EVALUATING THE EFFECT OF LARVAL PERIOD DURATION UPON THE SUBSEQUENT PERFORMANCE OF CULTURED EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*

by

Kelsey Allyson Bisker

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 8, 2020

Copyright 2020 by Kelsey Allyson Bisker

Approved by

William C. Walton, Chair, Associate Professor, School of Fisheries, Aquaculture and Aquatic Sciences
Ian A.E. Butts, Assistant Professor, School of Fisheries, Aquaculture and Aquatic Sciences

James Stoeckel, Associate Professor, School of Fisheries, Aquaculture and Aquatic Sciences

Jan A. Pechenik, Professor, Department of Biology

Abstract

With the increase in oyster aquaculture in the United States, it is important to understand the potential effects of larval experiences on the field performance of Eastern oysters (*Crassostrea virginica*) to maximize hatchery production and field grow-out performance. To test the relationship between larval duration period and subsequent performance, three different groups of oyster larvae were produced from a single spawn at the Auburn University Shellfish Laboratory (Dauphin Island, AL) based on the number of days required by larvae ($n \geq 900{,}000$) to become pediveligers. The performance of these three groups was monitored at 1) metamorphosis & setting, 2) in land-based early nursery culture and 3) in field grow-out at three different sites in Alabama. Initially, larval duration did not affect performance of post-metamorphic growth ($p = 0.35$), but there was an affect in growth rate in the land-based nursery culture ($p = 0.014$). However, in the field grow-out culture, there was no significant effect of larval duration period on quality variables at the experimental sites ($p > 0.05$). This evaluation and understanding factors that affect larval performance will further help commercial hatchery production and farming yields.

Acknowledgments

First and foremost, I would like to thank my advisor and mentor, Bill Walton, who answered all my questions, reassured me when I had concerns, and supported me through this journey.

Thank you to the Auburn University Shellfish Lab crew: Scott Rikard, Glen Chaplin, Sarah Spellman, Caitlin Robitaille, Victoria Purente, Sarah Hensey, Mary Collier Eastburn, John Lewis, James Kelly, Rusty Grice, and Becky Wasden for all the advice, encouragement, and assistance in the hatchery, field, and processing samples. I would like to thank Ian Butts, Jim Stoeckel, and Jan Pechenik for their patience and contributions to this manuscript. Thanks to Andy DePaola, Steve Crockett, Hugh McClure, Chuck Wilson, John Supan, Cullan Duke, and Aaron Fowler for dedicating time and effort into growing oysters for part of my project. A special thanks to Ian Butts, Todd Steury, and Victoria Purente for statistical assistance. Also, thank you Josh Goff at the Dauphin Island Sea Lab for assisting me with the water quality long-term monitoring logger.

Last, but not least, special thanks to my roommate, Madison McGough, and my dog, Mya, for all the encouragement and support.

This thesis is dedicated to my late father, Bob Bisker, who taught me to love the outdoors and the marine environment, which inspired my interest in mariculture. Also, to Peggy Kellam, a late friend that encouraged me to follow my dreams. Finally, to my mother, who has supported me in every endeavor since day one.

Table of Contents

Abstract ... *ii*

Acknowledgments ... *iii*

List of Tables ... *vii*

List of Figures ... *viii*

CHAPTER ONE: A BRIEF REVIEW OF HATCHERY PRODUCTION OF THE EASTERN OYSTER *Crassostrea virginica* AND HOW LARVAL EXPERIENCES CAN AFFECT SUBSEQUENT PERFORMANCE ... 1

Overview of Aquaculture ... 2

A Declining Oyster Fishery ... 2

Overview of Oyster Aquaculture ... 4

Importance of Oyster Hatcheries ... 5

Overview of Oyster Hatchery Production ... 6

Larval Production ... 9

Overview of Mechanisms for Larval Growth Differences ... 10

1.Environmetnal Factors ... 11

2.Physiological Differences ... 12

3.Genetic Differences ... 13

Overview of Stress on Larval Experiences ... 13

Literature Cited ... 18

CHAPTER TWO: EFFECTS OF DIFFERENCES IN LARVAL DURATION PERIOD UPON FIELD PERFORMANCE OF CULTURED EASTERN OYSTER (*Crassostrea virginica*) ...24

Introduction ... 25

Methods ... 28

Spawning Procedure 28
Larval Culture 29
Determining Treatment Groups 31
Determining Metamorphic Success 32
Land-Based Nursery Culture 34
Field Nursery Procedure 36
Field Grow-Out Experimental Design 37
Sampling 39
Environmental Data 41
Commercial Farmer's Field Grow-Out Production 42
Statistical Analysis 42
Results.... 44
Metamorphic Success 44
Land-Based Nursery Culture Growth Rate 46
Land-Based Recruitment to 4 mm and 6 mm....48
Field Grow-Out Sample Time 49
Field Grow-Out Larval Duration Period 53
Commercial Oyster Farm Site 55
Discussion.... 56
Metamorphosis and Setting 57
Land-Based Nursery Culture 58
Field Grow-Out Culture 58
Commercial Farm Grow-Out 61

Conclusion 62

Literature Cited 64

CHAPTER THREE: ONCE AN OYSTER RUNT, ALWAYS AN OYSTER RUNT? A Summary for the Commercial Oyster Industry 68

A Rise in the Commercial Oyster Farming Industry 69

In The Hatchery 69

Setting Day 70

The Study 70

What Did We Find? 72

Going Forward 72

Literature Cited 74

APPENDIX A 75

Larval Growth 75

Land-Based Nursery Culture 75

Field Grow-Out Culture 76

List of Tables

Table Page

CHAPTER TWO: EFFECTS OF DIFFERENCES IN LARVAL DURATION PERIOD UPON FIELD PERFORMANCE OF CULTURED EASTERN OYSTER (*Crassostrea virginica*)

1. Grading schedule for each larval duration period group in the land-based nursery culture 35

2. Stocking densities for each upwelling replicate silo for the land-based nursery culture 35

3. Field grow-out culture sample dates and number of days between sampling 37

4. Restricted maximum likelihood variance components as percentages for field grow-out growth rate, dry shell weight, condition index, and cup ratio..........51

APPENDIX A

A1. Restricted maximum likelihood variance components as percentages for field grow-out fan ratio, whole wet weight, and dry tissue weight..........77

List of Figures

Figure Date

CHAPTER TWO: EFFECTS OF DIFFERENCES IN LARVAL DURATION PERIOD UPON FIELD PERFORMANCE OF CULTURED EASTERN OYSTER (*Crassostrea virginica*)

1. Larval shell height measurement illustration ……………………………………….31

2. Number of eyed larvae that retained on mesh screen per day to determine the larval duration period treatment groups ……………………………………………………….32

3. Illustration of oyster shell metrics used for land-based nursery and field grow-out growth ……………………………………………………………………………………36

4. Map of Mobile Bay and Mississippi Sound ……………………………………… 38

5. Diagram of BST basket arrangement on the adjustable longline system …..................39

6. Post-metamorphic growth rate and metamorphic success for larval duration period treatment groups ……………………………………………………………..45

7. Growth rate for larval duration period treatment groups at each land-based nursery culture grade ……………………………………………………………………………47

8. Overall growth rate for larval duration period treatment groups in the land-based nursery culture ………………………………………………………………………….48

9. Percent retention for the 4 mm and 6 mm grade for the larval duration period treatment groups ………………………………………………………………………..49

10. Growth rate, dry shell weight, condition index, and cup ratio for sample time at field grow-out 52

11. Shell height of live and dead oysters at each sample time for field grow-out …...........53

12. Growth rate, dry shell weight, condition index, and cup ratio for larval duration period treatment groups at field grow-out ………………………………….................54

13. Cumulative survival for larval duration period treatment groups at field grow-out …………………………………………………………………………………….....55

14. Growth rate for larval duration period treatment groups at DePe's Oyster farm ……..56

CHAPTER THREE: ONCE AN OYSTER RUNT, ALWAYS AN OYSTER RUNT? A Summary for the Commercial Oyster Industry

1. Life cycle of the Eastern oyster ……………………………………………………..70

2. Number of eyed larvae that retained on mesh screen per day to determine treatment groups …………………………………………………………………...71

APPENDIX A

A1. Shell height over 11 days of larval culture ……………………………………....75

A2. Shell height for larval duration period treatment groups at land-based nursery culture ……………………………………………………………………......76

A3. Fan ratio, whole wet weight, and dry tissue weight for sample time at field grow-out ……………………………………………………………………………......78

A4. Fan ratio, whole wet weight, and dry tissue weight for larval duration period treatment groups at field grow-out ………………………………………….…...79

CHAPTER ONE:

A BRIEF REVIEW OF HATCHERY PRODUCTION OF THE EASTERN OYSTER *Crassostrea virginica* AND HOW LARVAL EXPERIENCES CAN AFFECT SUBSEQUENT PERFORMANCE

Overview of Aquaculture

Globally, freshwater and marine fisheries stocks have decreased because of anthropogenic impacts such as overharvesting, habitat loss, and environmental degradation. With these declines, the importance of farming shellfish and fish species have increased. According to Godfray et al. (2010), the human population is predicted to reach around 9 billion before the 21st century concludes, intensifying the demand for food. The increasing dependence upon aquaculture is expected to continue with all aspects of aquaculture production.

The production of cultured aquatic species depends heavily on support from hatchery systems. Hatcheries can be designed specifically for the type of larvae being reared, in addition to the production goals. Lee and Ostrowski (2001) describe how laboratories were first used to culture aquatic larvae; however, as the interest for commercializing species for production increased, hatcheries advanced and grew to be able to produce larger quantities that were applicable for marketable operations. The use of hatcheries in aquaculture can decrease the bottleneck problem of seed availability due to the advanced techniques that have recently developed, such as the ability to culture broodstock and conduct selective breeding, manipulate reproduction, and culture live feed for nutritional and necessary diets for proper organismal development (Phelps 2010).

A Declining Oyster Fishery

Oyster farming methods date back to ancient Rome where oyster grounds were created (Günther, 1897). Globally, oysters were abundant along coastal waters since the Roman empire, and eventually a market began to increase for the nutritious bivalve. The Eastern oyster (*Crassostrea virginica* Gmelin, 1791) is a species of oyster that is found in North America along the coasts of Canada to the Gulf of Mexico. This bivalve is part of the Mollusca phylum and

Ostreidae family. Long before the voyages of Christopher Columbus, this species was once referred to as the American oyster and was harvested in the United States by the Native American Indians (Wheaton, 2007).

In the late 1800s, harvesting from oyster beds was at its all-time high in order to satisfy the growing human population in the United States (MacKenzie 2007). However, throughout the East Coast, the oyster industry's total landings of oyster bushels began to rapidly decrease in the early 1900s. Factors that contributed to the decrease in oyster landings included: pollution that led to strict harvest regulations, habitat damage from harvesting practices like dredging, spread of diseases, fluctuation in water salinity, excess nutrient and sediment inputs in the environment, and overharvesting (e.g. NRC 2004, MacKenzie 2007, Wilberg et a. 2011). These factors led to an almost 60% loss of oysters that were harvestable for the market in 1940; this decline in oyster landings continued through the early 21st century along the United States' East Coast (MacKenzie 2007).

The Chesapeake Bay region had dominated the US fishery; however, as the oyster harvest began to rapidly decrease, this opportunity gave rise to the Gulf Coast region's oyster industry. Within 50 years, the Gulf Coast region went from barely harvesting 20% of the nation's oysters to 90% in the early 2000s (NRC 2004). However, in 2012, the Apalachicola Bay region located along Florida's Gulf Coast began experiencing the stress of the decline in oyster populations, which affected the employment in that fishery dependent region (Havens et al. 2013, Camp et al. 2015). Like other east coast regions, the natural oyster production and viability declined greatly compared to that of the initial oyster landing records for the coastal states that border the Gulf of Mexico. Now, restoration efforts have been in effect in each of the five states in order to rebuild the natural populations. However, due to the economics around

oyster harvesting, some of the states began to investigate other opportunities to produce oysters for their economy, while simultaneously repopulating their wild oysters through restoration efforts (Bendick et al. 2018**).**

Overview of Oyster Aquaculture

Therefore, in order to meet consumer demands, the technique of farming oysters began to evolve for the commercial oyster industry. During the 20th century, watermen in Maryland and Virginia began farming oysters by deploying benthic shell bags that provided a substrate for oyster larvae to set, which eventually produced seed for the farmers (Haven and Garten 1972, NRC 2004). Since then, the number of oyster farms have increased greatly along the coasts of the United States, including the Gulf of Mexico region. In 2010, oyster farming began in Alabama and Louisiana, and after a decade there are more than 100 farms throughout the states of: Alabama, Louisiana, Florida, and recently Mississippi (Petrolia & Walton 2018, Walton, pers. comm., 2020). In Alabama alone, the industry increased from a single farm in 2012 to 22 commercially operated oyster farms in just six years (Grice & Walton 2019).

Oyster aquaculture is valued now at over 185 million dollars and accounts for over 46% of the total value of farmed marine species produced in the United States (NOAA 2018). In the 2018 world review, oysters from the genus *Crassostrea* made up 28% total of the percentage of molluscan species that were produced worldwide through aquaculture efforts in 2016, which was the greatest proportion in that phylum (FAO 2018). Locally, in 2018, Alabama brought in a farm gate value of over 1 million dollars and provided over 60 job opportunities for the state through the expansion of the commercial oyster farm industry (Grice & Walton 2019).

In Alabama, wholesale oysters can have a price range between 30 cents to more than 70 cents per oyster (Grice & Walton 2019). Recently, the demand for oysters have increased due to

a rise of oyster bars throughout the nation that are specializing in the half-shell market (Sackton 2013, as cited in Mizuta & Wikfors 2019). The half-shell market consists of live oysters that are fresh and easy for customers to eat without the possibility of injury during the shucking process and these oysters make it simple for special preparations made by chiefs (Loose et al. 2013). In order to reach the demands of the market, farmers have started to change their growing techniques from a bottom-culture to an off-bottom culture that suspends oysters in the water column, which allows production of high quality oysters to sell in the competitive half-shell market (e.g. Mizuta & Wikfors 2019, Thomas et al. 2019, Davis 2013, Walton et al. 2013).

Importance of Oyster Hatcheries

The growth of oyster farms throughout the United States has produced a high demand for oyster seed. Shellfish hatcheries aim to produce high quality larvae and seed by selectively breeding broodstock oysters that have a phenotype of a deep cup, wide fan, teardrop shape, and no backbend at the hinge for easier shuckability. However, for growers to make a profit, they must be able to culture an oyster to market size in a reasonable timeframe to provide for the demands of their customers. Even if a hatchery can breed high-quality oysters in terms of aesthetics, if the oysters do not grow or survive, farmers will not have any economic gain toward their business or to the local and state economy.

In commercial hatcheries, the personnel will typically cull out slow growing larvae and keep the larger larvae that develop quickly, reducing total production. Additionally, growers want to be sure that they are obtaining seed that will exhibit high quality performance during field grow-out, so that the oysters can be harvested for the market in an expected time with good survival. Therefore, understanding how larval performance may affect field performance is essential for all stages in oyster husbandry. Due to the cost that it takes to successfully run a

hatchery, it is essential to ensure that the least amount of problems will occur within the oyster production processes. Understandably, hatcheries strive to maximize their profits and minimize their costs, but may not be aware of the effects of their decisions on subsequent grow-out. Thus, it is important to understand larval experiences because the insight can help hatcheries produce and provide high quality oyster seed for the industry, which in turn can thrive and support the hatchery sector.

Overview of Oyster Hatchery Production

The Eastern oyster belongs to the genus *Crassostrea*, which are cupped oysters, meaning that the left valve is convex and the right is usually flat. The *Crassostrea* spp will release their eggs into the water column, so that their eggs will be fertilized outside the individuals, which characterizes these species as nonincubatory (Galtsoff 1964). One way to tell the sex of a *Crassostrea* spp is during the broadcast spawning event, as they will release their gametes into the water column. The Eastern oyster is a protandrous hermaphrodite, which means that the younger and smaller oyster is most likely a male, but eventually will switch the gonad to that of a female due to ovarian development and cytolysis of the sperm cells; however, sex reversal can occur multiple times within an oyster's lifetime (Coe 1943, Thompson et al.1996).

To successfully produce oyster larvae, a hatchery must have the correct systems to be able to provide a controlled setting that mimics the natural environment that will signal the release of gametes for oyster reproduction. Oyster hatcheries are usually located adjacent to seawater that is optimal for oyster larvae and seed rearing. These hatcheries must be able to pump in large volumes of the available seawater in order to create an estuarine like environment for larval growth and further production. Oysters that are spawned in the Gulf of Mexico region will begin to release their gametes when the temperature of the water rises above 25°C, which

begins during the spring and can continue through the summer and into the fall as the decrease in temperature can trigger oysters to spawn again (Thompson et al. 1996, Hayes and Menzel 1981, Wallace et al. 2008). Wallace et al. (2008) recommends the addition of sperm to the ambient water to also help trigger the release of gametes as another spawning stimulate that can be used simultaneously with temperature changes. Hatcheries will usually sacrifice oysters in order to find sperm to heat up to ensure that the sperm is not mobile in order to be used as the other chemical stimulant (Wallace et al. 2008). Galtsoff (1938) discovered that when sperm was added to ripe oysters at 22ºC, there was no effect; but, when temperature increased to about 25ºC, spawning occurred about an hour later. However, in a separate experiment, females spawned when the temperature reached 20.5 ºC (Galtsoff 1938). These tests conclude that spawning is dependent on the condition of each female in order to determine if a higher or lower temperature gradient is necessary for the chemical cue to assist in the spawning process (Galtsoff 1938). Therefore, ensuring the optimal temperature and environment is important to have successful spawns since hatchery production does come with several costs, like the expenses of: heating, water filtration, and pumping, as well as, the destructive oyster practices like strip-spawning that involves sacrificing oysters for spawning purposes.

The location of a hatchery is an important aspect in order to help eliminate expenses by ensuring that the salinity of the seawater the hatchery pumps in, is optimal for oyster growth and survival. Oysters can survive in an extensive salinity range; however, optimal salinity conditions for spawning are from 10 to 30 parts per thousand (ppt) (Cake 1983). Galtsoff (1964) mentioned that if the salinity becomes less than 10 ppt, eggs can experience cytolysis as they are released, which can result in a high number of unfertilized eggs. Thus, 15 ppt is recommended to be the lowest optimal salinity measurement for rearing (Wallace 2001). Juvenile and adult oysters have

been found to tolerate salinity ranges from 5 to 40 ppt, but recommended salinity for physiological reasons is between 15 to 18 ppt, which can also vary depending on factors like geographical locations (Galtsoff 1964, R. Newell, (pers. comm,), as cited in Shumway, 1996). These conditions are important for ensuring optimal growth and survival during the larval period, so that the culture can produce the quantity and quality of seed for farming demands.

