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Improvements in Triploid Crassostrea virginica Production: Characterizing the Diploid Parent

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Improvements in triploid *Crassostrea virginica* production: Characterizing the diploid parent

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This thesis is submitted in partial fulfillment of

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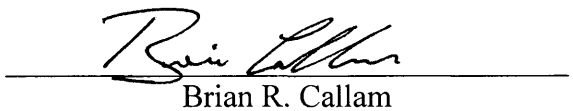

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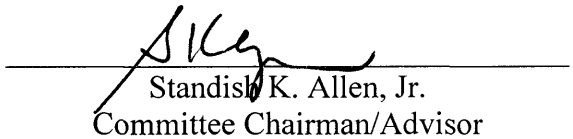

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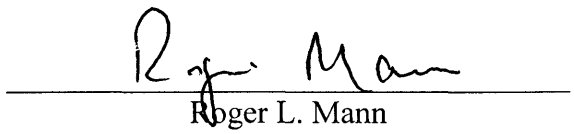

Roger L. Mann

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ABSTRACT

The effect of triploidy to effect faster growth and increased survival compared to diploid oysters is called the 'triploid advantage' and this advantage in the Chesapeake Bay for *Crassostrea virginica* is the principal reason for the value of triploid *C. virginica* in the Chesapeake Bay, USA. The triploid advantage is hypothesized to be the result of genetic effects, physiological changes, or a combination of both. The causative genetic mechanisms at play may include additive genetic effects and heterosis while the physiological changes obtain from triploid sterility. The triploid advantage was examined by comparing 13 diploid and 13 triploid crosses across three environments. The genotypes used in this study consisted of wild stocks from both the Virginia and Maryland portions of the Chesapeake Bay, four lines from ABC's 2006-year class of selected lines, and four ABC Superlines. Three experimental sites, ranging in salinity and disease pressure (Choptank River – low salinity and no disease pressure; Rappahannock River – moderate salinity and occasional disease pressure; York River – higher salinity and consistent disease pressure) were chosen to investigate the influence of environment on triploid advantage. Growth metrics (shell height, whole wet weight, and wet tissue weight) and survival rates among diploid and triploid *C. virginica* were recorded.

The triploid advantage for growth and survival ranges from positive to negative depending on environmental factors. In the low salinity environment, triploidy proved disadvantageous regardless of the genotype of the diploid parent. As salinity increased so did the triploid advantage, which was greater for the more disadvantaged (wild) groups. In the Rappahannock River, with moderate salinity and no disease pressure, selected diploids performed equivalently to their triploid counterparts showing that breeding efforts can improve diploid field performance to rival triploids. In the York River, under disease pressure, triploids offered the greatest advantage. Triploids from both wild-type and selected diploids had higher growth and survival than their diploid counterparts under disease pressure. In addition to greater survival, triploids also had lower Dermo infection prevalence than diploids indicating that there is a triploid advantage for Dermo disease resistance, perhaps as a result of triploid sterility.

Variation in the effect of triploidy on field performance follows the notion that triploidy may be thought of as a tool useful in some applications but not in others. For low salinity, it appears that triploidy may not be the appropriate tool for providing benefits for oysters but for oysters grown under disease pressure, it certainly is. The triploid advantage appears to be caused by both genetic effects and physiological changes, with the environment influencing the expression of each in manifesting the triploid advantage.

Improvements in triploid *Crassostrea virginica* production:

Characterizing the diploid parent

Chapter One: Introduction

Decline of *Crassostrea virginica* in Chesapeake Bay

The eastern oyster (*Crassostrea virginica*) has a large natural distribution, ranging from Canada to Brazil throughout many bays and estuaries, including Chesapeake Bay (Carriker and Gaffney, 1996). Within Chesapeake Bay, *C. virginica* has played and continues to play an important socio-economic role. Prior to European colonization of the Chesapeake Bay watershed, fishing oysters was primarily for subsistence. As colonization of areas surrounding Chesapeake Bay began so too did the economic exploitation of this species (Kirby, 2004). Goulletquer et al. (1994) described a dramatic decrease in oyster catches within Chesapeake Bay over the past century and attributed this decline to a number of sources: overfishing, removal of juveniles for transplanting projects, which reduces local populations, and increased disease pressure. Attempts to remedy the severely overfished oyster populations within Chesapeake Bay through numerous restoration efforts have had paltry success, due in part to extensive disease impacts (Mann and Powell, 2007).

Recorded declines in oyster stocks throughout the entire Chesapeake Bay began in the 19th century (Figure 1.1). Wild catches in the Maryland and Virginia waters of Chesapeake Bay have declined dramatically to approximately 3% and 5%, respectively, since 1980 (VMRC, 1996; VMRC, 2008; Tarnowski, 2010). Given this level of decline in landings and subsequent increases in economic value, it is logical that oyster aquaculture, or the farming of oysters, ought to have expanded rapidly. The lag between

general overfishing and the inception of widespread aquaculture in Chesapeake Bay may be attributed more to a cultural aversion to aquaculture than to a lack of technological capacity as oyster aquaculture has been well established. Biological and logistical competences were developed as early as the first half of the 20th century. Galtsoff (1964) characterized, in great detail, the life history and mode of living for *C. virginica.* Multiple patents are filed with the US Patent Office detailing both larval and seed culture methods (Wells, 1933; Glancy, 1965). Fishing wild oysters in Chesapeake Bay has been a long established practice, to the point that many would consider it part of the regional heritage and any deviation from the practice of harvesting wild native oysters would be considered foreign and thus unnatural. The cultural aspect of the oyster industry in Chesapeake Bay has been studied in detail (*cf.* Paolisso et al., 2005).

Culturing *Crassostrea virginica* in Chesapeake Bay

Only recently, aquaculture using hatchery-produced oysters has become established and continues to grow in the Virginia portion of Chesapeake Bay. This can be most clearly seen through rapid increases in the planting of cultured *C. virginica* in Virginia, which has increased from 6.2 million oysters in 2005 to 76.6 million oysters in 2010 (Murray and Hudson, 2011). This recent boom in cultured *C. virginica* can probably be at least partly attributed to a series of industry trials conducted by the Virginia Seafood Council (VSC) that exposed many non-oyster growers to oyster culturing (Allen, 2005). The purpose of these trials was to compare, in a commercial setting, the native *C. virginica* with a non-native species (*Crassostrea ariakensis*), which at the time was being considered for introduction into Chesapeake Bay to ease the pressures of a severely reduced fishery. Since many of the growers in the VSC trials did

not have extensive experience growing oysters, these trials provided first-hand exposure to the economic potential of culturing oysters. Due to the potential negative impacts of introducing a non-native species, these trials were heavily scrutinized by other industry members not directly involved in the trials as well as policy makers. This in turn placed the potential impact that oyster aquaculture may have on Chesapeake Bay, in terms of generating revenues as well as creating valuable jobs, in the public spotlight. The introduction of *C. ariakensis* as a candidate replacement species for aquaculture has since been ruled out as an option for Chesapeake Bay. In light of this conclusion, there was one perceived hurdle that had to be overcome for the native *C. virginica* to succeed in an aquaculture setting: disease.

Disease

In Chesapeake Bay there are two principal diseases affecting oysters: parasitism by *Perkinsus marinus* (Dermo) and by *Haplosporidium nelsoni* (MSX), the combination of which has at times resulted in losses of up to 80% of planted seed (Burreson, 1991). Dermo was most likely well established in Chesapeake Bay before its discovery in 1949; however it did not significantly colonize the Maryland portion of Chesapeake Bay until the 1980s, when intense droughts increased the salinity in these northern waters (Andrews, 1996). The presence and spread of Dermo is influenced by temperature and salinity as well as geographic proximity of infected oyster populations. Oysters typically begin to acquire infections during their first summer but do not develop serious disease until water temperatures reach 20°C the next year, when the parasite begins to proliferate once more, this time to often lethal infection levels. The dispersal of this parasite may occur through release in feces or upon the death infected oysters (Burreson and Ragone

Calvo, 1996, Villalba et al., 2004). This long exposure to Dermo negatively impacts both growth and gametogenesis in sexually mature *C. virginica* (Kennedy et al., 1995; Barber, 1996; Dittman et al., 2001).

MSX infections can be prevalent among *C. virginica* populations in polyhaline regions of Chesapeake Bay. The distribution of this parasite in *C. virginica* populations is limited mainly by salinity, with the parasite inhibited by salinities below 10ppt (Ford, 1985). While the life cycle of MSX is unknown, typical infection development within oysters is generally understood. Oysters are usually infected in the summer months with the initial infection being restricted to the gill tissue before becoming systemic via distribution through the circulatory system (Ford and Haskin, 1982; Ford, 1985). Mortality can occur within the first year of exposure (Ford and Haskin, 1982). Barber et al. (1988a) demonstrated that reductions in condition index and relative fecundity were directly proportional to the level of infection intensity. The authors observed decreases in relative fecundity ranging from 35-81%. Decreased feeding rates have been observed as well in oysters infected with MSX, which may contribute to declines in condition and reproductive output (Newell, 1985).

Both Dermo and MSX are major sources of mortality that have contributed to the present state of decimated oyster populations throughout Chesapeake Bay since the 1950's (Goulletquer et al., 1994). As a result of the contribution of these diseases to mortality events, a need arose to combat the negative effects of Dermo and MSX. Through selective breeding, strains of *C. virginica* were developed that show reduced susceptibility to both Dermo and MSX (Ford and Haskin, 1987; Ragone Calvo et al., 2003; Harding, 2007).

Selective Breeding

Reduced susceptibility to the deleterious effects of MSX, Dermo, and the combination of both diseases is heritable in *C. virginica* (Haskin and Ford, 1979; Burreson, 1991, Ragone Calvo et al., 2003). Ragone Calvo et al. (2003) demonstrated a dual resistance of *C. virginica* to both MSX and Dermo indicating that selection for resistance to both diseases can occur simultaneously. In the Virginia portion of Chesapeake Bay, testing of various genetic lines selected for disease resistance and high growth rate has been carried out at the Virginia Institute of Marine Science, College of William & Mary (VIMS) (Ragone Calvo et al., 2003) and at the Aquaculture Genetics and Breeding Technology Center (ABC) at VIMS (Dégremont et al., 2006; ABC, 2010). Breeding lines are populations whose gene frequencies have experience artificial selective pressures. The major goal of these selective breeding efforts has been to explore solutions to the endemic disease problem that has continued to cause significant mortality. Through this program, significant gains in survival were realized in oysters exposed to these two diseases. Survival of ABC's 2006-year class lines after two years of exposure to both Dermo and MSX was 2-3 times higher than that of wild controls (ABC, unpublished). In the Maryland portion of Chesapeake Bay, measures to enable and promote oyster aquaculture have recently been adopted (MdDNR, 2010). Hatchery-produced oysters selected for disease resistance had significantly greater survival and growth rates than wild stocks obtained from testing sites in these Maryland waters (Abbe et al., 2010). These studies are promising because the gains already obtained in established genetic lines may also manifest in the upper Chesapeake Bay as well making the development of lines appropriate for Maryland's up-and-coming oyster aquaculture

industry more readily achievable. With the achievement of these increases in survival, the main focus of the breeding program has now been shifted onto other traits that may be of economic importance as well, such as increased growth rates.

Prior to arriving at this recent shift in breeding strategies from survival to growth, researchers were already exploring ways to increase the economic value of oysters. In some areas, the peak market for oysters occurs during the months when oysters are sexually mature, which renders oysters unpalatable. To overcome this, a technique was developed to prevent oysters from reaching sexual maturity. This technique was to induce triploidy, which prevented oysters from successfully completing gonadogenesis (Allen and Downing, 1991). Triploidy is the state of having three sets of chromosomes in each cell. Inducing triploidy in oysters significantly reduced gametogenesis, and, therefore, improved meat quality during summer months, but it also had another side effect: increased growth rate. For simplicity throughout this thesis, triploid oysters will be referred to as 'triploids' and diploid oysters as 'diploids'.

Triploidy

Triploidy was first developed in *C. virginica* (Stanley et al., 1981) and later in the Pacific oyster (*Crassostrea gigas*), where it was developed for commercial use on the North American West Coast (Allen et al., 1989). There are two principal methods for the creation of triploid *C. virginica.* The first is by inducing the triploid condition by treating fertilized eggs with a chemical solution that impairs the completion of meiosis. The second makes use of another ploidy level, tetraploid (having four chromosome sets). Crossing tetraploids with diploids results in triploid offspring (Guo et al., 1996). After the establishment of tetraploid broodstock, this is the method used for producing

triploids, both for research and private enterprise.

Triploid production

Chemical induction

Chemical induction is typically achieved by treating embryos with either cytochalasin-B (CB) or 6-dimethylaminopurine (6-DMAP), though there are a variety of other treatments that provide similar results (Allen and Bushek, 1992; Desrosiers et al., 1993; Guo et al., 1996; Piferrer et al., 2009). Eggs of bivalve molluscs, including *Crassostrea* spp., are arrested at metaphase of meiosis I providing the opportunity to manipulate the release of polar bodies in either meiosis I or meiosis II, both of which will produce triploid progeny (Figure 1.3), although meiosis II treatments are more common (Guo et al., 1992; Piferrer et al., 2009). Chemical induction of triploidy, by any means, is difficult because of the variable nature of the treatments. There are a variety of considerations that must be made to ensure a successful treatment: temperature of egg development, dosage, time of initiation, duration of treatment, and possibly salinity variations (Desrosiers et al., 1993). These difficulties, compounded with toxicity of chemical inductions and the typical 40-80% triploid induction rate (Allen and Downing, 1987; Allen and Bushek, 1992), led to the development of a safer and more consistent method for inducing triploidy in oysters using tetraploids (Guo et al., 1996).

Mated triploids

Mating tetraploids with diploids will also produce triploid offspring (Guo et al., 1996; Piferrer et al., 2009). Tetraploids are obtained by treating eggs from a triploid female with a similar chemical treatment as described above after fertilization with sperm from a diploid male (Guo and Allen, 1994; Eudeline et al., 2000a, 2000b) (Figure 1.4).

Obtaining eggs from triploids is possible because triploid are not 100% sterile (Guo and Allen, 1994). Triploid *C. gigas* larvae were the first triploid oysters to be obtained by mating tetraploids and diploids. While using tetraploids to produce triploids through tetraploid × diploid crosses was not new (this process is used in agricultural crops), tetraploid × diploid crosses were not previously possible with oysters for lack of tetraploids (Guo et al., 1996). The primary advantage of mating tetraploids and diploids is that the success rate (the percentage of larvae that become triploids) is greater than 97%, compared to 40-80% in chemically induced triploids (Allen and Downing, 1987; Allen and Bushek, 1992; Guo et al., 1996). Another advantage is higher oyster growth rates than is observed in chemically induced triploid cultures (Wang et al., 2005). The authors showed that while the magnitude of oyster growth and survival rates varied by environment, these rates were consistently superior to both chemically induced triploids as well as diploids (Wang et al., 2006).

The spread of commercial triploid oyster production to areas that previously did not use triploids was enabled by the development of tetraploid oysters. Breeding programs in Europe and Australia began using the technique of mating tetraploids with diploids for triploid oysters production around 1999-2000 (Nell, 2001). With the rapid expansion of tetraploid broodstock availability in these areas, triploids now compromise about half of hatchery-reared oysters worldwide. In Chesapeake Bay, however, demand for triploids has developed significantly only in recent years, which may have been impelled by research on the proposed introduction of the non-native *C. ariakensis* (Allen, 2005). These studies compared triploid *C. ariakensis* and *C. virginica* for use in an aquaculture setting (Calvo et al., 2001; Grabowski et al., 2004; Paynter et al., 2008;

Kingsley-Smith et al., 2009). It was mainly because of the previously mentioned trials conducted by the VSC and ABC that many oyster farmers were exposed to triploid oysters (Hudson et al., 2005). Due to restrictions set for these trials, all *C. ariakensis* tested in the field had to be certified as triploid through flow cytometry. To make comparisons appropriate, both triploid and diploid *C. virginica* were used in these studies as well. It was because of these trials that growers experienced firsthand the qualities of triploids. The effect of triploidy on oysters may influence field performance relative to diploids grown to market size in three ways: no difference, triploids performing better than diploids, or triploids performing worse than diploids. When triploidy affects a trait in a positive way relative to diploid performance, this effect is referred to as the *triploid advantage*. When triploidy affects a trait in a negative way relative to diploid performance, this effect is referred to as the *triploid disadvantage*. In Chesapeake Bay, the ability to survive to market size in the presence of major disease obstacles, is the main concern in oyster culture. It is through this propensity for survival that triploids may gain much of their value in these waters. This advantage is hypothesized to be the result of genetic effects, physiological changes, or a combination of both. The causative genetic mechanisms at play may include additive genetic effects and heterosis while the physiological changes result from reduced gametogenesis. Throughout this thesis, physiological changes will refer to those that obtain due to triploid sterility.

Triploid advantage: Genetic effects

Gains from both additive genetic improvement and heterosis have been demonstrated through established breeding programs (Hand et al., 2004; Hedgecock and David, 2007). Both are also likely to play a major role in the 'triploid advantage'. The

phenotypic value of a trait (its actual measured value) is influenced by genetic effects as well as environmental effects. The genetic effects may be further divided into additive genetic effects and non-additive effects (gene dominance and interactions). The additive effects are a measure of the breeding value of an individual. The breeding value of an individual is the part of the deviation of an individual phenotype from the population mean that is due to the cumulative effects of alleles. Breeding value is essentially a measure of the individual as a parent for improving a trait in the next generation.

Non-additive effects can be both deleterious as well as beneficial. Heterosis is an example of beneficial non-additive effects, which occur through dominance of one locus over another, interaction among loci, and epistatic effects, where one locus affects or changes the gene products of another.

Additive effects

Additive genetic effects describe the portion of the phenotypic value of an individual for a given trait, from which dominance and interaction deviations are subtracted. These estimates are the basis of quantitative genetics. Additive genetic gains are the hallmark of all selective breeding programs, including the breeding of diploids in the ABC's breeding program. In triploids, additive genetic gains obtain through the addition of another set of optimal alleles. This additional set of alleles provides an increase in the dosage of beneficial effects from the optimal alleles, thus resulting in better performance of a given trait. Additive genetic gains from selective breeding have been realized in triploid aquatic animals from finfish to shellfish (Johnson et al., 2007; Piferrer et al., 2009). A hallmark study on additive gains in triploids was done with the Sydney rock oyster (*Saccostrea glomerata*) through a comparison of diploid and

chemically induced triploid progeny of a third generation breeding line and a control group (Hand et al., 2004). In this study, triploids were chemically induced, so all three sets of chromosomes had been selected for increased growth rate. The authors hypothesized that if observed gains in whole body weight were simply additive genetic gains, the triploids made from selected lines ought to be a minimum of 30% heavier than the selected diploids. Previous studies had already showed that selected diploids were 23% better than wild control oysters. In the Hand et al. (2004) et al. study, they predicted the selected diploids would be 23% better than the control; the chemically induced triploids would be 30% heavier than the selected diploids, and, therefore, approximately 60% heavier than the diploid control group. However, the authors observed that the whole body weight of the chemically induced triploid progeny was, on average, 74% greater than the control diploids. The observed improvement of the chemically induced triploids was 14% greater than the 60% hypothesized gain, possibly indicating another, more intangible factor to the triploid advantage, perhaps one due to non-additive effects, such as, heterosis.

In 2005 the ABC began a two-year field trial in an effort to determine the overall value of triploids in oyster culture. This trial consisted of four spawns each of diploids and triploids (eight in total) using broodstock selected for disease resistance. This comparison was conducted with grow out at three study sites in the upper, middle, and lower portion of Virginia waters in Chesapeake Bay. At all three sites triploids grew significantly faster than the diploid oysters, in some cases reaching market size in as much as a year faster than the diploids. At all three sites the whole tissue weight in triploids was 100% greater than in diploid oysters (ABC, unpublished). The difference

observed in whole tissue weight resulted in a line of questioning regarding the effect of triploidy. The differences observed in the ABC triploid comparison appeared to support the observations of Hand et al. (2004) with Sydney rock oyster that there is more than simply an additive component to the 'triploid advantage'.

Heterosis

Heterosis, also known as 'hybrid vigor', is an increase in the average performance progeny over and above the mean performance of the two parents, the so-called mid-parent value. It is usually manifest when two distinct inbred populations are bred together. It is the opposite of inbreeding depression and results from an increase in heterozygosity. In the Pacific oyster, *C. gigas*, heterosis has been observed as a mechanism in enhanced larval survival (Lannan, 1980) and in whole tissue weight at harvest size (14 to 40 months in age) (Hedgecock et al., 1991). Several mechanisms have been proposed as the source of heterosis in the Pacific oyster, *C. gigas*: dominance, overdominance, and epistasis (Hedgecock et al., 1995). Dominance is an interaction of alleles at a single locus, which affects the phenotype differently than if the two genes were considered singly. Overdominance, a form of dominance, occurs when the phenotypic value of a heterozygote is outside the range of either homozygote parent. Epistasis describes interactions among various loci. Another possible mechanism, increased heterozygosity, produces measurable gains in growth and survival in *C. gigas* (Hedgecock et al., 1996; Hawkins et al., 2000).

Triploid oysters possess an extra allele at every locus. Thus triploids may enjoy an added advantage from these non-additive interactions. An extra allele increases the chance that heterozygosity will occur at any given locus (Piferrer et al., 2009). While

having three distinct alleles at every locus provides an increase in heterozygosity, having other combinations of alleles may still provide beneficial effects on the phenotype by adding to dominance interactions among single or multiple loci.

Triploid advantage: Physiological changes

Triploids have one outstanding physiological distinction from their diploid counterparts: sterility. As an R-strategy organism, oysters devote a huge amount of their energy budget to reproduction (Pianka, 1970). The overall budget consists of three major components: somatic growth, gonadogenesis, and maintenance of biomass (both somatic and gametic) (Davis, 1994). Sterility among triploids is hypothesized to allow for an increase in the amount of time for somatic growth because of reduced gametogenesis, and, potentially, energy devoted to somatic growth. Increased time and energy for growth may account for some of the growth advantage triploids have over sexually mature diploids (Stanley et al., 1981; Guo et al., 1996). In Chesapeake Bay where disease pressures are, at times, significant causes of mortality for oysters, sterility may also allow for additional energy reserves that are available in defense of or in recuperation from deleterious effects of Dermo and MSX effectively making triploids more disease resistant.

Glycogen storage and utilization are important parts of oyster energy budgets, especially during gonadogenesis, when metabolic needs are high and glycogen can serve as a valuable energy source (Gabbott, 1983). Characteristic patterns of seasonal glycogen levels that correspond with gametogenic cycles were observed in a variety of marine bivalves: *Ruditapes philippinarum* and *Perna perna* (Benormar et al., 2010), *Mytilus galloprovincialis* (Moukrim et al., 2008), and *C. gigas* (Allen and Downing, 1986;

Chávez-Villalba et al., 2007; Dridi et al., 2007). In triploid *C. gigas*, depressed gonadogenesis was correlated with relatively depressed glycogen usage during peak-spawning months when compared to diploids during the same time period (Allen and Downing, 1990; Goulletquer et al., 1996). This is evident from the minor decrease in glycogen stores observed during the spawning period, compared to diploids (Allen and Downing, 1986). If glycogen is being utilized in the absence of major gametogenic activity, it follows that these reserves are being metabolized for either of the remaining allocations of energy in oysters (maintenance and growth) (Kesarcodi-Watson et al., 2001). When compared with diploid oysters, triploid *C. virginica* exhibit increased survival in the presence of both Dermo and MSX in Chesapeake Bay (Dégremont et al., 2012), which may be related to the increase in available energy stores observed in triploids (Allen and Downing, 1986). Since the 'triploid advantage' is thought to be the result of a combination of factors rather than one key mechanism (Nell, 2001), examining differences in the way diploid and triploid *C. virginica* utilize energy reserves should provide insight into the faster growth rate observed in triploids as well as their apparent ability to maintain greater survival rates in high levels of disease resistance than diploids. Matthiessen and Davis (1992) observed lower mortality in triploid *C. virginica* than diploids across three sites even though triploids had greater MSX infections. During a two-year field comparison of diploid and triploid oysters (produced from disease resistant broodstock) conducted by ABC, triploids showed a 20% increase in survival under high Dermo and MSX pressure in the York River, VA even though both were produced from disease resistant lines (Dégremont et al., 2012).

The literature is not conclusive regarding the comparative survivorship of triploid

and diploid *C. virginica* in the presence of Dermo and/or MSX. Matthiessen and Davis (1992) found that triploids had greater MSX infection prevalence, but had higher survival than diploids despite greater MSX infections. Meyers et al. (1991) exposed oysters to Dermo causing parasite and did not find a difference in survival between diploids and triploids. Triploid oysters have as high as 20% greater survival than diploids in the York River, VA where Dermo and MSX are enzootic (Dégremont et al., 2012). The relationship between the observed increases in survival of triploids under disease pressure and the resistance of triploids to these two diseases has not been explored in any great detail.

Conclusion

Culturing triploid oysters in Chesapeake Bay is becoming a widespread phenomenon because the faster growth rates and higher survival under disease pressure result in a product that can reach the market up to six months ahead of diploid stocks while maintaining high meat quality. A recent survey of growers in Virginia reported that 87% of the 66.7 million oyster seed planted in 2012 were triploid (Murray and Oesterling, 2013). Apparently, the 'triploid advantage' provides significant thrust to the growth of oyster aquaculture in Chesapeake Bay, especially because of lower mortality rates and also increased meat yield (for shucked product). The increasing economic importance of triploid oysters makes it important to further understand the mechanisms behind the triploid advantage. For example, is it more important to concentrate on improving the diploid, improving the tetraploid, or improving both? What are the most appropriate traits to target if improvement of the tetraploids is warranted?

The literature is sparse on the contributing factors of the triploid advantages for

most oyster species and is, for all intents and purposes, non-existent for *C. virginica*, especially for comparisons of triploid and diploid stocks of common lineages. This study provides comparisons of growth rates and survival rates among diploid and triploid *C. virginica.* It also compares these parameters among triploid groups produced from various genotypes, ranging from wild to highly selected lines.

Objectives

The first objective of this study is to compare field performance among wild and selected lines both as diploids and as triploids. Several aspects of these comparisons are of particular interest to further our knowledge of the triploid advantage. Comparing relative performance among diploids and their triploid counterparts may offer insight as to whether or not the genomic contribution of the diploid parent in a tetraploid x diploid cross is significant (i.e., did the triploids perform relative to their diploid counterparts). One unique comparison is between wild stocks originating in Maryland with both wild and selected Virginia stocks at each site, and in particular the Maryland study site. With few studies done to examine the potential of appropriate Virginia stocks in Maryland, and vice versa, this study provided valuable information for use of wild stocks in private aquaculture operations.

The second objective is to compare field performance among several generations of selected lines of oysters. While a comparison of a wild line and a line that has been selected for several generations may offer insight into the role of the diploid parent in the triploid cross, it is of great interest to determine if gains achieved through selection across generations carry in their triploid counterpart and to quantify these gains to determine how they translate from diploid to triploid. To achieve this, the selected 2006-year class

lines are compared with the 2008-year class lines, also known as 'Superlines'.

The third objective is to compare disease prevalence and intensities among triploids made from susceptible diploids and those from diploids selected for disease resistance. Triploid oysters typically have greater survival rates than diploid oysters under disease pressure when grown to market size and an examination of this comparison provided insight as to whether triploids have increased disease resistance than diploids or not.

