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A Comparison of Diploid and Triploid Eastern Oysters for Aquaculture Production Under Extreme Temperatures and Salinities

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A COMPARISON OF DIPLOID AND TRIPLOID EASTERN OYSTERS FOR AQUACULTURE PRODUCTION UNDER EXTREME TEMPERATURES AND SALINITIES

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Off-bottom aquaculture of Eastern oysters (*Crassostrea virginica)* is a nascent industry that is increasingly supported by the use of triploid oysters, which grow faster than diploids. Despite their growth advantage, elevated triploid mortality compared to diploids under high temperature, low salinity, or a combination of these conditions challenge consistent triploid production. Identifying the environmental thresholds at which differential triploid mortality occurs and predicting its economic impact are important to informing decision-making in oyster aquaculture. The goal of this thesis was to compare the biological and economic performance of diploid and triploid oysters under high temperature and low salinity conditions. To accomplish this goal, I first conducted a laboratory study to assess the differential biological performance between ploidies. Forty diploid and forty triploid oysters were placed in each of 12 replicate tanks, which were adjusted to three different temperatures (24, 29, 34ºC), held at a salinity of 18, and mortality checked daily. Salinity was then reduced to 2 in half the tanks at each temperature. Mortality was tracked daily, and samples were taken to assess physiological responses. Under low salinity (2), we found that triploids died faster than diploids at every temperature treatment, reflecting their reduced ability to osmoregulate. In contrast, we found that under medium salinity (18), diploids died faster than triploids at 34°C but at around the same rate at 24 and 29°C. To project the impact on farm profitability, we assessed the economic implications of differential ploidy performance under various salinity and temperature combinations using a bioeconomic model. The model combined outputs from the dynamic energy budget model with a modified enterprise budget to estimate profit for aquaculture operations under different salinity and temperature scenarios. Both salinity and temperature affected profits, and outcomes in all environmental scenarios varied by ploidy. These results can help inform seed and site selection, although future investigation using our bioeconomic approach can be applied to inform other best management practices including optimal husbandry techniques and stocking densities. Combined, these studies help reduce risk to triploid growers by assessing the potential biological and economic differences in ploidy performance when exposed to extreme environmental conditions.

CHAPTER 1. INTRODUCTION

Over the past 60 years, global seafood consumption increased at a faster rate (3.2%) than meat produced from all terrestrial animals combined (2.8%) (FAO, 2018), making fisheries production the fastest growing food sector in the world (Subasinghe et al., 2009). Global fish production peaked in 2016 at 171 million tons, with aquaculture contributing 47% of the production (FAO, 2018). The harvest of wild fish stocks has remained stagnant since the 1980's, with aquaculture growing to meet the increasing demand for human consumption (FAO, 2018). Although most of the world's aquaculture by weight and financial value is conducted in Asia, the FAO identifies the United States as one of the most promising nations for marine aquaculture development due to its extensive coastline (Kapetsky et al., 2013). In 2014, U.S. aquaculture production was 608 million pounds with a value of around $1.3 billion dollars. In 2018, the total U.S. aquaculture production grew to 680 million pounds, with a value of around $1.5 billion; a 12% increase of weight and 15% increase of value over 4 years (NMFS, 2022). The growth in aquaculture is fueled by significant focus and investment from several federal agencies and private investing companies in recent years that support research, development, and promotion of domestic aquaculture operations.

Within the U.S., the largest contributors to the marine aquaculture production are shellfish, making up around 75% of the marine aquaculture production by value between 2013 and 2018 (NMFS, 2022). Cupped oysters (*Crassostrea* spp.) are the most popular shellfish being farmed, which account for around half of the shellfish production in the US during the same time period (NMFS, 2022). Not only are oysters significant as a food source for humans, but they also serve as ecosystem engineers, providing structural habitat for resident and juvenile organisms, filtering water, and reducing wave energies to protect shorelines (Coen & Humphries, 2017). An economic evaluation of the ecological services that oyster reefs provide estimated a value of $5,500 to $99,000 per hectare of oyster reef per year, with shoreline stabilization being the most valuable potential service (Grabowski et al., 2012). Despite their cultural and ecological significance, oysters and the reefs they create were identified as the most imperiled marine habitat type with an 85% functional decrease from their historic extent (Beck et al., 2011).

The combined effects of numerous factors including overharvest, decreased water quality, and disease are major factors in the decline of oysters in the U.S and worldwide. While there is evidence that individual stressors including environmental damage, direct reef harvest, and subsequent reef destruction have led to this decline, the effects are often synergistic (Beck et al., 2011; Lenihan et al., 1999). Destruction of natural oyster reef structure from overharvest and bottom dredging methods, decreases the complexity and vertical relief of reefs and are cited as prevalent causes of decline around the United States (Rothschild et al., 1994). Bottom dredging with large oyster dredges was legalized with a special permit through Maryland's first oyster license law in 1854 (Kirby, 2004). The demand for oysters soared around this time, especially with new canning techniques that allowed oyster meat to be preserved and shipped around the country (MacKenzie, 1996). The wild harvest of Eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay peaked in the late 1800s, and by 1920 wild harvests began to decline (MacKenzie, 1996). These trends of historic oyster population decline from anthropogenic and environmental changes have been observed in estuaries nationwide, including in the northern Gulf of Mexico (nGoM), a notable oyster growing region spanning from Texas to Florida.

The nGoM supports extensive natural oyster reefs and a highly productive oyster industry that consistently leads the United States in Eastern oyster production. In 2014, the nGoM region harvested 8,731 metric tons of Eastern oysters through oyster farming, accounting for about 75% of the total production of the United States (Petrolia et al., 2017). Within the nGoM, Louisiana regularly leads US oyster production by weight (50% nationwide landings from 1999-2018) (LDWF, 2023) and market value (43% nationwide value from 1999-2018) (LDWF, 2023). However, in 2020, Louisiana produced only 16% of the nationwide annual landings, accounting for only 13% of the nationwide value (LDWF, 2024). This significant drop in landings is hypothesized to be due to climate changes and anthropogenic activities that decreased salinity in critical oyster growing areas. For example, flooding from the Mississippi River in 2019 resulted in the opening of the Bonnet Carré Spillway which diverted large amounts of freshwater into the Gulf to minimize damage to human communities. Mass oyster mortality (100% in most Mississippi Sound sites; Gledhill et al., 2020) along with reduced larval growth and survival (Pruett et al., 2021) were documented following the opening of the Spillway. Researchers attributed the mass oyster mortality following the second Spillway opening in 2019 to a prolonged period of extreme low salinity (0.18-4.21) (Gledhill et al., 2020) that was well below the range of oyster tolerance (5-40) (Galtsoff, 1964).

High temperature (>30°C), low oxygen (< 2.0 mg L^{-1}), and low salinity (<5) events are an increasing concern for oyster production and sustainability within the central nGoM. Climate change and anthropogenic activities are predicted to alter water quality in oyster growing areas through changes in temperatures, nutrients and freshwater inputs from altered river inflow, and more variable local precipitation (IPCC, 2023; Luo et al., 2024). Increased eutrophication and warming of coastal waters contribute to low dissolved oxygen events (< 2.0 mg L^{-1}; Justić et al., 2005; Rabalais et al., 2009) which pose a significant threat to oysters (Stickle et al., 1989; Coxe et al., 2023). High amounts of nutrient-rich freshwater from the Mississippi River and other large rivers contribute to coastal eutrophication which has subsequently resulted in large hypoxic zones and harmful algal blooms in the nGoM, the severity of which could worsen with climate change (Justić et al., 2005). Along with nutrients from riverine inputs, the freshwater inflow causes significant decreases and variation in salinity.

Although oysters generally can survive across a wide salinity range (Galtsoff, 1964), duration of exposure and timing of exposure to extreme low salinity events may reduce growth, reproduction, and survival of different life stages (La Peyre et al., 2013; Marshall et al., 2021). In some cases, low salinity events may help promote oyster survival by decreasing potential infections of diseases and by reducing predation (Mackin et al., 1950; Soniat, 1985). However, extended exposure to extreme low salinity (salinities < 5) can result in mass oyster mortality (Gledhill et al., 2020; Marshall et al., 2021). Therefore, the timing and length of low salinity exposure remains critical to ensuring negative effects of low salinity do not occur (La Peyre et al., 2013).

Exacerbating these issues is climate change, which is forecasted to increase temperatures and the frequency of low salinity events (IPCC, 2023; Wang et al., 2023). In the nGoM, sea surface water temperatures have already increased 1.0 ± 0.3°C from 1970 to 2020 and are expected to continue to rise in the future (Wang et al., 2023). Warming trends in sea surface waters in the nGoM also show a seasonal pattern, warming more during the summer (0.22°C

decade^{-1}) than in the winter (0.05°C decade^{-1}; Li et al., 2022). This pattern of seasonally disproportionate warming is expected to amplify the thermal stress that oysters in the nGoM already experience from high water temperatures during the summer months. Additionally, the potential for increased river management (CPRA, 2023), and changes in key environmental variables including temperature and salinity from climate change, are forecasted to increase the occurrence of stressful conditions for oysters (Masanja et al., 2023). To continue to support a viable oyster industry in the nGoM in face of these environmental changes and declines in wild oyster stock (LDWF, 2024), innovative oyster aquaculture strategies have been developed and popularized as an alternative method of production in this region.

Oyster aquaculture, or the farming of oysters, is conducted either through on-bottom (extensive) or off-bottom (intensive) culture. On-bottom aquaculture is the traditional method of in the United States where oysters are grown on the seafloor (Supan, 2002). On-bottom oyster farmers plant cultch, or small pieces of hard substrate, on private leases or public oyster grounds to which wild oyster larvae attach or set on (Supan, 2002). Farmers transplant the resulting oyster spat onto their own private leases where they are grown on the sea floor until market size. After market size is reached, oysters are finally harvested with bottom dredges. In contrast, off-bottom farming is the culture of oysters in a mesh container suspended over the sea floor. By suspending oysters in protective and removable bags that farmers can manipulate, off-bottom farming promotes faster growth, increases survival, allows control of biofouling, and increases product consistency (Walton et al., 2012).

Off-bottom production systems help manage risks associated with oyster farming and enable control over farm operations including grow-out methods and gear choice that can contribute to advantages over on-bottom farming. While oysters grown on-bottom are typically sold in the shucked market, oysters grown off-bottom are typically sold in the half-shell market for a higher price. In addition, off-bottom farming benefits from the use of selective breeding programs that are developing oyster seed with faster growth characteristics, enhanced disease tolerance, or greater resilience to unpredictable environmental stressors such as low salinity events (Leonhardt et al., 2017; Grice, 2018). Off bottom farming is a nascent industry that has quickly expanded in the nGoM from zero farms in 2009, to at least 50 farms in 2018 (Petrolia et al., 2017; Grice, 2018). In Louisiana, state funding through Louisiana Sea Grant has contributed to the development of 19 off-bottom farms and two oyster hatcheries to further jumpstart the state's participation in this budding industry (Melancon, 2023, as cited in Petrolia & Caffey, 2024).

One key approach used to support and promote consistent oyster production has been investment into the development of hatcheries and provision of seed for grow-out (Walton et al., 2013). Although traditional methods of on-bottom oyster aquaculture relied on the wild recruitment of oysters for production, the rise of aquaculture has enabled oysters to be reared in hatcheries, allowing for the artificial selection of desired traits for production. Today, many public and private hatcheries play a critical role in breeding disease-resistant oysters, triploid and tetraploid oysters, and oysters with tolerance to extreme environmental conditions (Wallace et al., 2008).

Oyster diseases such as MSX caused by the protisan *Haplosporidium nelsoni* and Dermo caused by *Perkinsus marinus* are leading causes of oyster mortality, and limit populations of Eastern oysters across their range (Soniat et al., 2006; Powell et al., 2011). Because of the typical open environment where oysters are cultured, it is not feasible to disinfect the environment or vaccinate individuals for disease (Dégremont et al., 2015). Therefore, the only way to reduce the impact of disease once the pathogenic organism is present in the environment is through selective breeding (Roch, 1999). Selective breeding for the development of disease-resistant oysters has successfully improved yield and growth rates while limiting mortality in oyster aquaculture (Frank-Lawale et al., 2014). One example of successful selective breeding of Eastern oysters for disease resistance can be seen in the Chesapeake Bay, where a breeding program was established in 1997 that aimed to create disease resistant oysters to support the oyster industry in Virginia. In recent years, disease-resistant cultivars account for approximately 90% of the oysters grown for production in the region (Frank-Lawale et al., 2014). Selective breeding of Eastern oysters has also allowed for artificial selection of other desirable traits including size, shape, and growth rates.

Cross breeding of broodstock diploid oysters over several generations for enhancement of traits such as disease resistance has been successful, but diploid oysters are fertile and apportion large amounts of energy to reproduction (Allen & Downing, 1986). An alternative to using diploids involves the development of triploid oysters. Triploidy is an attribute where an organism has 3 sets of chromosomes (3N) versus wild diploids that have 2 sets of chromosomes (2N). Although there are several methods for triploid induction, the use of sperm from tetraploid males (4N) to fertilize diploid (2N) eggs has proven itself to be the most reliable method to produce 100% triploids (Nell et al., 1996; Guo et al., 2009). Triploid seed has recently become popular for oyster farmers in the nGoM, accounting for more than 85% of the commercial seed orders from the Auburn University Shellfish Laboratory in 2017, while only 15% were for diploid seed (Wadsworth et al., 2019).

Triploid oysters are advantageous as they grow faster and have better meat quality particularly during spawning seasons (Nell, 2002; Yang et al., 2018). The faster growth rate of triploids may also help in reducing the disease exposure time that these susceptible oysters face during grow out, ultimately increasing survival and bottom-line yield for farmers (Barber & Mann, 1991). One study in the Chesapeake Bay demonstrated that triploid Eastern oysters had lower mortality than diploids (34% less), greater shell height (25%), greater whole weight (88%), higher yield (152%), yielding more market-size oysters (114%) when compared to diploids in a set time period (Dégremont et al., 2012). These advantages may come from reduced gamete production of triploids, which provides energy that can be applied to growth (Allen & Downing, 1986; Hawkins et al., 2000; Honkoop, 2003).

In the nGoM, the partial sterility of triploids allows farmers to have a summer harvest and provides income during a part of the year when oysters historically have poor meat quality (Bodenstein et al., 2021). On the Louisiana coast, diploid oysters typically undergo gametogenesis to prepare for spawning in April-May and again in October-November. Gametogenesis and spawning generally result in reduced body condition, especially during the summer months after the first spawning cycle in April-May (Normand et al., 2008). Historically, this caused diploid oysters to be unmarketable and prevent farmers harvesting during the

summer. However, with the development of triploid oysters, farmers can harvest year-round as the body condition of triploid oysters is much higher than diploid oysters especially during the summer when diploid body condition is heavily reduced (Nell, 2002).

Despite the many advantages for growing triploid oysters, recent reports from oyster farmers and studies in the nGoM report unexplained high spring and summer mortality of triploid oysters in the field compared to their diploid counterparts (Casas et al., 2017; Wadsworth et al., 2019), causing significant concern to triploid growers in the nGoM. One study found elevated mortalities of triploids across four study sites in Alabama compared to diploids (Wadsworth et al., 2019). The peaks of the mortality were associated with high temperature (>28°C) and low salinity (<5) that followed exceptional rainfall events (Wadsworth et al., 2019). A different study that explored differential triploid mortality in coastal Louisiana found a higher triploid mortality in the low salinity (<5) site when combined with high temperature (>28°C) (Bodenstein et al., 2023). Interestingly, a Chesapeake Bay study demonstrated that triploids had lower performance than diploids, measured by tissue weight and shell height in low salinity sites, but performed similarly with diploids in medium salinity sites (Callam et al., 2016). It was only in high salinity sites where triploids performed better than diploids and showed a greater potential for growth (Callam et al., 2016). Given that most nGoM sites fall within the low to medium salinity ranges (average salinity 8-20 at most grow-out areas; Swam et al., 2022) and water temperatures commonly exceed 30°C during the summertime, the reports of higher triploid mortality in these conditions pose a concern to farms and hatcheries producing triploids in the nGoM.

While triploids are hypothesized to better tolerate stressful conditions due to not having to deal with the energetic cost of gametogenesis or spawning, it remains unclear as to why nGoM triploid oysters experience higher mortality during environmentally stressful events. Differences in ploidy response to various environmental (i.e., temperature, salinity, food availability, disease) and physiological stressors (i.e., gametogenesis, cell regulation and balance) may be driving the elevated triploid mortality (Brianik & Allam, 2023). In terms of reproductive stress, it is possible that while triploids grow faster due to partial sterility, gametogenesis may still be acting as a stressor to triploids and contributes to mortality (Bodenstein et al., 2023). Differences in gonad development between diploids and triploids have been observed, and these differences may not be detectable using gonad development classification systems created for diploids (Matt et al., 2020). Development of a specific classification system for gametogenesis in triploid oysters may be required to accurately understand the relationship between triploid oyster mortality and gamete development.

Outside of gametogenesis, differential ploidy responses at the cellular level to coping with low salinities has been proposed as a potential contributor to elevated triploid mortality. Oysters deal with fluctuating salinities by osmoregulation or changing the concentration of ions within their blood to match the outside environment. A reduced ability to osmoregulate correlates to higher energy expenditure to maintain homeostasis with the changing environment, which could be lethal depending on the duration of the imbalance (La Peyre et al., 2013; Casas et al., 2024). A recent study investigating osmoregulation in diploid and triploid oysters found that triploid oysters were slower to osmotically conform and were less efficient in maintaining acid-base status and cell water content at decreased salinities as compared to diploid oysters (Casas et

al., 2024). Differences in physiological responses between ploidies may stem from structural differences such as larger cell sizes and lower cell surface-to-volume ratios in triploid organisms, which is known as triploid gigantism (Comai, 2005). The larger cell size of triploids is hypothesized to slow biochemical cellular processes across membranes required for osmoregulation in extreme salinities (Miettinen & Björklund, 2017).

Considering the biological tradeoffs between growing diploid or triploid oysters in different environmental conditions is important to maximizing profitability. For example, growing diploids in one site may be the most profitable choice because of their superior survival in adverse (low salinity) conditions, while growing triploids in another site may be more profitable due to higher growth rates. One method to help increase the understanding of these tradeoffs is the use of bioeconomic modelling. Bioeconomic modelling serves to combine economic and biological considerations and helps quantify the economic implications from changes in biological parameters (Llorente & Luna, 2016). The use of bioeconomic modeling in aquaculture can help farmers plan, monitor, quantify risk, and determine cost-effectiveness of aquaculture operations (Pomeroy et al., 2008; Llorente & Luna, 2016). Previous uses of bioeconomic modelling for oyster aquaculture have identified ideal harvest schedules and site selection (Ferreira et al., 2007; Silva et al., 2011; Kamiyama et al., 2021). However, bioeconomic modelling has not yet been conducted to serve as a decision-making tool for Eastern oyster ploidy selection based on various environmental factors.

Here, I use a laboratory study to explore elevated triploid mortality testing the effects of low salinity and high temperature exposure of half-sibling diploid and triploid oysters during the spawning season. Specifically, Chapter 2 of this thesis examines lethal (mortality) and sub-lethal (gametogenic progression and biomarkers) responses of diploid and triploid oysters to understand differences in ploidy responses to salinity and temperature stressors. This study was designed to gain a better understanding of salinity and temperature conditions that result in differential ploidy mortality. This information can be used to assist development of oyster lines that tolerate low salinity and high temperature, and also to help make decisions about the selection of ploidy by oyster farmers for grow-out under different predicted environmental conditions.

