highest being 29 L/hr/g, determined by Riisgård (1988) using a Coulter counter in a laboratory study, down to a maximum rate of 3 L/hr/g determined by Barrera-Escorcia et al. (2012) using a Nebauer chamber also in laboratory conditions. The range of measured clearance rates found in this study (0-10.69 L/hr/g) was similar to

Figure 25. Range of clearance rates from previous studies ordered by maximum clearance rate reported



the average range of clearance rate values (10.31 L/hr/g) from this non-exhaustive search of the literature, though some studies propose a small range (2 L/hr/g by Barrera-Escorcia et al. 2012), and some have a large range (20 L/hr/g by Grizzle et al. 2008). The measured rates in this study showed consistency with previous research about the feeding activity of oysters; however, the large range in clearance rates suggested the method chosen or parameters varied, could have a large impact on the results of the experiment.

One of the greatest benefits to the FOFS is the ability to examine temporally resolved variability in clearance rates, which should greatly improve clearance rate accuracy when scaling to estimate filtration services provide by oysters. To demonstrate the value of a high sampling rate in clearance rates, the average clearance rate and standard deviation for a trial in the month of September (9/9/2022 - 9/15/2022) was calculated based on a second, hour, and daily sampling rate (Table 9). The daily sampling rate was extracted from the second-by-second at both noon and midnight. To determine the clearance rate for the hour, the rate at the top of each hour was extracted from the second-by-second data. From these rates, the highest mean clearance rate for the trial in September results from the daily sampling rate at midnight (4.22 ± 0.91 L/hr/g). The lowest average clearance rate occurred at the daily sampling rate, taken at noon (3.29 ± 1.01 L/hr/g). The second and hourly sampling rate had a very similar mean clearance rate, 3.64 ± 0.98 L/hr/g and 3.64 ± 1.01 L/hr/g respectively, however all the averaged rates were not statistically different from one another.

If these rates were scaled up to calculate how many liters per day were cleared by a single oyster, the daily sampling rate at midnight estimated the largest volume of water cleared (101.21 L), and the daily sampling rate taken at noon estimated the smallest volume of water cleared (78.95 L). Both the second and hourly sampling rates scale up to the same average liters cleared in a day, 87 L/day. This analysis suggests that if one were to sample at each second, hour, or once daily across a 5-day trial, the overall average clearance rate would not be statistically different. This validates using hourly sampling timeframes when looking at clearance rates, which is an important consideration when accounting for computational resources (RAM, processing power, etc.) during long-term monitoring efforts. Nevertheless, high-resolution data collected at the per second level will still provide the most accurate estimates of clearance rates.

This sampling timescale analysis provides insight into possible bias that may arise based on the time of day that samples are taken. In Figure 26, rates at midnight and noon are plotted with the hourly and second-by-second sampling rates. Although the hour of day was not a significant driver of clearance rates across the entire experimental period (

Figure 14), a student's T-test was used to compare the clearance rates extracted at midnight and noon resulting in a p-value of 0.042, meaning the difference in the two datasets was significant. This suggests that low resolution data could misrepresent the clearance rates of oysters due to the variation that is present in clearance rates across daily to weekly timescales.

Figure 26. Clearance rate at different sampling timescales over 9/9/2022 - 9/15/2022 trial