An individual female oyster can produce a large quantity of eggs during a single spawn. Davis and Chanley (1956) found that season affects the total amount of eggs a female will release by assessing a mid-winter spawn to an early summer spawn, which had averages of 28.8 and 54.1 million eggs per female, respectively. When oysters spawn in a hatchery, multiple oysters are used to conduct spawns in order to maximize genetic diversity and to reach target production quantities to fulfil orders from farmers. Wallace et al. (2008) report that typically 10 female oysters can produce around 200 million eggs and from that, half will become fertilized and develop into free-swimming larval organisms in optimal conditions. From there, larval culling and natural morality will create an overall decrease in the number of total oysters that will be available to reach the next step in the oyster production process. When 100 million larvae begin their growth and development in larval tanks, the overall number of oysters that will successfully metamorphose, and therefore be able to grow in a land-based nursery with natural seawater pumped in, will only be around 10 million for that spawning event (Wallace et al. 2008). This extreme reduction from the overall number of oyster larvae produced from Wallace et al. (2008) example further emphasizes the importance of having the right environmental conditions to obtain optimal growth and survival, determined that multiple females are needed for a single spawn, and concluded that multiple spawns are desired within a season to be able to fill the demands of farmers and restoration efforts. Additionally, understanding larval

development and experiences can assist in the increase of larval production and potentially avoid high mortality in the early stages.

Larval Production

When sperm and eggs are released from adult oysters in independent containers during the spawning procedure, the process of fertilization and larval development can begin. Sperm can be added to the eggs up to 45 minutes after the female has released her gametes (Wallace et al. 2008). In order to see if fertilization has begun, a sample of the egg and sperm mixture can be examined in a microscope to check for the existence of polar bodies. It is critical to not introduce a high concentration of sperm to the egg container in order to avoid the occurrence of multiple spermatozoa fertilizing a single egg because that can result in abnormities during development and eventually embryonic death (Togo & Morisawa 1999). This phenomenon is known as polyspermy. Eggs of the Eastern oyster can prevent polyspermy from occurring when the sperm concentration is at a threshold of 1,000 sperm per egg; however, once when the sperm density increases, the block becomes repressed (Alliegro & Wright 1983).

Once the egg is fertilized, cell division begins and after 24 hours, the embryo has turned from a trochophore (with a mouth, stomach, intestine, anus, and locomotive cilia), into a veliger that has a structure known as a velum that consists of layers of cilia used to swim and direct food particles toward the mouth to be ingested during the planktonic stage (Galtsoff 1964). Initial shell growth occurs during this time, known as the Prodissoconch I stage. During this time, valves are formed, as well as a hinge that is straight, shifting the larvae into the D-shape larval stage (Carriker 1996). After a few days in culture, the Prodissoconch II stage forms deep valves that become rounded and develop umbones at the hinge, with the larger umbo located on the left valve (Galtsoff 1964, Carriker 1996).

The final stage of larval development occurs about two weeks post-fertilization. During this stage, the larvae have: a functioning mouth, esophagus, stomach, larger digestive tubules, intestine, heart, kidney, gills, a retractable foot, and a dark eyespot (Galtsoff 1964). This stage, known as Pediveliger, or eyed-larval stage, indicates that the larvae are ready to achieve metamorphosis. During the metamorphic period, it becomes a multi-step process where the larvae first become competent to begin settlement before they reach the ability to complete metamorphosis (Coon et al. 1990). The ciliated foot helps navigate the free-swimming larvae, but as the larvae stops swimming, the foot is used to crawl and direct the larvae to a substrate where the left valve will cement itself to the substrate by releasing fluid from the byssus gland that hardens within minutes (Nelson 1924, Galtsoff 1964). Once attached, the free-swimming larvae has become a sessile spat, or an 'oyster seed'. Thus, metamorphosis is achieved through the development and growth of organs, disappearance of specific larval structures, and new shell growth, which takes place in four phases: Settler, Prodissoconch postlarvae, Dissoconch postlarvae, and Juvenile (Baker & Mann 1994). During this stressful transition, the oyster relies upon stored lipids left over from the larval period while new adult feeding organs develop (Hickman & Gruffydd 1971; as cited in Newell & Langdon, 1996).

Overview of Mechanisms for Larval Growth Differences

Hatcheries can culture larvae to achieve higher production rates through the meticulous and careful procedures that are used in a controlled environment, which can largely avoid, manipulate, or fix many potential factors that could affect larval development and survival. Despite these efforts to control production, there are several factors that may affect larval growth and survival, such as environmental, physiological, and genetic factors. Same-age bivalve larvae naturally differ in size and siblings can reach the metamorphic stage at different times when

cultured in controlled conditions (for the Eastern oyster, for example: Newkirk et al. 1977, Haley & Newkirk 1977, and Losee 1979; zebra mussel: Stoeckel et al. 2004; and Northern quahog: Przeslawski & Webb 2009). This indicates that larvae express individual differences by the way each sibling will obtain energy and use that energy for development (Tamayo et al. 2014).

1.Environmental Factors

For wild oysters, food abundancy, salinity, and temperature are just a few factors that can affect the time and intensity of when adult oysters will spawn (Dekshenieks et al. 1993). Likewise, several studies have examined how many environmental conditions affect oyster larval growth and development, such as changes in salinity, turbidity, temperature, carbon dioxide, and dissolved oxygen concentrations. Larval duration period can be extended for multiple weeks depending on the temperature and the food condition and abundance that are available in the water column (Loosanoff 1965). Dekshenieks et al. (1993) concludes that larval duration period is affected by the seasonal changes that relates to warmer water temperatures, such as altering from late spring to summer to early fall. These seasonal changes correspond to the increase of algal concentrations, which can result in a shorter larval duration that can encourage earlier settlement (Dekshenieks et al. 1993). Additionally, oyster larvae have been found to extend their larval duration in order to find the optimal substrate for attachment (Hidu 1969).

Furthermore, low salinity can influence oyster larvae by slowing down growth and reducing survivorship (e.g. Davis 1958, Davis & Calabrese 1964, Dekshenieks et al. 1993). Similarly, a decline in ambient oxygen was found to reduce larval feeding, though larvae may have an anaerobic metabolic pathway to survive in anoxic conditions for a certain amount of time (Widdows et al. 1989). Lastly, the increasing levels of carbon dioxide are causing the oceans to become acidic. When exposed to higher levels of carbon dioxide, oyster larvae delayed

metamorphosis significantly compared to larvae that were reared in ambient conditions (Talmage & Gobler 2009).

2.Physiological Differences

Even under uniform environmental conditions, variation has been observed among individuals. Variation in the functional behavior between growth among individuals can rely on the differences in the rate of processing algae (Tamayo et al. 2011). In a controlled environment, Tamayo et al. (2011) found that growth differences that rely on processing food rates was explained by the ability of larvae to process food quickly because of the size of the filtration surface, not gill efficiency; clam (*Ruditapes philippinarum*) seed that had larger gill area processed food faster. Differences could be explained by morphological factors. Furthermore, Bayne (1999) described how physiological components could explain the variation among individuals that are reared in the same environment, such as: (1) faster consumption and absorption rates could result in faster growth; (2) variation in growth rates may be due to different distribution of energy for the higher energy cost activities such as, development, reproduction, and individual upkeep; and (3) faster growth may be because of low metabolic cost. For example, decreased energy used for individual maintenance in blue mussel (*Mytilus edulis*) juveniles was linked with better protein synthesis efficiency and generally lower nitrogen metabolism, which generated faster growth (Hawkins et al. 1986). In adults, the Pacific oyster (*Crassostrea gigas*) and Sydney rock oyster (*Saccostrea commercialis*) both illustrated that the slow growing line of oysters were found to have higher metabolic cost of growth (measured of joules respired per joule of growth) compared to the faster growing individuals (Bayne 1999; Bayne et al. 1999). Also, epigenetic mechanisms could potentially contribute to variations by influencing several physiological parameters as well (Burggren 2014).

3. Genetic Differences

Additionally, intraspecific variation may be explained by the difference in genotype. Since the larval period has a higher mortality rate compared to post-settlement, a faster growth rate in bivalves that leads to an early metamorphosis could be a significant fitness trait (Del Rio-Portilla & Beaumont 2000). In three different blue mussel crosses conducted by Del Rio-Portilla and Beaumont (2000), growth rate in the larval stage was not correlated with growth rate of juveniles. Additionally, they found no association between the amount of heterozygosity and larval growth rate; out of 202 cases, only two homozygotes differed significantly in shell length compared to the heterozygotes (Del Rio-Portilla & Beaumont 2000). In contrast, Hawkins et al. (1986) found that heterozygosity explained growth variation due to different genetic-dependent changes in the rate of protein breakdown and synthesis that ultimately affected food absorption rates, energetic constraints, and metabolic maintenance efficiencies. Furthermore, in Pacific oyster larvae, a cardioactive peptide precursor gene (*SCPb*) was expressed in the slow-growing larvae only (Meyer & Manahan 2010). This finding suggests that this gene potentially regulates feeding activities and ultimately growth of bivalves (Meyer & Manahan 2010). Additionally, heterosis linked with multi-locus heterozygosity is a genetic factor that can also control growth (Meyer & Manahan 2010). Finally, Pechenik et al. (1998) mentioned that variation in juveniles after metamorphosis is completed could be a result from a change in gene transcription or translation due to a stress factor that affected those processes that are needed for proper post-metamorphic development.

Overview of Stress on Larval Experiences

Given this variation in larval growth and survival and the potential for carryover effects post-metamorphosis, it is also important to understand how larval performance and experience

can affect subsequent performance. Organisms that have a metamorphic phase use large amounts of energy to achieve metamorphosis in order to reach the next stage in the cycle; therefore, the history of the nutrient intake for the individual may have an effect on the performance in later stages (Phillips 2002). Pechenik (2018) describes how the metamorphic stage for an organism is the time where the structure, physiology, habitat, and lifestyle of that individual changes, but certain experiences that occur during the larval stage could potentially create a latent effect. This could also explain some of the intraspecific variations that occurs in the growth rates of individuals (Pechenik 2018). Also referred to as "carryover effects", latent effects, as described by Pechenik (2006), occur when a treatment, such as some type of stressor (e.g. delay in metamorphosis, dietary stress, and environmental stress) is introduced during the embryotic and larval stage, but is only noticed in the later juvenile and adult phases. For example, during a several week period, L-3,4-dihydroxyphenylalanine and epinephrine were added to Pacific oyster larvae for the initiation of settlement and metamorphosis, respectively, and results concluded that larvae were able to delay metamorphosis and maintain competence throughout the experimental duration (Coon et al. 1990). However, feeding larvae do not usually demonstrate a reduction in fitness as a result of delayed metamorphosis (Pechenik et al. 1998). Nevertheless, lecithotrophic larvae of the small abalone (*Haliotis diversicolor*) that intentionally delayed metamorphosis had post-larval growth rates and survival that were noticeably decreased due to the prolonged larval periods (Onitsuka et al. 2010).

Furthermore, latent effects have been observed when larval nutrition was stressed. Zebra mussels (*Dreissena polymorpha*) were found to use storage for growth when temporarily starved, which resulted in a much slower growth rate compared to individuals that were fed a high quantity diet (Wacker & von Elert 2002). Similarly, if food quality was poor, then post-

metamorphic growth for zebra mussels was reduced compared to those larvae that consumed a mixed diet that met the metabolic demands for high growth rates (Wacker & von Elert 2002). Likewise, post-metamorphic performance for Mediterranean mussel (*Mytilus galloprovincialis*) larvae was found to be dependent on food supply (Phillips 2002). When these mussel larvae were exposed to large amounts of available food, it resulted in larger individuals that had high lipid content for settlement, which allowed them to maintain high performance in terms of faster growth rates in their juvenile stage (Phillips 2002). Additionally, latent effects have been examined in several other types of organisms (e.g. fish: McCormick and Molony 1992; birds: Merilä and Svensson 1997; and mammals: Desai and Hales 1997; Pechenik 2006).

In addition, it is important to be aware that there are components that occur before larval development, such as parental exposures and experiences, that also have an impact on subsequent performance. For example, two oyster species were separately observed for their carryover effects on the performance of juvenile offspring. Adult Sydney rock oysters that were exposed to high levels of CO_2 were mated to produce F1 offspring, that produced a F2 generation, and there was a positive carryover effect for elevated CO_2 levels (Parker et al. 2015). Transgenerational exposure enhanced performance for both the F1 and F2 generations (Parker et al. 2015). Likewise, Spencer et al. (2020) found that juvenile offspring, that were produced from adult Olympia oysters (*Ostrea lurida*) that were reared in elevated winter pCO_2, presented carryover effects of high survival when exposed to an environment with a lower mean pH and higher mean salinity compared to the other sites. The exposure indicates that perhaps when the adult oysters were reared in that stressful environment, the memory was passed along to their offspring to result in beneficial carryover effect, which depends on environmental conditions and genetics (Spencer et al. 2020).

Notably, while multiple examples demonstrate that larval experience can predict subsequent performance, there is also contrasting evidence that these outcomes don't always occur. Newkirk (1981) found that predicting growth rates from the relative size of juvenile European flat oysters (*Ostrea edulis*) could be inaccurate and believed that culling out the smallest oysters at 5 months old is a loss of total harvestable oysters in the future for farmers. Therefore, for management purposes, Newkirk (1981) recommended to not cull out more than a small percentage of juveniles; however, the potential cost of labor for grading a small amount of seed out may be greater than the overall benefit from sorting out the smallest sizes. Additionally, Newkirk and Haley (1982) found that even though the length of the larval period of European flat oysters did have an effect on juveniles that were, on average, a length of 23 mm, the effect disappeared by the time the oysters grew $\geq$60 mm in length. This indicated that individuals that were fast growing in the larval stage did not potentially demonstrate that performance for life (Newkirk and Haley 1982).

In conclusion, commercial oyster farms and the demand for oyster seed are steadily increasing nationwide. This enhances the importance of understanding the larval production process that occurs in the hatchery in order to fulfil seed demands and to reduce potential economic loss. For example, Pechenik et al. (1998) proposed that the clam and oyster aquaculture industry could suffer considerably if larvae postpone metamorphosis for long periods of time or experience dietary stress before reaching metamorphosis because those occurrences could affect growth rates of juveniles. Furthermore, the potential for understanding how larval performance may affect grow-out performance opens up opportunities for hatcheries to both increase production and quality of seed that they produce. Understanding larval performance may benefit hatcheries by potentially reducing culling practices and producing

more product per spawn. Additionally, oyster farmers need high survival and growth in order to provide product for the increasing worldwide population that have high demands for this elite and nutritious shellfish. Hence, it is important to understand the production that occurs in the larval tanks and the multiple factors that affect larval development and performance in order to produce high quality oyster seed with the confidence that the seed will produce high yields for the farmers.

LITERATURE CITED

Alliegro, M. C. & D. A. Wright. 1983. Polyspermy inhibition in the oyster, *Crassostrea virginica*. *J. Exp. Zoo*. 227(1):127-137.

Baker, S. M. & R. Mann. 1994. Description of metamorphic phases in the oyster *Crassostrea virginica* and effects of hypoxia on metamorphosis. *Mar. Ecol. Prog. Ser*. 104: 91-99.

Bayne, B. L. 1999. Physiological components of growth differences between individual oysters (*Crassostrea gigas*) and a comparison with *Saccostrea commercialis*. *Physiol. Biochem. Zool*. 72(6): 705-713.

Bayne, B.L., S. Svensson & J. A. Nell. 1999. The physiological basis for faster growth in the Sydney rock oyster, *Crassostrea commercialis*. *Biol. Bull*. 197(3): 377-387.

Bendick, R., B. DeAngelis & S. Blitch. 2018. Oyster Restoration in the Gulf of Mexico. The Nature Conservancy. https://www.nature.org/content/dam/tnc/nature/en/documents/OysterRestorationintheGulf.pdf (accessed December 2019).

Burggren, W.W. 2014. Epigenetics as a source of variation in comparative animal physiology-or-Lamarck is lookin' pretty good these days. *J Exp. Biol*. 217: 682-689.

Cake, E. W. 1983. Habitat suitability index models: Gulf of Mexico American oyster. U.S. Dept. Int. Fish Wildl. Serv. FWS/OBS-82/10.57. pp.1-37.

Camp, E. V., W. A. Pine III, K. Havens, A. S. Kane, C. J. Walters, T. Irani, A. B. Lindsey & J. G. Morris Jr. 2015. Collapse of a historic oyster fishery diagnosing causes and identifying paths toward increased resilience. Ecology and Society 20(3), 45. Retrieved from www.jstor.org/stable/26270266 (accessed December 2019).

Carriker, M.R. 1996. The shell and ligament. In: V. S. Kennedy, R. I. E. Newell, & A. F. Eble, editors. The Eastern Oyster Crassostrea virginica. College Park, MD: Maryland Sea Grant College Publication, pp. 75-168.

Coe, W. R. 1943. Sexual differentiation in mollusks. I. Pelecypods. *Quarterly Review of Biology*. 18:154-164.

Coon, S. L., W. K. Fitt & D. B. Bonar. 1990. Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. *Mar. Biol*. 106(3): 379-387.

Davis, H. C. & P. E. Chanley. 1956. Spawning and egg production of oysters and clams. *Biol Bull*. 110: 117-128.

Davis, H.C. 1958. Survival and growth of clam and oyster larvae at different salinities. *Biol Bull*. 114(3): 296-307.

Davis, H. C. & A. Calabrese.1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica. Fish. Bull*. 63(3): 643-655.

Davis, J. E. 2013. Effects of basket arrangement and stocking density when using adjustable long-line system for oyster grow-out. Master Dissertation for the Graduate Facility of Auburn University, Auburn, AL, pp.82.

Dekshenieks, M. M., E. E. Hofmann & E. N. Powell. 1993. Environmental effects on the growth and development of eastern oyster, *Crassostrea virginica* (Gmelin, 1791), larvae: A modeling study. *J. Shellfish Res*. 12(2): 241-254.

Del Rio-Portilla, M. A. & A. R. Beaumont. 2000. Larval growth, juvenile size and heterozygosity in laboratory reared mussels, *Mytilus edulis*. *J. Exp. Mar. Biol. Ecol*. 254: 1-17.

Desai, M. & C. N. Hales. 1997. Role of fetal and infant growth in programming metabolism in later life. *Biol. Rev.* 72: 329-348.

FAO. 2018. The State of World Fisheries and Aquaculture – meeting the sustainable development goals. Rome. License: CC BY-NC-SA 3.0 IGO. http://www.fao.org/3/i9540en/i9540en.pdf (accessed November 2019).