Equally important is the need to understand the potential trade-offs between selection of diploid and triploid oysters for aquaculture production. Biological factors such as time to harvest and mortality may vary between ploidies based on current and predicted environmental conditions such as salinity and temperature, which can have effects on economic factors such as cost, revenue, and predicted profit based on ploidy selection. Chapter 3 of this thesis develops a bioeconomic model that combines a mechanistic oyster growth and survival model with an enterprise budget. Here, I develop and use this model to project the expected differences in profit across different environmental conditions, based on initial selection of seed ploidy.

Combined, this work contributes to reducing risk on oyster farms by informing seed and site selection based on biological responses to environmental conditions. Risk, or the probability that some event or outcome adverse will occur, is inherent in all agricultural or aquacultural operations. To decrease the costs of risk, farmers must accurately identify and assess the effects on their farm. In terms of production risk management, farmers must understand the effects that

factors such as disease, predation, or change of environmental conditions could have on final production. This work acts as an assessment of the biological and economic performance of diploid and triploid oysters under a wide range of environmental conditions. The findings may illuminate connections between factors in oyster farming and help inform best management practices to maximize production. Increasing the understanding of how biological responses may vary under environmental conditions specific to a farm site is important to accurately predicting profit, and the connection between these factors can help inform effective decision making in oyster aquaculture operations.

CHAPTER 2. DIFFERENTIAL BIOLOGICAL RESPONSES BETWEEN DIPLOID AND TRIPLOID EASTERN OYSTERS UNDER LOW SALINITY AND HIGH TEMPERATURE CONDITIONS

2.1. Introduction

Aquaculture production has tripled in volume over the last two decades, with molluscan culture a key area of focus worldwide accounting for 26% of the marine aquaculture production by weight in 2020 (Naylor et al., 2021; FAO, 2022). Within molluscan culture, cupped oysters (*Crassostrea* spp.) are the most popular genus being farmed, contributing at least 30% of the total molluscan aquaculture production by weight in 2020 (FAO, 2022). The cultural and economic importance of bivalves, including oysters, generate significant attention on improving overall product quality and ensuring consistent growth and survival (Yang et al., 2019). Eastern oysters (*Crassostrea virginica*) support a valuable commercial aquaculture fishery in the northern Gulf of Mexico (nGoM), a region that produced 76% of the nation's oysters by volume in 2019 (NMFS, 2022). In nGoM, commercial oyster farming has traditionally depended on on-bottom culture (Supan, 2002), but advances in grow-out methods and gear, and improvements in hatchery-produced oyster seed have driven the growth of off-bottom production (Walton et al., 2013; Petrolia et al., 2017). Oysters grown in suspended off-bottom systems are tumbled by natural wave action, cultivating a product with a more desirable shell shape and appearance that tends to sell for a higher price in the half-shell market (Petrolia et al., 2017; Dame et al., 2019). Although the nGoM currently only contributes an estimated 12% of total off-bottom oyster production in the southern US (Walton & Swann, 2021), there has been a rapid rise in the number of farms and the total production of each farm in the nGoM since 2010 (Supan, 2014; Petrolia et al., 2017; Walton & Swann, 2021). However, increasing environmental variation from climate change (i.e., increased freshwater inflow from extreme precipitation events and heatwaves) (Myhre et al., 2019; Luo et al., 2024) and human activities (i.e., river management, Posadas, 2022) (CPRA, 2023) has resulted in increased risks to the industry, challenging consistent oyster production in coastal waters.

To address these risks, development of selective breeding programs that produce seed with enhanced salinity or temperature tolerance and growth characteristics has accelerated (Allen et al., 1993; Leonhardt et al., 2017; Yang et al., 2018). One approach involves the use of triploid oysters to provide enhanced growth and production. Triploidy is an attribute where an organism has three sets of chromosomes (3N) versus wild diploids that have two sets of chromosomes (2N). Triploid oysters are commonly induced by crossing a tetraploid (4N) male with a diploid female and are advantageous for aquaculture because they grow faster and have better meat quality particularly during spawning seasons (Nell, 2002; Guo et al., 2009). These advantages are a result of increased energy allocation from gametogenesis to somatic growth in triploid oysters, while other potential contributors can include increased cell size and genetic heterozygosity (Allen & Downing, 1986; Guo et al., 1996; Hawkins et al., 2000; Brianik & Allam, 2023). Triploid oysters have become popular in aquaculture (Brianik & Allam, 2023), accounting for 90% of oyster spat in France (Dégremont et al., 2010), and 90% of oysters sold in Virginia in 2017 (Hudson & Murray, 2014). Serving as the primary producer of oyster seed for Alabama off-bottom farmers, the Auburn University Shellfish Laboratory reported that 85% of their commercial seed orders were triploids in 2017 (Schneider, 2017; Wadsworth et al., 2019).

Despite the advantages and growing reliance on triploid oysters in off-bottom aquaculture, recent reports from oyster farmers and studies in the nGoM show elevated triploid mortality compared to diploids in late spring through early fall. These mortality events are marked by lowered salinity and elevated temperatures (Casas et al., 2017; Wadsworth et al., 2019; Bodenstein et al., 2023). Low salinity (>5) is hypothesized to be one of the primary drivers of the triploid mortality especially when combined with other stressors such as elevated temperature, disease from *Perkinsus marinus,* or desiccation although the exact mechanisms remain unclear (Callam et al., 2016; Wadsworth et al., 2019; Bodenstein et al., 2023; Casas et al., 2024). Given that most central nGoM sites fall within the low to medium salinity ranges (salinity means 8-20 at most grow-out areas) with temperatures that can exceed 33°C during the summertime (Lowe et al., 2017; Swam et al., 2022), this is especially of concern to growers and hatcheries producing triploids in the region. Studies conducted outside of the Gulf of Mexico report significant site variation in diploid and triploid mortality, attributing increased triploid mortality to gametogenesis and increasing temperatures when salinity is similar across sites (Guévélou et al., 2019; Matt et al., 2020). For example, despite similar salinity and temperature across three sites, one study investigating triploid mortality in the Chesapeake Bay found differential ploidy mortality at only one of three sites (Matt et al., 2020). Disease and poor husbandry were ruled out as causes, and the observed triploid mortality was attributed to additional stress from gamete production, or the changes in physiology associated with the process. Combined, the literature indicates elevated triploid mortality during periods of gonad development, and rising temperatures; in the central nGoM, this period often also coincides with increasing freshwater inflow and decreasing salinity.

These findings are of particular concern to triploid farmers in the nGoM where low salinity (salinity < 5) and high temperature (>30°C) conditions are common during the summer months. Exacerbating the issues with triploid responses to low salinities and high temperatures are changes in environmental conditions from global climate change and anthropogenic activities in this region. The occurrences of marine heat waves are projected to increase over the 21st century with further climate change and anthropogenic activity (IPCC, 2023; Luo et al., 2024). Forecasted increases in rainfall volume and frequency of extreme precipitation (Prein et al., 2017) are other effects of climate change that can negatively impact oyster production through their impact on estuarine salinity. Examples of extreme, low salinity, “freshet” events in important oyster growing areas can be seen in coastal Louisiana with the openings of manmade Mississippi River diversions, such as the Bonnet Carré spillway openings in 2019 and 2020 (LDWF, 2024). The drastic changes in environmental conditions following these events resulted in mass oyster die-offs in affected Mississippi waters (Gledhill et al., 2020). These mass oyster die-offs were also observed in Galveston Bay after a drastic decrease in salinity from extreme rainfall following Hurricane Harvey (Du et al., 2021). Although oysters generally can survive across a wide salinity range (Pruett et al., 2021), duration of exposure and timing of exposure to freshwater may reduce growth, reproduction, and survival of different life stages of oysters (La Peyre et al., 2013; Rybovich et al., 2016; Marshall et al., 2021; McFarland et al., 2022). With continued river management, including river diversions, along with an increase in the frequency of large precipitation events, diploid and triploid oyster exposure to extreme environmental conditions remains a concern.

Differences in ploidy responses at the cellular level to environmental (i.e., temperature, salinity, food availability, disease) and physiological stressors (i.e., gametogenesis, cell regulation and balance) may explain observed mortality patterns (Li et al., 2021; Brianik & Allam, 2023; George et al., 2023). Of these stressors, salinity is one of the most critical factors that controls survival, growth, and general success of oyster aquaculture (Brianik & Allam, 2023). Therefore, differences in how triploid organisms cope with changes in salinity through osmoconformation can have major implications on their survival (Brianik & Allam, 2023). Although conflicting information exists on the effect of salinity variation on triploids, one study found that triploid oysters were slower to osmoconform and adjust their internal salinity to match the environment (Casas et al., 2024). Triploids were also found to be less efficient in maintaining acid-base status and cell water content at decreased salinities as compared to diploids (Casas et al., 2024). The differences in physiological responses may stem from differences in cell sizes and lower cell surface-to-volume ratios in triploid organisms (Comai, 2005). Larger cell sizes may slow down biochemical cellular processes across membranes required for osmoregulation (Miettinen & Björklund, 2017). Cellular metabolism, intracellular ion regulation, and ultimately use of energy are negatively affected in oysters during decreasing salinities (Paparo & Dean, 1984; Ballantyne & Berges, 1991; Brianik & Allam, 2023; Bodenstein et al., 2023), highlighting a potential mechanism explaining the differential physiological responses of diploid and triploid oysters to decreasing salinities.

This work examined the synergistic effects of low salinity and high temperature on diploid and triploid mortality. Diploid and triploid oysters were exposed to low salinity and high temperature conditions in a phased laboratory experiment enabling testing of response to temperature, salinity, and temperature by salinity interactive effects. Diploid and triploid oysters were exposed to 3 different temperature treatments (24, 29, and 34°C) with a salinity of 18 to assess the effect of a single stressor (high temperatures) on mortality amongst ploidies. I then exposed diploid and triploid oysters to two different salinities (2 and 18) within the same temperature treatments established in the previous phase to assess the effects of co-stressors (elevated temperature and decreased salinity) on lethal and sub-lethal (biomarkers) responses. Sublethal responses included osmolality, hemolymph pH, protein concentration, hemocyte density, percent granulocyte, and condition index, and were measured to gain an understanding of the underlying physiological responses that may contribute to differences in mortality.

2.2. Methods

2.2.1. Oysters

Half-sibling diploid and triploid oysters were produced in May 2021 by the Auburn University Shellfish Laboratory (AUSL) in Dauphin Island, Alabama. Diploid oysters (2M2LAFT21) used in this study were the progeny of the 2MLAFT19 diploid oyster line and were produced by fertilizing the eggs of 56 females with the sperm of 26 males. Triploid oysters (3MLAFTFL21) were produced by fertilizing the eggs from the same 56 2MLAFT19 diploid females with the sperm of 8 tetraploid males of the 4MAPCK19 tetraploid oyster line. The oysters were grown in mesh baskets suspended on adjustable long lines (ALS, BST Oyster Co., Cowell, South Australia) at the Grand Bay Oyster Park (GBOP), AL (30° 22’ 19” N, 88° 18’ 58” W).

On April 19, 2023, approximately 550 diploid (mean ± standard deviation unless otherwise noted, 58 ± 6 mm shell height, SH) and 550 (59 ± 6 mm SH) of the triploid oysters were collected and brought to the Louisiana State University Agricultural Center Animal and Food Sciences Laboratory (AFL) in Baton Rouge, Louisiana. About forty-five (45) oysters of each ploidy were cleaned and placed on individual trays that were submerged into twelve 400-L tanks (45 diploids and 45 triploids per tray per tank), equipped with bio-filters and filled with aerated artificial seawater (Crystal Sea Marinemix, Marine Enterprises International, Baltimore, Maryland, USA) adjusted to a salinity of 18 and a temperature of 24°C, similar to field conditions at the time of collection. Water temperature was controlled using submersible heaters (Hygger Saltwater Tank Titanium Tube Submersible 500 W).

Oysters were acclimated for 3 weeks while maintaining salinity (18) and temperature (24°C) relatively constant to the target parameters (Table 2.5). Throughout acclimation and all experiments (Phase 1, Phase 2 below), salinity, temperature (°C), and dissolved oxygen (DO, mg L^{-1}) were measured daily with a YSI-Pro30 handheld multimeter (YSI Incorporated, Yellow Springs, OH). Water quality (ammonia, nitrite, and nitrate) in the tanks was checked once a week using test strips (Lifeguard Aquatics 5-2ay Test strips and ammonia test strips, Santa Fe Springs, CA). Oysters were fed daily with 3 mL of Shellfish Diet 1800® (Reed Mariculture Inc, Campbell, CA) per 80 individual oysters. Oysters were checked daily for mortality. The acclimation period concluded on May 21, 2023 and all trays were adjusted to hold 40 oysters each such that each tank held 40 diploid and 40 triploid oysters. Oyster mortality ranged from 0-10% during acclimation.

2.2.2. Experimental Design

Between May and October 2023, oysters were exposed to three different temperatures (24°C, 29°C, 34°C) and two salinity (2, 18) treatments through a phased experiment (Figure 2.1). Phase 1 examined ploidy response to high temperatures at a moderate salinity (18); Phase 2 examined ploidy response to the combined effects of high temperatures and low salinity (2), similar to salinity decreases that occur in the summer in the central nGoM.

2.2.3. Phase 1

After initial acclimation, on May 23, 2023, water temperature was increased at a rate of 2°C every four days to the target temperature of 29°C in four tanks and 34°C in another four tanks. Water temperature was held constant at 24°C in the remaining four tanks. Salinity was maintained at 18 in all tanks (Figure 2.1). Day zero of Phase 1 was defined as the day when the 34°C designated tanks reached their target temperature. Throughout Phase 1, oyster mortality was recorded daily, and dead oysters were removed from each tank with no replacement. Cumulative mortality was then calculated following procedures from Ragone Calvo et al., (2003).

2.2.4. Phase 2

Beginning June 27, 2023, salinity was decreased by 4 every two days to the target salinity of 2 in two out of the four tanks at each temperature. The salinity in the remaining tanks, at each temperature, was maintained at 18. Temperatures were held constant in all tanks as described in Phase 1 (Figure 2.1). Day zero for Phase 2 (temperature x salinity) was set as the day when salinity reached 2 in designated tanks (Date; 7/8/23). Throughout Phase 2, oyster mortality was recorded daily, and dead oysters were removed from each tank with no replacement. Cumulative mortality was calculated as described above for Phase 1. Between July 13, 2023 and July 20, 2023, eight 2N and eight 3N oysters were removed from each tank to measure sublethal oyster responses.

2.2.5. Sublethal Responses

The whole weight and shell height of each of the eight triploid and diploid oysters mentioned in the previous section were measured and recorded. The oysters were notched on their dorsal side using an angle grinder, and hemolymph was withdrawn from the adductor muscle sinus of each oyster through the notch using a 3-ml syringe equipped with a 25-gauge 11/2" (3.8 mm) needle. The sinus fluid was then placed in 1.5-mL Eppendorf tubes. Hemolymph pH was immediately measured with a Thermo Scientific Orion PerpHecT ROSSTM combination pH Micro electrode (Fisher Scientific, Suwanee, Georgia, USA). The Eppendorf tubes were then quickly immersed in an ice slurry to limit hemocyte clumping. Hemocyte density and granulocyte percentage were determined with improved Neubauer hemocytometers (Reichert, Buffalo, NY). Hemolymph samples were centrifuged at 400 x g for 15 minutes at 4°C, and the resulting supernatant, or plasma, was aliquoted into two 1.5-mL Eppendorf tubes. Eppendorf tubes were stored at -20°C to determine osmolality and protein concentration. Plasma protein concentration was measured with Pierce Biotech Micro BCA Protein Assay Kit (Rockford, IL, USA) using bovine serum albumin (BSA) as a standard. Plasma osmolality was measured with a Precision Systems Inc. 5010 OSMETTE III Fully Automatic 10 µL Osmometer (Natick, MA, USA). Oysters were carefully opened, and shell and meat weight were recorded. The meat of each oyster was placed into individual aluminum cups and dried in an oven for 48 hours at 65°C. The oysters were then placed into a muffle furnace at 500°C for 12 hours to determine their ash weight and calculate their ash free dry meat weight. The ash-free dry weight-based condition index of each oyster sampled was determined by dividing its ash-free dry weight by the weight of the whole wet oyster minus its shell wet weight and multiplying by 100 using a modification of the formula of Abbe & Albright, (2003).

2.2.6. Statistical Analyses

All analyses were conducted using R 4.2.1 (R Foundation for Statistical Computing 2022). Phase 1 and Phase 2 data were analyzed separately, and each phase was analyzed by temperature (24, 29, 34ºC). In Phase 1, chi squared tests were used to examine differences in final cumulative mortality between diploid and triploid oysters at each temperature treatment. Probit analysis using the R package 'ecotox' was used to calculate lethal median time (LT_{50}) from mortality data (Wheeler et al., 2006). Lethal median time was defined as the number of days that it took for half the population to die. In Phase 2, an overlap of 95% confidence intervals

were used to compare median lethal times (LT_{50}) between diploid and triploid oysters within each temperature treatment.

Hemolymph pH, plasma osmolality and protein concentration, hemocyte density, % granulocyte, and condition index data were examined for normality and homogeneity of variance. Data that fulfilled ANOVA requirements were analyzed with a two-factor (ploidy, salinity) ANOVA, by temperature treatment. When significant differences were found ($p < 0.05$), Tukey's HSD was used for pairwise multiple comparisons. Data that did not fulfill the ANOVA requirements of homogeneity of variance were analyzed using Kruskal-Wallis nonparametric rank-sum test, and a pairwise t-test with a Bonferroni correction when significant differences were found.

2.3. Results

2.3.1. Phase 1: Ploidy effects at different temperatures

Water Quality

Heaters were used to warm tanks to target temperatures starting on May 23, 2023, and target temperatures in the highest target temperature tanks were reached on June 5, 2023 (Figure 2.1). From that day (Day 0) to the end of Phase 1 on June 26, 2023, over a three-week period, water temperature and salinity remained at the target levels (Table 2.1). Throughout the study, ammonia and nitrite never exceeded 0.5 and nitrate never exceeded 40 in any of the tanks.