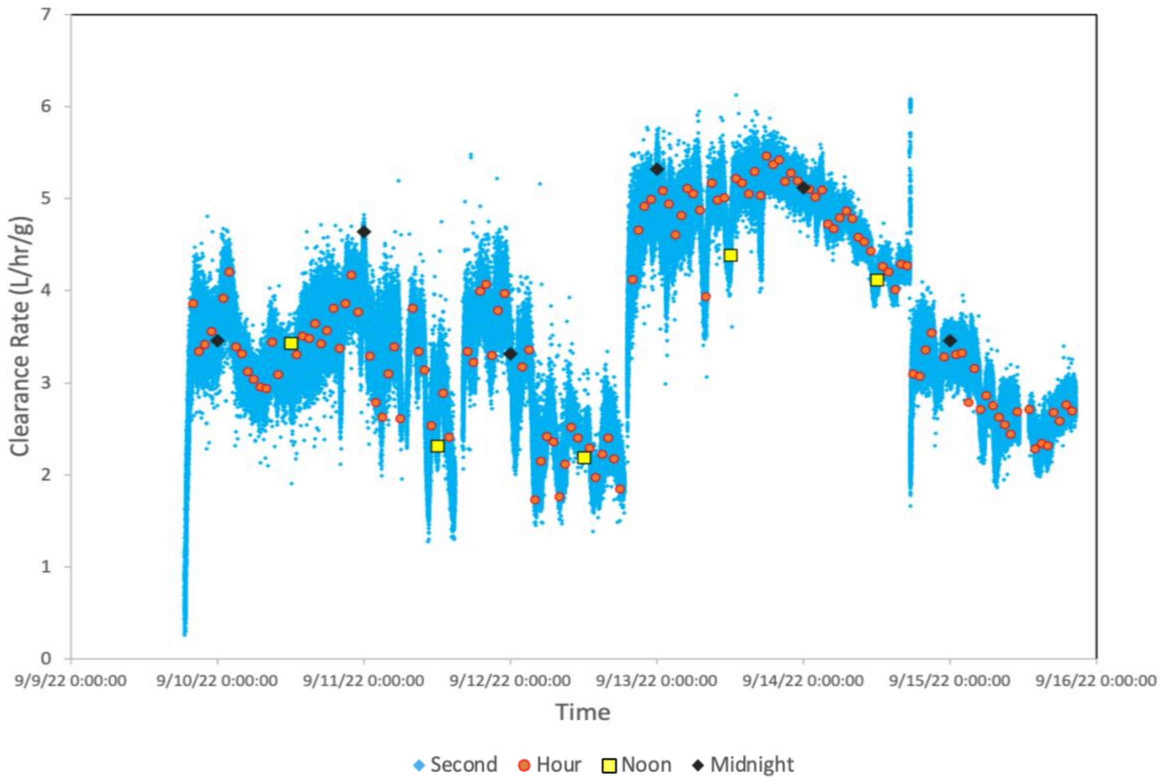


Table 9. Mean and standard deviation of clearance rates, average liters cleared per day, and total volume cleared from 9/9/2022 to 9/15/2022 using multiple sampling rates

Sampling Rate	Mean CR ± SD	Avg L/Day	Total Integrated Volume
Second	3.64 ± 0.98	87.38	502.39
Hour	3.64 ± 1.01	87.46	474.27
Noon	3.29 ± 1.01	78.95	304.11
Midnight	4.22 ± 0.91	101.21	421.41

Allometric Relationships

The allometric relationships collected throughout this study could be used in future studies to predict the dry tissue weight of the experimental oysters based on the length without the need to sacrifice the organism (

Figure 16). A positive linear relationship was present between condition index and time, shown in

Figure 16a. This was most likely due to the lower condition index normally presented by eastern oysters at the end of the spawning season due to loss of gonadal tissue (Lawrence & Scott, 1982; Casas et al., 2018), and then a slow increase over the winter as oysters regain their condition. Condition index is a site and oyster specific measurement due to the impact of age and gonadal development on dry tissue weight (Rainer & Mann, 1992; Lawrence & Scott, 1982). However, the change in condition index overtime can be an important indicator of the state of the oysters used throughout the experimental period. The addition of summer condition index measurements would be an interesting factor to include in this analysis, and could be used as a possible predictor variable in a model.

Dry tissue weight (g) and shell length (mm) are two common ways to standardize clearance rates for oyster size. An equation to relate shell length, a non-destructive measurement, with dry tissue weight, a destructive measurement, would allow for multiple trials with the same organism. Running multiple trials with the FOFS with the same organism may be able to tease out inter-seasonal variability, without the influence of intraspecies variability. From the experimental period, a positive linear relationship was present between oyster dry tissue weight and length. Equation 7 was derived from this linear relationship. Although this equation is site and oyster specific, similar to condition index and time, this equation could be vital to estimate dry tissue based on the shell length for FOFS experiments in the Choptank River going forward.

Method Design

The goal of this study was to design a system that would be able to measure oyster clearance rates under conditions that *in-situ* oysters would experience, with minimal human input. This system collected 1,457 hours of usable data across 9 months. Based on the average amount of time spent for set-up, daily system checks, and take-down there was 6 hours spent monitoring the system per trial. There were 25 total trials, therefore 150 hours were spent monitoring the system across 9 months, not including system development and troubleshooting. This system provides 9.7 hours, or 34,968 instantaneous clearance rate values for every 1 hour of human input. Therefore, this system is able to produce a large amount of high temporal-scale clearance rate values per human input hour.

In order to limit experimental error or drift in *ex situ* conditions, measures were put in place to monitor the conditions in the FOFS and *in situ*. Confirming that environmental conditions used in the analysis and the conditions of the water bath were representative of river conditions.