Galtsoff, P. S. 1938. Physiology of reproduction of *Ostrea virginica*." II. Stimulation of spawning in the female oyster. *Biol. Bull.* 75:286-307.

Galtsoff, P. S. 1964. The American Oyster, *Crassostrea virginica* (Gmelin). *U. S. Fish. Wildl. Serv. Fish. Bull.* 64: 1-480.

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas & C. Toulmin. 2010. Food security: the challenge of feeding 9 billion people. *Science*. 327:812-818.

Grice, R. & B. Walton. 2019. Alabama shellfish aquaculture situation & outlook report 2018. Alabama Cooperative Extension System. https://www.aces.edu/blog/topics/aquaculture/alabama-shellfish-aquaculture-situation-outlook-report-2018/ (accessed November 2019).

Günther, R. T. 1897. The oyster culture of the ancient Romans. *J. Mar. Biol. Ass. U. K*. 4: 360-365.

Haley, L. E. & G. F. Newkirk. 1977. Selecting oysters for faster growth. *Proc. Wld Maricult. Soc*. 8: 557-565.

Haven, D. & D. Garten. 1972. Shell bags for catching oyster spat. Marine Resources Advisory No. 6. Virginia Institute of Marine Science, College of William and Mary. http://dx.doi.org/doi:10.21221/m2-dc7x-z812.

Havens, K., M. Allen, E. Camp, T. Irani, A. Lindsey, J. G. Morris, A. Kane, D. Kimbro, S. Otwell, B. Pine & C. Walters. 2013. Apalachicola Bay oyster situation report. Technical Publication 201. University of Florida Sea Grant, Gainesville, FL, USA.

Hawkins, A. J., B. L. Bayne, A. J. Day & E. J. Denton. 1986. Protein turnover, physiological energetics and heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific growth. Proceedings of the Royal Society of London. Series B. Biological sciences, 229(1255): 161-176.

Hayes, P. F. & R. W. Menzel. 1981. The reproductive cycle of early setting *Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico, and its implications for population recruitment. *Biol. Bull.* 160:80-88.

Hidu, H. 1969. Gregarious setting in the American oyster *Crassostrea virginica* Gmelin. *Chesapeake Science*. 10:85-92.

Lee, C. -S. & A. C. Ostrowski. 2001. Current status of marine finfish larviculture in the United States. *Aquaculture*. 200:89-109.

Loosanoff, V. L. 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. *Biol. Bull*. 129(3): 546-561.

Loose, S. M., A. Peschel & C. Grebitus. 2013. Quantifying effects of convenience and product packaging on consumer preferences and market share of seafood products: the case of oysters. *Food Qual. Prefer*. 28: 492-504.

Losee, E. 1979. Relationship between larval and spat growth rates in the oyster (*Crassostrea virginica*). *Aquaculture*.16: 123-126.

MacKenzie, C. L., Jr. 2007. Causes underlying the historical decline in eastern oyster (*Crassostrea virginica* Gmelin, 1791) landings. *J. Shellfish Res*. 26(4): 927-938.

McCormick, M. I. & B. W. Molony. 1992. Effects of feeding history on the growth characteristics of a reef fish at settlement. *Mar. Biol.* 114: 165-173.

Merilä, J. & E. Svensson. 1997. Are fat reserves in migratory birds affected by condition in early life? *J. Avian Biol.* 28: 1276-1286.

Meyer, E. & D. T. Manahan. 2010. Gene expression profiling of genetically determined growth variation in bivalve larvae (*Crassostrea gigas*). *J. Exp. Biol*. 213(5): 749-758.

Mizuta, D. D. & G. H. Wikfors. 2019. Seeking the perfect oyster shell: a brief review of current knowledge. *Reviews in Aquaculture* 11: 586-602.

[NRC] National Research Council. 2004. Non-native Oysters in the Chesapeake Bay. Washington D.C.: National Academy Press, pp. 1-326. https://www.nap.edu/read/10796/chapter/1 (accessed December 2019).

Nelson, T. C. 1924. The attachment of oyster larvae. *Biol. Bull.* 46(3): 143-151.

Newell, R. I. E. & C. J. Langdon. 1996. Mechanisms and physiology of larval and adult feeding. In: V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editors. The Eastern Oyster Crassostrea virginica. College Park, MD: Maryland Sea Grant College Publication, pp. 185-229.

Newkirk, G. F. 1981. On the unpredictability of bivalve growth rates: is a slow growing juvenile oyster a runt for life. In: C. Claus, N. de Pauw and E. Jaspers, editors. Nursery Culturing of Bivalve Molluscs. *European Mariculture Society*, Bredene, Belgium, pp. 211-218.

Newkirk, G. F. & L. E. Haley. 1982. Phenotypic analysis of the European oyster *Ostrea edulis* L.: relationship between length of larval period and postsetting growth rate. *J. Exp. Mar. Biol. Ecol.*, 59: 177-184.

Newkirk, G. F., L. E. Haley, D. L. Waugh & R. Doyle. 1977. Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica*. *Mar. Biol.* 41: 49-52.

NOAA Fisheries. 2018. Aquaculture Production Highlights Infographic, 2017. Fisheries of the United States. https://www.fisheries.noaa.gov/national/aquaculture/us-aquaculture (accessed March 2020).

Onitsuka, T., T. Kawamura, S. Ohashi, S. Iwanaga, T. Horii & Y. Watanabe. 2010. Effects of delayed metamorphosis and delayed post-settlement feeding on post-larval survival and growth of the abalone *Haliotis diversicolor*. *Aquaculture.* 298: 239-244.

Parker, L. M., W. A. O'Connor, D. A. Raftos, H. O. Pörtner & P. M. Ross. 2015. Persistence of positive carryover effects in the oyster, *Saccostrea glomerate*, following transgenerational exposure to ocean acidification. *PLoS One*. 10(7): e0132276 (accessed March 2020).

Pechenik, J. A. 2006. Larval experience and latent effects—metamorphosis is not a new beginning. *Integr. Comp. Biol*. 46(3): 323-333.

Pechenik, J. A. 2018. Latent effects: surprising consequences of embryonic and larval experience on life after metamorphosis. In: T. J. Carrier, A. M. Reitzel, and A. Heyland, editors. Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford. pp. 208-225.

Pechenik, J. A., D. E. Wendt & J. N. Jarrett. 1998. Metamorphosis is not a new beginning: larval experience influences juvenile performance. *Bioscience*. 48(11): 901-910.

Petrolia, D. R. & W. C. Walton. 2018. Hurricanes and water wars threaten the Gulf Coast's new high-end oyster industry. The Conversation. https://theconversation.com/hurricanes-and-water-wars-threaten-the-gulf-coasts-new-high-end-oyster-industry-105479 (accessed November 2019).

Phelps, R. P. 2010. Recent advances in fish hatchery management. *Revista Brasileira de Zootecnia.* 39: 95-101.

Phillips, N. E. 2002. Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. *Ecology*. 83(9): 2562-2574.

Przeslawski, R. & A. R. Webb. 2009. Natural variation in larval size and developmental rate of the northern quahog *Mercenaria mercenaria* and associated effects on larval and juvenile fitness. *J. Shellfish Res*. 28(3): 505-510.

Shumway, S. E. 1996. Natural environmental factors. In: V. S. Kennedy, R. I. E. Newell, & A. F. Eble, editors. The Eastern Oyster Crassostrea virginica. College Park, MD: Maryland Sea Grant College Publication, pp. 467-511.

Spencer, L. H., Y. R. Venkataraman, R. Crim, S. Ryan, M. J. Horwith & S. B. Roberts. 2020. Carryover effects of temperature and pCO_2 across multiple Olympia oyster populations. *Ecological Applications*. 30(3): e02060. 10.1002/eap.2060.

Stoeckel, J. A., D. K. Padilla, D. W. Schneider & C. R. Rehmann. 2004. Laboratory culture of *Dreissena polymorpha* larvae: spawning success, adult fecundity, and larval mortality patterns. *Can. J. of Zool*. 82(9):1436-1443

Talmage, S. C. & C. J. Gobler. 2009. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virignica*). *Limnol. Oceanogr.* 54(6): 2072-2080.

Tamayo, D., I. Ibarrola, M. B., Urrutia & E. Navarro. 2011. The physiological basis for inter-individual growth variability in the spat of clams (*Rduitapes philippinarum*). *Aqaculture*. 321: 113-120.

Tamayo, D., I. Ibarrola, I. Urrutxurtu & E. Navarro. 2014. Physiological basis of extreme growth rate differences in the spat of oyster (*Crassostrea gigas*). *Mar. Biol.* 161(7): 1627-1637.

Thomas, L. L., S. K. Allen Jr & L. V. Plough. 2019. The effect of aquaculture gear on the growth and shape of the oyster *Crassostrea virginica* during a "finishing period" in Chesapeake Bay, USA. *Aquaculture.* 508: 1-9.

Thompson, R. J., R. I. E. Newell, V. S. Kennedy & R. Mann. 1996. Reproductive Processes and Early Development. In: V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editors. The Eastern Oyster Crassostrea virginica. College Park, MD: Maryland Sea Grant College Publication, pp. 335-370.

Togo, T. & M. Morisawa. 1999. Mechanisms for blocking polyspermy in oocytes of the oyster *Crassostrea gigas*. *J. Exp. Zool*. 283(3): 307-314.

Wacker, A. & E. von Elert. 2002. Strong influences of larval diet history on subsequent post-settlement growth in the freshwater mollusk *Dreissena polymorpha*. *Proc R Soc Lond B*. 269: 2113-2119.

Wallace, R. K. 2001. Cultivating the Eastern Oyster, *Crassostrea virginica*. *Southern Regional Aquaculture Center* Publication No. 432.

Wallace, R. K., P. Walters & F. S. Rikard. 2008. Oyster Hatchery Techniques. *Southern Regional Aquaculture Center* Publication No. 4302.

Walton, William, 2020. Oyster Aquaculture Extension Specialist. Auburn University Shellfish Laboratory, Dauphin Island, Alabama. Personal comment.

Walton, W. C., F. S. Rikard, G. I. Chaplin, J. E. Davis, C. R. Arias & J. E. Supan. 2013. Effects of ploidy and gear on the performance of cultured oysters, *Crassostrea virginica*: survival, growth, shape, condition index and *Vibrio* abundances. *Aquaculture*. 414-415: 260-266.

Wheaton, F. 2007. Review of the properties of eastern oysters, *Crassostrea virginica*: Part I. Physical properties. *Aquaculture Engineering*. 37: 3-13.

Widdows, J., R. I. E. Newell & R. Mann. 1989. Effects of hypoxia and anoxia on survival, energy metabolism, and feeding of oyster larvae (*Crassostrea virginica*, Gmelin). *Biol. Bull.* 177(1): 154-166.

Wilberg, M. J., M. E. Livings, J. S. Barkman, B. T. Morris & J. Robinson. 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. *Mar. Ecol. Prog*. 436: 131-144.

CHAPTER TWO:

EFFECTS OF DIFFERENCES IN LARVAL DURATION PERIOD UPON FIELD PERFORMANCE OF CULTURED EASTERN OYSTER (*Crassostrea virginica*)

Introduction

Globally, freshwater and marine fisheries stocks have decreased because of overexploitation from the rapid increase in human population. According to Godfray et al. (2010), the human population is predicted to reach around 9 billion before the 21st century concludes. In order to reach production goals for the future, farmed raised seafood can be used to restock natural populations for conservation, harvesting efforts, and consumer consumption. The Eastern oyster (*Crassostrea virginica* Gmelin, 1791) is a nutritious bivalve that has been harvested continuously for its large consumer demand. Nationwide, oyster aquaculture is valued at over 185 million dollars and accounts for over 46% of the total value of farmed marine species produced (NOAA 2018). In the 2018 world review, oysters from the genus *Crassostrea* made up 28% total of the percentage of molluscan species that were produced worldwide through aquaculture efforts in 2016, which was the greatest proportion in that phylum (FAO 2018). In 2018, Alabama brought in a farm gate value of over 1 million dollars and provided over 60 job opportunities for the state through the expansion of the commercial oyster farm industry (Grice and Walton 2019). In order to be successful in this evolving industry, farmers have begun to change their growing techniques from a bottom-culture to an off-bottom culture that suspends oysters in the water column, which allows production of high quality oysters to sell in the competitive half-shell market (e.g. Mizuta and Wikfors 2019, Thomas et al. 2019, Davis 2013, Walton et al. 2013). To assist in the production of selling marketable oysters in the competitive oyster industry, shellfish hatcheries aim to produce high quality larvae and seed by selectively breeding broodstock oysters that have a phenotype of a deep cup, wide fan, teardrop shape, and no backbend at the hinge for easier shuckability.

The production of cultured aquatic species depends heavily on the support from hatchery systems. Hatcheries are receiving an increase of demand for seed by growers because of the steady increase of oyster farms. These farmers want to be sure that the seed they pay a hatchery full price for is excellence seed that will demonstrate high quality performance during field grow-out. Oyster farmers want to be sure that their oysters will have high survival and growth, so that they can be harvested for the market in an expected timeframe with good survival. Therefore, it is important to understand if larval performance during hatchery production affects the performance of cultured oysters during the field grow-out stage.

Hatcheries culture larvae to achieve higher production rates through the meticulous and careful procedures that are used in a controlled environment, which can largely avoid, manipulate, or fix any potential factors that could affect larval development and survival. However, same-age bivalve larvae naturally differ in size and siblings can reach the metamorphic stage at different times when cultured in controlled conditions (for the Eastern oyster, for example: Newkirk et al. 1977, Haley & Newkirk 1977, and Losee 1979; zebra mussel: Stoeckel et al. 2004; and Northern quahog: Przeslawski & Webb 2009). This intraspecific development and growth variations extend larval duration period within the larval culture. Size variations among two commercial aquaculture species, Eastern oyster and Northern quahog (*Mercenaria mercenaria*), were examined, and both studies found that the larger larvae continued to have better performance in the juvenile stage compared to the slow growing larvae (Losee 1979, Przeslawski & Webb 2009, respectively). Additionally, unfavorable conditions for aquatic invertebrates, such as a stressful environment, can potentially cause a delay in achieving metamorphosis, which creates a latent effect that ultimately affects performance later in the

juvenile and adult stages (Pechenik 2006, Pechenik 2018). Therefore, larval performance and experiences have been found to affect growth and development of juveniles.

However, for the commercial oyster industry, understanding how larval variation affects performance beyond the juvenile stage is important for commercial production purposes. For example, female European flat oysters (*Ostrea edulis*) will incubate their larvae in the pallial cavity up to 10 days, depending on temperature, and release the larvae into the water column to continue larval development before metamorphosis occurs (Lapègue et al. 2006). For this species, Newkirk and Haley (1982) found that even though the duration of the larval period did have an effect on growth rate of juveniles that were, on average, a length of 23 mm, the effect disappeared when the oysters measured >60 mm in length. This indicated that individuals that were fast growing in the larval stage do not potentially demonstrate that performance for life (Newkirk and Haley 1982).

As the commercial oyster industry increases along with seed demand, farmers want to know if they are receiving high quality seed. Prompted by inquiries from local farmers concerned that they had gotten seed produced from the 'tail end' of a larval culture and are concerned that those seed could be 'runts' and slow growing, this study was conducted to determine if Larval Duration Period has an effect on subsequent performance in the Eastern oyster, *C. virginica*, a broadcast spawner. The objectives were to determine if Larval Duration Period affects metamorphic success, performance in land-based nursery culture, and performance in field grow-out culture. All Larval Duration Period treatment groups were grown under uniform conditions to ensure each group had the same rearing experience. Ultimately, this study will provide insight to hatchery managers, commercial growers, and researchers on how to maximize the quantity and quality of Eastern oyster larvae production.

Methods

Spawning Procedure

Two distinct Auburn University Shellfish Laboratory (AUSL) diploid Eastern oyster (*Crassostrea* virginica) broodstock lines were mated on July 10, 2018. Spawning was induced using thermal shock by pumping over 1 µm filtered ambient saltwater from cool and warm 4,000 L tanks that measured 27.5 and 34.6 ° C, respectively. Salinity in the two tanks measured ~21 ppt. After three hours of alternating the water temperature and adding immobile sperm to the individual chambers, a total of 29 and 8 females released eggs and a total of 8 and 2 males released sperm from the two broodstock lines, AL16A and AL16B, respectively.

Since spawning occurred randomly within the three hours, a total of four buckets were used for fertilization purposes. Three buckets had eggs from the AL16A broodstock oysters and one bucket combined the eggs from the AL16B broodstock line. Sperm from AL16B were pooled in equal proportions and added to the AL16A egg buckets. Sperm concentrate was added at conservatively low volumes in order to control fertilization and to reduce chances of polyspermy. To ensure sufficient fertilization, after 20 min, a sample of the mixed gametes were examined under a compound microscope (Olympus, Tokyo, Japan). Similarly, the same procedure was used for the introduction of AL16A broodstock sperm to the single bucket of AL16B eggs.

In order to create a random and even distribution of all the fertilized eggs from the two lines, the four fertilized buckets were filled with filtered saltwater to 8 L and then 2 L from each mixed bucket were added to 4 new buckets. After filling the new buckets up to 14 L each, a 100 µL sample was taken with a micropipette from each bucket while simultaneously plunging gently in order to evenly mix the eggs for a precise sample to calculate the total density.

Proportion fertilized and total number of fertilized eggs were calculated as follows (Equation 1-2):

(Equation 1)

Proportion fertilized = [No. of developing eggs (polar bodies) + No. of dividing eggs] / total No. of eggs

(Equation 2)

Total number of fertilized eggs in bucket = [(No. of developing eggs + No. of dividing eggs) / mL] × bucket volume (mL)

The spawn had a 91.38% fertilization success and produced a total of 256.62 million fertilized eggs. Six 4,000 L tanks were stocked with 10.69 fertilized eggs/mL (42.77 million fertilized eggs/tank) to begin larval culture (designated 'Day 0').

Larval Culture

Normal hatchery procedures were conducted to mimic commercial hatcheries. Larval tanks were aerated with the use of aeration lines attached to the bottom of a centered standpipe. This allowed air to rise with just enough pressure to circulate the tank without disturbing the free-swimming larvae. Salinity and temperature ranged from 15.3 – 25.6 ppt and 27.6 – 31.3º C, respectively through the 18 days of larval culture. The first day after the spawn was considered Day 1 of culture and larvae achieved the D-hinge larval stage. Larvae were batch fed a calculated feed ration of Reed Mariculture Inc.'s Shellfish Diet 1800® twice a day depending on larval culture day and stocking density (Rikard and Walton 2012). Larval drain downs began on Day 2 and were done every other day until Day 8, which were then conducted daily to ensure the maximum number of pediveligers would be culled for setting purposes. During drain downs, new 1 µm filtered seawater was added into clean 4,000 L tanks with 4 g of VWR® EDTA

Disodium Salt Dihydrate for Biotechnology to ensure optimal water quality conditions in larval tanks. Larvae were carefully drained down into 20 µm (for Day 2) and 40 µm (all subsequent drain downs) mesh bags and rinsed with 1 µm filtered ambient saltwater into buckets in order to allow calculation of the total number of larvae per tank, obtain samples for height measurements, and determine larval stocking densities. Tank densities were reduced as larvae grew throughout the culture, according to Wallace et al. (2008). Tanks were also consolidated when the densities decreased due to harvesting pediveligers for setting.

