EFFECTS OF EXTREME FRESHWATER EVENTS AND *PERKINSUS MARINUS* ON *CRASSOSTREA VIRGINICA* STRESS RESPONSE

A Thesis

by

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Estuarine salinity is one of the most important factors affecting oyster (*Crassostrea virginica*) growth and *Perkinsus marinus* disease characteristics. The combined effect of increased freshwater inflows and *P. marinus* infection on oyster physiology is important to understand for improving the predictions of oyster response to increasing climate variability. This study determined the effects of rapid declines in salinity, such as those oysters experience after a strong storm or flood, and *P. marinus* infection on the scope for growth of oysters from the Laguna Madre, a hypersaline estuarine system. Scope for growth was assessed by determining clearance rate, absorption efficiency, ammonia excretion rate, and oxygen consumption rate for oysters at six salinity treatments: 10, 15, 20, 25, 30, and 35. Salinity did not have a significant effect on clearance rate, absorption efficiency, or oxygen consumption rate, but did significantly affect the rate of ammonia excretion. Scope for growth ranged from 37.25 J hr^{-1} g dry $weight^{-1}$ to 867.46 J hr^{-1} g dry $weight^{-1}$, and demonstrated a decreasing trend from the lowest to highest salinity treatments, indicating reduced growth potential with increasing salinity. *Perkinsus marinus* infection intensity ranged from low to moderately heavy, but did not have a significant effect on oyster scope for growth. Oyster condition index increased with increasing salinity treatment, likely reflecting Laguna Madre oyster tolerance for high salinities. Regardless, oysters experiencing rapid reductions in salinity demonstrated increased physiological function compared to oysters that remained at the control salinity 35, indicating that the normally high salinities of the Laguna Madre may not present optimal conditions for oyster growth. Results of

this study improve understanding of oyster response to rapid decreases in salinity conditions influenced by human and climate-driven changes.

DEDICATION

To Mom and Dad, thank you for believing in me, no matter what.

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INTRODUCTION

Estuaries are unique ecosystems that represent transitional zones where freshwater and saltwater environments converge. Freshwater inflows are an important source of nutrients and organic matter to estuaries, and the quantity of freshwater entering an estuary affects estuarine salinity (Alber 2002; Sahoo and Smith 2009; Tolley et al. 2006). The delivery of freshwater and nutrients is influenced by landuse patterns, as well as by climate and precipitation gradients within the watershed surrounding the estuary. As human populations continue to increase, upstream water withdrawals are becoming more significant, resulting in less water and fewer nutrients being transported to estuaries (Alber 2002; Sahoo and Smith 2009).

In addition to localized anthropogenic changes on freshwater inflow, large scale climatic features such as El Niño-Southern Oscillation (ENSO) can affect the salinity structure and water quality of Gulf of Mexico estuaries (Lipp et al. 2001; Tolan 2007). El Niño-Southern Oscillation is a predictable climatic signal that originates in the Pacific Ocean as a result of sea surface temperature anomalies (Gershunov and Barnett 1998). Precipitation anomalies caused by ENSO can affect both evapotranspiration rates and runoff/stream flow throughout the Mississippi River basin, which is a major source of freshwater for the Gulf of Mexico and many of its estuaries (Childers et al. 1990; Twine et al. 2005; Tolan 2007; Sharda et al. 2012; Lipp et al. 2001; Schmidt and Luther 2002). As estuarine salinity fluctuates, the biotic community structure of the estuary also changes (Wells 1961). It is important to understand the effects of changes in freshwater inflows on estuarine community structure in order to ensure the continued function of these ecosystems.

The eastern oyster, *Crassostrea virginica*, is an important component to estuarine ecosystems. As a foundation species, oysters generate three-dimensional reef structure that fish and other invertebrate species utilize as habitat, in addition to providing hard substrate for juvenile oysters to colonize (Lenihan and Peterson 1988). Oyster reefs help to protect other nearshore habitats such as marsh and wetlands, and help to prevent the erosion of shorelines by stabilizing sediments (Meyer et al. 1997). As filter feeders, oysters help maintain estuarine water quality and clarity by removing phytoplankton, particulate organic carbon, pollutants, and sediments from the water column, improving the health of other estuarine habitats, including seagrasses (Tolley et al. 2005).

Crassostrea virginica oyster reefs have a wide distribution in North American estuaries, ranging from the northwestern Atlantic Ocean to the Yucatán Peninsula (MacKenzie et al. 1997). As indicated by this extensive distribution, oysters are able to tolerate a wide range of water temperatures and salinities. Oysters have been shown to survive in water temperatures around 1°C during winter in the northern limits of their distribution to 36°C in the southern limits of their distribution (Galstoff 1964). Oysters are also able to survive in salinities ranging from 3.0 – 43.5, with optimal growth occurring when salinities range from 16 – 24 (Boyd et al. 1992). Previous research indicates that a change in freshwater inflow to estuaries has the potential to affect the structure of oyster reefs (Tolley et al. 2006; Montagna et al. 2008; Turner 2006), and although the effects of temperature on oyster populations have been well documented, freshwater inflows, which control salinity, have a greater effect on oyster populations (Dekshenieks et al. 2000).

The protozoan parasite, *Perkinsus marinus*, is the causative agent for the oyster disease commonly referred to as Dermo (Mackin et al. 1950). *Perkinsus marinus* cells are released into

the water column from dead and decaying oyster tissue, as well as from the feces of live oysters (Ford and Tripp 1996). There are several methods proposed for the infection of oysters by *P. marinus*, including infection through the digestive tract (Mackin 1951), the pallial organs (Dungan et al. 1996), and specifically the pseudofeces discharge area of the mantle (Allam et al. 2013). Once an oyster is infected with *P. marinus*, proliferation of the parasite occurs, resulting in the formation of ulcers and extensive tissue lysis in the mantle tissue. Oyster growth and reproduction decreases as a result of *P. marinus* infection, eventually leading to death (Ford and Tripp 1996). *Perkinsus marinus* distribution is influenced by temperature and salinity patterns, with the parasite proliferating at both high temperatures and salinities (Ford and Tripp 1996).

Previous studies have documented the effects of *P. marinus* on the growth of oysters using various condition indices (Baird 2006; La Peyre et al. 2003; La Peyre et al. 2009); however, few studies have isolated the specific effects of *P. marinus* on the energetic processes involved in oyster growth. For example, Willson and Burnett (2000) found that *P. marinus* infection had no effect on the oxygen uptake of *C. virginica* gill tissue. Powell et al. (1996) simulated the effects of *P. marinus* on oyster population growth, and reported that epizootics can be triggered by different combinations of temperature and salinity, in addition to variability in food availability. Scope for growth (SFG) is a physiologically based index that reflects the balance between energy acquisition and energy expenditure processes (Warren and Davis 1967). These studies are conducted in order to provide instantaneous estimates of metabolic responses of individual organisms to changes in the environment (Smaal and Widdows 1994). Scope for growth index values are positive under optimal environmental conditions and negative under stressful conditions. Sessile organisms tend to be ideal study organisms for changes in

environmental quality because when environmental quality degrades, these species are not able to relocate to areas with more suitable conditions.

The physiology of bivalve species (Class: Bivalvia) has been widely researched, with studies encompassing numerous species from both Subclass Pteriomorphia and Heterodonta. Within Subclass Pteriomorphia, the physiology of bivalve species including *Mytilus edulis*, *Ostrea edulis*, *Saccostrea commercialis*, *Crassostrea angulata*, *C. ariakensis*, *C. corteziensis*, *C. gigas*, *C. rhizophorae*, and *C. virginica* has been investigated (Widdows et al. 1995; Buxton et al. 1981; Bayne 2000; Haure et al. 2003; Guzmán-Agüero et al. 2013; Barillè et al. 2003; Kelly et al. 2011). Additionally, within Subclass Heterodonta, the physiology of *Argopecten irradians*, *Chione elevata*, *Rangia cuneata*, and *Mercenaria mercenaria* have been investigated (Palmer 1980; Schoech 2013; Hartwell et al. 1991; Srna and Baggaley 1976; Espinosa and Allam 2006). Full scope for growth analyses have been conducted on *M. edulis*, *O. edulis*, *Chione elevata*, *Crassostrea corteziensis*, *C. gigas*, *C. virginica*, and *A. irradians* (Widdows et al. 1995; Buxton et al. 1981; Guzmán-Agüero et al. 2013; Barillè et al. 2003; Kelly et al. 2011; Schoech 2013). Although bivalves appear to be the model organism for scope for growth studies, the initial response of bivalves when stressful conditions are encountered is to close their valves (McFarland et al. 2013). The main disadvantage to using bivalves in scope for growth experiments is that valve closure inhibits normal physiological processes, and can lead to death if conditions do not improve (McFarland et al. 2013).