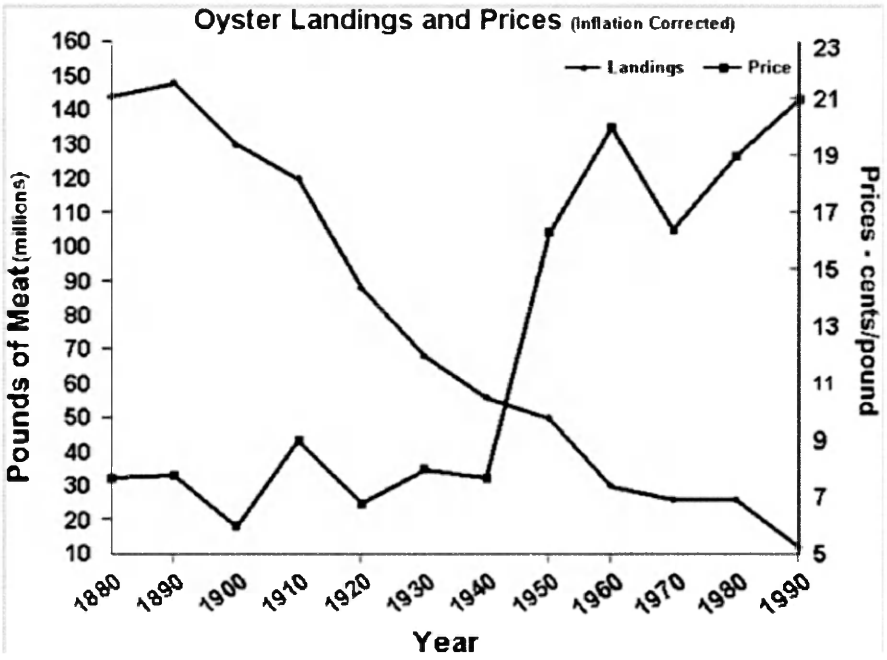

Figure 1.1. East Coast (USA) landings (millions of pounds of meat) and inflation-corrected prices (cents·pound^{-1}) of oysters, 1880 to 1990. Data from Lyles (1969) and NMFS landings statistics (from MacKenzie, 2007). Landings are indicated with triangles and inflation-corrected prices with squares.

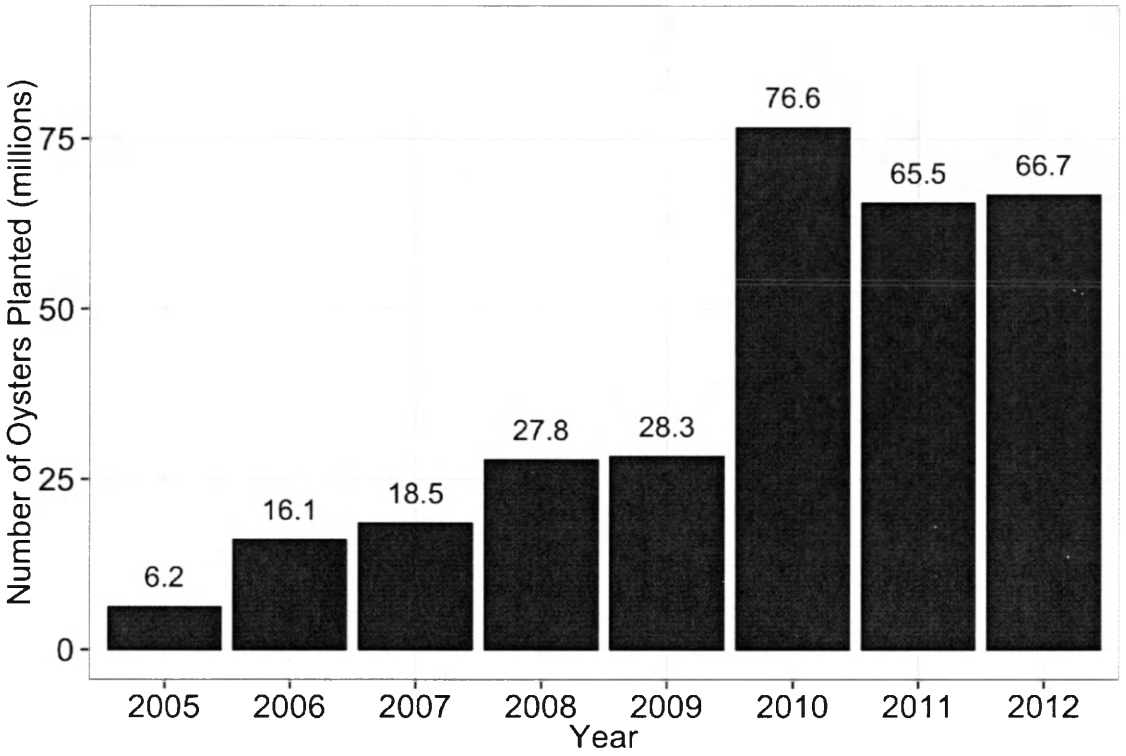

Figure 1.2. Number of Oysters Planted (in millions) by Virginia Aquaculturists from 2005-2010. Plantings were reported through Virginia Sea Grant industry survey (from Murray and Hudson, 2011).

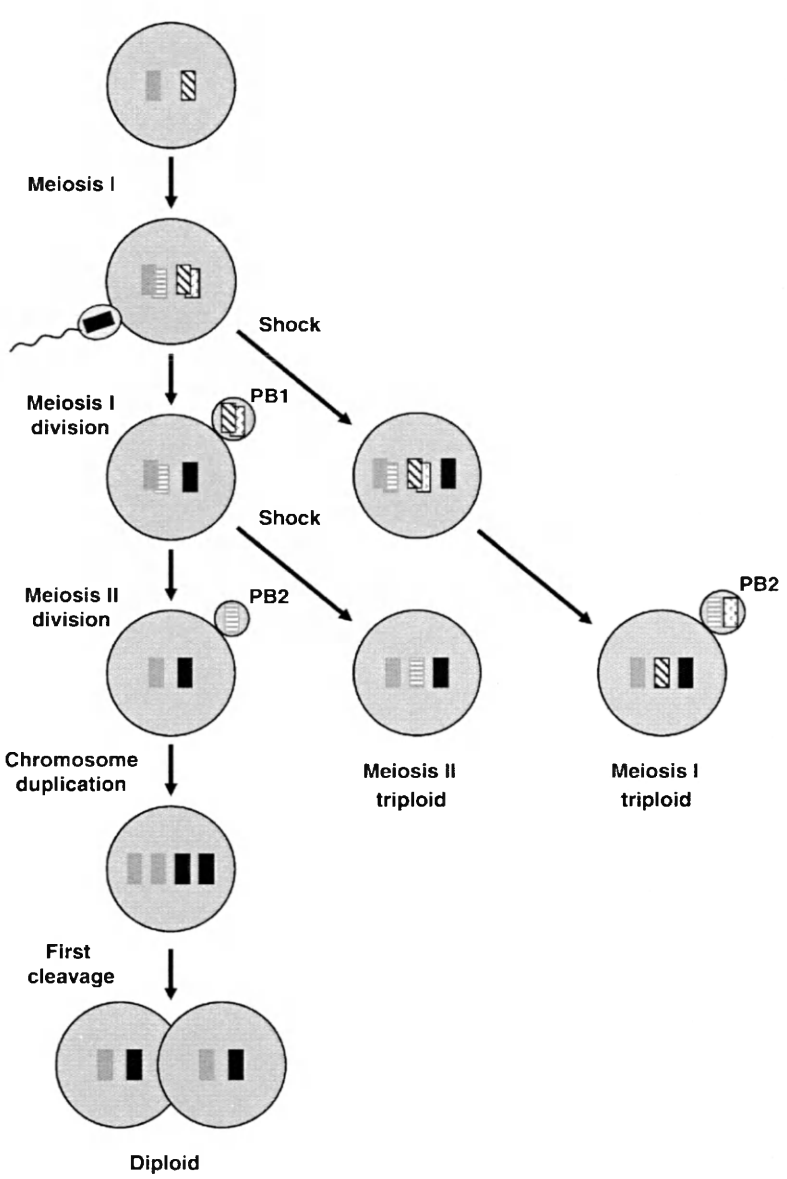

Figure 1.3. Ploidy manipulation in shellfish. Eggs are released at metaphase of meiosis I. Fertilization resumes meiosis. Physical or chemical shock applied during meiosis I or meiosis II can suppress cell division, producing triploids by retention of the first (PB1) or second (PB2) polar body. For simplicity, in this hypothetical species $2n=2$. Thus, each bar inside the cell represents one chromosome and overlapping bars indicate the sister chromatids after DNA replication during meiosis I. (from Piferrer et al., 2009)

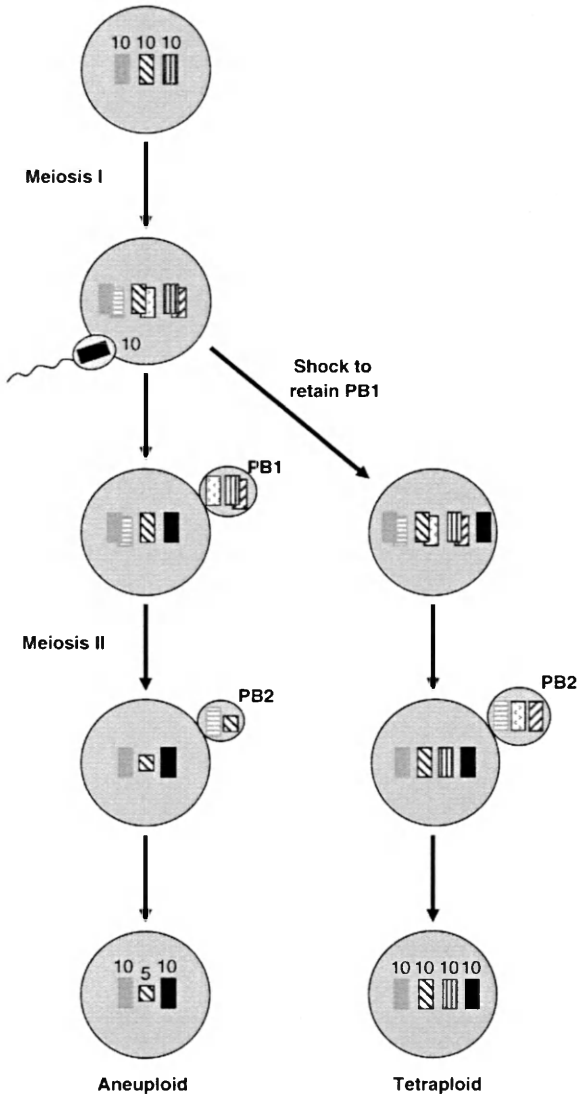

Figure 1.4. Production of tetraploid Pacific oysters, Crassostrea gigas, from a cross between eggs from a triploid female and spermatozoa from a diploid male with suppression of first polar body (PB1) extrusion. The haploid number for Pacific oyster is 10, and 15 chromosomes are indicated in the triploid egg after meiosis II, which along with the 10 chromosomes provided by the sperm result in an aneuploid embryos ($2n$=25). Here, each bar inside the cell represents an entire haploid complement of 10 chromosomes (from Piferrer et al., 2009).

Chapter Two: Triploid advantage for growth in *C. virginica* produced from wild stocks and breeding lines

1. INTRODUCTION

Adoption of triploid *C. virginica* for aquaculture in the Chesapeake Bay has only occurred recently. Arguably it started when farmers were exposed to polyploidy through growth trials (Allen, 2005) conducted by the Virginia Seafood Council and ABC in which triploid *C. virginica* were used as a comparative group to test the feasibility of growing non-native oysters (*C. ariakensis*) (Calvo et al., 2001; Grabowski et al., 2004; Paynter et al., 2008; Kingsley-Smith et al., 2009). This exposure enabled growers to gain firsthand experience with how triploids growth in the lower Chesapeake Bay. In the lower portion of the Chesapeake Bay, the major obstacle for survival was disease pressure from two protozoan parasites: *Perkinsus marinus* (cause of Dermo disease) and *Haplosporidium nelson* (cause of MSX disease) (Burreson, 1991). The triploid advantage observed in the presence of these diseases likely led to the increased popularity of triploid *C. virginica* for aquaculture in the Virginia portion of the Chesapeake Bay. Triploid oysters now comprise 89% of all farmed oysters in Virginia estuaries in the Chesapeake Bay (Murray and Hudson, 2013). Various explanations for the triploid advantage have been proposed: energy allocation differences due to gametogenic suppression, additive genetic effects from selective breeding, and even greater heterozygosity (heterosis) (Allen and Downing, 1986; Barber and Mann, 1991; Hand et al, 1998).

Energy allocation is a major aspect of the triploid advantage. Oyster energy budgets consist of three major components: somatic growth, gonadogenesis, and biomass maintenance (somatic and gametic) (Bayne and Newell, 1983; Hawkins et al., 1989; Widdows and Hawkins, 1989). Several studies have observed that growth rate of triploids overtakes that of diploids after the first year, when diploids start to allocate significant annual energy resources to reproduction (Stanley et al., 1984; Barber and Mann, 1991). Davis (1994) observed, through a series of laboratory experiments on metabolic rates of Pacific oysters, that standard and routine metabolic rates of diploids and triploids were similar even though gametogenic development of diploids in the study was significantly higher than that of triploids. Similar metabolic rates of reproductively active diploids and actively growing triploids suggest the relative cost of production and maintenance of gametic tissue is similar to that of somatic tissue. In environments characterized as "less suited for germinal production" because of environmental factors, Davis (1994) observed similar metabolic rates between diploids and triploids again. The lack of differences in metabolic activity between diploids and triploids may be an important factor in why no differences in growth between diploid and triploid oysters were observed in poor growing areas. If diploids are not utilizing energy reserves for producing gametic tissue, then that energy may otherwise be used for somatic growth (Davis, 1994; Nell, 2001; Racotta et al., 2008), like a sterile triploid.

For additive effects of the triploid advantage, Hand et al. (2004) conducted a hallmark study with Sydney rock oysters (*Saccostrea glomerata*) by comparing diploid and chemically induced triploid progeny of a third generation breeding line along with an unselected control group (Hand et al., 2004). In this study, triploids were chemically

induced, so all three sets of chromosomes originated from the selectively bred population. This is distinct from the triploids used in this thesis because, as described in greater detail below, triploids from $4n \times 2n$ crosses contain chromosomes from two separate populations (the tetraploid population and the diploid population) rather than from one source (the population of the diploid parents used for triploid induction). The authors hypothesized that if observed gains in whole body weight were simply additive genetic gains, the triploids made from selected lines ought to be a minimum of 30% heavier than the selected diploids. With previous data on the whole weight gains of selected diploids over the control, the authors predicted the additive gains expected from triploids would be 60%. What Hand et al. (2004) found was that whole body weight of the chemically induced triploid progeny was, on average, 74% greater than the control diploids. The observed improvement of the chemically induced triploids was 14% greater than the predicted gain. Essentially, then, the additive gain was realized, and then some. The additional 14% improvement over the prediction indicates another component to the triploid advantage.

Research on the benefit of triploids has shown similar effects in *C. virginica*. In an effort to determine the overall value of triploid *C. virginica* in oyster culture, ABC conducted a two-year field trial, beginning in 2005, consisting of four spawns of diploids and triploids using disease resistant broodstock (Dégremont et al., 2012). This comparison was conducted at three study sites in the upper, middle, and lower portion of Virginia waters in Chesapeake Bay. At all three sites triploids grew significantly faster than diploid oysters, in some cases reaching market size a year faster than diploids. At all three sites the whole tissue weight was 100% greater than the diploid oysters. The

difference in whole weight – that metric used by Hand et al. – was 88% greater for triploids across all three sites. Thus, findings of the ABC trials with Eastern oysters confirmed observations of Hand et al. (2004) with Sydney rock oysters, that there may be several components to the triploid advantage.

A third possible explanation for triploid advantage may be heterosis. Heterosis describes an increase in the average performance of progeny over and above the mean performance of the two parents (i.e., the mid-parent value). It is usually manifest when two distinct inbred populations are bred together. It is the opposite of inbreeding depression and results from an increase in heterozygosity. In oysters, heterosis has been observed as a mechanism in enhanced diploid larval survival for *C. gigas* (Lannan, 1980) and in adult whole tissue weight (Hedgecock et al., 1991). Increased heterozygosity in diploid oysters has been linked with gains in growth and survival in *C. gigas* (Hedgecock et al., 1996; Hawkins et al., 2000). Because heterosis is well documented by these and other studies in oysters, it seems reasonable to propose a role for heterosis as part of the triploid advantage. That is, by virtue of having another set of alleles, more interaction is possible in triploids than diploids. With a genotype of AB, there is one heterozygote combination; with a genotype of ABC, three.

The genetic contribution from both the tetraploid and diploid parents ($4n \times 2n \rightarrow 3n$) of triploids may also be critical for analyzing the potential contribution of heterosis to the triploid advantage. Mated triploids have three sets of chromosomes, two from the tetraploid parent and one from the diploid. Clearly, the choice of parental stock and the relative contribution of them in the triploid will influence heterosis. Given two distinct genetic groups, for example, wild *versus* selected as in this study, there are four 'types' of

mated triploids possible based on the genetic contribution of the tetraploid and diploid parents. Note, however, commercial triploids are made from tetraploid males and diploid females, and never vice versa. Thus, technically – with reciprocal crosses – there are eight different types of triploid combinations possible using different genotypes from the tetraploid and the diploid, but only the following four are relevant. True wild triploids (+++) have a wild-type tetraploid (++++) father and wild-type diploid (++) mother. (male parent first). Selected (S) triploids (SSS) have a selected tetraploid (SSSS) father and selected diploid (SS) mother. "Hybrid" triploids can result from a cross of a selected tetraploid (SSSS) father with a wild-type diploid (++) mother yielding a double dose of selected chromosomes in an SS+ triploid. Or, a hybrid can originate from a wild-type tetraploid (++++) father with a selected diploid (SS) mother yielding only one dose of chromosomes that have been through selection (++S) (Table 2.1). However, ABC does not produce wild-type tetraploids. All of the triploids in this study are either the true selected type (SSS) or hybrid (SS+). Producing wild-type tetraploids (++++) is impractical in the Chesapeake Bay. Inducing tetraploidy, in general, is a difficult process typically resulting in low survival of larvae (<1%) and variable proportions of tetraploids in surviving larvae (Guo and Allen, 1994; Eudeline et al., 2000a, b; Guo et al., 2002). In addition, tetraploids can be difficult to maintain under the best of circumstances and trying to maintain them in disease free locations is challenging, especially in the lower portion of Chesapeake Bay. These factors make pursuing the development of ++++ tetraploids impractical and unlikely.

The triploid advantage attributable to heterosis may be more difficult to demonstrate than that from sterility or additive gains in the absence of more extensive test

crosses. Nonetheless, this study could provide a better understanding of the triploid advantage overall and begin to dissect the effect of the tetraploid vs. the diploid contribution. Specifically, for this study, we examined the role of the diploid female in the tetraploid × diploid. By comparing triploids produced from wild-type females ($3n$ = SS+) from a variety of environments as well as females from selected diploid lines ($3n$ = SSS), the roles of additive gains, physiological differences, and possibly heterosis may become clear. Of direct practical import, these comparisons will yield important information about broodstock choice for triploid crosses. This information is of great value to industry hatcheries and farmers seeking the most economically and biologically sound decisions for their operations.

2. MATERIALS AND METHODS

2.1 Crosses

Diploid and triploid oysters were produced at the ABC Oyster Hatchery, Gloucester Point, VA through June and July 2010. Broodstock used to produce these oysters were collected from five wild populations in the Chesapeake Bay as well as from two groups of selectively bred disease resistant (DR) lines – 2006-year class lines and Superlines – from ABC's breeding program.

Of the five wild stocks used, three were collected from different estuaries in Virginia, which range in environmental conditions. The Great Wicomico River (WIC) has low salinity (range 10-15 ppt) and only sporadically intense disease pressure, therefore oysters from this location show high susceptibility to disease pressures, both MSX and Dermo (Southworth et al., 2010; ABC, unpublished data). The Rappahannock River (RAP) has moderate salinity (range 13-20 ppt) and disease pressure from Dermo and, in addition, is a common source of broodstock used in commercial hatcheries, allowing for a comparison between a commonly used industry product (diploids and triploids generated from Rappahannock brood) and selected ABC lines. Wild oysters from Mobjack Bay (MBY) are the standard control used within ABC's breeding program because of their higher salinity origin and frequent disease exposure to both MSX and Dermo. The remaining two wild stocks were collected from estuaries in Maryland: Chester River (CHES) and Patuxent River (PATX). The two wild stocks from Maryland

were chosen because wild oyster populations in the Patuxent River experience consistent Dermo pressure (Albright et al., 2007; McCollough et al., 2007) while oysters in the Chester River do not (Abbe et al., 2010).

Four lines from ABC's 2006-year class lines were used: LGT, OBOY, DBY, and XB. The LGT line was derived from wild oysters in Grande Terre, LA in 2000 and selected by ABC for disease resistance since then for four generations. OBOY was introduced into ABC's breeding program in 2002 as an F_3 generation derived from wild oysters in Oyster Bayou, LA and subsequently selected for Dermo resistance by Dr. Jerome LaPeyre's program at Louisiana State University. XB was developed in Delaware Bay, NJ at Rutgers University by S. Allen from a consolidation of many lines produced by Ford and Haskin (1987) prior to 1988. They were brought to Chesapeake Bay in 1998 and propagated within ABC (Dégremont et al., 2012). Due to limited availability of the 2006-year classes of LGT, OBOY, and XB, 2009-year classes of these three lines were used. These were propagated from the 2006-year class of the corresponding line via random pooled spawns through an effort to preserve the germplasm of these lines. Pooled spawns are those in which gametes from multiple dams and sires are each combined and then added together to initiate fertilization. The DBY line was developed from wild oysters collected from Delaware Bay, NJ in 1987 and selected for Dermo and MSX resistance for four generations in the York River, VA (Ragone Calvo et al., 2003). Subsequent selection and generations were produced by ABC's breeding program. The 2006 DBY year class is an F_7 generation.

The four Superlines are Lola, hANA, SL-DBY, and SL-XB. Lola was produced using progenitors from Louisiana, known to be Dermo resistant (Ragone Calvo et al.,

2003), and selected in Virginia for MSX resistance for three generations. Since 2007, however, this line had been further selected for low salinity tolerance in a mesohaline site (Yeocomico River, VA). hANA was also developed using progenitors from Louisiana; however, since 2007 these animals were selected for increased MSX disease resistance in a polyhaline site (York River, VA). The SL-DBY line was developed from wild oysters from Delaware Bay, NJ that were collected in 1987 (Ragone Calvo et al., 2003). SL-XB was created in Delaware Bay, NJ then transferred to Chesapeake Bay for selection under ABC's breeding program beginning in 1998 (Ford and Haskin, 1987; Dégremont et al., 2012). A detailed pedigree of the Superlines can be found in ABC's breeding manual (ABC, 2010).

Broodstock were conditioned in a flow-through system at ABC's conditioning facility, the Kauffman Aquaculture Center (KAC) on Locklies Creek, VA. In the flow-through system, water temperature was held constant at 23°C. Broodstock were batch fed cultured algae cocktails containing *Isochrysis* sp., *Tetraselmis chui*, and *Chaetoceros muelleri*. When all stocks had conditioned, they were transferred to ABC's research hatchery in Gloucester Point, VA for spawning and larval rearing.

Eggs obtained from at least 10 dams per stock (wild) or line (selected) were stripped from gonad tissue and pooled in plastic beakers. The pools of eggs were then divided into two groups containing 3×10^6 eggs each, one for diploids and one for triploids. To produce diploids, one group of eggs was fertilized with sperm pooled from at least 10 sires of the same stock/line when available. To produce triploids, the remaining groups of eggs were fertilized with sperm pooled from 11 sires from a single tetraploid family following the methods of Guo et al. (1996). The number of dams and

sires from each group are outlined in Table 2.2. Several crosses were made with reduced dam and sire numbers due to the limited availability ripe broodstock. This produced 26 groups: 13 diploid and 13 triploid (Figure 2.1). While all 26 groups were spawned, the analysis of the 2006-year class lines will appear in Chapter 3, this chapter details the wild stocks and the Superlines.

2.2 Larval Rearing

Larvae were reared through settlement following the ABC protocol adapted from Helm et al. (2004) in 60L flat-bottom larvae tanks, consisting of daily batch feeding of microalgae and complete water exchanges three times a week (Monday, Wednesday, and Friday). Larval tank densities were adjusted based on age (in days) post-fertilization, such that, on days two, seven, and 14, the densities were adjusted to 10-larvae·mL^{-1}, 5-larvae·mL^{-1}, 2.5-larvae·mL^{-1}, respectively. Eyed-larvae (i.e., larvae competent to metamorphose) were collected on 212µm for diploids or 250µm nylon screen for triploids. Eyed-larvae were transferred to 16cm^2 downwellers for settlement. After two weeks in this downwelling system, the recruited juveniles (i.e., spat) were moved into a flow-through upweller based nursery until field deployment.

2.2.1 Ploidy Determination

Ploidy was determined at various stages of rearing by flow cytometry to determine the frequency of triploidy within crosses (Allen, 1983). Prior to pooling sperm from the 11 tetraploid sires, sperm from each individual were confirmed 100% di-haploid by analyzing gametes dissected from gonad tissue. Ploidy was analyzed again at the prodissoconch I larval stage on larvae collected on a 48µm nylon screen 48hrs post-fertilization by sampling 2000 larvae and prior to field deployment by sampling 50 spat

from each group. At each of these sampling points all groups were confirmed 100% diploid or triploid.

2.3 Experimental Sites and Design

The timing of deployment was not ideal, although all groups were treated identically. Spawning near the end of the summer leads to longer growth time in the nursery prior to deployment. This led to a deployment later than is typical (i.e., late spring-early summer).

Oysters were deployed at three sites in the Chesapeake in November 2010 (Figure 2.2). In the Virginia portion of the Chesapeake Bay, the two sites were the York River (13-25 ppt) and the Rappahannock River (13-20 ppt). The York River site is opposite VIMS on a private lease operated by Tommy Leggett of Chessie Seafood Company. The grow-out location in the Rappahanock River is on a lease owned by the Rappahannock River Oysters, LLC in Topping, VA. These sites were chosen in order to perform the experiment under environmental conditions of commercial operations. In the Maryland portion of the Chesapeake Bay, oysters were deployed in the Choptank River (5-12 ppt) adjacent to the University of Maryland Horn Point Environmental Laboratory (Figure 2.2).

Oysters were deployed in off-bottom cages at each of the Chesapeake Bay sites for evaluating growth. The off-bottom cages were designed and manufactured by the Chesapeake Bay Oyster Company. A single cage can hold three full-sized oyster grow-out bags (60cm x 91cm) with 1.27cm mesh size. Off-bottom cages were chosen because it is the most common method of commercial culture in the Chesapeake Bay. Diploid

and triploid MBY had unexplained low survival in the field nursery and as a result were not deployed to the Choptank River site.

2.3.1 Sampling

2.3.1.1 Growth Parameters

Two ADPI bags (approx. 0.6m x 0.9m polyethylene oyster bags) per group (thirteen diploid groups and thirteen triploid groups) were stocked at 500 oysters per bag. The 52 bags (two replicates per group) were randomly placed into 15 cages. Density in the bags was not periodically reduced since destructive sampling and mortality kept densities suitable until June 2012. In June 2012, bag densities were reduced to occupy 1/3 of the bag by splitting groups into additional replicates in both the York and Rappahannock Rivers. Splitting was not necessary in the Choptank River. Samples of 25 oysters per replicate per group (50 oysters per group total) were sampled every spring, fall, and winter beginning in the spring of 2011 ending winter 2012 for measurements of shell height (distance between the umbo and the ventral valve margin), whole wet weight, wet tissue weight, and meat yield (calculated as wet tissue weight ÷ whole wet weight). Wet tissue weight was measured after the body tissues were drained on a mesh screen. The percentages of sampled oysters that were harvest size (≥76mm) were also recorded at each point as well. From the growth measurements at the end of the study (December 2012), triploid advantage was calculated as the percent difference in growth of triploids relative to diploid performance of a given line/stock (Equation 1):

(1)

Triploid effect = (3*n* line/stock performance – 2*n* line/stock performance) ÷ 2*n* line/stock performance * 100

where a positive triploid effect indicates triploid outperformed diploids and a negative triploid effect indicates diploids outperforming triploids.