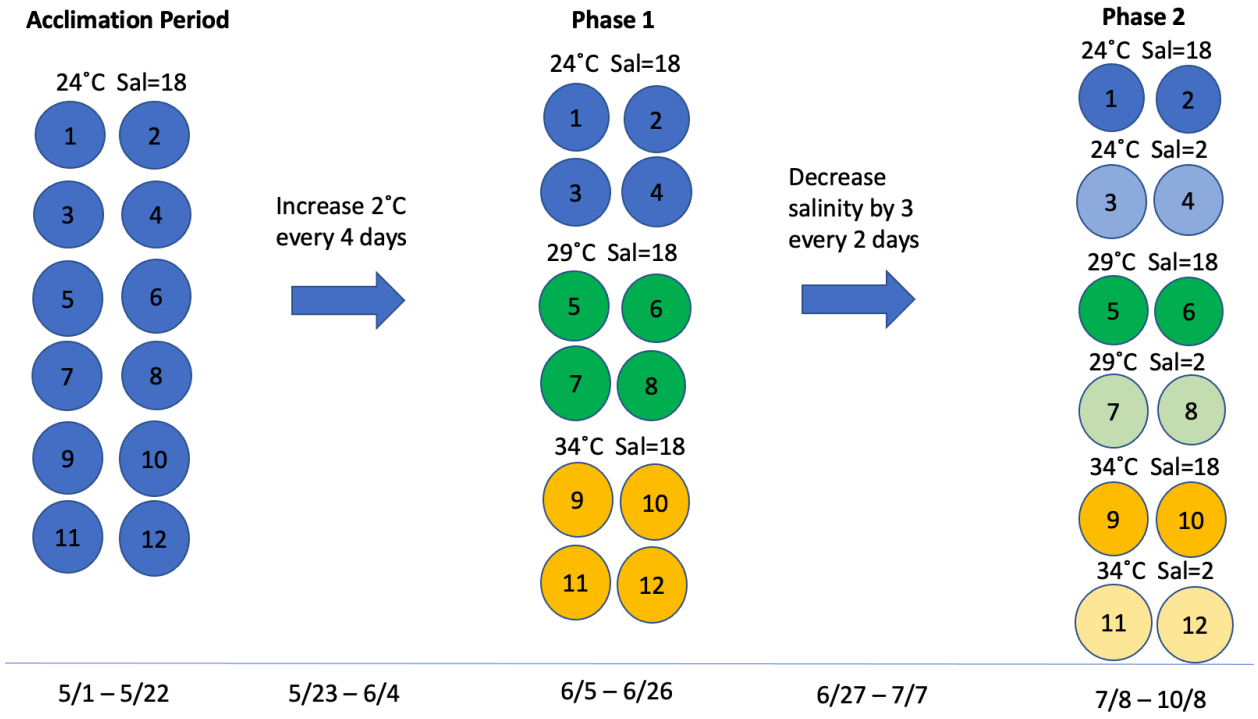


Figure 2.1. Flow chart illustrating timing and conditions for all phases in the laboratory study. Each circle represents one 400-L tank. Color changes denote different treatments at each phase

of the experiment. Start and end dates displayed on the bottom of the figure describe how long each phase of the experiment took. Acclimation Period is defined as the time water conditions were maintained at 24ºC and a salinity of 18 in all tanks to acclimate oysters to the laboratory environment. Phase 1 began after temperature was increased to target levels in certain tanks (24, 29, and 34 ºC), while salinity was maintained at 18 across all tanks. Phase 2 began after salinity was decreased to target levels in certain tanks (2, 18), while temperature was maintained at levels established in Phase 1.

Table 2.1. Mean ± standard deviation of temperature and salinity throughout the experiment. Treatment reflects the target temperature (Temp; °C) and salinity, listed as temperature/salinity. Data presented are the measured temperature (°C; mean ± standard deviation) and salinity (mean ± standard deviation) during experiments. Initial acclimation represents the first 2 weeks after placement of oysters in tanks (n = 12); Phase 1 represents measured temperature and salinity after temperature was adjusted at a rate of 2°C every 4 days until target temperature treatment was reached and salinity was maintained at 18 (n = 4 per treatment); Phase 2 represents measured temperature and salinity after salinity was adjusted at a rate of 3 every 2 days until target salinities were reached and salinity was maintained at 2 and 18 (n = 2 per treatment).

Initial Acclimation		
Treatment	Temperature	Salinity
24°C/18	23.2 ± 0.8	18.1 ± 0.1
29°C/18	23.5 ± 1.0	18.1 ± 0.1
34°C/18	23.5 ± 0.9	18.1 ± 0.1
Phase 1		
Treatment	Temperature	Salinity
24°C/18	24.1 ± 0.7	18.1 ± 0.1
29°C/18	28.5 ± 0.2	18.1 ± 0.1
34°C/18	33.4 ± 0.1	18.2 ± 0.1
Phase 2		
Treatment	Temperature	Salinity
24°C/2	24.4 ± 0.3	2.1 ± 0.1
24°C/18	24.3 ± 0.3	19.8 ± 0.3
29°C/2	28.7 ± 0.1	2.1 ± 0.1
29°C/18	28.6 ± 0.4	19.9 ± 0.8
34°C/2	33.4 ± 0.3	2.1 ± 0.1
34°C/18	33.5 ± 0.2	19.9 ± 0.7

Oyster Mortality

Cumulative mortality in Phase 1 remained below 15% for both ploidies at all temperatures. At all treatment temperatures (24, 29, 34 ºC), triploid oysters had higher cumulative mortalities at the end of Phase 1 as compared to diploid oysters at 24 ºC (D: 6.4 ±

1.3%; T: 9.6 ± 1.8%; P < 0.001), 29ºC (D: 5.2 ± 2.8%; T: 13.3 ± 2.5%; P < 0.001), and at 34ºC (D: 6.4 ± 1.3%; T: 9.6 ± 1.8%; P < 0.001) (Figure 2.2). LT_{50} values tended to be lower in triploids than in diploids at every temperature treatment (Table 2.2).

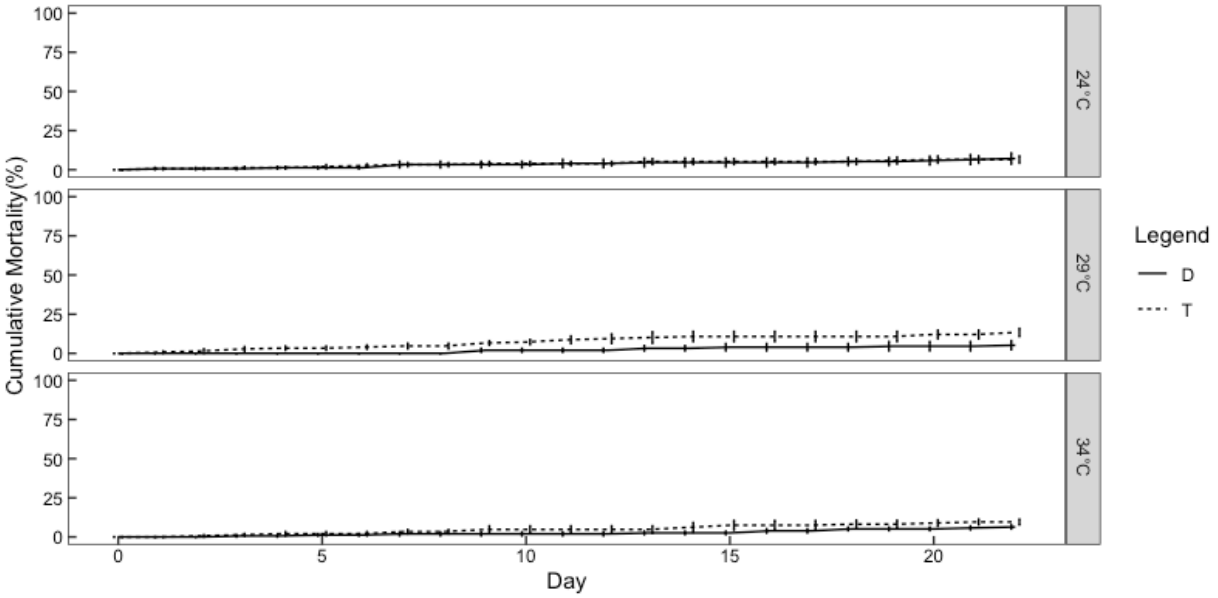


Figure 2.2. Mean ± standard error of cumulative mortality of diploid (D) and triploid (T) oysters in Phase 1 experiment (n = 4). Each line represents a mean of the 4 representative tanks of each target temperature (24, 29, and 34°C). Dashes on lines represent standard error of cumulative mortality for each day. Mortality was recorded daily. Each tank contained all populations of diploids and triploids.

Table 2.2. Median lethal time (LT_{50}; days) from Phase 1 of the experiment with 95% confidence interval in parentheses. Diploid (D) and triploid (T) oysters when exposed to three temperatures (24, 29, and 34°C) while maintained at a salinity of 18.

	Phase 1 LT_{50}					
	24˚C		29˚C		34˚C	
Ploidy	LT_{50}	95% CI	LT_{50}	95% CI	LT_{50}	95% CI
D	83.2	(69.0, 109.0)	76.2	(63.7, 98.7)	66.8	(59.9, 76.7)
T	75.7	(59.3, 74.8)	56.1	(50.1, 64.0)	56.6	(52.1, 62.5)

2.3.2. Phase 2: Ploidy effects at different temperature and salinity combinations

Water Quality

Target salinities for all tanks in Phase 2 were reached on July 8, 2023. From that day (Day 0) to the end of the study on October 9, 2023, water quality remained consistent to target conditions (Table 2.1).

Oyster Mortality (24, 29, 34 °C)

At 24°C, triploids died in about half the time of diploids at the low salinity treatment (Table 2.3). At a salinity of 2, cumulative mortality at the end of Phase 2 was higher in triploids than in diploids (D: 63.1% ± 3.0%; T: 89.3% ± 3.2%) (Figure 2.3). At a salinity of 18, only one of the two replicates showed triploids dying faster than diploids, while cumulative mortality was similar in diploids and in triploids (D: 45.5% ± 3.9%; T: 50.8% ± 10.6%).

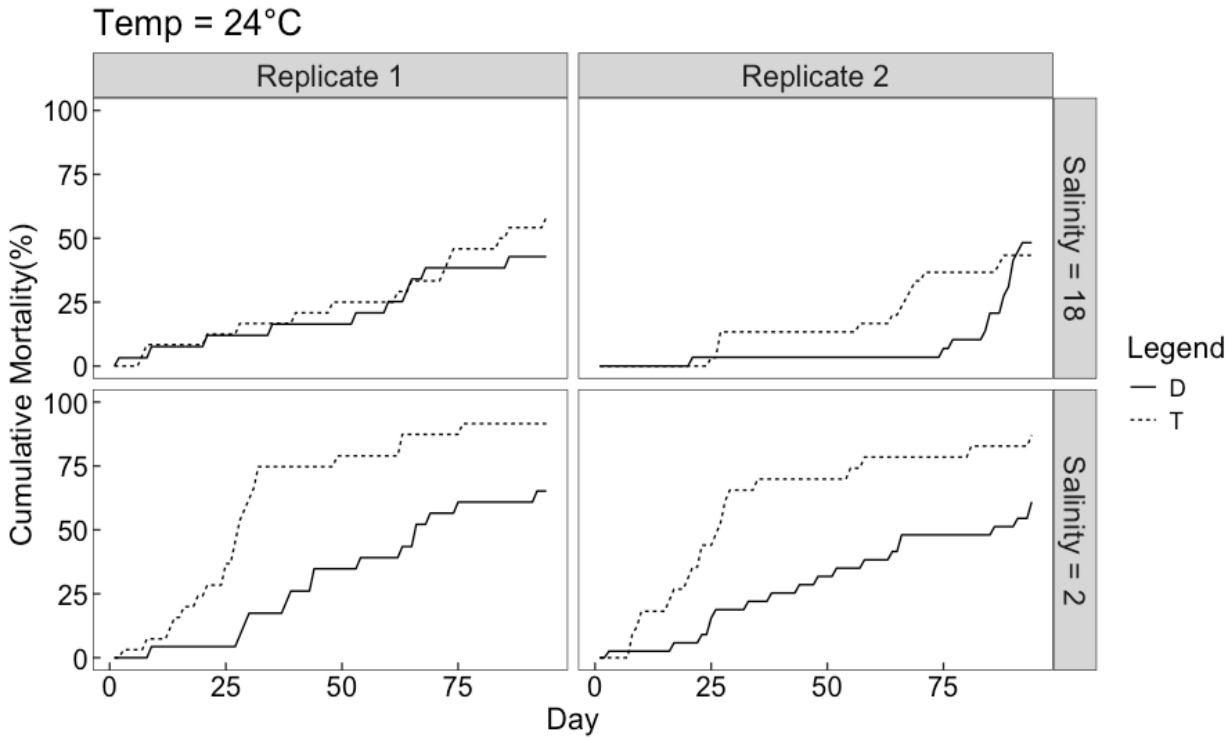


Figure 2.3. Cumulative mortality of diploid (D) and triploid (T) oysters in the Phase 2 experiment exposing diploids and triploid oysters to once target temperature conditions were reached in Phase 2. Each line represents the cumulative mortality of either diploids or triploids in one tank in the study. This figure shows 2 replicates for both salinity treatments (2, 18) at 24°C. Mortality was recorded daily. Each tank contained all populations of diploids and triploids.

Table 2.3. Median lethal time (LT_{50}; days) with 95% confidence intervals (CI) of diploid (D) and triploid (T) oysters when exposed to temperatures of 24, 29, and 34°C, and a salinity (Sal) of 2 or 18 in Phase 2.Replicate 1 (Rep 1) and replicate 2 (Rep 2) provide results for individual tanks for each treatment. n = the total number of days for which oyster mortality was tracked and used to calculate LT_{50}.

24°C												
	Sal = 2						Sal = 18					
	Rep 1			Rep 2			Rep 1			Rep 2		
Ploidy	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n
D	67.6 ± 1.7	(64.5, 71.2)	94	74.9 ± 1.98	(71.3, 79.1)	94	95 ± 4	(87.9, 104.0)	94	124 ± 5.7	(114, 137)	94
T	34.2 ± 1.3	(31.9, 36.8)	94	38.1 ± 1.46	(35.3, 41.1)	94	83.8 ± 3.0	(78.6, 90.4)	94	91.8 ± 2.5	(87.4, 97.2)	94

29°C												
	Sal = 2						Sal = 18					
	Rep 1			Rep 2			Rep 1			Rep 2		
Ploidy	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n
D	39.3 ± 1.1	(37.2, 41.6)	94	39.2 ± 1.2	(37, 41.7)	94	72.7 ± 2.6	(68.1, 78.4)	94	49.3 ± 1.9	(45.3, 53.7)	94
T	23.7 ± 1.6	(20.7, 26.9)	94	19.7 ± 1.4	(15.5, 24.8)	94	41.6 ± 1.5	(38.8, 44.7)	94	47.3 ± 1.3	(44.9, 50.1)	94

34°C												
	Sal = 2						Sal = 18					
	Rep 1			Rep 2			Rep 1			Rep 2		
Ploidy	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n	LT_{50}	CI 95%	n
D	6.9 ± 0.4	(5.2, 11.1)	22	7.7 ± 0.5	(6.9, 8.8)	20	43.3 ± 1.4	(40.7, 46.2)	94	51 ± 1.3	(48.5, 53.8)	94
T	4.7 ± 0.3	(4.2, 5.5)	13	4.3 ± 0.3	(3.9, 5.0)	8	63 ± 1.2	(60.7, 65.5)	94	58.8 ± 1.4	(56.2, 61.7)	94

At 29°C, triploids died in about half the time of diploids at the low salinity treatment (Table 2.3; Figure 2.4). At a salinity of 2, cumulative mortality was similar between diploids and triploids (D: 96.5% ± 5.0%; T: 95.1% ± 0.5%). At a salinity of 18, cumulative mortality was higher in triploids than in diploids (D: 64.0% ± 10.5%; T: 87.9% ± 0.53%), while only one replicate showed triploids dying faster than diploids (Rep 1; Table 2.3).

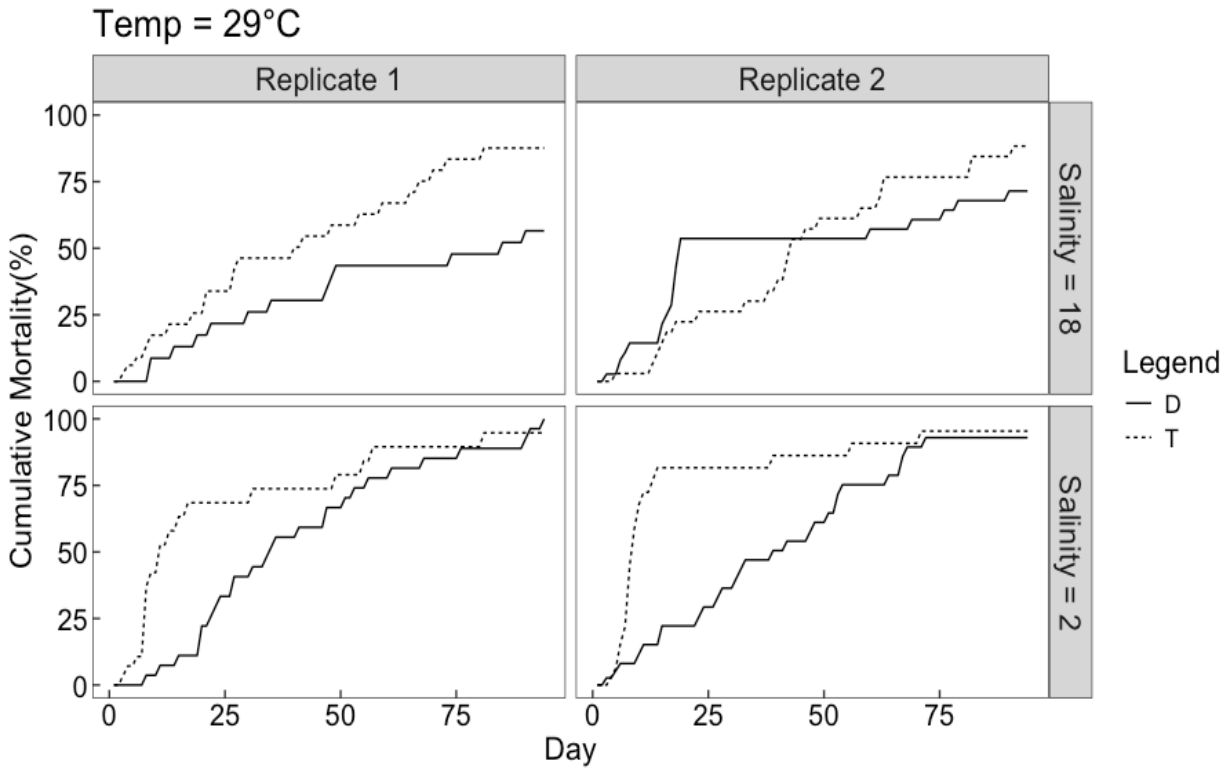


Figure 2.4. Cumulative mortality of diploid (D, solid line) and triploid (T, dotted line) oysters once target temperature conditions were reached in Phase 2. Each line represents the cumulative mortality of either diploids or triploids in one tank in the study. This figure shows 2 replicates for both salinity treatments (2, 18) at 29°C. Mortality was recorded daily. Each tank contained all populations of diploids and triploids.

At 34°C, cumulative mortality of both ploidies exceeded 75% regardless of salinity treatment in all replicates (Figure 2.5). At a salinity of 2, triploids died faster than diploids (Table 2.3). Cumulative mortality of both ploidies in the low salinity treatment reached 100% within three weeks of when conditions were met. At a salinity of 18, cumulative mortality was similar between diploids (D: 85.7% ± 8.3%) and triploids (T: 90.1% ± 2.3%). At the moderate salinity treatment, diploids died faster than triploids in both replicates (Table 2.3).