Throughout larval culture, tanks were treated uniformly and stocked at low densities to ensure optimal growth and larval performance. At drain downs, larvae were counted by obtaining two 100 µL formalin fixed samples that were obtained by gently plunging each larval bucket for an even distribution while samples were taken with a micropipette. These samples were examined on a Sedgewick rafter under a compound microscope for counts. Two 1 mL destructive samples of larvae were taken daily from each larval tank and pipetted into microcentrifuge tubes for larval height measurements.

Larval height measurements were obtained with the use of a biological compound microscope or dissecting microscope (depending on size) and Infinity 2 Microscope Camera connected to a desktop computer with iMT iSolution Lite (Martin Microscope, Easley, SC) program for hand-measuring pictures of each larva under the scopes. This program was standardized with a calibration slide for each objective lens that was used in order to correctly examine larval growth. Twenty-five individual height measurements per tank were taken each day during larval culture (Fig 1). Measurements for larvae stopped on the first day ≥900,000 pediveligers were culled out for setting purposes (Day 11).

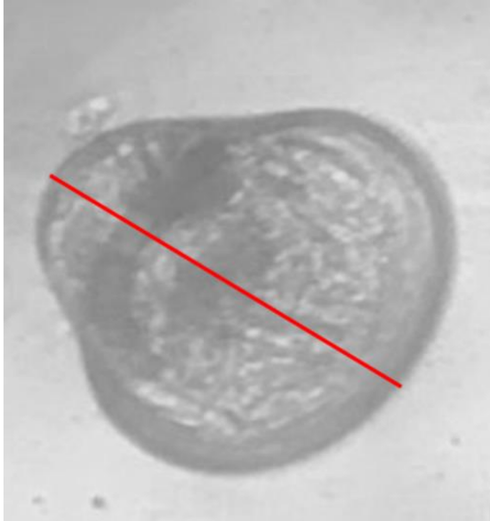

Figure 1. Larval shell height measurement illustrated by the red line.

Determining Treatment Groups

When larvae reached the pediveliger stage, eyed larvae were collected with a 200 µm mesh sieve to obtain individuals that measured ≥282.8 µm from the diagonal length measurement of the mesh, similar to the methods of Wallace et al. (2008). Shell height measurements of the pediveligers (n=10) that retained on the 200 µm mesh sieve per tank were obtained to establish the initial average shell height for the onset of the metamorphic period. The larvae that fell through but were retained on a 75 µm mesh sieve screen were counted and returned to the larval tanks. The "Early" Larval Duration Period was determined by the first day that a minimum number of eyed larvae sufficient to stock 3 setting downwellers (≥900,000 eyed larvae) were collected, which was 11 days post-fertilization. The last day in larval culture that had ≥900,000 eyed larvae was 18 days post-fertilization and designated as the "Late" Larval Duration Period treatment group. The "Peak" treatment group was designated as the day that produced the greatest quantity of eyed larvae, which was 13 days post-fertilization (Fig. 2). Since the Peak and Late treatment groups could not be established a priori, eyed larvae from

each day (days 11-18) were set in static recirculating downweller systems at 300,000 pediveligers per 150 µm size mesh replicate silos (~122 eyed larvae per cm^2 per silo, n = 3 silos) on their respective days. Each treatment group was given 4 days to complete metamorphosis in the downwelling system. In order to produce single oysters (typically used for off-bottom culture), a thin layer of microcultch (oyster shell particles 250-300 µm) was spread on the bottom of each silo (Wallace et al. 2008). Standard daily maintenance of gently scrapping the sides of the silos, spraying screens to detach any larvae that set on the mesh, and water changes were done to all downweller tanks. The Shellfish Diet was batch fed twice a day to each system.

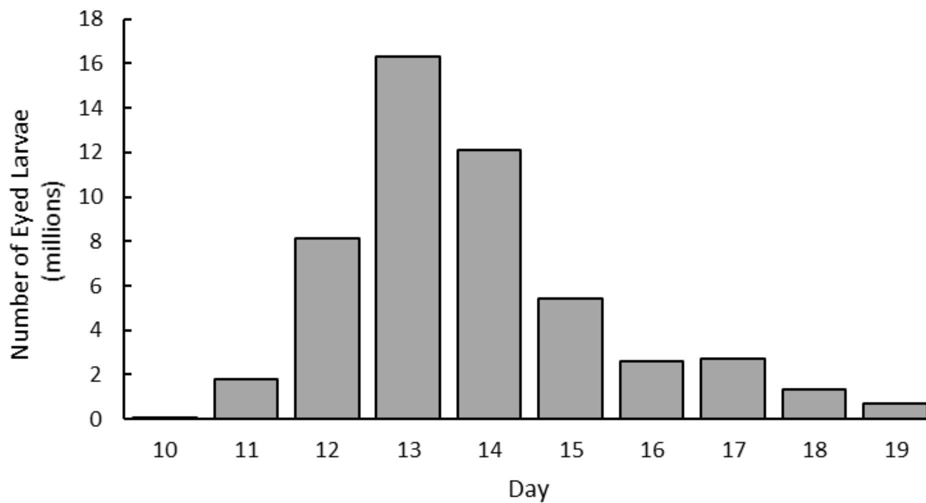

Figure 2. Number of pediveligers that retained on a 200 µm mesh sieve screen each day to determine which days represented the three Larval Duration Period treatment groups: Early (Day 11), Peak (Day 13), and Late (Day 18). Days represent the number of days post-fertilization.

Determining Metamorphic Success

After each group spent four days in the downweller system, the oyster seed were graded on a 300 µm mesh sieve. The seed that retained for each treatment group were placed back in

their respective silos and moved to the flow-through upweller tank system to continue the experiment in the nursery stage. A destructive sample of seed was taken after each nursery grade for shell height measurements (n=20) of the retained seed for each replicate silo (n=3) for only the Early, Peak, and Late treatment groups to determine the average shell height per replicate for the analysis (Fig. 3). The average change in shell height (growth rate (mm/day)) over the metamorphic period was determined for the Larval Duration Period treatment groups (Equation 3).

(Equation 3)

Average post-metamorphic change in shell height = [mean shell height retained on 300 µm mesh screen (oyster seed) – mean shell height retained on 200 µm mesh screen (pediveliger)] / four days

After a standard of five days of culture in the upweller land-based nursery system, all Larval Duration Period groups were graded on a 600 µm mesh sieve and the retained oysters were counted (Table 1). These counts were used to estimate the average metamorphic success percentage for each replicate silo of the Larval Duration Period and determined recruitment to 600 µm (Equation 4). Only seed from the designated Early, Peak, and Late treatment groups that retained were returned to the upwelling system in their individual 500 µm mesh replicate silos. All other Larval Duration Period days were removed from the study at this stage.

(Equation 4)

Metamorphic success percentage = (quantity of seed retained on 600 µm mesh / 300,000 eyed larvae) × 100%

Land-Based Nursery Culture

During the land-based nursery culture, the three Larval Duration Period treatment groups were treated uniformly under standard nursery procedures. As oyster seed grew, stocking densities were reduced after each successful grade and water flow was increased to provide ambient food ad libitum and adequate aeration for optimal growing conditions.

To maintain optimal growth, each group went through a series of grades every 7th day of the nursery culture to reduce stocking densities and increase mesh grade size (Table 2). To ensure that treatments were not treated differently due to differences in growth rates, a "move up" rule was determined. Thus, seed were only moved up if ≥75% of the average amount of seed among the replicates retained on the next size up mesh grade. If not, the group was returned to the upweller system on the previous mesh grade and given two more days before grading again. The group was then graded on the next size mesh grade to determine if ≥75% of the group retained. In these cases, the groups were again sieved in five days to see if they could return to the every 7th day schedule, following the same rule. This occurred twice in the study; both the Peak and Late groups needed two extra days before the 1.4 mm mesh grade was completed, but both were able to move up to the 2 mm mesh grade (the next scheduled grade) in five days. The weekly grading procedure continued until groups retained on a 6 mm mesh sieve. Seed that retained and fell through each grade screen were counted for each of the nursery grades. Destructive subsamples (n=25) for each replicate silo per Larval Duration Period were obtained from each grade and shell height measurements were completed with the IMT iSolution Lite (Martin Microscope, Easley, SC) program in micrometers and with digital calipers to the nearest 0.01 mm as the seed retained on ≥2 mm mesh (Fig 3). Each of the three groups grew in the land-

based nursery culture for a total of 37 days (Table 1), albeit with different start dates: Early 07/21 – 08/27, Peak 07/23 – 08/29, and Late 07/28 – 09/03.

Table 1. The grading schedule for each Larval Duration Period treatment group. Grading for Day 0, 4, and 9 were standard for all groups, but the remaining days in the land-based nursery culture were determined weekly by the "move up" rule.

	Days in Land-Based Nursery Culture for Each Treatment Group		
Sieve Screen Size for Grade	**Early**	**Peak**	**Late**
200 µm	0	0	0
300 µm	4	4	4
600 µm	9	9	9
1.4 mm	16	18	18
2 mm	23	23	23
4 mm	30	30	30
6 mm	37	37	37

Table 2. Stocking densities for each replicate silo (n=3) for the seed that retained on the respective size sieve screen used in the weekly grading procedure.

Grade	Density of Retained Seed per Silo
600 µm	80,000
1.4 mm	40,000
2 mm	20,000
4 mm	10,000

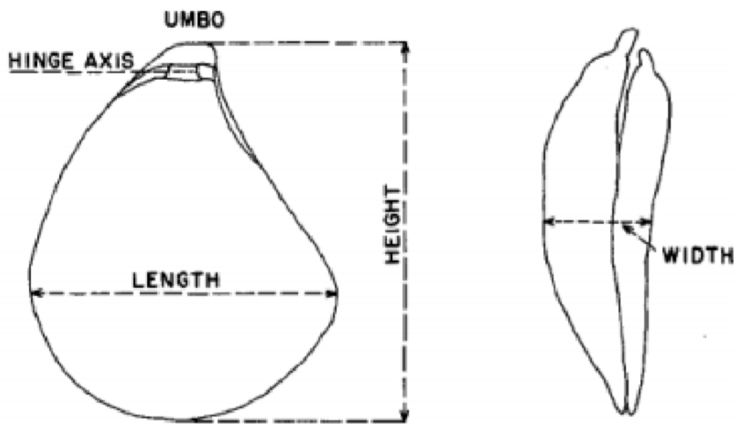

Figure 3. Illustration of the shell metrics used to determine growth for land-based nursery and field grow-out cultures (Galtsoff 1964).

Field Nursery Procedure

Once each group achieved the 6 mm grade, the three replicate silos for each group were combined, mixed, and evenly distributed among eleven 4 mm BST^{TM} hexagonal mesh baskets (n=~1,636 oysters) to be deployed at Grand Bay Oyster Park (GBOP), located in Grand Bay, AL on an Adjustable Longline System (ALS). The baskets were hung at an intertidal height to help control biofouling. At the nursery field grow-out site, oysters in the three Larval Duration Period treatment groups were allowed to grow so that all groups would have $\geq$75% retained on a 12 mm grade. On October 9th, seed from all groups were brought back to AUSL to be graded. On October 10th, each of the three groups were mechanically graded separately by use of the QuickTube Sorter^{TM} manufactured by the Chesapeake Bay Oyster Company in order to sort out seed that retained on 12 mm mesh, with each group retaining $\geq$75%. Seed that both fell through the 19 mm diameter hole section and came out the end of the tumbler were combined to generate the seed (that did not fall through 12 mm holes in the tumbler) used for field grow-out. Shell

height, length, and width oyster measurements were obtained for each group post-grade (n=75) with digital calipers to the nearest 0.01 mm (Fig. 3). For each group, seed were combined, mixed, and distributed among the field grow-out replicates.

Field Grow-Out Experimental Design

The three groups were deployed at three different AUSL field grow-out sites: GBOP, Bayou Sullivan Oyster Park (BSOP), and Dauphin Island Sea Lab's Dock (Fig. 4). Seed from each group were grown in six replicate 12 mm hexagonal mesh BST™ baskets on the ALS at an intertidal height and hung within each 'bay' (Fig. 5). For deployment, six replicate baskets were stocked with ~400 live single oysters per treatment group, using a weighing sub-sample method (Alabama Cooperative Extension System 2019).

Sampling (described below) was conducted 4, 7 and 10 months after the October 11th deployment date (Table 3). At the 4 month (February) sample date, the density in each basket was reduced to 100 oysters/basket (hand-counted) for all replicates at the three sites. Grow-out techniques were uniform for the Larval Duration Period treatment groups at each site and mimicked oyster farming methods.

Table 3. Monthly sample dates and the corresponding number of days between each sample period for each of the three sites were used to calculate the change in shell height per day for the Larval Duration Period treatment groups. Deployment for all sites was 10/11/2018.

	February Sample Date	No. of Days from Deployment	May Sample Date	No. of Days from Previous Sample	August Sample Date	No. of Days from Previous Sample
GBOP	02/25/3019	137	05/28/2019	92	08/28/2019	92
BSOP	02/26/2019	138	05/29/2019	92	08/28/2019	91
AUSL Dock	02/27/2019	139	05/30/2019	92	08/28/2019	90

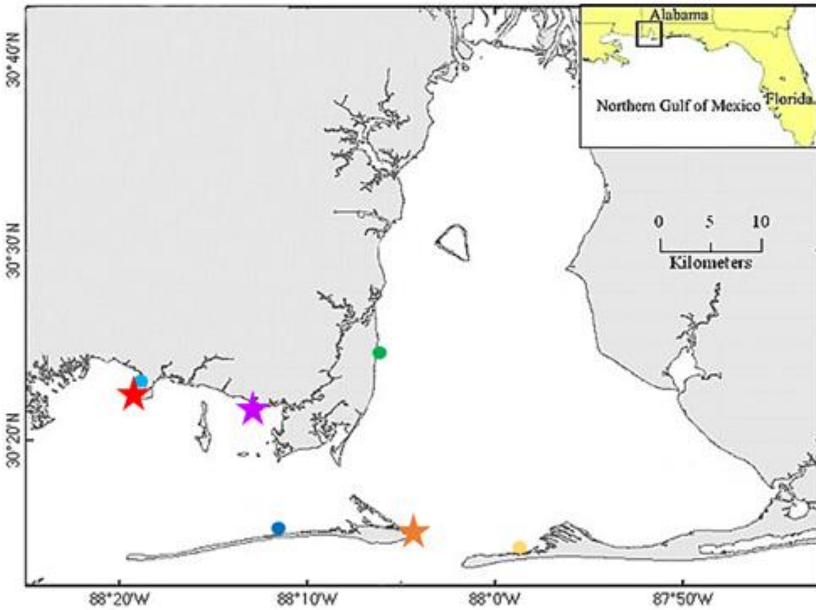

Figure 4. A map of the Mobile Bay and the Mississippi Sound regions located in the Northern Gulf of Mexico (adopted from Kim et al. 2010). The stars represent the Auburn University Shellfish Laboratory Oyster Park grow-out sites where the three treatment groups were deployed: Grand Bay Oyster Park (red), Bayou Sullivan Oyster Park (purple), and Dauphin Island Sea Lab's Dock (orange). The circles denote the four commercial oyster farms spanning a range of growing conditions along the Alabama coast that grew the three treatment groups, which were: Point aux Pins Oyster Farm (bright blue), Mobile Oyster Company (dark blue), DePe's Oysters (green), and Navy Cove Oyster Company (yellow).

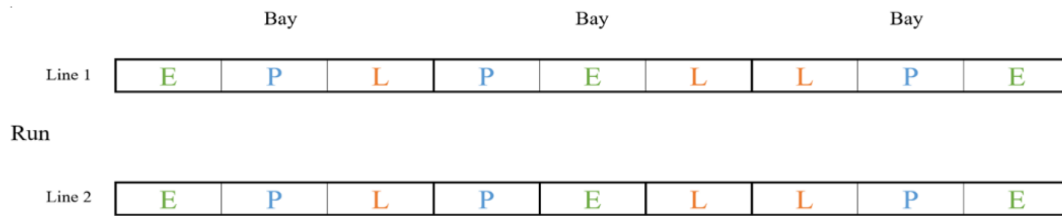

Figure 5. A diagram of the replicate bag arrangement within 'bays' for the Larval Duration Period treatment groups that were cultured on the Adjustable Longline System (ALS) at each of the Auburn University Shellfish Lab grow-out farm sites. One ALS run consists of two lines. On both lines, each of the three Larval Duration Period treatment groups had one bag arranged at each of the three bays. Three bays were used per line to hold the replicate bags (n=6) per treatment group: green "E" (Early), blue "P" (Peak), and orange "L" (Late).

Sampling

Destructive, haphazard random sub-samples of oysters (n=30) were collected from each replicate (n=6) per Larval Duration Period (3 groups) and taken from each of the field grow-out sites (3 sites) during each Sample Time (3 sample periods). Live and dead oysters were counted during the 7 and 10 month post-deployment sampling dates (May and August, respectively) for each treatment group. Dead oysters were collected in May and August for shell height measurements of the bottom valve (to minimize double counting of dead oysters). Survival was calculated with similar methods as described by Ragone Calvo et al. (2003). Interval survival (Equation 5) was determined for both February to May and May to August, with dead oysters removed. Cumulative survival (Equation 6) was used to determine the average survival of each Larval Duration Period group, accounting for the destructive sampling that occurred in May and

August, in order to determine the probability of surviving to August. Shell height, length, and width measurements were taken of each live oyster from the sub-samples by the use of digital calipers to the nearest 0.01 mm (Fig. 3). Cup and fan ratios were obtained as well (Equation 7 and 8, respectively).

Condition index was assessed on 15 of the 30 oysters from the sub-sample. Those oysters were individually weighed on a Metler Toledo AL204 balance to quantify whole wet weight to the nearest 0.0001 g. Oysters were then shucked and tissue was removed from the shell. The tissues were independently placed in individually labeled VWR® aluminum dishes. After 48 hours in a Fisher Scientific Isotemp oven set at 80°C, the dry tissue weight was obtained to the nearest 0.0001 g. The shells of the oysters were left to air dry for 48 h in individual Petri dishes with the same tag as their corresponding tissue. The dry shells were weighed on the balance to obtain dry shell weight to the nearest 0.0001 g. Condition index (Equation 9) was calculated as described by Abbe & Sanders (1988), where the shell cavity volume is essentially the difference between whole wet weight (g) and dry shell weight (g). Average change in shell height per day (Equation 10) was obtained for all of the sample oysters at to the nearest 0.001 mm/day in order to find the mean "growth rate" between October – February, February – May, and May – August intervals for each replicate basket at the three sites. Individual growth rates were not obtained because the same oysters were not measured over time.

