ENVIRONMENTAL EFFECTS ON CULTURED OYSTER *Crassostrea virginica*: IMPLICATIONS FOR FILTRATION CAPACITY AND PRODUCTION

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A Thesis Submitted to the
University of North Carolina Wilmington in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

Center for Marine Science

University of North Carolina Wilmington

2019

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ACKNOWLEDGEMENTS

I would first and foremost like to thank Dr. Elizabeth Darrow. Thank you for taking me onto this project and for being such a great support system through this entire process. Who knew we would end up here four years ago when I was grinding away at midden shells? You have taught me so much since we first met, and I appreciate the confidence you have had in me to help lead your lab. I am honored to be your first graduate student.

I would also like to thank my committee members, Dr. Aswani Volety and Dr. Suzanne Bricker. It means so much to have you trust in my abilities and guide me through decisions. You have together shown me the "big picture", both in my thesis and for my future and I cannot begin to explain how significant that has been.

Thank you to our growers, Matt Schwab, Al Smeilus, and Tim Holbrook. It is a unique opportunity to have people trust you enough to allow you to work with their livelihood. Your experiences have put so much perspective into this project that I will always take with me. Also, how lucky am I to have the freshest oysters right from the boat?

Next, I want to thank everyone in the Estuarine Ecology lab. I genuinely could not have done this without you all. I know oyster sampling was dreadful at times, but each of you was so supportive. A special thanks to Conor Murphy, Jessica Carlton, Michelle Weatherford, Nicole Webster, Emily Manuel, Kirsten Zitkus, Sesalie Rhinehart, Kimberly Gaston, Samantha Mendicino, and Connor Kelly.

Thank you to the other members of the ShARES project, including Dr. Martin Posey, Troy Alphin, Dr. Joao Ferreira, Dr. Alhambra Cubillo, Dr. Brandon Puckett, and Dr. Susanne Brander. You have all been so helpful in so many ways, but mainly, thank you for all of your

knowledge. Madison Lytle and Kelsey Billet, I am grateful to have had the both of you on this project to work through problems, share fieldwork, and overall experience grad school together.

Thank you to the Benthic Ecology Lab, the UNCW Shellfish Hatchery, Dr. Rob Whitehead, the administrative staff at CMS, Steve Hall, Rob Deanes, and Ron Moore. It is such a privilege to have so many resources at the Center for Marine Science. It is even more of a privilege to have people as generous and accommodating as all of you. A special shout-out to the HVAC crew who had to share Wetlab #3 with me during my long experiment days.

Finally, I thank my family and friends. It has been incredible sharing this experience with so many smart and caring peers. I would personally like to thank Alexis Martisomeone who knows *exactly* what you're going through sure does help. I am so grateful to have such an unbelievable support system outside of the walls of CMS too. Erin and Alex, we all finally did it. Thank you for talking me through grad school from hundreds of miles away. To my friends from home, thank you for being the strong women you are who push me to be better every day. To everyone else who has supported me, thank you and I love all of you from the bottom of my heart.

Mom, Dad, Krysta, and Paul. You have never doubted me. Even through my roughest times when I doubted myself most, you have had so much confidence in me. I cannot thank each of you enough for all you have done. Most people grow out of their dream of being a marine biologist, but you stuck through it with me. You have accepted me for my strange, nerdy, tree-hugging ways. You have made me the person I am today, someone I am proud to be. I love you all forever (and to you Krysta, to the moon and back over and over again).

Juan, thank you. I cannot talk about support without talking about you and Kora. Coming home to you both everyday has made this whole experience worth it. You listened to me when I needed it most and laughed with me every single day. I genuinely do not know how I would have done this without you.

DEDICATION

This thesis is dedicated to Wilmington, the place where I learned who I am.

My home.

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ABSTRACT

Throughout the United States, shellfish aquaculture is being incorporated into best management practices due to the demonstrated positive effects bivalves have on water quality through suspension feeding. Farm-scale and ecosystem-scale models are useful tools that allow resource managers to better understand filtration capacity and shellfish aquaculture's effect on water quality, while also helping growers make farm practice decisions to improve production. To generate the most accurate model predictions, it is preferred to collect data specific to the cultivated species and site. This is the first study to examine a full individual energy budget for the triploid Eastern oyster, *Crassostrea virginica,* with implications for shellfish aquaculture in the southeastern United States.

The goal of this study was to examine the effects of temperatures and salinities specific to southeastern North Carolina on physiological processes of the cultured Eastern oyster, *Crassostrea virginica.* Physiological rates, such as: clearance rate (CR), egestion rate (ER), oxygen consumption rate (OCR), and ammonia excretion rate (AER), were determined for cultivated oysters in laboratory experiments supplied with estuarine water at varying temperatures and salinities. These rates were then combined to examine "scope for growth" to estimate under which environmental conditions these oysters had maximum energy for growth. I hypothesized that physiological rates would be maximized at higher temperatures (25-30 °C) and higher salinities (25 and 35 psu), typical of the cultivated environment in this region.

Temperature had a stronger effect on physiological rates than salinity. Oysters performed best at 30 °C, with highest scope for growth due to high clearance rates and low egestion rates. Energetically, oysters optimum temperature was at a slightly lower temperature of 25°C, demonstrated by high oxygen consumption rates at 30°C and maximum O:N at 25 °C. Salinity

results support that oysters optimum salinity is between 15 and 25 psu. These physiological rates paired with growth rates and farm practice data collected directly from the aquaculture sites will be used to optimize farm-scale models for this region that will allow resource managers to make informed decisions on leasing and nutrient mitigation techniques for shellfish aquaculture in North Carolina.

1. Introduction

Oyster aquaculture is an environmentally-sustainable way to fulfill the needs of municipalities in the southeastern United States for both local food sources and improved water quality. Understanding how environmental factors affect the physiological rates of ecologically and commercially valuable bivalves is necessary for the development of farm-scale and ecosystem-scale carrying capacity models used to evaluate this sustainability. These models are useful tools that allow managers and farmers to make informed decisions on shellfish aquaculture siting and scale (Ferreira et al., 2007). When determining whether to approve a shellfish aquaculture lease, resource managers must first establish geographical constraints (i.e. access), whether water quality conditions are appropriate for the intended species, what negative effects aquaculture may have on the surrounding environment or, conversely, what ecosystem services, or natural processes that serve human benefits, shellfish aquaculture could provide (Silva et al., 2011; Mitra and Zaman, 2016).

The ecosystem service of biofiltration, or top-down control of phytoplankton by shellfish aquaculture, has been proposed for nutrient mitigation in various programs (Ferreira et al., 2007; Rose et al., 2014; Bricker et al., 2018). In water bodies throughout the world, eutrophication, or "an increase in the rate of supply in organic matter to an ecosystem" (Nixon, 1995), can be detrimental when an overproduction of phytoplankton limits light to the benthos and creates hypoxic or anoxic conditions from increased bacterial decomposition (Johannesse and Dahl, 1996; Chislock et al., 2013). Therefore, resource managers are implementing innovative ways to mitigate the introduction of nutrients. In several regions of the U.S., programs are using "nutrient bioextraction", or growing shellfish or algae to harvest for nutrient reduction (Rose et al., 2014; Galimany et al., 2017; Bricker et al., 2018). Chesapeake Bay Program's Oyster Best

Management Practice Panel provided recommendations for effective nitrogen and phosphorous reduction via oyster tissue assimilation from on-bottom and off-bottom oyster aquaculture (Cornwell et al., 2016). The Mashpee Sewer Commission is using shellfish aquaculture in their Mashpee River nutrient mitigation plan to help promote the Massachusetts aquaculture industry, reach their goal of removing 5.01 metric tons of N per year, and cut capital costs by $90 million (Town of Mashpee Sewer Commission, 2015). In Washington State, it was concluded that bioextraction of the blue mussel, *Mytilus edulis*, could assist in water quality improvements in areas with sufficient water circulation (Pacific Shellfish Institute, 2014). It is estimated that just from oyster aquaculture, 125 kg N $acre^{-1}$ yr^{-1} can be removed from the Long Island Sound (Bricker et al., 2018). Before implementing similar projects in varying locations throughout the country, it is necessary to produce accurate estimates of this ecosystem service for specific regions. This study will contribute to these estimates for southeastern North Carolina by providing physiological data that will be incorporated into a farm-scale model to predict ecosystem services and impacts of oyster aquaculture in this region.

This is the first study to examine a full individual energy budget for triploid Eastern oysters, *Crassostrea virginica*, with the implications for shellfish aquaculture in the southeastern United States. Many researchers have examined the energy budget of commercially viable filter-feeders, such as the Eastern oyster, yet most studies were performed in the Gulf of Mexico or in northern regions of the North American coast, such as New England or Canada (Comeau et al., 2008; Hoellein et al., 2015; Lavaud et al., 2017; Casas et al., 2018a). Few have investigated the physiological ecology of bivalves in the southeastern U.S., and these experiments focus on the implications of oyster reef restoration instead of shellfish aquaculture (Grizzle et al., 2008; Galimany et al., 2017). This study is also unique in that the oysters provided for physiological

experiments were grown from triploid seed and may display different responses to environmental stressors due to the absence of their reproductive cycle, including the energy expenditure of gametogenesis. Since organisms can acclimate to different environments and climates and studies have shown regional differences in physiological rates, determining feeding behavior for the specific cultured species and site is essential for accurate model predictions (Cranford et al., 2011).

Temperature is arguably the most important environmental stressor on an organism's physiological processes (Angilletta et al., 2006). Changes in temperature can influence several aspects of an oyster's physiology, such as mitochondrial function, circulation, or shell growth (Bayne, 2017). *Crassostrea virginica* is a eurythermal species distributed throughout a large latitudinal gradient of North America, ranging from the Gulf of St. Lawrence to the Gulf of Mexico (Dame, 2012; Casas et al., 2018a). It has been suggested that physiological rates of oysters will vary at different latitudes due to thermal acclimation (Shumway, 1996; Dame, 2012). While the reported optimum thermal range for *C. virginica* is 20 °C to 30 °C (Shumway 1996), subtropical populations have shown to survive body temperatures of 49 °C (Galtsoff, 1964). In shallow estuaries of North Carolina, such as the Masonboro Island National Estuarine Research Reserve (NERR), it is common for temperatures to reach 36 °C (NOAA NERRS, n.d.), the upper limits of *C. virginica*'s thermal range (Galtsoff, 1964). Exposure to extreme temperature changes paired with other environmental stressors, like hypoxia or extreme salinities, is common to intertidal areas, where many oyster aquaculture sites and oyster reefs within the subtropical regions of the United States are located (Bahr and Lanier, 1981; Stanley and Sellers, 1986; Kingsley-Smith et al., 2013). At low tide, oyster physiological response to these extreme

conditions is unpredictable, with high heat causing decreased aerobic capacity and in some circumstances, mortality (Bayne, 2017).

Estuarine species face the added challenge of salinity fluctuations. Bivalves are osmoconformers, with internal osmotic concentrations that vacillate with environmental osmotic concentrations, which can affect cellular function and volume due to the balance of water, solutes, and macromolecules (Bayne, 2017). *C. virginica* is a typical euryhaline species, with a wider salinity tolerance than most other bivalves, surviving from 5 to 40 psu with an optimum of 15 to 25 psu (Shumway, 1996). Most studies have examined the short-term responses to these changes with mixed results that focus primarily on oyster response to low salinities (Shumway and Koehn, 1982; Casas et al., 2018b). They concluded that feeding and the degree to which an organism can regulate its oxygen uptake is reduced at lower salinities (Shumway and Koehn, 1982; Casas et al., 2018b). Masonboro Island NERR experiences much higher salinities than these locations due to its proximity to the ocean and is prone to abrupt salinity changes during frequent storm events (NOAA NERRS, n.d.). Limited research is available regarding the influence of salinity fluctuations on oysters acclimated to high salinity.

The goal of this study is to examine the effects of temperatures and salinities typical of southeastern North Carolina on physiological processes of the cultured Eastern oyster, *Crassostrea virginica.* Physiological rates, such as: clearance rate (CR), egestion rate (ER), oxygen consumption rate (OCR), and ammonia excretion rate (AER), were determined for cultivated oysters in laboratory experiments supplied with estuarine water at varying temperatures and salinities. These rates were then combined to examine "scope for growth" to estimate under which environmental conditions these oysters were maximizing their energy for growth, allowing farmers to gain a better understanding of growth for harvest. I hypothesized

that physiological rates would increase with increasing temperatures to an optimum temperature of 25-30 °C. I also hypothesized that oyster physiological rates would be lower at lower salinities (15 psu) than 25 and 35 psu due to their acclimation to local higher-salinity conditions. These physiological rates paired with growth rates and farm practice data collected directly from the aquaculture sites will be used to optimize farm-scale models for this region that will allow resource managers to make informed decisions on leasing and nutrient mitigation for shellfish aquaculture in North Carolina.

2. Materials and methods

2.1 Study Site

Two oyster aquaculture sites ("Farm 1" and "Farm 2" for this study) were located in Big Bay, a central intertidal lagoon within the Masonboro Island NCNERR (34°09'51"N 77°49'56"W) in New Hanover County, North Carolina (Figure 1). Although adjacent to developed areas, such as Wrightsville Beach and Carolina Beach, Masonboro Island is the largest undisturbed barrier island in southeastern North Carolina (Buerger et al., 2000). It receives inputs from both the Atlantic Ocean and the Intracoastal Waterway, with influences from the Cape Fear River during periods of high river flow. Big Bay has a tidal range of approximately 1-2 meters and average depth of 1 meter. Due to its large oceanic influence, Big Bay typically has high salinities ranging 25 psu to 35 psu. The water temperature in this lagoon ranges from about 10 °C to 30 °C. Since Masonboro Island is relatively undisturbed, it is considered oligotrophic, with nutrient levels (NO_4^+, NO_3^-, PO_4^{3-}) regularly below detection limits (Fear, 2008).

Farms 1 and 2 were the first oyster leases to be approved within the Reserve in 2015. Each farm was divided by gear type or seed plant date. Oyster seed (triploid only) for both farms was provided by the College of William and Mary Virginia Institute of Marine Science (VIMS). Farm 1 was separated into three different gear types (Figure 2). It contained four bottom cages ("Bay Bottom Cages"), racks that hold six bags on the sediment floor and exposed during low tide, containing DBY strain seed planted in August 2016. The suspended culture on Farm 1 ("Lentz"), or bags on longlines that flip with ebbing and flooding tidal flow, contained the DBY seed strain planted in November 2016. The third gear type was floating culture ("Floating bags"), with several lines containing floating bags containing the LoLA strain of seed planted in October 2015. Farm 2, on the other hand, consisted of all bottom culture, with bags sitting on 6" diameter irrigation piping (Figure 3). One group contained the LoLA strain of seed that was planted in October 2015. The other consists of the DEBY strain of seed planted in August 2016.

The third aquaculture site ("Farm 3") was located approximately 63 km north of Masonboro Island NERR in the New River Estuary, within Onslow County, North Carolina (Figures 4 and 6). Farm 3 is in one of the New River's meanders, Stone's Bay, approximately 13.5 km (8.4 mi) from the Atlantic Ocean. Due to barrier islands at the mouth of the estuary and broad, shallow lagoons, the New River Estuary has limited mixing with the Atlantic Ocean (Mallin et al., 2005; Currin et al., 2015). Unlike Farms 1 and 2, which are intertidal, Farm 3 is subtidal and is in water approximately 2 m deep. The middle region of the New River Estuary where Stone's Bay is located experiences mesohaline conditions with salinities ranging from 5-15 psu (Mallin and McIver, 2010). Temperature can range anywhere from 3.3 °C to 32 °C in the New River (Mallin et al., 1997). The New River Estuary is highly N-limited and primarily receives nutrients from non-point sources, such as agricultural activities (Mallin et al., 2005; C. Altman and Paerl, 2012).