The purpose of this study was to determine the effects of rapid reductions in salinity, such as those reflective of storms, flood events, and freshets, and *P. marinus* infection on the scope for growth of *C. virginica* oysters. Salinity and temperature are the two major factors that affect marine bivalve disease and overall growth potential (Beseres Pollack et al. 2011; Soniat et al.

2009). This study sought to answer two research questions: 1) Does salinity affect scope for growth of *C. virginica*? and 2) Within salinity treatments, does *P. marinus* infection level influence scope for growth? Study hypotheses were: 1) Scope for growth will differ among salinity treatments, and 2) Within salinity treatments, scope for growth will differ among *P. marinus* infection levels. Results of this study will help improve our knowledge of oyster response to salinity stress and will be useful for water resource management decision-making.

METHODS

Study Site

The Laguna Madre is the largest hypersaline estuarine system in the world, comprising of the Laguna Madre of Texas and the Laguna Madre de Tamaulipas (Figure 1; Tunnell, Jr. and Judd 2002). Of the seven estuarine systems along the Texas coast, the Laguna Madre of Texas is the largest, and is commonly divided into the Upper and Lower Laguna Madre (Diener 1975; Tunnell, Jr. and Judd 2002). Freshwater inflows into the Laguna Madre are highly dependent on rainfall events and the discharge of municipal or industrial waters (Tunnell, Jr. and Judd 2002). With an average depth of 1 meter, the major habitat types include seagrass meadows and wind-tidal flats (Tunnell, Jr. and Judd 2002). Although oyster reefs are not a major habitat type in the Laguna Madre, several natural reefs exist in the Port Isabel, Texas area, and in South Bay (Figure 1; King et al. 1994).

Field Methods

Market-sized (≥76mm shell height) oysters were hand collected every two weeks (September 2017 through November 2017; Table 1) from intertidal oyster reefs in the Lower Laguna Madre near Arturo Galvan Coastal Park in Port Isabel, Texas (Figure 1; N 26° 04’ 44.6”

W 97° 13’ 32.2”). Water quality measurements, including temperature, dissolved oxygen, salinity, pH and turbidity, were taken using a YSI Pro Digital Sampling System sonde Model 626910-4 at the time of collection. Large biofouling organisms such as mussels were removed in the field. Oysters were transported to the lab in coolers and scrubbed to remove remaining biofouling organisms, such as barnacles and polychaetes, and algae. Once clean, oyster shell height (mm) was measured and recorded. Oysters were drip acclimated to a control salinity of 35. Depending on collection salinity, drip acclimation occurred over one to eight hours. Drip acclimation methods are as follows: oysters were placed into water with the same salinity as the collection salinity, the number of hours required to raise the salinity no more than 2 units per hour were determined, and water with a salinity of 50 was slowly added over the pre-determined amount of time to bring the oysters up to a salinity of 35. Oysters were starved during drip acclimation and holding prior to initiation of the experiments.

Lab Methods

Each experiment began on the fourth day after oyster collection. Oysters were each randomly assigned into 1890 mL glass jars filled with artificial seawater at one of six salinity treatments: 10, 15, 20, 25, 30, and 35. The highest salinity (35) was the control, with lower salinity treatments used to determine the effects of rapid decreases in salinity. Four experiments were conducted, each with a sample size of thirty-six oysters (six replicate oysters for each of six salinity treatments). Oysters were fed 1mL of DT’s Live Marine Phytoplankton Reef Blend on the first day of the experiment, and then not again until the fourth day of the experiment during which samples were obtained to measure oyster clearance rates.

Oysters remained in the same salinity treatment throughout the entire experiment, and were held in an incubator at 25°C with12:12 (day:night) photoperiod and constant aeration. Each

experiment lasted 5 days, with daily water exchanges conducted at the beginning of each day. Oysters were rotated throughout the incubator daily to remove the potential effect of subtle temperature differences in the incubator. Physiological measurements for scope for growth calculations occurred on days 4 and 5 of each experiment as described in the following section.

Scope for Growth (SFG) Methods

Clearance Rate—Clearance rate measurements were obtained on day 4 in order to determine the clearance rate of individual oysters. A water exchange occurred before the start of each feeding trial. Oysters were kept under constant aeration and provided 1 mL of DT's Live Marine Phytoplankton Reef Blend, a mixture of *Nannochloropsis oculata*, *Phaeodactylum tricornutum,* and *Chlorella* spp. ranging from 2 – 20μm. A control jar filled with seawater and the same concentration of microalgae was used to determine algae settling or sticking throughout the feeding trial. Water samples (10 mL) were collected from each jar at 0, 60, and 120 minutes post-feeding and preserved with 200μl of 50% glutaraldehyde to a final concentration of 1%. Initially, cell counts were to be conducted using a Coulter Counter, however due to logistical constraints resulting from malfunction of the Coulter Counter, samples that were collected during feeding trials were not able to be processed. Instead, a weight standardized clearance rate was calculated following the methods of Casas et al. (2018), using a published clearance rate value for *C. virginica* (Shumway et al. 1985):

$$CR_w = (W_{std}/W_{exp})^b * CR_i \quad (1)$$

Where:

- CR_w = dry tissue weight (g) standardized clearance rate (L hr^{-1} g^{-1})
- W_{std} = dry tissue weight of standard oyster (1 g)
- W_{exp} = dry tissue weight of experimental oyster (g)

- b = dry tissue weight allometric exponent (0.58; Cranford et al. 2011)
- CR_i = clearance rate for individual oyster, set to 0.3962 L hr^{-1} (Shumway et al. 1985) due to malfunction of the Coulter Counter.

Absorption Efficiency—Absorption efficiency (%AE) was calculated using the equation defined by Conover (1966):

$$\%AE = [(F - E) / (1 - E)F] \text{ X } 100 \quad (2)$$

Where:

- F = fraction of organic content from the dry weight of the algae
- E = fraction of organic content in the feces produced by the oysters

The feces produced by individual oysters were collected using a Pasteur pipette, then placed in an aluminum boat, and finally dried in a convection oven at 60°C for 24 hours to determine dry weight. Feces were then incinerated in a muffle furnace at 450°C for 4 hours and weighed to obtain ash free dry weight. Organic content was calculated as the difference between the dry weight and ash free dry weight.

The water with remaining microalgae in each feeding trial jar was filtered onto precombusted Whatman ® GF/F glass fiber filters (0.7 μm porosity) using a vacuum pump. Filters were then dried at 60°C for 24 hours to determine dry weight, and then incinerated in a muffle furnace at 450°C for 4 hours to obtain ash free dry weight. Organic content was calculated as the difference between the dry weight and ash free dry weight.

Ammonia Excretion—Once the feeding trials were complete, each oyster was transferred into a 950 mL jar at the same salinity, and placed back into the incubator without aeration to allow for ammonia production. Initial ammonia measurements occurred before placing oysters into the incubator, and final ammonia measurements occurred the following morning, around 20

hours later. Ammonia samples (10 mL) were stored in 20 mL scintillation vials, and fixed with six drops of 1.0M hydrochloric acid (HCl). Ammonia concentration (mg L^{-1}) was measured using a Thermo Orion 7 model 95-12 Ammonia probe. Ammonia excretion rate was calculated, and then standardized to a standard 1 gram dry weight oyster.

$$AER_w = (W_{std} / W_{exp})^b * AER_i \quad (3)$$

Where:

- AER_w = dry tissue weight (g) standardized ammonia excretion rate (mg NH_3 hr^{-1} g dry $weight^{-1}$)
- W_{std} = dry tissue weight of standard oyster (1 g)
- W_{exp} = dry tissue weight of experimental oyster (g)
- b = dry tissue weight allometric exponent (0.57; Kelly et al. 2011)
- AER_i = experimentally derived ammonia excretion rate for individual oyster

Oxygen Consumption—Change in oxygen concentration (mg L^{-1}) was measured in the static environment of a sealed 950 mL glass jar using an OXY-4 SMA oxygen meter (PreSens, Regensburg, Germany). Change in oxygen concentration was determined via discrete oxygen measurements when oysters were first placed in the jar and one hour later. Oxygen consumption rate was calculated, and then standardized to a standard 1 gram dry weight oyster, following the methods of Casas et al. (2018).

$$OCR_w = (W_{std} / W_{exp})^b * OCR_i \quad (4)$$

Where:

- OCR_w = dry tissue weight (g) standardized oxygen consumption rate (mg O_2 hr^{-1} g dry $weight^{-1}$)
- W_{std} = dry tissue weight of standard oyster (1 g)
- W_{exp} = dry tissue weight of experimental oyster (g)
- b = dry tissue weight allometric exponent (0.58; Casas et al. 2018)
- OCR_i = experimentally derived oxygen consumption rate for individual oyster

To obtain the calorie content (g dry weight^{-1}) for DT's Live Marine Phytoplankton Reef Blend, algae samples were combusted in a Parr ® 6200 bomb calorimeter. Salt content was removed prior to calorimetry by centrifuging 15 mL vials of algae at 4000rpm for seven minutes, pouring off the supernatant, rinsing the pellet with DI water and re-centrifuging for seven additional minutes (McGlaun 2012). The resulting supernatant was poured off and the pellet was dried using a vacuum freeze dryer (Labconco FreeZone 2.5, Kansas City, Missouri). Calorie content was used in determining the energy gained and lost by the oysters.