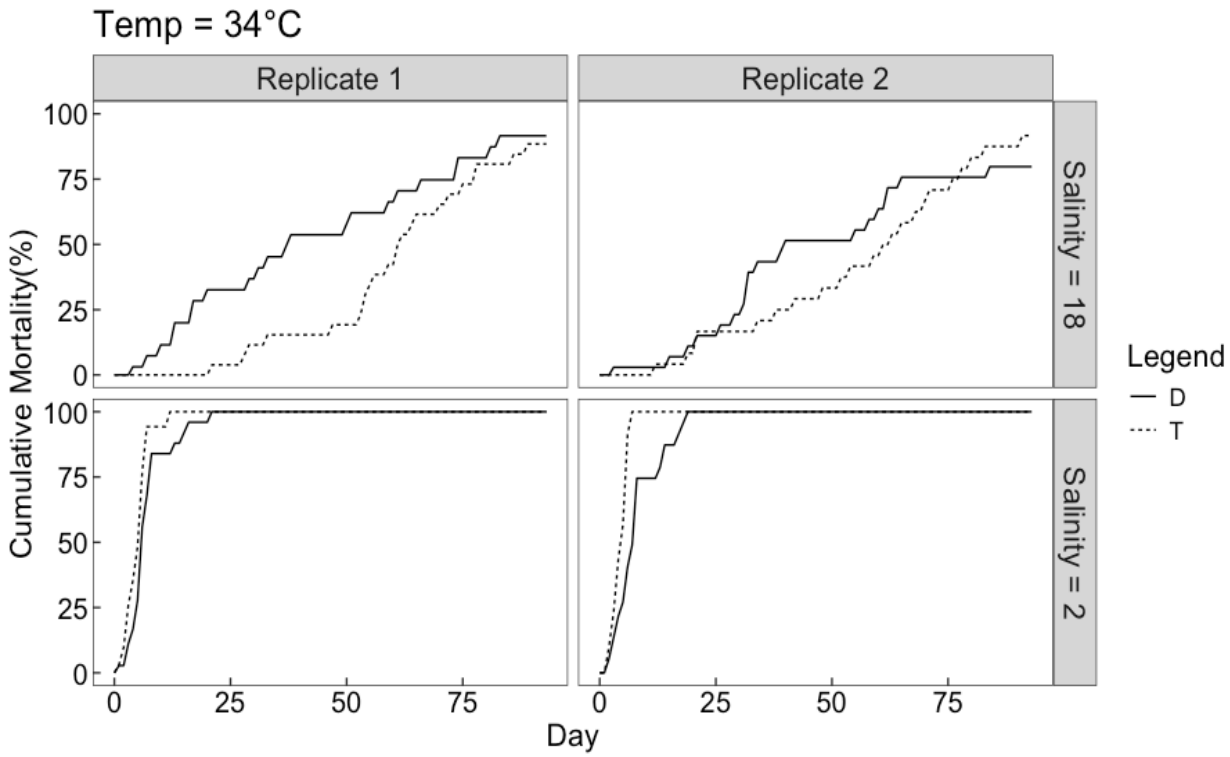


Figure 2.5. Cumulative mortality of diploid (D) and triploid (T) oysters once target temperature conditions were reached in Phase 2. Each line represents the cumulative mortality of either diploids or triploids in one tank in the study. This figure shows two replicates for both salinity treatments (2, 18) at 34°C. Mortality was recorded daily. Each tank contained all populations of diploids and triploids.

Physiological Responses (24, 29, 34°C)

At 24°C, plasma osmolality showed a significant interaction of ploidy and salinity (P = 0.008), with diploids having a lower plasma osmolality than triploids at a salinity of 2 (D: 105.6 ± 53.8, T:175.4 ± 55.3) but not at a salinity of 18 (D: 561.1 ± 10.8; T: 569.3 ± 13.0) (Table 2.4). Hemolymph pH showed a significant difference in ploidy and salinity combination treatments (Kruskal Wallis: P = 0.004; Table 2.5) with triploids having a lower hemolymph pH than diploids at a salinity of 2 (D: 7.4 ± 0.8, T: 6.7 ± 0.6) but not at a salinity of 18 (D: 6.9 ± 0.3, T: 6.8 ± 0.4). Plasma protein concentration differed significantly only by ploidy (P < 0.001), with diploids (D: 4.8 ± 2.4) having lower protein concentration than triploids (T: 8.2 ± 3.7). There were no differences in hemocyte density by ploidy, salinity, or their interaction. Percent granulocyte varied significantly by ploidy (P < 0.001) with diploids (having lower percent granulocytes as compared to triploids (D: 27.5% ± 10.8%; T: 46.9% ± 15.5%). Condition index varied significantly by ploidy (P < 0.001) with diploids (D: 4.4 ± 2.7) having lower condition index as compared to triploids (T: 9.6 ± 1.2).

Table 2.4. Osmolality, hemolymph pH, plasma protein, hemocyte density, granulocyte percentage (%), and condition index of diploid (D) and triploid (T) oysters. Oysters were sampled after approximately 2 weeks after reaching their experimental temperatures (24, 29, and 34°C) and salinity (Sal; 2, 18). Sample size (n) of each assay is provided for all treatment groups. Different letters denote statistical differences ($p<0.05$) using an ANOVA, or Kruskal-Wallis if data did not pass the minimum assumptions of an ANOVA. When no letters are present, no significant difference was detected.

24°C								
	Sal = 2				Sal = 18			
	D	n	T	n	D	n	T	n
Osmolality (mOsm/kg)	105.6 ± 53.8^{C}	14	175.4 ± 55.3^{B}	14	561.1 ± 10.8^{A}	12	569.3 ± 13.0^{A}	13
Hemolymph pH	7.4 ± 0.8^{A}	14	6.7 ± 0.6^{B}	14	6.9 ± 0.3^{AB}	12	6.8 ± 0.4^{A}	13
Protein (mg mL^{-1})	5.3 ± 1.8^{B}	14	9.1 ± 3.7^{A}	14	5.0 ± 2.5^{B}	12	7.9 ± 3.1^{A}	13
Hemocyte density (10^6 cells mL^{-1})	1.4 ± 1.3	14	1.3 ± 0.6	14	1.2 ± 0.3	12	1.8 ± 1.1	13
% Granulocyte	26.2 ± 10.8^{B}	14	47.4 ± 14.0^{A}	14	29.0 ± 11.1^{B}	12	46.5 ± 17.5^{A}	13
Condition Index	4.1 ± 3.3^{B}	14	8.9 ± 0.9^{A}	14	4.7 ± 1.9^{B}	14	10.3 ± 1.1^{A}	14
29°C								
	Sal = 2				Sal = 18			
	D	n	T	n	D	n	T	n
Osmolality (mOsm/kg)	90.2 ± 53.2^{B}	13	128.1 ± 61.5^{B}	14	551.5 ± 11.5^{A}	12	549.8 ± 13.3^{A}	14
Hemolymph pH	7.6 ± 0.6^{A}	13	7.0 ± 0.5^{B}	14	7.4 ± 0.3^{A}	12	6.9 ± 0.3^{B}	14
Protein (mg mL^{-1})	2.5 ± 1.6^{B}	13	6.1 ± 3.6^{A}	14	2.9 ± 1.9^{B}	12	6.3 ± 2.6^{A}	14
Hemocyte density (10^6 cells mL^{-1})	2.7 ± 2.2	13	1.7 ± 0.7	14	1.1 ± 0.7	12	2.7 ± 4.7	14
% Granulocyte	27.7 ± 12.4^{B}	13	45.3 ± 7.8^{A}	14	39.8 ± 15.5^{B}	12	49.3 ± 14.1^{A}	14
Condition Index	2.3 ± 0.6^{B}	14	6.3 ± 1.5^{A}	14	2.7 ± 0.9^{B}	13	6.8 ± 3.1^{A}	13
34°C								
	Sal = 2				Sal = 18			
	D	n	T	n	D	n	T	n
Osmolality (mOsm/kg)	83.6 ± 23.9^{B}	14	91.4 ± 19.9^{B}	14	554.3 ± 3.0^{A}	14	556.9 ± 7.4^{A}	14
Hemolymph pH	7.5 ± 0.6^{A}	14	7.1 ± 0.5^{C}	14	7.6 ± 0.1^{A}	14	7.3 ± 0.1^{B}	14
Protein (mg mL^{-1})	3.1 ± 1.8^{B}	14	5.3 ± 2.0^{A}	14	2.9 ± 1.4^{B}	14	4.7 ± 2.4^{A}	14
Hemocyte density (10^6 cells mL^{-1})	1.1 ± 0.8^{A}	14	0.9 ± 0.5^{B}	14	1.8 ± 0.9^{A}	14	1.0 ± 0.6^{B}	14
% Granulocyte	36.8 ± 7.9^{BC}	14	57.5 ± 12.1^{AB}	14	48.9 ± 19.7^{B}	14	67.9 ± 9.1^{A}	14
Condition Index	2.8 ± 0.9^{C}	14	4.8 ± 1.2^{B}	14	2.1 ± 0.7^{C}	13	5.6 ± 1.3^{A}	13

Table 2.5. Results of ANOVA and Kruskal-Wallis tests for assays during Phase 2 of the experiment including measurement of protein concentration (PC; mg ML^{-1}), hemocyte density (HD; 10^6 cells mL^{-}), condition index (CI), hemolymph pH (pH), osmolality (O; mOsm/kg), and percent granulocyte (PG). Kruskal-Wallis nonparametric rank-sum test was used when ANOVA conditions were not met, and results are indicated in italics. Results of ploidy is the significance of the single effect of ploidy on the assay, results of salinity is the significance of the single effect of salinity on the assay, and finally results of Ploidy:Salinity is the significance of the interactive effects of ploidy and salinity.

24°C						
	PC	HD	CI	pH	O	PG
Ploidy	1.52E-04	0.316	8.50E-13	-	5.55E-05	4.00E-06
Salinity	0.346	0.552	0.0961	-	2.00E-16	0.8
Ploidy:Salinity	0.615	0.191	0.475	*0.0043*	0.0082	0.62

29°C						
	PC	HD	CI	pH	O	PG
Ploidy	9.73E-06	0.717	1.22E-12	1.10E-04	-	2.50E-04
Salinity	0.644	0.7763	0.598	0.232	-	0.02935
Ploidy:Salinity	0.946	0.0723	0.473	0.558	*2.33E-09*	0.25589

34°C						
	PC	HD	CI	pH	O	PG
Ploidy	0.0003	0.0156	1.21E-13	-	-	-
Salinity	0.412	0.08	0.847	-	-	-
Ploidy:Salinity	0.66	0.1116	0.01	*0.02665*	*3.94E-09*	*8.13E-06*

At 29°C, plasma osmolality was significantly impacted by the interaction of ploidy and salinity (Kruskal Wallis: $P < 0.001$), with oysters in a salinity of 18 (D: 551.5 ± 11.5, T: 549.8 ± 13.3) having higher osmolality than oysters in salinity of 2 (D: 90.2 ± 53.2, T: 128.1 ± 61.5) regardless of ploidy (Table 2.4). Hemolymph pH was significantly different ($P < 0.001$) between ploidies at 29°C with diploids (7.5 ± 0.5) having higher pH than triploids (6.9 ± 0.4). Plasma protein concentration varied significantly by ploidy ($P < 0.001$) with diploids (2.7 ± 1.7) having lower protein concentration than triploids (6.2 ± 3.1). There were no differences in hemocyte density by ploidy, salinity, or their interaction. Granulocyte percentage was significantly affected by ploidy (D: 33.5% ± 15.0, T: 47.3% ± 11.4%) and by salinity (Sal=18: 44.9% ± 15.3%, Sal=2: 36.8% ± 13.4%), but not by the interaction of the two. Condition index showed a significant ploidy effect with diploids (D: 2.5 ± 0.8) having lower condition index as compared to triploids (T: 6.6 ± 2.4).

At 34°C, plasma osmolality showed a significant difference in ploidy and salinity combination treatments (Kruskal Wallis: $P < 0.001$) with oysters at a salinity of 2 (2N: 83.6 ± 23.9, 3N: 91.4 ± 19.9) having a lower osmolality than oysters at a salinity of 18 (2N: 554.3 ± 3.0, 3N: 556.9 ± 7.4). Hemolymph pH showed a significant difference in ploidy and salinity combination treatments (Kruskal Wallis, $P = 0.27$), with diploids in both salinities being nearly identical (Sal=2: 7.5 ± 0.6, Sal=18: 7.6 ± 0.1), but triploids having a higher value in a salinity of 18 (3N: 7.3 ± 0.1) than in a salinity of 2 (3N: 7.1 ± 0.5). Plasma protein concentration was significantly different by ploidy ($P = 0.003$), with triploids (3N: 5.0 ± 2.2) having higher plasma protein concentration than diploids (2N: 2.9 ± 1.6). Hemocyte density varied significantly by ploidy ($P=0.016$), with diploids (2N: 145.3 ± 96.8) having higher hemocyte density than triploids (3N: 96.8 ± 53.5). Granulocyte percentage showed a significant difference in ploidy and salinity combination treatments (Kruskal Wallis: $P < 0.001$), as triploids at a salinity of 18 had the highest percent granulocytes (67.9% ± 9.1%), followed by triploids at a salinity of 2 (57.5 ± 12.1), then diploids at a salinity of 18 (48.9% ± 19.7%), and finally diploids at a salinity of 2 (36.8 ± 7.9) (Table 2.4). Condition index showed a significant ploidy by salinity interaction ($P=0.01$), with triploids at a salinity of 18 having the highest condition index (5.6 ± 1.3), followed by triploids at a salinity of 2 (4.8 ± 1.2), and finally the diploids at a salinity of 2 (2.8 ± 0.9) and 18 (2.1 ± 0.7).

2.4. Discussion

Triploid oysters experienced more rapid mortality compared to diploids when exposed to low salinity (salinity = 2) regardless of temperature. Across all temperatures, the LT_{50} (number of days) for triploids was 30-50% lower compared to diploids when held at low salinity (2). Differences in LT_{50} between diploids and triploids decreased as temperature increased. The higher rate of triploid mortality in low salinity may reflect a reduced ability of triploids to osmoconform to low salinity (Casas et al., 2024). Interestingly, at moderate salinity (18) and high temperature (34°C), diploids had a lower LT_{50} as compared to triploids, suggesting potential advantages of triploids with rising temperatures.

At low salinity, triploids died faster than diploids at all temperature treatments, although both ploidies were increasingly affected with rises in temperature. Specifically, triploid LT_{50} values were half that of diploid LT_{50} values across all temperature treatments (24, 29 34°C) when

at a salinity of 2 (Table 2.3). Triploid oysters exposed to 24°C and a salinity of 2 had significantly higher cumulative mortality compared to diploids under the same treatment, but cumulative mortalities of both ploidies remained below 100%. At 29 and 34°C, cumulative mortalities of both ploidies reached approximately 100%, showing how the effects of low salinity on oysters are amplified when combined with high (>30°C) temperatures. Similar to the findings in this study, combined stressors of extended low salinity (<5) with high temperatures (>25C) have been shown to negatively impact oyster survival in previous studies (La Peyre et al., 2013; Rybovich et al., 2016). Here, the rate of mortality was higher for both ploidies in a salinity of 2 as compared to a salinity of 18, and increased with the rises in temperature from 24, to 29, to 34°C. Despite high mortality of both ploidies in low salinity and high temperature conditions, triploids reached 100% cumulative mortality about twice as quickly as diploids. Field studies demonstrate similar patterns with high interval mortalities associated with extended periods of low salinity (<5) and high temperatures (>30°C) (Wadsworth et al., 2019; Bodenstein et al., 2023); in both cases, triploid mortality exceeded that of diploid mortality.

To cope with stressful environmental conditions such as low salinity and high temperatures, oysters temporarily close their valves to isolate their internal conditions from the environment (Casas et al., 2024). Although valve closure can be effective under short durations of environmental stress, extended valve closure can lead to a variety of physiological issues, including acidosis. By preventing the release of metabolic byproducts (i.e. CO_2, lactic acid) into the environment, extended valve closure often results in decreases of hemolymph pH (Casas et al., 2024). Triploids have been shown to take longer to reopen their valves after exposure to extreme environmental conditions (Casas et al., 2024), which could explain the lower hemolymph pH of triploids in our study. The physiological mechanisms that affect pH such as length of valve closure, rate of metabolism, and mobilization of carbonate from the shell to act as a buffer were largely unexplored in our experiment. However, in all temperature treatments, we found that hemolymph pH was significantly lower in triploids than in diploids (Table 2.3). Acidic hemolymph pH negatively affects internal biochemical processes such as acid-base regulation, shell maintenance in oysters, and immunoregulation in oysters (Dwyer, III & Burnett, 1996; Lombardi et al., 2013). In addition to potential starvation from extended valve closure, the negative physiological effects of lower hemolymph pH could have acted as another co-stressor that contributed to the elevated triploid mortality in our study. However, extended valve closure and corresponding low hemolymph pH did not always correlate with higher mortality in our study. For example, in the 34°C treatment at a salinity of 18, diploids died faster than triploids but had a higher hemolymph pH. There are other physiological factors that could have led to this observation, such as differences in internal salinity regulation.

The higher susceptibility of triploids to low salinity conditions may be attributed to their reduced ability to osmoconform (Sokolova et al., 2012; Jones et al., 2019). Exposure to low salinity has been shown to result in metabolically costly active transport of inorganic ions, which can impact bioenergetic pathways and lead to energetic tradeoffs and shifts in lethal tolerances (Paparo & Dean, 1984; Sokolova et al., 2012; Lombardi et al., 2013; Jones et al., 2019; Casas et al., 2024). In oysters, the reduced ability to osmoconform in low salinity environments has been shown to correlate with higher mortality in the field (La Peyre et al., 2009; Marshall et al., 2021). In this study, diploid and triploid oysters osmoconformed similarly at a salinity of 18 at all temperature treatments. In a salinity of 2 however, diploids tended to osmoconform better than

triploids in all temperature treatments, particularly when low salinity was the only stressor at 24°C. A study observing osmoconformation of diploids and triploids in decreasing salinities showed that triploids consistently had slower osmoconformation rates within the low salinity range tested (5-1.5) (Casas et al., 2024). Mortality has been shown to be high when oysters were unable to osmoconform and match the surrounding low salinity environment, while mortality was low when oysters successfully osmoconformed (La Peyre et al., 2013). Slower osmoconformation rates and high mortality rates particularly in triploids may be consequences of extended valve closure, which can result in hypoxia, acidosis, or starvation that can lead to mortality (Lombardi et al., 2013; La Peyre et al., 2013). Differential osmoconformation rates and success between ploidies may be rooted in differences at the cellular level between diploids and triploids (Casas et al., 2024).

At reduced salinities, triploids have been shown to take longer to open their valves, osmoconform, and regulate hemolymph pH and tissue water content (Casas et al., 2024). Taking longer to conduct these physiological processes may be attributed to the larger cell size of triploids compared to diploids, which is known as triploid cell gigantism (Comai, 2005). Triploids have more cell volume than diploids, as having more genomic material usually causes increases in cell volume in triploid organisms (Child & Watkins, 1994; Comai, 2005). Having a larger cell volume corresponds to increased intracellular distance for molecule transport, which slows down cellular processes across membranes (Miettinen & Björklund, 2017). In decreasing salinities, cellular metabolism along with intracellular ion and acid base regulation are negatively affected in oysters (Paparo & Dean, 1984; Ballantyne & Berges, 1991). Therefore, triploid cells with disproportionate increases in cell volume are expected to be correspondingly disproportionately affected in their ability to metabolize and conduct ion and acid base regulation at lower salinities, which could explain why triploids had a higher mortality than diploids at a salinity of 2 (Guo & Allen, 1994). Other endogenous stressors impacting mortality may include the energetic investment into gonadal development (Casas et al., 2024).

Under extreme high temperature (34^{o}C) at moderate salinity, diploid mortality rates increased and died faster than triploids. During the study, spawning occurred in the oysters held in the high temperature (29, 34°C) tanks, which may account for the faster diploid mortality as diploids were likely the ones that spawned. In the 34°C moderate salinity treatment, diploids may have used more energy for spawning than triploids, depleting ATP sooner and causing mortalities to occur faster (Huvet et al., 2010; Sokolava et al., 2012). The increased energetic demand of spawning in diploids could also explain their lower condition index and protein concentration values (Table 2.4). Condition index has been shown to closely follow the reproductive patterns of oysters, reaching the highest values during the pre-spawn in March and dropping to the lowest values in the summer during the peak spawning months (Chávez-Villalba et al., 2008; Manley & Walker, 2011). This suggests that the low condition index values observed in diploids could be derived from an elevated energy apportionment into spawning, which has been shown to correspond with elevated mortality in previous studies (Matt et al., 2020). However, this connection does not explain why triploids died faster in other treatments where spawning was also observed. Although triploids are widely considered partially sterile, they have been shown to produce gametes and spawn, albiet to a lesser degree than diploids (Allen & Downing, 1986, Houssin et al., 2019).