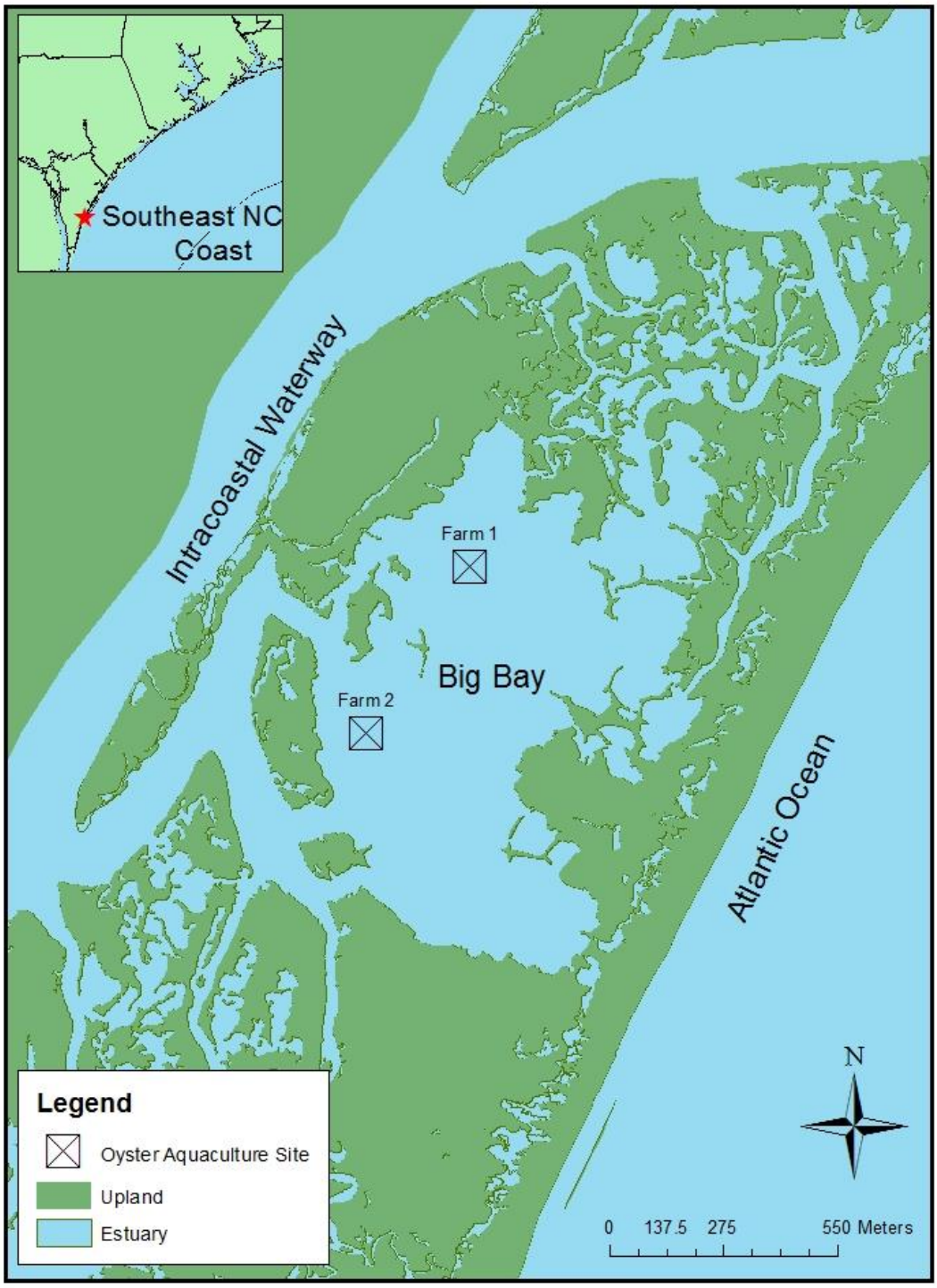

Figure 1. Oyster aquaculture sites within Masonboro Island North Carolina National Estuarine Research Reserve (NCNERR)

Figure 2. Three gear types on Farm 1

A. “Bay Bottom Cages”
B. “Lentz”
C. “Floating Bags”

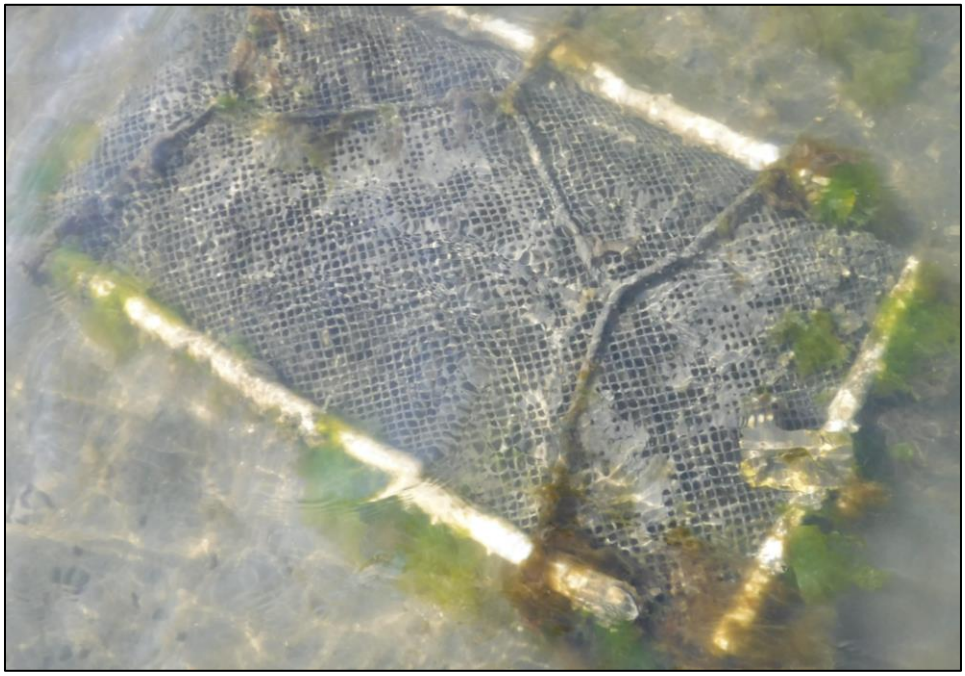

Figure 3. Bottom culture on Farm 2

All of Farm 2 contained the same bottom culture, mesh bags sitting on 6” diameter irrigation piping.

Figure 4. Oyster aquaculture site within the New River Estuary ("Farm 3")

Figure 5. Bottom culture on Farm 3

One example of bottom culture, “Cage”. Each “Cage” is divided into two sections with two layers stacked on top of one another. Other two gear types (“Cage w/ Bag” and “Tray”) are different versions of this bottom culture

A. Outside view of “Cage”
B. Inside view of “Cage”

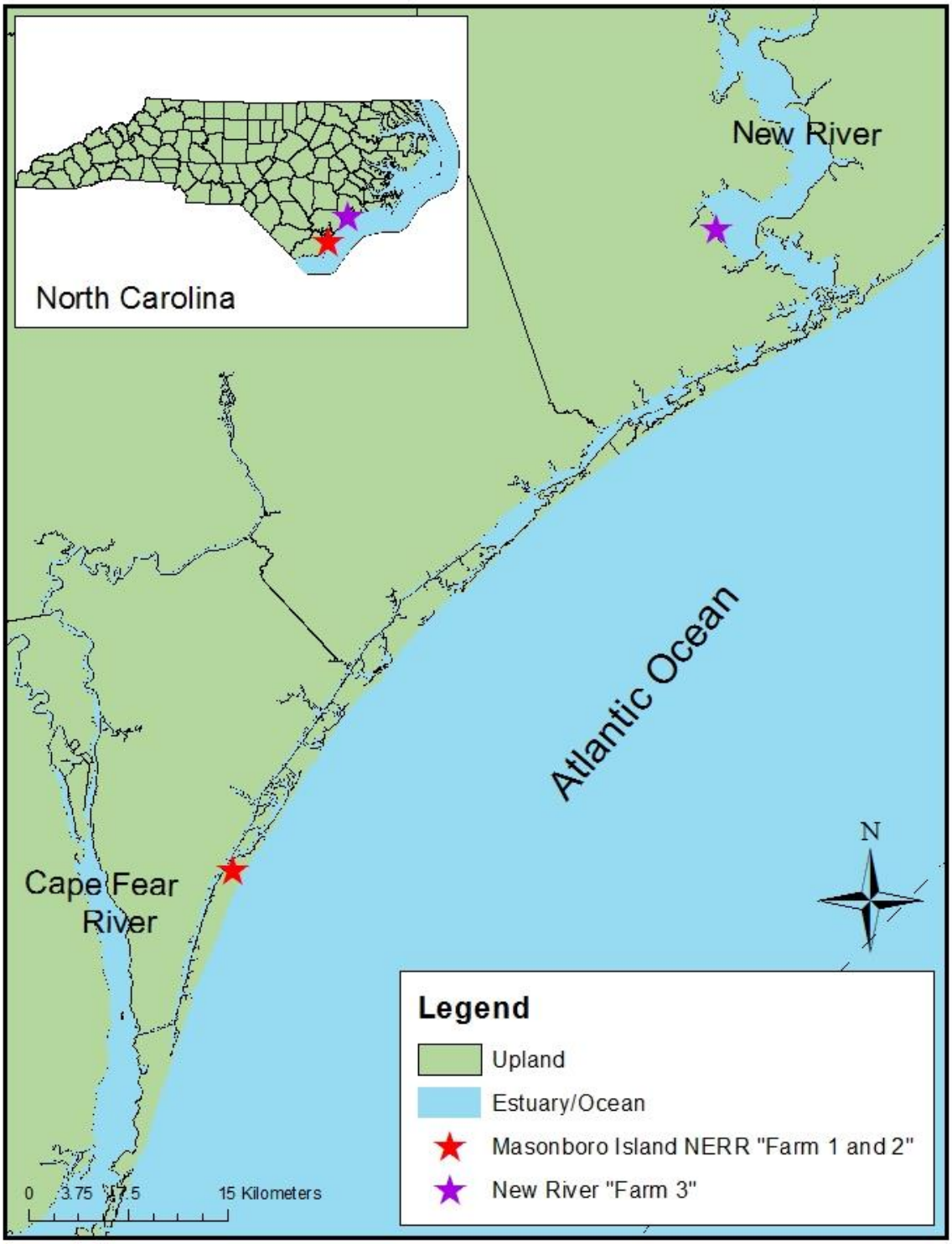

Figure 6. Oyster aquaculture sites within southeastern, North Carolina

Historically, it is one of the most eutrophic estuaries in the southeastern U.S. and is prone to phytoplankton blooms (Mallin et al., 2005).

Farm 3 consisted of three different types of bottom culture. The LoLA strain of seed planted in November 2017 was in large cages divided into four sections containing mesh bags ("Cage with Bag") (Figure 5). The DBY strains both started in this gear type in July 2017. One group was then moved into bottom trays divided into two sections ("Tray") and the other was moved into large cages divided into four sections ("Cage").

2.2 Experimental Design (Figure 7)

Oysters from Farm 1 and Farm 2 were collected to perform laboratory physiological experiments based on *in situ* temperatures. They were transported to University of North Carolina Wilmington's Center for Marine Science to be used for physiological rate measurements, including clearance, egestion, respiration, and ammonia excretion rates (Table 1, Figure 4). Oysters were first cleaned of any epibionts, measured for height (longest axis), length, and width, then placed into 5 L temperature-controlled, oxygenated tanks to be acclimated to experimental temperature and/or salinity for at least two weeks (Galtsoff, 1964). During their acclimation period, oysters were fed 0.3 – 1.2 ml phytoplankton mixture, Shellfish Diet 1800® (Reed Mariculture, Campbell, CA) per adult animal per day. Temperature and salinity were checked using a YSI Professional Plus Multiparameter instrument every other day. If temperature needed to be adjusted, it was changed +/- 1 ˚C per day. Salinity was adjusted +/- 1 psu per day. Prior to feeding trials, five randomly selected oysters were placed in an adjacent temperature-controlled tanks with 1 μm filtered estuarine water and to clear their guts for 24-48 hours. After each experiment, oysters were dissected to collect whole wet weight (g), tissue wet weight (g), and tissue dry weight (g).

Table 1. Descriptions of experimental physiological rates

Physiological Rates	Description	Calculation
Clearance Rate (L hr^{-1})	Volume of water cleared of suspended algal cells per unit time	(flow rate) x [(outflow concentration – inflow concentration) / (inflow concentration)]
Egestion Rate (mg DW feces hr^{-1})	Production of feces per unit time	(mg of dry weight of feces) / (length of experiment in hours)
Oxygen Consumption Rate (mg O_2 hr^{-1})	Amount of oxygen consumed by tissues	[background O_2 concentration] – [(final O_2 concentration) – (initial O_2 concentration)] x volume of O_2 in static chamber) / (length of experiment in hours)
Ammonia Excretion Rate (Lhr^{-1})	Amount of ammonia excreted per time	[(final NH_3-N concentration) – (initial NH_3-N concentration)] x volume of O_2 in static chamber) / (length of experiment in hours)

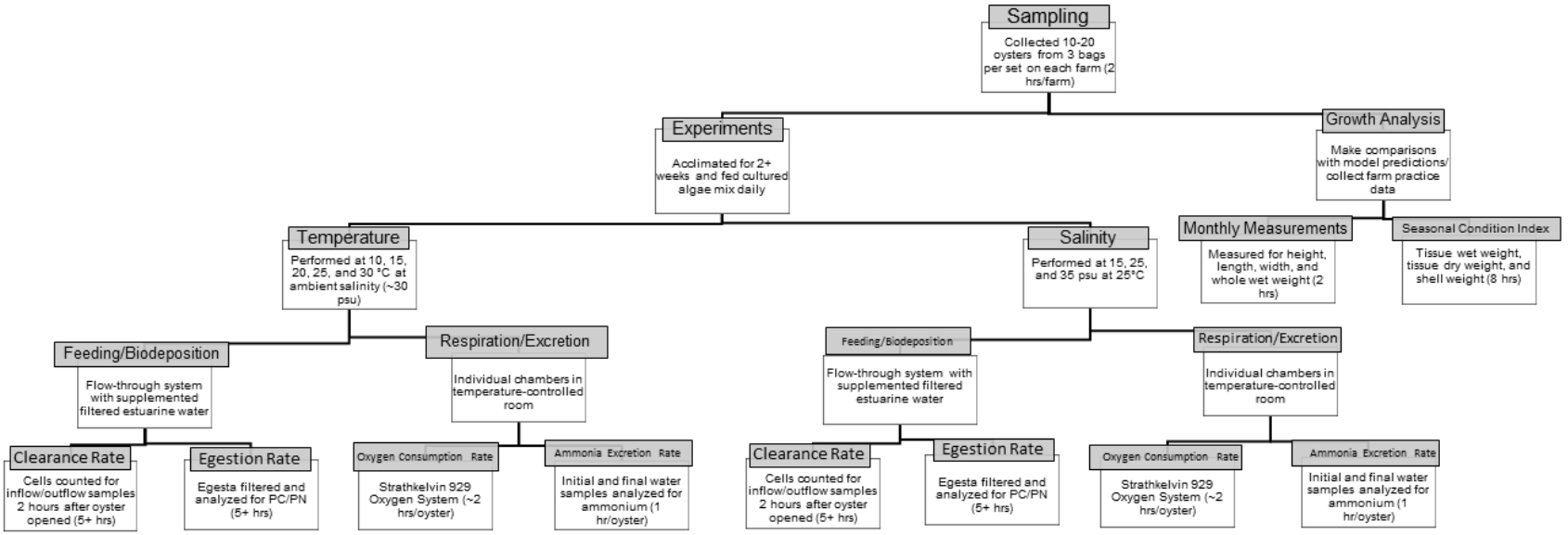

Figure 7. Flow chart of experimental design

Brief description of each component of data collection. (Time) represents the amount of time required for each experimental run unless specified otherwise. Time required does not include setup/clean up nor travel time.

Each type of physiological rate was determined at five temperatures ranging from 5 ˚C to 30 ˚C, typical of the cultivated environment in this region. The experiments were repeated at 25 ˚C for *C. virginica* at three different salinities (15, 25, and 35 psu). Each of the physiological rates was standardized per gram of dry tissue weight, a proxy for gill size, using the following formula:

$$Y_s = Y_e(1/W_e)^b,$$

in which Y_s represents the corrected physiological rate, Y_e is the experimental rate, W_e is the weight of the individual, and b is the allometric exponent (0.6265 for temperature experiments, 0.6565 for salinity experiments; Appendix 1) (Cranford et al., 2011).

2.3 Oyster Feeding and Biodeposition

Clearance rates, defined as the amount of particles cleared per unit time (Widdows, 1985), were determined using a flow-through system (Figure 8). A mixture of fresh cultured algae *Tisochrysis lutea* and *Chaetoceros muelleri* was provided by the University of North Carolina Wilmington Shellfish Hatchery and diluted to approximately 2.1 x 10^4 cells mL^{-1} in 60 µm filtered estuarine water from the Intracoastal Waterway in a 275 L head tank. The tank was aerated to ensure particles were well-mixed. Temperature was controlled in the head tank using heaters and chillers and salinity was controlled by adding freshwater or Instant Ocean®. This water was pumped into seven 2 L feeding chambers, with flow rate to each feeding chamber individually controlled to rates between 160-220 mL min^{-1}. These rates were determined in preliminary experiments to achieve maximum oyster clearance rates, or the "independency phase" when clearance rates remain constant as flow rates increase (Filgueira et al., 2006). To reduce temperature fluctuations throughout the experiment, feeding chambers were slightly immersed in a surrounding water bath that was also temperature-controlled by heaters and

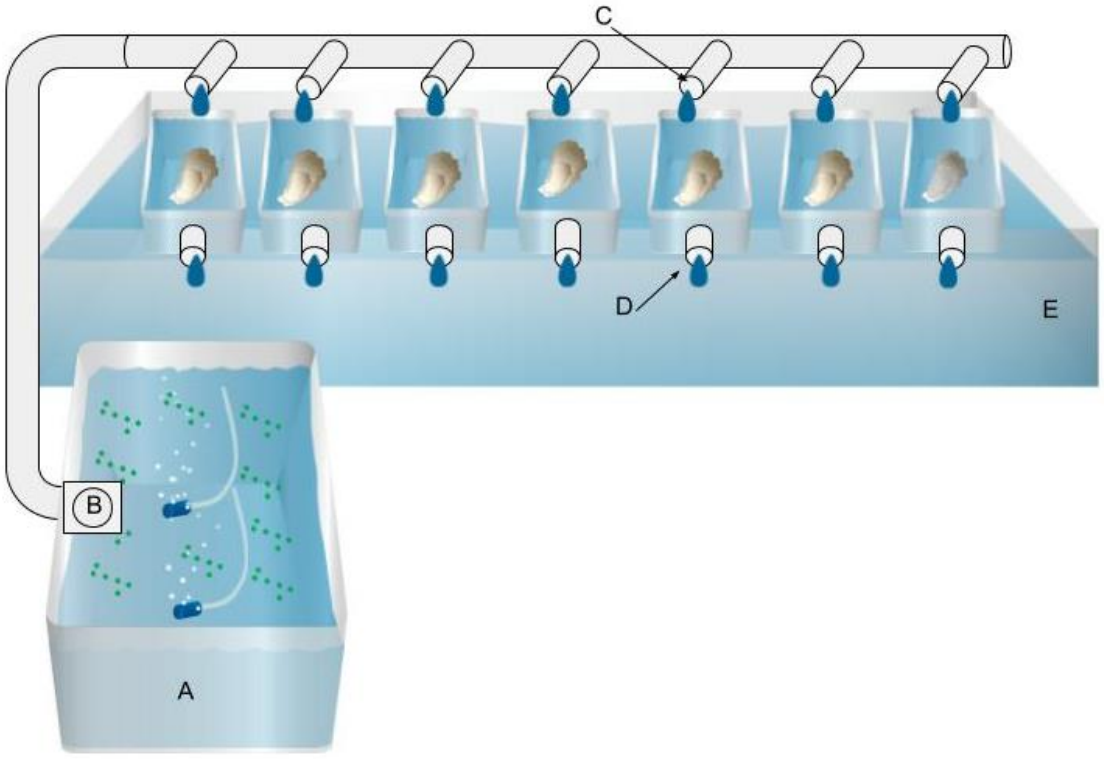

Figure 8. Flow-through experimental setup for laboratory feeding experiment

A. Temperature/salinity-controlled head tank containing aerated filtered estuarine water supplemented with cultured algae mix
B. Pump feeding water into flow-through system
C. Inflow
D. Outflow
E. Temperature-controlled water bath

chillers. Once chambers were filled with water, five oysters were placed into separate chambers with two control chambers containing only shells to control for particle settlement.