Finally, this system was able to capture two distinct seasons to represent the long temporal scale trends present in oyster clearance rate experiments (Casas et al., 2018). The system was able to capture large variation in oyster clearance rates that were hypothesized to occur instantaneously throughout the day, which cannot be represented using certain laboratory experiments that only measure an average clearance rate, such as the widely used indirect static system clearance method.

Modeled Clearance Rates

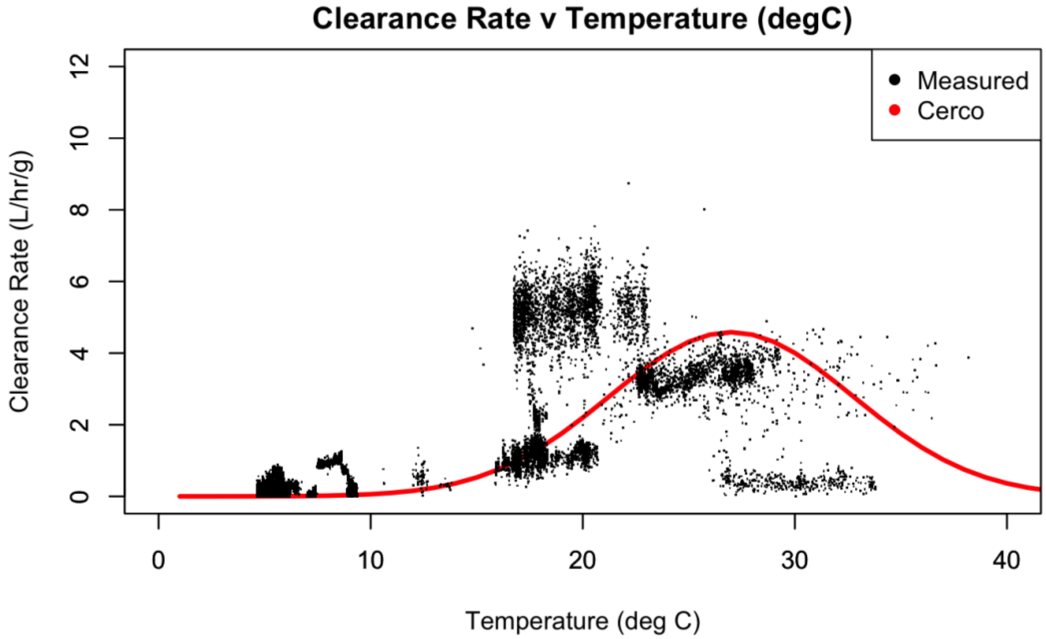
A multiple linear regression model for clearance rates was used to output a linear regression for each environmental parameter that can be used in conjunction to predict the overall clearance rate. When creating a larger ecosystem model, these equations would be informative as to the clearance rates of oysters in a modeled system.

The multiple linear regression in this study did not have a normal distribution of residuals, but all parameters are significant (Table 6). The training and tests sets and their diversion from the measured rates can be seen in Figure 18. A problem that arises with a linear regression model was that it was assumed that the predictor value follows a linear regression. However, Jordan (1987) has shown in laboratory experiments that clearance rates have a temperature maximum with a steady decrease in rate at either extreme. An optimum temperature maximum cannot be accurately represented in a linear regression because of the nature of the model and instead should be modeled using a Gaussian distribution with the temperature maximum being the peak of the curve.

A plot of the temperature versus measured rates demonstrate that the clearance rates do follow a curve with the influence of temperature (

Figure 27). However, when comparing to the estimated curve for the Cerco and Noel model, the optimum temperature for the oysters in this experiment was skewed left. This suggests that the Cerco & Noel curve may represent the clearance rates better than the linear regression, however the oysters in this experiment may be acclimated to a different optimum temperature maximum than the oysters used in experiments by Jordan (1987), which informs Cerco and Noel's model. The optimum temperature maximum for this experiment was 19°C, which was below the commonly accepted optimum of 27°C that has been applied on numerous modeling studies (zu Ermgassen 2013, Gray et al. in prep.). This shift could also represent additional conditions that could be affecting oysters at high temperatures, such as harmful algal blooms or low salinity.

Figure 27. Clearance rate across a temperature range with Cerco & Noel's predictive curve plotted in red and the measured clearance rates in black



The experimental period was primarily in colder months, excluding May through July. Oysters may adapt to temperature (Pernet et al., 2008), which could result in higher clearance rates at lower temperatures after acclimation, such as at 19 °C in this case. However, this trend calls attention to the need to collect clearance rates across multiple seasons. The lower clearance rate at the 27 °C temperature maximum could also be the result of additional suboptimal environmental conditions that only occur at high temperatures, such as decreased DO. Previous laboratory studies into temperature have kept all other parameters constant (e.g., Casas et al., 2018; Vajedsamiei et al., 2021), and therefore would not be able to capture the combined effects that an *ex-situ* study, such as this would measure.