Contrasting evidence exists as to whether a relationship exists between gametogenesis and mortality in triploids (Samain et al., 2007; Guévélou et al., 2019; Wadsworth et al., 2019), however these studies used gonad assessment methods designed for diploid oysters (Matt et al., 2020). Since triploid gonad development is different from diploids, the precision required to detect and quantify the relationship between mortality and gametogenesis may not have been reached (Matt et al., 2020). In this study, the level to which gametogenesis contributed to triploid mortality is unknown as gametic progression was not tracked. However, gamete production in both ploidies requires significant energy investment, and correspondingly increases metabolism, which can lead to oxidative stress and eventually cell death (Lesser, 2006; Hulbert et al., 2007). Like other stressors, oxidative stress is aggravated when combined with another stressor such elevated temperatures. Differential susceptibility to stress from reproduction in addition to environmental stressors may play a role in the observed differential mortality between ploidies in our study. Further investigation into the bioenergetics of triploid gametogenesis compared to diploids, especially when coupled with additional stressors, is required to confirm this theory.

In this study, triploids died faster than diploids in low salinities, especially when combined with another stressor such as high temperatures. In contrast, at moderate salinity, diploids experienced more rapid mortality when exposed to higher temperatures (34°C), which could partially be attributed to the larger cell size of triploids. Triploids with a larger cell size may have more energy reserves to cope with the single stressor of high temperature. However, under low salinity, larger triploid cells may be disproportionately negatively affected due to lower surface to volume ratios. Theoretically, larger triploid cells have more volume to regulate with less cell membrane surface area to conduct the regulatory processes on, which could contribute to the observed elevated triploid mortality under low salinity conditions. Further investigation into the differential physiological effects that larger cell sizes have on triploid oysters under extreme temperature and salinity conditions is required to confirm this hypothesis.

In conclusion, investigating diploid and triploid biological responses under low salinity and high temperature conditions is important as these conditions are projected to occur more frequently in the nGoM. Climate change along with the projected increases in environmental variation that impact key water quality parameters such as temperature and salinity are expected to exacerbate the effects that these environmental factors have on diploid and triploid oysters. Future studies investigating the role of elevated triploid cell sizes along with other physiological differences between ploidies such as differences in gametogenesis will be helpful in confirming the observed differences in mortality under these environmental conditions. In addition to physiological differences, exploring different grow-out practices such as optimal tumbling frequency and stocking density to reduce additional stress on oysters under extreme environmental conditions can be important to maintaining consistent production in the region in face of climate change. Understanding how environmental factors, including projected future conditions and extreme heat and precipitation events, may impact diploid and triploid oyster mortality and physiology provides a first step to developing approaches to minimize mortality and maximize production. After these relationships are understood, we can then use this information to quantify the effects of extreme environmental factors on the profitability of growing diploids versus triploids. This biologically based approach to profit prediction would be helpful to site farm operations, identify ploidy seed selection, and inform selective breeding programs that would aim to minimize risk to oyster producers.

CHAPTER 3. BIOECONOMIC MODELLING OF DIPLOID AND TRIPLOID EASTERN OYSTERS ACROSS A RANGE OF CURRENT AND FUTURE WATER TEMPERATURES AND SALINITIES

3.1. Introduction

Historically, the northern Gulf of Mexico (nGoM) contributed more than 50% of the U.S. production of Eastern oysters (*Crassostrea virginica*) (NMFS 2023). Over the last two decades, this production has been increasingly supported by a nascent off-bottom aquaculture industry (Posadas, 2022). Off-bottom oyster farming, or culture of oysters in mesh containers suspended above the seafloor, gives farmers more flexibility in operations, enabling them to maximize profit and reduce risk associated with oyster culture. In addition to tumbling from natural wave action that promotes a more marketable shell shape, off-bottom oyster culture allows farmers to use artificially selected seed produced in hatcheries. Hatchery breeding programs aim to increase production on off-bottom oyster farms by selecting for traits such as disease resistance, environmental tolerance, growth rates, ploidy, and broodstock parentage (Walton et al., 2013). In the nGoM, current selective breeding programs focus on development of oysters with increased growth, disease and environmental stressor tolerance including tolerance to low (<10) salinity events (Leonhardt et al., 2017; Grice, 2018).

The high primary productivity and temperate climate found in the nGoM supports some of the fastest growth rates in the country with oysters reaching market size within a year (Davis 2017; Lowe et al. 2017; Lavaud et al. 2023). However, oysters grown in this region also face an elevated risk of mortality from increasing environmental variation due to climate change and river diversions, often resulting in large freshwater inputs into estuarine areas. These low salinity events often coincide with periods of high water temperatures, and result in mass oyster mortalities (e.g., Gledhill et al., 2020; Du et al., 2020). Reducing risk of high mortality and ensuring production on oyster farms in the nGoM requires consideration of the effects that local environmental factors, particularly temperature and salinity, have on biological factors such as oyster growth and mortality. Using bioeconomic modeling, biological information integrated into economic models can inform farm operation decisions and maximize profit. Here, bioeconomic modelling was used to assess the performance and profitability of diploid and triploid oysters in varying environmental conditions common to the nGoM.

One contributing factor to the success of off-bottom oyster aquaculture is the development and use of triploid oysters through selective breeding programs in oyster hatcheries. Triploid oysters have three sets of chromosomes instead of the typical two sets of chromosomes found in wild diploids (Allen & Downing, 1986b). Compared to diploid oysters, triploids are considered advantageous in aquaculture due to faster growth rates and superior meat quality during the spawning season (Yang et al., 2018). These advantages result from reduced gamete development of triploid oysters, as triploids are hypothesized to use the energy, otherwise used for gametogenesis in diploids, for somatic growth (Allen & Downing, 1986; Hawkins et al., 2000; Honkoop, 2003; Fraser et al., 2021). These advantages result in extensive farming of triploid oysters, which accounted for 85% of the commercial seed (juvenile oysters sold to farms for grow-out) orders from the Auburn University Shellfish Laboratory (AUSL) in 2017 (Wadsworth et al., 2019). Despite the popularity and advantages of triploids, previous studies

show high variation in grow-out success. This variation is hypothesized to result from higher triploid vulnerability to husbandry techniques, disease, and environmental stressors (Matt et al., 2020; Bodenstein et al., 2020, 2023; Casas et al., 2023). Of particular concern in the nGoM is the observation that triploid oysters have higher mortality compared to diploids during the reproductive period when exposed to low salinity (<5) and high temperature (>33°C) (Wadsworth et al., 2019; Bodenstein et al., 2023). The potentially large effects of this differential mortality on aquaculture production and ultimately farm profit emphasize the need to understand differential ploidy response to these combined high temperature and low salinity stressors. Quantifying the relationships between these environmental, biological, and economic factors in oyster farming can help identify potential risks associated with ploidy selection, or to help inform ploidy selection for grow-out at different locations.

Salinity and temperature are two of the most important environmental factors affecting oyster growth and mortality (Shumway, 1996). Oysters survive in locations where temperature ranges from -2° to 36°C, and salinity ranges from 5 to 40, with optimal salinities from 14 to 28 (Galtsoff, 1964; Shumway, 1996). In the nGoM, oysters routinely experience high temperatures (>30°C) during the summer months, and certain areas are at risk of extended periods of low salinities (<5) from large river inputs and extreme precipitation events that often tend to coincide with warmer temperatures (La Peyre et al., 2013; Rybovich et al., 2016; Gledhill et al., 2020). In addition, interannual variation of wet, dry, and normal years also drive changes in salinity that affect oyster reefs in the region (Lowe et al., 2017; Gledhill et al., 2020; Du et al., 2020; Swam et al., 2022). These issues are exacerbated by the effects of climate change, which has increased surface sea temperature in the Gulf of Mexico at a rate of around 0.2 °C per decade from 1970 to 2020, and is expected to continue to increase in the future (Wang et al., 2023).

Climate change is also increasing the frequency of marine heat waves, heavy precipitation, and river and pluvial flooding (IPCC 2023). These more extreme conditions increase the challenges to the oyster industry by contributing to higher oyster mortality, labor time, and repair of gear and equipment (Dame et al., 2019). The resulting changes in environmental conditions and particularly the forecasted decreases in salinity has been hypothesized to differentially impact the growth and survival of triploid and diploid oysters (Callam et al., 2016; Matt et al., 2020; Wadsworth et al., 2019; Bodenstein et al., 2023). Prior studies investigating triploid response to environmental stressors in the nGoM indicate that triploids have higher mortality compared to diploids during periods of low salinity (<5) and high temperatures (>33°C; Wadsworth et al., 2019; Bodenstein et al., 2023). Predicting the effects of varying salinities and temperatures on diploid and triploid oyster growth rates and mortality is therefore important to enable better decision making with the goal of minimizing farm profit susceptibility to environmental stressors.

Numerous models have been developed that enable predictions of oyster performance outcomes (i.e., growth, mortality) under different environmental conditions (Dekshenieks et al., 2000; Wang et al., 2008; La Peyre et al., 2021). One numerical model, the Dynamic Energy Budget (DEB) provides a mechanistic approach that incorporates physiological and environmental data to predict an individual organisms' growth and mortality (Lavaud et al., 2017; Augustine & Kooijman, 2019). A DEB model has been validated for diploid oysters across the nGoM with temperature, salinity, and food availability as key variables that control energy

apportionment to metabolic processes including growth, feeding, respiration, and reproductive investment (Lavaud et al., 2017, 2021). In this model, water temperature affects all physiological rates while salinity impacts feeding and survival rates (Lavaud et al., 2017); food is assumed to not be limiting in this region. This model has been used to develop an aquaculture index for diploid oysters in six nGoM estuaries that calculated time to harvest (number of days; based on growth rates) and mortality under current and future conditions (Lavaud et al. 2023). An expansion of this DEB model that assesses biological performance can be used to project mortality and growth rates for triploid oysters. Together, the DEB models for diploids and triploids can be used to examine trade-offs resulting from ploidy selection when exposed to different environmental scenarios, and ultimately help predict farm profit.

Mortality and growth rates have direct implications on the economics of an oyster farm. Production volume, or how many units a farm produces in a set amount of time, is the most important factor that impacts profit, so changes in production will be mirrored in change of profit (Abduhalli et al., 2017; Sharp & Kaan, 1999). Understanding how revenue, how much money a farm earns in a set amount of time, is affected by changes in production volume and costs is critical for decision-making regarding budget, product selection, and sale price (Lulaj & Iseni, 2018; Jonick, 2018). Enterprise budgets are a common method used to project costs and revenue for the purpose of estimating profit by categorizing all estimated income and expenses (Callam, 2018; Sahs 2022). Using enterprise budgets can help business owners see differences in their costs and profit based on production volume changes. For oyster farming, several enterprise budgets have been created, but these rely on average growth and mortality rates for off-bottom oyster farming in the nGoM (Callam, 2018; Hensey et al., 2020; Petrolia & Caffey, 2024). These previous budgets were useful as guidelines for expected profit based on husbandry techniques, gear choice, production volume, and ploidy. However, the use of fixed mortality and growth values fails to capture the known effects that environmental conditions can have on the production of diploid and triploid oysters. The exclusion of this consideration limits the accuracy of profit prediction, and fails to provide site or condition-specific predictions. These models also fail to capture the potential risk to profit due to interannual variation of environmental factors.

Understanding the effects of environmental conditions on biological factors (i.e. growth, mortality) and how these components influence the economics of an oyster farming operation is critical for maximizing production and reducing risk (Pomeroy et al., 2008; Llorente & Luna, 2016). Bioeconomic modeling provides a means to integrate economics and biology and quantify the economic implications (i.e. change in profit) from changes in biological parameters. By wholistically considering all the factors that affect profit, such as changes in production and environmental variables, bioeconomic modelling can increase the accuracy of profit prediction for an aquaculture operation (Llorente & Luna, 2013). Bioeconomic modelling is a methodological approach that can help farmers analyze the complex interactions among these factors and aid in decision making for operation management and production system design (Pomeroy et al., 2008). The use of bioeconomic modeling in aquaculture operations can help farmers plan, monitor, quantify risk, and determine cost-effectiveness of aquaculture operations (Pomeroy et al., 2008; Llorente & Luna, 2016). Previous uses of bioeconomic models for shellfish aquaculture have merged environmental and economic considerations to optimize culture practice such as timing of seed deployment and harvest, and site selection (Ferreira et al., 2007; Silva et al., 2011; Kamiyama et al., 2021). However, these studies were conducted outside

of North America, focused on the Pacific oyster (*Crassostrea gigas*), and did not consider the effects of ploidy in their modelling.

The goal of this chapter was to develop a bioeconomic model that analyzes the effect of ploidy and environmental conditions on profit by using biological results from a DEB model to inform an enterprise budget. By testing the developed bioeconomic model with a range of environmental scenarios, this study provides proof-of-concept for bioeconomic modelling that could aid in ploidy selection for production while considering environmental conditions at the farm site. Farmers are not recommended to forecast their own profit margins based on the outputs of this analysis, but rather to see how using different ploidies grown in sites with different environmental conditions can result in a difference in annual profit.

3.2. Methods

Here, I used the DEB model to predict oyster performance (time to harvest, mortality) across a range of current and projected future environmental conditions in nGoM waters. The existing diploid DEB model from Lavaud et al., (2017; 2023) was used to generate diploid performance data. I then modified the existing DEB model to generate triploid performance data (time to harvest, mortality) based on available laboratory and field data from triploids (Chap. 2 of this thesis; Eastburn et al,. 2021; Bodenstein et al., 2023). To capture the effects that a range of environmental scenarios would have on diploid and triploid oysters, six environmental scenarios were developed and tested in a DEB model (Figure 3.1). The performance outputs (time to harvest, mortality) for diploids and triploids were then used as inputs in a modified enterprise budget, which were used to identify potential profits based on ploidy and environmental scenarios.

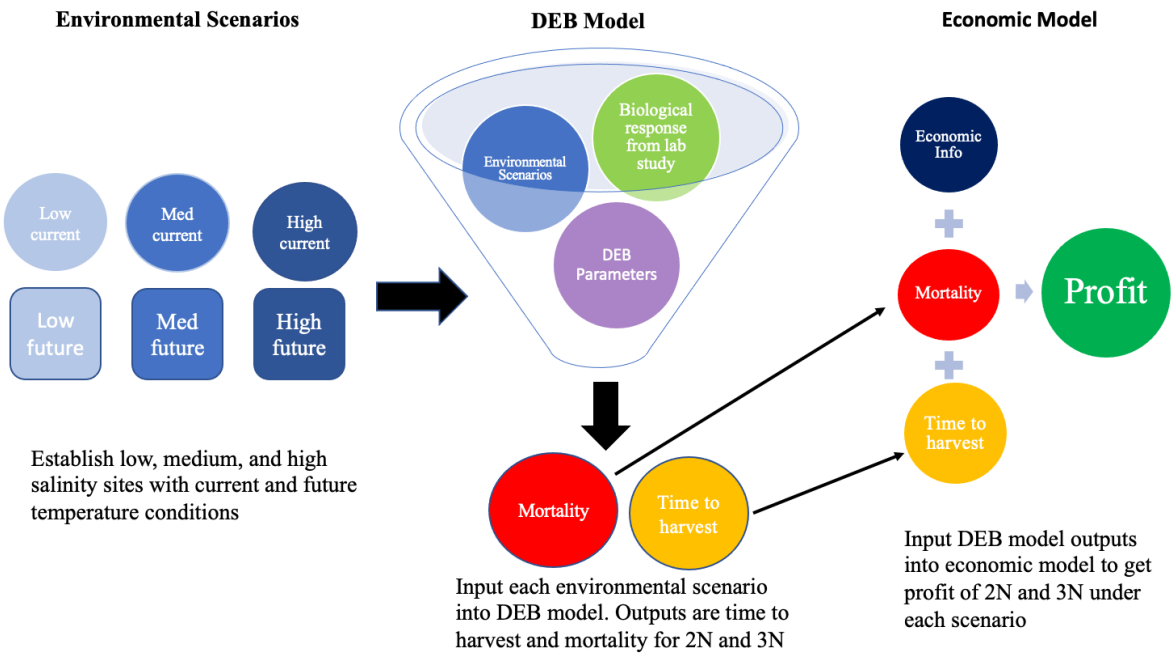


Figure 3.1. Overviewing schematic of the bioeconomic analysis. Six environmental scenarios were developed and used in a biological DEB model to quantify mortality and time to harvest for diploid and triploid oysters under each environmental scenario. Time to harvest and mortality results for diploid and triploid oysters specific to each environmental scenario were then used in an economic model to estimate profit.

Environmental Scenarios

Of the six total environmental scenarios, three represented existing water quality conditions in the nGoM, while the other three scenarios represented potential future conditions under global warming (Table 3.1). Daily salinity and temperature regimes for a calendar year (January 1 - December 31) were identified from interpolated environmental data from 2014-2020 for coastal Louisiana (Swam et al., 2022; Lavaud et al., 2024). We selected datasets with salinity regimes that were representative of what existing oyster reefs can experience in the nGoM (Lowe et al., 2017; Swam et al., 2022). The selected salinity regimes were low salinity (mean $\pm$ standard error; 6.8 $\pm$ 0.3), moderate salinity (11.3 $\pm$ 0.2), and high salinity (22.9 $\pm$ 0.1) (Figure 3.2).

The corresponding temperature regime at the same location and year of the selected salinity regime was used, together representing the three current environmental scenarios (low, medium, and high salinity; Figure 3.3). Because the salinity regimes were selected from different calendar years and locations, the corresponding temperature regime also varied between scenarios. To create the future environmental scenarios, we incorporated the potential effects of increased temperatures from climate change in 100 years by increasing the water temperature by 2°C for each the current environmental scenarios , matching average projections (Wang et al. 2023). Daily salinity regimes remained the same between current and future scenarios, although we acknowledge that changes in the amount, timing, and variation of precipitation are likely to impact future salinity scenarios. Including the three current environmental scenarios, the addition of the future environmental scenarios resulted in a total of six different environmental scenarios.

Table 3.1 Yearly mean, standard deviation (SD), and range for each environmental scenario. Salinity is unitless, while temperature is represented in °C. Environmental scenarios represented the combination of the selected salinity regime and the current vs. future temperature regimes. Salinity regime represented the selected daily salinity regimes for a calendar year in coastal Louisiana, and are classified as low, medium, and high salinity regimes. Time period represents the interpolated temperature regimes and are classified as current or future conditions.