2.3.1.2 Environmental Parameters

Temperature was recorded every hour using iBCod submersible temperature data loggers (Alpha Mach Inc., Ste-Julie, Qc, Canada). Cumulative day degrees (CDD) were calculated from average daily temperatures at each site as an indicator of time spent in growth-favorable temperature ranges. Individual day degrees (DD) were calculated using average daily temperatures (Equation 2):

$$DD = T_{avg} - T_{base} \qquad (2)$$

where T_{base} is 8C, and when DD is greater than zero, otherwise DD is zero. CDD was calculated by the summation of DD over a given time period (Equation 3):

$$CDD = \sum_{i=1}^{i} DD_i \qquad (3)$$

where DD_i is the DD for day *i*.

Salinity data for the Rappahannock and York Rivers were taken from long-term monitoring stations from the Virginia Estuarine and Coastal Observing System. The Rappahannock River data were taken from station LE3.4 and the York River data from LE4.3. The University of Maryland Center for Environmental Science's Oyster Hatchery provided salinity data for the Choptank River.

2.4 Analyses

2.4.1 Time Frame

Shell height, whole weight, and meat yield were analyzed in December 2011 (17 months post-spawn) and December 2012 (29 months post-spawn).

2.4.2 Model Equation

The following model was used for analysis (Equation 4):

(4)

$$Y_{ijkl} = \mu + site_i + ploidy_j + stock_k + (site_i \text{ X } ploidy_j) + (site_i \text{ X } stock_k) + (ploidy_j \text{ X } stock_k) + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable (shell height, whole wet weight, wet tissue weight, and meat yield), μ is the overall mean, $site_i$ is the site effect (York River, Rappahannock River, Choptank River), $ploidy_j$ is the ploidy effect in *C. virginica* (diploid or triploid), $stock_k$ is the genotype effect, 'X' indicates interactions, and ε_{ijkl} is the residual error.

2.4.3 Statistical Procedures

Normality and the homogeneity of variance for shell height, whole weight, and meat yield were confirmed with the Shapiro-Wilk's *W* test for normality with the statistical programming language R (R Core Team, 2012).

ANOVA was performed in R using the nlme package (Pinheiro et al., 2013). Following significant findings from the ANOVA, multiple comparisons were conducted using Tukey's Honest Significant Differences test in R. When a significant interaction between site and group was found, the site was dropped from the analyses to test the group effect within the site. Replicates were not a significant source of variation and as such were not included in the ANOVA model. Due the equivalency of replicates, confidence intervals reported were calculated from the combined individual measurements from both replicates.

3. RESULTS

Results in this chapter are restricted to the relative performance of the wild stocks versus the Superlines.

3.1 Environmental Parameters

Average daily temperature was calculated from hourly measurements at each site. The three experimental sites were similar in range of temperatures observed (Figure 2.3). Temperature in the Choptank River ranged from (-0.5) - 31.2°C, in the Rappahannock River, (-1.4) - 30.1°C, and in the York River, 1.6 - 31.9°C. The ranges of temperature observed at each site were similar, but the CDD varied slightly. CDD in December 2011 in the Choptank River was 5771, in the Rappahannock River, 5605, and in the York River, 5831. At the end of the trial the CDD in the Choptank River was 9172, the Rappahannock River, 8964, and in the York River, 9350 (Figure 2.4). There was only a 4% difference between the highest and lowest CDD values by the end of the study. Average salinity and SD was 18.2 ± 2.9 in the York River, 13.9 ± 3.2 in the Rappahannock River, and 9.6 ± 2.6 in the Choptank River during the study period (Figure 2.5).

3.2 Growth Parameters

3.2.1 Shell Height

Overall growth trends are depicted in Figure 2.6. Oysters were deployed from the field nursery into the experimental design at each site in April 2011. Table 2.3 reports

initial shell height measurements (mm ± 95%CI) of individual stocks and lines. Diploid and triploid shell height increased from May 2011 to September 2011 at each site. From September 2011 until May 2012, growth plateaued in the Choptank River. Growth in the Choptank River began again after May 2012. This plateau in growth was not observed in the Rappahannock and York Rivers, but rather a reduced but consistent growth rate for the remainder of the study occurred (Figure 2.6). In both December 2011 and 2012, the site effect, ploidy effect, stock effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 2.4).

3.2.1.1 Choptank River, MD

Wild Virginia Stocks

By December 2011 (17 months) diploid WIC was the only wild stock from Virginia to have significantly greater shell height (mean ± 95%CI) than its triploid counterpart (46.9 ± 1.6mm and 38.9 ± 1.8mm, respectively, $p<0.05$). There were no significant differences between the individual diploid or triploid wild stocks from Virginia by 17 months ($p>0.05$) (Figure 2.7).

By December 2012 (29 months), the effect of triploidy had diminished from WIC and there were no significant differences among shell heights of the diploid and triploid groups ($p>0.05$). The triploid effect of wild Virginia stocks, though not significant, ranged from a disadvantage of -2% to 0% (Table 2.6). There were no significant differences between the individual diploid or triploid wild stocks ($p>0.05$) (Figure 2.8).

Wild Maryland Stocks

By 17 months, diploid PATX was the only wild stock from Maryland that had lower shell height than its triploid counterpart, and a triploid disadvantage (43.8 ± 2.0mm

and 46.5 ± 2.0mm, respectively, $p<0.01$). There were no significant differences between the individual diploid or triploid wild stocks from Maryland ($p>0.05$) (Figure 2.7).

By 29 months, the triploid disadvantage had diminished and there were no significant differences among shell heights of the diploid and triploid groups ($p>0.05$). The triploid advantage of wild Maryland stocks, though not significant, ranged from 2-3% (Table 2.6). There were also no significant differences between the individual diploid or triploid wild stocks ($p>0.05$) (Figure 2.8).

Superlines

By 17 months, diploid Superline SL-XB had greater shell height than triploid SL-XB (42.8 ± 2.1mm and 36.9 ± 1.5mm, respectively, $p<0.05$). There were no significant difference between diploid and triploids of the remaining Superlines in the Choptank River ($p>0.05$). The Superline SL-XB had significantly lower shell height than the hANA, Lola, and SL-DBY Superlines as diploids and triploids (diploid: SL-XB 42.8 ± 2.1mm, hANA 48.1 ± 2.1mm, Lola 46.9 ± 2.3mm, and SL-DBY 48.3 ± 1.5mm and triploid: SL-XB 36.9 ± 1.5mm, hANA 43.4 ± 1.8mm, Lola 46.4 ± 2.1mm, and SL-DBY 44.8 ± 2.0mm) (Figure 2.7).

By 29 months, the effect of triploidy had diminished and there were no significant differences among shell heights of the diploid and triploid lines ($p>0.05$). The triploid advantage of Superlines, though not significant, was greater than the wild stocks and ranged from 4% to 8% (Table 2.6). Diploid Superlines Lola and SL-DBY (77.6 ± 3.9mm and 72.8 ± 3.0mm, respectively) had significantly higher shell heights than diploid hANA and SL-XB (69.8 ± 3.5mm and 65.2 ± 3.9mm, respectively, $p<0.05$). Of the triploid Superlines, Lola had higher shell height than hANA, SL-DBY, and SL-XB (77.6 ±

3.9mm, 69.8 ± 3.5mm, 72.8 ± 3.0mm, and 65.2 ± 3.9mm, respectively, $p<0.05$). Triploid hANA and SL-DBY did not have significantly different shell heights ($p>0.05$). Triploid SL-XB had the lowest shell height of the four triploid Superlines (Figure 2.8).

3.2.1.2 Rappahannock River, VA

Wild Virginia Stocks

In the Rappahannock River, by 17 month, only one diploid:triploid comparison differed in size: diploid WIC had significantly lower shell height than its triploid counterpart (55.1 ± 2.2mm and 63.5 ± 2.5mm, respectively, $p<0.01$). Of the individual diploid wild stocks from Virginia, RAP had higher shell height than both the WIC and MBY stocks (60.2 ± 2.5mm, 55.1 ± 2.2mm, and 56.1 ± 1.9mm, respectively, $p<0.05$). Diploid WIC and MBY stocks had equivalent shell heights ($p>0.05$). There were no significant differences between the individual triploid wild stocks ($p>0.05$) (Figure 2.7).

By 29 months, the effect of triploidy was larger triploids than diploid for WIC and MBY equating to a triploid advantage of 10% for WIC and 15% for MBY (WIC: diploid – 82.7 ± 4.1mm and triploid – 90.7 ± 2.8mm, MBY: diploid – 78.1 ± 2.7mm and triploid – 90.0 ± 4.5mm, $p<0.05$) (Table 2.6). The triploid RAP did not survive to the end of the study. Diploid wild stocks WIC and RAP had equivalent shell heights (82.7 ± 4.0mm and 82.3 ± 3.5mm, $p>0.05$) and both had greater shell heights than MBY (78.1 ± 2.7mm), though this difference was not significant ($p>0.05$). There were no significant differences between the individual triploid wild stocks ($p>0.05$) (Figure 2.8).

Wild Maryland Stocks

There were no differences among diploid-triploid counterparts by 17 months in the Rappahannock River. There were no significant differences between the individual diploid or triploid wild stocks ($p>0.05$) (Figure 2.7).

At 29 months, the triploid advantage was present for CHES (diploid – 77.4 ± 2.7mm and triploid – 85.7 ± 2.8mm) and PATX (diploid – 76.0 ± 3.1mm and triploid – 83.5 ± 3.3mm) ($p<0.05$). The triploid advantage for the CHES stock was 11% and 10% for the PATX stock (Table 2.6). There were no significant differences between the individual diploid or triploid wild stocks ($p>0.05$) (Figure 2.8).

Superlines

There was no triploid effect in the Superlines at 17 months and no diploid:triploid counterparts were significantly different. Among the diploid Superlines there were no differences in shell heights ($p>0.05$). Triploid Superlines Lola and hANA had equivalent shell heights (63.3 ± 2.3mm and 60.5 ± 2.2mm, respectively, $p>0.05$) and triploid SL-DBY and SL-XB had equivalent shell heights (58.4 ± 2.7mm and 54.4 ± 1.5mm, respectively, $p>0.05$). Triploid Lola and hANA had greater shell heights than SL-DBY and SL-XB ($p<0.05$) (Figure 2.7).

By 29 months, there was still no triploid effect on shell height as there were no significant differences among shell heights of diploid and triploid Superlines ($p>0.05$). The triploid advantages were low (as the differences from ploidy were not significant): hANA – 2%, Lola – (-1%), SL-DBY – 3%, and SL-XB – 6% (Table 2.6). Diploid Superline Lola had the greatest shell height of the diploid Superlines (92.7 ± 4.3mm), followed by hANA (88.7 ± 3.1mm), and then SL-DBY and SL-XB (83.9 ± 3.7mm and 80.6 ± 3.1mm, respectively), both of which had equivalent shell heights. Triploid hANA

and Lola had similar shell heights (90.9 ± 3.0mm and 92.1 ± 2.9mm, respectively) and were both significantly greater than triploid SL-DBY and SL-XB (86.3 ± 3.6mm and 85.3 ± 2.6mm, respectively), whose shell heights were not significantly different from one another (Figure 2.8).

3.2.1.3 York River, VA

Wild Virginia Stocks

In the York River, at 17 months, there was no triploid effect on shell height for any of the wild Virginia stocks ($p>0.05$). There were no differences among the individual diploid or triploid wild Virginia stocks ($p>0.05$) (Figure 2.7).

By 29 months, triploidy had affected growth positively in two of the wild Virginia stocks. Diploid WIC and MBY had significantly smaller shell height than their triploid counterpart (WIC: diploid – 73.2 ± 2.3mm and triploid – 87.7 ± 3.6mm, MBY: diploid – 82.4 ± 3.3mm and triploid – 90.2 ± 3.2mm, $p<0.05$) equating to a triploid advantage of 20% and 9%, respectively (Table 2.6). Among the diploid wild Virginia stocks, shell heights were equivalent ($p>0.05$). Triploid wild stocks WIC and MBY had significantly greater shell heights than RAP (87.7 ± 3.6mm, 90.2 ± 3.1mm, and 81.5 ± 3.7mm, respectively, $p<0.05$) (Figure 2.8).

Wild Maryland Stocks

In the York River, by 17 months, triploidy had an effect on the PATX stocks. Diploid PATX had significantly lower shell height than its triploid counterpart (45.9 ± 3.1mm and 61.3 ± 2.5mm, respectively, $p<0.001$). Of the diploid wild Maryland stocks, CHES had significantly greater shell height than PATX (56.3 ± 2.5mm and 45.9 ±

3.1mm, respectively, $p<0.05$). There was no significant difference in the individual triploid stocks ($p>0.05$) (Figure 2.7).

By 29 months, triploidy had affected growth positively in the CHES crosses. Diploid CHES had significantly smaller shell height than triploid CHES (66.4 ± 2.8mm and 89.2 ± 3.2mm, respectively, $p<0.05$) and a triploid advantage of 34% (Table 2.6). Diploid PATX oysters did not survive to the end of the study. Triploid CHES had greater shell height than PATX (89.2 ± 3.2mm and 80.7 ± 3.4mm, respectively, $p<0.05$) (Figure 2.8).

Superlines

By 17 months, there were no other significant differences between diploid and triploids of the Superlines in the York River ($p>0.05$). The diploid Superlines hANA, Lola, SL-DBY, and SL-XB all had equivalent shell heights as did the triploid Superlines ($p>0.05$) (Figure 2.7).

By 29 months, triploidy had affected growth positively in two of the Superline crosses. Of the Superlines, diploid hANA had significantly smaller shell height than its triploid counterpart (83.3 ± 3.3mm and 95.4 ± 3.6mm, respectively, $p<0.05$) and diploid Lola being significantly smaller than triploid Lola (80.8 ± 3.6mm and 90.8 ± 3.5mm, respectively, $p<0.05$). These differences equate to a triploid advantage of 15% for hANA and 12% for Lola (Table 2.6). The shell heights of diploid hANA, Lola, and SL-DBY were not significantly different (83.3 ± 3.3mm, 80.8 ± 3.6mm, and 82.2 ± 3.1mm, respectively, $p>0.05$) and all were greater than diploid SL-XB (76.2 ± 3.1mm, $p<0.05$). Triploid Lola, SL-DBY, and SL-XB had equivalent shell heights (90.8 ± 3.5mm, 89.3 ±

3.0mm, and 88.5 ± 2.8mm, respectively, $p>0.05$). Triploid hANA (95.4 ± 3.6mm) had greater shell height than triploid SL-XB only ($p<0.05$) (Figure 2.8).

3.2.2 Whole Wet Weight

Overall growth trends are depicted in Figure 2.9. Initial whole wet weight measurements (mean ± 95%CI) of individual stocks are reported in Table 2.5. Diploid and triploid whole wet weight followed the increase in shell height of increased from May 2011 to September 2011 at each site. In the Choptank River, from September 2011 until May 2012, growth plateaued and then increased again after May 2012. The Rappahannock and York Rivers did not exhibit this plateau (Figure 2.9). In both December 2011 and 2012, the site effect, ploidy effect, stock effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 2.4).

3.2.2.1 Choptank River, MD

Wild Virginia Stocks

In the Choptank River, at 17 months, there were several groups that showed a significant difference between diploids and triploids for whole wet weight. From the wild Virginia group, both diploid WIC and RAP were heavier than their triploid counterparts (WIC: diploid 12.7 ± 1.0g and triploid 9.8 ± 1.1g and RAP: diploid 14.6 ± 1.5g and triploid 11.3 ± 1.1g, $p<0.05$). Of the individual diploid wild stocks, RAP was significantly heavier than WIC (14.6 ± 1.5g and 12.7 ± 1.0g, respectively, $p<0.05$). There were no differences among the individual triploid stocks ($p>0.05$) (Figure 2.10).

By 29 months, the effect of triploidy had diminished and there were no significant differences among whole wet weights of the diploid and triploid groups ($p>0.05$). Triploid advantages, though not significant, were small ranging from 3-5% (Table 2.6).

There were no significant differences between the individual diploid or triploid wild stocks or the Superlines by 29 months ($p>0.05$) (Figure 2.11).

Wild Maryland Stocks

There were no significant difference between diploid and triploids in the Choptank River by 17 months ($p>0.05$). Diploid PATX was significantly heavier than diploid CHES (14.2 ± 1.4g and 12.1 ± 1.1g, respectively, $p<0.05$). Similarly, of the individual triploid wild stocks, PATX was significantly heavier than CHES (14.3 ± 1.3g and 11.9 ± 1.1g, respectively, $p<0.05$) (Figure 2.10).

By 29 months, the triploid effect on the wild Maryland stocks was and advantage of 5% for CHES and disadvantage of (-9%) for PATX, but the differences due to ploidy were not significant (Table 2.6). There were also no significant differences between the individual diploid or triploid wild by 29 months ($p>0.05$) (Figure 2.11).

Superlines

By 17 months, the Superlines hANA, SL-DBY, and SL-XB were all heavier as diploids than triploids (hANA: diploid 15.2 ± 1.3g and triploid 12.2 ± 1.3g, SL-DBY: diploid 14.7 ± 1.0g and triploid 12.1 ± 1.1g, and SL-XB: diploid 12.8 ± 1.4g and triploid 7.9 ± 0.7g, $p<0.05$). There were no significant differences in whole wet weight between any of the diploid Superlines ($p>0.05$). Triploid hANA, Lola, and SL-DBY had equivalent whole wet weights (12.2 ± 1.3g, 13.5 ± 1.4g, and 12.1 ± 1.1g, respectively, $p>0.05$) and were all significantly heavier than SL-XB (7.9 ± 0.7g, $p<0.05$) (Figure 2.10).

By 29 months, the effect of triploidy had diminished and there were no significant differences among whole wet weights of the diploid and triploid groups ($p>0.05$). Both Superlines of Louisiana origin had positive triploid advantages (hANA – 14% and Lola –

9%) while SL-DBY and SL-XB had triploid disadvantages (-5% and -12%, respectively), though these differences were not significant (Table 2.6). There were no significant differences between the individual diploid or triploid wild stocks or the Superlines by 29 months ($p>0.05$) (Figure 2.11).

3.2.2.2 Rappahannock River, VA

Wild Virginia Stocks

In the Rappahannock River, at 17 months, there were several groups that showed a significant difference between diploids and triploids for whole wet weight. From the wild Virginia group, both diploid WIC and MBY were lighter than their triploid counter parts (WIC: diploid 25.8 ± 2.3g and triploid 34.5 ± 2.8g and MBY: diploid 27.1 ± 2.0g and triploid 36.3 ± 2.8g, $p<0.05$). There were no significant differences in the whole wet weight among the individual diploid wild Virginia stocks. Of the individual triploid wild stocks, RAP was significantly heavier than WIC (36.3 ± 2.8g and 34.5 ± 2.8g, respectively, $p<0.05$) (Figure 2.10).

By 29 months, the effect of triploidy in the wild stocks was heavier triploids than diploids for both stocks (WIC: diploid 76.8 ± 6.6g and triploid 98.4 ± 7.8g, MBY: diploid 83.9 ± 8.2g and triploid 118.9 ± 14.3g, $p<0.05$). The triploid advantage was greatest for MBY (42%) followed by WIC (28%) (Table 2.6). Triploid RAP did not survive to 29 months. All of the diploid wild stocks had equivalent whole wet weights. Triploid MBY was significantly heavier than WIC ($p<0.05$) (118.9 ± 14.3g and 98.4 ± 7.8g, respectively) (Figure 2.11).

Wild Maryland Stocks

By 17 months, both diploid CHES and PATX from the wild Maryland group were significantly lighter than their triploid counterparts (CHES: diploid 22.0 ± 1.6g and triploid 31.7 ± 2.6g and PATX: diploid 23.1 ± 1.7g and triploid 29.2 ± 2.3g, $p<0.05$). There were no significant differences in the whole wet weight among the individual diploid or triploid wild Maryland stocks (Figure 2.10).

By 29 months, triploid wild stocks were heavier than diploids for both stocks (CHES: diploid 73.8 ± 6.1g and triploid 97.4 ± 7.1g, and PATX: diploid 74.0 ± 5.2g and triploid 92.9 ± 6.8g, $p<0.05$). The triploid advantage of the CHES stock was 32% and 26% for the PATX stock (Table 2.6). All of the diploid and triploid wild stocks had equivalent whole wet weights (Figure 2.11).

Superlines

By 17 months, only Superline Lola was lighter as diploid than triploid (30.1 ± 2.5g and 37.2 ± 3.2g, respectively, $p<0.05$). There were no significant differences between diploid and triploids of the remaining genotypes in the Rappahannock River ($p>0.05$). Of the diploid Superlines, Lola, SL-DBY, and SL-XB had equivalent whole wet weights (30.1 ± 2.5g, 27.6 ± 2.0g, and 26.0 ± 2.3g, respectively, $p>0.05$). Diploid hANA (34.2 ± 2.5g) was significantly heavier than both diploid SL-DBY and SL-XB ($p<0.05$). Triploid hANA, Lola, and SL-DBY had equivalent whole wet weights (34.1 ± 3.3g, 37.2 ± 3.2g, and 30.4 ± 3.0g, respectively, $p>0.05$), while hANA and Lola were significantly heavier than SL-XB (26.7 ± 1.6g, $p<0.05$) (Figure 2.10).

By 29 months, SL-XB was the only Superline in which triploidy affected whole wet weight. Triploid SL-XB was significantly heavier than its diploid counterpart (97.9 ± 6.4g and 79.9 ± 7.6g, respectively, $p<0.05$) and had a triploid advantage of 23% (Table

2.6). The diploid Superline hANA was significantly heavier than both SL-DBY and SL-XB (100.2 ± 7.2g, 81.8 ± 8.3g, and 79.9 ± 7.6g, respectively, $p<0.05$), but not Lola (93.2 ± 8.3g, $p>0.05$). Lola, SL-DBY, and SL-XB whole wet weights were not significantly different. The triploid Superline SL-DBY was the lightest of the Superlines (hANA: 109.6 ± 8.0g, Lola: 103.5 ± 7.2g, SL-DBY: 89.6 ± 9.9g, and SL-XB: 97.9 ± 6.4g), but any of the Superlines were not significantly different from one another ($p>0.05$) (Figure 2.11).

3.2.2.3 York River, VA

Wild Virginia Stocks

In the York River, at 17 months, the wild Virginia group, diploid WIC was lighter than its triploid counterpart (27.4 ± 2.9g and 37.0 ± 3.6g, respectively, $p<0.05$). From the diploid wild Virginia stocks, WIC and MBY had equivalent whole wet weights (27.4 ± 2.9g and 31.8 ± 3.8g, respectively, $p>0.05$), but only RAP was significantly lighter than MBY (24.5 ± 1.9g, $p<0.05$). Of the individual triploid wild stocks, WIC and MBY had equivalent whole wet weights (37.0 ± 3.6g, and 37.1 ± 3.3g, respectively, $p>0.05$) and were both significantly heavier than triploid RAP (25.5 ± 3.4g, $p<0.05$) (Figure 2.10).

By 29 months, the effect of triploidy in the wild stocks was heavier triploids than diploids for all stocks except RAP (WIC: diploid 68.8 ± 5.3g and triploid 111.8 ± 9.4g, MBY: diploid 92.7 ± 9.5g and triploid 125.5 ± 11.0g, RAP: diploid 81.9 ± 7.0g and triploid 87.5 ± 9.7g, $p<0.05$, for RAP $p>0.05$). The triploid advantage for the wild Virginia stocks was estimated to be 62% for WIC, 35% for MBY, and 7% for RAP (Table 2.6). The diploid MBY and RAP stocks had equivalent whole wet weights (92.7 ± 9.5g and 81.9 ± 7.0g, $p>0.05$), and were both significantly heavier than the diploid WIC

stock (68.8 ± 5.3g, $p<0.05$). Triploid WIC and MBY had equivalent whole wet weights ($p>0.05$) and were both heavier than RAP ($p<0.05$) (111.8 ± 9.4g, 125.5 ± 11.0g, and 87.5 ± 9.7g, respectively) (Figure 2.11).

Wild Maryland Stocks

By 17 months, both triploid CHES and PATX from the wild Maryland group were significantly heavier than their diploid counterparts (CHES: diploid 24.2 ± 2.2g and triploid 34.2 ± 3.4g and PATX: diploid 19.0 ± 3.3g and triploid 29.9 ± 3.0g, $p<0.05$). The diploid CHES stock was heavier than PATX, but this difference was not significant (24.2 ± 2.2g and 19.0 ± 3.3g, respectively, $p>0.05$) (Figure 2.10).

By 29 months, the triploid advantage was present for CHES (diploid 52.3 ± 5.3g and triploid 108.8 ± 9.0g, $p<0.05$) resulting in an advantage of 108% (Table 2.6). PATX diploids did not survive to 29 months, $p<0.05$). Triploid CHES was significantly heavier than PATX (108.8 ± 9.0g and 88.3 ± 7.3g, respectively, $p<0.05$) (Figure 2.11).

Superlines

By 17 months, the Superlines, Lola, SL-DBY, and SL-XB were heavier as triploid than diploid (Lola: diploid 27.9 ± 3.3g and triploid 36.5 ± 3.5g, SL-DBY: diploid 28.1 ± 2.5g and triploid 35.2 ± 2.8g, and SL-XB: diploid 28.8 ± 2.4g and triploid 36.5 ± 3.2g, $p<0.05$). There were no significant differences in the whole wet weight among the individual diploid Superlines ($p>0.05$). There were no significant differences in the whole wet weight among the individual triploid Superlines ($p>0.05$) (Figure 2.10).

By 29 months, triploidy affected whole wet weight for all four Superlines: hANA, Lola, SL-DBY, and SL-XB (hANA: diploid 91.9 ± 7.1g and triploid 121.2 ± 10.4g, Lola: diploid 89.7 ± 8.3g and triploid 108.1 ± 9.3g, SL-DBY: diploid 79.3 ± 6.8g and triploid

112.9 ± 8.0g, and SL-XB: diploid 80.9 ± 7.1g and triploid 107.7 ± 8.2g, $p<0.05$). The estimated triploid advantage for the Superlines was 32% for hANA, 20% for Lola, 42% for SL-DBY, and 33% for SL-XB (Table 2.6). There were no significant differences in whole wet weight of the diploid or triploid Superlines (Figure 2.11).

3.2.3 Wet Tissue Weight

Measurements of wet tissue weight were taken beginning in October 2011. For overall trends, wild stocks and the Superlines in the Choptank River exhibited a period of suppressed tissue growth ending in the spring of 2012. The increase in growth rate corresponded with an increase in salinity in the spring of 2012. For the Rappahannock and York Rivers, wild stocks and Superlines showed similar growth trends. That is, diploids and triploids stocks showed a steady increase in tissue weight until May 2012 when the growth rate of the diploid stocks fell off. Diploid Superlines maintained seemingly higher growth rates through the spawning period than did the wild stocks (Figure 2.12). In both December 2011 and 2012, the site effect, ploidy effect, genotype effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 2.4).