TSS was another important variable in the models. The Cerco and Noel (2007) model scales the clearance rate to zero when TSS is greater than 100 mg/L. Cerco and Noel made the assumption that oysters did not clear water when the TSS concentration was over 100 mg/L, however, Suedel et al. (2014) has shown that oysters are very tolerant to elevated TSS levels, even up to 500 mg/L. This suggests the need for more studies into the impacts of natural fluctuations and spikes in TSS on clearance rates as *in situ* oysters may handle increased TSS better than oysters in the laboratory.

The Cerco & Noel model tended to over predict the clearance rates of oysters when the environmental conditions were within the ideal mechanistic ranges (

Figure 24). Conversely, during suboptimal conditions, such as low temperatures or high TSS, this model under predicted oyster clearance rates, often estimating 0 L/hr/g. The Cerco & Noel rates agreed with the measured clearance rates most closely in the winter because the low temperature scales the modeled clearance rate to 0. The measured clearance rates were close to zero, but there was some variation occurring that the Cerco and Noel model was not able to capture because of the nature of mechanistic models (Baker et al., 2018). In the warmer months, when temperature no longer becomes a limiting factor, TSS became the driving force behind the clearance rates. This can be seen when the clearance rate droped from >20 L/hr/g down to ~5 L/hr/g and even 0 L/hr/g in the month of August and September (

Figure 24). The step-wise function that controled the scaling of clearance rate based on TSS concentrations controlled that scaling. When the TSS concentration was at the middle step of this function, the clearance rate from the Cerco & Noel model agreed most closely with the measured clearance rate during warmer months. Based on the results of this study, the Cerco & Noel would be best representative of clearance rates for *in situ* oysters in warmer months when the TSS is between 25 and 100 mg/L. The range of the environmental conditions for this experimental period and the functions of the Cerco & Noel model can be found in the appendix of this thesis.

The MLR model has its own drawback, focused on the relationship between the predictor and the clearance rate being assumed linear. It has been shown that there are certain parameters, such as temperature, that do not follow this trend (

Figure 27). In the future, a multiple regression model with nonlinear relationships would most likely be able to capture relationships that are not represented using the MLR model. This would become even more important with the inclusion of summer data, which would result in larger extremes in environmental conditions. However, the overall residuals and summary statistics indicate that the MLR does predict the clearance rates well for the experimental timeframe that the model was trained on. With the addition of more data, and the inclusion of nonlinear regressions, a multiple linear regression would be a good option for researchers looking to predict clearance rates based on environmental conditions.

The random forest was able to model 97% of the variation in the clearance rate data using the environmental predictor variables, and had a low MSE for the testing set (Testing MSE= 0.08). This model had a better performance to the MLR (Testing MSE= 1.23) for the testing set. However, this model does have problems predicting clearance rates during changes in seasonal conditions, such as the transition from fall to winter and winter to spring. Machine learning models are not able to predict what they have not seen, such as transition periods. Including time as a predictor variable across a full year worth of data may improve this model. If this study were to continue across a full year, the day of year may prove to be a valuable predictor variable. In addition, due to the nature of machine learning, this model would benefit from the addition of more predictor variables. With the increase availability of environmental monitoring tools and frequency of sampling, this will likely become more viable in the future.

Each of the models were able to make predictions as to the clearance rate across the experimental period based on the environmental period. Each model was also able to predict which variable was most important towards estimating clearance rates at any given time. These differing modeling methods could be used based on the preferences of the researcher, and the parameters that they are most interested in.

Comparison of Modeled Rates

All of the methods for modeling clearance rates have their benefits and drawbacks. Similar to the benefits and drawbacks associated with each approach to measuring oyster clearance rates, the resulting models from the experiments presented here also must be balanced. Each of the models may be preferred in different scenarios depending on the focus of a study.

The importance of each predictor variable and the forecasted clearance rates under natural environmental parameters becomes most influential when scaling up the model for ecosystem-wide filtration services estimates. The MLR and random forest have contradicting most important predictor variables with the MLR ranging temperature and salinity as most important and the random forest ranking TSS and salinity. From the measured rate analysis, we found that temperature was an important factor, and therefore this leads one to trust the ranking of the MLR predictor variables. However, salinity was ranked highly for both models, leading to the conclusion that salinity is an important predictor variable. This could be due to the Choptank River being on the lower limit of oyster salinity tolerance range, causing oysters to moderate their feeding based on the salinity at any given time.