Energy Budget Calculations—The scope for growth of oysters was calculated by determining the energy budget for individual oysters using the physiological measurements: clearance rate (C), absorption efficiency (Ab), ammonia excretion (U), and oxygen consumption (R). The values obtained from these measurements were converted to energy expenditures using the calorie contents of the algae provided to the oysters and then entered into the formula defined by Winberg (1960).

$$C - F = Ab = R + U + P \quad (5)$$

Where:

- C = energy obtained through food consumption
- F = energy lost as feces
- P = energy available for growth and reproduction (SFG)

The equation can be balanced to give the following equation:

$$P = Ab - (R + U) \quad (6)$$

Where:

- Ab = energy absorbed from food
- R = energy lost as respiration (oxygen consumption)
- U = energy lost as excretion (ammonia excretion)

Perkinsus marinus Infection Intensity and Condition Index Methods

Perkinsus marinus Infection Intensity—Following completion of the scope for growth procedures, oysters were sacrificed to determine *P. marinus* infection intensity using the culture method of Ray (1966). A 10 mm x 10 mm section of mantle tissue was removed and incubated in fluid thioglycollate medium for 7 days. Tissue was then placed on a glass microscope slide, teased apart using stainless steel probes, stained with Lugol's solution, and observed with the aid of a compound microscope (Nikon Eclipse E200, Melville, New York). The entire tissue sample was observed under 10x magnification. Infection intensity was ranked and recorded from 0 (uninfected) to 5 (heavily infected) (Mackin 1962; Craig et al. 1989). *Perkinsus marinus* prevalence (proportion infected) was calculated as the number of infected oysters per salinity treatment divided by the total number of oysters per salinity treatment. *Perkinsus marinus* weighted prevalence (severity) was calculated by multiplying the mean infection intensity (Soniat et al. 2012) of oysters in a salinity treatment by the prevalence.

Condition Index—Condition index was measured for each oyster using the dry flesh weight: dry shell weight ratio (Mann 1978; Lucas and Beninger 1985). Because a small section of mantle tissue was removed for the *P. marinus* assay, a wet weight was obtained for each individual sample before it was placed in the fluid thioglycollate medium. Oyster tissue and valves were dried in a convection oven at 60°C for 48 hours to determine dry weight.

$$\text{Condition Index} = [\text{Dry weight of oyster tissue (g)} / \text{Dry weight of oyster shell (g)}] * 100 \quad (7)$$

Statistical Approach

Scope for growth variables were analyzed using one-way ANOVAs to determine the effects of salinity treatment. Ammonia excretion rate data were transformed by taking the square root of the data, while oxygen consumption rate data were log-transformed to meet the

assumptions required for performing ANOVA. Overall energy gained, energy lost, and scope for growth were also analyzed using a one-way ANOVA in order to determine the effect of salinity treatment. Data for energy lost to respiration were log-transformed to meet the assumptions required for performing ANOVA. Tukey's HSD post-hoc test was conducted if a significant ANOVA ($p=0.05$) was found to separate means.

One-way ANOVAs were conducted on oyster shell height, condition index and *P. marinus* infection intensity data in order to determine effects of salinity treatment. Condition index data were log-transformed in order to satisfy normality assumptions. A one-way ANOVA was also conducted on scope for growth in order to determine the effects of *Perkinsus marinus* infection intensity. All statistical analyses were conducted using R.3.3.1 (R Foundation for Statistical Computing 2016).

RESULTS

Water Quality Measurements

Salinities during oyster collection ranged from 15.59 to 35.76 (n=4; mean ± standard error 29.84 ± 4.78), with the lowest value occurring on 11 October 2017 after a strong thunderstorm passed through the area (Table 1). Temperature ranged from 20.3°C to 29.4°C (mean 24.35 ± 2.17°C). Dissolved oxygen was always high, ranging from 5.88 mg L^{-1} to 9.88 mg L^{-1} (mean 7.47 ± 0.87 mg L^{-1}). pH ranged from 7.64 to 8.03 (mean of 7.88 ± 0.08). Turbidity ranged from 9.3 NTU to 122.2 NTU; the highest turbidity occurred after the strong October storm (mean 46.88 ± 25.54 NTU).

Crassostrea virginica Size by Salinity Treatment

A total of 144 oysters were collected from September 2017 to November 2017. Oyster shell height (mm) ranged from 72 – 124 mm (mean 90.35 ± 0.99 mm). Oyster size was similar among salinity treatments (Appendix 2.1; p=0.06189).

Calorimetry Results

Two bottles of DT's Live Marine Phytoplankton were analyzed for calorie content. Bottle 1 was used during Experiment #1, and had a calorie content of 4752.9559 calories g dry weight^{-1}. The oxycaloric equivalent used in calculating the energy lost from respiration for oysters in Experiment #1 was 318.16 KJ mol^{-1}. Bottle 2 was used for Experiments #2-4, and the calorie content was 5260.2520 calories g dry weight^{-1}. The oxycaloric equivalent used to calculate energy losses from respiration in Experiments #2-4 was 352.12 KJ mol^{-1}.

Physiological Measurements

Clearance rate was calculated for all 144 oysters. Mean clearance rate was 0.42 ± 0.008 L hr^{-1} g dry weight^{-1}. Clearance rate was not significantly different among salinity treatments (Appendix 2.2; p=0.2496), although oysters at a salinity of 30 had the lowest mean clearance rates (Figure 2; 0.39 ± 0.02 L hr^{-1} g dry weight^{-1}).

Absorption efficiency was calculated for the 42 oysters that produced feces across the four experimental trials. Mean absorption efficiency was 54.32 ± 5.36%. There were no significant differences in absorption efficiency based on salinity treatment (Appendix 2.3; p=0.6696), however mean absorption efficiency generally decreased with increasing salinity (Figure 3). Variation in absorption efficiencies was highest at salinities > 20.

Ammonia excretion was measured for all 144 oysters. Only oysters with a positive change in ammonia were included in analyses (n=139). The mean rate of ammonia excretion was 0.02 ± 0.001 mg NH_3 hr^{-1} g dry $weight^{-1}$. There were significant differences in the rate of ammonia excretion by salinity treatment (Figure 4; Appendix 2.4; p=0.00631), but no obvious overall trend. While the overall rate of ammonia excretion was significant according to salinity treatment, Tukey's HSD post-hoc analysis found no significant comparisons (Appendix 2.4).

Oxygen consumption was measured for all 144 oysters. Only oysters for which a decrease in oxygen concentration between t_0 min and t_{60} min was obtained, were included in analyses (n=111). The mean rate of oxygen consumption was 0.12 ± 0.02 mg O_2 hr^{-1} g dry $weight^{-1}$. There were no significant differences in the rate of oxygen consumption by salinity treatment (Appendix 2.5; p=0.4675), however, oxygen consumption rates were highest at the two moderate salinities (20 and 25; Figure 5).

Energy budget calculations were completed for oysters that had positive absorption efficiency, ammonia excretion, and oxygen consumption values (n=31). Overall scope for growth of oysters was determined using energy gains and energy losses. Mean energy gains by salinity treatment ranged from 112.30 ± 73.89 J hr^{-1} g dry $weight^{-1}$ to 517.20 ± 67.89 J hr^{-1} g dry $weight^{-1}$ (mean 410.31 ± 32.20 J hr^{-1} g dry $weight^{-1}$). There were no significant differences in energy gains between salinity treatments (Appendix 2.6; p=0.05446), however energy gains generally decreased as salinity increased (Figure 6).

Ammonia excretion and oxygen consumption rate data were used to calculate energy losses. Mean energy loss from excretion was 0.54 ± 0.06 J hr^{-1} g dry $weight^{-1}$. There were no significant differences in energy loss from excretion between salinity treatments (Appendix 2.7;

p=0.4932). The energy loss from excretion was consistently low for oysters at salinity 15 (0.37 ± 0.06 J hr^{-1} g dry $weight^{-1}$; Figure 7), and showed a decreasing trend for oysters from salinity 20 to 35.

Mean energy loss from respiration was 3.13 ± 1.01 J hr^{-1} g dry $weight^{-1}$. There were no significant differences in energy losses from respiration between salinity treatments (Appendix 2.8; p=0.8375), however there was a general decrease in energy losses from respiration with increasing salinity, with the exception of salinity treatment 25, which also had the largest variation (Figure 8).