3.2.3.1 Choptank River, MD

Wild Virginia Stocks

By 17 months, diploids were significantly heavier (all $p<0.05$) than their triploid counterparts for two of the wild stocks WIC (2.2 ± 0.2g and 1.6 ± 0.2g, respectively, $p<0.05$) and RAP (1.7 ± 0.2g and 6.2 ± 0.7g, respectively, $p<0.05$). There were no significant differences in the whole wet weight among the individual diploid or triploid wild stocks ($p>0.05$) (Figure 2.13).

By 29 months, the effect of triploidy among stocks was absent and though the difference in wet tissue weight was not significant, WIC had an estimated triploid disadvantage of (-13%) and for RAP a triploid advantage of 2% (Table 2.6). The wet tissue weights of individual diploid or triploid wild stocks were not significantly different (Figure 2.14).

Wild Maryland Stocks

By 17 months, there were no significant difference between diploid and triploids of the remaining genotypes in the Choptank River ($p>0.05$). Of the individual diploid wild stocks, PATX was significantly heavier than CHES (2.4 ± 0.3g and 1.9 ± 2.0g, respectively, $p<0.05$). There were no significant differences in the whole wet weight among the individual triploid wild stocks ($p>0.05$) (Figure 2.13).

By 29 months, the effect of triploidy among groups was absent (Figure 2.11). The triploid advantage of CHES was estimated to be 3% and PATX had a triploid disadvantage of (-13%) (Table 2.6). The wet tissue weights of diploid wild stocks were not significantly different. The wet tissue weights of diploid wild stocks were not significantly different (Figure 2.14).

Superlines

By 17 months, three of the Superlines were heavier as diploids than triploids: hANA (2.8 ± 0.3g and 1.8 ± 0.4g, respectively), SL-DBY (2.9 ± 0.2g and 1.8 ± 0.2g, respectively), and SL-XB (2.3 ± 0.3g and 1.1 ± 0.1g, respectively). The two heaviest diploid Superlines were hANA and SL-DBY and these were not significantly different from each other ($p>0.05$), but were significantly heavier ($p<0.05$) than both Lola and SL-XB which were equivalent to one another (hANA 2.8 ± 0.3g, SL-DBY 2.9 ± 0.2g, Lola

2.3 ± 0.3g, and SL-XB 2.3 ± 0.3g). Triploid hANA, Lola, and SL-DBY had equivalent whole wet weights (1.8 ± 0.2g, 2.0 ± 0.3g, and 1.8 ± 0.2g, respectively, $p>0.05$) and were all significantly heavier than SL-XB (1.1 ± 0.1g, $p<0.05$) (Figure 2.13).

By 29 months, the Superline SL-DBY was the only line that was significantly heavier as a diploid than triploid (7.8 ± 0.7g and 5.9 ± 0.7g, respectively, $p<0.05$) with a -25% triploid advantage (Table 2.6). Diploid Superlines Lola, SL-DBY, and SL-XB had equivalent wet tissue weights ($p>0.05$), but only Lola and SL-DBY were significantly heavier ($p<0.05$) than hANA (hANA 6.0 ± 0.8g, Lola 7.8 ± 0.9g, SL-DBY 7.8 ± 0.7g, and SL-XB 7.5 ± 1.2g). Triploid Superlines hANA, Lola, and SL-DBY had equivalent wet tissue weights ($p>0.05$), but only Lola was significantly heavier ($p<0.05$) than SL-XB (hANA 6.3 ± 0.9g, Lola 7.3 ± 0.8g, SL-DBY 5.9 ± 0.7g, and SL-XB 5.5 ± 0.7g) (Figure 2.14).

3.2.3.2 Rappahannock River, MD

Wild Virginia Stocks

In the Rappahannock River, at 17 months, triploids got off to a faster start in several of the wild stocks. Diploid WIC and MBY each had significantly lighter wet tissue weights than their triploid counterparts (all $p<0.05$, WIC diploid 4.8 ± 0.5g and triploid 6.3 ± 0.6g; MBY diploid 4.7 ± 0.4g and triploid 6.0 ± 0.5g). There were no significant differences among the wet tissue weights of the diploid or triploid wild stocks (Figure 2.13).

By 29 months, the triploid advantage in wet tissue weight at 17 months was maintained for WIC and MBY (WIC diploid 8.7 ± 1.0g and triploid 10.5 ± 0.8g; MBY diploid 8.1 ± 1.0g and 12.4 ± 1.7g). The triploid advantage for the WIC stock was 20%

and 53% for the MBY stock (Table 2.6). There were no significant differences among the wet tissue weight of diploid or triploid wild stocks from Virginia (Figure 2.14).

Wild Maryland Stocks

By 17 months, there were no differences between diploid and triploid stocks in the Rappahannock River ($p>0.05$). Among the individual diploid stocks there were also no differences in wet tissue weight ($p>0.05$). Triploid wild stock CHES was significantly heavier than triploid PATX (5.8 ± 0.6g and 4.8 ± 0.5g, respectively, $p<0.05$) (Figure 2.13).

By 29 months, the triploid advantage was present for CHES (diploid 7.8 ± 1.0g and triploid 11.6 ± 0.9g, $p<0.05$). The triploid advantage of the CHES stock was 49% (Table 2.6). There were no significant differences among the wet tissue weight of wild stocks from Maryland. Of the triploid wild stocks, CHES was significantly heavier than PATX (11.6 ± 0.9g and 9.1 ± 0.9g, respectively, $p<0.05$) (Figure 2.14).

Superlines

By 17 months, there were no differences between diploid and triploid counterparts from the Superlines. Diploid Superlines hANA, Lola, and SL-DBY did not have significantly different wet tissue weights ($p>0.05$), but only hANA was significantly heavier ($p<0.05$) than SL-XB (hANA 6.9 ± 0.6g, Lola 5.7 ± 0.6g, SL-DBY 5.9 ± 0.5g, and SL-XB 5.2 ± 0.5g). Triploid Superlines hANA, Lola, and SL-DBY did not have significantly different wet tissue weights ($p>0.05$), and were all significantly heavier ($p<0.05$) than SL-XB (hANA 5.8 ± 0.7g, Lola 6.2 ± 0.7g, SL-DBY 5.5 ± 0.7g, and SL-XB 4.3 ± 0.2g) (Figure 2.13).

By 29 months, notably, there were no differences in tissue weight between diploid and triploid Superline counterparts. While these differences were not significant, the triploid advantage ranged from 8-16% (Table 2.6). There were also no differences among the individual diploid Superlines or individual triploid Superlines by 29 months (Figure 2.14).

3.2.3.3 York River, VA

Wild Virginia Stocks

In the York River, by 17 months, there was no significant difference in wet tissue weight between diploid and triploid stocks. There were also no significant differences in the wet tissue weights of the individual diploid wild stocks (Figure 2.13).

By 29 months, triploids in two of the wild stocks were heavier than their diploid counterparts: WIC and MBY (all $p<0.05$, WIC diploid 6.9 ± 0.6g and triploid 9.8 ± 1.0g; MBY diploid 8.6 ± 1.2g and triploid 12.3 ± 1.2g) with triploid advantages of 43% each (Table 2.6). Of the individual diploid stocks from Virginia, MBY and RAP had equivalent wet tissue weights (8.6 ± 1.2g and 9.2 ± 1.0, respectively, $p>0.05$), and were both significantly heavier than diploid WIC (6.9 ± 0.6g, $p<0.05$). Triploid MBY was significantly heavier than both triploid WIC and RAP ($p<0.05$), which had equivalent wet tissue weights ($p>0.05$) (12.3 ± 1.2g, 9.8 ± 1.0g, and 8.6 ± 1.3g, respectively) (Figure 2.14).

Wild Maryland Stocks

By 17 months in the York River, there was no significant difference in wet tissue weight between diploid and triploid stocks. There were also no significant differences in the wet tissue weights of the individual diploid wild stocks (Figure 2.13).

By the end of the study (29 months), triploids in the CHES stock were heavier than diploid CHES (diploid 5.2 ± 0.6g and triploid 11.4 ± 1.3g, $p<0.05$) with a triploid advantage estimated at 120% (Table 2.6). No diploid PATX animals survived to 29 months. There were no differences among the individual diploid or triploid stocks (Figure 2.14).

Superlines

By 17 months in the York River, there was no significant difference in wet tissue weight between diploid and triploid lines. There were also no significant differences in the wet tissue weights of the individual diploid Superlines (Figure 2.13).

There were no significant differences in wet tissue weight among the diploid and triploid Superlines by 29 months. Although these differences were not significant, the triploid advantage ranged from an estimated 11-23% (Table 2.6). Triploid Superlines hANA, Lola, and SL-DBY did not have significantly differing wet tissue weights among them (12.1 ± 1.3g, 10.7 ± 1.2g, and 11.1 ± 1.1g, respectively, $p>0.05$). Only triploid hANA was significantly heavier than triploid SL-XB (9.5 ± 0.9g, $p<0.05$) (Figure 2.14).

3.2.4 Meat yield

Meat yield is a derived parameter: meat weight ÷ whole wet weight. In all groups in all sites, meat yield declined over the course of the study, with only a few differences between diploid and triploid. The diploid Superlines, at all sites, showed a pattern of greater tissue growth than shell growth during gametogenic periods, as evidenced by increases in meat yield (Figure 2.15). In both December 2011 and 2012, the site effect, ploidy effect, genotype effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 2.4).

3.2.4.1 Choptank River, MD

Wild Virginia Stocks

In the Choptank River, by 17 months, in the wild Virginia stocks, there was no significant effect of ploidy on meat yield. The individual diploid and triploid stocks from Virginia all had equivalent meat yields (Figure 2.16).

By 29 months, diploid WIC was the only wild stock to have greater meat yield than its triploid counterpart (0.143 ± 0.009 and 0.121 ± 0.007, respectively, $p<0.05$). There were no differences in meat yield between the individual diploid or triploid wild stocks (Figure 2.17).

Wild Maryland Stocks

In the wild Maryland stocks, by 17 months, there was no significant effect of triploidy on meat yield. The individual diploid and triploid stocks from Maryland all had equivalent meat yields (Figure 2.16).

By 29 months, there was no triploid advantage observed in the wild Maryland stocks. There were also no differences in meat yield between the individual diploid or triploid wild stocks (Figure 2.17).

Superlines

By 17 months, in the Superlines, diploids had significantly higher meat yields than their triploid counterparts for all four Superlines (hANA: diploid 0.177 ± 0.008 and triploid 0.145 ± 0.008, Lola: diploid 0.163 ± 0.008 and triploid 0.145 ± 0.006, SL-DBY: diploid 0.195 ± 0.006 and triploid 0.149 ± 0.006, and SL-XB: diploid 0.179 ± 0.011 and triploid 0.139 ± 0.007, $p<0.05$). Diploid Superlines hANA, Lola, and SL-XB did not have significantly different meat yields ($p>0.05$), and all three had significantly lower

meat yield than SL-DBY (0.177 ± 0.008, 0.163 ± 0.008, 0.179 ± 0.011, and 0.195 ± 0.006, respectively, $p<0.05$) (Figure 2.16).

By 29 months, diploid lines Lola, SL-DBY, and SL-XB all had significantly greater meat yields than their triploid counterparts (Lola: diploid 0.149 ± 0.007 and triploid 0.127 ± 0.006, SL-DBY: diploid 0.152 ± 0.006 and triploid 0.121 ± 0.006, and SL-XB: diploid 0.151 ± 0.008 and triploid 0.127 ± 0.008). Diploid Superlines Lola, SL-DBY, and SL-XB had similar meat yields (0.149 ± 0.007, 0.152 ± 0.006, 0.151 ± 0.008, respectively, $p>0.05$) and all three had significantly greater meat yields than diploid hANA (0.126 ± 0.008) (Figure 2.17).

3.2.4.2 Rappahannock River, VA

Wild Virginia Stocks

In the Rappahannock River, by 17 months, the meat yield of all diploid wild stocks did not significantly differ from the meat yields of their triploid counterparts. Diploid RAP had significantly greater meat yield than diploid MBY (0.191 ± 0.006 and 0.172 ± 0.008, respectively, $p<0.05$). There were no significant differences among the diploid or triploid wild stocks from Virginia ($p>0.05$) (Figure 2.16).

By 29 months, RAP was the only wild stock to demonstrate an effect of triploidy on meat yield. The meat yield of the diploid RAP stock was significantly greater than its triploid counterpart (0.123 ± 0.006 and 0.103 ± 0.008, respectively, $p<0.05$). Of the individual diploid wild stocks from Virginia, RAP and WIC had equivalent meat yields ($p>0.05$) and had significantly greater meat yields than MBY ($p<0.05$) (0.123 ± 0.006, 0.111 ± 0.007, and 0.096 ± 0.007, respectively) (Figure 2.17).

Wild Maryland Stocks

By 17 months, the meat yield of all diploid wild stocks did not significantly differ from the meat yields of their triploid counterparts. There were no differences between any of the remaining diploid or triploid wild Maryland stocks in the Rappahannock River (Figure 2.16).

By 29 months, CHES was the only wild stock to demonstrate a triploid advantage for meat yield. The meat yield of the diploid CHES stock was significantly less than its triploid counterpart (0.104 ± 0.008 and 0.121 ± 0.006, respectively, $p<0.05$). There were no differences between the wild diploid stocks from Maryland. From the triploid wild stocks, only CHES and PATX were significantly different from one another, with CHES having greater meat yield (0.121 ± 0.006 and 0.096 ± 0.004, respectively, $p<0.05$) (Figure 2.17).

Superlines

By 17 months, all four of the diploid Superlines had greater meat yields than their triploid counterparts (hANA: diploid 0.202 ± 0.008 and triploid 0.168 ± 0.007, Lola: diploid 0.189 ± 0.007 and triploid 0.164 ± 0.007, SL-DBY: diploid 0.214 ± 0.007 and triploid 0.175 ± 0.008, and SL-XB: diploid 0.200 ± 0.009 and triploid 0.162 ± 0.007, $p<0.05$). There were no significant differences in meat yield between individual diploid Superlines with the exception of SL-DBY having a greater meat yield than Lola ($p<0.05$) (hANA: 0.202 ± 0.008, Lola: 0.189 ± 0.007, SL-DBY: 0.214 ± 0.007, and SL-XB: 0.200 ± 0.009). There were no differences among the individual triploid Superlines ($p>0.05$) (Figure 2.16).

At 29 months, all four of the diploid Superlines still had greater meat yields than their triploid counterparts (hANA: diploid 0.109 ± 0.007 and triploid 0.108 ± 0.006, Lola:

diploid 0.110 ± 0.004 and triploid 0.110 ± 0.005, SL-DBY: diploid 0.124 ± 0.006 and triploid 0.132 ± 0.007, and SL-XB: diploid 0.121 ± 0.009 and triploid 0.112 ± 0.005, $p<0.05$). Diploid Superlines hANA, Lola, and SL-XB did not have significantly different meat yields (0.109 ± 0.007, 0.110 ± 0.004, and 0.121 ± 0.009, respectively, $p>0.05$). The meat yield of SL-DBY (0.124 ± 0.006) was significantly greater than that of hANA and Lola only ($p<0.05$). Triploid Superlines hANA, Lola, and SL-XB all had equivalent meat yields (0.108 ± 0.006, 0.110 ± 0.005, and 0.112 ± 0.005, respectively, $p>0.05$). Triploid SL-DBY had greater meat yield (0.132 ± 0.007, $p<0.05$) than the three other Superlines: hANA, Lola, and SL-XB (Figure 2.17).

3.2.4.3 York River, VA

Wild Virginia Stocks

In the York River there were no significant differences between diploid and triploid groups by 17 months. There were no significant differences in meat yield between the individual diploid wild stocks. Unlike the diploid stocks, triploid RAP had significantly greater meat yield than MBY (0.110 ± 0.008 and 0.110 ± 0.008, respectively, $p<0.05$) (Figure 2.16).

By 29 months, there were no difference in diploid:triploid comparisons for the wild Virginia stocks in the York River ($p>0.05$). Diploid RAP had greater meat yield than both WIC and MBY (0.112 ± 0.006, 0.100 ± 0.005, and 0.091 ± 0.005, respectively, $p<0.05$). Among the individual triploid wild stocks there were no significant differences in meat yield ($p>0.05$) (Figure 2.17).

Wild Maryland Stocks

In the York River there were no significant differences between diploid and triploid stocks by 17 months. There were no significant differences in meat yield between the individual diploid wild stocks. Triploid PATX had a greater meat yield than CHES (0.126 ± 0.004 and 0.107 ± 0.005, respectively, $p<0.05$) (Figure 2.16).

By 29 months, there were remained no significant differences in diploid:triploid comparisons. Diploid PATX did not survive to 29 months so no comparison of diploid wild Maryland stocks was performed. Of the triploids, CHES had significantly greater meat yield than PATX (0.104 ± 0.006 and 0.085 ± 0.004, respectively, $p<0.05$) (Figure 2.17).

Superlines

In the York River there were no significant differences between diploid and triploid groups by 17 months. The diploid Superline SL-XB had the lowest meat yield, while hANA, Lola, and SL-DBY all had equivalent meat yields (hANA: 0.124 ± 0.006, Lola: 0.126 ± 0.007, SL-DBY: 0.131 ± 0.007, and SL-XB: 0.110 ± 0.008). There were no significant differences in meat yield between the triploid Superlines (Figure 2.16). By 29 months, diploid Superlines SL-DBY and SL-XB were the only two stocks or lines in which triploidy had an effect on meat yield. SL-DBY and SL-XB had meat yields greater than their triploid counter parts (SL-DBY: diploid 0.116 ± 0.008 and triploid 0.096 ± 0.005, SL-XB: diploid 0.102 ± 0.005 and triploid 0.097 ± 0.004, $p<0.05$). There were no other differences between individual diploid or triploid lines (Figure 2.17).

4. DISCUSSION

This study examined the effect of the diploid female on triploids created from tetraploid × diploid cross. Five populations of wild oysters were used to create SS+ genotypes (selected tetraploid × wild-type diploid) and four lines of selected oysters were used to create SSS genotypes (selected tetraploid × selected diploid). Examined metrics of growth were restricted to shell height, whole weight, tissue weight, and the derived metric of meat yield. The results show that these metrics are influenced by both the genetic contribution of the diploid parent and the environment. Triploids had an advantage for these growth metrics, but not everywhere. Generally, the triploid advantage occurred in each metric and is more pronounced, in ascending order from shell height, whole wet weight, to wet tissue weight. Previous studies of triploid *C. virginia* mostly compared induced (meiosis I or II) triploids (Stanley et al., 1984; Barber & Mann, 1991; Matthiessen & Davis, 1992), with only a few studies comparing mated triploids (produced from $4n \times 2n$ crosses) with diploids (Wang et al., 2005, 2006; Harding, 2007; Dégremont et al., 2012). The distinction between induced and mated triploids is important because the origin of the extra set of chromosomes in triploids differs between these types. Induced triploids obtain a third chromosome set from the diploid female whereas mated triploid obtain a third chromosome set from the tetraploid male. Furthermore, the two sets of chromosomes from the female parent of an induced triploid are identical (by pre-meiotic duplication) except for regions where there were crossovers.

For this research, triploids were all mated triploids. The genes were inherited by random segregation of four sets of alleles (Curole and Hedgecock, 2005). The inbreeding coefficient is likely less in mated than induced triploids. Mated triploids have greater growth rates than induced triploids across a variety of environments (Wang et al., 2006) making the distinction between induced and mated triploids critical when comparing the observations of this study with previous studies on triploid performance.

4.1 Choptank River

Throughout this study there was an absence of triploid advantage in the Choptank River for all parameters measured (shell height, whole wet weight, and wet tissue weight) for both the wild-type groups and Superlines (Table 2.6). With no apparent differences from genotype, the influence of the environment must be considered. Water temperature measured at the three study sites was similar (Figures 2.3 and 2.4) reducing the defining characteristics of the study sites to salinity and the presence/absence of disease pressures (Dermo and MSX). The Choptank River is characterized by lack of disease pressure and low salinity. The average salinity in the Choptank River during the study period was 9, ranging from 6 to 13 (Figure 2.5). The optimum salinity range for oysters is approximately 14 - 28 (Galstoff 1964; Loosanoff 1965), but oysters are known to survive prolonged exposure to salinity as low as 0.2 – 3.5 (Butler, 1952) or as high as 32 – 42 (Breuer, 1962).

Salinity affects many aspects of oyster biology including valve activity, feeding, respiration, reproduction, and growth. Most studies that have investigated the effect salinity on oysters focused on acute fluctuations in salinity; however, some of these effects last even after oysters have generally acclimated to the salinity change. Abnormal

valve movement and pumping activity were reported when oysters were exposed to low salinity (0-5 ppt), but oysters resume normal activity after an acclimation period (Loosanoff, 1952; Galtsoff, 1964). Oyster feeding is also affected by low salinity and has been observed to stop at salinities below 3 ppt while at a salinity of 5 ppt oysters exhibit abnormal activity and produce white/pale pseudofeces (Loosanoff, 1952). The ability to regulate respiration rate decreases with decreased salinity and as salinity decreases respiration regulation becomes more sensitive to temperature increases (Shumway and Koehn, 1982). Gametogenesis is depressed at low salinities. Reported lower limits of salinity for normal gonadal development were estimated near 7.5 ppt (Butler, 1949, Loosanoff, 1952) and 10 ppt (Calabrese and Davis, 1970). Butler (1949) and Loosanoff (1952) suggest the variation and suppression of gonadal activity at lower salinities may be a result of variations in food quality and availability in these environments. Chanley (1958) observed juvenile oysters with reduced growth rates below 12 ppt (60% reduction relative to salinity greater than 15 ppt) and no growth below 5 ppt. This reduction in juvenile growth is consistent with observations of adult oyster growth in similar salinity conditions leading to a suggested minimum salinity of 10 ppt for normal adult growth (Loosanoff, 1952). Growth of triploid Pacific oysters relative to diploids, measured as whole volume rather than whole wet weight, has been observed to change with the quality of growing conditions (Davis, 1994). Triploids in environments characterized by poor growth (low salinity, low temperature, and poor food quality and availability) had less, and in some cases no advantage over diploids, compared to triploids grown in environments characterized as conducive for fast growth (high salinity and high food quality and availability).

Reduced growth, relative to oysters grown in higher salinities, was observed in this study. Diploids and triploids in the Choptank River did not reach harvest size (76mm) on average by the end of the study, which was not the case in the higher salinity sites (York and Rappahannock Rivers). There is one difference that sets wet tissue weight apart from the other growth parameters in the Choptank River: the diploid Superlines had a greater wet tissue weight than their triploid counterparts, though this difference is not significant (Figure 2.12). This suggests that, while all oysters in this environment suffered suppressed growth, the triploid Superlines had a greater disadvantage from the stress of low salinity than did the diploids. Meat yield indicates how oysters are partitioning their growth, either as shell or tissue. Changes in meat yield over time provide an insight into overall condition and because meat yield is highly dependent on changes in condition due to reproduction, interesting aspects of the relationship between diploid and triploid oysters are revealed through changes in meat yield through gametogenic periods. Overall, in all groups at all sites, meat yield declined over the course of the study (Figure 2.15).

The diploid Superlines showed a pattern of greater tissue growth than shell growth prior to the second spawning period, as evidenced by increases in meat yield, then decreased, presumably due to loss of tissue mass from spawning, to a meat yield similar to the triploid Superlines. Meat yield of triploid Superlines increased, indicating the triploids are undergoing some gonadogenesis, but not to the same level as the diploids, which is expected due to the limited gametogenic activity in triploids. The increase in meat yield of the diploid Superlines leading up to spawning was not observed in the two groups of wild stocks.

While the diploid wild stocks did not display the same increase in meat yield leading up to spawning and the subsequent decrease thereafter, triploid wild stocks followed the same pattern across time as the Superline triploids. Even though growth of triploid groups varied, it appears that the way triploids partition growth (i.e., as tissue mass or shell mass) across time is consistent and results from the general sterility of triploid oysters. The difference in the meat yield of wild and selected diploids may be from improved health in selected diploids from selective breeding when grown under low salinity stress.

In Choptank River, the Superline Lola was the largest as both diploid and triploid (for all growth parameters). The Lola diploid performance confirms that selection of Lola for growth in low salinity environments has succeeded. As triploid, this indicates that selection for low salinity environments is transferable to the triploid construct. With growth in the Choptank River being lower than the remaining sites, this offers the potential of further increasing performance in this type of environment.

4.2 Rappahannock River

In the Rappahannock River the Superlines exhibited no triploid advantage for shell height (Figure 2.6). The Superlines did, however, show a triploid advantage for the remaining growth parameters (whole wet weight and tissue weight). The absence of the triploid advantage from the Superlines' shell height can be explained by the selective breeding of these lines that focused on enhanced growth (using shell height) and disease resistance. The Rappahannock River is a 'good' growing site as it falls within the optimum salinity range for oysters of 14 – 28 (Figure 2.5) and had no disease pressure during this study. The selection of the diploid Superlines have enhanced growth (shell

height) performance to be comparable with triploid oysters. Unlike the Superlines, the triploid advantage was observed in groups of wild-type origin.

Both groups of wild stocks had a greater triploid advantage than the Superlines for shell height (Maryland – 13%, Virginia – 11%, Superlines – no advantage), whole wet weight (Maryland – 29%, Virginia – 38%, Superlines – 13%), and wet tissue weight (Maryland – 33%, Virginia – 37%, Superlines – 12%). If the triploid advantage was driven by additive gains, then triploids made from Superlines (SSS) would have had an advantage over diploid Superlines for shell height as they do for the other growth parameters. SSS triploids consistently have a lower triploid advantage than triploids made from wild groups (SS+) suggesting that there are more contributing factors to the triploid advantage than simple additive gains. These disproportionate triploid advantages may be explained partly by heterosis and sterility or a combination of both.

The greater triploid advantage in triploids from wild-type females (SS+) than triploids from selected females (SSS) may by due to heterosis. Heterosis is the increase in average performance above the mid-parent value, increases with heterozygosity, and generally occurs when two inbred groups are crossed (Griffing, 1990; Hedgecock et al., 1996; Hawkins et al., 2000). Polyploids have an increased chance for greater heterozygosity from more possible varieties of allelic combinations at a given loci than diploids (Piferrer et al., 2009). Triploids, for example, have three alleles present at each locus (e.g. ABB, AAB, or ABC) compared to diploids. Triploids from wild-type diploids (SS+) in this study have an increased potential for heterosis over the Superline triploids (SSS) because the 'S' set of chromosomes provided by the diploid parent in the $4n \times 2n$ cross originated from the same founder populations as the 'SS' from the tetraploid parent

whereas the '+' from the wild-type parent is from a separate genetic source.

Whole wet weight and wet tissue weight are physiological indicators that differ from shell height because they are sensitive to health and gametogenic activity. Triploid sterility is likely to be a contributing factor to the triploid advantage because energy that would otherwise be utilized for gametogenic activity can be used for growth. There is one difference that sets wet tissue weight apart from whole wet – the diploid Superlines have a greater wet tissue weight than the triploids in the Rappahannock River entering into the second reproductive season, though this difference is not significant (Figure 2.12). Superior wet tissue weight of diploid Superlines produced greater meat yield than their triploid counterparts (Figure 2.15). At the second reproductive period, the triploid advantage for whole wet weight and wet tissue weight began to increase (Figures 2.9 and 2.12). The advantage triploids had over their diploid counterpart increases after the first year coinciding with when diploids start to allocate significant annual energy resources to reproduction (Stanley et al., 1984; Barber and Mann, 1991).