The MSE represents the amount of error within each model, with zero being no error present. Overall, the MLR model had the lowest MSE (1.26) with the random forest model as a close second (2.92). The Cerco & Noel model had the highest MSE (22.72) (Table 8). By comparing the MSE values, the MLR model has the best fit to represent this experimental period and should be chosen to forecast clearance rates.

The average clearance rate predicted by each model (Table 8) was used to project the total water cleared over one hour, one month, and one year by a single 1 g oyster (Table 10). This was repeated for an oyster reef in the Tred Avon River, a Maryland Department of Natural Resources restoration site, located approximately 4.1 km from the confluence with the Choptank River, with an area of 0.038 km^2. The total oyster biomass of the site based on the target restoration goals (30% of site with 50g biomass) is 570 kg (oysterrecovery.org).

Table 10. Estimates of the volume of water cleared over time using the Cerco & Noel, multiple linear regression, and random forest models and the measured values for a single oyster and restored Tred Avon River oyster reef

Biomass	Volume/Time	Cerco & Noel	MLR	Random Forest	Measured
Single Oyster (1 g)	L/Hour	3.21	1.46	1.46	2.45
	L/Day	77.04	35.04	35.04	58.8

	L/Month	2,344.87	1,066.51	1,066.51	1,789.70
	L/Year	28,119.6	12,789.6	12,789.6	21,462
Tred Avon River (570 kg)	Million L/Hour	1.83	0.83	0.83	1.40
	Million L/Day	43.91	19.97	19.97	33.52
	Million L/Month	1336.57	607.91	607.91	1020.13
	Million L/Year	16028.16	7290.07	7290.07	12233.33

Even over the course of an hour for an entire reef, the Cerco & Noel model would have overestimated the volume cleared by oysters by 430,000 L. If this is scaled to across a day, this difference becomes 10.39 million L of water. The MLR and random forest model both have a smaller average clearance rate across the experimental period than the measured rate, resulting in underestimates in the volume cleared over time. Both models underestimated the measured volume cleared over an hour by 570,000 L. This scales up to 13.55 million L of water across a day for a reef. This analysis highlights how the choice of model can impose a large impact on the scale of perceived filtration services that oysters provide. However, when using the instantaneous clearance rates based on the environmental parameters of each model, ones could remove the need for an average rate and most likely result in closer estimates to the measured value at any given time.

In order to determine the impact of oysters on the ecosystem through filtration services, a trustworthy model must be employed that is able to accurately estimate the clearance rate under both ideal and non-ideal conditions. The goal of the two models proposed in this thesis is to create a framework for future models that could be trained on the environmental and clearance rates collected using this innovative flow-through system. However, these models were trained specifically for the Choptank River during cooler months outside of the spawning season. In the future, replicating this system for various regions as well as across a full calendar year would provide valuable insights into the clearance rate and filtration services of oysters.

Conclusion

The purpose of this thesis was to outline and implement a new system for measuring clearance rates in bivalves to better understand the variability of clearance rates at various timescales and the impacts of environmental conditions on the filtration services of oysters. The system described in this chapter was able to capture high-frequency instantaneous clearance rates across a range of environmental conditions. In total, 60.7 days of data were collected from August, 2022 to April, 2023.

This system ran for approximately 5 days for each of the 25 trials, allowing for the comparison of clearance rates by hour of day. Oysters have not been found to rely on diurnal cycling of clearance rates (Loosanoff & Nomejko, 1946; Palmer, 1980), and this was supported by this study. However, the influence of tidal cycle on clearance rates was not analyzed and could be an informative predictor variable for clearance rates.

As described in chapter 1, traditional methods for collecting clearance rates, such as the in-direct method generally produce an average rate at the end of the experiment, not able to capture the second-by-second variation that oysters have when feeding. This system was able to capture that variation at a second-by-second scale. The difference in this variation at different timescales was analyzed to find that there is not a significant difference between an hourly timescale and the second-by-second timescale across the experimental period. However, clearance rates collected every 24 hours, either at midnight or noon, proved to be statistically different data sets. Therefore, conducting instantaneous clearance rates studies at the hourly timescale could be a suitable way to reduce the volume of data collected and consequently reduce computing power, while still capturing variation in clearance rates. However, collecting data at the daily timescale could prove problematic based on variation in clearance rates throughout the day.