Scope for growth (n=31) ranged from 37.25 J hr^{-1} g dry $weight^{-1}$ to 867.46 J hr^{-1} g dry $weight^{-1}$ (mean 406.64 ± 32.01 J hr^{-1} g dry $weight^{-1}$). There was no significant relationship between salinity treatment and scope for growth (Appendix 2.9; p=0.05492), however, scope for growth demonstrated a decreasing trend as salinity increased (Figure 9).

Perkinsus marinus Infection

Perkinsus marinus infection was assessed for all oysters (n=144). Among salinity treatments, infection intensity ranged from low (0.00) to moderately heavy (3.67) with a prevalence of 79-92% (Table 2). Infection severity among salinity treatments ranged from 1.35 to 1.56. Salinity treatment did not have a significant effect on *P. marinus* infection intensity (Appendix 2.10; p=0.9813), and scope for growth was not significantly different among *P. marinus* infection intensities (Appendix 2.11; p=0.2513).

Condition Index

Condition index was assessed for all oysters (n=144), and ranged from 0.64 – 3.52 (mean 1.70 ± 0.04). Salinity had a significant effect on condition index (Appendix 2.12; p=0.03609),

however, Tukey's HSD post-hoc analysis found no significant comparisons. Condition index increased with salinity treatment (Figure 10).

DISCUSSION

A key outcome of this project is to understand how oysters respond to rapid reductions in salinity, reflective of increased freshwater inflows due to storms, floods, and water management practices. Results indicate that oysters from the Lower Laguna Madre have enough energy for maintenance and growth, regardless of *P. marinus* infection level, throughout salinity variations that are common to estuarine habitats. However, results also indicate that oyster condition index decreases after oysters experience rapid reductions in salinity. One of the most important environmental variables affecting the growth and abundance of oysters in their highly variable estuarine habitats is salinity (Tolley et al. 2006; Montagna et al. 2008; Turner 2006). The relationship between freshwater inflow and oyster population dynamics is complex and results are equivocal (Buzan et al. 2009; Turner 2006; Savage 2017), warranting additional research, particularly into the individual and combined effects of *P. marinus* and salinity on energetic processes at the individual level that influence population-level changes (e.g. Casas et al. 2018; Lavaud et al. 2017; Baird 2006).

This study determined the scope for growth of *C. virginica* oysters, standardized to 1 g dry tissue weight, across six salinity treatments. Scope for growth was highest at salinity 10 (867.46 J $hr^{-1}g^{-1}$) and lowest at salinity 35 (37.25 J $hr^{-1}g^{-1}$). Previous investigations into the scope for growth of various *Crassostrea* species indicates the influence of season, temperature, salinity, and the microalgae diet provided to oysters (Kelly et al. 2011; Guzmán-Agüero et al. 2013; Barillé et al. 2003). Kelly et al. (2011) compared seasonal differences in the physiology and

growth of *C. virginica* and *C. ariakensis*, and results indicate that scope for growth of both species was influenced by season. Scope for growth, standardized to 1 g dry tissue weight, for *C. virginica* ranged from 46.3 J $g^{-1}h^{-1}$ in the spring to -4.5 J $g^{-1}h^{-1}$ in the winter (Kelly et al. 2011). For *C. ariakensis*, scope for growth standardized to 1 g dry tissue weight, ranged from -36.2 J $g^{-1}h^{-1}$ in the summer to -1.02 J $g^{-1}h^{-1}$ in the winter (Kelly et al. 2011). Scope for growth values of *C. gigas* varied based on the species of microalgae provided to the oysters, and ranged from 163 to 282 J $h^{-1}g^{-1}$ (Barillé et al. 2003). Observed at different combinations of temperature and salinity, the scope for growth of *C. corteziensis* ranged from 15.49 ± 17.46 to 472.92 ± 69.28 J h^{-1} (Guzmán-Agüero et al. 2013). Comparatively, *C. virginica* oysters from the Lower Laguna Madre had high scope for growth values. Further research is necessary in order to understand the implications of salinity and the overall effect changes in environmental quality have on the scope for growth of *C. virginica* oysters.

Absorption efficiency of oysters decreased with increasing salinity, while variation increased (salinities 25, 30, and 35). Similar trends in absorption efficiency with respect to salinity were observed for *C. corteziensis* (Guzmán-Agüero et al. 2013). Absorption efficiency rates for *C. virginica* may depend on the algal species consumed (Romberger and Epifano 1981). Algal species provided to oysters in this study included the chlorophyte *Chlorella* sp., the diatom *Phaeodactylum tricornutum*, and the heterokont *Nannochloropsis oculata*. Oysters fed the diatom *Thalassiosira pseudonana* and the flagellate *Isochrysis galbana*, had higher absorption efficiency (Romberger and Epifano 1981), than oysters fed chlorophyte species with thick cell walls (Webb and Chu 1982). Mean absorption efficiencies for *C. virginica* in the current study are likely conservative due to the combination of algal species provided.

In the current study, methods were designed to determine the amount of food particles obtained by oysters over a two-hour period however, the majority of oysters remained closed for the extent of each feeding trial. This was likely in response to handling stress the oysters experienced in the water exchange prior to clearance rate measurements (McFarland et al. 2013), rather than to the salinity itself. The optimal salinity range over which oyster feeding occurs has been cited as 15 – 25 (Galstoff 1964; Casas et al. 2018), although it is possible that variations in optimal salinity for feeding exist between oyster populations. In the current study, technical difficulties with the Coulter Counter meant that clearance rates were not able to be measured empirically. As a result, estimated clearance rates (standardized to 1g dry tissue weight) were calculated using the published value 0.3962 L hr^{-1} (Shumway et al. 1985; Casas et al. 2018).

Among the four physiological processes used to calculate oyster scope for growth (clearance rate, absorption efficiency, ammonia excretion, and oxygen consumption), the only one significantly affected by salinity was the rate of ammonia excretion. However, there were no discernible trends in the mean ammonia excretion rate data. Extreme variation in ammonia excretion has previously been reported among oysters kept in the exact same conditions (Hammen et al. 1965). Valve closure during the time allotted for ammonia excretion could result in alternative excretion pathways being utilized and the production of different excretion compounds (Srna and Baggaley 1976).

Oxygen consumption rate in the current study was not affected by salinity, in support of previous research demonstrating little to no effect on oyster respiration (Galstoff 1964; Van Winkle 1968). Oysters remained closed for the majority of time allotted for oxygen consumption to occur, indicating that valve closure likely influenced oxygen consumption measurements. Season may play a role in oxygen consumption, with *C. virginica* oysters from Louisiana

consuming less oxygen in winter than in summer (Casas et al. 2018). Oysters in this study were collected during fall, and may have consumed less oxygen than if experiments had been conducted during the summer months.

Perkinsus marinus Infection Analysis

Periodic low salinity events can be harmful to oyster populations (Loosanoff 1952), but also play an important role in their reestablishment by limiting *P. marinus* infections (Beseres Pollack et al. 2011; La Peyre et al. 2003; La Peyre et al. 2009). Previous research indicates that *P. marinus* infection intensity had no effect on the physiological response of oysters (Willson and Burnett 1999; Paynter 1996; Newell et al. 1994), including those subjected to extreme low salinity events (Baird 2006), and that oysters may have the ability to reduce *P. marinus* infection levels during extremely low salinity events (La Peyre et al. 2003). Historically, light *P. marinus* infections have been recorded from the Port Isabel, TX area (Breuer 1960), but a subsequent study indicated that oysters from reefs around Port Isabel, TX were no longer infected with *P. marinus* (Osborn 1962). No other contemporary studies have monitored the status of *P. marinus* infections in oysters of the Lower Laguna Madre. The proliferation of *P. marinus* infection in oysters has been linked to high salinities (Andrews and Ray 1988; Ford and Trip 1996; Soniat et al. 2009; Savage 2017). Therefore, the consistent exposure of Lower Laguna Madre oysters to hypersaline conditions (Breuer 1962; Diener 1975) could facilitate proliferation of the *P. marinus* parasite. Results from the current study indicate that *P. marinus* infection intensity had no influence on oyster scope for growth, and that oysters were able to survive extreme reductions in salinity, regardless of *P. marinus* infection.

Oyster Condition Analysis

Condition index is a measure commonly used to determine overall fitness of the oyster. This metric can also be used to assess an oyster's physiological status (Lucas and Beninger 1985; Casas et al. 2017) and to evaluate the effects of changes in environmental conditions such as salinity (Mercado-Silva 2005). In the current study, mean condition index showed a significantly positive relationship with salinity treatment, indicating that Lower Laguna Madre oysters function better at higher salinities. An alternative explanation is that oysters with the highest condition index values were exposed to the least extreme decreases in salinity compared to the control salinity of 35.