4.3 York River

Superlines made into triploids showed no effect of an extra set of chromosomes on shell height unless exposed to disease pressure in the York River (Figure 2.6). Dermo and MSX are endemic to the York River. The triploid advantage for the Superlines was an average growth advantage of 13% for shell height (Table 2.6). The triploid advantage for shell height has been observed in several studies in the York River comparing selected diploids with both induced and mated triploids from selected diploids. After 1.5 years of growth in the York River, induced triploids had an 8% shell height advantage over diploid shell height (Barber & Mann, 1991) and mated triploids have been observed

to have a 13% and 26% advantage over diploid shell height (Harding 2007; Dégremont et al., 2012, respectively).

The triploid advantage was greatest for the most disadvantaged groups (i.e., those most susceptible to disease pressures) – wild Maryland group – for all growth parameters under disease pressure in the York River (Table 2.6). The wild Virginia group showed a similar triploid advantage as the Superlines for shell height (10% and 13%, respectively) and whole wet weight (35% and 32%, respectively). In the Rappahannock River, the larger triploid advantage in the wild groups was attributed largely to heterosis. This, however, does not fully explain why the Maryland group experienced a significantly larger triploid advantage in the York River than the wild Virginia group. That difference is likely due to disease resistance imparted from the tetraploid parent.

Growth has been documented to be inhibited by the two diseases likely to be encountered in the York River: Dermo and MSX (disease analysis is presented in Chapter 4). Shell deposition rates may be lower in oysters with Dermo infections than those without and nonexistent in those oysters with heavy infections (Paynter and Burreson, 1991). With MSX infection, highly susceptible oysters typically die within several weeks of infection but some oysters that may be more resistant have shown signs of reduced growth (Barber et al., 1988a). The great advantage the wild Maryland group shows at a site with disease pressure stems from the tetraploid parent's origin. The tetraploid parent originated from lines that have experienced intense growth and survival selection under Dermo and MSX pressures. It is likely that some disease resistance is inherited from the disease resistant tetraploid parent in the triploid cross ($4n \times 2n$) and the group that can benefit the most from this contribution of disease resistance is the susceptible wild

Maryland group. The triploid advantage for shell height of the wild Virginia group was 3% lower than the Superlines in the York River, but is 3% greater for whole wet weight. Whole wet weight is composed of tissue weight as well as shell weight and it is tissue weight that is the most affected by disease pressure. The 3% greater triploid advantage in whole wet weight of the wild Virginia group over the Superlines compared to shell height may be because, while the wild stocks experience periodic disease exposure, they benefited from the disease resistance of the tetraploid parent.

The pattern of meat yield change across time in the York River is different from either the Choptank or Rappahannock Rivers (Figure 2.15) and is largely attributed to the tumbling the oyster received in this environment. The York River, relative to the Choptank and Rappahannock River, which are sheltered, is a high-energy site with significant wave action and because of this the oysters are frequently tumbled within the grow-out cages.

4.4. Conclusions

The first objective of this study was to compare field performance among triploid from wild-type females ($3n$ = SS+) and selected females ($3n$ = SSS). The comparison of relative performance among diploids and their triploid counterparts showed that the contribution of the diploid parent in a tetraploid × diploid cross is significant. The differences of relative performance across the study sites showed that the effect of the environment is significant as well.

The Choptank River is, in the context of this study, a quite different environment. There are no disease pressure stresses but there is stress from low salinity. The lack of triploid advantage under low salinity stress in the Choptank River and increase in triploid

advantage with salinity and the addition of disease pressure highlights the importance of the environment on the triploid advantage. Clearly the triploid advantage is adversely affected by the stress of low salinity in both wild and selected groups. As salinity increased the triploid advantage manifested and was typically greater for the groups that have not been selectively bred (triploids from wild-type diploid females).

Unlike the Choptank and York Rivers, the site in the Rappahannock was an environment more conducive to oyster growth because of the lack of disease pressure during this study and salinity falling in the physiological optimum range for oysters. Essentially, this site offered oysters the opportunity to express genetic potential with minimal environmental stress. The results showed that breeding efforts could improve diploids to perform as well as triploids as evidenced by the Superlines, at least for shell height. Triploid Superlines had a 13% and 12% advantage over diploid Superlines for whole wet weight and wet tissue weight indicating that. Overall triploid advantages were lower in the Rappahannock River than in the York River suggesting that the greatest value of triploidy is not the ability to outperform diploids regardless of environmental factors, but rather the ability to outperform diploids under specific stresses (e.g., disease pressure).

In the York River, as in the Rappahannock River, the triploid advantage was greatest for the most disadvantaged group (wild Maryland group). This difference in the triploid advantage across groups, especially under disease pressure as the tetraploid parent comes from a disease resistant origin, informs on the role of disease resistance in the tetraploid parent and the possibility of heterosis through increased heterozygosity from using wild-type diploids over selected diploids. Additive gains provided the disease

resistance in the tetraploid population and are manifest in triploids under disease pressure, especially in groups that have experienced little selection for Dermo and MSX. The increased performance of many of the triploid groups in the presence of disease may also be due to a shift in energy expenditure in lieu of gametogenesis due to triploid sterility, which were not measured in this study.

		Tetraploid male	
		++++	SSSS
Diploid female	++	Not possible	SS+
	SS	Not possible	SSS

Table 2.1. Possible types of triploid combinations from male tetraploid × female diploid crosses from parents of either wild (+) or selected (S) origin. Triploids from ++++ tetraploids are not possible because ABC does not produce this type of tetraploid.

		Diploid		Tetraploid
Stock	Type	No. dams	No. of sires	No of sires
WIC	++	10	8	11
MBY	++	6	12	10
RAP	++	10	7	11
CHES	++	10	6	10
PATX	++	10	11	11
hANA	SL	10	9	10
Lola	SL	15	11	11
SL-DBY	SL	10	10	11
SL-XB	SL	10	10	11

Table 2.2: Number of *C. virginica* broodstock used per stock (wild) or line (selected) to generate diploid and triploid offspring. For diploids, diploid dams and sires were used; for triploids, diploid dams and one set of 11 tetraploid sires (pooled sperm). ++ = wild stock; SL = selected Superlines; Rivers systems for wild stocks: WIC = Wicomico, Virginia; MBY = Mobjack Bay, Virginia; RAP = Rappahanock, Virginia; CHES = Chester, Maryland; PATX = Patuxent, Maryland.

	Diploid			Triploid		
Stock	**Shell height (mm)**		**95%CI**	**Shell height (mm)**		**95%CI**
Choptank River						
WIC	20.1	±	1.2	16.8	±	1.0
MBY	NA	±	NA	NA	±	NA
RAP	15.0	±	1.0	15.8	±	0.8
CHES	18.4	±	0.8	18.7	±	1.1
PATX	18.9	±	1.0	19.2	±	0.9
hANA	20.9	±	0.9	20.9	±	0.9
Lola	20.4	±	1.1	22.0	±	0.8
SL-DBY	16.2	±	0.9	19.6	±	0.9
SL-XB	18.6	±	0.9	19.6	±	1.1
Rappahannock River						
WIC	22.4	±	1.3	23.6	±	1.0
MBY	29.5	±	1.4	22.0	±	0.8
RAP	22.6	±	1.1	18.8	±	0.9
CHES	22.9	±	1.0	22.9	±	0.9
PATX	23.0	±	1.0	21.8	±	1.1
hANA	23.7	±	1.0	24.4	±	1.2
Lola	24.4	±	1.1	27.2	±	1.2
SL-DBY	22.6	±	0.9	25.3	±	1.1
SL-XB	22.3	±	1.1	25.5	±	1.2
York River						
WIC	18.6	±	1.0	18.2	±	1.2
MBY	23.8	±	1.6	20.0	±	0.8
RAP	18.2	±	0.9	14.3	±	1.0
CHES	20.6	±	1.3	21.4	±	1.0
PATX	21.9	±	1.4	19.1	±	1.1
hANA	19.4	±	1.1	24.3	±	1.1
Lola	18.8	±	1.0	23.3	±	0.7
SL-DBY	17.5	±	0.9	22.1	±	0.8
SL-XB	19.2	±	0.7	23.0	±	0.8

Table 2.3: Initial shell heights (mean ± 95%CI) at deployment into the experimental design (April 2011) of diploid and triploid wild stocks and Superlines in the Choptank, Rappahannock, and York Rivers. See Table 2.1 for wild stock designation (WIC, MBY, RAP, CHES, and PATX).

	Growth December 2011				Growth December 2012			
Source	*df*	**MS**	**F**	**P**	*df*	**MS**	**F**	**P**
Shell height								
Site	2	65152	906.77	<0.001	2	33329	227.93	<0.001
Ploidy	1	2211	30.77	<0.001	1	21553	147.40	<0.001
Stock	8	1366	19.01	<0.001	8	2693	18.42	<0.001
Site × Ploidy	2	5022	69.89	<0.001	2	3444	23.56	<0.001
Site × Stock	15	490	6.82	<0.001	15	747	5.11	<0.001
Ploidy × Stock	8	583	8.12	<0.001	8	525	3.59	<0.001
Error	2513	72			2452	146		
Whole wet weight								
Site	2	81353	1060.03	<0.001	2	520670	753.56	<0.001
Ploidy	1	9426	122.82	<0.001	1	159802	231.28	<0.001
Genotype	8	1265	16.48	<0.001	8	11418	16.53	<0.001
Site × Ploidy	2	5197	67.721	<0.001	2	43255	62.60	<0.001
Site × Stock	15	580	7.55	<0.001	15	2711	3.92	<0.001
Ploidy × Stock	8	400	5.21	<0.001	8	2364	3.42	0.001
Error	2513	77			2452	691		
Wet tissue weight								
Site	2	2197.9	903.12	<0.001	2	3100	240.60	<0.001
Ploidy	1	17.2	7.06	<0.001	1	768.7	59.66	<0.001
Genotype	8	28.5	11.72	<0.001	8	176.3	13.68	<0.001
Site × Ploidy	2	100.6	41.33	<0.001	2	528.3	41.00	<0.001
Site × Stock	15	12.5	5.13	<0.001	15	39.3	3.05	<0.001
Ploidy × Stock	8	18.7	7.66	<0.001	8	85.4	6.63	<0.001
Error	2463	2.4			2452	12.9		
Meat yield								
Site	2	1.79	1404.80	<0.001	2	0.38	223.41	<0.001
Ploidy	1	0.13	208.11	<0.001	1	0.03	38.08	<0.001
Stock	8	0.07	14.37	<0.001	8	0.12	17.43	<0.001
Site × Ploidy	2	0.05	42.55	<0.001	2	0.02	11.64	<0.001
Site × Stock	15	0.04	3.90	<0.001	15	0.04	3.07	<0.001
Ploidy × Stock	8	0.06	11.81	<0.001	8	0.03	4.81	<0.001
Error	2463	1.57			2452	2.09		

Table 2.4: Analysis of variance for growth traits (shell height, whole wet weight, wet tissue weight, and meat yield) at 17 months (December 2011) and 29 months (December 2012).

Stock	Diploid Whole wet weight (g)		95%CI	Triploid Whole wet weight (g)		95%CI
Choptank River						
WIC	1.3	±	0.5	0.8	±	0.1
MBY	NA	±	NA	NA	±	NA
RAP	0.7	±	0.1	0.7	±	0.1
CHES	0.8	±	0.1	1.1	±	0.1
PATX	1.3	±	0.2	0.8	±	0.1
hANA	1.6	±	0.2	1.5	±	0.2
Lola	1.5	±	0.2	1.5	±	0.1
SL-DBY	0.8	±	0.1	1.2	±	0.1
SL-XB	0.9	±	0.1	1.1	±	0.1
Rappahannock River						
WIC	1.4	±	0.2	1.4	±	0.2
MBY	3.3	±	0.5	1.1	±	0.1
RAP	1.5	±	0.2	0.9	±	0.1
CHES	1.5	±	0.2	1.7	±	0.2
PATX	1.7	±	0.2	1.2	±	0.2
hANA	1.7	±	0.2	1.8	±	0.1
Lola	1.9	±	0.3	2.3	±	0.3
SL-DBY	1.3	±	0.1	1.6	±	0.3
SL-XB	1.5	±	0.2	1.8	±	0.2
York River						
WIC	1.2	±	0.2	1.1	±	0.2
MBY	2.3	±	0.3	1.4	±	0.1
RAP	1.1	±	0.1	0.7	±	0.1
CHES	1.6	±	0.2	1.2	±	0.2
PATX	1.6	±	0.3	1.3	±	0.2
hANA	1.6	±	0.5	1.5	±	0.2
Lola	1.3	±	0.2	1.7	±	0.1
SL-DBY	0.9	±	0.1	1.4	±	0.1
SL-XB	1.0	±	0.1	1.4	±	0.1

Table 2.5: Initial whole wet weights (mean ± 95%CI) at deployment into the experimental design (April 2011) of diploid and triploid wild stocks and Superlines in the Choptank, Rappahannock, and York Rivers. See Table 2.1 for wild stock designation (WIC, MBY, RAP, CHES, and PATX).

		Shell height			Whole wet weight			Wet tissue weight		
		Experimental site			Experimental site			Experimental site		
Group	Line	MD	RR	YR	MD	RR	YR	MD	RR	YR
VA++	WIC	(2)	*10	*20	3	*28	*62	(13)	*20	*43
	MBY	ND	*15	*9	ND	*42	*35	ND	*53	*43
	RAP	0	dead	0	5	dead	7	2	dead	(6)
MD++	CHES	3	*11	*34	5	*32	*108	3	*49	*120
	PATX	2	*10	No 2n	(9)	*26	No 2n	(13)	17	No 2n
Superlines	hANA	8	2	*15	14	9	*32	4	8	23
	Lola	4	(1)	*12	9	11	*20	(6)	12	11
	SL-DBY	4	3	9	(5)	10	*42	*(25)	16	22
	SL-XB	6	6	16	(12)	*23	*33	(27)	10	15

Table 2.6: Estimated triploid effect (%) at 29 months (December 2012) of wild stocks and Superlines in the Choptank, Rappahannock, and York Rivers. Parentheses indicate negative values. '*' Indicates the difference between diploid and triploid stocks/lines are significant at the alpha = 0.05 level. 'ND' signifies the stock or line was not deployed in a given site.

DIPLOID

♂ \ ♀	VA++			MD++		F_1 Superlines			
	WIC	MBY	RAP	CHES	PATX	hANA	Lola	SL-DBY	SL-XB
WIC	2*n*								
MBY		2*n*							
RAP			2*n*						
CHES				2*n*					
PATX					2*n*				
hANA						2*n*			
Lola							2*n*		
SL-DBY								2*n*	
SL-XB									2*n*

TETRAPLOID

4B	3*n*	3*n*	3*n*	3*n*	3*n*	3*n*	3*n*	3*n*	3*n*
	MD++		VA++			F_1 Superlines			

Figure 2.1: Mating design for diploid and triploid crosses of *C. virginica*. Solid boxes indicate which crosses were made. Aliquots of pooled eggs were split in half for diploid and triploid crosses. Diploids were produced from sperm of each stock or line and triploids were produced from the other half of eggs fertilized with pooled sperm from tetraploid males of family 4B. For wild stock designation, see Table 2.1 (WIC, MBY, RAP, CHES, and PATX).

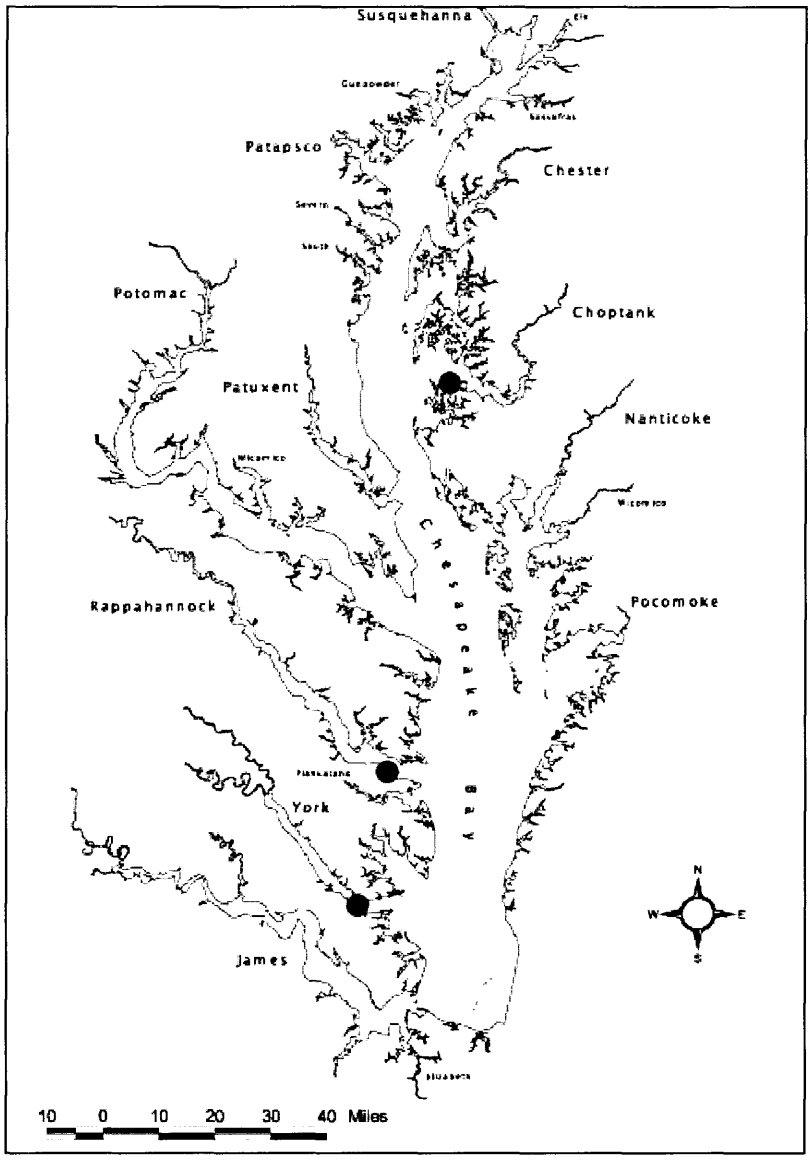

Figure 2.2: Map of the experimental grow-out sites in the Chesapeake Bay. Oysters were grown in three estuaries: York River, VA, Rappahannock River, and Choptank River, MD. Specific experimental site locations are marked with black circles.

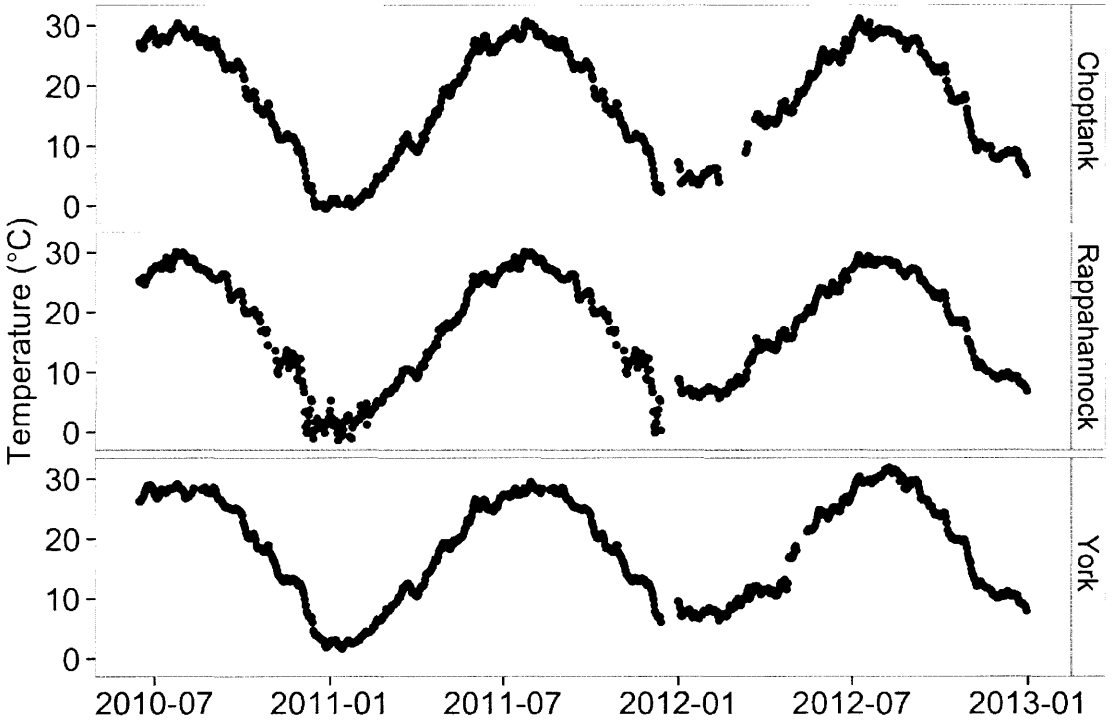

Figure 2.3: Daily average water temperature (°C) in the Choptank River, Rappahannock River, and York River from June 2010 (2010-06) to December 2012 (2012-12). Oysters were spawned in June and July 2010. All stocks and lines were moved to field nurseries at each site in December 2011.

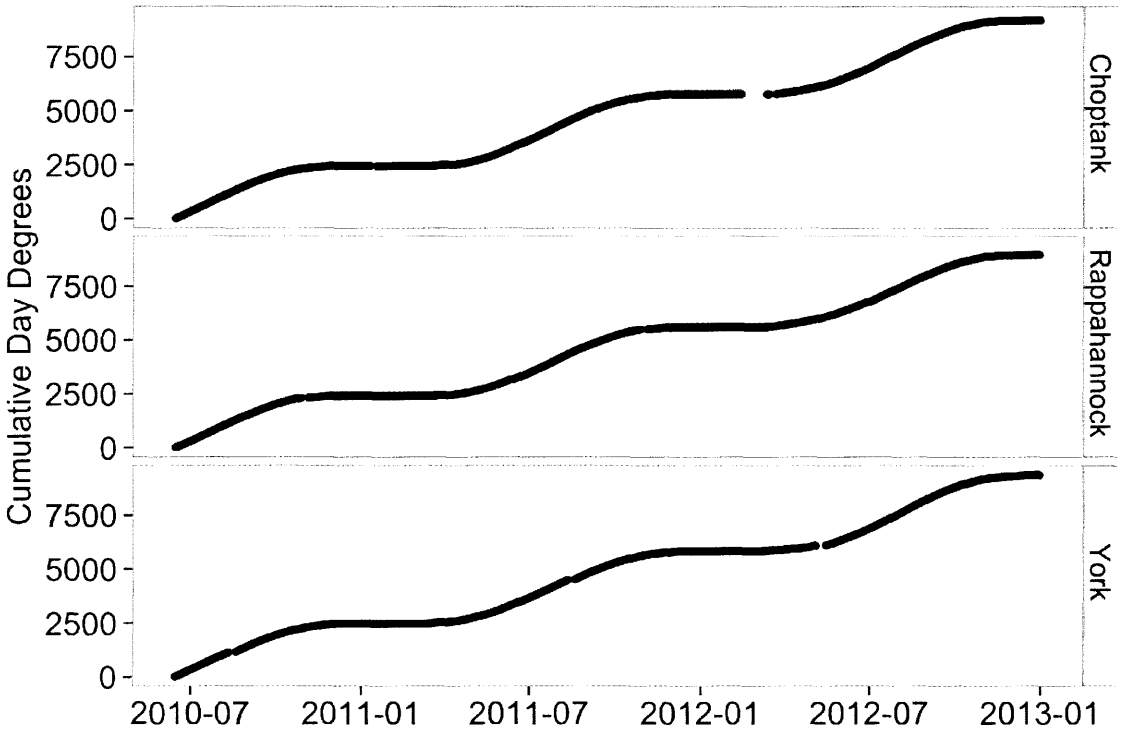

Figure 2.4: Cumulative day degrees for oysters grown in the Choptank River, Rappahannock River, and York River from spawning (June 2010) to 29 months (December 2012). Cumulative day degrees are adjusted for time spent in the nursery system of the VIMS oyster hatchery on the York River prior to deployment to field nurseries at the final grow-out sites.

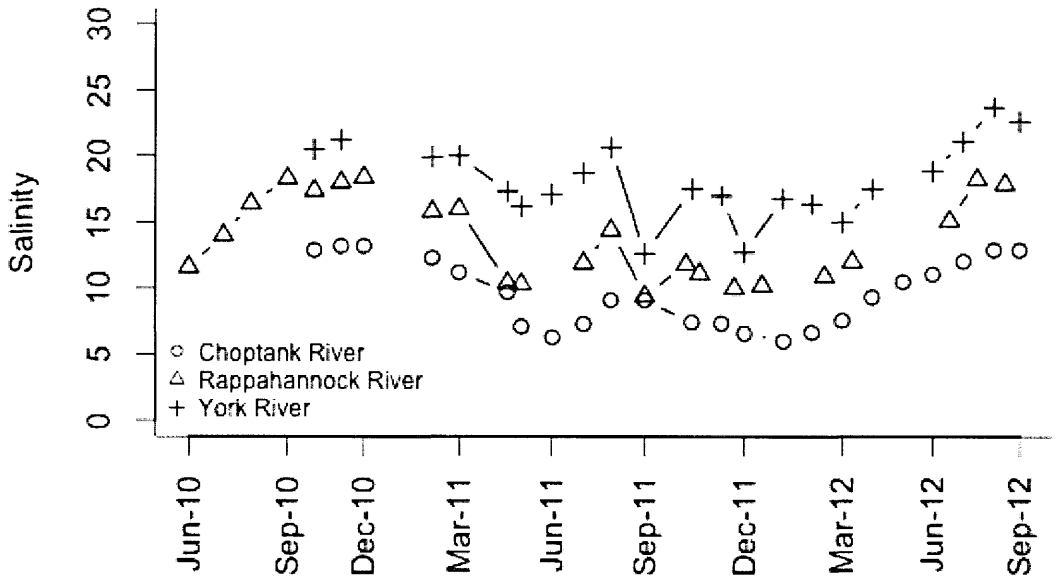

Figure 2.5: Salinity in the Choptank River (○), Rappahannock River (Δ), and York River (+) from June 2010 to December 2012. Salinity data for the Rappahannock and York Rivers were obtained from the Virginia Estuarine and Coastal Observing System stations LE3.4 and LE4.3, respectively. Choptank River data was provided by the University of Maryland Center for Environmental Science's Horn Point Oyster Hatchery.