For each experimental chamber, feeding was considered to begin once the individual oyster opened its valve. The percentage of oysters open during each experiment was noted to estimate how on a population level, individual maximum clearance rates would be modulated by percent time feeding. Water samples were collected after 90 minutes and 2 hours from the inflow (1.5 cm in diameter) and outflow (2.5 cm in diameter). The flow rate for each tank was determined at the time the water sample was taken. Water samples were analyzed on a Beckman Multisizer 4e Coulter Counter within 3 hours of the experiment for counts of particles of diameters 3 to 10 µm, which is the size range retained by the oyster gill with 100% efficiency (Bayne and Newell, 1983).

Under flow-through conditions in which the individual oyster was exposed to the inflow concentration only and the exhalent current mixes with the inflowing particles "downstream" of the organism, individual clearance rate was determined using the calculation:

$$CR_i\ (L\ h^{-1}) = f \cdot \frac{[C_i - C_0]}{C_i}$$

where *f* represents the flow rate, C_i is the particle concentration of the inflow, C_o is the particle concentration of the outflow (Filgueira et al., 2006). As a proxy for population clearance rate (CR_p) , each of individual clearance rates (CR_i) was multiplied by the percentage of oysters that were open during each experiment. Water samples were collected from the head tank and individual tank inflow to be filtered onto pre-ashed 0.7 µm nominal pore sized GF/F filters. They were analyzed using a CE Instruments NC 2100 elemental analyzer (ThermoQuest) for particulate carbon/particulate nitrogen concentrations of the incoming food.

Egestion rates were determined during feeding experiment. Feces and pseudofeces produced by each oyster during the experiment were separated and collected with pipettes. The oysters were then held for 24 hours in individual aerated chambers of 1 µm filtered water at acclimated temperatures to collect any remaining biodeposits from the feeding experiment. All feces and pseudofeces and water samples from the inflowing water and head tank were filtered onto separate pre-ashed and pre-weighed GF/F filters (Hoellein et al., 2015). The filters were then dried for 24 hours at 45°C and weighed. These filters were analyzed using elemental analysis to determine total particulate carbon/particulate nitrogen of egesta.

Ingestion rate (IR) was calculated using the following equation:

$$IR\ (mg\ C\ g\ DW^{-1}\ h^{-1}) = (mg\ C\ from\ inflow) \cdot V \cdot CR_i$$

where V is the volume of inflow water filtered onto GF/F filters (L) and CR_i is the individual clearance rates standardized per 1 gram of tissue dry weight (L g DW^{-1} h^{-1}).

2.4 Respiration and Ammonia Excretion

Oxygen consumption and ammonia excretion rates were determined simultaneously in static, stirred chambers within an environmental chamber to control air temperature. Oysters had been pre-acclimated to the experimental temperature in aerated tanks. Estuarine water filtered to 1 µm was aerated and pre-acclimated to the experimental temperature using heaters and chillers. Oxygen consumption was measured with a Strathkelvin 929 Oxygen System. Each respiration chamber was placed on a stir plate and air bubbles were removed prior to sealing the top of the chamber. Respirometers ran for 1-2 hours prior to adding oysters to obtain background respiration rates. Each oyster was then added to an individual chamber and an initial water sample was collected for ammonia excretion measurements.

Oxygen consumption and ammonia production were measured over two hours. Once the oyster was removed from the chamber, the volume of water from each chamber was measured and a final water sample was collected for ammonia excretion measurements. Initial and final water samples were filtered immediately after collection using Whatman polyethylsulfone membrane filters with a 0.45 um pore size. Water samples were frozen until analysis for ammonium on a Bran+Luebbe AutoAnalyzer 3.

The calculations for absolute oxygen consumption rate collected from the Strathkelvin 929 software were based on the formula:

$$VO_2\ (mg\ O_2\ h^{-1}) = \frac{([O_2]_{t_f} - [O_2]_{t_i}) \cdot V}{t}$$

where VO_2 represents the oxygen consumption rate (mg O_2 h^{-1}), $[O2]_{t_i}$ is the initial oxygen concentration, $[O2]_{t_f}$ is the final oxygen concentration, V is the volume of water in the chamber without the volume of the oyster (L), and t is the length of the experiment (h). This rate was then subtracted from the background rate collected from the Strathkelvin 929 software using the same equation.

Ammonia excretion rate was determined using the formula:

$$AER = \frac{\left([NH_3 - N]_{t_f} - [NH_3 - N]_{t_i}\right) \cdot V}{t}$$

where AER represents the ammonia excretion rate (mg NH_3 h^{-1}), $[NH_3 - N]_{t_i}$ is the initial ammonium concentration, $[NH_3 - N]_{t_f}$ is the final ammonium concentration, V is the volume of water in the chamber without the volume of the oyster (L), and t is the length of the experiment (h).

O:N are energy production indices used observe catabolism of proteins, lipids, and carbohydrates (Bayne, 2017). Since these oysters were starved prior to respiration and ammonia excretion experiments, O:N is calculated using standard metabolic rate, or the post-absorptive stage of an organism. When an oyster is feeding, ammonia excretion is influenced by the quality of food in addition to metabolic needs, which can bias O:N. Therefore, in a fasted stated, O:N can reliably predict stress based on the utilization of carbohydrates and lipids versus proteins (Bayne, 2017). O:N was calculated by dividing the molar oxygen consumption rates by ammonia excretion rates.

The van't Hoff temperature coefficient (Q_{10}) is a useful tool to observe the temperature sensitivity of a physiological rate (Bayne, 2017). It describes how a physiological rate changes as the temperature of the individual's surrounding environment increases by 10 ºC. Most physiological rates double with a 10 ºC temperature increase, represented as a Q_{10} value of 2. Any values much greater than 2 represent biochemical process changes (Bayne, 2017). It can be calculated using the formula:

$$Q_{10} = \frac{k_2}{k_1}^{10\,(t_2 - t_1)}$$

where k_2 and k_1 are the physiological rates at temperatures t_2 and t_1 (Bayne, 2017). Oxygen consumption rates (OCR) collected during temperature experiments were used to calculate Q_{10} values for this study.

After collecting each of the physiological rates, scope for growth was calculated to distinguish under which southeastern North Carolina environmental conditions the triploid Eastern oyster would be expected to perform best and have the highest growth rates. To measure scope for growth, each of the physiological parameters was converted to units of carbon (µmol C g DW^{-1} h^{-1}). The molar ratio of 1 µmol O_2: 1 µmol CO_2 during respiration was used to convert

oxygen consumption rate. Ammonia excretion rates were converted to carbon based on the molar ratio for respiration of proteins (16 µmol N: 106 µmol C) . The following formula was used to calculate scope for growth:

$$Scope\ For\ Growth\ =\ IR - (OCR\ +\ ER\ +\ AER)$$

where IR is ingestion rate, OCR is oxygen consumption rate, ER is egestion rate, and AER is ammonia excretion rate.

2.5 Field Growth

Growth data were collected to make comparisons between scope for growth and the actual growth patterns occurring within the aquaculture sites. Farm 3 growth and condition data were collected to make comparisons on a regional scale. Each month, farming practice data (harvest/planting) were provided by the growers and a sample of oysters were collected to measure growth rates. Three bags or trays were randomly selected from each set, designated by gear type or seed planting date. From each bag, all oysters were counted for mortality calculations and 10-20 live oysters were randomly collected to be brought back to the laboratory for analysis. Each month, oysters were measured for height, length, width, and whole wet weight. Each season, they were further examined to determine condition index.

Condition index can be used to observe responses to environmental change as it measures the amount of soft tissue that occupies that shell cavity of the oyster, acting as a proxy for the amount of carbohydrates stored for nutrient reserve (Bayne, 2017). Condition index was calculated using the following formula:

$$CI\ =\ (DW\ /Shell\ Cavity\ Volume)\ \times 100$$

in which DW is the tissue dry weight of the oyster (g) and shell cavity volume is the dry shell weight subtracted from the whole wet weight of the oyster (g).

2.6 Statistical Analysis

All statistical analysis was performed using SigmaPlot 13.0 (© 2019 Systat Software Inc.). Clearance rate, egestion rate, respiration rate, and ammonia excretion rate were examined for normality and homogeneity of variance. Nonlinear regression was used to determine relationships of temperature to clearance, egestion, and respiration rate. The thermal performance curves (relationship between temperature and physiological rates, such as clearance rate and respiration rate), were modeled using a Gaussian function (Angilletta, 2006). Generalized linear modelling (GLM) with a poisson distribution was used to determine the effect of the interaction of season and set of oysters ("Gear Type" or "Plant Date") on condition index. All data are represented as mean rate (+/- standard error).

3. Results

3.1 Effect of temperature on physiological rates

Oysters were most active at higher temperatures during feeding experiments. There was a strong positive relationship between temperature and individual clearance rate ($CR_i = 20153 * e^{\left(-0.5\left(\frac{t-346}{80}\right)^2\right)}$, $F_{2,44} = 17.0$, $p < 0.01$) (Figure 9) and population clearance rate ($CR_p = 86189 * e^{\left(-0.5\left(\frac{t-301}{63}\right)^2\right)}$, $F_{2,44} = 25.2$, $p < 0.01$) (Figure 10). A similar trend was found for ingestion rate, where IR increased with increasing temperature, but at a slower rate ($IR = 5.31 * e^{\left(-0.5\left(\frac{t-47}{26.43}\right)^2\right)}$,

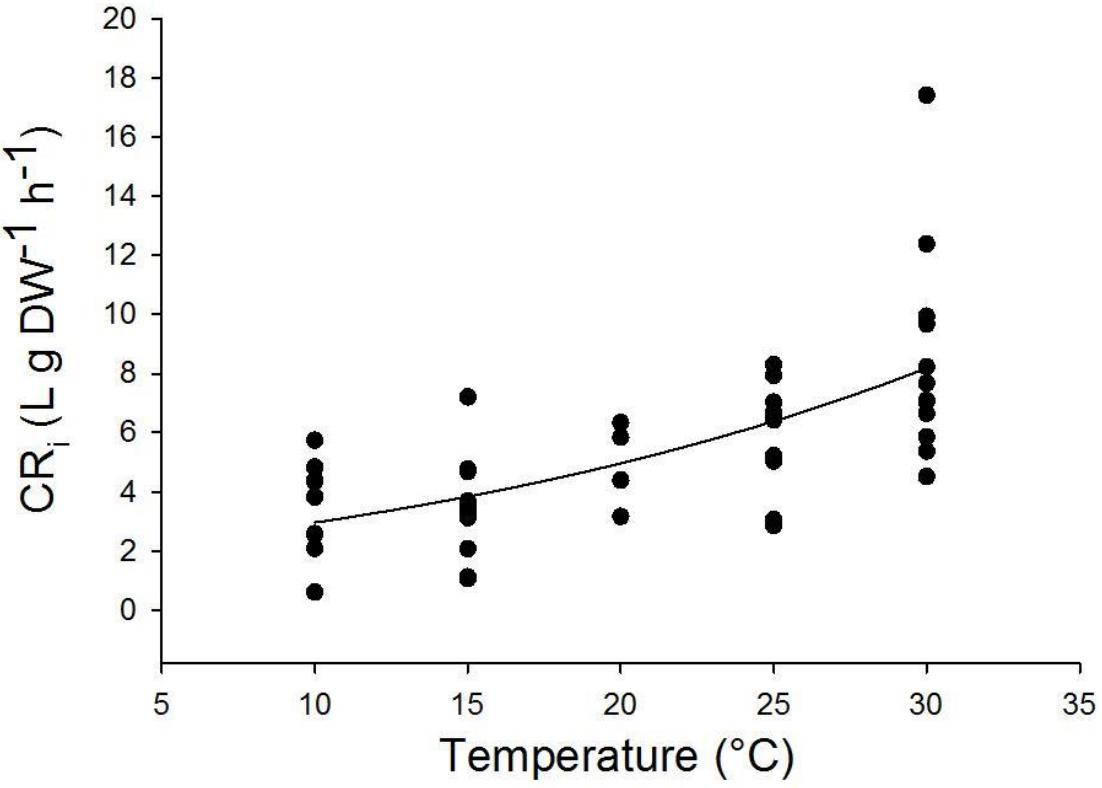

Figure 9. Individual clearance rate (CRi) versus temperature (°C)

Temperature had a positive significant effect on CR_i for experimental temperatures. Each point represents clearance rate for an individual oyster.

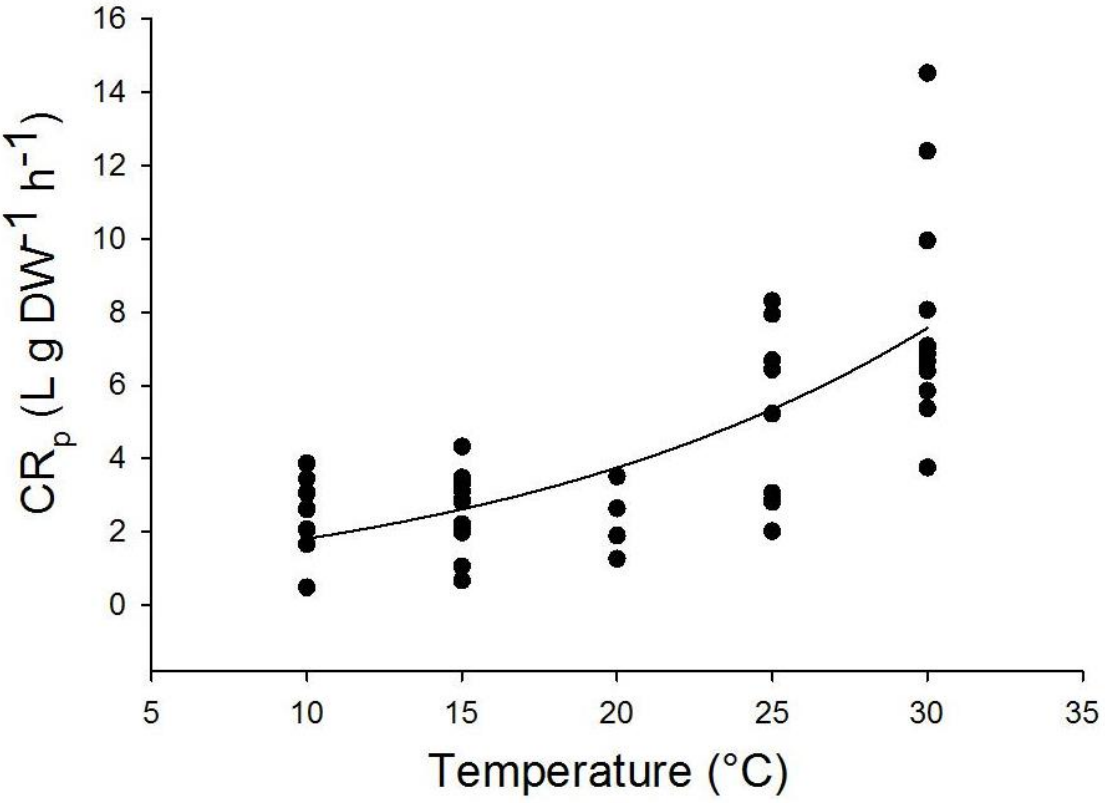

Figure 10. Population clearance rate (CRp) versus temperature (°C)

Temperature had a positive significant effect on CR_p for experimental temperatures. Each point represents an individual clearance rate (CR_i) multiplied by the percentage of open oysters during that experiment.

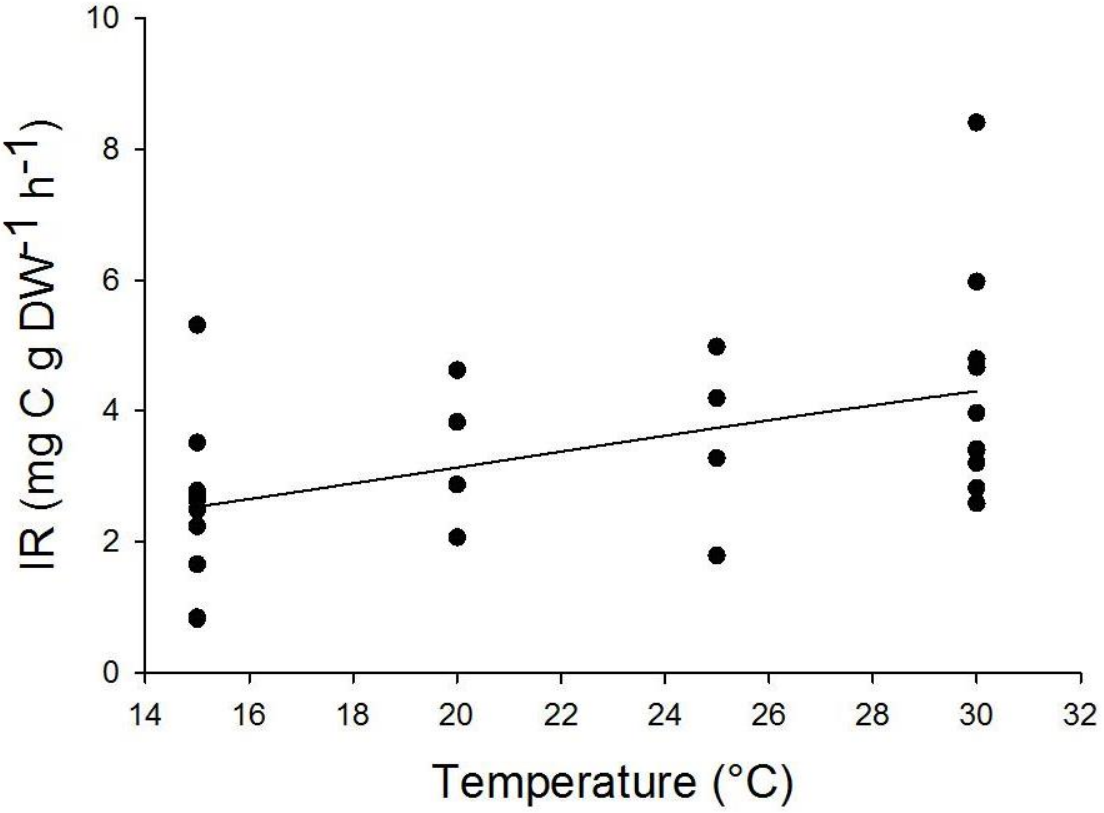

Figure 11. Ingestion rate (IR) versus temperature (°C)

Temperature had a positive significant effect on IR for experimental temperatures. Each point represents an individual egestion rate.