Overall, the clearance rates collected in this study aligned well with previously measured rates. However, there is a large range in clearance rates measured using different methods, and the maximum measured clearance rate in this study was well below the rates measured by laboratory studies such as Riisgård (1988) and Jordan (1987). This difference displays the influence of the method on the clearance rates measured. This system was able to capture the environmental conditions along with feeding rates, similar to how laboratory studies monitor environmental conditions. However, the FOFS was able to keep oysters in natural seston conditions to remove the influence of near perfect laboratory conditions that may produce overestimated clearance rates for *in situ* oysters.

From the measured values and environmental conditions collected by the FOFS, two models were built, a multiple linear regression and a random forest model, and compared to an existing mechanistic model currently in use to estimate the filtration services of oysters by the Chesapeake Bay Foundation. These models all present different approaches to forecasting clearance rates. The Cerco & Noel mechanistic model is able to predict clearance rates well in warmer months when the TSS is between 25 mg/L and 100 mg/L, but does not capture any of the variation present during colder months when the clearance rate is predicted to be zero.

The multiple linear regression model is able to predict the measured clearance rates well, but will likely fail at extreme environmental conditions because not all conditions fit a linear regression. These conditions would benefit from a non-linear regression. With the addition of a full 12 months of data, a multiple regression model that captures non-linear relationships is needed.

Finally, a machine learning random forest model was created to demonstrate the benefits of a machine learning algorithm for estimating clearance rates. This model was able to predict clearance rates using environmental predictor variables, however machine learning models are most beneficial when encapsulating many predictor variables in a model, therefore, in the future, this model would benefit from the addition of more environmental data. With the lower cost and size of environmental monitoring equipment, collecting additional predictor variables alongside feeding rate experiments becomes more viable.

Overall, temperature proved to be a strong influence on the measured values, causing clearance rates to be low in colder months when the range of DO and salinity were in more ideal ranges for oysters. In the warmer months, clearance rates stayed high even when other environmental parameters' condition decreased. This is consistent with the influence of temperature on metabolism, and the need for food when metabolism is high.

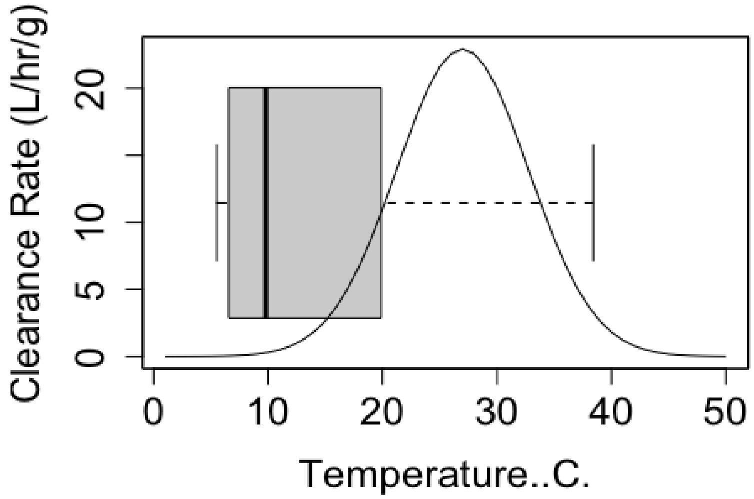
Based on these findings, this system was able to help better represent the variability in feeding activity at various timescales using second-by-second high resolution data. The resulting measured clearance rates and predictive models help to understand the impacts of environmental conditions on oyster feeding rates. Going forward, this system will be collecting a full 12 months of data, completing in August of 2023. Additional high resolution clearance rates for the Choptank River could help validate the models produced by the measured clearance rate. Additionally, the system could be recreated in other regions to compare clearance rates for oysters acclimated to different ecosystems. This system could also be modified to measure the feeding rates of other bivalves or estimate the clearance rates for additional life stages, such as larval oysters.