(Equation 5)

Interval Survival = total live oysters at end of interval / [total live + dead oysters at end of interval]

(Equation 6)

Cumulative Survival = [survival of the February to May interval × survival of the May to August interval] × 100 (%)

(Equation 7)

Cup Ratio = shell width (mm) / shell height (mm)

(Equation 8)

Fan Ratio = shell length (mm) / shell height (mm)

(Equation 9)

Condition index= [dry tissue weight (g) / shell cavity volume (ml)] × 100

(Equation 10)

Change in shell height per day = [current sample month's shell height (mm) – previous sample month's replicate's mean shell height (mm)] / No. of days since previous measurement

Environmental Data

Temperature and salinity data were examined using multiple water quality monitoring loggers. At GBOP, environmental data was first taken from an In-Situ Inc. Aqua TROLL 600 Multi-Parameter Sonde for the field nursery portion till February 2019. However, this probe began to have missing data points. A HOBO® U24-002 C Conductivity Logger (Onset Computer Corporation, Bourne, Massachusetts) was used in replace to log data for the duration of the experiment. BSOP was monitored with YSI 6600 VS Multi-Parameter Sonde from the beginning of field grow-out till May 9, 2019 (Yellow Springs Instruments, Yellow Spring, Ohio). Some data from this probe became unreliable due to sensor drift. A HOBO® U24-002 C Conductivity Logger was then deployed at this site to replace the YSI. However, the HOBOs at both locations had data points that were questionable for several periods of the deployments, which made the use of the data hard to examine. The hydrographic data for the DISL dock site

was collected through Alabama's Real-Time Coastal Observing System with the use of a long-term monitoring station on Dauphin Island, which is maintained by DISL and the Mobile Bay National Estuary Program.

Commercial Farmers' Field Grow-Out Production

Oysters from each treatment group that were left over after the distribution to the field sites in October were distributed evenly to four local Alabama commercial oyster farms: DePe's Oysters, Navy Cove Oyster Company, Point aux Pines oyster farm, and Mobile Oyster Company (Fig. 4). Farmers were allowed to perform their own farming techniques with the Larval Duration Period treatment groups to see how each group performed at commercial farm operations. Two of the farms used OysterGro™ floating cages and two used ALS for grow-out. E-mails were exchanged with farmers to get feedback on any observations they had during the field grow-out period. Shell height measurements were obtained in the beginning of August 2019 for each Larval Duration Period group grown at three of the four commercial farm sites. Random, haphazard sub-samples (n=10 oysters) per replicate bag were used to collect height data. However, only one cooperator farm had replicates for each treatment that were handled uniformly in order to be properly analyzed for the average change in shell height per day.

Statistical Analysis

Land-based nursery response variables were analyzed using one-way analysis of variance (ANOVA) and *lsmeans* package in the statistical software program, RStudio (R Development Core Team 2019). Statistical significance was determined among Larval Duration Period treatment groups (2 degrees of freedom (df)) and the following response variables: post-metamorphic growth rate (change in shell height (mm) during metamorphic period), growth rate for each grade, growth rate for the overall time spent in the land-based nursery culture and

recruitment to 4 mm and 6 mm. Each nursery grade (600 µm, 1.4 mm, 2 mm, 4 mm, and 6 mm) was analyzed separately. To determine the metamorphic success response variable, statistical significance was analyzed among the treatment groups (7 df) for each of the days (11-18) the pediveligers were collected to achieve metamorphosis. The mean data for each of the replicate silos (n=3) per Larval Duration Period were analyzed. Post-hoc comparisons of the treatment means were completed by Tukey's Honest Significant Difference Test. Shapiro-Wilk normality test and Fligner-Killeen test of homogeneity of variances were used to validate the assumptions of normality and homogeneity of variance, respectively. The data were considered normally distributed and variances homogenous when $p > 0.05$. Only the growth rate data for the 2 mm grade was found to be non-normal, so the data were exponentially transformed to meet the assumptions of normality.

Field grow-out response variables were analyzed using SAS University Edition statistical software program (SAS Institute Inc. 2018). A mixed model repeated measures ANOVA was used to determine the statistical significance based on the mean values of each replicate BST basket for each Larval Duration Period treatment group at the AUSL experimental field sites (GBOP, BSOP, and DISL Dock) (PROC MIXED; SAS 2018) for the following field grow-out response variables: cup and fan ratios, growth rate (change in shell height (mm) per day), condition index, dry shell weight (g), dry tissue weight (g), whole wet weight (g), and cumulative survival (%). Site was considered a random factor because the three field sites were used as a representation of aquaculture locations across the Northern Gulf of Mexico and to exemplify the different environmental conditions farmers experience at those locations (2 df). Larval Duration Period treatment groups (2 df), Sample Time (2 df), and the interaction between Larval Duration Period and Sample Time (4 df) were considered fixed effects, with Sample Time

as a repeated factor. Since Site was a random factor, Site × Larval Duration Period, Site × Sample Time, and Site × Larval Duration Period × Sample Time were considered random interactions and their variance components were generated. Variance components were calculated as percentage from the restricted maximum likelihood (REML) in the SAS PROC MIXED. For variance components that were >0, likelihood ratio statistics were constructed by determining the difference of the -2 Res[tricted] log-likelihood from the full model to a model that changed the given variance component to zero with the use of the PARMS statement (Butts & Litvak 2007). This allowed for the determination of χ^2, which was then halved to determine the statistical significance of each variance component (Littell et al. 1996; Messina & Fry 2003; Fry 2004). For statistical significance, alpha was set at 0.05. Shapiro-Wilk tests were done to test the normality assumption and plots of residuals versus fit values were run to test for homogeneity of variances (PROC GPLOT, SAS Institute 2018). When data did not achieve normality and/or homoscedasticity assumptions, data were transformed by arcsine square-root and $\log_{10}$ (Zar 1996). A multiple comparison was run on the least square means of the fixed effects by an adjusted Tukey-Kramer post-hoc test to determine statistical significance. The commercial farm data was analyzed with the same process as the data from the land-based nursery grades.

Results

Metamorphic Success

The average post-metamorphic growth rate (mm/day) was not significantly different among the Larval Duration Period groups ($p = 0.35$, Fig. 6A). On average (mean ± SEM) Early, Peak, and Late grew 0.08 ± 0.0063, 0.069 ± 0.0063, and 0.066 ± 0.0063 mm/day, respectively during the metamorphic setting period. For metamorphic success (percent recruitment to 600

µm), there were no significant differences among Early, Peak, and Late groups ($p = 0.19$, Fig 6B). On average (mean ± SEM), the metamorphic success was lowest for the Early group (51.1 ± 4.23 %) compared to the Peak and Late treatment groups which had 59.8 ± 4.23 % and 63.3 ± 4.23 % retention, respectively. However, when all Larval Duration Days (days 11-18) were considered, Larval Duration Days had a significant effect on metamorphic success ($p < 0.001$, Fig. 6C). Percent recruitment to 600 µm for Day 12 was significantly lower than Days 11, 13-14 and 16-18 by at least 22% ($p < 0.05$).

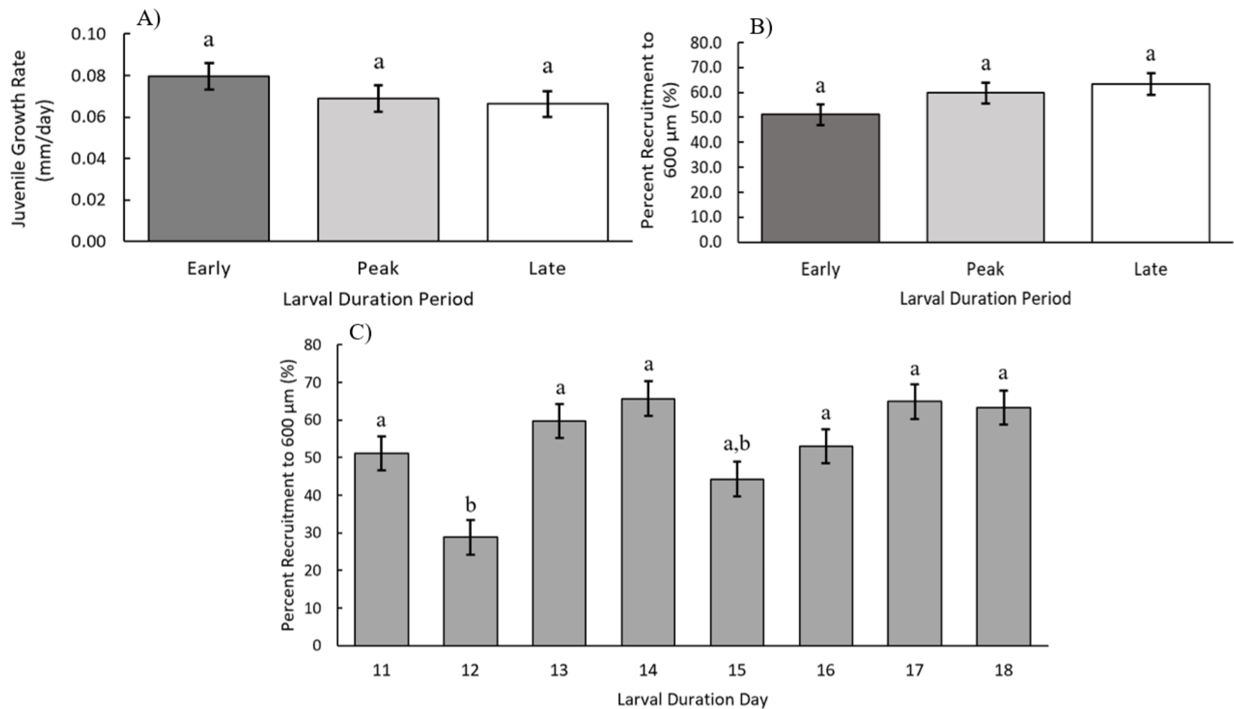

Figure 6. Average growth rate (mm/day) (A) and average metamorphic success (%) (B) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period. Average metamorphic success (%) for all Larval Duration Days (C). Error bars represent standard error of the mean. Sample size included the averages of three replicate silos for each larval duration group. Different letters indicate a significant difference ($p < 0.05$).

Land-Based Nursey Culture Growth Rate

Average growth rate (mm/day) varied for the Larval Duration Period throughout the land-based nursery system. There was a significant effect of Larval Duration Period treatment groups on the growth rate of the seed that retained on the 600 µm grade sieve screen ($p = 0.004$, Fig. 7A). The Early group had a growth rate that was significantly faster compared to the Peak and Late groups ($p \leq 0.011$); with no significant difference between Peak and Late ($p = 0.64$, Fig. 7A). Nevertheless, for the next greater size grade screen (1.4 mm), the seed that retained for each group had similar average growth rates of around 0.2 ± 0.0075 mm/day and there was no significant difference among LDP ($p = 0.67$, Fig. 7B). The Late group had a significantly faster average growth rate compared to the other two groups for the ending on the 2 mm size grade screen ($p \leq 0.001$, Fig. 7C). For the 4 mm sieve grade, Late average growth rate was significantly slower compared to the Early group ($p = 0.0048$) and Peak did not significantly differ from either Early or Late ($p \geq 0.07$ Fig. 7D). At the last grade in the land-based nursery culture (6 mm), there was a significant effect of Larval Duration Period on the oysters ($p = 0.023$, Fig. 7F); the Peak group had a significantly faster average growth rate compared to the Early and Late treatment groups ($p \leq 0.043$, Fig. 7F). Overall, the growth rate from the start (300 µm) to the end (6 mm) of the land-based nursery system suggests that Late had a significantly slower growth rate compared to the other two groups ($p \leq 0.046$, Fig. 8). This indicates that the Late group has a disadvantage, in terms of growth rate, at the end of land-based nursery culture. Furthermore, all groups increased growth rate from the 600 µm to 2 mm grade. The Early group had the largest, on average, shell height (mm) at the field nursery deployment time compared to the other groups (Appendix A).

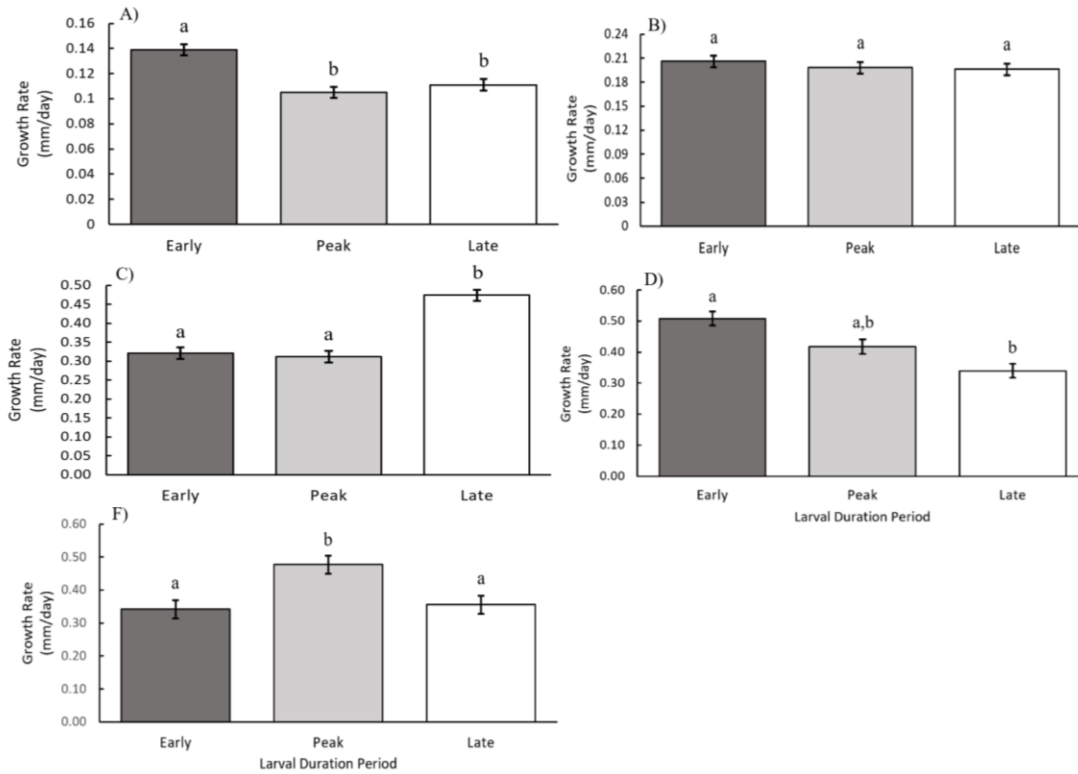

Figure 7. Average growth rate (mm/day) of the seed that retained on a 600 µm grade screen (A), 1.4 mm grade screen (B), 2mm grade screen (C), 4mm grade screen (D), and 6mm grade screen (F) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups. Error bars represent standard error of the mean. Sample size included the averages of three replicate silos for each larval duration period. Different letters indicate a significant difference ($p < 0.05$).

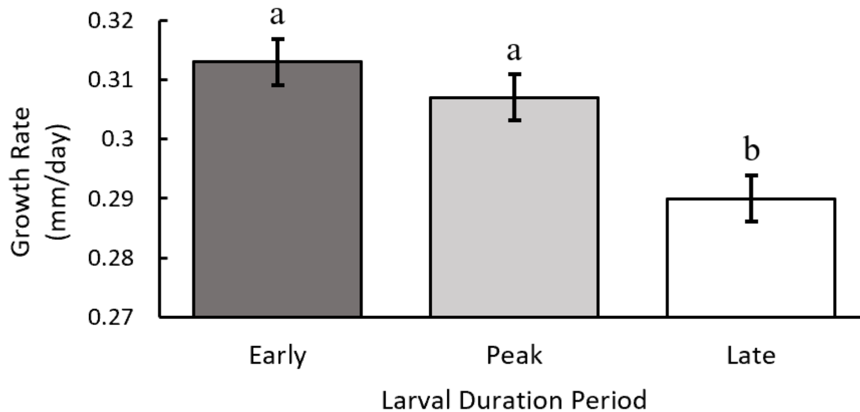

Figure 8. Average growth rate (mm/day) of the seed from the start (300 µm grade) to end (6 mm grade) of the land-based nursery culture for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups. Error bars represent standard error of the mean. Sample size included the averages of three replicate silos for each larval duration period. Different letters indicate a significant difference ($p < 0.05$).

Land-Based Recruitment to 4 mm and 6 mm

Seed of each Larval Duration Period were estimated using the weighing method at each grade to find the average percent retention for each of the respective grades. Due to the differences in days required for the different Larval Duration Period groups to reach 1.4 mm, only the 4 mm and 6 mm retention percentages were analyzed because all groups remained on the respective mesh size for the same amount of days, unlike the other grading periods (Table 1). The three treatment groups each had an average of >80% of seed that retained on the 4 mm grade screen. The Early group had a significantly greater percentage compared to Peak and Late ($p \leq 0.022$), while Peak and Late did not significantly differ from each other ($p = 0.32$, Fig. 9A). For the 6 mm grade, the Peak group had a significantly higher percent recruitment compared to the Late group ($p = 0.009$, Fig. 9B), while Early did not significantly differ from either ($p \geq 0.093$).

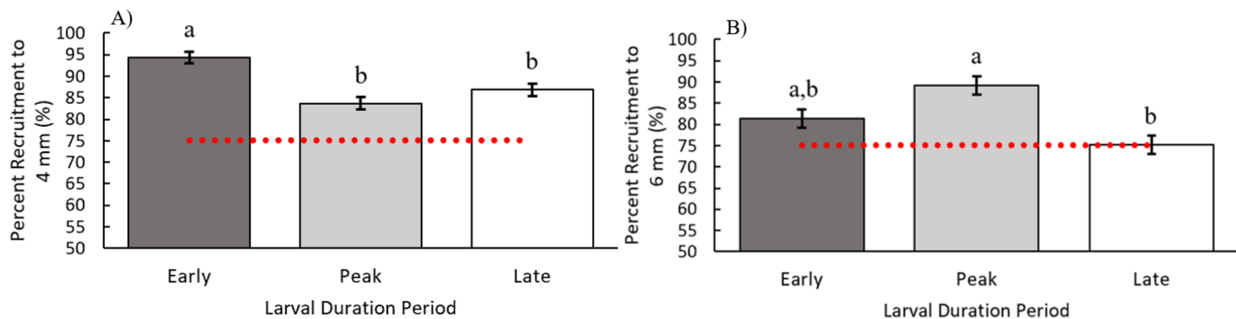

Figure 9. Average recruitment (%) to the 4 mm grade (A) and 6 mm grade (B) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups. Red dashed line illustrates the minimum requirement of retention for the grade (75%). Error bars represent standard error of the mean. Sample size included the averages of three replicate silos for each larval duration period. Different letters indicate a significant difference ($p < 0.05$).