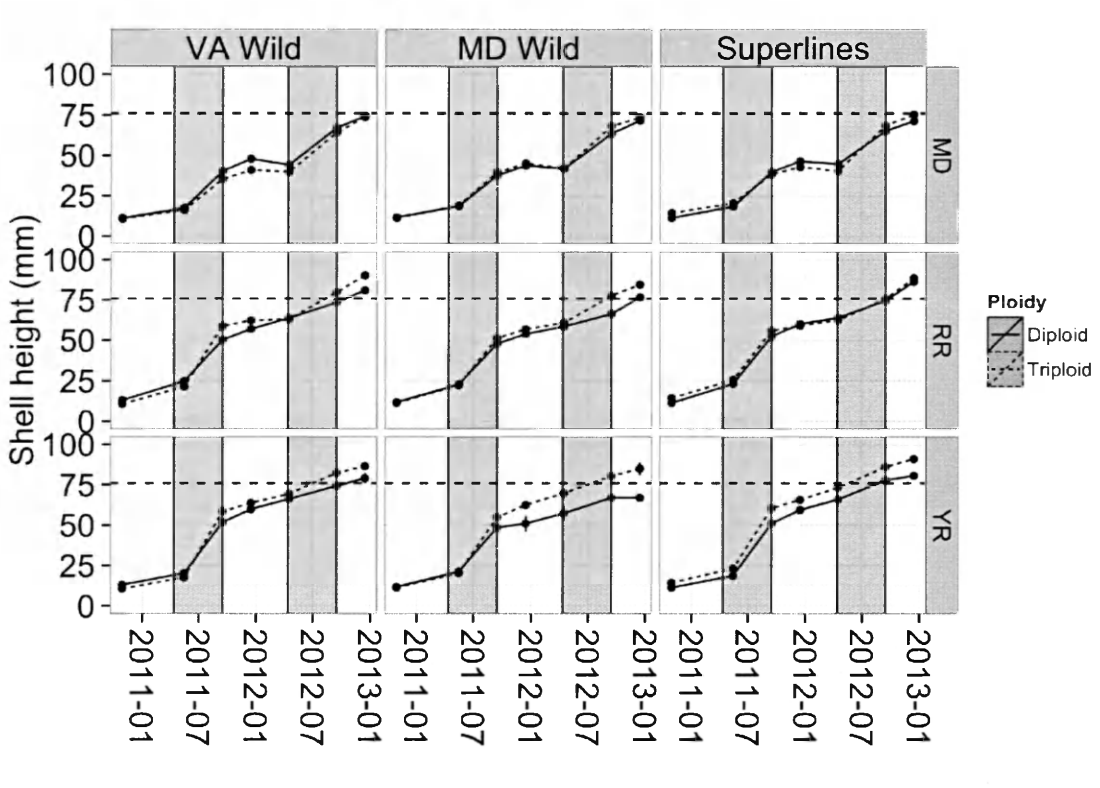

Figure 2.6: Average shell height (mm ± SEM) growth curves of diploids (solid line) and triploids (dashed line) of the wild Virginia stocks (VA Wild), wild Maryland stocks (MD Wild), and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Growth curves begin with measurements taken from animals at the deployment to field nurseries at the final grow-out sites. Dashed lines indicate typical harvest size (shell height ≥76mm). Shaded area indicates typical reproductive period in Chesapeake Bay.

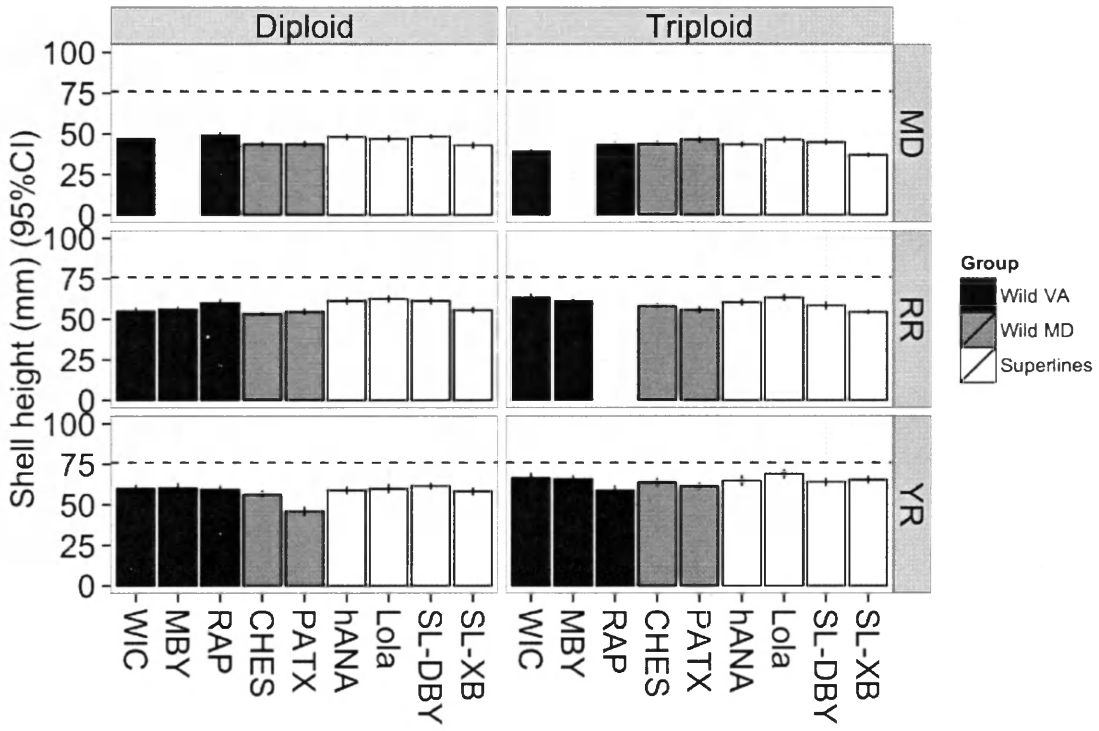

Figure 2.7: Shell height (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months). For wild stock designation, see Table 2.1 (WIC, RAP, MBY, CHES, and PATX). Dashed lines indicate typical harvest size (shell height ≥76mm).

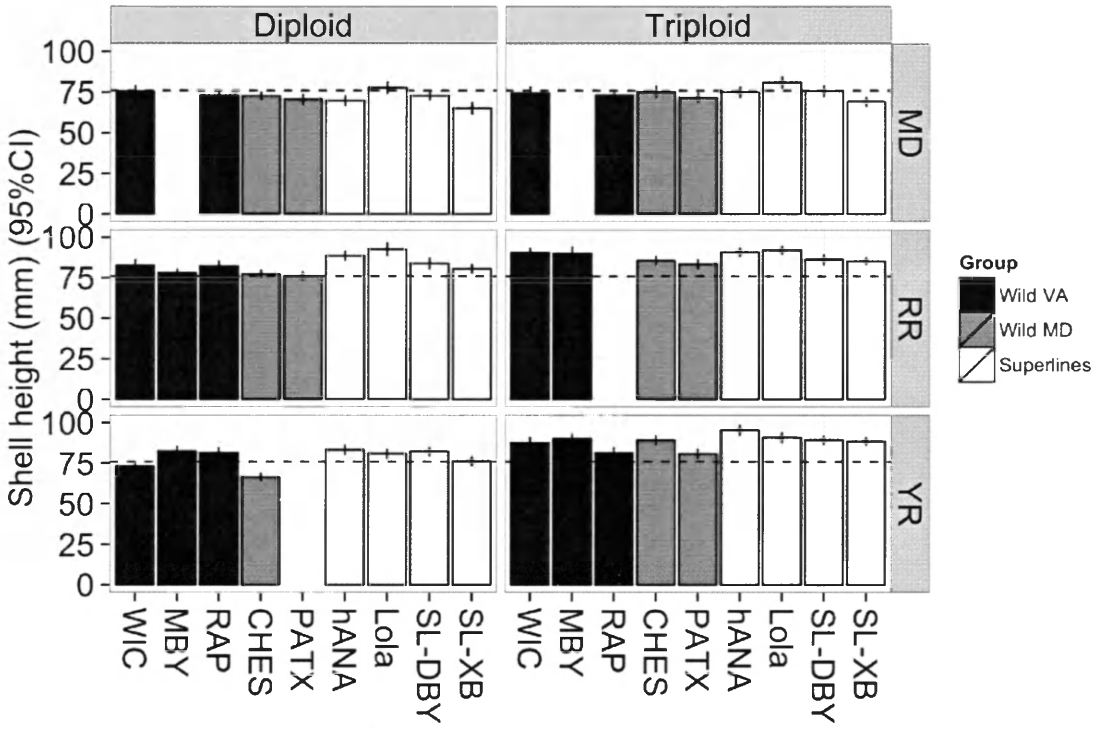

Figure 2.8: Shell height (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months). For wild stock designation, see Table 2.1 (WIC, RAP, MBY, CHES, and PATX). Dashed lines indicate typical harvest size (shell height ≥76mm).

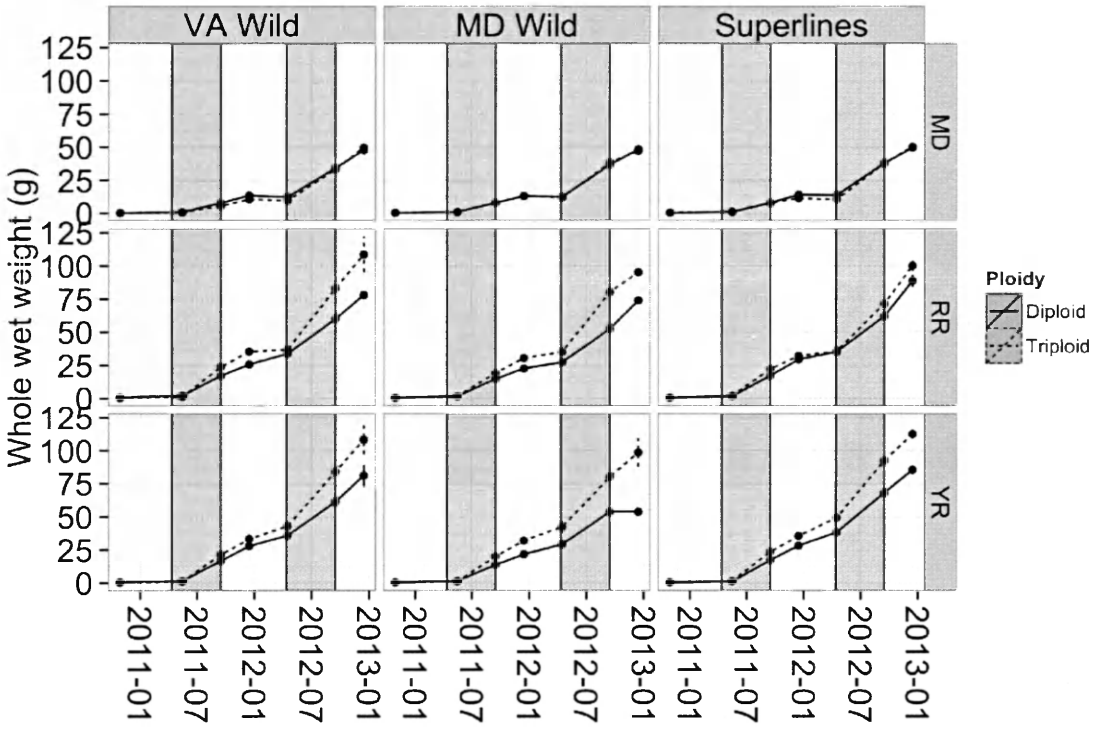

Figure 2.9: Average whole weight (g ± SEM) growth curves of diploids (solid line) and triploids (dashed line) of the wild Virginia stocks (VA Wild), wild Maryland stocks (MD Wild), and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Growth curves begin with measurements taken from animals at the deployment to field nurseries at the final grow-out sites. Shaded area indicates typical reproductive period in Chesapeake Bay.

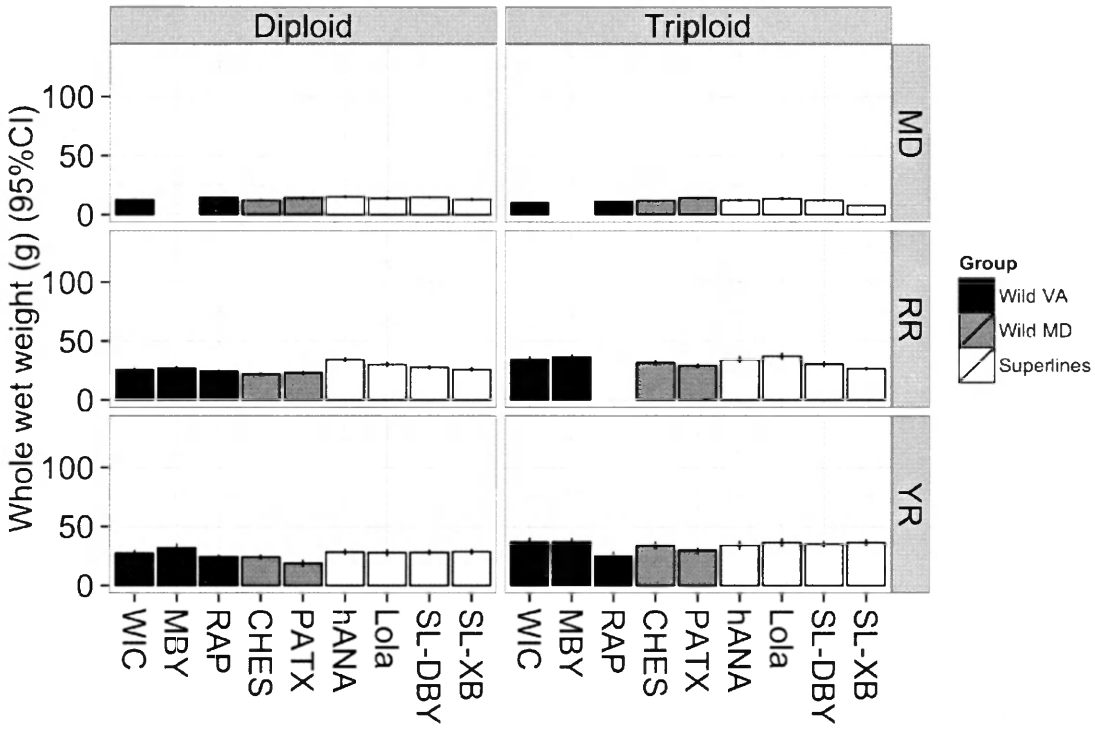

Figure 2.10: Whole wet weight (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months). For wild stock designation (WIC, RAP, MBY, CHES, and PATX), see Table 2.1.

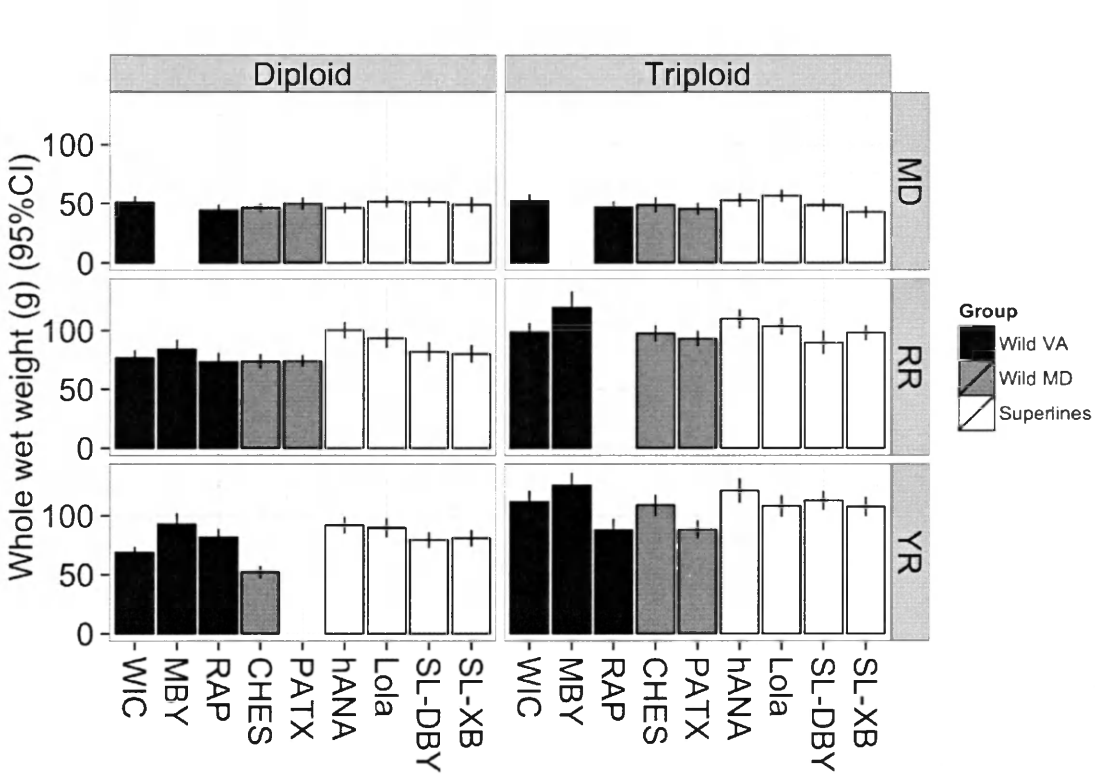

Figure 2.11: Whole wet weight (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months). For wild stock designation (WIC, RAP, MBY, CHES, and PATX), see Table 2.1.

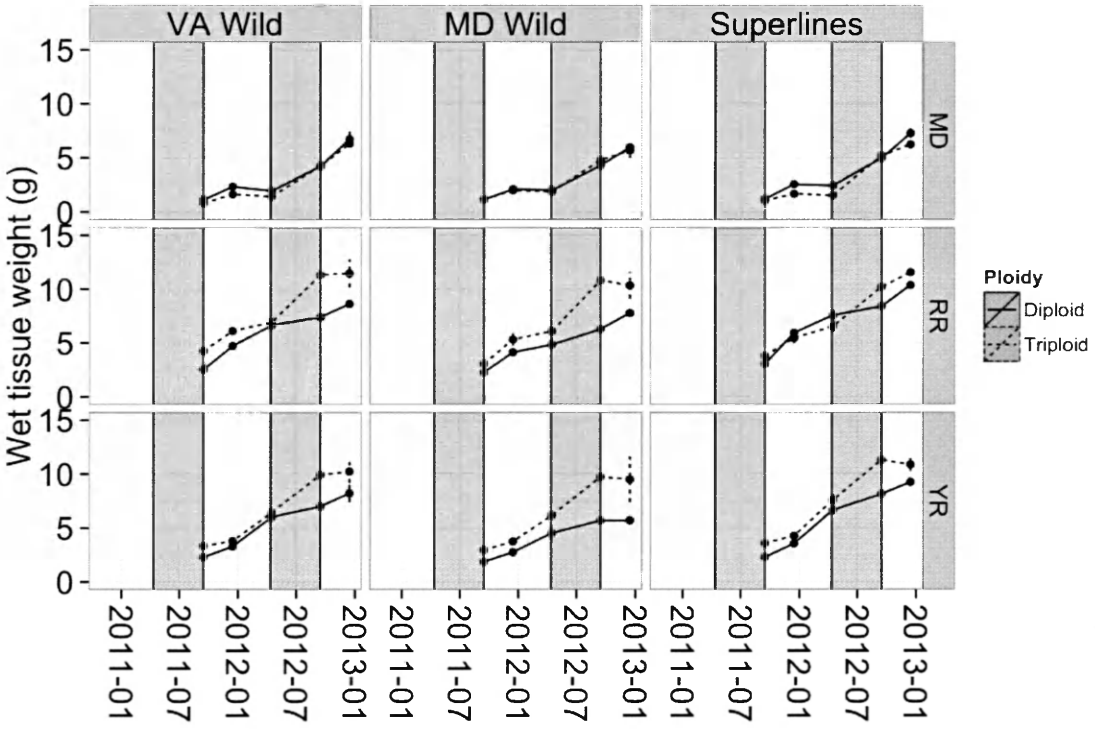

Figure 2.12: Average wet tissue weight (g ± SEM) growth curves of diploids (solid line) and triploids (dashed line) of the wild Virginia stocks (VA Wild), wild Maryland stocks (MD Wild), and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Growth curves begin with measurements taken in September 2011. Shaded area indicates typical reproductive period in Chesapeake Bay.

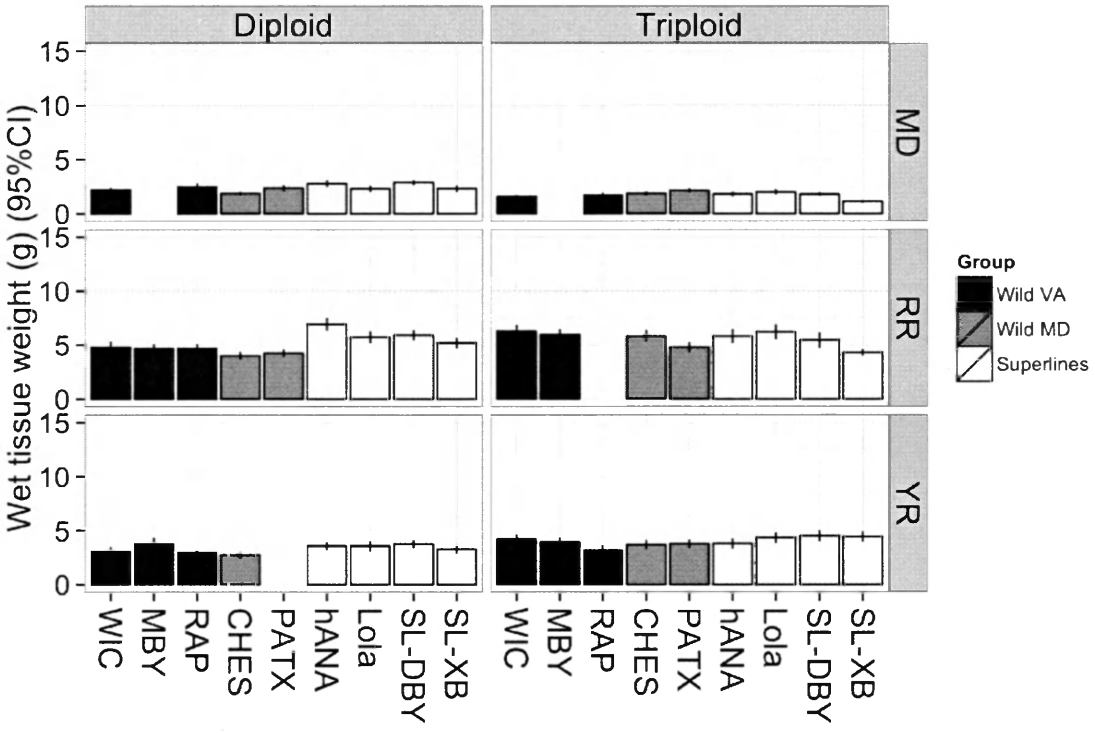

Figure 2.13: Wet tissue weight (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months). For wild stock designation (WIC, RAP, MBY, CHES, and PATX), see Table 2.1.

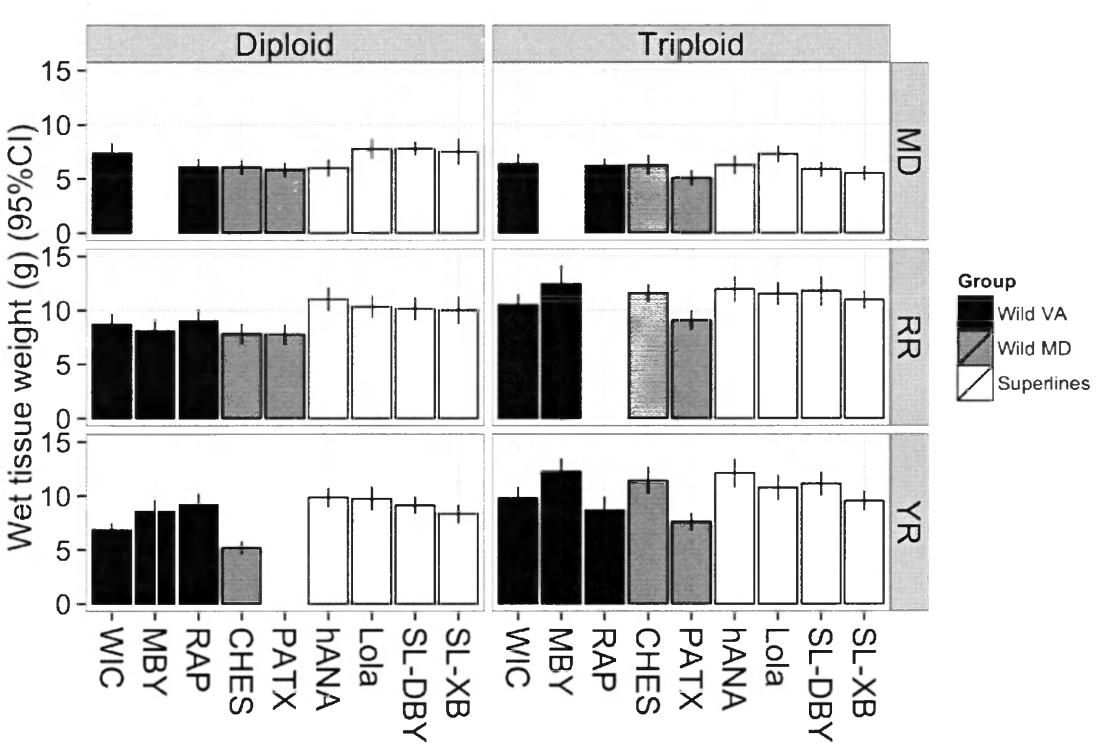

Figure 2.14: Wet tissue weight (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months). For wild stock designation (WIC, RAP, MBY, CHES, and PATX), see Table 2.1.

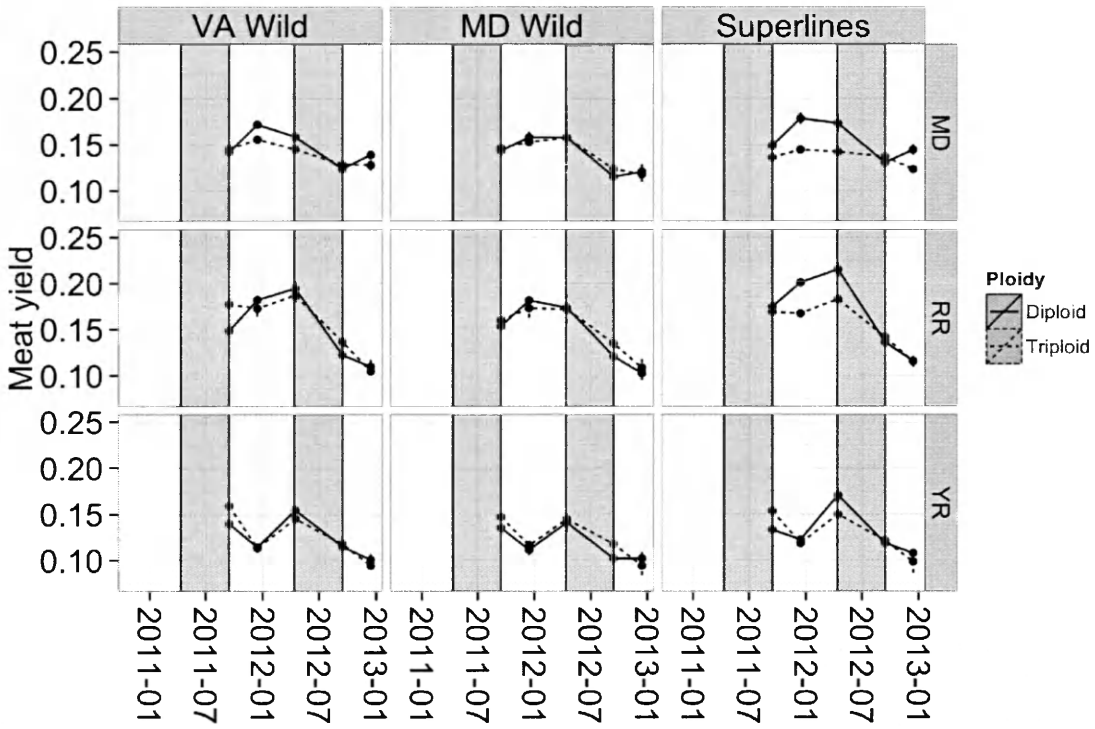

Figure 2.15: Meat yield curves of diploids (solid line) and triploids (dashed line) of the wild Virginia stocks (VA Wild), wild Maryland stocks (MD Wild), and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Curves begin at September 2011 when wet tissue weight was first measured. Shaded area indicates typical reproductive period in Chesapeake Bay.