Environmental Scenarios		Salinity			Temperature		
Salinity Regime	Time Period	Mean	SD	Range	Mean	SD	Range
Low	Current	6.8	4.9	0.1 - 22.8	22.9	7.2	4.5 - 31.5
Low	Future	6.8	4.9	0.1 - 22.8	24.9	7.2	6.5 - 33.5
Medium	Current	11.3	3.5	5.9 - 20.5	23.1	6.7	9.2 - 32.5
Medium	Future	11.3	3.5	5.9 - 20.5	25.1	6.7	11.2 - 34.5
High	Current	22.9	2.8	16.6 - 32.2	23.8	5.3	8.3 - 31.7
High	Future	22.9	2.8	16.6 - 32.3	25.8	5.3	10.3 - 33.7

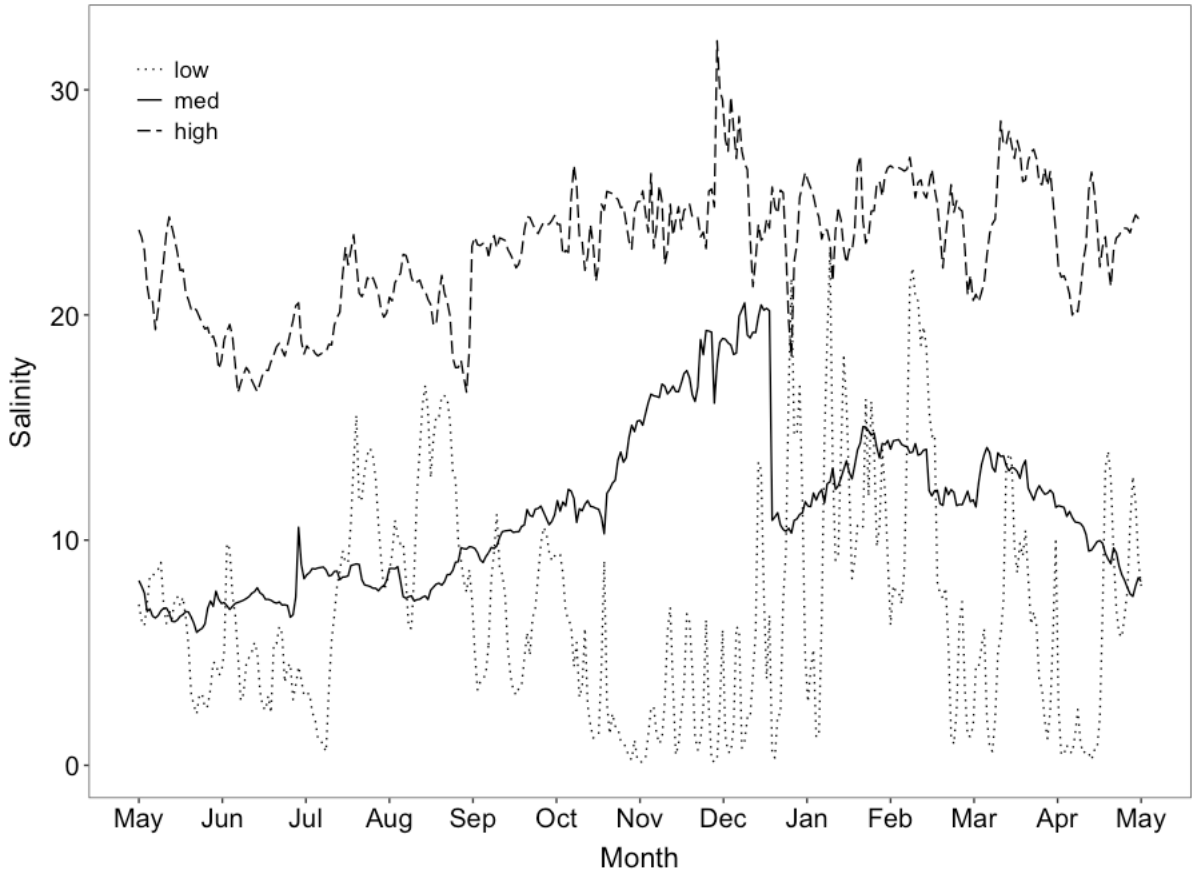


Figure 3.2. Interpolated daily salinity regimes selected from Lavaud et al., (2024) to represent the current low, medium, and high salinity environmental scenarios. Start date was May 15 to represent the date of initial planting, and the model simulated a one-year (365 days) timespan.

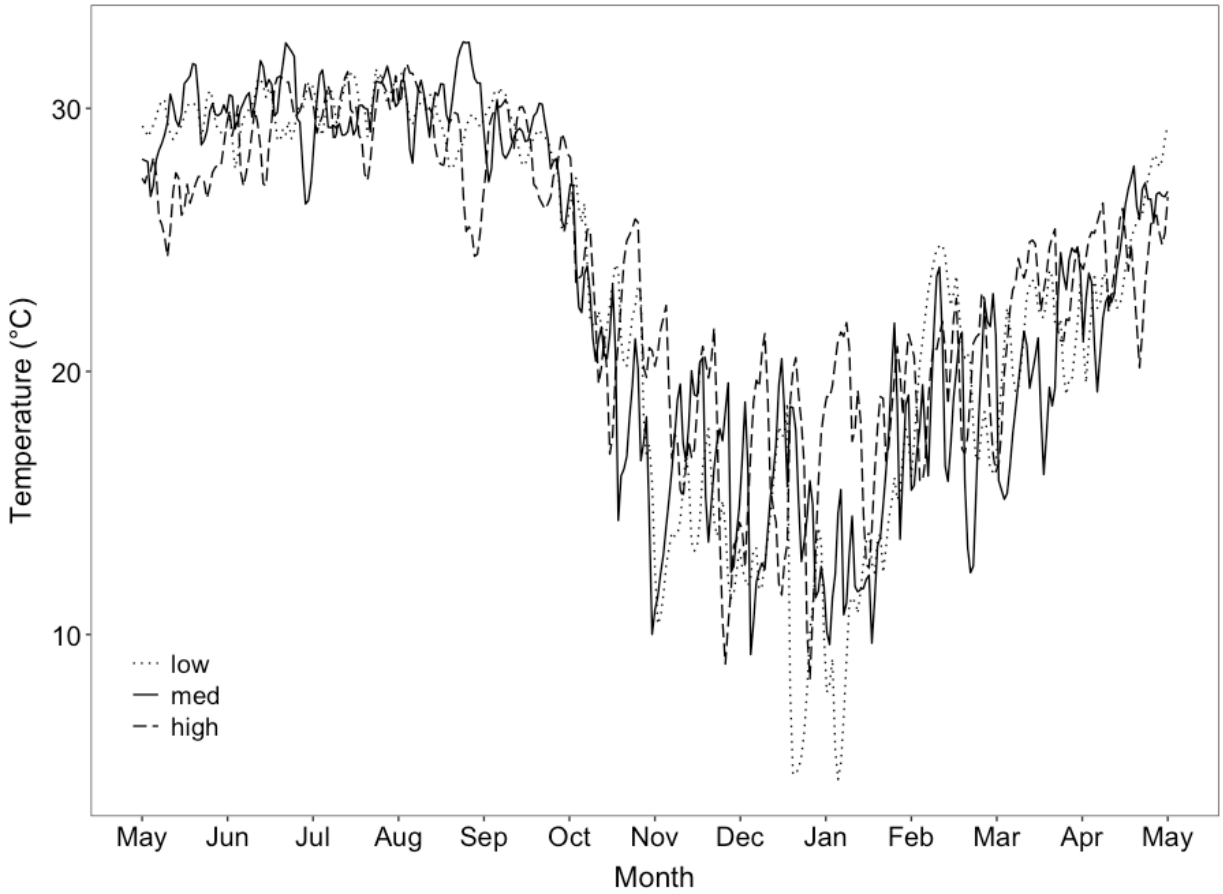


Figure 3.3. Daily temperatures regimes selected from Lavaud et al., (2024) to represent the low, medium, and high salinity environmental scenarios. The high variation in temperature observed between December and January were likely due to differences in the depth of the site. Start date was May 15 to represent the date of initial planting, and the model simulated a one-year (365 days) timepsan. Future conditions increased each temperature regime by 2°C daily to reflect future climate change predictions.

Dynamic Energy Budget Model

Using the published DEB model developed for nGoM diploid oysters (Lavaud et al. 2017, 2024), mortality and time to market were simulated in Matlab (Version 2022b) for diploid and triploid oysters under the six environmental scenarios. Differences between diploid and triploid metabolism were implemented in the DEB model through a modification of energy allocation rules. In triploids, the fraction of the available energy allocated to reproduction ($1 - \kappa$; Lavaud et al., 2017, Figure 3.1) was divided by half, resulting in more energy available for growth.

The DEB model was run as an individual-based model. Each model run outputted growth and survival for 21 individual oysters which was assumed to represent a population. The growth rate was computed in the DEB model as the change in shell length through time, based on the influence of environmental conditions (temperature, salinity) on the dynamics of energy allocation fluxes (see Lavaud et al., 2017 for further details). Percent survival (using mortality),

and percent harvestable (using time to harvest) were calculated from averaging model outputs from the 21 individuals after one year (365 days) of simulation. Oyster shell height was used to determine time to market, which was the number of days for the oyster to reach a shell height greater than 75 mm. Percent harvestable was then calculated as the percent of the oyster population of harvestable size within 365 days. All simulations started on May 15th with 6-mm oysters and ran for one year, which replicates a realistic start date and seed size for planting in off-bottom culture in the nGoM (Lavaud et al. 2024). The DEB model requires daily temperature, food availability and salinity inputs to perform simulations. As chlorophyll-a concentrations, representing available phytoplankton food for oysters, are not limiting along the Louisiana coast (D'Sa, 2014; Turner et al., 2019; Lavaud et al., 2024), the functional response to food availability in the model (varying between 0 and 1; 0 = no food, 1 = unlimited food), was set to 1 (Lavaud et al., 2024).

Mortality was incorporated into the DEB model both mechanistically (as a function of state variables defining the DEB model, i.e. individual volume), and empirically (knowledge from field and laboratory experiments) (Lavaud et al., 2024). Both mechanistic and empirical data were used to modify the validated diploid model to model triploids. In terms of mechanistic data, two state variables, aging acceleration (q, dimensionless) and hazard rate (h, d^{-1}), were added to the existing model following general practice grounded in DEB theory (Kooijman, 2010); see Chap. 6.1 in Kooijman (2010) and Martin et al. (2012) for details and equations. Briefly, the aging acceleration implements the accumulation of damaging compounds from general metabolism, and the hazard rate translates this accumulation of damaging compounds into survival probabilities. The faster the metabolism, the more damaging compounds accumulate. In terms of empirical data, data from Chapter 2 of this thesis, Eastburn et al., (2021), and Bodenstein et al., (2023) were used to calibrate the background mortality rate (1×10^{-6} d^{-1}) (Supplementary Data A.2; A.3; A.4). The background mortality rate adds to the hazard rate, which lowers survival.

Enterprise Budget

Percent harvestable and percent survival data from each environmental scenario were used in an adapted version of a published enterprise budget by Petrolia & Caffey, (2024) to estimate revenue, costs, and profit of farms using diploids and triploids. The profit equation used was:

$$\text{Profit} = (SP \times PV) - (VC \times X) - TFC$$

Profit was defined as the money generated from the business after subtracting costs, and in this analysis was equal to the revenue minus variable and total fixed costs. Revenue was defined as the money generated from the business without subtracting costs, and in this analysis was equal to the sale price per unit multiplied by the production volume (Sahs, 2022). Sale price per unit (SP) was defined as the money an oyster farmer will receive for selling one oyster. Production volume (PV) was defined as the quantity of oysters produced and ready for sale in the market (Sahs, 2022). In this analysis PV was equal to the number of seed multiplied by percent harvestable and percent survival. Variable costs (VC) was defined as costs that vary based on the level of production (Sahs, 2022), and in this analysis only included the cost of purchasing seed.

Number of seed (X; 1,000's of seed) was the number oyster seed purchased for grow-out at the beginning of the analysis. Total variable cost (TVC) was calculated by multiplying the variable costs (VC; cost per 1,000 seed) by the number of seed purchased (X). Finally, total fixed costs (TFC) was defined as the sum of costs that do not change based on the level of production (Sahs, 2022). In this analysis TFC was equal to the sum of all costs except for cost of seed. Costs included in TFC were the money spent to buy gear, expenses, and overhead. To investigate the effects of ploidy and environmental conditions on profits, enterprise budgets were created to compare diploids and triploid farm profit within each environmental scenario. Comparisons of profit were also made across current scenarios, and across current and future scenarios.

Economic assumptions were set to maintain consistency among treatments in the enterprise budget. I used partial accounting when adapting the enterprise budget from Petrolia & Caffey, (2024) into this analysis. I included the out-of-pocket costs only and excluded managerial labor, money spent on boat, motor, truck, and trailer, and the depreciation and interest associated with this gear (Petrolia & Caffey, 2024). Partial accounting was more representative of an existing grower that already owns a boat, truck, trailer, and motor. An enterprise budget with partial accounting was appropriate for existing farmers deciding to grow diploids or triploids based on an existing farm site, as opposed to new farmers without a farm site already designated.

The bioeconomic model simulated one year of operation to show proof of concept for incorporating outputs from a biological model into an economic analysis to create a bioeconomic model. Given that the analysis only covered a one-year timespan, depreciation of gear was not considered. I also did not include loans or associated annual interest. Including these factors would have affected final profit between ploidies and taken emphasis away from the main purpose of this analysis which was to show differences in profit resulting from ploidy selection and environmental conditions.

I assumed a farm size of two acres with an expected planting of 480,000 oysters, which was the smallest farm size that was the closest to “breaking even” (profit of $0) in Petrolia & Caffey, (2024). The average farm size across Gulf and Atlantic coasts ranges from 2-10 acres (Petrolia & Caffey, 2024). Twelve lines (for hanging bags of oysters) were assumed to fit in a farm size of 2-acres, and 200 bags could fit on each line (Grice et al., 2023). The estimated holding capacity of 2,400 bags was between 320,000 and 640,000 oysters, therefore, 480,000 oysters was selected as a midpoint value in the analysis.

Costs were split between variable costs and fixed costs. Variable costs, defined in this analysis as the money spent to purchase R6-sized (retained on a 6-mm screen during grading) seed, varied based on ploidy. Diploid seed was cheaper than triploid seed, which likely was due to the licensing fee charged to the hatcheries for the use of improved stocks to produce triploid seed. In 2023, the Auburn University Shellfish Laboratory (AUSL) sold R6 diploid seed at a rate of US $24.00 for 1,000 seed, while R6 triploid seed sold at a rate of US $28.00 for 1,000 seed (S. Rikard pers. comm. 2024, AUSL Hatchery Manager). Total fixed costs for a two-acre farm with 12 lines were sourced from Petrolia & Caffey, (2024), which included cost of gear and overhead (Table 3.2). Cost of gear included all required gear such to operate a farm size of two-acres with an expected planting of 480,000 oysters such as hanging bags, anchor lines, and buoys. Overhead

costs included the costs for labor, gas for the boat and truck, and lease rent, permits, licenses, insurance, and marketing and sales. Traditionally, overhead costs like labor and gas would be assigned as variable costs because they vary based on year-to-year production volume and intensity. However, this analysis has fixed assumptions of production and time, therefore, these overhead costs were assigned to fixed costs because they do not change within this enterprise budget.

Table 3.2 Fixed costs for 2-acre farm using partial accounting from Petrolia & Caffey, (2024). All costs were represented in dollars ($). One line represented the total cost for each item to create one oyster-growing line on an oyster farm, while two acres represented the total cost for each item to create 12 lines that fits on a two-acre farm.

Fixed Costs		
Item	One line	Two Acres
6-mm Bags	$683.00	$8,196.00
12-mm Bags	$5,465.00	$65,580.00
Bag Closures	$19.00	$228.00
Screw Anchors	$97.00	$1,164.00
Floating Buoys	$99.00	$1,188.00
Anchor Lines, 300'	$68.00	$816.00
Truck Refrigeration Unit	$4,471.00	$4,471.00
Power Washer	$373.00	$373.00
Harvest Baskets	$87.00	$1,044.00
HACCP Training	NA	$390.00
Contingency	NA	$625.00
Fuel	NA	$21,163.00
Lease Rent, Permits, and Licenses	NA	$2,632.00
Insurance	NA	$2,000.00
Labor	NA	$64,000.00
Marketing and Sales	NA	$2,500.00
Total Fixed Costs		$176,370.00

Labor hours were based on estimates from Hudson et al., (2013), which was the same source used to estimate labor hours in Petrolia & Caffey, (2024). In this analysis, I assumed 5,120 total hours of labor to grow 480,000 oysters, which was the total labor hours estimated for a range of 400,000-700,000 oysters in Hudson et al., (2013). Partial accounting dictated that managerial labor is excluded from the analysis, so all labor costs were assumed to be general labor at the rate of $12.50 per hour (Petrolia & Caffey, 2024). For our one-year analysis, the $12.50 per hour general labor rate multiplied by the expected 5,120 total hours of labor equaled $64,000 spent on labor. I excluded employer-paid taxes and costs associated with added labor for direct sales from this analysis to emphasize the any differences in profit on the effects of using different ploidies. Realistically, labor would be split amongst multiple workers and concentrated during the growing season portion of the year, but because this analysis only covered one year, labor estimated from Hudson et al., (2013) was represented as a fixed cost.

Boat time was defined as the number of hours spent running the boat for one year of operation on a two-acre farm. Boat running hours was assumed to be 10% of labor hours (Petrolia & Caffey, 2024). For a two-acre farm, I assumed 5,120 total labor hours, and therefore the boat running hours were estimated to be 512 hours. To calculate the miles per gallon to use the outboard motor, the motor's horsepower was divided by ten (Johnson 2011). I assumed a 50 hp motor to match the assumptions in Petrolia & Caffey, (2024), which resulted in five gallons per hour. Gallons per hour (five) was then multiplied by the boat running hours (512) to calculate total number of gallons spent on boat fuel for the year, which resulted in 2,586 gallons of fuel for the boat. The sum of boat fuel and truck fuel was used to calculate total fuel.

Truck time was defined as the number of hours spent running the truck for one year of operation on a two-acre farm. Truck time will vary based on the distance between the farmers' home, farm, and delivery location. To show an example of truck time for an off-bottom farmer in Louisiana, I assumed the same home, farm, and delivery location as Petrolia & Caffey, (2024). The farm was located in Grand Isle, LA, the home was located in of Thibodaux, LA (192 miles round trip to Grand Isle), and the delivery was located in New Orleans, LA (214 round trip). I assumed the farmer would drive round trip from Thibodaux to Grand Isle five times a week and deliver to New Orleans from Grand Isle once a week. With a 15-mpg truck, I calculated the farmer would use 3,328 gallons of gas each year travelling between Grand Isle and Thibodeaux, and 741 gallons travelling from Grand Isle to New Orleans. Combined with the estimated 2,586 gallons to run the boat for one year, I estimated a total of 6,655 gallons used between the truck and the boat for the year. The average price per gallon of all grades of gasoline for the Gulf of Mexico was $3.18 in 2023 (EIA 2024). The price per gallon ($3.18) was multiplied by the total gallons used between the truck and boat (6,655 gallons) to calculate the money spent on fuel for one year of operation, which resulted in a total of $21,162.90 spent on boat and truck fuel.

Lease rent, permits, licenses, insurance, and marketing and sales costs were taken directly from Petrolia & Caffey, (2024). I assumed the farmer would be leasing property in a Louisiana off-bottom farming park, which costs $2,000 per year for a two-acre farm site. Required licenses included an Oyster Harvester License, Commercial Fisherman License, Wholesale/Retail Seafood Dealer License, and a Seafood Transport License. The sum of these licenses for one year of operation was $632. General liability insurance was based on the rate from Petrolia & Caffey, (2024), who used a rate of $1,000 per acre. For the two-acre farm size, general liability insurance was estimated to be $2,000 for the year. The fixed cost of $2,500 for marketing, promotion, and website maintenance used in Petrolia & Caffey, (2024) was also applied in this analysis.