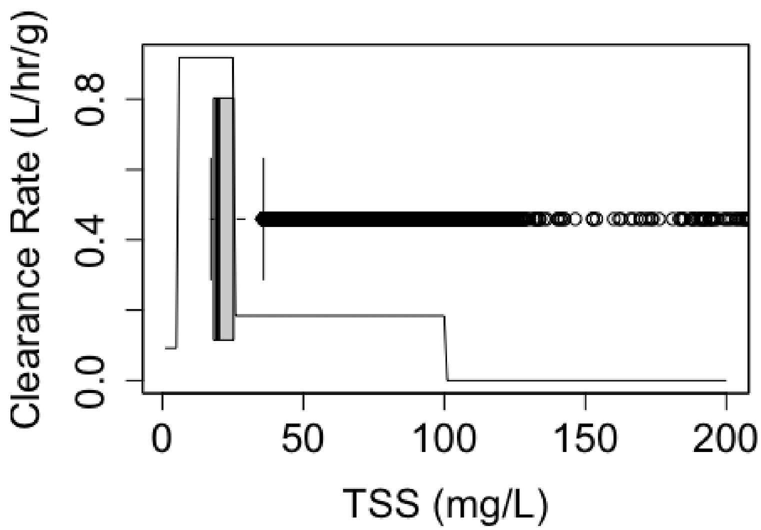
Ecological Implications

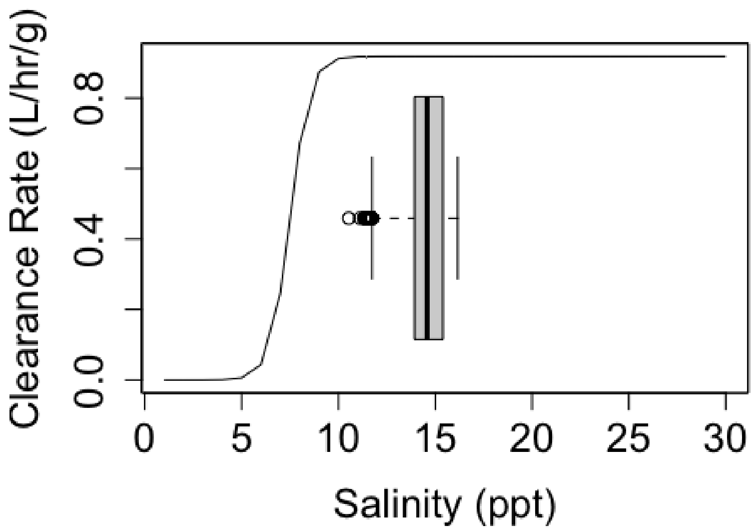
The clearance rates collected during this *ex situ* study are influential in representing the short-term trends present in feeding oysters. Bivalve clearance rate studies have been conducted over the last century, but only with recent innovations in technology have the fine-scale variation, that has been thought to be present in organisms like oysters for decades, really come to light. As anthropogenic influences continue to change the environmental conditions of large ecosystems, like the Chesapeake Bay, filter-feeding activity will likely change with it. This study has shown that oyster clearance rates in the Choptank River through the months of August to April are highly influenced by temperature. Clearance rates drop to almost zero in the cooler months. This results in majority of water being cleared by oysters to occur in warmer months. However, as overall water temperature increases, low winter temperatures will not be as low, resulting in a possible increase in clearance rates during the winter. As filter-feeding organisms are being looked to for their ecosystem benefits, it is important to note their filtration services are most valuable during warmer months. In addition, when declaring the scale of influence of oysters on removing nutrients and primary production from an ecosystem, it is important to take into account the condition of that system. This study has shown that oysters are not machines that can constantly clear water. *In situ* oyster feeding rates are variable and cannot be represented using optimal conditions alone.

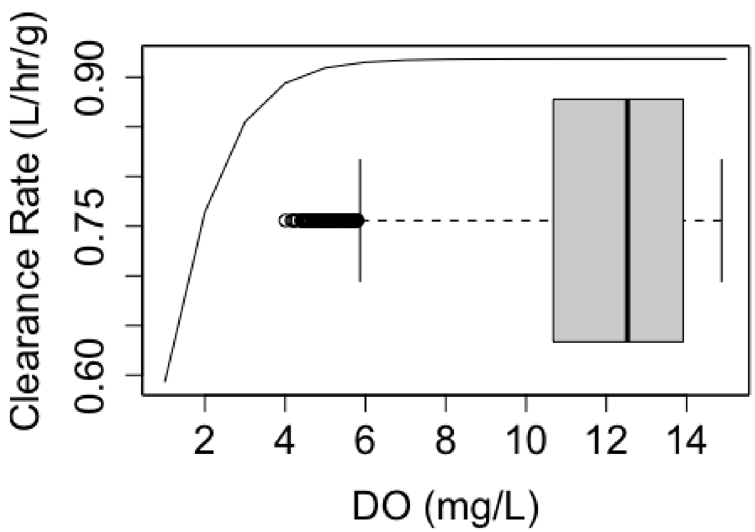
Appendices

Appendix 1 - 4. Cerco and Noel Scaling with boxplots of the measured environmental parameters