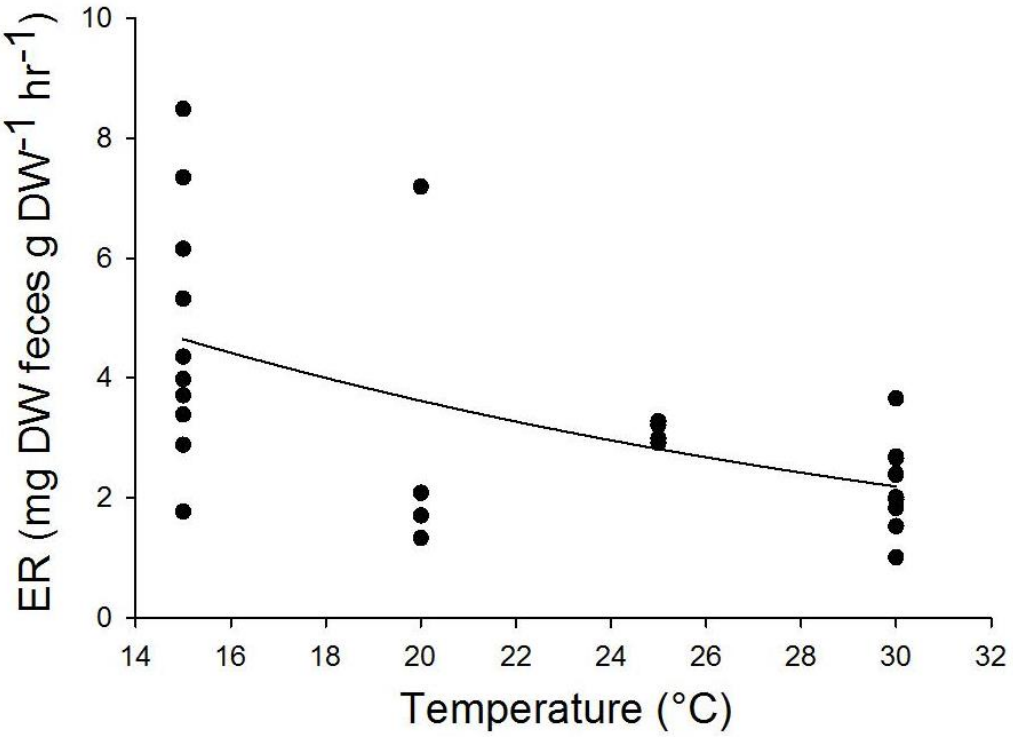

Figure 12. Egestion rate (ER) versus temperature (°C)

Temperature had a negative significant effect on ER for experimental temperatures. Each point represents an individual egestion rate.

Table 2. Pseudofeces collected during feeding experiments

Pseudofeces samples collected when visible. Sample collected at 20 °C was contaminated. Bolded values represent experimental condition.

Experiment	Temp (°C)	Salinity	Pseudofeces wt. (mg)
Temperature	**20**	30	
	30	30	6.40
Salinity	25	**15**	0.41
	25	**15**	0.08
	25	**15**	0.55
	25	**25**	0.79

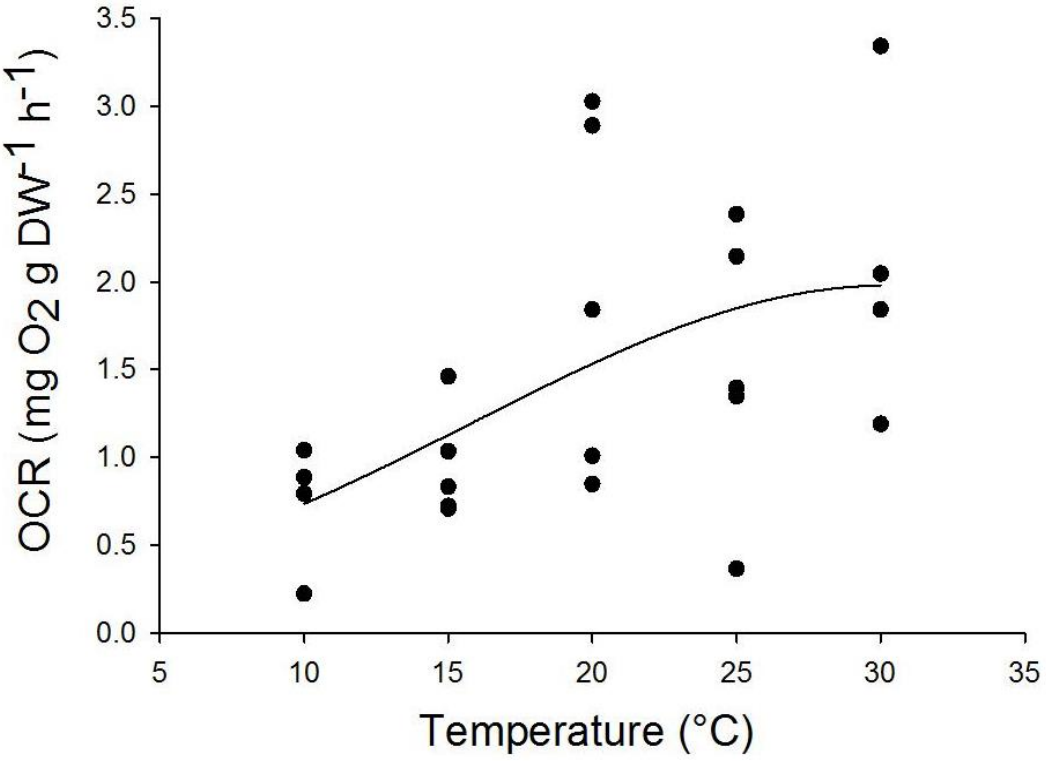

Figure 13. Oxygen consumption rate (OCR) versus temperature (°C)

Each point represents an individual oxygen consumption rate. Oxygen consumption rate increased as temperature increased until 30.33 ºC.

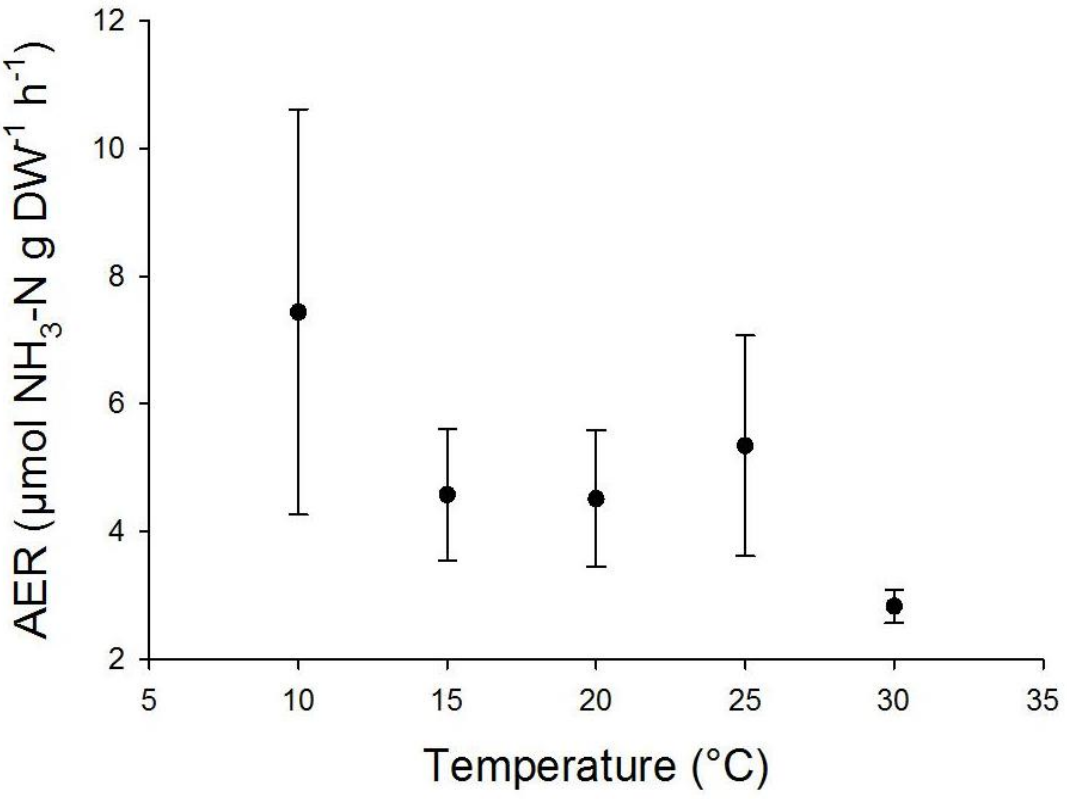

Figure 14. Ammonia excretion rate (AER) versus temperature (°C)

There was no significant effect of temperature on AER. Each point represents the mean ammonia excretion rate for each temperature. Error bars represent standard error.

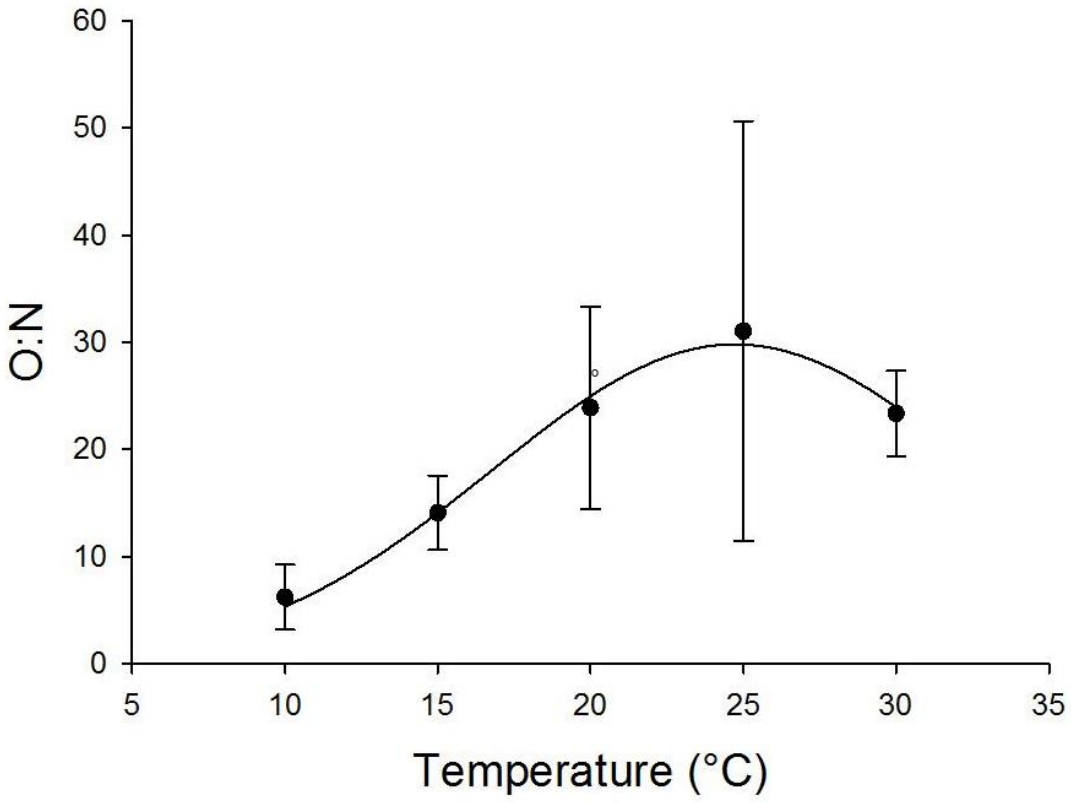

Figure 15. O:N versus temperature (ºC)

Maximum O:N is at 24.73 ºC. Each point represents the mean O:N for each temperature. Error bars represent standard error.

Table 3. Q10 values calculated from oxygen consumption rates (OCR).

Bolded value represents Q_{10} >2, indicative of high temperature dependence.

T_1	T_2	R_1	R_2	Q_{10}
10	15	0.73	0.95	1.67
15	20	0.95	1.92	**4.08**
20	25	1.92	1.53	0.63
25	30	1.53	2.11	1.90

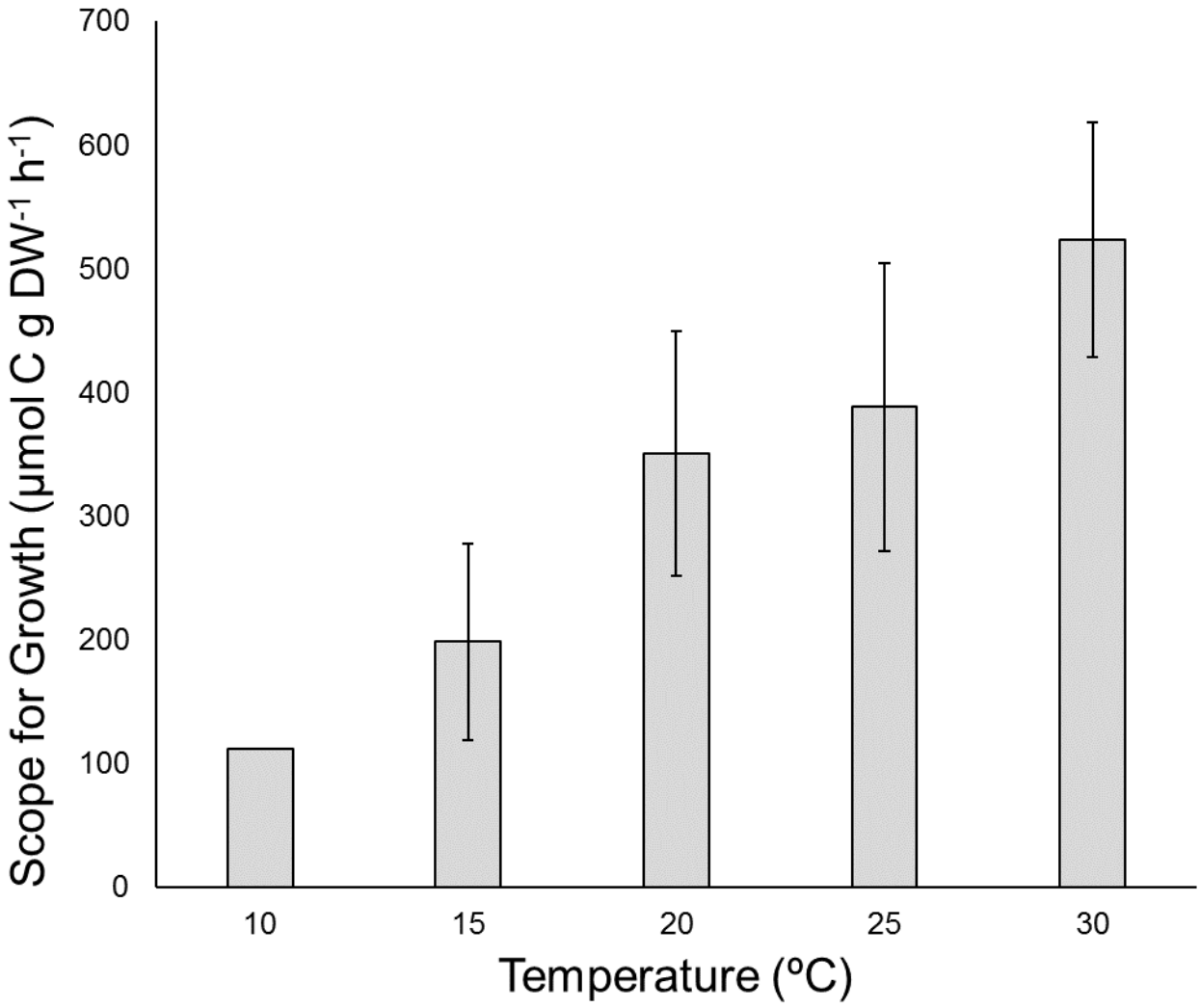

Figure 16. Scope for Growth versus temperature (ºC)

Scope for growth increases with increasing temperature. Error bars represent standard error. Samples for egestion rate at 10 ºC were lost. Scope for Growth at 10 ºC was calculated by linear regression ($f(x) = 20.24x - 90.36$, $r^2 = 0.96$).

Table 4. Mean physiological rates collected during temperature experiments.

Experiments were conducted at five different temperatures typical of the cultivated environment ranging from 10 ºC to 30 ºC at approximate ambient salinities (30 psu). Each of these rates was standardized using the equation $Y_s = Y_e(1/W_e)^b$ (Cranford et al., 2011). The constant "b" was determined during preliminary experiments (Appendix 1).

Parameter	b	Rate	Temp (°C)	Salinity	n	Mean	Std. Err.	Mean DW (g)	Std. Err.
Temperature	0.6265	CR_i	10	30	11	3.60	0.45	0.61	0.09
			15		11	3.43	0.53	0.66	0.07
			20		4	4.93	0.72	0.37	0.05
			25		9	5.84	0.65	0.42	0.11
			30		12	8.48	1.03	0.49	0.10
		CR_p	10	30	11	2.62	0.31		
			15		11	2.53	0.32		
			20		4	2.33	0.48		
			25		9	5.04	0.80		
			30		12	7.82	0.88		
		ER	15	30	10	4.74	0.66	0.70	0.07
			20		4	3.08	1.38	0.37	0.05
			25		4	3.10	0.09	0.33	0.09
			30		10	1.95	0.31	0.52	0.12
		OCR	10	30	4	0.74	0.18	0.60	0.22
			15		5	0.95	0.14	0.40	0.10
			20		7	1.92	0.42	0.25	0.08
			25		5	1.53	0.35	0.26	0.07
			30		4	2.11	0.36	0.17	0.06
		AER	10	30	2	7.44	3.17	0.86	0.40
			15		6	4.60	1.03	0.45	0.11
			20		5	4.51	1.07	0.25	0.08
			25		4	5.34	1.73	0.31	0.08
			30		3	2.83	0.26	0.12	0.05
		O:N	10	30	2	11.71	5.74	0.86	0.40
			15		6	26.51	6.54	0.45	0.11
			20		5	44.92	19.47	0.25	0.08
			25		4	58.44	36.88	0.31	0.08
			30		3	43.96	8.71	0.12	0.05

$F_{2,25}$ = 3.85, p = 0.03) (Figure 11). The opposite relationship was found between temperature and egestion rate with highest egestion rates observed at lower temperatures ($ER = 9.86 * e^{(-0.05t)}$, $F_{1,26}$ = 11.87, p < 0.01) (Figure 12). Pseudofeces collections from all experiments can be found in Table 2.