These results indicate that rapid reductions in salinity have a significant effect on the condition index of oysters from the Lower Laguna Madre. Low condition index values result after adverse environmental conditions have been experienced, or after spawning (Lucas and Beninger 1985; La Peyre et al. 2003; La Peyre et al. 2009). Only one observation of spawning occurred throughout the experimental trials, at salinity treatment 30. As spawning was not observed at any of the lower salinities, the reduction in condition index can most likely be attributed to oysters utilizing energy reserves in response to stressful salinity conditions. Recent studies indicate that oysters in the Lower Laguna Madre represent a genetically distinct population (King et al. 1994; Anderson et al. 2014) being adapted to more extreme salinity conditions (Breuer 1962; Diener 1975). The restricted salinity variability experienced by Lower Laguna Madre oyster populations may select for oysters adapted to higher salinities, but with lower plasticity than oyster populations frequently exposed to wider salinity ranges. It is possible that condition index results in the current study reflect this oyster population's high tolerance for salinity extremes.

Future Recommendations

Methodologies employed in the current study have revealed areas of improvement for future experiments. First, oysters were held in individual jars to prevent the spread of *P. marinus*. When water exchanges were conducted prior to each physiological measurement, oysters were moved around and subjected to physical stress that resulted in valve closure for indefinite periods of time. Future experiments could be conducted in an experimental system such as the one used by Guzmán-Agüero et al. (2013), where oysters were held in flow-through experimental chambers. Physiological measurements were obtained in two phases, the first for energy gains (filtration rate, clearance rate, and absorption efficiency), and the second for energy losses (oxygen consumption and ammonia excretion) (Guzmán-Agüero et al. 2013). Samples used to determine filtration rate, clearance rate, ammonia excretion, and oxygen consumption were collected from samples of outflow water (Guzmán-Agüero et al. 2013), and so oysters were not disturbed by physical movement once in the experimental chambers, unlike in this study. Additionally, this study was not able to monitor the change in *P. marinus* infections over the course of the experiment due to a single measurement of *P. marinus* infection using sacrificial methods. Multiple determinations of *P. marinus* infection level would be possible by analyzing oyster hemolymph (Gauthier and Fisher 1990; Nickens et al. 2002), to determine if a change in *P. marinus* infection level occurred as a result of reductions in salinity, as suggested by La Peyre et al. (2003) and (2009). Freshet events lasting between 2-3 weeks have been documented to reduce *P. marinus* infection intensities in oysters (La Peyre et al. 2009). Finally, oysters in the Lower Laguna Madre frequently experience hypersaline conditions, salinities potentially ranging from 35 – 60 (Diener 1975). A control salinity of 35 was chosen because it represents the lower end of hypersaline conditions this oyster population experiences. Future studies could

incorporate salinity treatments of ≥40 in order to determine if even larger salinity variation has an effect on the stress response of oysters.

CONCLUSION

The response of estuarine species to changes in environmental quality, especially salinity, as a result of climate variability is important to understand (Childers et al. 1990; Soniat et al. 2005; Dekshenieks et al. 2000). Freshwater inflows are a major determinant of estuarine salinity (Alber 2002; Sahoo and Smith 2009; Tolley et al. 2006), with low salinity events, such as those simulated in this study, resulting from increased freshwater inflows (Tolan 2007). Future climate predictions indicate the potential for increased climate variability, including an increase in the frequency and severity of rainfall events associated with El Niño-Southern Oscillation (Ropelewski and Halpert 1986).

Salinity has been shown to be an important environmental factor affecting the growth and distribution of *C. virginica* oysters (Ford and Tripp 1996; Dekshenieks et al. 2000). This study observed the effects of rapid changes in salinity, such as those likely to occur as a result of El Niño-Southern Oscillation, on oysters from the Lower Laguna Madre, Texas. Results indicate that the scope for growth of *C. virginica* oysters from a hypersaline estuary was not affected by salinity, and that within salinity treatments, *P. marinus* infection intensities did not influence scope for growth. However, the condition index of oysters exposed to rapid and extreme decreases in salinity was significantly lower than oysters exposed to less extreme variations in salinity. These results could indicate that while oysters have enough energy for maintenance and growth after extreme decreases in salinity occur, their overall condition is decreased.

As the Lower Laguna Madre has no direct source of freshwater inflow, and salinities are influenced by climatic conditions, an increase in storm frequency and severity could significantly impact estuarine salinity. This study demonstrated the ability of oysters from the Lower Laguna Madre, Texas to tolerate a wide range of salinities resulting from increases in freshwater inflow. Results improve our understanding of individual oyster response to future climate conditions, which is important in order to effectively manage oyster populations and to guide water management decision-making.

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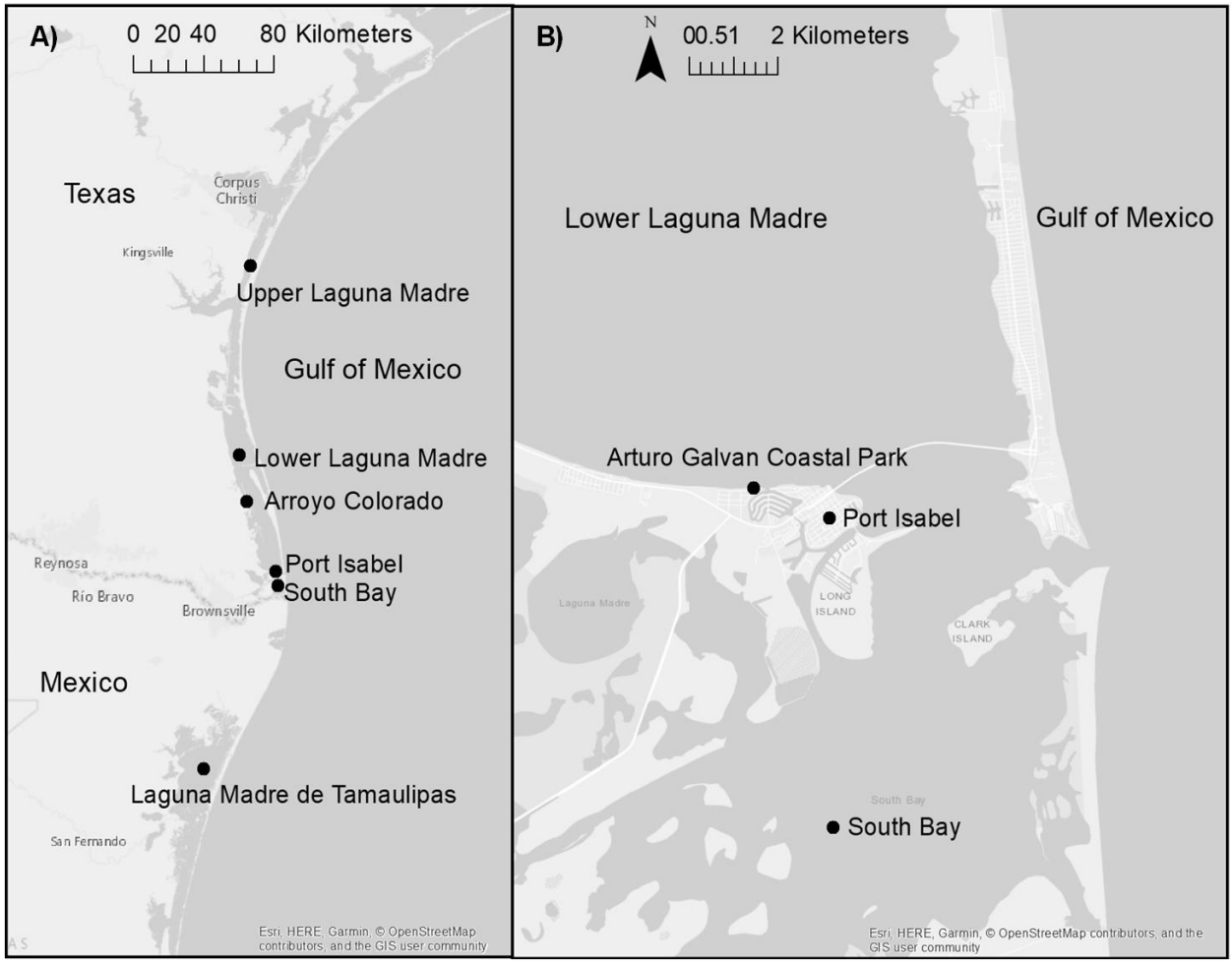
FIGURES