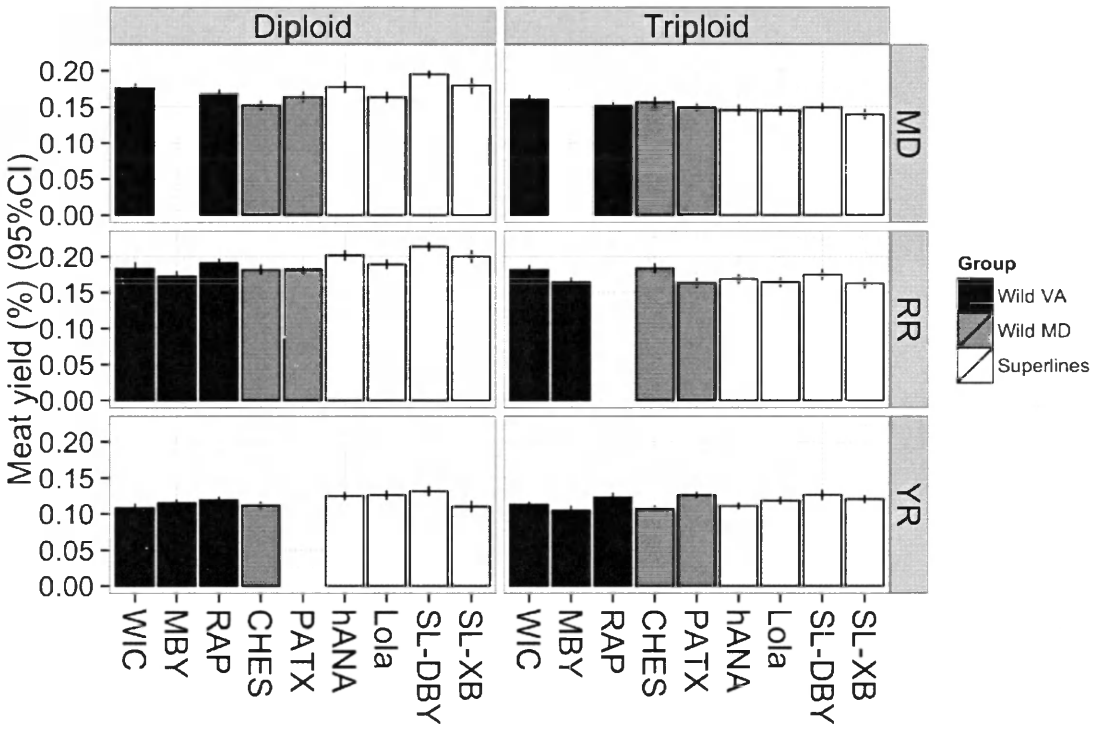

Figure 2.16: Meat yield (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months). For wild stock designation (WIC, RAP, MBY, CHES, and PATX), see Table 2.1.

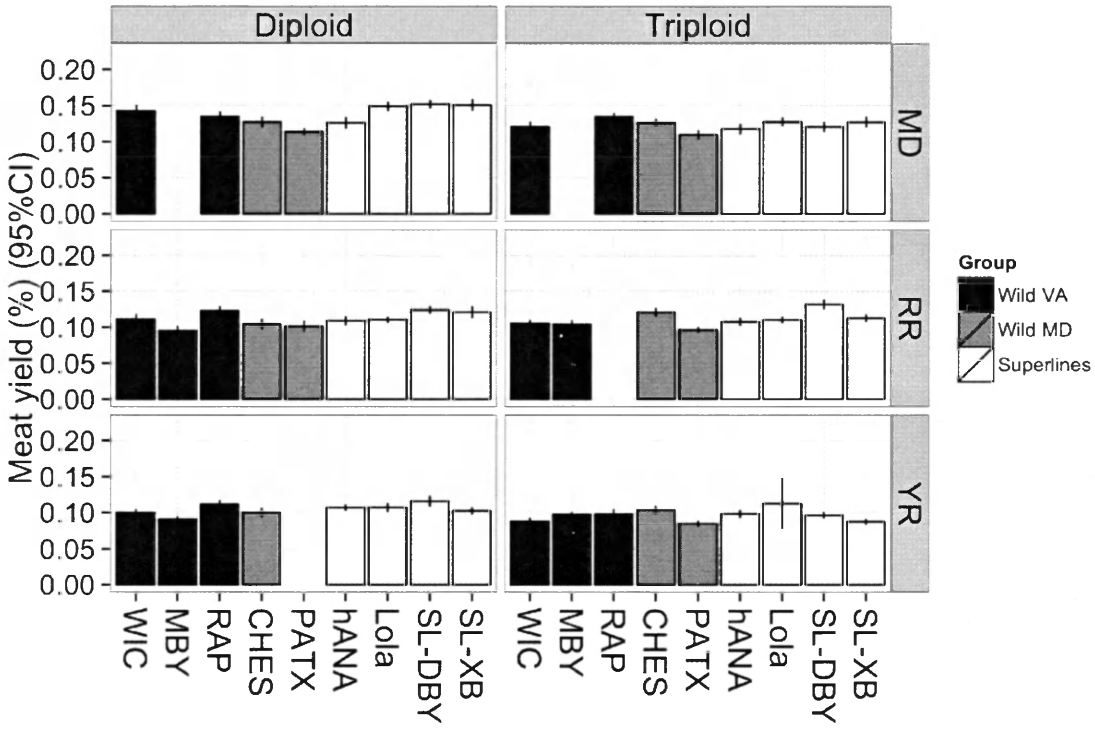

Figure 2.17: Meat yield (mean ± 95%CI) of diploid and triploid wild stocks and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months). For wild stock designation (WIC, RAP, MBY, CHES, and PATX), see Table 2.1.

Chapter Three: Triploid advantage for growth in *C. virginica* produced from two generations of breeding lines

1. INTRODUCTION

The 'triploid advantage', as defined in the previous Chapters, describes the faster growth and increased survival observed in triploids when compared to diploid oysters grown in similar conditions. This quality of triploids is hypothesized to be a result of several aspects: energy allocation differences due to suppressed reproduction, greater heterozygosity, and additive genetic effects from selective breeding (Allen and Downing, 1986; Barber and Mann, 1991; Hand et al, 1998). This chapter mainly examines the role of additive gains in the triploid advantage by comparing field performance of two groups of selectively bred triploid lines.

Additive genetic gains are one of the hallmarks of all selective breeding programs, including the breeding of diploid oysters (*Crassostrea virginica*) in ABC's breeding program. Additive genetic effects describe the breeding value of an individual. The breeding value of an individual is the part of the deviation of an individual phenotype from the population mean that is due to the cumulative effects of alleles. Essentially, this is a measure of the individual, as a parent, for improving a trait in the next generation. In triploids, additive genetic gains are likely to obtain through the addition of another set of optimal alleles. If additive gains obtain in triploids, it would be from an increase in the dosage of beneficial effects from the optimal alleles, thus resulting in better performance of a given trait. For example, additive gains in triploids were studied in the Sydney rock oyster (*Saccostrea glomerata*) through a comparison of

diploid and chemically induced triploid progeny of a third generation breeding line and an unselected control group (Hand et al., 2004). In Hand et al.'s study, triploids were chemically induced, so all three sets of chromosomes had been selected for increased growth rate. The authors showed that improvement in whole body weight of the breeding line was still present over the control group when triploidy was chemically induced showing that gains made from selective breeding carry into triploidy.

There are no studies examining how the improvements through selective breeding, in successive generations of diploid lines, translate in triploids in *C. virginica*. Guo et al. (2009) point out that mated triploid *C. virginica* have been shown to improve over diploid controls with successive generations of the tetraploid parent. The authors describe two studies in which triploids produced from second generation tetraploids grew more than 88% faster than diploids while those from first generation tetraploids only grew 34% faster (Wang et al., 2005; Guo et al., 2008). Guo et al. (2009) put forth a hypothesis called *genome adaption* to explain the advantages observed in successive tetraploid generation. This hypothesis suggests that one of the reasons triploids from second-generation tetraploids grew faster is that the tetraploids have undergone intense growth and survival selection relative to the enormous genetic variation new tetraploid populations must have. If gains in triploid performance are possible from selection of tetraploids, it follows that gains are also achievable through selection of diploid parents and should be measurable with successive generations in the diploids.

In Chapter 2, the performance of triploids made from wild-type oysters and the current version of ABC selected lines, so-called Superlines, were compared. The comparison of relative performance among diploids and their triploid counterparts

showed that the contribution of the diploid parent in a tetraploid × diploid cross is significant. The differences of relative performance across the study sites showed that the effect of the environment is significant as well.

The major objective of this Chapter was to compare field performance among successive generations of selected lines (2009 lines vs. Superlines). To achieve this, the selected lines from the 2006-year class were compared with the 2008 Superlines as both diploids and triploids. This comparison is not a comparison of successive generations.

In 2008, ABC's breeding program changed its strategy from one based primarily on increasing disease resistance to regionally based selection for growth traits. There were 15 lines derived from three base populations: DBY, XB, and, Louisiana origin. The creation of each Superline (SL) consisted of combining over 100 pair-mated crosses of lines with common origin from the 25 previous lines, including several hybrids of the base populations. To create SL-DBY in 2008, nine lines from the 2006-year class were crossed together; for SL-XB, nine lines from the 2006-year class were used; and for hANA and Lola, 11 lines were used, based on whether selection sites were in high salinity (hANA) or low salinity (Lola). As a result of this line consolidation, the Superlines are not truly successive generations for the four 2006-year class lines in this study, but rather composites of lines (including the four 2006-year class lines here) based on three main base populations. The four lines from the 2006-year class were chosen as representatives of those base populations.

Considering the triploids of interest in this Chapter are all produced by $2n \times 4n$ crosses and both the $2n$ and $4n$ parent are from selected populations, all triploids have three sets of selectively bred chromosomes (SSS). In Chapter 2, wild triploids (+SS)

were compared to selected triploids (SSS) and differences were attributed to the difference in the contribution of the wild-type parent (+) *versus* the selectively bred Superline parent (S). In this Chapter, the comparison is subtler, that is, between two selectively bred sources: SSS_{2006} *versus* $SSS_{Superline}$.

2. MATERIALS AND METHODS

Diploid and triploid oysters were produced at the ABC Oyster Hatchery, Gloucester Point, VA through June and July 2010. Broodstock used to produce these oysters were collected from five wild populations in the Chesapeake Bay as well as from two groups of selectively bred disease resistant (DR) lines – 2006-year class lines and Superlines – from ABC's breeding program.

The five wild stocks used, Great Wicomico River (WIC), Rappahannock River (RAP), Mobjack Bay (MBY), Chester River (CHES), and Patuxent River (PATX) are described in detail in Chapter 2. These populations were chosen because the estuaries from which they originate range in salinity and disease exposure.

Four lines from ABC's 2006-year class lines were used: LGT, OBOY, DBY, and XB. The LGT line was derived from wild oysters in Grande Terre, LA in 2000 and selected by ABC for disease resistance since then for four generations. OBOY was introduced into ABC's breeding program in 2002 as an F_3 generation derived from wild oysters in Oyster Bayou, LA and subsequently selected for Dermo resistance by Dr. Jerome LaPeyre's program at Louisiana State University. XB was developed in Delaware Bay, NJ at Rutgers University by S. Allen from a consolidation of many lines produced by Ford and Haskin (1987) prior to 1988. They were brought to Chesapeake Bay in 1998 and propagated within ABC (Dégremont et al., 2012). Due to limited availability of the 2006-year classes of LGT, OBOY, and XB, 2009-year classes of these

three lines were used. These were propagated from the 2006-year class of the corresponding line via random pooled spawns through an effort to preserve the germ plasm of these lines. The DBY line was developed from wild oysters from Delaware Bay, NJ that were collected in 1987 and selected for Dermo and MSX resistance for four generations in the York River, VA (Ragone Calvo et al., 2003). Subsequent selection and generations were produced by ABC's breeding program. The 2006 DBY year class is an F_7 generation.

The four Superlines are Lola, hANA, SL-DBY, and SL-XB. Lola and hANA were produced using progenitors from Louisiana, known to be Dermo resistant (Ragone Calvo et al., 2003), and selected in Virginia for MSX resistance. The DBY and XB lines were developed from wild oysters from Delaware Bay, NJ (Ragone Calvo et al., 2003; Ford and Haskin, 1987; Dégremont et al., 2006). The Superlines are described in detail in Chapter 2 and a detailed pedigree of the Superlines can be found in ABC's breeding manual (ABC, 2010).

Although all the lines were spawned simultaneously, only diploids and triploids made from 2009 lines and Superlines were compared in this Chapter.

Broodstock were conditioned in a flow-through system at ABC's conditioning facility, the Kauffman Aquaculture Center (KAC) on Locklies Creek, VA. In the flow-through system, water temperature was held constant at 23°C. Broodstock were batch fed cultured algae cocktails containing *Isochrysis* sp., *Tetraselmis chui*, and *Chaetoceros muelleri*. When all stocks had conditioned, they were transferred to ABC's research hatchery in Gloucester Point, VA for spawning and larval rearing.

2.1 Crosses

Eggs obtained from at least 10 dams per stock (wild) or line (selected) were stripped from gonad tissue and pooled in plastic beakers. The pools of eggs were then divided into two groups containing 3×10^6 eggs each, one for diploids and one for triploids. To produce diploids, one group of eggs was fertilized with sperm pooled from at least 10 sires of the same stock/line when available. To produce triploids, the remaining groups of eggs were fertilized with sperm pooled from 11 sires from a single tetraploid family following the methods of Guo et al. (1996). The number of dams and sires from each group are outlined in Table 3.1. Several crosses were made with reduced dam and sire numbers due to insufficient numbers of ripe broodstock. In this manner, 26 groups were produced: 13 diploid and 13 triploid (Figure 3.1). While all 26 groups were spawned, the analysis of the wild stocks appears in Chapter 2. This chapter details relative performance of the 2006-year class lines versus the Superlines.

2.2 Larval Rearing

Larvae were reared through settlement following the ABC protocol adapted from Helm et al. (2004) in 60L flat-bottom larvae tanks, consisting of daily batch feeding of microalgae and complete water exchanges three times a week (Monday, Wednesday, and Friday). Larval tank densities were adjusted based on age (in days) post-fertilization, such that, on days two, seven, and 14, the densities were adjusted to 10-larvae·mL^{-1}, 5-larvae·mL^{-1}, 2.5-larvae·mL^{-1}, respectively. Eyed-larvae were collected on 225μm for diploids or 230μm nylon screen for triploids. Competent eyed-larvae were transferred to 16cm^2 square downwellers for settlement. After two weeks in this downwelling system, the spat were moved into a flow-through upweller based nursery until field deployment.

2.2.1 Ploidy Determination

Ploidy was determined at various stages of rearing by flow cytometry to confirm the success of triploid crosses (Allen, 1983). Prior to pooling sperm from the 11 tetraploid sires, sperm from each individual were confirmed 100% di-haploid by analyzing gametes dissected from gonad tissue. Ploidy was analyzed again at the prodissoconch I larval stage on larvae collected on a 48μm nylon screen 48hrs post-fertilization by sampling 2000 larvae and prior to field deployment by sampling 50 spat from each group. At each of these sampling points all groups were confirmed 100% diploid or triploid.

2.3 Experimental Sites and Design

Oysters were deployed at three sites in the Chesapeake in November 2010 (Figure 3.2). In the Virginia portion of the Chesapeake Bay, the two sites were the York River (13-25 ppt) and the Rappahannock River (13-20 ppt). The York River site is opposite VIMS on a private lease operated by Tommy Leggett of Chessie Seafood Company. The grow-out location in the Rappahanock River is on a lease owned by the Rappahannock River Oysters, LLC in Topping, VA. These sites were chosen in order to perform this experiment under environmental conditions of commercial operations. In the Maryland portion of the Chesapeake Bay, oysters were deployed in the Choptank River (5-12 ppt) adjacent to the University of Maryland Horn Point Environmental Laboratory (Figure 3.2).

Stocks were deployed in off-bottom cages at each of the Chesapeake Bay sites for evaluating growth. The off-bottom cages were designed and manufactured by the Chesapeake Bay Oyster Company (Irvington, VA). A single cage can hold three full-sized oyster grow-out bags. Off-bottom cages were chosen because it is the most

common method of commercial culture in the Chesapeake Bay. Due to low survival in the field nursery, there were several limitations in the deployment of all lines at all sites. Diploid 2006-year class line OBOY09 was not deployed to the Choptank River site and diploid and triploid 2006-year class line XB06 was not deployed to the Choptank or Rappahannock Rivers.

2.3.1 Sampling

2.3.1.1 Growth Parameters

Two ADPI bags (approx. 0.6m x 0.9m polyethylene oyster bags) per group (thirteen diploid groups and thirteen triploid groups) were stocked at 500 oysters per bag. The 52 bags (two replicates per group) were randomly placed into 15 cages. Density in the bags was not periodically reduced since destructive sampling and mortality kept densities suitable until June 2012. In June 2012, bag densities were reduced to occupy 1/3 of the bag by splitting groups into additional replicates in both the York and Rappahannock Rivers. Splitting was not necessary in the Choptank River. Samples of 25 oysters per replicate per group (50 oysters per group total) were sampled every Spring, Fall, and Winter – beginning in the Spring of 2011 and ending Winter 2012 – for measurements of shell height (distance between the umbo and the ventral valve margin), whole wet weight, wet tissue weight, and meat yield (calculated as wet tissue weight ÷ whole wet weight). Wet tissue weight was measured after the body tissues drained on a mesh screen. The percentages of sampled oysters that were harvest size was determined from shell height measurements at each sampling point as well. Harvest size is typically ≥76mm following harvesting conventions of wild oysters.

2.3.1.2 Environmental Parameters

In the Choptank, Rappahannock and York Rivers, average daily temperatures were estimated from hourly temperature measurements using submersible temperature data loggers. Individual and cumulative day degrees were calculated from average daily temperatures. Salinity data for the Rappahannock and York Rivers were taken from long-term monitoring stations from the Virginia Estuarine and Coastal Observing System. The University of Maryland Center for Environmental Science's Oyster Hatchery provided salinity data for the Choptank River. These data are reported in Chapter 2.

2.4 Analyses

2.4.1 Time Frame

Cumulative mortality, shell height, whole weight, and meat yield were analyzed in December 2011 (17 months post-spawn) and December 2012 (29 months post-spawn). Proportion of market-size oysters (≥76 mm) was analyzed at each sampling point.

2.4.2 Model Equation

The following model was used for analysis (Equation 3):

(3)

$$Y_{ijkl} = \mu + site_i + ploidy_j + line_k + (site_i \text{ X } ploidy_j) + (site_i \text{ X } line_k) + (ploidy_j \text{ X } line_k) + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable (shell height, whole wet weight, wet tissue weight, or meat yield), μ is the overall mean, $site_i$ is the site effect (York River, Rappahannock River, Choptank River), $ploidy_j$ is the ploidy effect in *C. virginica* (diploid or triploid), $line_k$ is the genotype effect, 'X' indicates interactions, and ε_{ijkl} is the residual error.

2.4.3 Statistical Procedures

Normality and the homogeneity of variance for shell height, whole weight, and meat yield were confirmed with the Shapiro-Wilk's W test for normality with the statistical programming language R (R Core Team, 2012).

ANOVA was performed in R using the nlme package (Pinheiro et al., 2013). Following significant findings from the ANOVA, multiple comparisons were conducted using Tukey's Honest Significant Differences test in R. When a significant interaction between site and group was found, the site was dropped from the analyses to test the group effect within the site. Replicates were not a significant source of variation and as such were not included in the ANOVA model. Because of the equivalency of replicates, confidence intervals reported were calculated from the combined individual measurements from both replicates.

3. RESULTS

As a reminder, the results in this chapter are restricted to the relative performance of the 2006-year class lines versus the Superlines. Environmental parameters measured at the sites are shown in Chapter 2.

3.1 Growth Parameters

3.1.1 Shell Height

Overall growth trends are depicted in Figure 3.3. Oysters were deployed from the field nursery into the experimental design at each site in April 2011. Table 3.2 reports initial shell height measurements (mm ± 95%CI) of individual lines. Diploid and triploid shell height increased from May 2011 to September 2011 at each site. From September 2011 until May 2012, growth plateaued in the Choptank River. Growth in the Choptank River began again after May 2012. The Rappahannock and York Rivers did not show this plateau, but rather a seemingly slower growth rate for the remainder of the study was occurred. In both December 2011 and 2012, the site effect, ploidy effect, line effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 3.3).

3.1.1.1 Choptank River, MD

2006-Year class

By December 2011 (17 months), there were no significant differences between diploids or triploids in any of the 2006-year class lines ($p>0.05$). Diploid line OBOY09 and diploid and triploid lines XB06 were not deployed because of survival limitations in

the field nursery. There were no differences between the individual diploid or triploid 2006-year class lines in the Choptank River ($p>0.05$) (Figure 3.4).

After 29 months, there was no triploid effect on shell height for the 2006-year class lines. The triploid advantage of 2006-year class lines, though not significant, ranged from 2% to 3% (Table 3.5). There were also no significant differences between the individual diploid or triploid lines at 29 months ($p>0.05$) (Figure 3.5).

Superlines

At the December 2011 (17 month) sampling point, there was only one group that showed a significant difference between diploids and triploids (Figure 3.4). Diploid Superline SL-XB had significantly greater shell height (mean ± 95%CI) than its triploid counterpart (42.8 ± 2.1mm and 36.9 ± 1.5mm, respectively, $p<0.05$). There were no significant differences between diploids and triploids for the remaining genotypes in the Choptank River ($p>0.05$). SL-XB Superline had significantly lower shell height than the hANA, Lola, and SL-DBY Superlines as both diploid and triploid (diploids: SL-XB – 42.8 ± 2.1mm, hANA – 48.1 ± 2.1mm, Lola – 46.9 ± 2.3mm, and SL-DBY – 48.3 ± 1.5mm and triploids: SL-XB – 36.9 ± 1.5mm, hANA – 43.4 ± 1.8mm, Lola – 46.4 ± 2.1mm, and SL-DBY – 44.8 ± 2.0mm) (Figure 3.4).

In December 2012 (29 months), the triploid advantage was no longer present for the Superline SL-XB and there were no significant differences among shell heights of the remaining diploid and triploid groups ($p>0.05$, Figure 3.5). The triploid advantage of Superlines, though not significant, was greater than the 2006-year class lines and ranged from 4% to 8% (Table 3.5). The diploid Lola and SL-DBY Superlines (77.6 ± 3.9mm and 72.8 ± 3.0mm, respectively) had significantly higher shell heights than diploid hANA

and SL-XB (69.8 ± 3.5mm and 65.2 ± 3.9mm, respectively, $p<0.05$). Of the triploid Superlines, Lola had higher shell height than hANA, SL-DBY, and SL-XB (77.6 ± 3.9mm, 69.8 ± 3.5mm, 72.8 ± 3.0mm, and 65.2 ± 3.9mm, respectively, $p<0.05$). Triploid hANA and SL-DBY did not have significantly different shell heights ($p>0.05$). Triploid SL-XB had the lowest shell height of the triploid Superlines (Figure 3.5).

3.1.1.2 Rappahannock River, VA

2006-Year class

In the Rappahannock River, at 17 months, diploids and triploids were the same size, statistically (Figure 3.4). Diploid and triploid 2006-year class lines XB06 were not deployed in the Rappahannock River because of survival limitations in the field nursery. Of the individual diploid 2006-year class lines, DBY09 was significantly smaller than OBOY09 (58.2 ± 1.9mm and 64.8 ± 2.8mm, respectively, $p<0.05$). Diploid OBOY09 was larger than LGT09, though this difference was not significant ($p>0.05$). Triploid OBOY09 was the largest of the 2006-year class lines ($p<0.05$) and LGT09 and DBY09 did not have significantly different shell heights (65.7 ± 1.9mm, 59.3 ± 2.0mm, and 59.7 ± 2.0mm, respectively) (Figure 3.4).

By 29 months, the triploid advantage was present for LGT09 and DBY09 of the 2006-year class lines (Table 3.5). Triploid LGT09 was 15% larger than its diploid counterpart and triploid DBY09 was 14% larger than its counterpart (LGT09: diploid – 82.7 ± 4.1mm and triploid – 95.2 ± 2.7mm and DBY09: diploid – 80.2 ± 2.8mm and triploid – 91.2 ± 3.5mm, $p<0.05$). There were no significant differences between the individual triploid 2006-year class lines ($p>0.05$) (Figure 3.5).

Superlines

At 17 months, no diploid:triploid comparison differed in size (Figure 3.4). Among the individual diploid Superlines there were no significant differences in shell height ($p>0.05$). Triploid Superlines Lola and hANA had equivalent shell heights (63.3 ± 2.3mm and 60.5 ± 2.2mm, respectively, $p>0.05$) and triploid SL-DBY and SL-XB had equivalent shell heights (58.4 ± 2.7mm and 54.4 ± 1.5mm, respectively, $p>0.05$). Triploid Lola and hANA had higher shell heights than SL-DBY and SL-XB ($p<0.05$) (Figure 3.4).

By 29 months, the effect of ploidy was still absent and there were no significant differences among shell heights of diploid and triploid Superlines ($p>0.05$). The triploid advantages were low (as the differences from ploidy were not significant): hANA – 2%, SL-DBY – 3%, and SL-XB – 6%. Lola had a triploid disadvantage (-1%) (Table 3.5). Among the diploid Superlines, Lola had the highest shell height (92.7 ± 4.3mm), followed by hANA (88.7 ± 3.1mm), and then SL-DBY and SL-XB (83.9 ± 3.7mm and 80.6 ± 3.1mm, respectively), both of which had equivalent shell heights. Triploid hANA and Lola had equivalent shell heights (90.9 ± 3.0mm and 92.1 ± 2.9mm, respectively) and were both significantly larger than triploid SL-DBY and SL-XB (86.3 ± 3.6mm and 85.3 ± 2.6mm, respectively), whose shell heights were not significantly different (Figure 3.5).

3.1.1.3 York River, VA

2006-Year class

In the York River, by 17 months, triploidy had an effect on all four of the 2006-year class lines (Figure 3.4). All of these lines were larger as triploids than as diploids (LGT09: diploid – 60.8 ± 2.9mm and triploid – 68.0 ± 2.6mm; OBOY09: – diploid 45.2

± 1.6mm and triploid – 65.5 ± 2.8mm; DBY09: diploid – 59.9 ± 2.3mm and triploid – 66.4 ± 2.6mm; XB06: diploid – 65.0 ± 2.6mm and triploid – 72.2 ± 2.7mm). The diploid 2006-year class line OBOY09 was significantly smaller than all of the remaining diploid lines (OBOY09 – 45.2 ± 1.6mm; LGT09 – 60.8 ± 2.9mm; DBY09 – 59.9 ± 2.3mm; XB06 – 65.0 ± 2.6mm). There were no significant differences among the individual triploid 2006-year class lines ($p>0.05$) (Figure 3.4).

By 29 months, triploidy had affected growth positively in all of the 2006-year class lines and two of the Superline crosses (Figure 3.5). LGT09 had significant triploid advantage of 20% (diploid – 79.4 ± 4.1mm and triploid – 95.4 ± 3.1mm, $p<0.05$), DBY09 had a triploid advantage of 11% (diploid – 82.7 ± 3.0mm and triploid – 91.7 ± 3.1mm, $p<0.05$), and XB06 had a triploid advantage of 17% (diploid – 80.7 ± 3.1mm and triploid – 94.6 ± 2.5mm, $p<0.05$) (Table 3.5). Diploid OBOY09 did not survive to the end of the study in the York River. There were no differences among the triploid 2006-year class line shell heights ($p>0.05$) (Figure 3.5).