Temperature also had a positive significant relationship with oxygen consumption rate (Figure 13). Oxygen consumption rate gradually increased until it reached a peak at approximately 30.33 ºC ($OCR = 1.98 * e^{\left(-0.5\left(\frac{t-30.33}{14.45}\right)^2\right)}$, $F_{2,20}$ = 4.09, p = 0.03). The largest sensitivity to temperature change was observed between 15 ºC and 20 ºC, where there was a Q_{10} value of 4.08 (Table 3). Although there was no significant effect of temperature on ammonia excretion rate (AER) ($F_{4,15}$ = 0.92, p = 0.48) (Figure 11), there was a significant effect of temperature on O:N ($f = 56.13 * e^{\left(-0.5\left(\frac{x-24.73}{7.95}\right)^2\right)}$, $F_{2,2}$ = 95.0, p = 0.0104), with highest O:N at 24.7 ºC (Figure 15).

The combined effects of temperature on each of the physiological rates is explained in scope for growth, which increased as temperature increased (Figure 16). All mean physiological rates for temperature experiments are found in Table 4.

3.2 Effect of salinity on physiological rates

Salinity had no significant effect on individual clearance rate (CR_i) ($F_{2,28}$ = 1.06, p = 0.36) nor population clearance rate (CR_p) ($F_{2,28}$ = 0.01, p = 0.99) (Figure 17). Individual clearance rate ranged from 0.59-12.78 L g DW^{-1} h^{-1} and population clearance rate ranged from 0.45-8.64 L g DW^{-1} h^{-1}. Salinity did not have a significant effect on ingestion rate (Figure 18) nor egestion rate ($F_{2,15}$ = 1.01, p = 0.39) (Figure 19). Egestion rate ranged from 0.16 to 15.29 mg feces g DW^{-1} h^{-1}.

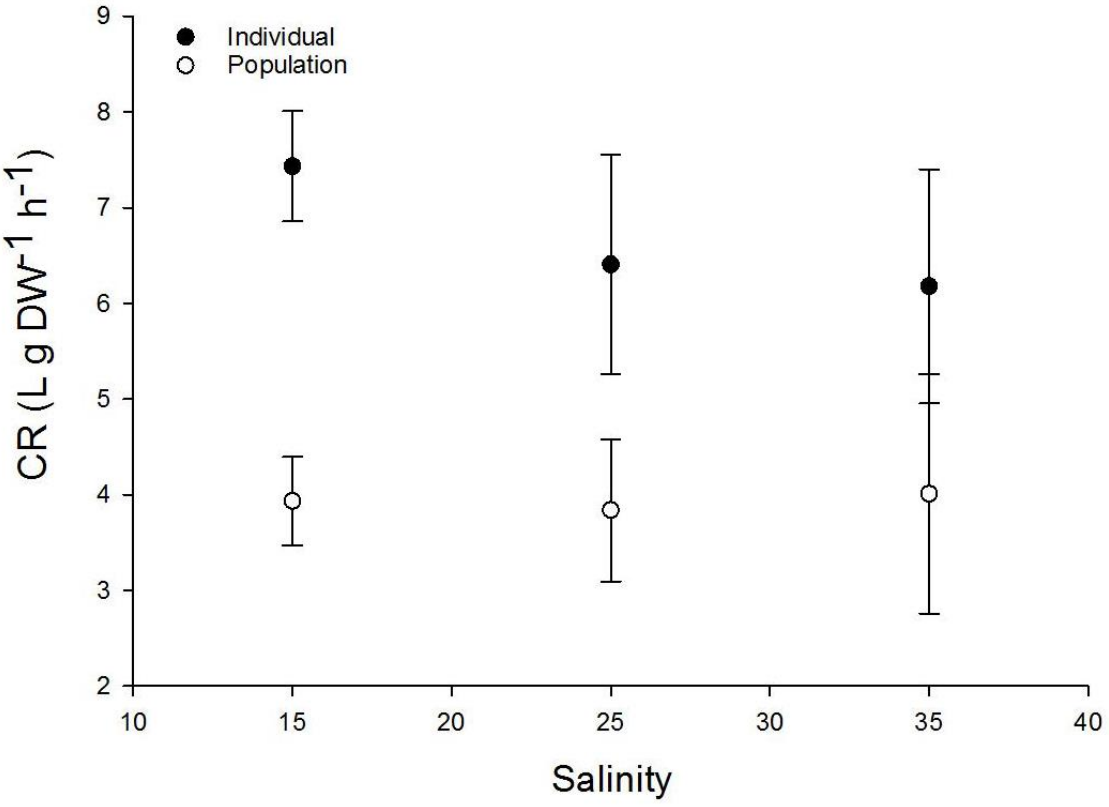

Figure 17. Clearance rate (CR) versus salinity

There was no significant effect of salinity on CR. Each point represents mean CR. Error bars represent standard error.

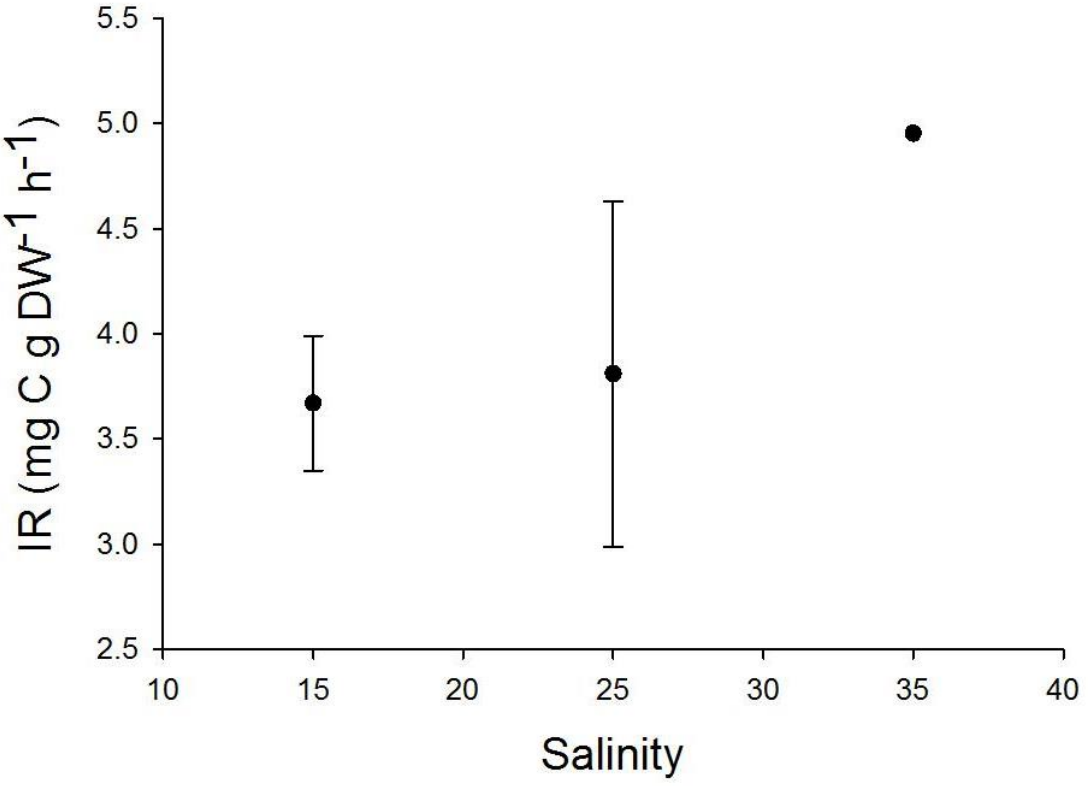

Figure 18. Ingestion rate (IR) versus salinity.

Points at 15 and 25 psu represent mean IR. Only one sample was collected at 35 psu. Error bars represent standard error.

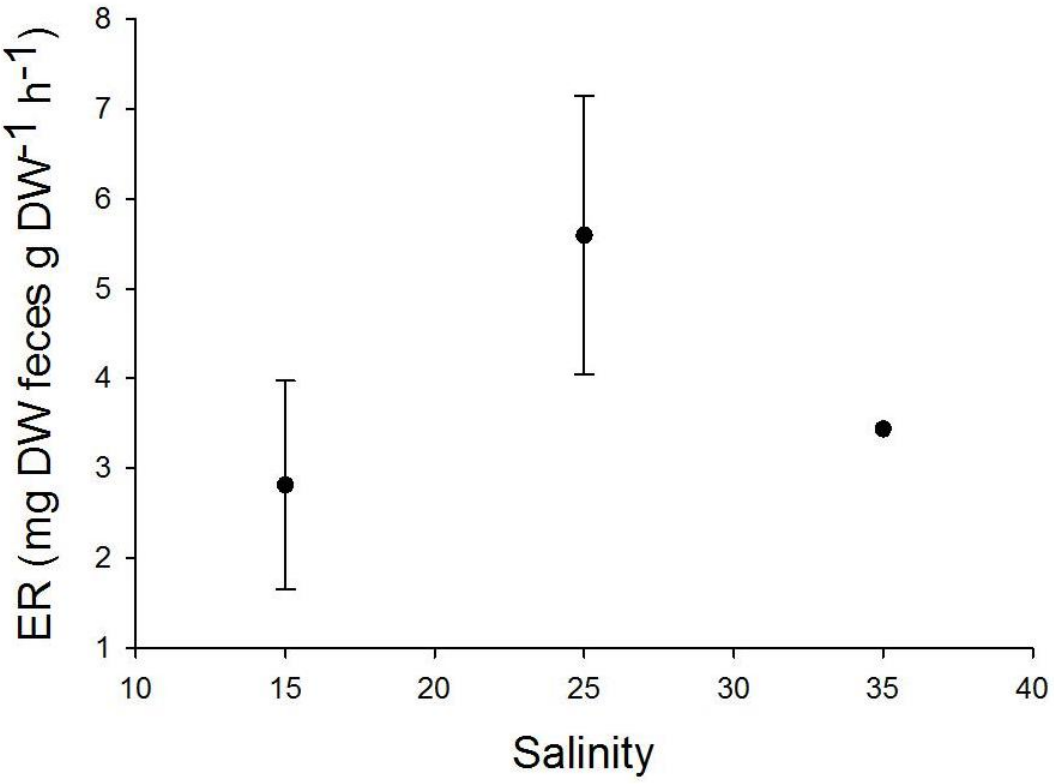

Figure 19. Egestion rate (ER) versus salinity.

Points at 15 (n=8) and 25 (n=9) psu represent mean ER. Only one sample was collected at 35 psu. Error bars represent standard error.

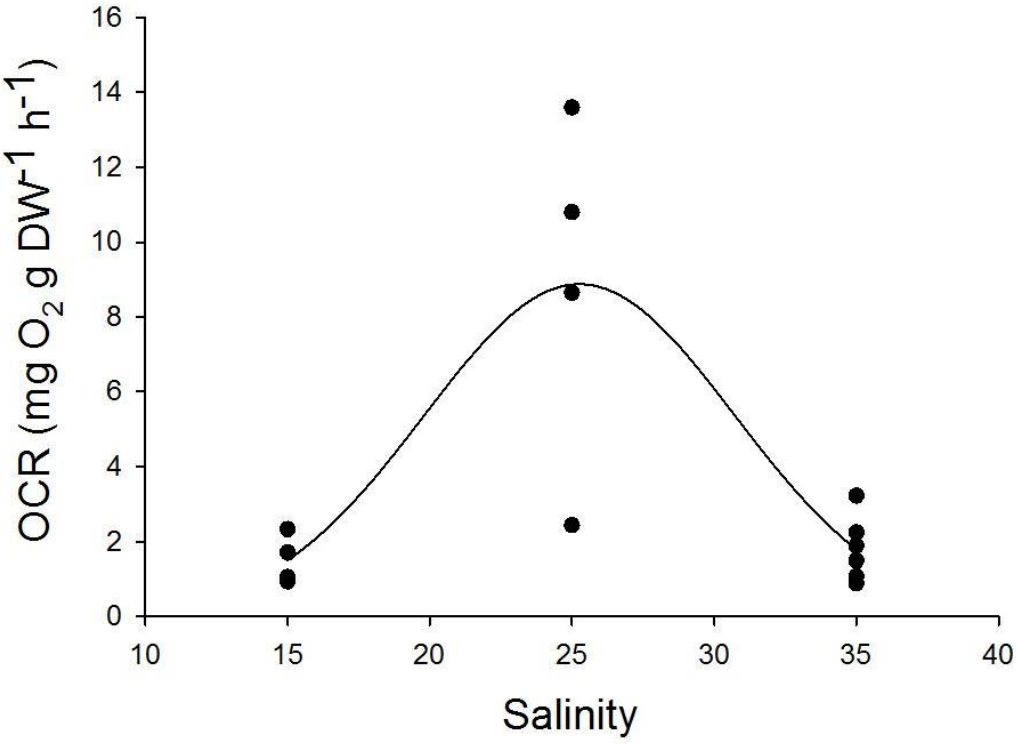

Figure 20. Oxygen consumption rate (OCR) versus salinity

Salinity had a significant effect on oxygen consumption rate, with highest rates at 25 psu. Each point represents an individual oxygen consumption rate.

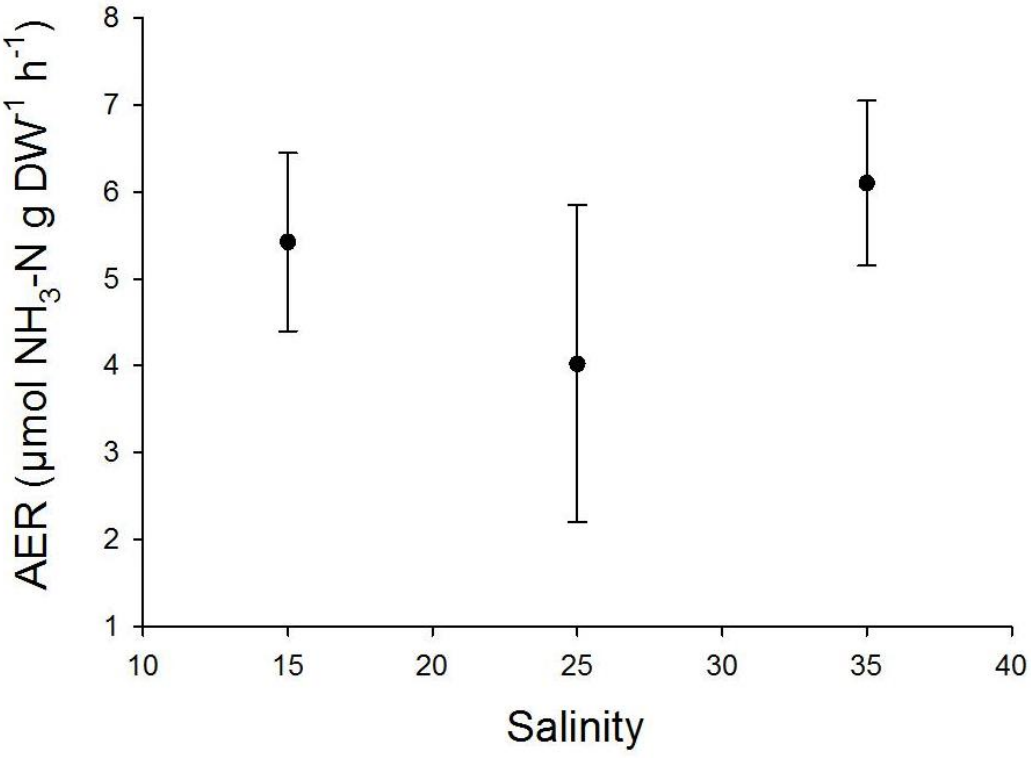

Figure 21. Ammonia excretion rate (AER) versus salinity

Points represent mean AER. From 15 to 35 psu, n = 4, 4, and 6 respectively. Error bars represent standard error.

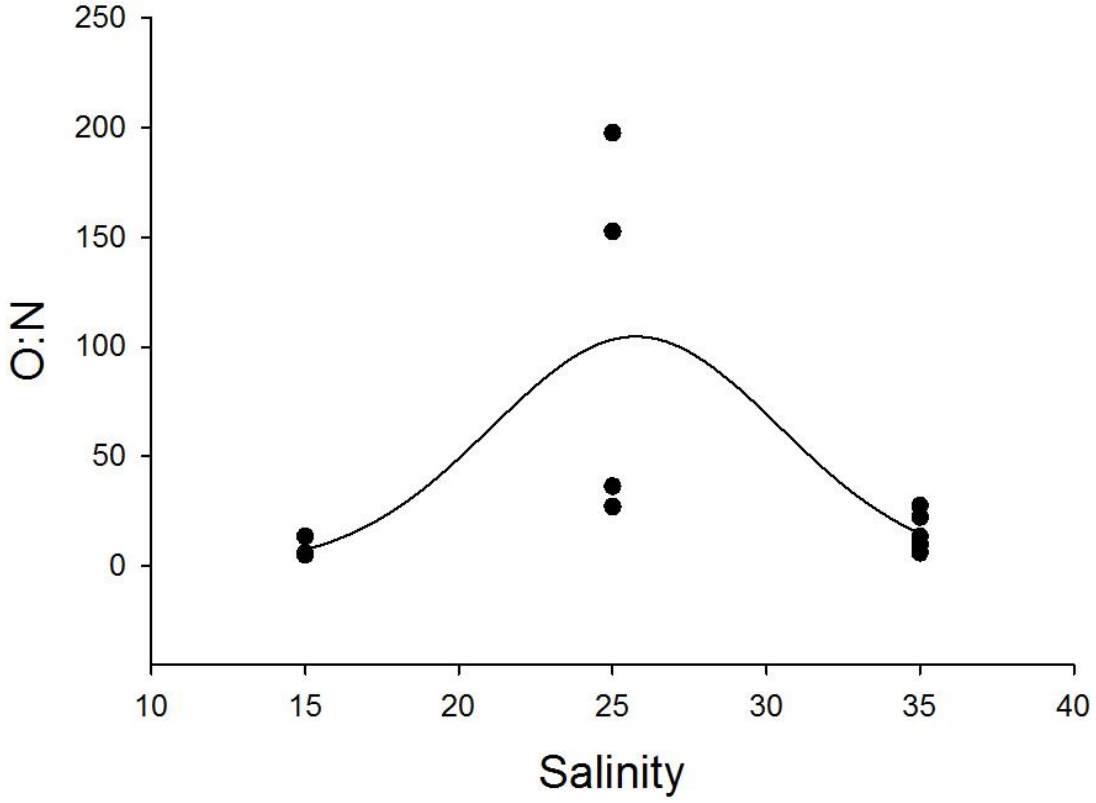

Figure 22. O:N versus salinity

High OCR caused high O:N at 25 psu. Each point represents one individual.