Field Grow-Out Sample Time

The Site by Sample Time interaction was significant for the response variables (Table 4). However, since the interactions of Site by Sample Time were not of main interest for this research, for simplicity, only the results of Sample Time are discussed for each response variable. For the field grow-out analyses, the means of the Larval Duration Period treatment groups across all three AUSL field sites were averaged together for each time point. There was no significant interaction effect between Larval Duration Period and Sample Time for all response variables ($p \geq 0.42$), so each factor is addressed separately, beginning with Sample Time. Sample Time did not have a significant effect on average growth rate (change in shell height (mm) per day) ($p = 0.25$, Fig. 10A). However, there was a significant effect of Sample Time on the average dry shell weight (g) ($p = 0.003$, Fig. 10B). Oysters in August had a significantly heavier dry shell weight compared to oysters in February and May ($p \leq 0.017$). Dry

shell weight (g), on average, increased from February to August. Likewise, there was a significant effect of Sample Time on the average condition index of the oysters ($p = 0.008$, Fig. 10C). On average, oysters in February had a significantly higher condition index compared to the other sampling months ($p \leq 0.011$), while May and August did not significantly differ in condition ($p = 0.999$). There was also a significant effect of Sample Time on the average cup ratio ($p = 0.023$, Fig. 10D). On average, oysters in August had a significantly larger cup ratio compared to February ($p = 0.019$). However, the average cup ratio of the oysters in May did not significantly differ from either February or August ($p \geq 0.15$). Sample Time significantly affected average whole wet weight ($p = 0.004$) and average fan ratio (p = 0.033) of the oysters, but did not affect dry tissue weight ($p = 0.12$). The whole wet weight and dry tissue weight are variables that help make up the condition index and complete graphs can be found in Appendix A for those variables, as well as for the fan ratio.

Table 4. Restricted maximum likelihood (REML) variance components given as percentages for growth rate (mm/day) (GR), dry shell weight (g) (DSW), condition index (CI), cup ratio, and cumulative survival (%). LDP represents Larval Duration Period. Significant variation is illustrated by asterisks.

Variance component	GR	DSW	CI	Cup	Survival
Site	0	41.45*	0	0	83.93**
Site × LDP	0	0.37	0	13.28**	1.94
Site × Sample Time	70.44***	45.08***	70.64***	29.69***	.
Site ×LDP × Sample Time	2.11	0	1.87	0	.
Residual	27.45	13.10	27.50	57.03	14.14

*$P < 0.05$
** $P < 0.001$
*** $P < 0.0001$

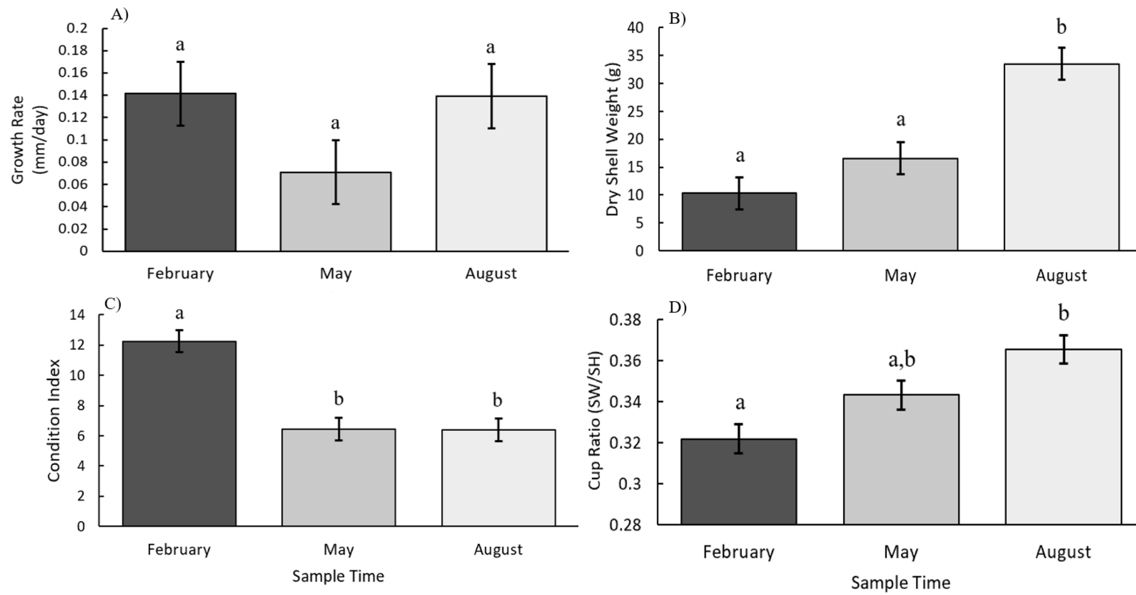

Figure 10. Average growth rate (mm/day) (A), average dry shell weight(g) (B), average condition index (C), and average cup ratio (D) for February (dark gray), May (moderate gray), and August (light gray) Sample Times. Error bars represent standard error of the mean. February, May, and August (4, 7 and 10 months after deployment, respectively) had 54, 54, and 52 replicates respectively, to calculate the shell metric analyses and 54, 52, and 52 replicates respectively, to determine the condition index analyses. Different letters indicate a significant difference ($p < 0.05$).

Additionally, as a complement to growth rate, on average, the shell height (mm) of the Larval Duration Period increased from the October deployment to the last sample month (Fig. 11). Average shell heights were similar at each of the four sample months among the three Larval Duration Period treatment groups. Dead oysters were obtained and measured in May and August (Fig. 11). The total amount of dead oysters that were measured across the three sites for Early, Peak, and Late in May were 190, 189, and 138, respectively. For August, the total amount of dead oysters that were measured across the three sites for Early, Peak, and Late were 27, 35,

and 39, respectively. The average shell height of the dead oysters in May were, on average 5mm smaller than that of the dead oysters in August (Fig. 11). Overall, on average (mean ± SD) the shell height of the dead oysters for Early, Peak, and Late group were 46.79 ± 8.40 mm, 45.48 ± 7.94 mm, and 47.07 ± 8.15 mm, respectively.

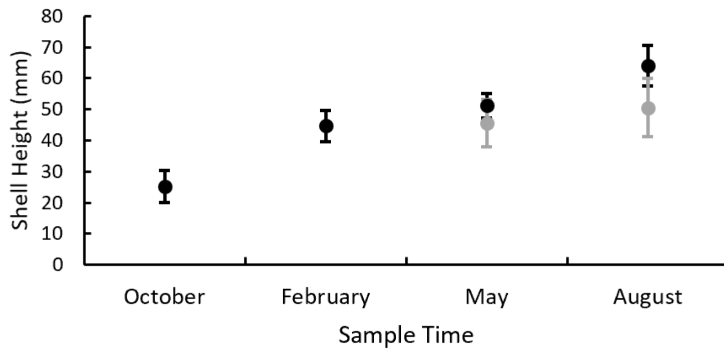

Figure 11. Mean shell height (mm) of the three Larval Duration Period treatment groups for the live oysters measured during each sample month, including the initial shell height taken before on the October deployment date (black) and the dead oysters that were collected May and August (gray). Error bars represent standard deviation. Sample size of live oysters measured for October, February, May, and August were: 75,54, 54, and 52, respectively. A total of 517 and 101 dead oysters were measured in May and August, respectively.

Field Grow-Out Larval Duration Period

The Site by Larval Duration Period interaction did not account for any variability in growth rate and condition index, but did account for an extremely low variation percentage for dry shell weight. Additionally, the interaction did account for a significant variation in the cup ratio ($p < 0.001$) only (Table 4). Therefore, since Site by Larval Duration Period interaction influenced variability for one response variable, Larval Duration Period was analyzed as a single

factor. For the analyses determining if Larval Duration Period has an effect, the mean of each treatment group includes the means of the Sample Time at all three AUSL experimental field sites. Larval Duration Period had no significant effect on any tested response variable: average growth rate, dry tissue weight, condition index, or cup ratio ($p \geq 0.17$ Fig. 12A-D). The three groups resulted in similar amounts for the response variables of: growth rate, dry shell weight, condition index, and cup ratio. Likewise, Larval Duration Period did not have a significant effect on average fan ratio, dry tissue weight and whole wet weight ($p \geq 0.12$, Appendix A).

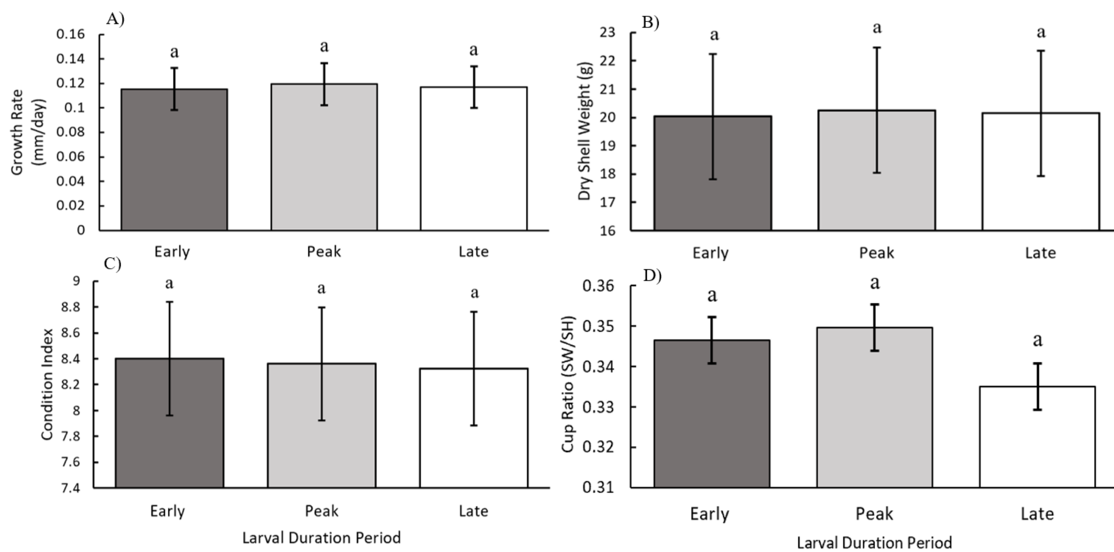

Figure 12. Average growth rate (mm/day) (A), average dry shell weight (g) (B), average condition index (C), and average cup ratio (D) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups. Error bars represent standard error of the mean. Early, Peak, and Late had 54, 54, and 52 replicates respectively, to calculate the shell metric analyses; 54, 53, and 51 replicates respectively, to determine the condition index analyses; and 18, 18, and 16 replicates respectively to determine cumulative survival. Letters indicate non-significance ($p < 0.05$).

Cumulative survival was used to determine the chance of surviving to August for each Larval Duration Period. Site accounted for about 84% of the variability in cumulative survival ($p < 0.001$); however, Site by Larval Duration Period variance component was not significant (Table 4.) Therefore, Larval Duration Period was analyzed as a single factor. Again, Larval Duration Period did not have a significant effect on average cumulative survival ($p = 0.67$, Fig. 13). The overall average cumulative survival to August was 88%.

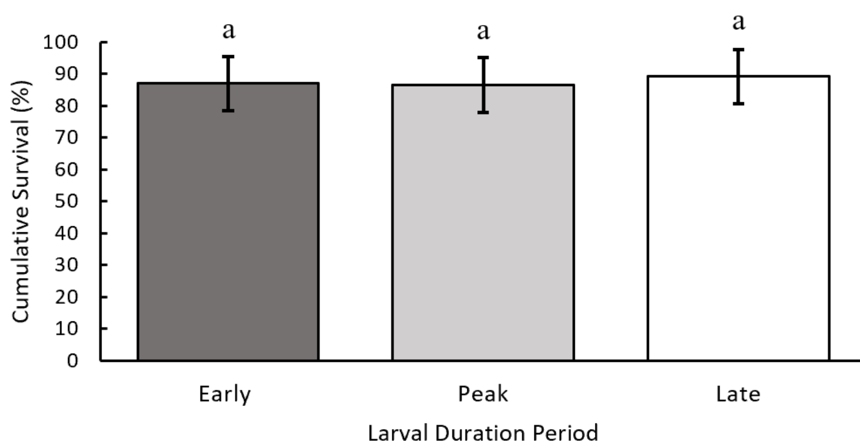

Figure 13. Average cumulative survival (%) (probability of survival to August) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups. Error bars represent standard error of the mean. Early, Peak, and Late had 18, 18, and 16 replicates respectively, to calculate the cumulative survival percentage. Letters indicate non-significance ($p < 0.05$).

Commercial Oyster Farm Site

Lastly, there was a significant effect of Larval Duration Period on the average growth rate for the oysters that were grown at the commercial oyster farm, DePe's Oysters (from

October 11, 2018 to August 6, 2019), located in Coden, AL ($p < 0.001$, Fig. 14). This was the only commercial site that had replicates suitable for analysis. The farm site is in Mobile Bay, just south of Fowl River (Fig. 4, green dot). At this site, survival data were not obtained; however, for the growth analysis, the Late group oysters grew significantly more slowly compared to Early and Peak ($p < 0.001$, Fig. 14).

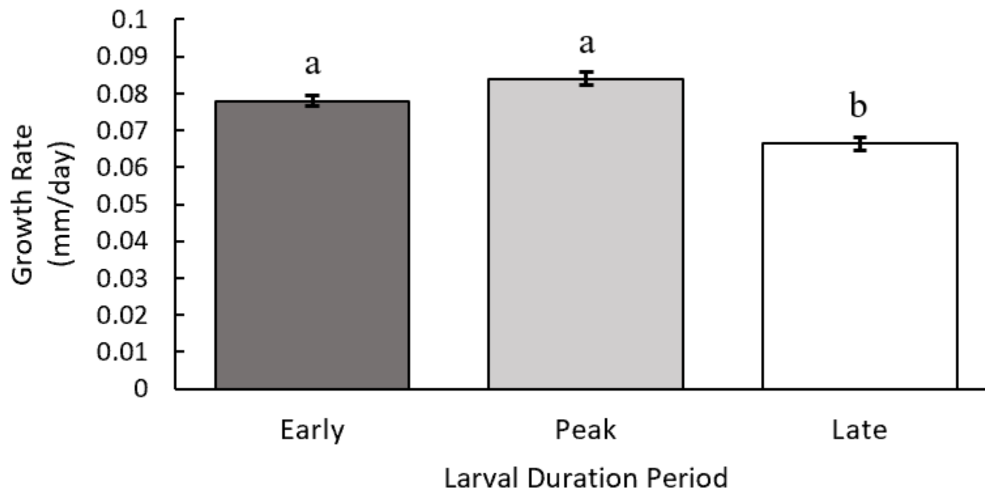

Figure 14. Average growth rate (mm/day) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups reared at DePe's Oyster farm. Error bars represent standard error of the mean. Early, Peak, and Late had 6, 4, and 4 replicates respectively, to calculate growth rate. Different letters indicate a significant difference ($p < 0.05$).

Discussion

Oyster farmers are concerned about the quality of the seed they are receiving from commercial suppliers, including the effects of using larvae from the 'tail end' of a larval culture. To determine if Larval Duration Period has an effect on subsequent quality and performance,

performance was observed among three Larval Duration Period treatment groups through metamorphosis/setting, land-based nursery, and field grow-out cultures.

Metamorphosis and Setting

The clear differentiation among larvae by shell height throughout culture period (Fig. A1) led to the expected pattern of pediveliger production for an entire week (with ≥900,000 pediveliger oysters that measured ≥282.8 µm each day after Day 10 post-fertilization). The variation that occurred in the larval tanks is similar to variations that have been found in several bivalve species of the same-age culture (for the Eastern oyster, for example: Newkirk et al. 1977, Haley & Newkirk 1977, Losee1979; zebra mussel: Stoeckel et al. 2004; and Northern quahog: Przeslawski & Webb 2009).

There was no significant difference among the three treatment groups for either the average post-metamorphic growth rate (mm/day) or metamorphic success (Fig. 6). Time to successfully complete metamorphosis varied among individual pediveligers. Baker and Mann (1994) suggests that some pediveligers are ready to achieve metamorphosis within 24 hours; however, on average, most will complete the transition between 1 to 3 days of the setting culture and those that fail to complete metamorphosis perish. Therefore, the four days allowed for setting in the downweller tank system should have been sufficient to insure maximum settlement across all Larval Duration Period groups. There was no clear trend in the effect of Larval Duration Period on metamorphic success (Fig. 6B-C), with a depression on the second day (Day 12, Fig. 6C). During the vulnerable larval stage, individuals use a lot of energy during their planktonic phase. Przeslawski and Webb (2009) suggested that the longer the duration period is for larvae to reach metamorphosis, the greater chance that the cost of energy that is constantly being used could result in a decrease in metamorphic success and even juvenile fitness of those

individuals. However, our metamorphic success (recruitment to 600 μm) results show no decrease in metamorphic success for the Late Larval Duration Period group or longer larval periods, in general. Furthermore, Larval Duration Period did not significantly affect the post-metamorphic growth rate during the 4 day metamorphic period, which indicates that new shell growth for the oysters in the three groups occurred in the third metamorphic phase, dissoconch postlarvae, which usually occurs around 30 h after settlement (Baker & Mann 1994).

Land-Based Nursery Culture

The differences in average growth rate (mm/day) and percent recruitment to 4 mm and 6 mm varied among the Larval Duration Period treatment groups throughout the land-based nursery (Fig. 7 & 9, respectively). Differences among ambient seawater flowrates and nursery management practices could potentially be factors that assisted in the differences observed among the Larval Duration Period treatment groups from grade to grade. However, the Late group had the disadvantage on growth rate for the overall land-based nursery culture (Fig. 8). Losee (1979) found that Eastern oyster pediveligers that set in the first 3 days of the metamorphic period continued to have faster growth rates in their juvenile phase. Early and Peak demonstrated similar results by having a significant growth advantage in the nursery culture compared to the Late group (Fig. 8). These results further indicate that Larval Duration Period did affect average growth rate in the land-based nursery culture, which may be relevant to commercial hatcheries and nurseries when seed orders at a high quantity need to be filled quickly. However, it does not impact growers as long as the seed does not demonstrate similar variation in performance during the grow-out culture.