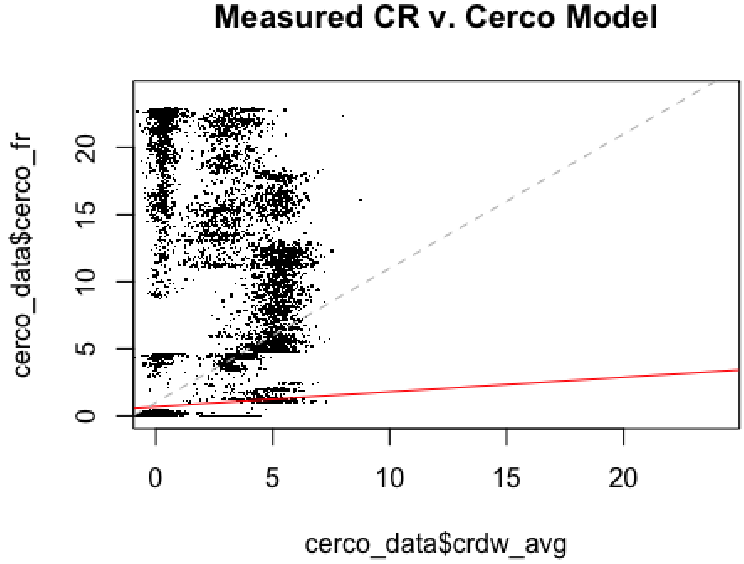




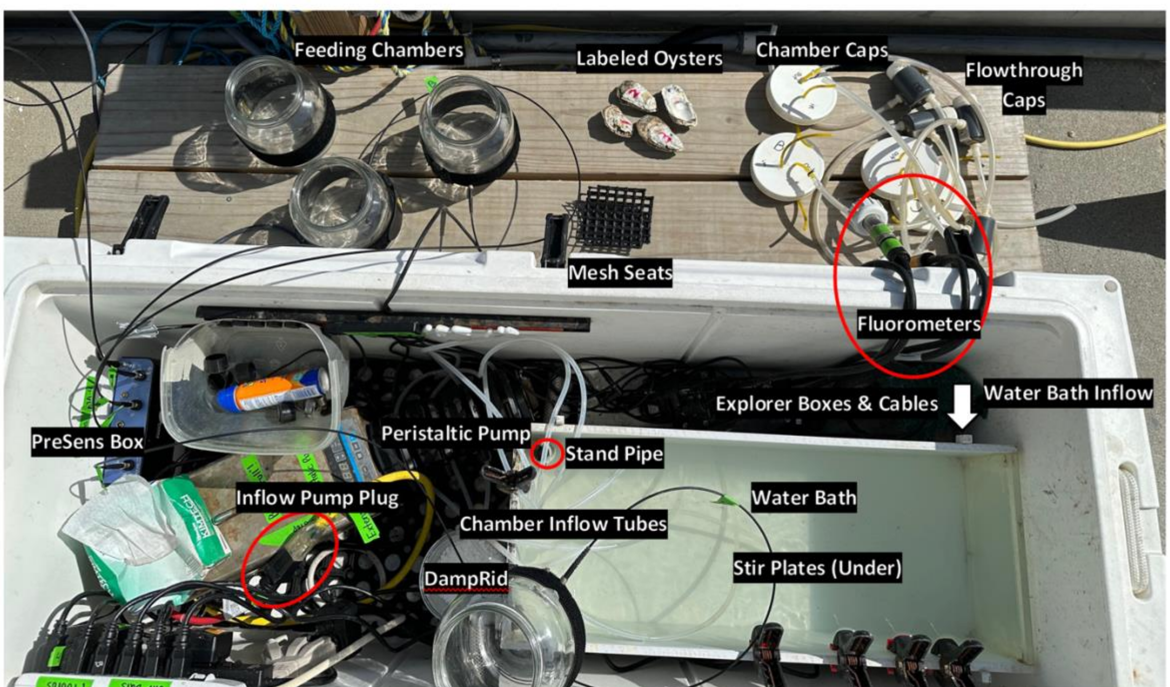

Clearance Rate (L/hr/g)
0.0
0.4
0.8
0
5
10
15
20
25
30
Salinity (ppt)


Clearance Rate (L/hr/g)
0.60
0.75
0.90
2
4
6
8
10
12
14
DO (mg/L)

Appendix 5. Measured clearance rates (x axis) versus the Cerco and Noel Modeled clearance rates (y axis). Linear regression line in red, 1:1 line in gray.



Appendix 6. Photograph of FOFS in the field.



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