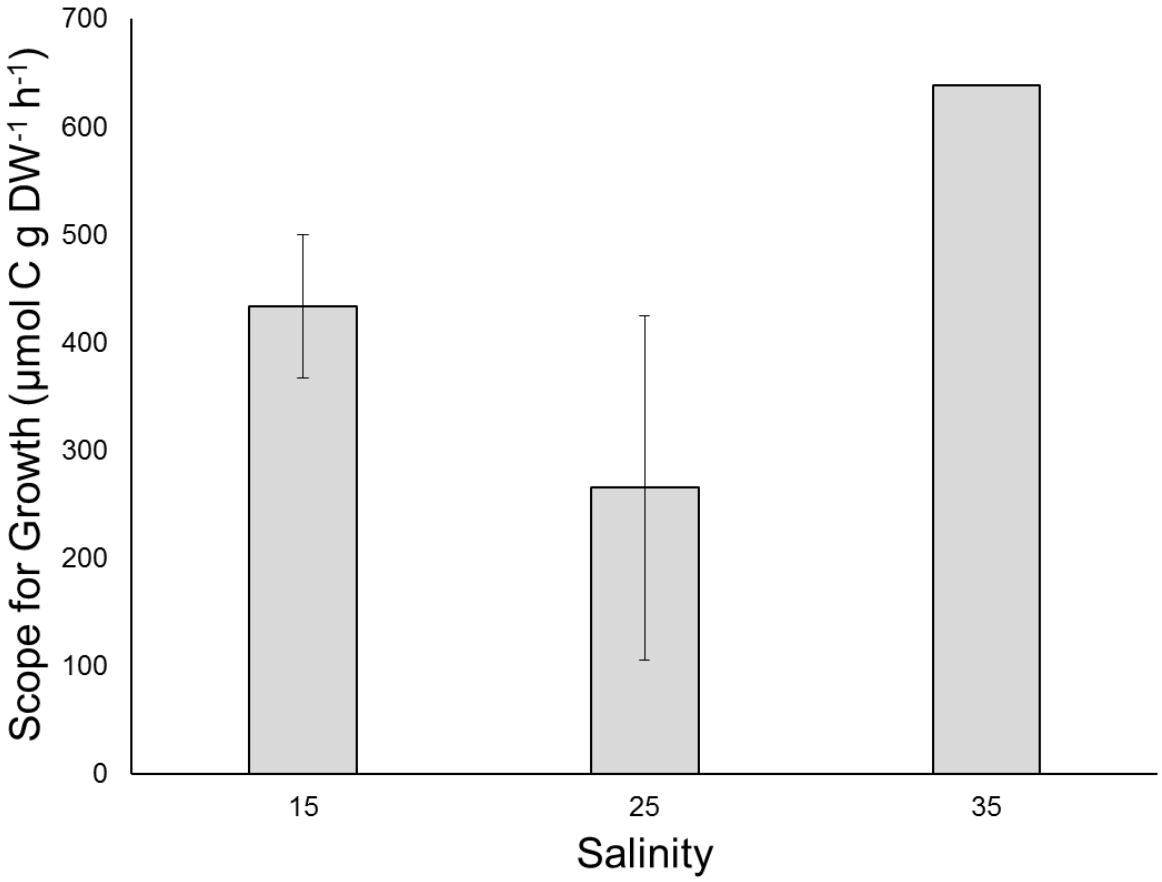

Figure 23. Scope for Growth versus salinity

High OCR caused low scope for growth at 25 psu. Therefore, scope for growth at 25 psu may not be representative of true value. Error bars represent standard error. Only one sample was collected for egestion rate (ER) and ingestion rate (IR) at 35 psu.

Table 5. Mean physiological rates collected during salinity experiments.

Experiments were conducted at three different temperatures typical of the cultivated environment ranging from 15 psu to 35 psu at approximate optimum temperature (25ºC). Each of these rates was standardized using the equation $Y_s = Y_e(1/W_e)^b$ (Cranford et al., 2011). The constant "b" was determined during preliminary experiments (Appendix 1).

Variable	b	Rate	Salinity	Temp (°C)	n	Mean	Std. Err.	Mean DW (g)	Std. Err.
Salinity	0.6565	CR_i	15	25	13	7.43	0.58	0.29	0.06
			25		12	6.41	1.15	0.52	0.14
			35		6	5.25	1.22	0.52	0.15
		CR_p	15	25	13	3.93	0.46		
			25		12	3.84	0.74		
			35		6	4.01	1.25		
		ER	15	25	8	2.82	1.16	0.33	0.06
			25		9	5.59	1.55	0.46	0.07
			35		1	3.44		0.26	
		OCR	15	25	4	1.50	0.32	0.29	0.08
			25		4	5.45	2.40	0.56	0.16
			35		6	1.79	0.35	0.26	0.06
		AER	15	25	4	5.42	1.03	0.29	0.08
			25		4	4.02	1.82	0.56	0.16
			35		6	6.10	0.95	0.26	0.06
		O:N	15	25	4	24.61	10.80	0.29	0.08
			25		4	336.24	203.52	0.56	0.16
			35		6	25.63	9.32	0.26	0.06

Table 6. Comparison of experimental physiological rates to literature rates

Mean rates from recent physiological studies of *C. virginica*. *Rates in which standard error was not provided.

Rate	Author	Location	Temp (°C)	Salinity	Mean ± SE
CR ($L\ g^{-1}\ h^{-1}$)	Kinsella, 2019	Wilmington, NC	10	30	3.60 ± 0.45
			20	30	4.93 ± 0.72
			30	30	8.48 ± 1.03
			25	15	7.43 ± 0.58
			25	25	6.41 ± 1.15
			25	35	5.25 ± 1.22
	Casas et al., 2018a	Louisiana	10	25	0.20 ± 0.09
			20	25	4.45 ± 0.71
			30	25	4.65 ± 1.78
	McFarland et al., 2013	Estero Bay, Florida	21-23	15	*0.41
			21-23	25	*0.31
			21-23	35	*0.30
	Hoellein et al., 2015	Squamscott River	23		7.31 ± 1.02
		Nannie Island	23		3.51 ± 0.25
OCR ($mg\ O_2\ g^{-1}\ h^{-1}$)	Kinsella, 2019	Wilmington, NC	10	30	0.74 ± 0.18
			20	30	1.92 ± 0.42
			30	30	2.11 ± 0.36
	Casas et al., 2018a	Louisiana	10	25	0.50 ± 0.15
			20	25	0.90 ± 0.09
			30	25	2.33 ± 0.29
AER ($\mu mol\ NH_3\text{-}N\ g^{-1}\ h^{-1}$)	Kinsella, 2019	Wilmington, NC	10	30	7.44 ± 3.17
			20	30	4.51 ± 1.07
			30	30	2.83 ± 0.26
	Kelly et al., 2011	Fort Piece, Florida	5	30	*0.15
			27	30	*5.52

Salinity had a significant effect on oxygen consumption rate ($(f = 9.83 * e^{\left(-0.5\left(\frac{x-25.25}{5.29}\right)^2\right)}$, $F_{2,11} = 8.73$, $p = 0.01$), with a maximum at 25.25 psu (Figure 20). Salinity did not have a significant effect on ammonia excretion rate ($F_{2,11} = 0.13$, $p = 0.88$) (Figure 21). Salinity did have an effect on O:N ($f = 105 * e^{\left(-0.5\left(\frac{x-25.75}{4.68}\right)^2\right)}$, $F_{2,11} = 6.02$, $p = 0.02$) .O:N displayed a similar relationship to OCR, with a maximum at 25.75 psu (Figure 22).

3.3 Field Growth

The interaction of month and gear type had a significant effect on the condition indices for Farm 1 (Table 7). Highest mean condition index for "Floating" and "Bay Bottom Cages" was found in the Fall (Figure 24). Mean condition index for Farm 1 ranged from 4.35 to 9.06 (Table 8). The interaction of month and plant date had no significant effect on the condition indices for Farm 2 (Table 9). Farm 2 had highly variable condition indices, with mean values that ranged from 3.42 to 6.67 (Figure 25 and Table 10). The interaction of month and gear type had a significant effect on the condition indices for Farm 3 (Table 11). Highest mean for all three gear types was found during spring (Figure 26). Mean condition index on Farm 3 ranged from 4.74 to 12.29 (Table 12).

Growth curves were collected for two groups of oysters on Farm 3. "Cage with Bag" growth curve is represented as: $f(x) = 65 / (1 + e^{-(\frac{x-3239)}{42}})$ ($F_{2,299} = 665$, $p < 0.01$) (Figure 27). The growth curve for "Tray" is: $f(x) = 71 / (1 + e^{-(\frac{x-43107)}{58}})$ ($F_{2,220} = 26.7$, $p < 0.01$) (Figure 28). Growth curves for "Cage" on Farm 3 (Figure 29 and Table 13), Farm 1, and Farm 2 could not be calculated because many individuals had already reached harvest size before sampling had begun.

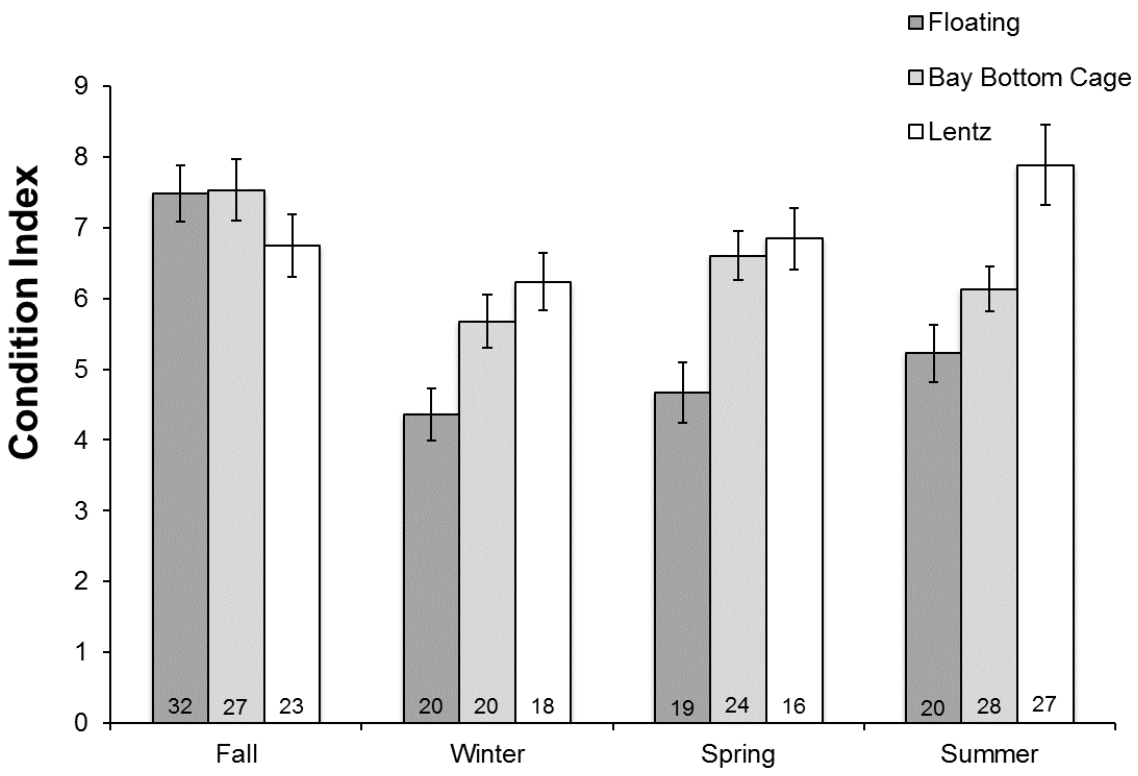

Figure 24. Condition index for each of the different gear types on Farm 1.

Samples taken in September 2017, January 2018, April 2018, and July 2018. Condition index = (dry weight of tissue (g)/shell cavity volume) x 100 (Abbe and Albright, 2003). Shell cavity volume = Whole oyster wet weight (g) – Weight of empty valve (g) (Abbe and Sanders, 1988). Numbers represent number of oysters used to calculate condition index (n). Error bars represent standard error.

Table 7. Results from GLM predicting Month and Gear Type on Condition Index for Farm 1

Variable	d.f.	χ^2	p
Season	3	20.24	**<0.01**
Gear Type	2	18.16	**<0.01**
Season*Gear Type	6	17.44	**0.01**

Table 8. Mean condition index for each gear type on Farm 1.

Samples collected from September 2017 to July 2018.

Month	Gear Type	Mean DW (g)	Mean Shell Cavity Vol.	Mean C.I.	Std. Dev.	Std. Err.	n
September	Floating	1.21	15.06	9.06	9.24	1.63	32
	Bay Bottom Cages	0.32	4.58	7.53	2.26	0.43	27
	Lentz	0.16	3.04	6.75	2.15	0.45	23
January	Floating	0.77	18.26	4.35	1.64	0.37	18
	Bay Bottom Cages	0.28	5.01	5.68	1.69	0.38	20
	Lentz	0.34	5.59	6.23	1.71	0.40	20
April	Floating	0.82	17.73	4.67	1.86	0.43	16
	Bay Bottom Cages	0.46	7.57	6.61	1.72	0.35	24
	Lentz	0.56	8.70	6.84	1.76	0.44	19
July	Floating	0.90	17.34	5.22	1.78	0.40	20
	Bay Bottom Cages	0.65	10.77	6.13	1.68	0.32	27
	Lentz	0.84	10.91	7.89	2.91	0.56	28

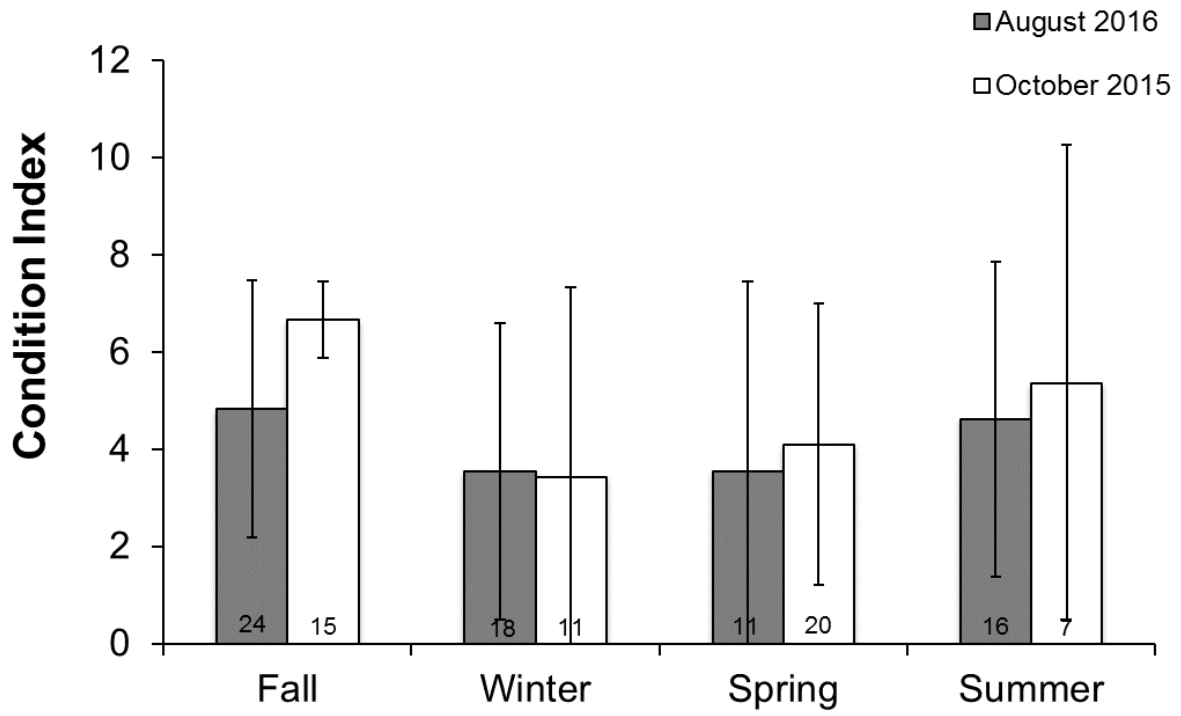

Figure 25. Condition index for each of the groups of oysters ("Plant Date") on Farm 2.

Samples taken in September 2017, January 2018, April 2018, and July 2018. Condition index = (dry weight of tissue (g)/shell cavity volume) x 100 (Abbe and Albright, 2003). Shell cavity volume = Whole oyster wet weight (g) – Weight of empty valve (g) (Abbe and Sanders, 1988). Numbers represent the number of oysters used to calculate condition index (n). Error bars represent standard error.

Table 9. Results from GLM predicting Month and Plant Date on Condition Index for Farm 2

Variable	d.f.	χ^2	p
Season	3	21.64	**<0.01**
Plant Date	1	2.45	0.12
Season*Plant Date	3	2.27	0.52

Table 10. Mean condition index for each group of oysters ("Plant Date") on Farm 2.

Samples collected from September 2017 to July 2018.