Field Grow-Out Culture

Ultimately, oyster farmers want to know if the Larval Duration Period of seed they obtain affects performance of oysters during the grow-out culture, which could affect yields and profits. Before assessing the effect of Larval Duration Period on performance, we first evaluated whether field performance in this study was a reasonable test of commercial production conditions. In this study, the growth rates, condition indices and shell metrics (Fig. 10) performed in a manner consistent with commercial oyster farms in the northern Gulf of Mexico, with good survival (Fig. 13). Despite a depression of growth rate in May (Feb-May), relative to February (Oct-Feb) and August (May-Aug), growth rates were comparable and varied over time similar to results from other observed growth rates of diploid oysters grown along Alabama's coast (Casas et al. 2017). The field sites are in locations subject to heavy rainfall that occurs across the coast, rivers from the Mobile Bay estuary flood the region, and with low tidal fluctuation, these sites will experience low salinity. Therefore, low salinity could be one factor that affected this insignificant reduction in average growth rate that occurred between February and May. In the Apalachicola Bay region, which is located in Florida's Gulf Coast, Wang et al. (2008) finds that the simulated growth rates show that the slowest oyster growth rate occurs between March and April when the salinity decreased due to high freshwater inflows. This change could also explain why more dead oysters were collected at the May sample, compared to the August sample time (Fig.11). Adult oysters in the Breton Sound Estuary, Louisiana were found to have increased mortality as the salinity became < 9 ppt, and the addition of temperature increase contributed to further mortality (La Peyre et al. 2016). The Eastern oyster has been found to tolerate short-term salinity changes; however, if the fluctuations are extended for long periods, valve closures will occur (Hand & Stickle 1977). Therefore, these unfavorable conditions could have contributed to the lower growth rates and higher mortality that was found for the May Sample Time and explain

the significant variability from the Site by Sample Time interaction (Table 4). By the August sample, on average, the oysters had shell heights measured >63 mm (Fig. 11). Previous research on reefs located in the northern Gulf of Mexico estimated that oyster shell height is typically >60 mm in one year of culture, therefore, the three treatment groups exhibit performance that is typically found in the Gulf (Casas et al. 2015). This further supports that, in general, oysters grew and adequately performed as expected at the experimental farm sites.

In terms of absolute response variables, dry shell weights and whole wet weights increased over the study as was expected (Fig. 10B and A3 B, respectively). The decrease in tissue mass in May indicates that these oysters may have spawned between the February and May sampling period, which corresponded to a loss in the condition index for May (Fig. 10C) (Abbe & Sanders 1988). The condition index in August remained low, but the dry tissue weight increased over time (Fig. A3 C), indicating that the oysters are consuming food supply and growing at the field sites because of the increase in whole wet weight and dry shell weights (Fig. A3 B, and 10B, respectively), but the oysters could have spawned again to result in a low meat condition (Fig. 10C).

Consumers tend to prefer oysters that have a deep cup and wide fan (Brake et al. 2003). As time increased, the cup ratio (shell width to shell height) significantly increased (Fig. 10, D), while the fan ratio (shell length to shell height) significantly decreased (Fig. A3 A). This indicates that the oysters are putting on new shell growth. Both cup and fan ratio had measurements that were above the established threshold values for cup (> 0.25) and fan (> 0.63) to determine “good” oyster depth and width, respectively (Brake et al. 2003).

Given the apparent adequate performance of the study sites to approximate commercial production conditions, we assessed the effect of Larval Duration Period on field grow-out culture

performance. Across all response variables examined, Larval Duration Period did not have a significant effect upon any measure (Fig. 12). Grow-out performance did not benefit from pediveligers set from the Early group, nor did grow-out performance suffer from pediveligers set from the Late group. The three treatment groups had, on average, high cumulative survival and again, no significant difference among the three for their probability of surviving to August (Fig. 13).

These results are similar to those observed by Newkirk and Haley (1982), who examined the effect of Larval Duration Period for the European flat oyster. They determined that the length of larval period does have an effect on the size of the seed early on; however, the effect disappeared later in age. This suggests further that average performance shown earlier on is a poor predictor of performance later in life and culling out small individuals may produce an overall loss in harvestable oysters later in the season (Newkirk 1981).

Commercial Farm Grow-Out

In contrast to our results, Larval Duration Period did have an effect on average growth rate at the commercial oyster farm, DePe's Oysters. At this site, the Late treatment group was found to have a disadvantage with a significantly slower average growth rate compared to Early and Peak groups (Fig. 14). Notably, the oysters in all three Larval Duration Period treatment groups also had substantially lower average growth rates compared to the growth rates of the oysters grown at the three AUSL experimental field sites. The mean (± SEM) growth rate for the Peak oysters grown at the experimental sites was 0.119 ± 0.017 mm/day compared to approximately 30% lower growth rate (0.084 ± 0.0018 mm/day) observed from the Peak oysters that were grown at the commercial farm site. This particular farm site is subject to substantial freshwater input, with salinities often below 10 ppt and periodically between 0-5 ppt (DePaola,

pers. comm. 2019). We propose that the difference in results may result from different growing conditions, where stressful growing conditions may lead to effects of Larval Duration Period that were not observed during more favorable conditions. The reduction in growth can impact the time and quantity of producing marketable oysters in order to sell for profit, which can potentially hurt both the farmer and retailer.

Site by Larval Duration Period interaction was not of concern in the field grow-out analyses since there were only 2 out of 8 response variables that varied significantly due to the interaction (Table 4 & A1). Thus, the three experimental sites that were chosen (BSOP, GBOP, and DISL Dock) confined the experiment to grow the oysters in viable and dependable field sites. However, if the experiment expands the field site range to account for more variability, rather than culturing in mid-salinity sites, a large interaction of Site by Larval Duration Period could exist from unfavorable conditions. Thus, those results may support the findings that were determined from the commercial farm site culture.

Conclusion

In conclusion, Larval Duration Period did not have a significant effect on metamorphic success, had inconsistent effects during the land-based nursery culture phase, and there were no differences in the experimental field grow-out culture. The experimental sites were typical of culture conditions for commercial farms in the northern Gulf of Mexico, and we expect this result to hold true across most farms. However, the contrasting results from one commercial farm in low salinity conditions suggests that future work needs to be completed to determine if the effect of Larval Duration Period on performance depends on growing conditions at certain sites. Additionally, other factors in larval production that might affect subsequent performance need to be tested in order to support the growing oyster industry. In particular, water quality conditions,

such as salinity and nutrient stressors, should be investigated in future studies that examine larval development and production on subsequent performance. Hatchery production can be optimized by a better understanding of these effects, allowing production of sufficient quantities of high-quality seed. Nonetheless, the results from this research suggests that the concerns that farmers had of receiving seed from the 'tail end' of the larval culture are not warranted in most cases.

Literature Cited

Abbe, G. R. & J. G. Sanders. 1988. Rapid decline in oyster condition in the Patuxent River, Maryland. *J. Shellfish Res.* 7: 57-59.

Alabama Cooperative Extension System. 2019. Weighing method to estimate number of oyster seed. [Video file]. Retrieved from https://www.youtube.com/watch?v=AvPSsvZ5tRE.

Baker, S. M. & R. Mann. 1994. Description of metamorphic phases in the oyster *Crassostrea virginica* and effects of hypoxia on metamorphosis. *Mar. Ecol. Prog. Ser*. 104: 91-99.

Brake, J., F. Evans & C. Langdon. 2003. Is beauty in the eye of the beholder? Developent of a simple method to describe desirable shell shape for the Pacific oyster industry. *J. Shellfish Res*. 22(3): 767-771.

Butts, I. A. E. & M. K. Litvak. 2007. Stock and parental effects on embryonic and early larval development of winter flounder *Pseudopleuronectes americanus* (Walbaum). *J. Fish. Biol.* 70: 1070-1087.

Casas, S. M., J. La Peyre & M. K. La Peyre. 2015. Restoration of oyster reefs in an estuarine lake: population dynamics and shell accretion. *Mar. Ecol. Prog. Ser*. 524: 171-184.

Casas, S., W. Walton, G. Chaplin, S. Rikard, J. Supan & J. La Peyre. 2017. Performance of oysters selected for dermo resistance compared to wild oysters in norther Gulf of Mexico estuaries. *Aquacult. Environ. Interact.* 9: 169-180.

Davis, J. E. 2013. Effects of basket arrangement and stocking density when using adjustable long-line system for oyster grow-out. Master Dissertation for the Graduate Facility of Auburn University, Auburn, AL, pp.82.

DePaola, A. 2019. Owner of DePe's Oysters. Coden, Alabama. Personal comment.

FAO. 2018. The State of World Fisheries and Aquaculture – meeting the sustainable development goals. Rome. License: CC BY-NC-SA 3.0 IGO. http://www.fao.org/3/i9540en/i9540en.pdf (accessed November 2019).

Fry, J. D. 2004. Estimation of genetic variances and covariances by restricted maximum likelihood using PROC MIXED. In: A. M. Saxton, editor. Genetic Analysis of Complex Traits Using SAS®. Cary, NC: SAS Institute Inc, pp. 11-34.

Galtsoff, P. S. 1964. The American Oyster, *Crassostrea virginica* (Gmelin). *U. S. Fish. Wildl. Serv. Fish. Bull.* 64: 1-480.

Grice, R. & B. Walton. 2019. Alabama shellfish aquaculture situation & outlook report 2018. Alabama Cooperative Extension System.

https://www.aces.edu/blog/topics/aquaculture/alabama-shellfish-aquaculture-situation-outlook-report-2018/ (accessed November 2019).

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas & C. Toulmin. 2010. Food security: the challenge of feeding 9 billion people. *Science*. 327:812-818.

Haley, L. E. & G. F. Newkirk. 1977. Selecting oysters for faster growth. *Proc. Wld Maricult. Soc*. 8: 557-565.

Hand, S. C. & W. B. Stickle. 1977. Effects of tidal fluctuations of salinity on pericardial fluid composition of the American oyster *Crassostrea virginica*. *Mar. Biol*. 42: 259-271.

Kim, C.-K., K. Park, S. P. Powers, W. M. Graham & K. M. Bayha. 2010. Oyster larval transport in coastal Alabama: Dominance of physical transport over biological behavior in a shallow estuary. *J. Geophys. Res.* 115. doi:10.1029/2010JC006115

La Peyre, M.K., J. Geaghan, G. Decossas & J. F. La Peyre. 2016. Analysis of environmental factors influencing salinity patterns, oyster growth, and mortality in Lower Brenton Sound Estuary, Louisiana, using 20 years of data. *J. Coast. Res.* 32(3): 519-530.

Lapègue, S., A. Beaumont, P. Boudry & P. Goulletquer. 2006. European flat oyster – *Ostrea edulis*. *Genimpact Final Scientific Report*. 70-75. https://archimer.ifremer.fr/doc/2006/acte-3321.pdf (accessed May 2020).

Littell, R. C., G. A. Milliken, W. W. Stroup & R. D. Wolfinger. 1996. *SAS System for Mixed Models*. Cary, NC: SAS Institute Inc.

Losee, E. 1979. Relationship between larval and spat growth rates in the oyster (*Crassostrea virginica*). *Aquaculture*.16: 123-126.

Messina, F. J. & J. D. Fry. 2003. Environmental-dependent reversal of life history trade-off in the seed beetle *Callosobruchus maculates*. *J. Evol. Biol.* 16: 501-509.

Mizuta, D. D. & G. H. Wikfors. 2019. Seeking the perfect oyster shell: a brief review of current knowledge. Reviews in Aquaculture 11: 586-602.

Newkirk, G. F. 1981. On the unpredictability of bivalve growth rates: is a slow growing juvenile oyster a runt for life. In: C. Claus, N. de Pauw and E. Jaspers, editors. Nursery Culturing of Bivalve Molluscs. *European Mariculture Society*, Bredene, Belgium, pp. 211-218.

Newkirk, G. F. & L. E. Haley. 1982. Phenotypic analysis of the European oyster *Ostrea edulis* L.: relationship between length of larval period and postsetting growth rate. J. Exp. Mar. Biol. Ecol., 59: 177-184.

Newkirk, G. F., L. E. Haley, D. L. Waugh & R. Doyle. 1977. Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica*. *Mar. Biol.* 41: 49-52.

NOAA Fisheries. 2018. Aquaculture Production Highlights Infographic, 2017. Fisheries of the United States. https://www.fisheries.noaa.gov/national/aquaculture/us-aquaculture (accessed March 2020).

Pechenik, J. A. 2006. Larval experience and latent effects—metamorphosis is not a new beginning. *Integr. Comp. Biol*. 46(3): 323-333.

Pechenik, J. A. 2018. Latent effects: surprising consequences of embryonic and larval experience on life after metamorphosis. In: T. J. Carrier, A. M. Reitzel, and A. Heyland, editors. Evolutionary ecology of marine invertebrate larvae. Oxford University Press, Oxford. pp. 208-225.

Przeslawski, R. & A. R. Webb. 2009. Natural variation in larval size and developmental rate of the northern quahog *Mercenaria mercenaria* and associated effects on larval and juvenile fitness. *J. Shellfish Res*. 28(3): 505-510.

R Development Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Ragone Calvo, L. M., C. F. Dungan, B. S. Roberson & E. M. Burreson. 2003. Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. *Dis Aquat Org.* 56: 75-86.

Reed Mariculture, 2020. Cambell, California. https://reedmariculture.com/. Accessed April 30, 2020.

Rikard, F. S. & W. C. Walton. 2012. Use of microalgae concentrates for rearing oyster larvae, *Crassostrea virginica*. *Mississippi-Alabama Sea Grant* Publication No. MASGP-12-048.

SAS Institute Inc. 2018. SAS Studio 3.8/SAS9.4. Cary, NC, USA.

Stoeckel, J. A., D. K. Padilla, D. W. Schneider & C. R. Rehmann. 2004. Laboratory culture of *Dreissena polymorpha* larvae: spawning success, adult fecundity, and larval mortality patterns. *Can. J. of Zool*. 82(9):1436-1443.

Thomas, L. L., S. K. Allen Jr & L. V. Plough. 2019. The effect of aquaculture gear on the growth and shape of the oyster *Crassostrea virginica* during a “finishing period” in Chesapeake Bay, USA. *Aquaculture.* 508: 1-9.

Wallace, R. K., P. Walters & F. S. Rikard. 2008. Oyster Hatchery Techniques. *Southern Regional Aquaculture Center* Publication No. 4302.

Walton, W. C., F. S. Rikard, G. I. Chaplin, J. E. Davis, C. R. Arias & J. E. Supan. 2013. Effects of ploidy and gear on the performance of cultured oysters, *Crassostrea virginica*: survival, growth, shape, condition index and *Vibrio* abundances. *Aquaculture*. 414-415: 260-266.

Wang, H., W. Huang, M. A. Harwell, L. Edmiston, E. Johnson, P. Hsieh, K. Milla, J. Christensen, J. Stewart & X. Liu. 2008. Modeling oyster growth rate by coupling oyster population and hydrodynamic models for Apalachicola Bay, Florida, USA. *Ecological Modelling*. 211: 77-89.

Zar, J. H. 1996. Biostatical Analysis. 3rd edition. Upper Saddle River, NJ: Prentice-Hall. 662 pp.

CHAPTER THREE:

ONCE AN OYSTER RUNT, ALWAYS AN OYSTER RUNT?

A Summary for the Commercial Oyster Industry

A Rise in the Commercial Oyster Farming Industry

The demand for high quality seed production has risen over the recent years as oyster aquaculture has increased along the East, West, and Gulf coasts of the United States. The increase in demand has brought attention to the quantity and quality of oyster seed produced in commercial hatcheries. As hatcheries strive to maximize their production, growers too want to maximize their farming yields. This means that farmers expect the seed they purchase from hatcheries to have high survival and growth in order to reach production goals that are profitable in the competitive half-shell market.

In The Hatchery

When microscopic oyster larvae reach the eyed larval (pediveliger) stage (Fig. 1), they are collected from the larval tanks (by catching them on specific size mesh screens) to be set in order to achieve a single oyster seed that will be grown for the half-shell market. There is evidence that faster growing larvae of commercially important aquaculture species perform better in the juvenile stage compared to their smaller, slow growing larval siblings of the same age (for the Eastern oyster, for example: Newkirk et al. 1977, Haley & Newkirk 1977, Losee 1979; and Northern quahog: Przeslawski & Webb 2009); in other words, hatcheries could potentially select only the fastest growing larvae by culling out slow growers and potentially improve performance of the juvenile production (seed). Practically, however, this technique could substantially reduce the quantity of hatchery production as it relies on discarding larvae. To maximize hatchery production while also maximizing the quantity and quality of seed, there is a need for a better understanding of how performance and larval experience in the hatchery affects field performance.

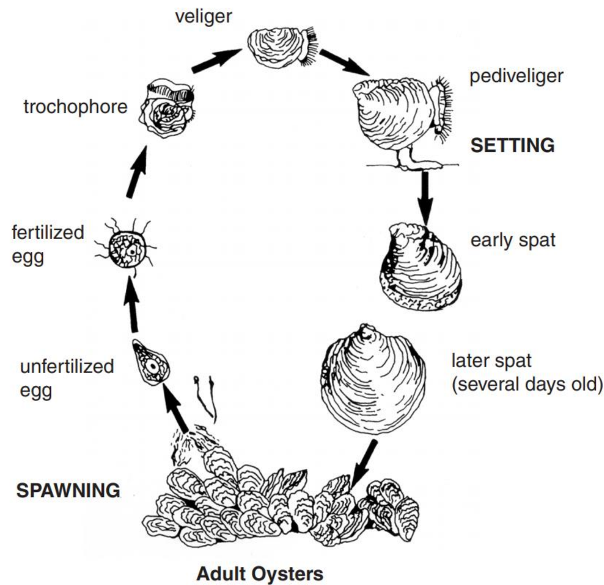

Figure 1. The Eastern oyster (*Crassostrea virginica*) life cycle from: Wallace 2001.

Setting Day

Any given larval culture can last several weeks due to size variation. Harvesting for eyed larvae will occur typically over multiple days in order to get as many eyed larvae as possible from a single spawn; it wouldn't be unusual to start harvesting eyed larvae around ten days after spawn and continue harvesting them each day for a week. Growers have raised concerns that the seed produced from the eyed larvae harvested at the tail end of that time might be poor performers or 'runts'. This made us ask if the age of the larvae affected how the oysters grew later.