Figure 1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Kingsville
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Gulf of Mexico
Lower Laguna Madre
Arroyo Colorado
Reynosa
Port Isabel
South Bay
Rio Bravo
Brownsville
Mexico
Laguna Madre de Tamaulipas
San Fernando
Esri, HERE, Garmin, © OpenStreetMap contributors, and the GIS user community
B)
N
00.51 2 Kilometers
Lower Laguna Madre
Gulf of Mexico
Arturo Galvan Coastal Park
Port Isabel
Laguna Madre
LONG ISLAND
CLARK ISLAND
South Bay
South Bay
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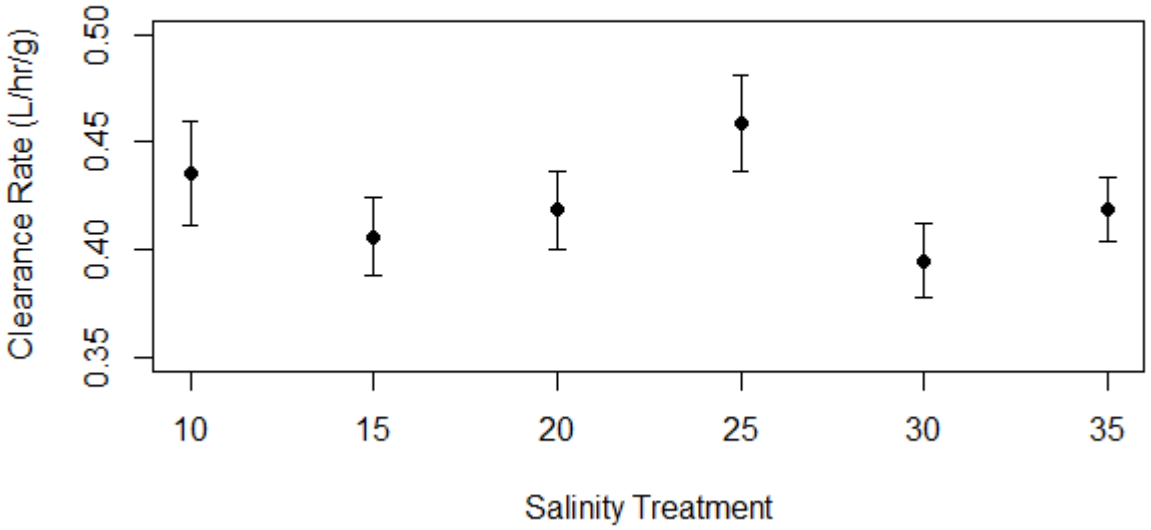
Figure 2



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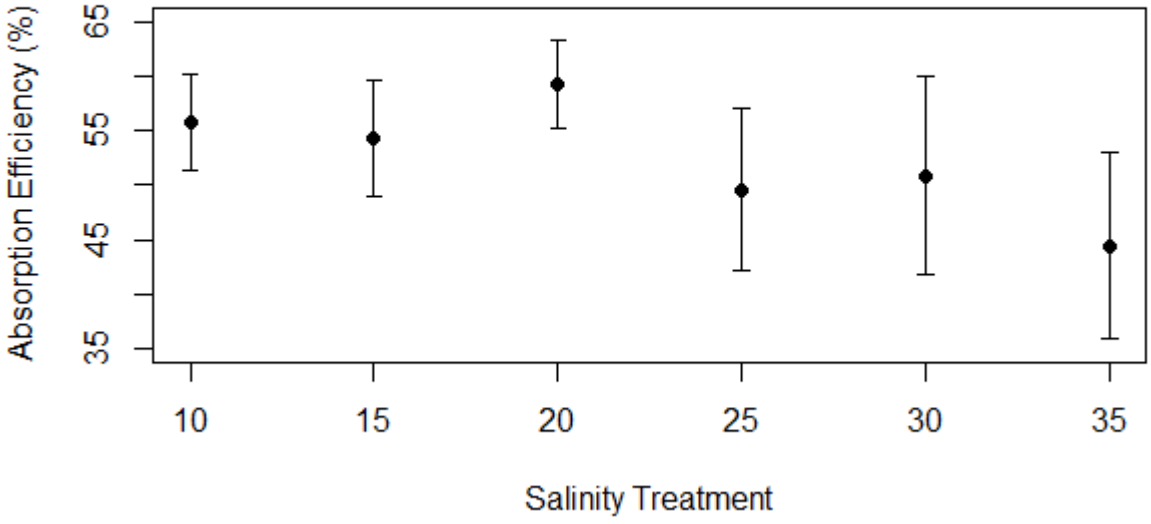

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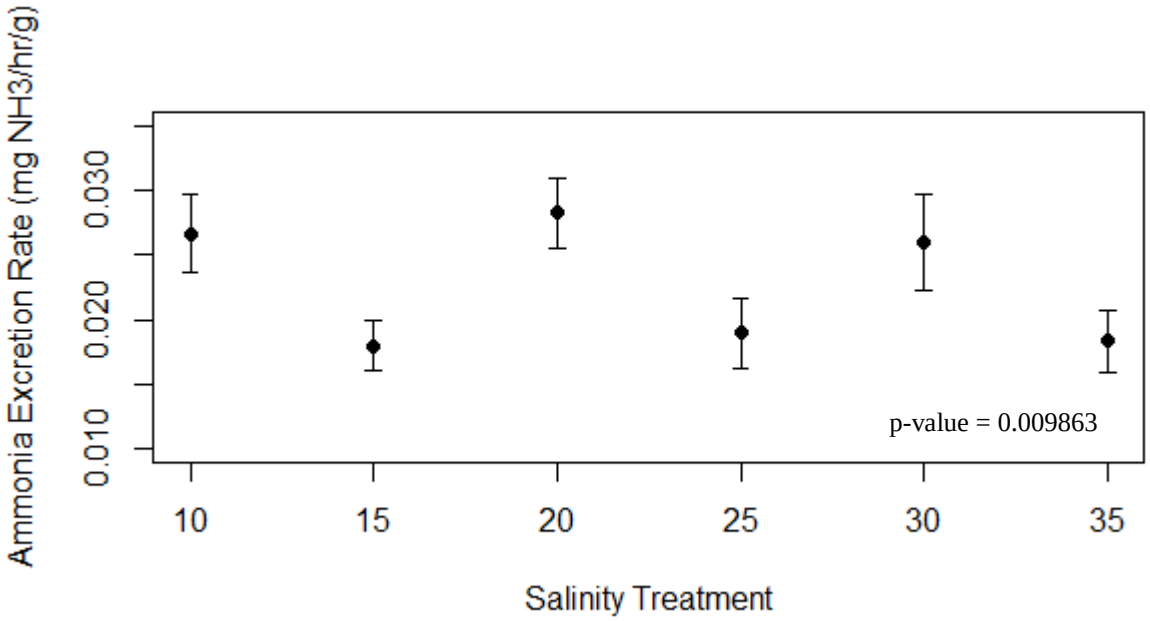

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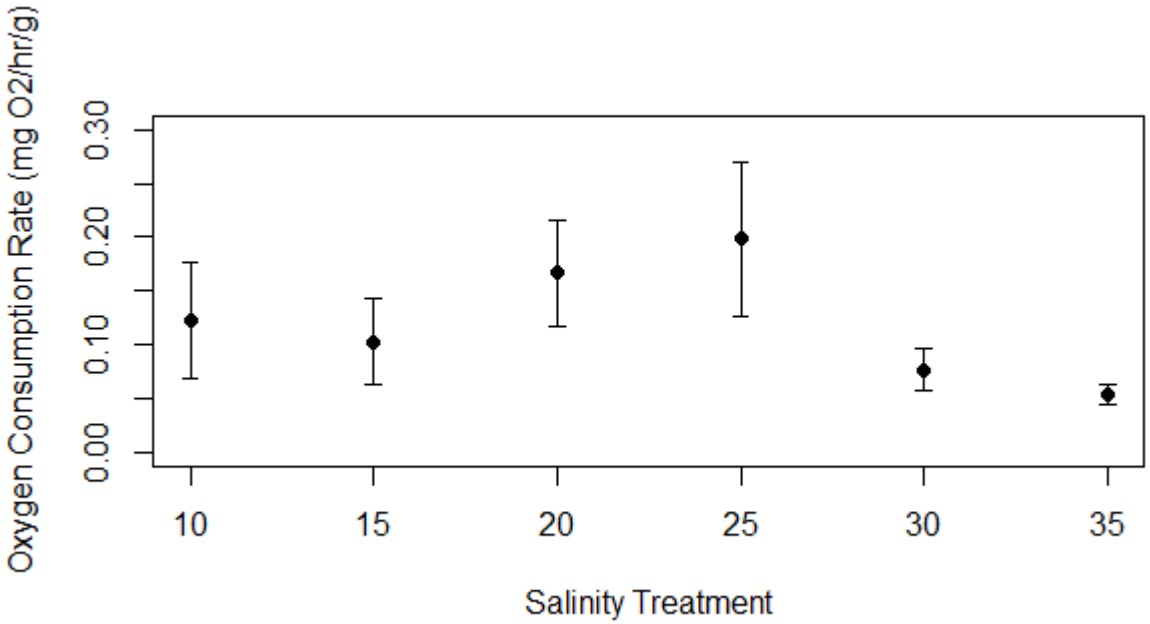