Data Analysis

Using the DEB model outputs (Supplementary Data A.5; A.6; A.7), we calculated one value each for percent survival and percent harvestable value for diploids and triploids in each environmental scenario. Using the calculated percent survival and percent harvestable values, I then estimated profit for each ploidy in each environmental scenario through the modified enterprise budget. These output values were compared between each of the ploidy and environmental scenarios using a calculation of percent change, where:

$$Percent\ (\%)\ change = \frac{(X_{2N} - X_{3N})}{X_{3N}} \times 100$$

X represents the percent harvestable, percent survival, or profit value of diploids (X_{2N}) and triploids (X_{3N}). Using this formula, we calculate the percent change when comparing from diploids to triploids.

3.3. Results

Diploid and triploid biological outcomes and effects on profit were compared across and within environmental scenarios. Results include a comparison of percent survival, percent harvestable, and profit between each of the ploidies, environmental scenarios, and time periods using a calculation of percent change.

DEB Outputs

Regardless of ploidy and time period, percent survival was lowest under the low salinity scenarios and highest under the high salinity scenarios (Table 3.3). Percent survival under low salinity was below 45% across all ploidies and time periods, with diploid survival slightly more than double that of triploids under the current (percent change; 126%) and future time period (percent change; 140%). In the medium salinity scenario, percent survival was slightly higher (percent change; 6%) for diploids than for triploids under the current time period, but both ploidies were predicted to have 100% survival under the future time period. In the high salinity scenario, percent survival was predicted at 100% for both ploidies.

Table 3.3. DEB (Percent survival and percent harvestable) and enterprise budget (Profit) outcomes for diploids and triploids in current and future environmental scenarios. Average time to harvest (TTH; days) was calculated by taking the average of oysters' time to harvest (TTH, number of days to reach market size, 75- mm shell height) that were less than 365 days. Results are shown for the low salinity (mean ± SE; 6.8 ± 0.3); medium salinity (11.3 ± 0.2), and high salinity (22.9 ± 0.1) environmental scenarios under current and future time periods. Future time period temperature regimes were paired with the same salinity regimes as the current time period, but temperatures were increased by 2°C for each day. Negative profits are displayed in italics.

Salinity	Ploidy	Survival (%)	Harvestable (%)	TTH	Profit ($)
Current Environmental Scenarios					
Low	2N	43	29	349	*-158,502*
	3N	19	19	333	*-181,102*
Medium	2N	95	90	343	18,913
	3N	90	90	330	6,653
High	2N	100	100	319	52,110
	3N	100	100	297	50,190
Future Environmental Scenarios					
Low	2N	24	0	>365	*-187,890*
	3N	10	5	348	*-188,721*
Medium	2N	100	81	352	6,396
	3N	100	95	340	38,762
High	2N	100	100	296	52,110
	3N	100	100	280	50,190

Similar to percent survival, percent harvestable remained low (below 30%) under the low salinity scenarios, and above 80% for both ploidies for the medium and high salinity scenarios (Figure 3.5; Table 3.3). Under the current low salinity scenario, diploids had higher percent harvestable than triploids (percent change; 53%), but both ploidies had low (≤ 5%) percent harvestable values under the future time period. Under the current medium salinity scenario, diploid and triploids had similar (90%) percent harvestable values, however under the future time period, diploid percent harvestable decreased (80%), while triploid percent harvestable increased (95%). Under the high salinity scenarios, both ploidies were predicted to be 100% harvestable within a year regardless of time period. Notably, although there were no differences in percent harvestable for diploids and triploids when comparing current to future time periods in high salinity, the average TTH (time to harvest) for both ploidies decreased by approximately 2-3 weeks.

Enterprise Budget

Regardless of ploidy, farms were profitable (positive profit) under both time periods in the medium and high salinity environmental scenarios but were unprofitable (negative profit) under both time periods in the low salinity environmental scenarios (Table 3.3). In medium

salinity, diploids were more profitable than triploids in the current time period (percent change; 184%). However, when comparing within ploidies from current to future time periods in medium salinity, diploid profit decreased by 185% while triploid profit increased over 500%. Therefore, triploids were more profitable under the future medium salinity environmental scenario compared to diploids. In all high salinity environmental scenarios, profit was greater than $50,000, although profit was slightly higher (4%; percent change) in diploids than in triploids under both time periods.

3.4. Discussion

Profitability of an oyster farm is driven by environmental, biological, and economic factors. Bioeconomic modelling facilitates the integration of environmental and biological outcomes with economic costs, providing a tool to visualize how changes in one component can lead to corresponding changes in another. In this study, I developed and tested a novel bioeconomic model to quantify the biological and economic performance of diploid and triploid Eastern oysters across a range of current and future projected environmental conditions. The results indicated that farms were unprofitable in all low salinity scenarios regardless of time period, despite the low salinity scenarios matching environmental conditions of current oyster reef distributions in Louisiana. Under medium salinity scenarios, maximum profit changed from current to future environmental conditions based on ploidy; triploid performance increased while diploid performance decreased with the elevated temperature. Oysters of both ploidies performed the best under the high salinity scenarios, resulting in the highest profits estimated in the analysis. The development and use of a bioeconomic model to estimate profit based on predicted oyster performance in range of environmental conditions could be applied to inform the selection of seed (i.e., ploidy, genetically selected lines), sites, and grow-out gear to maximize production.

While oysters can survive in low salinity environments across Louisiana (Lowe et al., 2017), these areas are unlikely to support profitable farm operations regardless of ploidy. The use of annual and monthly salinity means is common in identifying suitable areas for oyster production and restoration (i.e., Swam et al., 2022). However, this method fails to account for temporary extremes in environmental conditions which can negatively affect oyster populations (La Peyre et al., 2013). Therefore, the inclusion of duration and exposure to extreme low salinity events (<5) can be critical in accurately forecasting mortality and growth in areas with high variation, such as the central nGoM (La Peyre et al., 2013; Lowe et al., 2017; Lavaud et al., 2024). Although the mean salinity of the low salinity scenarios (6.8 ± 4.9 SD) in this study reflected areas of current oyster reefs in the central nGoM (Lowe et al., 2017; Swam et al., 2022), the daily salinity regime indicated that over 40% of days were below a salinity of 5. Increases of the number of days with a salinity of less than 5 are associated with increased oyster mortality and reduced growth rates (i.e., Lowe et al., 2017; Gledhill et al., 2020; Marshall et al., 2021; Bodenstein et al., 2023), including the study conducted in Chapter 2 of this thesis.

Changes in salinity trigger responses in oyster physiology, such as the upregulation of stress response genes and the closure of valves to isolate internal tissues (Lowe et al., 2017; Casas et al., 2024). Extended valve closure reduces food intake and the aerobic scope available for somatic growth. These responses could account for the low percent harvestable and survival values by limiting the amount of available energy in the organism (Sokolava et al., 2012;

Lombardi et al., 2013; Rybovich et al., 2016; Lowe et al., 2017; Casas et al., 2024). Modelling these effects of daily salinity and temperature regimes on oyster performance variables is important to accurately assessing sites for oyster aquaculture due to the differences in biological responses to environmental extremes such as low salinity. In addition to low salinity variation, oysters are more susceptible to low salinity during periods of high temperatures (Rybovich et al. 2016; Marshall et al. 2021). The interaction between these co-stressors could explain the decreased survival of oysters predicted under future scenario conditions in a low salinity site (Table 3.3).

The effects of biological responses and ploidy on profits are highlighted under medium salinity scenarios. Under medium salinity, diploids were more profitable (increase of 184%) than triploids in the current time period, while triploids were more profitable (increase of 506%) than diploids in the future time period. This large difference in profit under current conditions occurred despite a seemingly minimal (5%) difference in percent survival between ploidies (PS; 2N: 95%; 3N; 90%), and similar percent harvestable values, highlighting the significance of scale on an oyster farm operation. This difference in survival translated to 20,680 more oysters harvested by using diploids compared to triploids when starting with 480,000 oyster seed. Economically, using diploid oysters was projected to increase profit by a total of $12,260 compared to triploid oysters; $10,340 from sales and $1,920 saved from buying less expensive diploid seed. At a larger farm scale, the cascading effects of differences in percent survival and harvestable are even more pronounced. For example, a 5% increase of percent survival for a larger operation growing 1,000,000 oyster seed would yield 50,000 more oysters for sale (assuming 100% harvestable), which would translate to a profit increase of $25,000 for this larger farm. At this farm scale, the profit increase of $25,000 could completely cover the costs of buying 1,000,000 diploid seed for the following year. This bioeconomic model helps highlight how seemingly minimal differences in percent survival and harvestable can significantly affect profit differences, particularly “at scale” for the larger farms (>500,000 oysters) commonly seen in off-bottom farming (Petrolia & Caffey, 2024).

Farms were most profitable in the high salinity scenarios, with diploids being slightly more profitable than triploids (Table 3.3). At high salinity, percent survival and percent harvestable values were the same regardless of ploidy or time period. This suggested that farmers were more profitable growing diploids compared to triploids due to the cheaper diploid seed price. However, the differences in profits were slim (4%), and may be negligible when factoring in the advantages of growing triploids in a realistic farm simulation. One advantage of triploids is their higher growth rates in high salinity sites (Callam et al., 2016). This advantage was also observed in our study as evidenced by the faster average time to harvest of triploid oysters in both the current and future conditions (Current 3N: 12 days faster than 2N; Future; 3N: 16 days faster than 2N). Although these differences in average time to harvest are insignificant in a one-year analysis, having a slightly earlier time to harvest could help growers sell to the market before competitors, or avoid stress and disease pressure associated with warming water temperature.

This analysis did not consider disease pressure from the protistan parasite *Perkinsus marinus* (Dermo) or predation pressure from oyster drills or crabs, which are likely to increase in higher salinity sites (>15) (La Peyre et al., 2016; Casas et al., 2017). Previous studies have

indicated that triploids are advantageous in the presence of predation or disease due to their faster growth rates and corresponding reduced exposure time to disease and predation (Yang et al., 2018). In the high salinity scenario, the benefits of increased survivability and yield in face of disease pressure of triploid oysters may offset the slight profit loss compared to diploids observed in our analysis. Based on available data, the inclusion of the effects of disease in the DEB model could disproportionately increase the mortality of diploids. This addition could help refine the bioeconomic model in a scenario of high salinity and temperatures. A final advantage of using triploids that could offset the observed profit difference in our analysis is the superior meat quality during the summer, which could allow for a summer harvest during a time when diploids are unmarketable due to spawning (Guo et al., 2009; Walton et al., 2012; Yang et al., 2018).

This novel example of bioeconomic modelling for diploid and triploid oyster aquaculture in the Gulf of Mexico integrated variable biological and economic considerations to predict farm profit. Previous economic models and analyses in the Gulf of Mexico aimed to assess differences in profitability from farm decisions including ploidy, farm size and grow-out scale, and grow-out method and gear (Callam et al., 2018; Hensey et al., 2020; Petrolia & Caffey, 2024). While effective in analyzing changes in profit based on a suite of farm decisions, the prior economic models assumed set survival and harvest rates independent of constraints such as environmental data. Petrolia & Caffey, (2024) for example, provided an overview of off-bottom farming economics in Louisiana at several production scales common to the nGoM, providing a decision-making tool for farmers to better understand how scale can affect profitability on their farms.

Petrolia & Caffey, (2024) accounted for different levels of crop loss to model how changes in this variable could affect the profit. However, crop loss values in Petrolia & Caffey, (2024) were fixed in nature to represent different severities of losses in expected harvest and were not tied to environmental conditions. The bioeconomic model developed here provides a unique method to incorporate the known effects that environmental conditions (salinity and temperature) have on the biological performance of oysters and predict the corresponding changes in profitability. By allowing the biological performance to vary based on site specific environmental conditions, our bioeconomic approach ultimately increases the spatial and temporal applicability and accuracy of the enterprise budget developed in Petrolia & Caffey, (2024).

Bioeconomic modeling provides a powerful tool to inform oyster aquaculture decision-making in a wide range of environmental conditions, including current and future conditions. The bioeconomic model developed here provides a flexible approach where numerous factors can be modified to improve the accuracy of prediction outputs. However, improvements in the economic and biological sub-models within this bioeconomic model can expand the applicability of the model and increase the validity of the outputs. For the economic sub-model, expanding the analysis to simulate multiple years would enable oyster farmers to more accurately project profit by spreading costs over multiple years instead of just one. Costs that recur each year (e.g., seed, labor, fuel), but more importantly, costs that do not recur every year (e.g., gear, licenses, equipment upkeep) will be better represented in a temporally expanded model that enables the prediction of profit over multiple years. Another future modification to the economic sub-model can include a mechanism to analyze different production scales like in Petrolia & Caffey, (2024),

as the scale in our analysis was a fixed value. This modification would increase the applicability of oyster farms by capturing the effects that scale has on profitability.

For the biological sub-model, additional studies that show the response (mortality and growth) of triploids particularly under low (<5) salinity and high temperature (>30°C), would help in developing more accurate results of what farms can expect. Furthermore, this model simulated farms using either diploids or triploids exclusively. It would be relevant to current farming practices to model how growing a mix of ploidies could influence profit under different environmental conditions. Finally, the biological sub-model can also be modified to allow for the economic analysis of different genetic lines of oysters based on their performance under site-specific environmental conditions. Customizing these types of parameters can help increase the application of this model to eventually be used to test and evaluate individual farm sites.

While I only analyzed six environmental scenarios across three different salinities, further customization of the bioeconomic model will increase the range of application to temperature and salinity regimes found in oyster aquaculture sites across the US. Additionally, the preemptive use of bioeconomic modelling before a site is selected can help in identifying a site with high potential for oyster production. The wide range of applicability makes bioeconomic modelling a valuable tool for users ranging from individual farmers to state agencies like LDWF (Louisiana Department of Fish and Wildlife) that are currently looking for places to permit off-bottom leases. By providing sound estimates of production based on a wide range of environmental, economic, and biological constraints, bioeconomic modelling can be a powerful approach to inform off-bottom oyster aquaculture both before and during production.

CHAPTER 4. SUMMARY AND CONCLUSIONS

Off-bottom oyster aquaculture is a fast-growing industry in the United States that is increasingly supported by the use of triploid oysters. However, studies suggest an elevated mortality of triploids compared to diploids under severe environmental conditions, particularly low salinity, high temperature, or a combination of the two. Tradeoffs between the faster growth of triploid oysters, but also a higher mortality of triploids in extreme environmental conditions challenges effective seed selection that maximizes production. To further examine these tradeoffs and help inform ploidy selection based on farm site criteria, I quantified the biological and economic performance of triploid oysters under extreme temperatures and salinities.

This work integrated environmental and biological factors to inform risk management strategies in aquaculture operations. One strategy currently used in production risk management for aquaculture and livestock culture is the use of "budget calculators" (i.e. oyster FARM calculator) that model changes in variable costs, mortality, and profitability based on management decisions. Most existing budget calculators integrate the effects of production risk on final production by using assumed mortality and growth rates in various levels of pressure from disease, predation, environmental stress, or whatever the source of risk may be. While sufficient for demonstrating production trends in face of these risks (i.e. decrease of salinity, decrease in production), the use of set assumed values does not quantify the extent to which production is affected per unit of change in production risk. By quantifying the effects of environmental risk (changes in salinity and temperature) on diploid and triploid oyster production, this work helped increase the accuracy of predicting the effects of environmental risk under a wider range of environmental conditions compared to previous budget calculators. Overall, our bioeconomic model could help improve management decisions on oyster farms, particularly regarding seed and site selection, and could serve as a method to assessing other forms of risk in aquaculture in the future. The future use of bioeconomic modelling may help inform other farm management decisions such harvest schedule, selective breeding, local microalgae (food) abundance; all of which have been done for bioeconomic modelling of other aquaculture species.

The close relationships among the environmental, biological, and economical factors in oyster farming emphasize the importance of collaboration between stakeholders in aquaculture and coastal management. I found that production is closely coupled with the environmental conditions of a farm site. Therefore, ensuring consistent production in mariculture will likely require collaboration with coastal managers to continue to manage for environmental conditions that are conducive for aquacultural production. Although initial seed and site selection can be guided with results from this bioeconomic model, increased variability of environmental conditions from climate and anthropogenic (river management) changes in the central nGoM may challenge site selection and quality in the future. Further investigation into the cascading effects that even slight changes in water quality factors can have on oyster production will help inform coastal management decisions that could benefit the oyster aquaculture industry. Another aspect of increasing awareness of the relationship between water quality and production would be further emphasis on research and extension through agencies such as NOAA Sea Grant. At the management level, extension could help guide coastal management decisions and regulations that continue to support aquacultural production. At the farm level, extension and further

research could help farmers understand what to look for when picking a farm site, and guide best management practices to minimize susceptibility to risk. Finally, extension and collaboration among stakeholders could guide genetic selection at the hatchery level to produce robust seed that can withstand greater changes in environmental conditions and satisfy growers' needs.

Equally important to extension is the accuracy of the data itself. While bioeconomic modelling and other extension tools can help support the aquaculture industry by identifying action areas and informing best management practices, the effectiveness of extension depends on the quality of scientific data that it communicates. For example, I found that profit estimates were highly sensitive to even slight shifts in biological responses. Due to this close relationship between the biological and economic sub-models, any inaccuracies in the biological response data used to calibrate the biological sub-model may be reflected in larger inaccuracies in profit predictions. Therefore, the development and use of accurate biological data was crucial to estimating profit in a realistic way that farmers could expect in the field. Maintaining the integrity of science extension and preventing misinformation will require continued investment into experimental work that increases the availability and accuracy of biological responses. Here, my experimental laboratory work yielded additional data documenting differences in triploid and diploid mortality in response to low salinity and high temperature conditions, providing critical inputs in the bioeconomic model. Continued investment into experimental work that increases the understanding of oyster biology and responses to farm factors, including environmental conditions, gear, and grow-out methods is an important preliminary step to informing future risk management strategies.

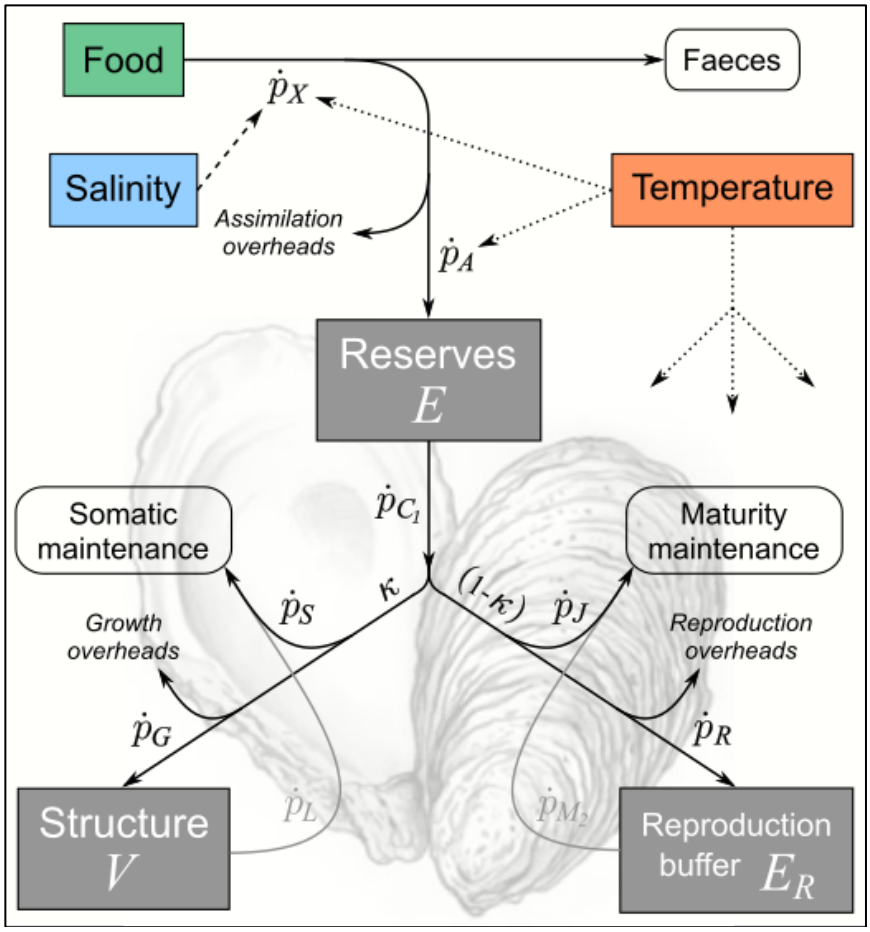
This work focused on integrating the biological, environmental, and economic factors in oyster farming to reduce risk associated with this industry. Reducing risk to aquaculturists in the future will require collaboration from stakeholders, environmental regulation and protection, and further research that demonstrates the consequences of changing environmental and economic factors on production. Understanding the relationships among these factors is important to guiding future regulation and research by demonstrating the extent to which these factors are intertwined. Through research and collaboration, stakeholders can work to ensure the longevity of aquaculture operations such as off-bottom oyster farming, which are critical maintaining fisheries production in spite of declines in wild fisheries stocks.