The Study

To test if growth rate of same age larvae affected performance, oysters were evaluated at three production stages: metamorphosis/setting culture, land-based nursery culture, and field grow-out culture. Three different groups of diploid Eastern oyster larvae were produced from a single spawn at the Auburn University Shellfish Laboratory (AUSL) located on Dauphin Island,

AL. Groups were based on when the larvae became eyed larvae; the first ones ready to set were Early, the ones ready when most others were ready to set were Peak, and the stragglers were Late, with larvae in the Late group reaching the eyed larval stage 7 days after the Early group (Fig. 2). The seed were set using typical methods and moved to land-based upwellers. Grading occurred regularly in the upwellers, but seed were only moved up if at least 75% of the batch was ready to be moved up (to ensure that groups were being treated ‘fairly’). Seed were moved from the land-based nursery and eventually to field grow-out in adjustable longline baskets at three sites along Alabama’s Gulf Coast, which were used to represent commercial oyster farm locations across the northern Gulf of Mexico. Oysters were sampled 4, 7 and 10 months after deployment to assess field performance, using growth (mm/day), survival, shell shape, and meat condition.

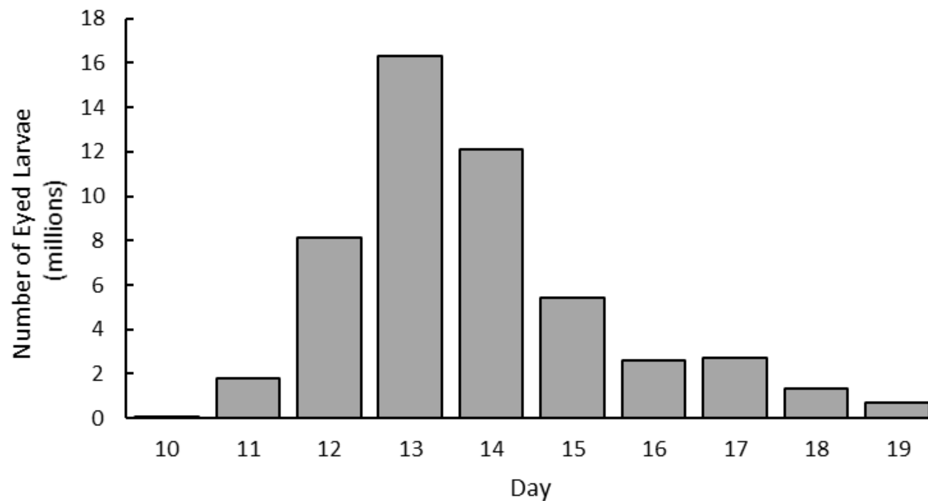

Figure 2. A graph of the number of eyed larvae that retained on a 200µm mesh sieve screen each day eyed larvae made an appearance to determine which days represented the three larval growth treatment groups: Early (Day 11), Peak (Day 13), and Late (Day 18). Days represent the number of days post-fertilization.

What Did We Find?

There was no difference in metamorphic success and post-metamorphic growth rate among the three larval treatment groups. For the land-based nursery culture, there were some differences in performance among the three groups, with the Late group having an overall growth disadvantage at the end of the nursery culture. The difference that was observed could not visibly be noticed; however, in terms of providing enough seed in the hatchery to fulfill an order, the growth difference may cause an issue. Critically, there was no effect on quality and performance in the field grow-out culture at the AUSL field sites in any of the measures related to growth or survival. The sites used in the study grew and performed well and appeared typical of many commercial operations. In contrast to our results, a commercial oyster farm at a low salinity site in Alabama found that the Late group grew slower compared to the Early and Peak groups. It is possible, that normally, larval growth rate doesn't affect performance, but differences may be revealed under poor growing conditions, though this theory needs to be tested.

Overall, though, farmers that rear oysters at field grow-out sites that maintain favorable oyster growing conditions should find that there is no difference in performance of the seed they receive, no matter if the seed was first or last to be set during the larval culture. For hatchery production, these results suggest that larvae of any growth rate may produce high performing oysters and that slow-growing larvae do not need to be discarded. Therefore, this practice should assist in maximizing larval and hatchery production.

Going Forward

Additionally, for hatchery production, it is important to consider factors in larval production that might affect performance in the field grow-out culture in order to support the

growing oyster industry. It is essential that hatchery managers, oyster farmers, and researchers work together to understand how factors that affect larval performance within the hatchery culture can affect subsequent performance on the farm. It is well recognized that a stress factor that occurs during larval culture can decrease performance in the juvenile or adult phase, a so-called latent effect (Pechenik 2006). By understanding latent effects like nutrient stress or water quality stressors, hatcheries can optimize larval rearing conditions in order to produce high quality seed for the industry and to avoid unwanted larval performance issues that carryover to grow-out culture.

Literature Cited

Haley, L. E. & G. F. Newkirk. 1977. Selecting oysters for faster growth. *Proc. Wld Maricult. Soc*. 8: 557-565.

Losee, E. 1979. Relationship between larval and spat growth rates in the oyster (*Crassostrea virginica*). *Aquaculture*.16: 123-126.

Newkirk, G. F., L. E. Haley, D. L. Waugh & R. Doyle. 1977. Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica*. *Mar. Biol.* 41: 49-52.

Pechenik, J. A. 2006. Larval experience and latent effects—metamorphosis is not a new beginning. *Integr. Comp. Biol*. 46(3): 323-333.

Przeslawski, R. & A. R. Webb. 2009. Natural variation in larval size and developmental rate of the northern quahog *Mercenaria mercenaria* and associated effects on larval and juvenile fitness. *J. Shellfish Res*. 28(3): 505-510.

Wallace, R. K. 2001. Cultivating the Eastern Oyster, *Crassostrea virginica*. *Southern Region Aquaculture Center* Pub. No, 432.

Appendix A.

Larval Growth

Larval shell height (µm) began to differ on Day 3 of larval tank culture and continued to increase in shell height (µm) variation through Day 11 (Fig A1).

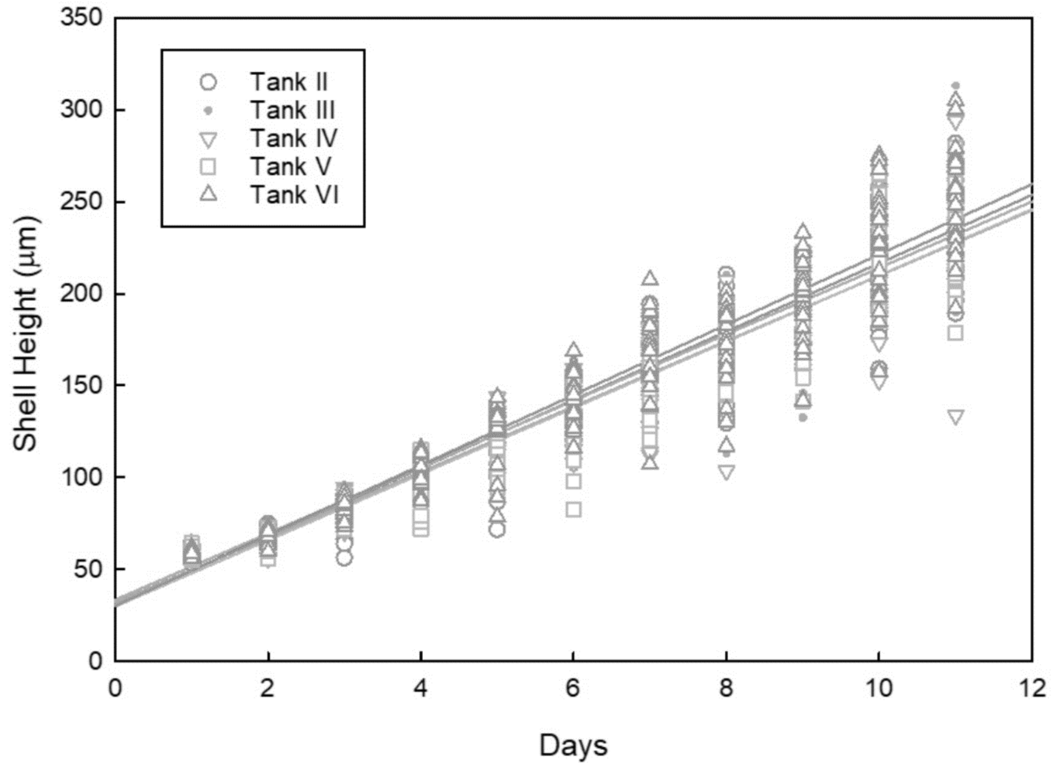

Figure A1. Larval shell height (µm) (n=25 larvae / tank) over the 11 days of culture.

Land-Based Nursery Culture

Variation among shell height (mm) across the three groups began to increase around day 9 of land-based nursery culture. (Fig. A2).

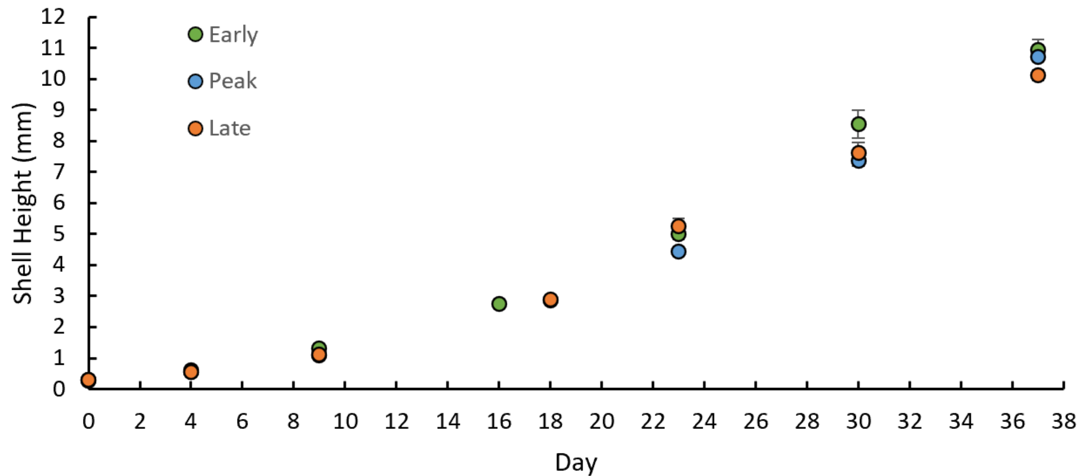

Figure A2. Average shell height (mm) that for the three Larval Duration Period treatment groups that retained on each grade during the land-based nursery culture. Green, blue, and orange circles represent Early, Peak, and Late, respectively. Day zero is the day the groups entered he downwelling system to achieve metamorphosis. Sample size included the averages of three replicate silos for each larval duration group. Error bars represent standard deviation of the mean.

Field Grow-Out Culture

Site variance components were not significant for fan ratio, whole wet weight (g), and dry tissue weight (g). Site by Sample Time interaction did cause a high degree of variation that was significant for all the response variables (Table A1). Sample Time significantly affected average whole wet weight ($p = 0.0038$) and average fan ratio ($p = 0.033$) of the oysters, but did not affect dry tissue weight ($p = 0.12$) (Fig. A3). On average, the fan ratio was significantly greater for February than August ($p = 0.037$), while the average whole wet weight (g) for February was significantly less than the whole wet weight for August ($p = 0.0032$).

Site by Larval Duration Period interaction accounted for a significant variation in the fan ratio ($p < 0.05$) (Table A1). Additionally, 4% of the variability in dry tissue weight (g) is explained by a significant three-way interaction of Site by Larval Duration Period by Sample Time (Table A1). As a single factor, Larval Duration Period did not have a significant effect on average fan ratio, dry tissue weight and whole wet weight ($p > 0.05$, Fig. A4). On average, the Early group had a heavier dry tissue weight (g) compared to the other treatment groups. However, average fan ratio, dry tissue weight, and whole wet weight were similar quantities between the Larval Duration Period treatment groups (Table A1).

Table A1. Restricted maximum likelihood (REML) variance components given as percentages for fan ratio, whole wet weight (g) (WWW), and dry tissue weight (g) (DTW). LDP represents Larval Duration Period. Significant variation is illustrated by asterisks.

Variance component	Fan	WWW	DTW
Site	0	34.89	16.10
Site × LDP	3.65*	0.60	0
Site × Sample Time	60.52***	48.71***	61.11***
Site × LDP × Sample Time	0	0.64	4.08*
Residual	35.83	15.17	18.71

*$P < 0.05$
** $P < 0.001$
*** $P < 0.0001$

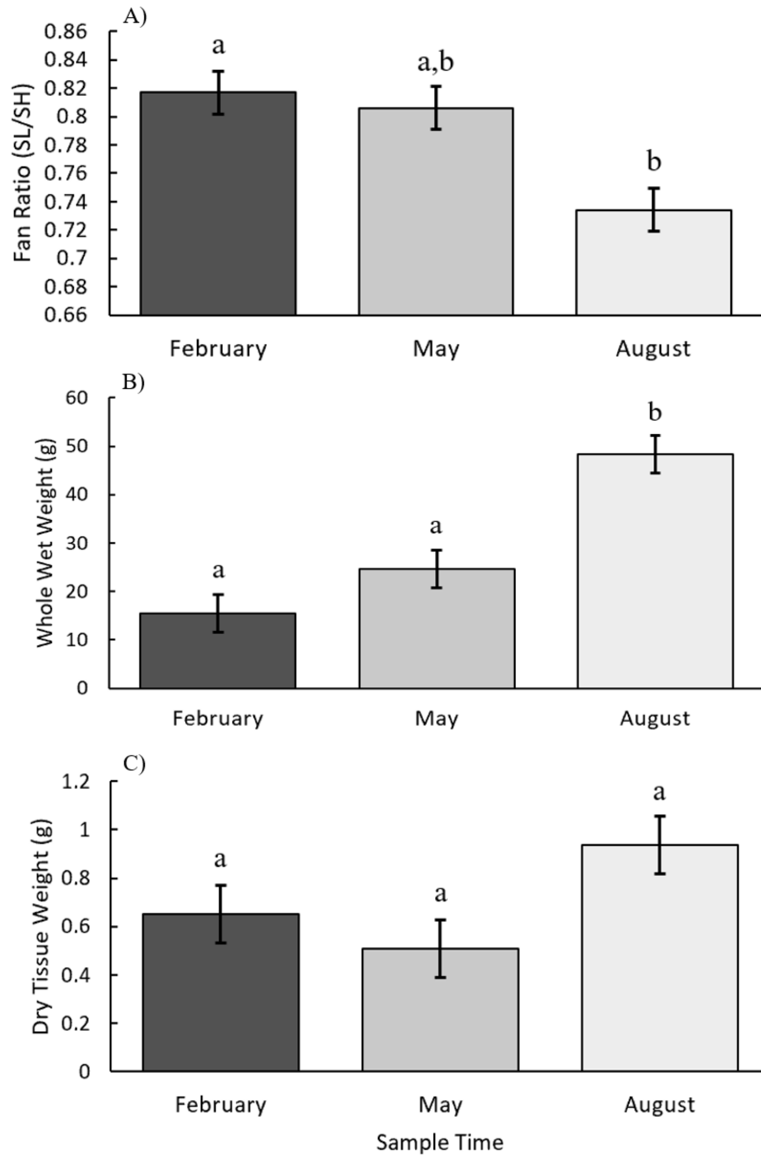

Figure A3. Average fan ratio (A), average whole wet weight weight(g) (B), and average dry tissue weight (C) for February (dark gray), May (moderate gray), and August (light gray) Sample Times. Error bars represent standard error of the mean. February, May, and August (4, 7 and 10 months after deployment, respectively) had 54, 54, and 52 replicates respectively, to calculate the fan ratio analysis and 54, 52, and 52 replicates respectively, to determine the condition index analyses. Different letters indicate a significant difference ($p < 0.05$).

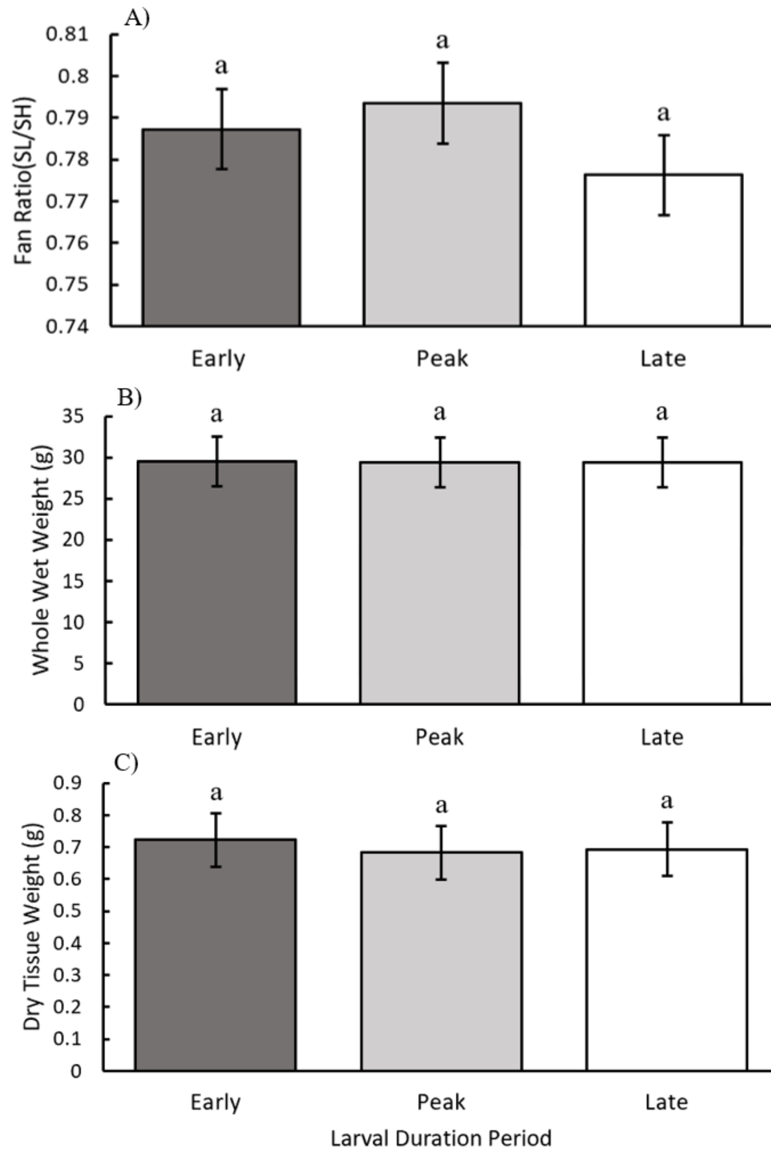

Figure A4. Average fan ratio (A), average whole wet weight weight(g) (B), and average dry tissue weight (C) for the Early (dark gray), Peak (light gray), and Late (white) Larval Duration Period groups. Error bars represent standard error of the mean. Early, Peak, and Late had 54, 54, and 52 replicates respectively, to calculate the fan ratio analysis and 54, 53, and 51 replicates respectively, to determine the condition index analyses. Letters indicate non-significance ($p < 0.05$).