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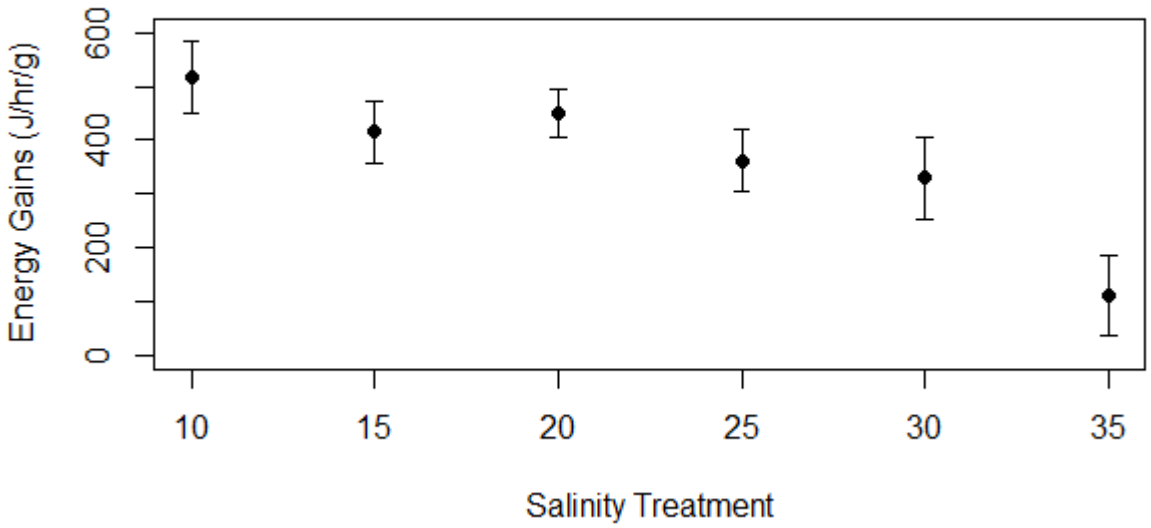

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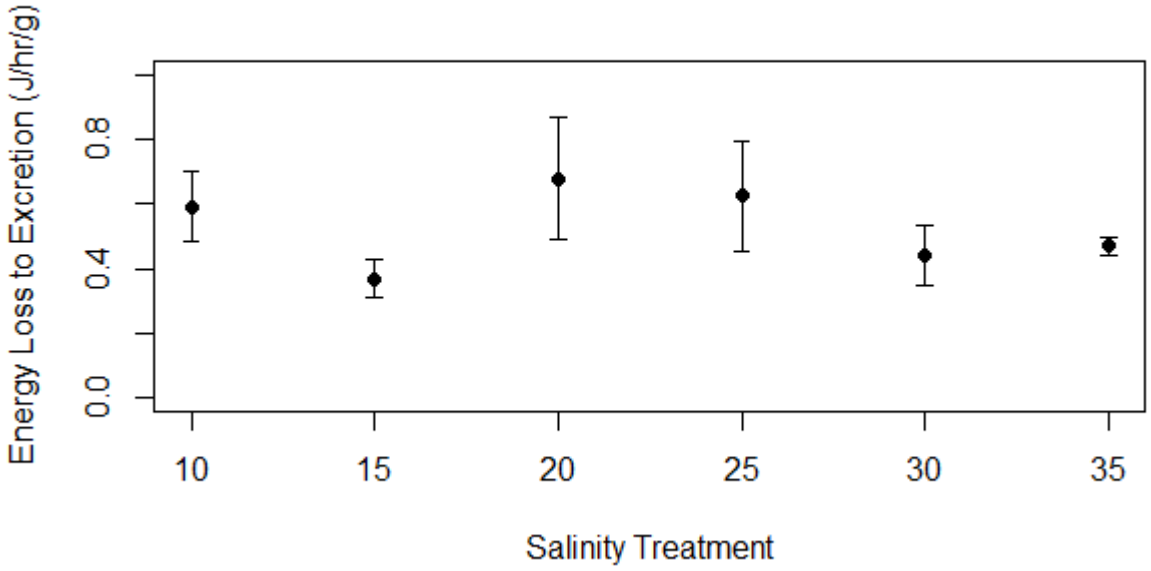

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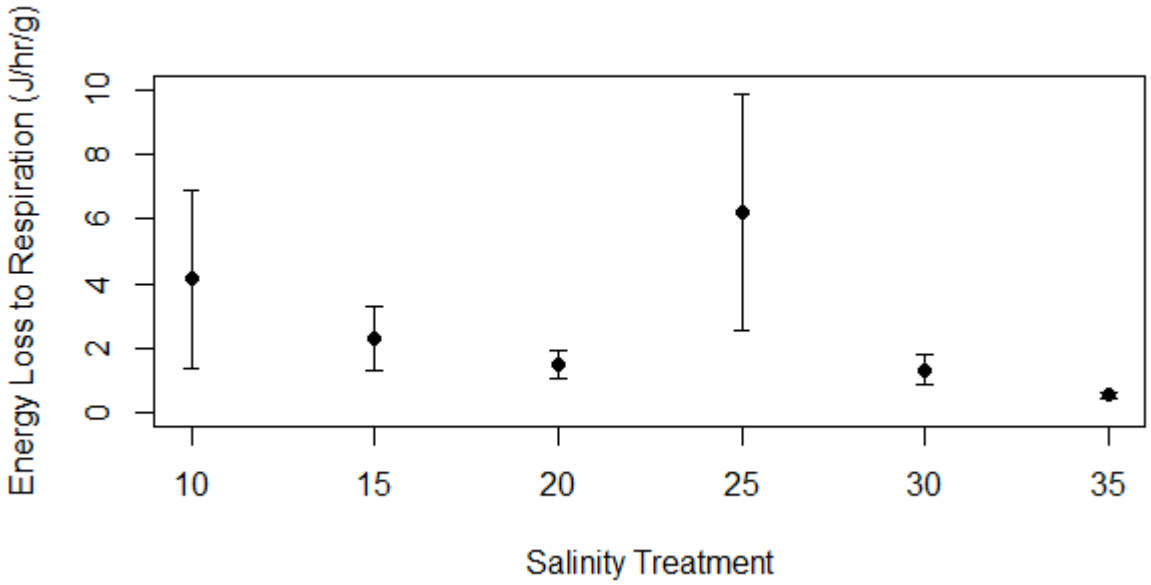

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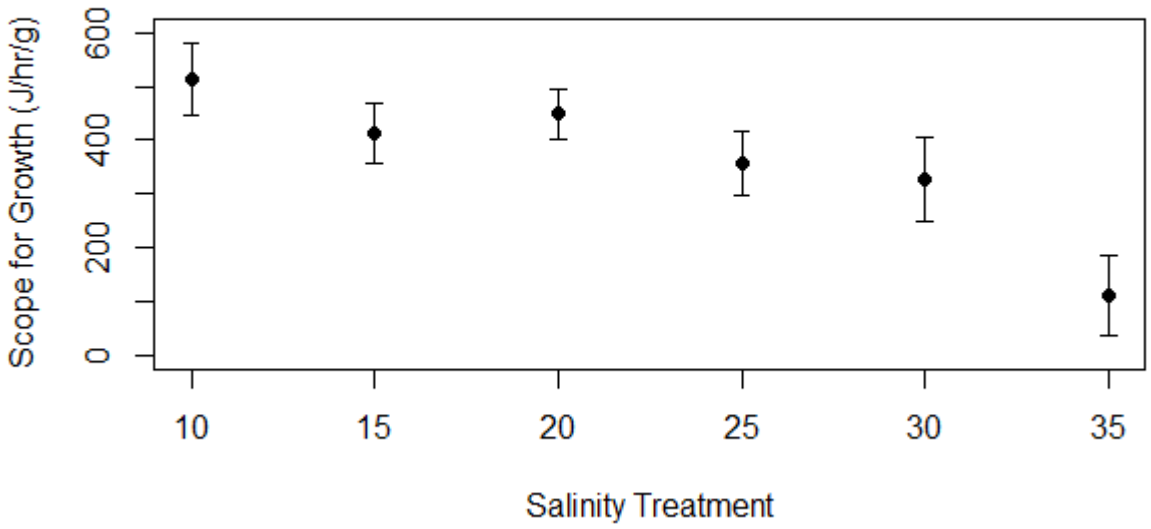
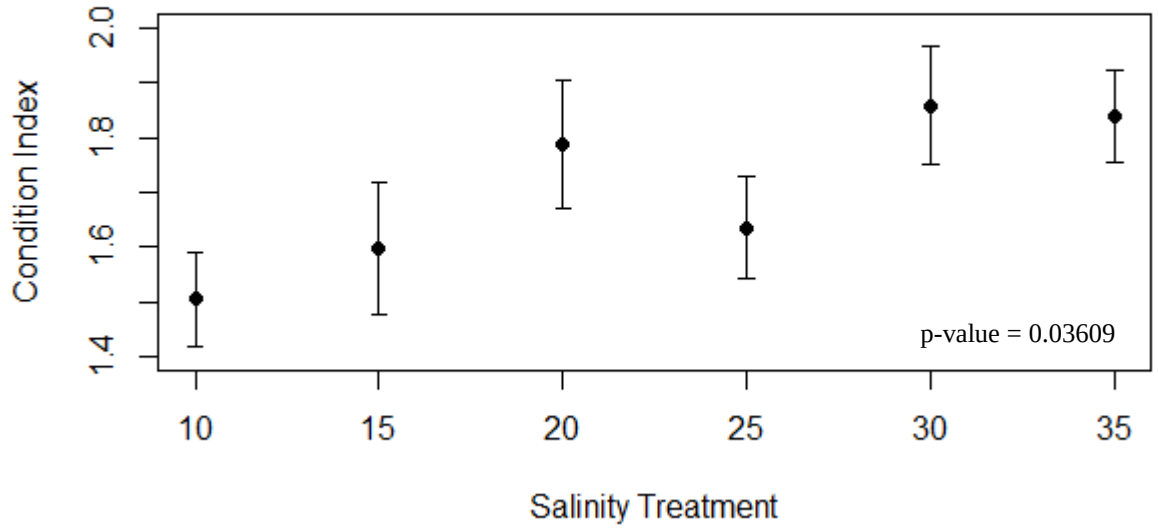