Month	Plant Date	Mean DW (g)	Mean Shell Cavity Vol.	Mean C.I.	Std Err	n
September	August 2016	0.21	4.25	4.83	2.65	31
	October 2015	0.95	14.05	6.67	0.78	27
January	August 2016	0.25	7.26	3.54	3.06	23
	October 2015	0.58	17.22	3.42	3.91	18
April	August 2016	0.55	15.68	3.53	3.91	20
	October 2015	0.22	6.29	4.10	2.90	20
July	August 2016	0.32	8.39	4.62	3.24	16
	October 2015	0.67	14.03	5.36	4.90	24

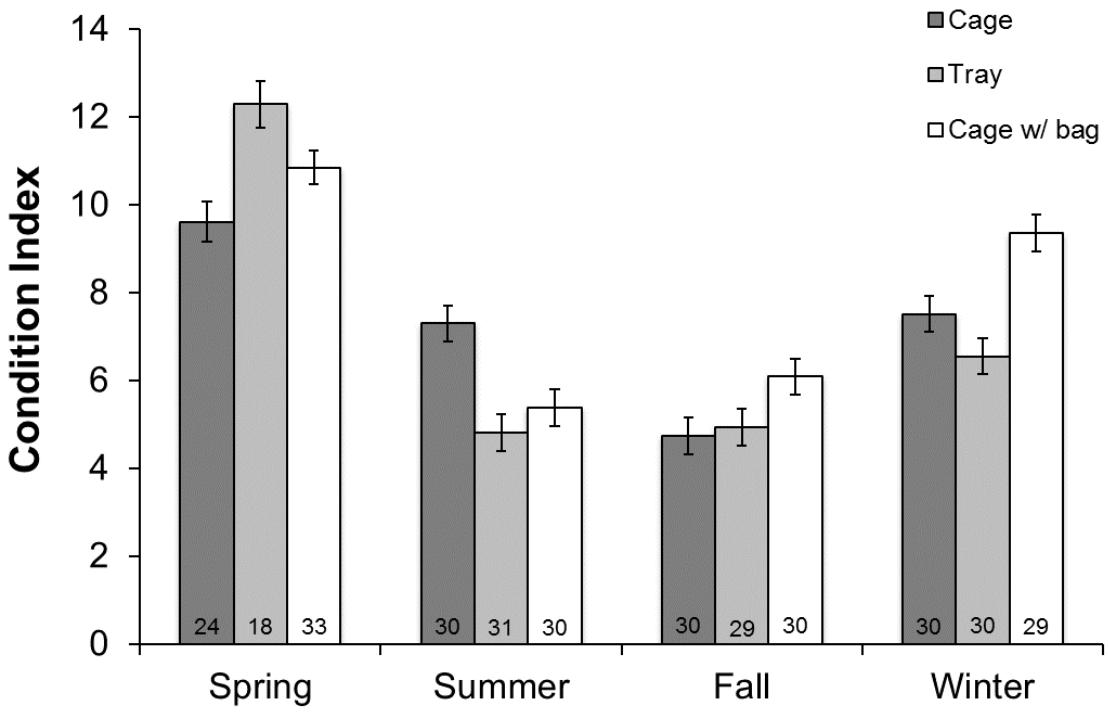

Figure 26. Condition index for each of the different gear types on Farm 3.

Samples taken in April 2018, July 2018, September 2018, and January 2019. Condition index = (dry weight of tissue (g)/shell cavity volume) x 100 (Abbe and Albright 2003). Shell cavity volume = Whole oyster wet weight (g) – Weight of empty valve (g) (Abbe and Sanders 1988). Numbers represent number of oysters used to calculate condition index (n). Error bars represent standard error.

Table 11.Results from GLM predicting Month and Gear Type on Condition Index for Farm 3

Variable	d.f.	χ^2	p
Season	3	198.86	**<0.01**
Gear Type	2	7.47	**0.02**
Season*Gear Type	6	37.61	**<0.01**

Table 12. Mean condition index for each gear type for Farm 3.

Samples collected from April 2018 to January 2019.

Month	Gear Type	Mean DW (g)	Mean Shell Cavity Vol.	Mean C.I.	Std. Err.	n
April	Cage	0.48	4.91	9.61	0.46	24
	Tray	0.93	7.65	12.29	0.53	18
	Cage w/ bag	0.04	0.34	10.85	0.39	33
July	Cage	1.07	15.11	7.30	0.41	30
	Tray	0.54	11.51	4.81	0.41	31
	Cage w/ bag	0.21	4.48	5.38	0.41	30
September	Cage	0.67	14.49	4.74	0.41	30
	Tray	0.83	17.03	4.94	0.42	29
	Cage w/ bag	0.52	8.61	6.09	0.41	30
January	Cage	0.95	12.75	7.51	0.41	30
	Tray	1.18	17.89	6.55	0.41	30
	Cage w/ bag	1.03	11.02	9.35	0.42	29

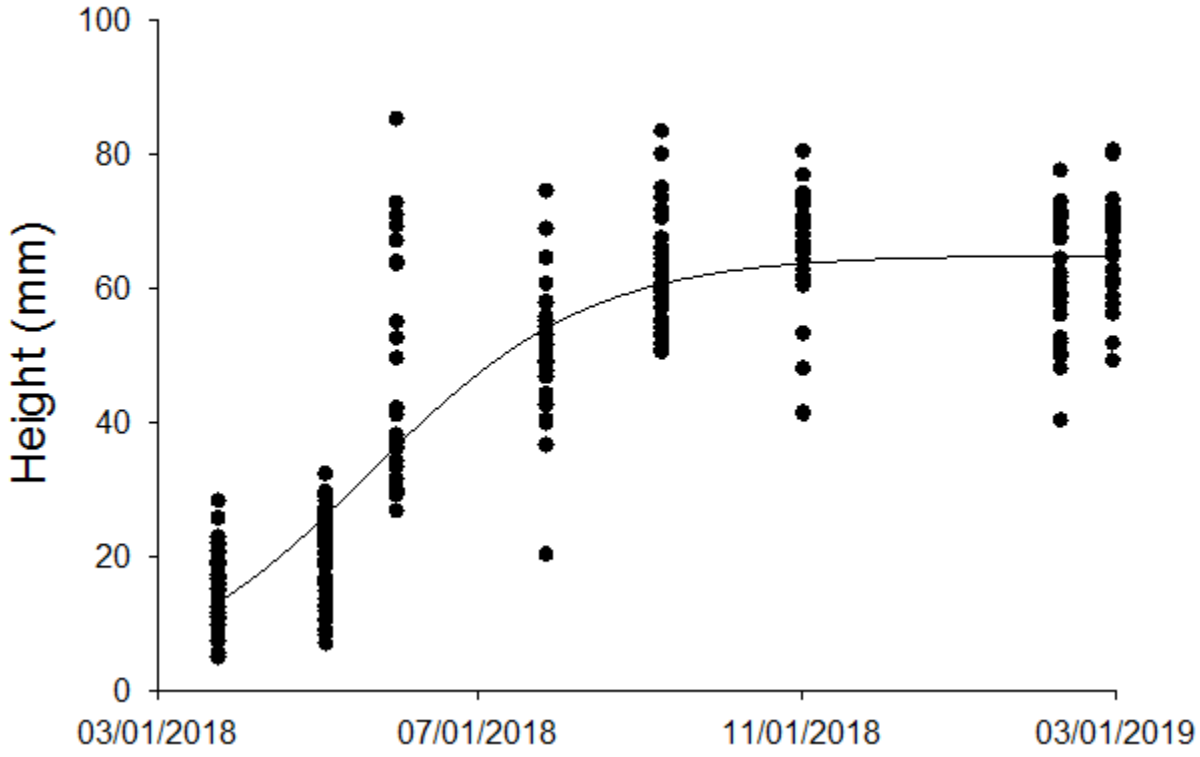

Figure 27. Change in mean oyster height over time in "Cage with bag" on Farm 3.

Points represent individual samples collected from "Cage with bag" gear type.

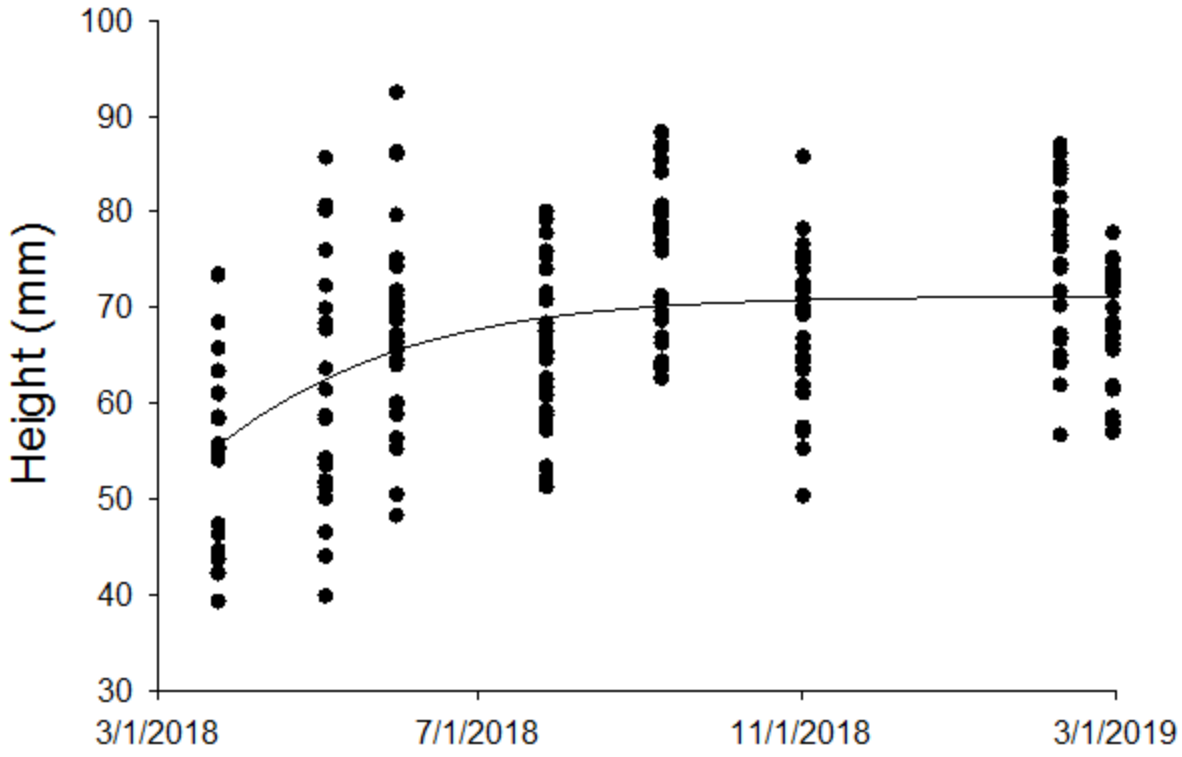

Figure 28. Change in mean oyster height in "Tray" over time on Farm 3.

Points represent individual samples collected from "Tray" gear type.

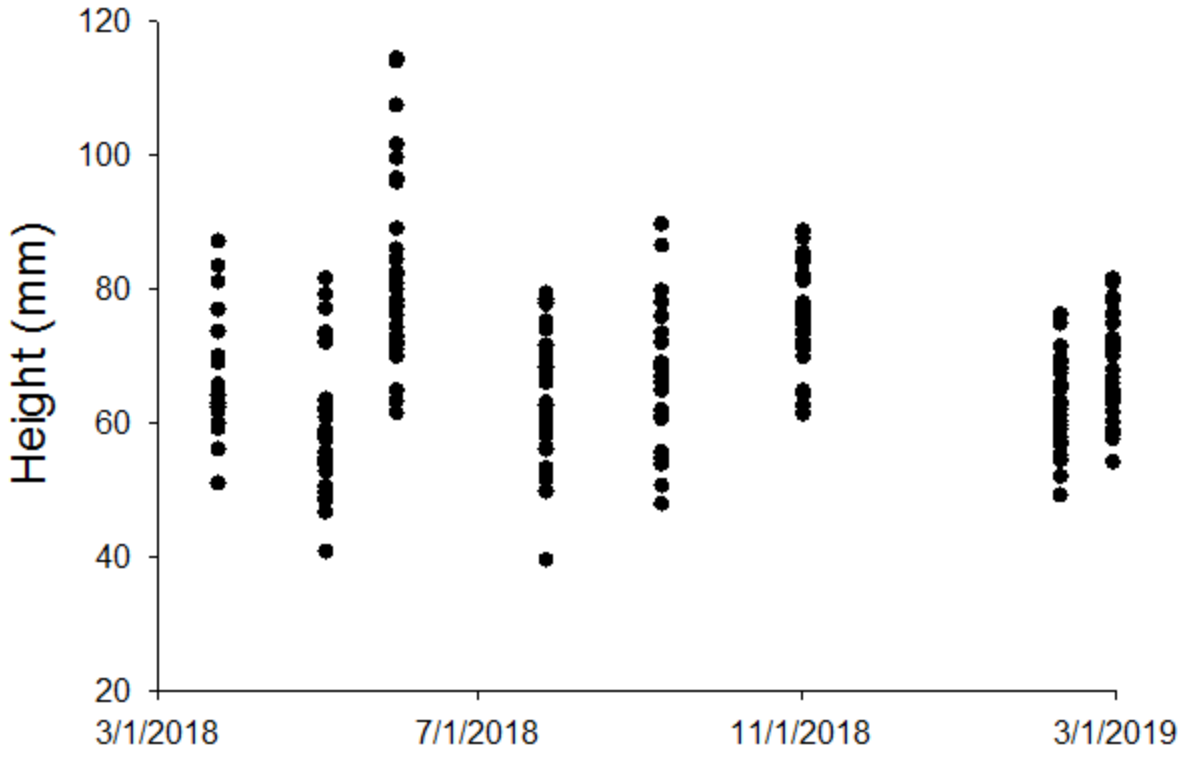

Figure 29. Change in mean oyster height in "Cage" over time on Farm 3.

Points represent individual samples collected from "Cage" gear type. Unable to develop growth curve because samples were harvest size when sampling began.

Table 13. Mean height (longest axis) of oysters from Farm 3 for each gear type per month.

H:L represents the proportion of oyster height to oyster length and H:W represents the proportion of oyster height to oyster width (Galtsoff 1964).

Month	Gear Type	Mean Height (mm)	Std. Err.	H:L	Std. Err.	H:W	Std. Err.
March	Cage w/ Bag	15.38	1.15	1.20	0.03	3.27	0.06
	Tray	55.56	2.03	1.53	0.04	3.41	0.12
	Cage	66.61	2.03	1.35	0.04	3.77	0.12
April	Cage w/ Bag	20.85	1.17	1.24	0.03	3.42	0.07
	Tray	60.39	1.86	1.39	0.04	3.23	0.11
	Cage	59.69	1.66	1.46	0.04	3.63	0.09
May	Cage w/ Bag	45.32	1.66	1.51	0.04	3.77	0.09
	Tray	68.09	1.66	1.63	0.04	3.34	0.09
	Cage	81.94	1.63	1.54	0.04	3.43	0.09
July	Cage w/ Bag	50.27	1.63	1.43	0.04	3.52	0.09
	Tray	64.74	1.63	1.42	0.04	3.01	0.09
	Cage	64.80	1.66	1.34	0.04	2.71	0.09
September	Cage w/ Bag	62.14	1.66	1.52	0.04	3.63	0.09
	Tray	75.92	1.66	1.58	0.04	3.08	0.09
	Cage	67.20	1.66	1.44	0.04	2.97	0.09
October	Cage w/ Bag	65.35	1.66	1.45	0.04	3.66	0.09
	Tray	67.83	1.66	1.40	0.04	2.86	0.09
	Cage	75.91	1.66	1.46	0.04	2.92	0.09
January	Cage w/ Bag	62.13	1.69	1.31	0.04	3.20	0.09
	Tray	74.42	1.66	1.37	0.04	2.92	0.09
	Cage	62.56	1.66	1.35	0.04	2.74	0.09
February	Cage w/ Bag	65.77	1.66	1.39	0.04	3.17	0.09
	Tray	68.17	1.66	1.44	0.04	2.83	0.09
	Cage	67.98	1.66	1.41	0.04	3.05	0.09

4. Discussion

Oysters in this study typically preferred higher temperatures ranging from 25-30ºC. In terms of performance, the preferred temperature is considered the point at which growth is maximized (Bayne, 2017). Highest scope for growth was at 30ºC (Figure 16), primarily due to high clearance rates and low egestion rates at higher temperatures. In a previous study, Kelly et al. (2011) found the highest scope for growth in spring compared to other seasons. This was likely due to increased primary productivity and food availability in the spring and energy losses due to gametogenesis occurring in late spring and early summer. However, my study focused on triploid oysters. Although some triploid individuals have shown to return to diploidy as they grow to harvest size, triploid oysters are dominantly sterile (Guo and Allen, 1994). Therefore, they are able to use the nutrients acquired during feeding to assimilate into their tissues and shell for growth instead of for development of gametes, making summer a high growth rate season for triploid compared to diploid oysters Providing this information will assist farmers in making decisions to maximize production and resource managers to better understand water quality implications of shellfish aquaculture.

Understanding the physiological parameters that contribute to growth helps shellfish aquaculturists make decisions on farm practices. For example, one of the first decisions a farmer must make is which type and size of seed to purchase. When deciding between diploid or triploid oysters, several factors must be considered including cost, anti-fouling techniques, or what the final product will look like. This choice will then influence other practice decisions, like plant date, that could determine when their oysters will reach harvest size. For triploid oysters in this region, greatest growth would occur during the summer, when temperatures are approximately 30ºC. Allen and Downing (1986) witnessed similar results when comparing diploid and triploid

growth between May and November, where triploids grew by 172% and diploids only grew by 34%. Another finding of this study was triploids had higher carbohydrate levels than diploids due to a decrease during gametogenesis and spawning (Allen and Downing, 1986). On Farm 1, condition indices were highest during July or September, depending on gear type (Figure 24). There was also evidence for growth at high temperatures on Farm 3, when the greatest rate of change in shell height for "Cage w/ bag" gear type occurred in May, when temperatures were approximately 26 °C (Figure 27).

Both growers and resource managers must consider water quality conditions when deciding on an aquaculture site, especially in coastal and estuarine ecosystems that are susceptible to changing environmental conditions. Temperature is one of the main stressors to consider for oysters that live in regions closer to the organisms' tolerance limits. Individuals acclimated to warmer environments are more vulnerable to temperature increases than individuals of the same species acclimated to colder ones (Kingsolver, 2009). An example of this occurred in Bodega Bay, California, when there was approximately 30% mortality of blue mussels in the surface layer due to high heat exposure during low tide (Castrodale, 2019). The oysters from this study are subject to temperatures between 25 ºC to 30 ºC for nearly six months out of the year (NOAA NERR). To combat extreme temperatures, oysters can synthesize heat shock proteins to bind with protein molecules to prevent denaturation, but doing so is energetically costly and can potentially inhibit growth (Bayne, 2017). Farmers should choose locations and gear types that limit the oysters' air exposure. For example, when using cages that become exposed at low tide, growers should regularly rotate bags from top to bottom, ensuring the same bags in the top rack aren't consistently exposed for longer periods of time. Doing so will also limit the amount of fouling organisms collecting on the bottom bags. For aquaculturists

using triploid oysters, regularly high temperatures paired with high food availability will have the benefit of faster growth, but they must be mindful of any long-term exposure that high heat may have on physiological processes, especially in intertidal zones.