APPENDIX. SUPPLEMENTAL DATA FOR CHAPTER 3

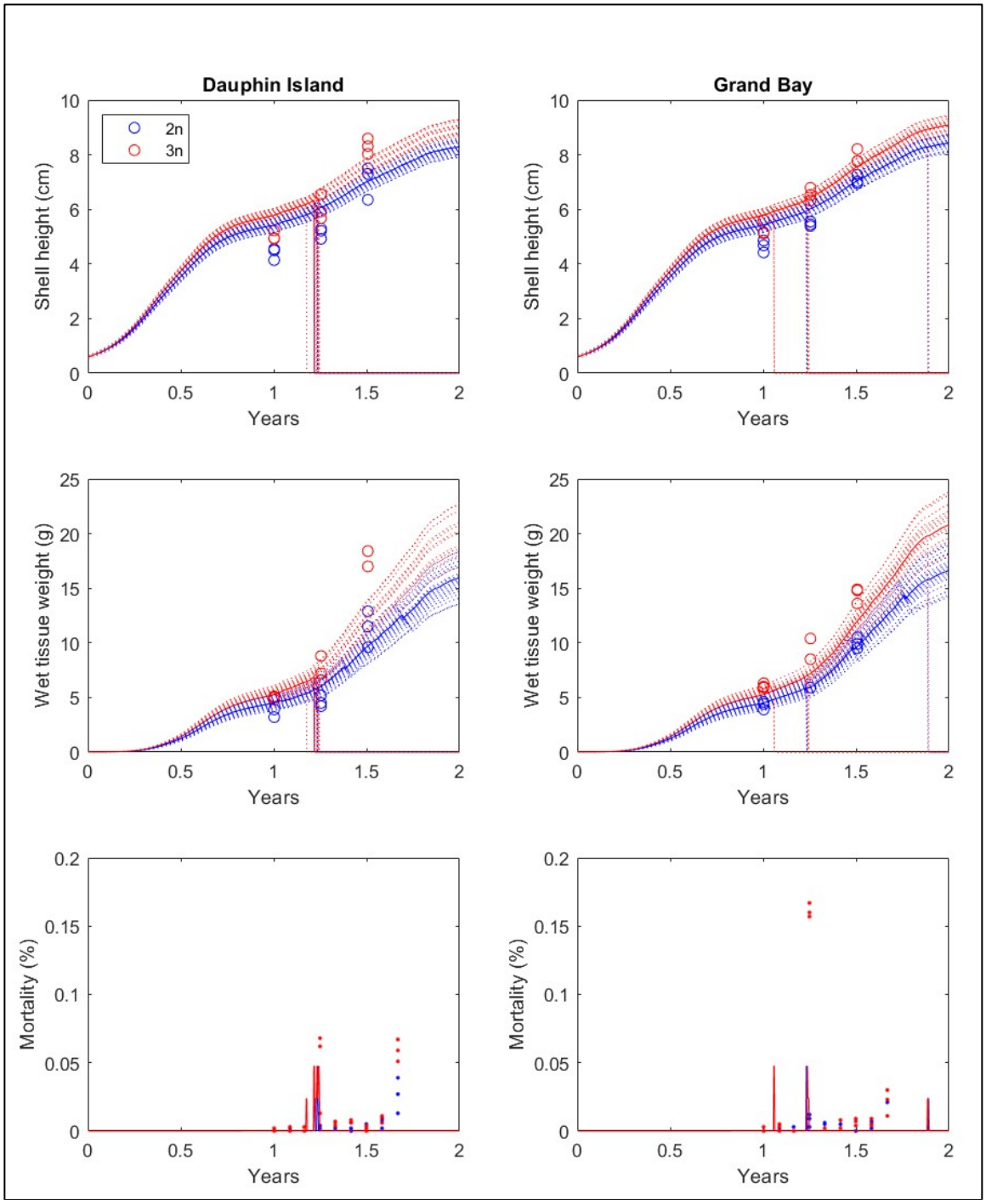
The figures included in this appendix identify the components of the DEB model (A.1.) along with the data used to calibrate specific parameters related to mortality rate that were added to the existing DEB model from Lavaud et al. (2024). Diploid and triploid growth and mortality data from two previous studies (Eastburn et al., 2021; Bodenstein et al., 2023) and from the experiment presented in Chapter 2 were used to fit the level of basal mortality (A.2., A.3., A.4.). The tables included in this appendix present DEB model results (A.5., A.6., A.7.). Mortality is defined in the DEB model as a function of temperature, salinity, and structural volume (see Lavaud et al., 2024 for details). In this work, I added a basal mortality risk to these rules based on a random parameter and a gamma distribution to compute a population survival rate (each individual has a chance to survive or die). Based on results from the Chapter 2 experiment, I define the basal mortality as follows:

Ploidy	2N	3N
T > 26 °C	$\begin{cases} M = 1 \text{ if } S < 6 \;\&\; \Gamma(x,a,b) > 0.385 \\ M = 0 \text{ otherwise} \end{cases}$	$\begin{cases} M = 1 \text{ if } S < 6 \;\&\; \Gamma(x,a,b) > 0.382 \\ M = 0 \text{ otherwise} \end{cases}$
T ≤ 26 °C	$\begin{cases} M = 1 \text{ if } S < 6 \;\&\; \Gamma(x,a,b) > 0.390 \\ M = 0 \text{ otherwise} \end{cases}$	$\begin{cases} M = 1 \text{ if } S < 6 \;\&\; \Gamma(x,a,b) > 0.385 \\ M = 0 \text{ otherwise} \end{cases}$

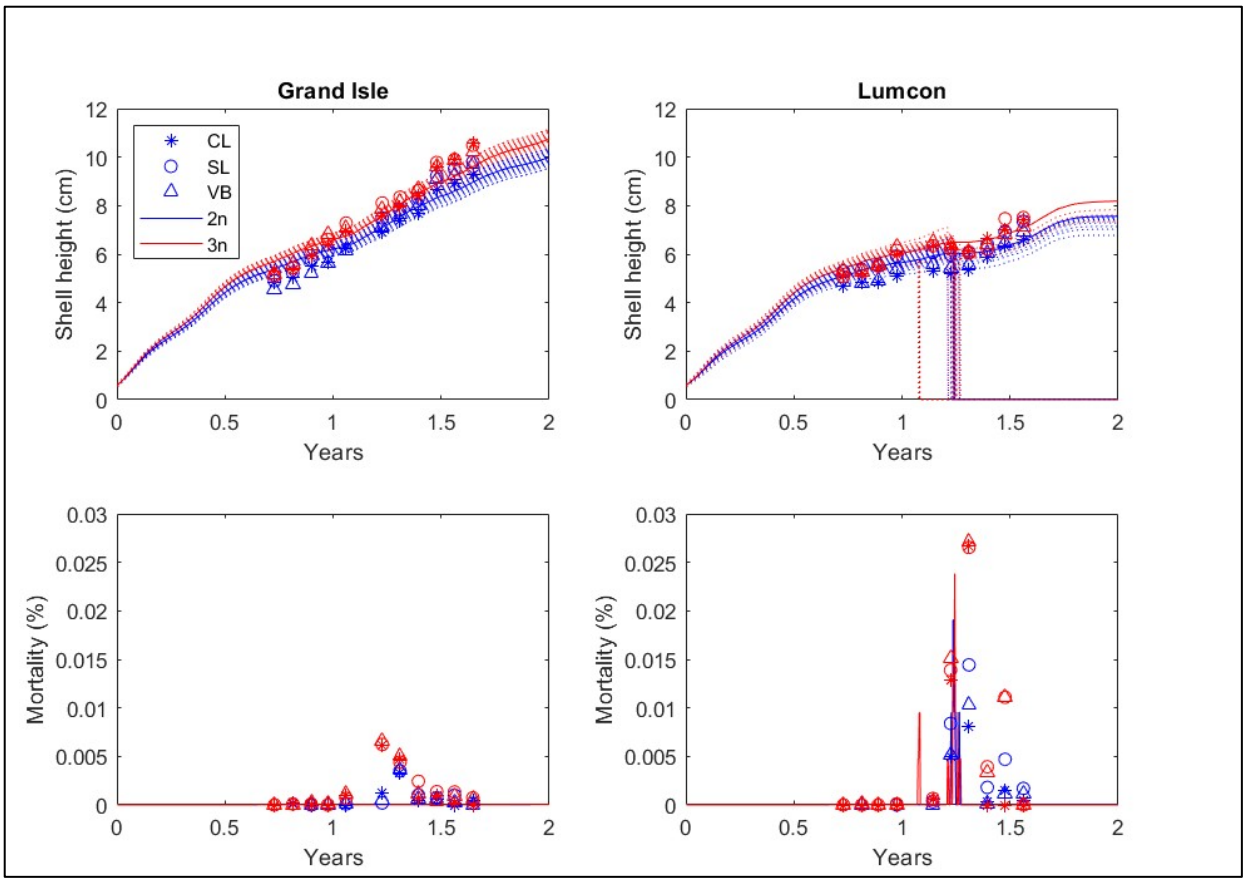
Where, S is the salinity, x is a random number drawn from a uniform distribution in [0,1], and $a = 1$ and $b = 2$, which represent the shape and rate parameters of a cumulative gamma distribution function.



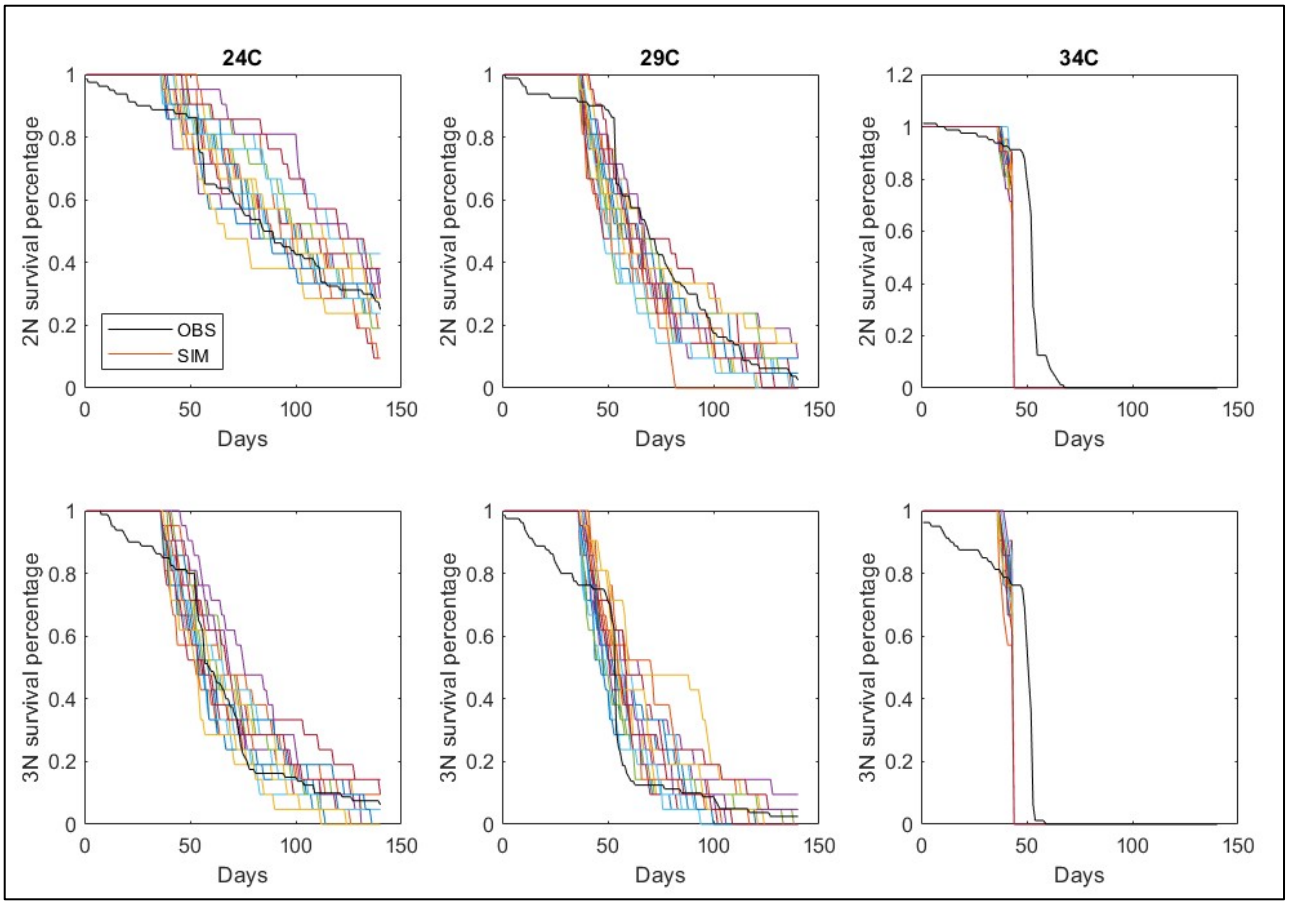
A.1. Scheme of the DEB model from Lavaud et al., (2017). Solid arrows illustrate energy fluxes, and dotted arrows represent the effect of environmental variables on energy fluxes. State variables are presented in square boxes. Gray arrows represent the energy mobilized from structure for somatic maintenance ($\dot{p}_L$) and from the reproduction buffer for maturity maintenance ($\dot{p}_{M2}$) when energy from reserves (E) are not sufficient.



A.2. Observations from Eastburn et al. (2021; circles) and DEB simulated (lines) shell height (top), wet tissue weight (center), and mortality rate (bottom) for diploid (blue) and triploid (red) oysters grown at Dauphin Island (top) and Grand Bay (bottom), AL. Each dotted line represents a modelled individual and plain lines represents population averages. When an individual died, its length and weight became null.



A.3. Observations from Bodenstein et al. (2023; circles) and DEB simulated (lines) shell height (top) and mortality rate (bottom) for diploid (blue) and triploid (red) oysters grown at Grand Isle (left) and LUMCON (right), LA. Each dotted line represents a modelled individual and plain lines represents population averages. When an individual died, its length and weight became null.



A.4. Observed (Chapter 2 experimental data; black line) and DEB-simulated (colored lines) survival curves of diploid (top) and triploid (bottom) oysters kept at 24 °C (left), 29 °C (center), and 34 °C (right) at a salinity of 20 for 36 days, then decreased to 2 for 106 days. Twenty simulations were run, starting with 21 individuals; each colored line represents a simulated population survival percentage.

A.5. Simulated DEB model results of mortality (0; dead, 1;alive) and time to harvest (TTH; days) for 21 individual diploid (2N) and triploid (3N) under the current and future low salinity environmental scenarios.

Low Salinity								
	Current 2N		Current 3N		Future 2N		Future 3N	
Ind	Mort	TTH	Mort	TTH	Mort	TTH	Mort	TTH
1	1	365	1	365	0	365	1	365
2	1	365	1	365	1	365	1	365
3	0	365	1	365	1	365	0	365
4	1	365	1	365	0	365	1	365
5	0	365	1	365	1	365	1	365
6	0	365	1	365	0	365	1	365
7	1	365	1	365	1	365	1	365
8	1	365	1	365	0	365	1	365
9	1	365	1	365	1	365	1	365
10	1	365	1	365	1	365	1	365
11	0	357	0	339	1	365	1	365
12	0	355	0	338	1	365	1	365
13	1	365	1	365	1	365	1	365
14	1	365	0	334	1	365	1	365
15	0	350	1	365	0	365	1	365
16	1	365	1	365	1	365	1	365
17	0	346	1	365	1	365	1	365
18	0	344	1	365	1	365	1	365
19	0	342	1	365	1	365	1	365
20	1	365	0	319	1	365	1	365
21	1	365	1	365	1	365	0	348

A.6. Simulated DEB model results of mortality (0; dead, 1;alive) and time to market (TTH; days) for 21 individual diploid (2N) and triploid (3N) under the current and future medium salinity environmental scenarios.

Medium Salinity								
	Current 2N		Current 3N		Future 2N		Future 3N	
Ind	Mort	TTH	Mort	TTH	Mort	TTH	Mort	TTH
1	0	365	0	361	0	365	0	365
2	0	364	0	349	0	365	0	356
3	0	360	0	345	0	365	0	352
4	0	357	0	365	0	365	0	350
5	0	365	0	339	0	364	0	348
6	0	353	0	337	0	362	0	347
7	0	351	0	365	0	360	0	346
8	0	350	0	334	0	359	0	344
9	0	349	0	333	0	358	0	343
10	0	347	0	331	0	356	0	342
11	0	346	1	330	0	355	0	341
12	0	344	0	329	0	354	0	340
13	0	343	0	328	0	353	0	339
14	1	341	0	326	0	351	0	338
15	0	339	0	325	0	350	0	337
16	0	337	0	322	0	349	0	336
17	0	336	0	320	0	347	0	334
18	0	333	0	317	0	346	0	333
19	0	331	1	314	0	344	0	331
20	0	328	0	310	0	341	0	329
21	0	313	0	292	0	334	0	321

A.7. Simulated DEB model results of mortality (0; alive, 1;dead) and time to harvest (TTH; days) for 21 individual diploid (2N) and triploid (3N) under the current and future high salinity environmental scenarios.

High Salinity								
	Current 2N		Current 3N		Future 2N		Future 3N	
Ind	Mort	TTH	Mort	TTH	Mort	TTH	Mort	TTH
1	0	335	0	314	0	310	0	293
2	0	335	0	314	0	310	0	292
3	0	331	0	310	0	307	0	289
4	0	328	0	308	0	304	0	287
5	0	326	0	306	0	302	0	286
6	0	325	0	303	0	300	0	285
7	0	323	0	301	0	299	0	284
8	0	322	0	300	0	299	0	283
9	0	321	0	299	0	298	0	282
10	0	320	0	297	0	297	0	281
11	0	318	0	296	0	296	0	281
12	0	317	0	295	0	295	0	280
13	0	316	0	294	0	294	0	279
14	0	315	0	292	0	293	0	277
15	0	314	0	290	0	291	0	276
16	0	313	0	289	0	290	0	275
17	0	312	0	288	0	290	0	274
18	0	310	0	286	0	288	0	273
19	0	308	0	284	0	287	0	272
20	0	304	0	281	0	285	0	270
21	0	303	0	281	0	284	0	269

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VITA

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