Figure 10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1.4
1.6
1.8
2.0
p-value = 0.03609
10
15
20
25
30
35
Salinity Treatment

TABLES

Table 1: Water quality measurements (temperature, salinity, dissolved oxygen, pH, and turbidity) obtained at the time of sample collection, listed by experiment and collection date.

Experiment	Date	Temperature	Salinity	Dissolved Oxygen	pH	Turbidity
Port Isabel #1	9/27/2017	29.4	35.8	5.9	7.9	24.1
Port Isabel #2	10/11/2017	21.2	15.6	6.7	7.6	122.2
Port Isabel #3	10/25/2017	20.3	33.1	7.5	7.9	9.3
Port Isabel #4	11/12/2017	26.5	34.9	9.9	8.0	31.9

Table 2: *Perkinsus marinus* infection calculations (prevalence and severity) by salinity treatment.

Salinity Treatment	*Perkinsus marinus* intensity range	Prevalence	Severity
10	0.00 – 3.67	0.83	1.49
15	0.00 – 3.67	0.79	1.35
20	0.00 – 3.33	0.92	1.56
25	0.00 – 3.33	0.92	1.43
30	0.00 – 3.33	0.92	1.50
35	0.00 – 3.33	0.88	1.36

LIST OF APPENDICES

APPENDICES

Appendix 1: Detailed scope for growth calculations

Energy Gained from Diet (Ab)

$Ab' = CR_w * E' * Ae$

CR_w = Clearance Rate (L $hr^{-1}g^{-1}$)
E = Energy of diet (calories g^{-1})
Ae = Absorption efficiency (%)

Conversion factors used:
$E' = E * 0.00166$ g * 0.004184 KJ cal^{-1} * 1000 J

Energy Lost from Respiration (R)

$R = O' * Y'$

O´ = Oxygen Consumption
Y´ = Oxycaloric Equivalent

Units of collected data
OCR_w = Oxygen Consumption Rate (mg O_2 hr^{-1} g^{-1})
Y = KJ mol $oxygen^{-1}$

Conversion factors used:
$O' = OCR_w * 0.001$ g mg^{-1} * 0.15999 mol g^{-1}
$Y' = Y * 1000$ J KJ^{-1}

Energy Lost from Excretion (U)

$U = A' * M'$

A´ = Ammonia Excretion
M´ = Ammonia Coefficient

United of collected data
AER_w = Ammonia Excretion Rate (mg NH_3 hr^{-1} g^{-1})
M = cal µmol $ammonium^{-1}$

Conversion factors used:
$A' = AER_w * 0.001$ g mg^{-1} * 0.017 mol g^{-1}
$M' = M * 4.1840$ J cal^{-1} * 0.000001 mol μmol^{-1}

Appendix 2: Supplementary Tables

Appendix 2.1: ANOVA output: effects of salinity treatment on oyster height (mm). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	1468.7	293.75	2.161	0.06189.
Residuals	138	18758.2	135.93		

Appendix 2.2: ANOVA output: effects of salinity treatment on clearance rate (L hr^{-1} g^{-1}). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	0.06114	0.0122288	1.3436	0.2496
Residuals	138	1.25602	0.0091016		

Appendix 2.3: ANOVA output: effects of salinity treatment on absorption efficiency (%). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	974.4	194.88	0.6414	0.6696
Residuals	36	10939.1	303.86		

Appendix 2.4: ANOVA output: effects of salinity treatment on ammonia excretion rate (mg NH_3 hr^{-1} g dry $weight^{-1}$); and Tukey's HSD results. Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	0.032047	0.0064094	3.4045	0.00631**
Residuals	133	0.250387	0.0018826		

			Estimate	Std. Error	t value	Pr(>|t|)
15 - 10	==	0	-0.0287	0.01308	-2.197	0.246
20 - 10	==	0	0.00518	0.01281	0.404	0.9986
25 - 10	==	0	-0.0292	0.01281	-2.277	0.211
30 - 10	==	0	-0.0035	0.01281	-0.275	0.9998
35 - 10	==	0	-0.0301	0.01294	-2.329	0.19
20 - 15	==	0	0.03392	0.01281	2.649	0.0929.
25 - 15	==	0	-0.0004	0.01281	-0.032	1
30 - 15	==	0	0.02523	0.01281	1.97	0.3651
35 - 15	==	0	-0.0014	0.01294	-0.107	1
25 - 20	==	0	-0.0343	0.01253	-2.741	0.074.

30 - 20	==	0	-0.0087	0.01253	-0.694	0.9823
35 - 20	==	0	-0.0353	0.01266	-2.789	0.0655.
30 - 25	==	0	0.02564	0.01253	2.047	0.3217
35 - 25	==	0	-0.001	0.01266	-0.077	1
35 - 30	==	0	-0.0266	0.01266	-2.102	0.2925

Appendix 2.5: ANOVA output: effects of salinity treatment on oxygen consumption rate (mg O_2 hr^{-1} g dry $weight^{-1}$). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	1.0833	0.21665	0.9257	0.4675
Residuals	105	24.5745	0.23404		

Appendix 2.6: ANOVA output: effects of salinity treatment on energy gains (J hr^{-1} g dry $weight^{-1}$). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	324798	64960	2.5393	0.05446.
Residuals	25	639537	25581		

Appendix 2.7: ANOVA output: effects of salinity treatment on energy loss from excretion (J hr^{-1} g dry $weight^{-1}$). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	0.3851	0.07702	0.9054	0.4932
Residuals	25	2.1266	0.08507		

Appendix 2.8: ANOVA output: effects of salinity treatment on energy loss from respiration (J hr^{-1} g dry $weight^{-1}$). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	0.5847	0.11694	0.4097	0.8375
Residuals	25	7.1361	0.28544		

Appendix 2.9: ANOVA output: effects of salinity treatment on scope for growth (J hr^{-1} g dry $weight^{-1}$). Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	320567	64113	2.533	0.05492.
Residuals	25	632774	25311		

Appendix 2.10: ANOVA output: effects of salinity treatment on *Perkinsus marinus* infection intensity. Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	0.801	0.16023	0.1447	0.9813
Residuals	138	152.817	1.10737		

Appendix 2.11: ANOVA output: effects of *Perkinsus marinus* infection intensity on scope for growth. Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Perkinsus marinus Intensity	7	285296	40757	1.4037	0.2513
Residuals	23	667814	29035		

Appendix 2.12: ANOVA output: effects of salinity treatment on condition index and Tukey's HSD results. Df = degrees of freedom.

	Df	Sum Square	Mean Square	F value	Pr(>F)
Salinity Treatment	5	0.18286	0.036571	2.4595	0.03609*
Residuals	138	2.05194	0.014869		

			Estimate	Std. Error	t value	Pr(>|t|)
15 - 10	==	0	0.01482	0.0352	0.421	0.998
20 - 10	==	0	0.0706	0.0352	2.006	0.344
25 - 10	==	0	0.03553	0.0352	1.009	0.914
30 - 10	==	0	0.0899	0.0352	2.554	0.116
35 - 10	==	0	0.09069	0.0352	2.576	0.11
20 - 15	==	0	0.05579	0.0352	1.585	0.61
25 - 15	==	0	0.02071	0.0352	0.588	0.992
30 - 15	==	0	0.07509	0.0352	2.133	0.277
35 - 15	==	0	0.07587	0.0352	2.155	0.265
25 - 20	==	0	-0.0351	0.0352	-0.996	0.918
30 - 20	==	0	0.0193	0.0352	0.548	0.994
35 - 20	==	0	0.02008	0.0352	0.57	0.993
30 - 25	==	0	0.05437	0.0352	1.545	0.636
35 - 25	==	0	0.05516	0.0352	1.567	0.622
35 - 30	==	0	0.00078	0.0352	0.022	1