Slight evidence of a stress response to high temperature was demonstrated in my respiration data. In this study, oxygen consumption rates increase with increasing temperature until approximately 30 ºC (Figure 13). Similar results have been found in previous studies, with oxygen consumption rates maximized at 30 ºC (Casas et al., 2018a). In terms of performance, optimal temperature, or the temperature range where oxygen delivered to tissues is maximized, may be different than the "preferred temperature" (Bayne, 2017). This is due to the relationship between ingestion and respiration. Aerobic respiration involves using organic carbon compounds as energy to perform all necessary survival functions of the organism (Dame 2012). When calculating scope for growth, ingestion is the input of energy into the organism, while respiration is an energy loss term (Dame 2012). According to my results at 30ºC, the slopes for clearance and ingestion rates were steeper than oxygen consumption rate, suggesting that growth was maximized at this temperature. On the other hand, oxygen consumption rate being highest at 30 ºC is indicative of the high amount of metabolic work required to achieve this growth (Figure 13). Performance curves are represented as a skewed gradual increase at lower temperatures and sharp decline when an individual approaches and then finally reaches its critical temperature (Huey & Stevenson 1979; Bayne, 2017). The relationship between oxygen consumption rate and temperature in my study demonstrates that these oysters were approaching their critical temperature, or the point where anaerobic processes begin (Bayne, 2017), at 30 ºC. Therefore, although highest scope for growth occurs at 30 ºC, 25 ºC is likely less stressful for these oysters.

While neither temperature nor salinity had an effect on ammonia excretion rate in this study, using this information to calculate O:N helped identify metabolic status of the oysters. Ammonia excretion rate is typically ignored when modelling metabolic loss since it is such a small part of the energy budget, but stressful conditions, like starvation or unfavorable conditions that cause oysters to stop feeding, may cause significant increases in ammonia excretion rate (Bayne, 2017). There have been mixed results in previous studies to support this, with some research showing higher ammonia excretion rates in the summer than autumn or when salinity decreases, while others had highly variable ammonia excretion rates (Sma and Baggaley, 1976; Kelly et al., 2011). This variability can be explained by an organism's size or changes in nutrient storage or utilization of reserves (Bayne et al., 1976; Sma and Baggaley, 1976). Calculating O:N, or the amount of oxygen consumed relative to the amount of ammonia being excreted gives insight on the catabolism of nutrient stores (carbohydrates and lipids versus proteins). O:N during this study was highest at about 25 ºC, suggesting this may actually be the optimum temperature for these oysters (Figure 15). Although ammonia excretion energy losses may be a relatively minimal component of the energy budget, collecting this data provides more information on the physiological processes of an organism affecting its overall performance in changing environments.

Oyster performance decreased as temperature decreased. Cold temperatures were stressful for oysters in this study. Overall, their performance decreased as temperature decreased. Physiological rates generally slow below the optimum temperature range because enzymatic activity driving these processes gradually decreases (van der Have, 2002). Clearance rates also tend to be lower at lower temperatures due to physical changes in the environment. Temperature has an inverse relationship with the viscosity of water and cilia within the bivalve must have

enough power to overcome this (Cranford et al., 2011; Humphries, 2013). Earlier research has noted physiological activity in oysters, such as feeding, ceased in winter months (Kelly et al., 2011). Oysters in southeastern North Carolina demonstrate a similar trend. Lowest scope for growth was found at 10 ºC due to clearance rates and oxygen consumption rates decreasing with decreasing temperature (Figure 16). Also, O:N fell below 13 at 10 ºC (Table 4), which is indicative exclusively of protein catabolism (Mayzaud and Conover, 1988). At temperatures lower than 10 ºC, it would be expected that there would be neutral or even negative scope for growth due to inactivity (Kelly et al., 2011), but scope for growth in this study was always positive. One reason for consistent positive growth is that this study did not consider the energetic loss for shell growth and pseudofeces. Although pseudofeces was collected separately from feces, there was not sufficient data for analysis (Table 2). Moreover, this study focused on temperatures between 10 ºC and 30 ºC. In extreme cases, water temperatures will drop to about 5 ºC or lower (NOAA NERR), in which scope for growth would become negative (Figure 16). Since these oysters are in the intertidal region, temperature fluxes will be even more extreme during low tide. For maximum or fastest growth, it is not suggested that growers plant their seed during winter months. Conversely, it is recommended to aim for a plant date in which oysters will reach harvest size as the temperature is dropping. Therefore, growth will slow down and oysters will consistently be the same size during the winter months when customers are consuming the most oysters.

Oyster physiological activity responded quickly to temperature changes at approximately 15 ºC. The highest Q_{10} value was observed between 15 ºC and 20 ºC, signifying when physiological rates are most sensitive to temperature change (Table 3). Also, below 15 ºC, O:N fell below 20 (Table 4), indicating stress (Mayzaud and Conover, 1988). Thus, this is the

threshold when feeding will become maximized, having the largest influence on eutrophication and causing fast growth. In southeastern North Carolina, water temperatures reach 15 ºC in early March (NOAA NERR). Therefore, early spring would be an ideal time to plant oyster seed to maximize growth and potentially help outcompete predators.

Since estuaries are fluid environments with several environmental factors that impact oyster growth processes, understanding the consequences of salinity changes is necessary for growers and resource managers when making siting or farm practice decisions. Although osmoconformity is both energetically and ecologically advantageous, it makes bivalves vulnerable to significant weather events. Oysters have limited cell volume regulation but must manage their osmotic concentration when salinity fluctuates for survival, by either increasing osmotic pressure during drought conditions or excreting solutes with increased rainfall (Bayne, 2017). To avoid this regulation, the most common way for *C. virginica* to respond to acute salinity changes in salinity is to shut their valve (Hand and Stickle, 1977), but doing so causes oysters to undergo anaerobic metabolism (Bayne, 2017). Consequently, growers and resource managers must be wary of long-term changes in salinity when siting.

Respiration was significantly higher at 25 psu than at lower (15 psu) and higher (35 psu) salinities. Multiple oxygen consumption rates determined at 25 psu were much higher than literature values (e.g., Willson and Burnett, 2000; Lannig et al., 2008) or rates collected during temperature experiments. This high mean OCR influenced both O:N and scope for growth. Thus, scope for growth at 25 psu was lower than expected and O:N was higher than expected. These values were thoroughly examined for any instrument or calculation error. One possible explanation for this increased respiration rate for the 25 psu experiments is due to the time of oyster collection, just one month after Hurricane Florence hit the southeastern N.C. coast. The

Cape Fear River, with Snow's Cut Inlet located 5 miles from the two aquaculture sites, had elevated water levels due to flooding and runoff for several weeks after the storm. There was a sharp salinity stratification within the aquaculture sites (Appendix 2), and dissolved oxygen (DO) was much lower than average at the time of sample collection. Intertidal oysters are typically hypoxia-tolerant, but the influence of multiple stressors increases stress on the mitochondria and oysters' ability to resist hypoxic conditions (Ivanina et al., 2012). Although the oysters were held in controlled conditions for at least two weeks after collection, they may have still been recovering from prolonged stress when the respiration experiments were conducted. This information provides insight to possible effects of climate change on cultured oysters that could influence long-term decisions of growers or resource managers. Due to Hurricane Florence and Tropical Storm Michael, $1.33 million in cultured oysters were lost between New Hanover County and Onslow county (Incremona, 2019). Moving forward, this region may experience more frequent and larger storms that will cause more drastic salinity changes due to predicted increased precipitation in subtropical regions (Trenberth, 2011). .

Salinity did not have a significant effect on clearance rates. It would be expected for clearance rates to be higher at 35 psu, which is typical of the farms in Masonboro Island NERR. These results may support that above 15 psu, oysters are in optimum salinity conditions and therefore, feeding is unaffected by any further salinity changes. Earlier studies have shown highest clearance rates between 15 and 25 psu (Casas et al., 2018), although most research examines the effect of salinity in estuaries that regularly are exposed to the lower limits of their salinity tolerance range. Previous studies were focused on maximum clearance rates for individual open oysters (Shumway & Koehn, 1982; Casas et al., 2018), rather than the population as a whole. In this study, although CR_i was not significantly different between

salinities, there was a much greater difference between CR_i and CR_p at 15 psu than at 25 and 35 psu (Figure 17). Also, there was almost no difference between population clearance rates for the experimental salinities ($p = 0.99$). This shows the ability of some oysters to perform better at 15 psu than others and the importance of considering the amount of time oysters are open and feeding. When trying to produce accurate model predictions, population-level physiological rates are a better estimate of ecological function of the farm.

5. Conclusions

Providing information to resource managers and growers on environmental conditions that affect the physiological processes of cultured oysters helps them make both short-term and long-term siting and practice decisions. Triploid oysters from southeastern NC intertidal oyster farms performed best higher temperatures around 30 ºC, but their optimal temperature is probably closer to 25 ºC. When deciding between diploid and triploid oysters, growers should continue to transition to using triploids on their farm for maximum growth. This data will then help inform farmers when making decisions on plant date. To optimize growth, the best time to plant oyster seed would be in early spring, as temperatures are beginning to warm up and food supply increases. Other factors each grower must consider, though, are conditions that favor predation, which varies with each farm, or how to manage their crop during hurricane season. Placing oysters in the water during spring when growth will occur the fastest will help them outcompete some of these predators and will allow some of the faster growing oysters to reach harvest size before early fall, when hurricanes are most common. Farmers should also be mindful of exposure time to high heat when making decisions on siting or gear types.

For resource managers, this study provides information on which conditions oyster filter-feeding will have the greatest influence on eutrophication. Triploid oysters would have the largest effect during summertime when size of the oyster and clearance rates are maximized. On the other hand, if temperatures are too high, this may lead to increased stress and limited growth. Incorporating this data into farm-scale models and performing scenario testing will help clarify the balance between maximum feeding and stress. These results also gave some understanding on long-term implications of salinity fluctuations, especially after severe storms. Since climate change favors increased and stronger storm events for this region of the United States, oysters acclimated to higher salinities may be more vulnerable to decreased salinity due to high levels of precipitation when paired with other stressors, like hypoxia. Resource managers should consider this when deciding on aquaculture leases and looking towards the future of the shellfish aquaculture industry. Moving forward, oyster aquaculture should be incorporated into nutrient mitigation techniques within North Carolina to support the growth of the industry, while also providing alternative best management practices. These oysters have high clearance rates to help improve water quality and have high growth rates to reach harvest size quickly, contributing to the local economy. Providing accurate predictions of farm production and filtration capacity helps generate this sustainable oyster aquaculture industry.

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APPENDIX 1: STANDARDIZATION OF PHYSIOLOGICAL RATES

Each of the physiological rates was standardized per gram of dry weight to make comparisons to literature rates using the following equation: $Y_s = Y_e(1/W_e)^b$. Y_s represents the corrected physiological rate, Y_e is the experimental rate, W_e is the weight of the individual, and b is the allometric exponent (Cranford et al. 2011). The allometric exponent "b" represents a proxy for gill surface area, since clearance rate is proportional to the cilia of the gill. This relationship is represented by the equation: $CR=aW^b$, in which CR is the clearance rate (L h^{-1}) and W is the weight of the organism (Cranford et al. 2011). Nonlinear regression was performed using CR and dry tissue weight (DW) from experimental individuals.

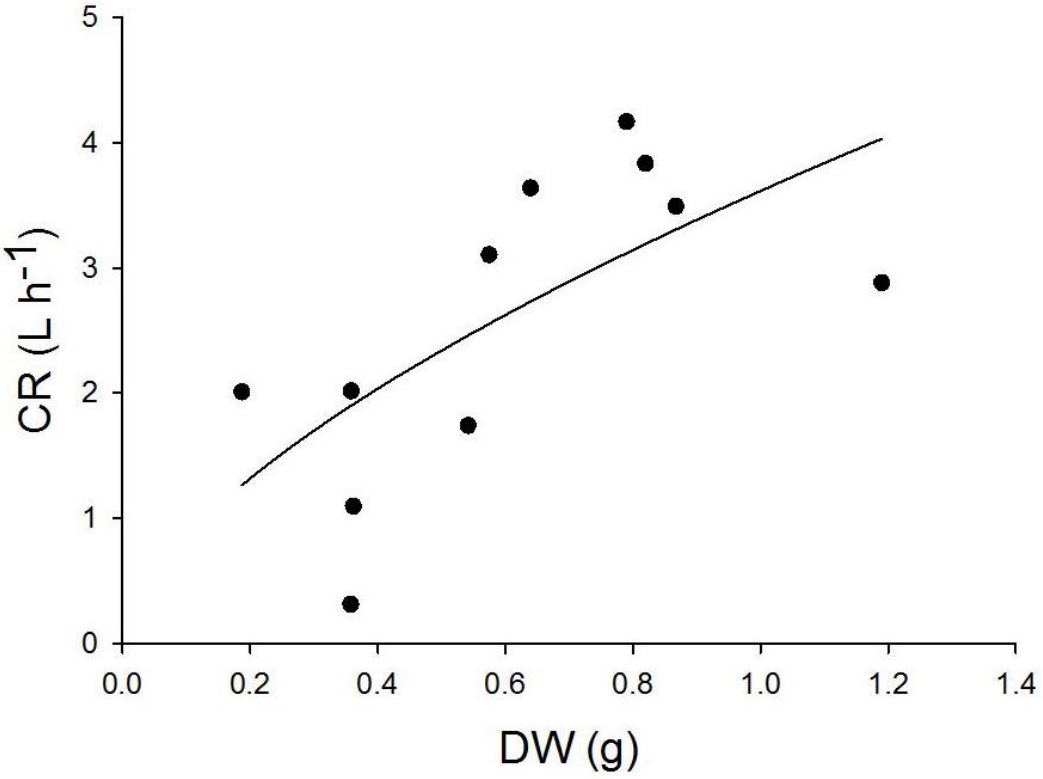

FIGURE 1. Nonlinear regression of individual clearance rate (CR) versus dry tissue weight (DW) for temperature experiments

For temperature experiments, nonlinear regression was conducted using data from the 10 ºC trials. Each point represents one individual. The relationship is represented by: $CR = 3.62W^{0.63}$ ($F_{1,9} = 7.48$, $p = 0.02$).

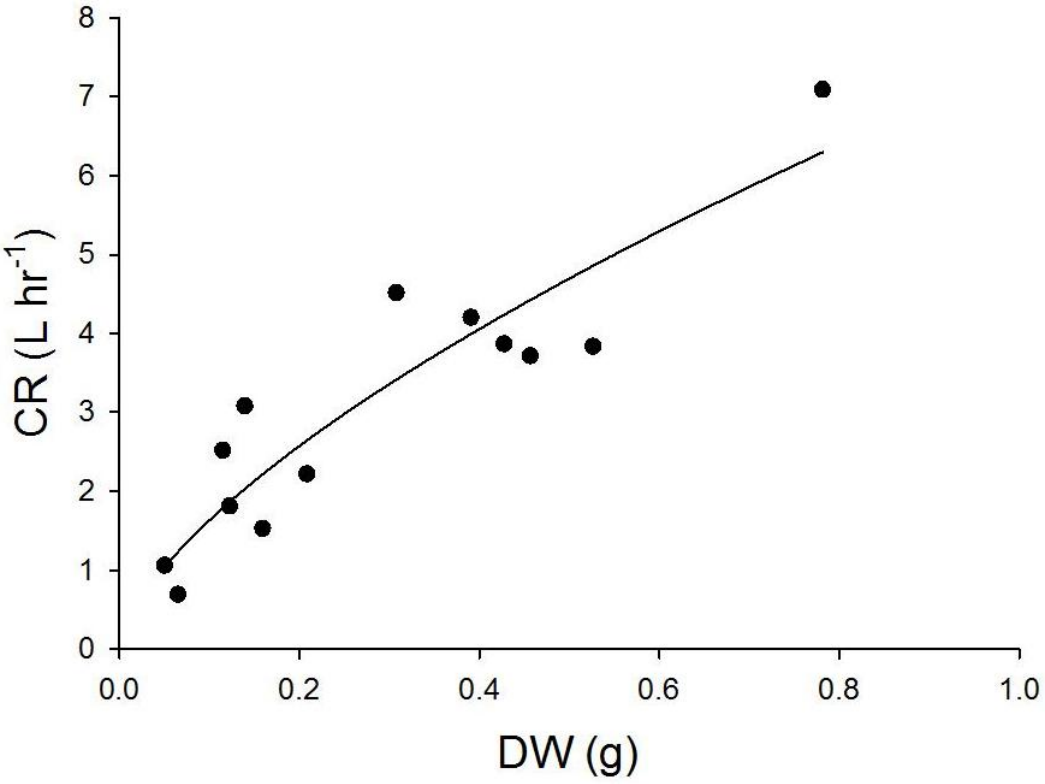

FIGURE 2. Nonlinear regression of individual clearance rate (CR) versus dry tissue weight (DW) for salinity experiments

For salinity experiments, nonlinear regression was conducted using data from the 15 ‰ trials. Each point represents one individual. The relationship is represented by: $CR = 7.4W^{0.66}$ ($F_{1,11} = 53.13$, $p < 0.01$).

APPENDIX 2: POST-HURRICANE FLORENCE WATER QUALITY DATA, MASONBORO ISLAND, NC

Table 1. Water quality conditions of aquaculture sites in Masonboro Island NCNERR post-Hurricane Florence (Darrow, unpublished). Data collected on September 27, 2018 during high tide.

Site	Depth (from surface) (ft)	Temp (°C)	Salinity	DO (%)	DO (mg/L)
Farm 1	0	28.1	16.75	49.5	3.48
	1	27.2	18.99	42.3	3.04
	2	27.3	19.95	45.6	3.23
	3	27.4	21.11	44.9	3.11
	4	27.5	22.99	56.6	3.97
	4.5	27.6	25.81	55.1	3.78
Farm 2	0	27.9	16.71	42.3	3.01
	1	27.8	17.49	43.7	3.12
	2	27.9	21.14	49.2	3.46
	3	27.9	22.15	58.7	4.08
	4	27.8	26.97	62.7	4.21