Superlines

By 17 months, from the Superlines, Lola and SL-XB were larger as triploids than diploids (Lola: diploid – 59.7 ± 2.9mm and triploid – 68.9 ± 3.0mm ; SL-XB: diploid – 58.1 ± 2.5mm and triploid – 65.3 ± 2.5mm) (Figure 3.4). There were no other significant differences between diploid and triploids of the remaining Superlines in the York River ($p>0.05$). The diploid Superlines hANA, Lola, SL-DBY, and SL-XB all had about the same shell heights (58.8 ± 2.5mm, 59.7 ± 3.0mm, 61.4 ± 2.2mm, and 58.1 ± 2.5mm, respectively, $p>0.05$). There were no significant differences between individual triploid Superlines (Figure 3.4).

By 29 months, diploid hANA had significantly smaller shell height than its triploid counterpart (83.3 ± 3.3mm and 95.4 ± 3.6mm, respectively, $p<0.05$) and diploid Lola being significantly smaller triploid Lola (80.8 ± 3.6mm and 90.8 ± 3.5mm, respectively, $p<0.05$) (Figure 3.5). These differences equate to a triploid advantage of 15% for hANA and 12% for Lola (Table 3.5). The shell heights of diploid hANA, Lola, and SL-DBY were not significantly different (83.3 ± 3.3mm, 80.8 ± 3.6mm, and 82.2 ± 3.1mm, respectively, $p>0.05$) and all were larger than diploid SL-XB (76.2 ± 3.1mm, $p<0.05$). Triploid Lola, SL-DBY, and SL-XB had equivalent shell heights (90.8 ± 3.5mm, 89.3 ± 3.0mm, and 88.5 ± 2.8mm, respectively, $p>0.05$). hANA (95.4 ± 3.6mm) had larger shell height than SL-XB only ($p<0.05$) (Figure 3.5).

3.1.2 Whole Wet Weight

Overall growth trends are depicted in Figure 3.6. Table 3.4 reports initial whole wet weight measurements (mean ± 95%CI) of individual lines. Diploid and triploid whole wet weight followed the increase in shell height from May 2011 to September 2011 at each site. In the Choptank River, from September 2011 until May 2012, growth plateaued and then increased again after May 2012. The Rappahannock and York Rivers did not show this plateau (Figure 3.6). In both December 2011 and 2012, the site effect, ploidy effect, stock effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 3.3).

3.1.2.1 Choptank River, MD

2006-Year class

In the Choptank River, at 17 months, there were several groups that showed a significant difference between diploids and triploids for whole wet weight (Figure 3.7).

From the 2006-year class lines, both diploid LGT09 and DBY09 were heavier than their triploid counter parts (LGT09: diploid – 14.2 ± 1.2g and triploid – 11.6 ± 1.1g and DBY09: diploid – 13.5 ± 1.3g and triploid – 10.7 ± 1.0g, $p<0.05$). Diploid OBOY09 and diploid and triploid XB06 were not deployed in the Choptank River. There were no significant differences in whole wet weight between any of the diploid 2006-year class lines ($p>0.05$). Of the individual triploid lines, OBOY09 was significantly heavier than both LGT09 and DBY09 ($p<0.05$), which had equivalent whole wet weights ($p>0.05$) (14.5 ± 1.4g, 11.6 ± 1.1g, and 10.7 ± 1.0g, respectively) (Figure 3.7).

By 29 months, the triploid effects were not significant but did range from and advantage of 3% to a disadvantage of (-6%) ($p>0.05$, Table 3.5, Figure 3.8). There were no differences between individual diploid 2006-year class lines ($p>0.05$). Triploid OBOY09 was significantly heavier than both LGT09 and DBY09 ($p<0.05$), which had equivalent whole wet weights ($p>0.05$) (OBOY09 – 76.6 ± 7.7g, LGT09 – 49.9 ± 5.9g, and DBY09 – 46.2 ± 4.7g) (Figure 3.8).

Superlines

By 17 months, the Superlines hANA, SL-DBY, and SL-XB were all heavier as diploids than triploids (hANA: diploid – 15.2 ± 1.3g and triploid – 12.2 ± 1.3g, SL-DBY: diploid – 14.7 ± 1.0g and triploid – 12.1 ± 1.1g, and SL-XB: diploid – 12.8 ± 1.4g and triploid – 7.9 ± 0.7g, $p<0.05$, Figure 3.7). There were no significant difference between diploid and triploids of the remaining genotypes in the Choptank River ($p>0.05$). There were no significant differences in whole wet weight between any of the diploid 2006-year class lines or the Superlines ($p>0.05$). Triploid hANA, Lola, and SL-DBY had

equivalent whole wet weights (12.2 ± 1.3g, 13.5 ± 1.4g, and 12.1 ± 1.1g, respectively, $p>0.05$) and were all significantly larger than SL-XB (7.9 ± 0.7g, $p<0.05$) (Figure 3.7).

By 29 months, the effect of ploidy had diminished and there were no significant differences among whole wet weights of the diploid and triploid groups ($p>0.05$). Both Superlines of Louisiana origin had positive triploid advantages (hANA – 14% and Lola – 9%) while SL-DBY and SL-XB had triploid disadvantages indicating diploids performed better than triploids (-5% and -12%, respectively), though these differences were not significant (Table 3.5). There were no significant differences between the individual diploid or triploid wild stocks or the Superlines by 29 months ($p>0.05$) (Figure 3.8).

3.1.2.2 Rappahannock River, VA

2006-Year class

In the Rappahannock River, at 17 months, there were several groups that showed a significant difference between diploids and triploids for whole wet weight (Figure 3.7). From the 2006-year class lines, both diploid OBOY09 and DBY09 were lighter than their triploid counter parts (OBOY09: diploid – 37.7 ± 2.6g and triploid – 47.6 ± 2.9g and DBY09: diploid – 23.6 ± 1.8g and triploid – 29.6 ± 2.3g, $p<0.05$). Diploid and triploid XB06 were not deployed to the Rappahannock River. The three 2006-year class lines all had significantly different whole wet weights from one another: DBY09 was the lightest, then LGT09, and the heaviest OBOY09 (23.6 ± 1.8g, 30.5 ± 2.5g, and 37.7 ± 2.6g, respectively, $p<0.05$). Of the individual triploid 2006-year class lines, OBOY09 was significantly heavier than both LGT09 and DBY09 ($p<0.05$), which had equivalent whole wet weights ($p>0.05$) (OBOY09 – 47.6 ± 2.9g, LGT09 – 32.8 ± 2.3g, and DBY09 – 29.6 ± 2.3g) (Figure 3.7).

By 29 months, the effect of triploidy in the 2006-year class lines was heavier triploids than diploids for LGT09 and DBY09 (LGT09: diploid – 83.2 ± 8.7g and triploid – 112.2 ± 8.8g, DBY09: diploid – 64.0 ± 4.5g and triploid – 101.9 ± 7.6g, $p<0.05$, Figure 3.8). This amounted to a triploid advantage of 35% for LGT09 and 59% for DBY09 (Table 3.5). The three 2006-year class lines all had significantly different whole wet weights from one another: DBY09 was the lightest, then LGT09, and the heaviest OBOY09 (64.0 ± 4.5g, 83.2 ± 8.8g, and 117.4 ± 7.6g, respectively, $p>0.05$). Triploid 2006-year class line OBOY09 was significantly heavier than both LGT09 and DBY09 ($p<0.05$), which had equivalent whole wet weights ($p>0.05$) (131.0 ± 10.8g, 112.2 ± 8.8g, and 101.9 ± 7.6g, respectively) (Figure 3.8).

Superlines

By 17 months, of the Superlines, only Lola was lighter as diploid than triploid (30.1 ± 2.5g and 37.2 ± 3.2g, respectively, $p<0.05$, Figure 3.7). There were no significant differences for diploid:triploid comparisons of the remaining genotypes in the Rappahannock River ($p>0.05$). Of the diploid Superlines, Lola, SL-DBY, and SL-XB had equivalent whole wet weights (30.1 ± 2.5g, 27.6 ± 2.0g, and 26.0 ± 2.3g, respectively, $p>0.05$). Diploid hANA (34.2 ± 2.5g) was significantly heavier than both diploid SL-DBY and SL-XB ($p<0.05$). Triploid hANA, Lola, and SL-DBY had equivalent whole wet weights (34.1 ± 3.3g, 37.2 ± 3.2g, and 30.4 ± 3.0g, respectively, $p>0.05$). hANA and Lola were significantly larger than SL-XB (26.7 ± 1.6g, $p<0.05$) (Figure 3.7).

For the Superlines, by 29 months, SL-XB was the only Superline in which triploidy affected whole wet weight (Figure 3.8). Triploid SL-XB was significantly

heavier than its diploid counterpart (97.9 ± 6.4g and 79.9 ± 7.6g, respectively, $p<0.05$) and had a triploid advantage of 23% (Table 3.5). The diploid Superline hANA was significantly heavier than both SL-DBY and SL-XB (100.2 ± 7.2g, 81.8 ± 8.3g, and 79.9 ± 7.6g, respectively, $p<0.05$), but not Lola (93.2 ± 8.3g, $p>0.05$). Lola, SL-DBY, and SL-XB whole wet weights were not significantly different. The triploid Superline SL-DBY was the lightest of the Superlines (hANA – 109.6 ± 8.0g, Lola – 103.5 ± 7.2g, SL-DBY – 89.6 ± 9.9g, and SL-XB – 97.9 ± 6.4g), but any of the Superlines were not significantly different from one another ($p>0.05$) (Figure 3.8).

3.1.2.3 York River, VA

2006-Year class

In the York River, by 17 months, three of the 2006-year class lines were heavier as triploids than as diploids: OBOY09 diploid – 15.8 ± 1.4g and triploid – 34.4 ± 3.3g, DBY09 diploid – 23.8 ± 1.9g and triploid – 35.2 ± 2.8g, and XB06 diploid – 35.1 ± 3.7g and triploid – 43.2 ± 3.3g ($p<0.05$, Figure 3.7). Diploid DBY09 was significantly heavier than diploid OBOY09 (28.1 ± 2.5g and 15.8 ± 1.4g, respectively, $p<0.05$). There were no significant differences in the whole wet weight among the individual triploid 2006-year class lines ($p>0.05$) (Figure 3.7).

By 29 months, the triploidy advantage was maintained for all of the 2006-year class lines, except for OBOY09, and ranged from 44% to 55% (Table 3.5). Diploid OBOY09 did not survive to 29 months. LGT09: diploid – 84.4 ± 9.7g and triploid – 129.2 ± 8.6g, DBY09: diploid – 76.2 ± 5.7g and triploid – 109.4 ± 7.2g, and XB06: diploid – 80.0 ± 6.9g and triploid – 123.7 ± 9.3g ($p<0.05$ for all, Figure 3.8). There were

no significant differences in whole wet weight of the diploid or triploid 2006-year class lines (Figure 3.8).

Superlines

From the Superlines, by 17 months, Lola, SL-DBY, and SL-XB were heavier as triploid than diploid (Lola: diploid – 27.9 ± 3.3g and triploid – 36.5 ± 3.5g, SL-DBY: diploid – 28.1 ± 2.5g and triploid – 35.2 ± 2.8g, and SL-XB: diploid – 28.8 ± 2.4g and triploid – 36.5 ± 3.2g, $p<0.05$, Figure 3.7). There were no significant differences between diploid and triploids of the remaining genotypes in the York River ($p>0.05$). There were no significant differences in the whole wet weight among the individual diploid Superlines ($p>0.05$) (Figure 3.7).

By 29 months, ploidy affected whole wet weight for all four Superlines: hANA, Lola, SL-DBY, and SL-XB (hANA: diploid – 91.9 ± 7.1g and triploid – 121.2 ± 10.4g, Lola: diploid – 89.7 ± 8.3g and triploid – 108.1 ± 9.3g, SL-DBY: diploid – 79.3 ± 6.8g and triploid – 112.9 ± 8.0g, and SL-XB: diploid – 80.9 ± 7.1g and triploid – 107.7 ± 8.2g, $p<0.05$). The estimated triploid advantage for the Superlines was 32% for hANA, 20% for Lola, 42% for SL-DBY, and 33% for SL-XB (Table 3.5). There were no significant differences in whole wet weight of the diploid or triploid diploid Superlines (Figure 3.8).

3.1.3 Wet Tissue Weight

Measurements of wet tissue weight were taken beginning in October 2011. In the Choptank River, the 2006-year class lines and the Superlines exhibited a period of suppressed tissue growth ending in the spring of 2012. The increase in growth rate corresponded with an increase in salinity in the spring of 2012. For the Rappahannock and York Rivers, the 2006-year class lines and Superlines showed similar growth trends.

That is, diploids and triploids lines showed a steady increase in tissue weight until May 2012 when the growth rate of the diploid lines fell off (Figure 3.9). In both December 2011 and 2012, the site effect, ploidy effect, genotype effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 3.3).

3.1.3.1 Choptank River, MD

2006-Year class

By 17 months, the 2006-year class diploid lines OBOY09 and XB06 and triploid XB06 were not deployed in the Choptank River due to survival limitations in the nursery (Figure 3.10). There were no significant difference between diploid and triploids of the remaining genotypes in the Choptank River ($p>0.05$). There were no significant differences among the diploid 2006-year class lines ($p>0.05$). Triploid 2006-year class line OBOY09 was significantly heavier than DBY09 (2.1 ± 0.2g and 1.7 ± 0.1g, respectively, $p<0.05$, Figure 3.10).

By 29 months, the difference between diploid and triploid counterparts was significant for only one 2006-year class line, DBY09. Diploid DBY09 was significantly heavier than triploid DBY09 (diploid – 7.4 ± 0.7g and triploid – 5.5 ± 0.7g, $p<0.05$) equating to a triploid disadvantage of (-26%) (Table 3.5). There were no significant differences among the diploid 2006-year class lines ($p>0.05$). Triploid 2006-year class line OBOY09 was significantly heavier than both LGT09 and DBY09 (9.6 ± 1.2g, 6.1 ± 0.8g, and 5.5 ± 0.7g, respectively, $p<0.05$). Triploids LGT09 and DBY09 did not differ in wet tissue weight ($p>0.05$) (Figure 3.11).

Superlines

By 17 months, triploid meat weight was lighter than diploid meat weight in the three Superlines hANA, SL-DBY, and SL-XB (hANA: diploid – 2.8 ± 0.3g and triploid – 1.8 ± 0.4g, SL-DBY: diploid – 2.9 ± 0.2g and triploid – 1.8 ± 0.2g, and SL-XB: diploid – 2.3 ± 0.3g and triploid – 1.1 ± 0.1g, $p<0.05$) (Figure 3.10). The two heaviest diploid Superlines were hANA and SL-DBY and were not significantly different from each other ($p>0.05$), but were significantly heavier ($p<0.05$) than both Lola and SL-XB, which had similar wet tissue weights (hANA – 2.8 ± 0.3g, SL-DBY – 2.9 ± 0.2g, Lola – 2.3 ± 0.3g, and SL-XB – 2.3 ± 0.3g). Triploid hANA, Lola, and SL-DBY had equivalent whole wet weights (1.8 ± 0.2g, 2.0 ± 0.3g, and 1.8 ± 0.2g, respectively, $p>0.05$) and were all significantly heavier than SL-XB (1.1 ± 0.1g, $p<0.05$) (Figure 3.10).

The Superline SL-DBY was the only line at 29 months that was significantly heavier as a diploid than triploid (7.8 ± 0.7g and 5.9 ± 0.7g, respectively, $p<0.05$) with a (-25%) triploid disadvantage (Table 3.5). Diploid Superlines Lola, SL-DBY, and SL-XB had equivalent wet tissue weights ($p>0.05$), but only Lola and SL-DBY were significantly heavier ($p<0.05$) than hANA (hANA 6.0 ± 0.8g, Lola 7.8 ± 0.9g, SL-DBY 7.8 ± 0.7g, and SL-XB 7.5 ± 1.2g). Triploid Superlines hANA, Lola, and SL-DBY had equivalent wet tissue weights ($p>0.05$), but only Lola was significantly heavier ($p<0.05$) than SL-XB (hANA – 6.3 ± 0.9g, Lola – 7.3 ± 0.8g, SL-DBY – 5.9 ± 0.7g, and – SL-XB 5.5 ± 0.7g) (Figure 3.11).

3.1.3.2 Rappahannock River, VA

2006-Year class

In the Rappahannock River, at 17 months, triploidy did not affect growth for the 2006-year class lines meaning diploid and triploid counterparts did not differ in wet

tissue weight (Figure 3.10). Diploid and triploid XB06 were not deployed in the Rappahannock River because of survival limitations in the nursery. Of the individual diploid 2006-year class lines, OBOY09 was significantly heavier than DBY09 (7.3 ± 0.7g and 4.5 ± 0.4g, respectively, $p<0.05$). DBY09 and LGT09 did not differ in wet tissue weight (7.3 ± 0.7g and 6.1 ± 0.5g, respectively, $p>0.05$). Triploid 2006-year class line OBOY09 was significantly heavier than both triploid LGT09 and DBY09 (8.2 ± 0.7g, 6.0 ± 0.5g, and 5.3 ± 0.5g, respectively, $p<0.05$) (Figure 3.10).

By 29 months, the triploid advantage was present for two of the 2006-year class lines: 35% for DBY09 and 25% for OBOY09 (Table 3.5, Figure 3.11). This advantage was heavier triploids than diploids (DBY09: diploid – 11.2 ± 1.0g and triploid – 8.3 ± 0.8g and OBOY09: diploid – 11.8 ± 1.1g and triploid – 14.7 ± 1.5g, $p<0.05$). Among the diploid lines, OBOY09 was the heaviest, but the difference between OBOY09 and LGT09 was not significant (OBOY09 – 11.8 ± 1.1g; LGT09 – 11.1 ± 1.6g; DBY09 – 8.3 ± 0.8g). There were no differences among the individual triploid 2006-year class lines by 29 months (Figure 3.11).

Superlines

In the Rappahannock River, at 17 months, ploidy did not affect growth for the Superlines meaning diploid and triploid counterparts did not differ in wet tissue weight (Figure 3.10). Diploid Superlines hANA, Lola, and SL-DBY did not have significantly different wet tissue weights ($p>0.05$), but only hANA was significantly heavier ($p<0.05$) than SL-XB (hANA – 6.9 ± 0.6g, Lola – 5.7 ± 0.6g, SL-DBY – 5.9 ± 0.5g, and SL-XB – 5.2 ± 0.5g). Triploid Superlines hANA, Lola, and SL-DBY did not have significantly different wet tissue weights ($p>0.05$), and were all significantly heavier ($p<0.05$) than

SL-XB (hANA – 5.8 ± 0.7g, Lola – 6.2 ± 0.7g, SL-DBY – 5.5 ± 0.7g, and SL-XB – 4.3 ± 0.2g) (Figure 3.10).

By 29 months, notably, there were no differences in tissue weight between diploid and triploid Superline counterparts. While these differences were not significant, the triploid advantage ranged from 8-16% (Table 3.5). There were no differences among the individual diploid or triploid Superlines by 29 months (Figure 3.11).

3.1.3.3 York River, VA

2006-Year class

By 17 months in the York River, the only significant difference in wet tissue weight between diploid and triploid lines was in DBY09 and XB06, and both were heavier as triploids than diploids (DBY09 diploid – 2.8 ± 0.3g and triploid – 3.9 ± 0.4g; XB06 diploid – 4.1 ± 0.5g and triploid – 5.5 ± 0.4g, both $p<0.05$, Figure 3.10). Diploid LGT09 and XB06 had similar wet tissue weights (4.3 ± 0.6g and 4.1 ± 0.5g, respectively, $p>0.05$), and were both significantly heavier than DBY09 (2.8 ± 0.3g, $p<0.05$). Diploid OBOY09 animals were not sacrificed for wet tissue weights measurements at 17 months because of low survival. There were no differences between the individual triploid 2006-year class lines ($p>0.05$) (Figure 3.10).

At 29 months, all of the triploid 2006-year class lines were heavier than their diploid counterparts: LGT09, DBY09, and XB06 (all $p<0.05$, LGT09 diploid – 9.2 ± 1.3g and triploid – 13.2 ± 1.2g; DBY09 diploid – 7.6 ± 0.7g and triploid – 10.8 ± 0.9g; XB06 diploid – 9.6 ± 1.0g and triploid – 13.1 ± 1.3g) (Figure 3.11). These differences equate to a triploid advantage of 43% for LGT09, 42% for DBY09, and 36% for XB06 (Table 3.5). No diploid OBOY09 animals survived to 29 months. All of the individual

diploid lines from the 2006-year class had equivalent wet tissue weights: LGT09, DBY09, and XB06 (9.2 ± 1.3g, 7.6 ± 0.7g, and 9.6 ± 1.0g,respectively, $p>0.05$). There were no significant differences among the individual triploid 2006-year class lines ($p>0.05$) (Figure 3.11).

Superlines

Diploid:triploid comparisons of the Superlines yielded no significant differences at 17 months ($p>0.05$). There were also no significant differences in the wet tissue weights of the individual diploid or triploid Superlines (Figure 3.10).

There were no significant differences in wet tissue weight among the diploid and triploid Superlines by 29 months. Although these differences were not significant, the triploid advantage ranged from an estimated 11-23% (Table 3.5). There were no significant differences in wet tissue weight among the diploid Superlines. Only triploid hANA was significantly heavier than triploid SL-XB (9.5 ± 0.9g, $p<0.05$) (Figure 3.11).

3.1.4 Meat yield

In all groups in all sites, meat yield declined over the course of the study, with only a few differences between diploid and triploid. The diploid 2006-year class lines and Superlines, at all sites, showed a pattern of greater tissue growth than shell growth during reproductive periods, as evidenced by increases in meat yield (Figure 3.12). In both December 2011 and 2012, the site effect, ploidy effect, genotype effect, and their interactions as described in section 2.4.2 were all significant ($p<0.001$) (Table 3.3).

3.1.4.1 Choptank River, MD

2006-Year class

In the Choptank River, by 17 months, in the 2006-year class lines, there was no significant effect of triploidy on meat yield. The individual diploid and triploid lines from 2006-year class all had similar meat yields ($p>0.05$) (Figure 3.13).

By 29 months, diploid XB06 was the only 2006-year class line to have greater meat yield than its triploid counterpart (0.0.120 ± 0.006 and 0.104 ± 0.005, respectively, $p<0.05$). There were no differences in meat yield between the individual diploid and triploid lines from the 2006-year class (Figure 3.14).

Superlines

In the Superlines, however, diploids had significantly higher meat yields than their triploid counterparts at 17 months (hANA: diploid – 0.177 ± 0.008 and triploid – 0.145 ± 0.008, Lola: diploid – 0.163 ± 0.008 and triploid – 0.145 ± 0.006, SL-DBY: diploid – 0.195 ± 0.006 and triploid – 0.149 ± 0.006, and SL-XB: diploid – 0.179 ± 0.011 and triploid – 0.139 ± 0.007, $p<0.05$, Figure 3.13). Diploid Superlines hANA, Lola, and SL-XB did not have significantly different meat yields ($p>0.05$), and all three had significantly lower meat yield than SL-DBY (hANA – 0.177 ± 0.008, Lola – 0.163 ± 0.008, SL-XB – 0.179 ± 0.011, and SL-DBY – 0.195 ± 0.006, $p<0.05$). Among the individual triploid lines there were no significant differences in meat yield ($p>0.05$) (Figure 3.13).

By 29 months, diploid Superlines Lola, SL-DBY, and SL-XB all had significantly larger meat yields than their triploid counterparts (Lola: diploid – 0.149 ± 0.007 and triploid – 0.127 ± 0.006, SL-DBY: diploid – 0.152 ± 0.006 and triploid – 0.121 ± 0.006, and SL-XB: diploid – 0.151 ± 0.008 and triploid – 0.127 ± 0.008) (Figure 3.14). Diploids Lola, SL-DBY, and SL-XB had equivalent meat yields (0.149 ± 0.007, 0.152 ± 0.006,

0.151 ± 0.008, respectively, $p>0.05$) and all three had significantly larger meat yields than hANA (0.126 ± 0.008). There were no differences between individual triploid Superlines at 29 months ($p>0.05$) (Figure 3.14).

3.1.4.2 Rappahannock River, VA

2006-Year class

By 17 months in the Rappahannock River, the meat yield of two diploid 2006-year class lines differed significantly from the meat yields of their triploid counterparts: LGT09 and OBOY09 (LGT09: diploid – 0.198 ± 0.007 and triploid – 0.180 ± 0.007, OBOY09: diploid – 0.192 ± 0.009 and triploid – 0.170 ± 0.006, $p<0.05$, Figure 3.13). Diploid and triploid XB06 were not deployed in the Rappahannock River because of survival limitations in the nursery. There were no differences between any of the 2006-year class diploid or triploid lines ($p>0.05$) (Figure 3.13).

By 29 months, the differences between diploid and triploid meat yield in the 2006-year class lines was no longer present (Figure 3.14). Diploid lines LGT09 and DBY09, from the 2006-year class, had equivalent meat yields (0.130 ± 0.008 and 0.128 ± 0.008, respectively, $p>0.05$) and both had greater meat yields than OBOY09 (0.099 ± 0.005, $p<0.05$). All of the triploid 2006-year class lines had equivalent meat yields ($p>0.05$) (Figure 3.14).

Superlines

By 17 months, all four of the diploid Superlines had greater meat yields than their triploid counterparts (hANA: diploid – 0.202 ± 0.008 and triploid – 0.168 ± 0.007, Lola: diploid – 0.189 ± 0.007 and triploid – 0.164 ± 0.007, SL-DBY: diploid – 0.214 ± 0.007 and triploid – 0.175 ± 0.008, and SL-XB: diploid – 0.200 ± 0.009 and triploid – 0.162 ±

0.007, $p<0.05$) (Figure 3.13). There were no significant differences in meat yield between individual diploid Superlines with the exception of SL-DBY having a greater meat yield than Lola (0.214 ± 0.007 and 0.200 ± 0.009, respectively, $p<0.05$). All of the four triploid Superlines had similar meat yields ($p>0.05$) (Figure 3.13).

By the end of the study at 29 months, all four of the diploid Superlines still had greater meat yields than their triploid counterparts (hANA: diploid – 0.109 ± 0.007 and triploid – 0.108 ± 0.006, Lola: diploid – 0.110 ± 0.004 and triploid – 0.110 ± 0.005, SL-DBY: diploid – 0.124 ± 0.006 and triploid – 0.132 ± 0.007, and SL-XB: diploid – 0.121 ± 0.009 and triploid – 0.112 ± 0.005, $p<0.05$, Figure 3.14). Diploid Superlines hANA, Lola, and SL-XB did not have significantly different meat yields (0.109 ± 0.007, 0.110 ± 0.004, and 0.121 ± 0.009, respectively, $p>0.05$). The meat yield of SL-DBY (0.124 ± 0.006) was significantly greater than that of hANA and Lola only ($p<0.05$). Triploid Superlines hANA, Lola, and SL-XB all had equivalent meat yields (0.108 ± 0.006, 0.110 ± 0.005, and 0.112 ± 0.005, respectively, $p>0.05$). Triploid SL-DBY had greater meat yield (0.132 ± 0.007, $p<0.05$) than the three other Superlines: hANA, Lola, and SL-XB (Figure 3.14).

3.1.4.3 York River, VA

2006-Year class

In the York River there were no significant differences between diploid and triploid 2006-year class line meat yields by 17 months (Figure 3.13). Meat yield estimation for the diploid OBOY09 line was not made because animals at 17 months were not sacrificed for wet tissue weights measurements because of low survival. 2006-year class diploid lines DBY09 and XB06 had equivalent meat yields (0.118 ± 0.006 and

0.128 ± 0.008, respectively, $p>0.05$) and the meat yield of these two lines was significantly less than LGT09 (0.138 ± 0.008, $p<0.05$). Triploid OBOY09 had a smaller meat yield than LGT09 and XB06 (0.112 ± 0.006, 0.131 ± 0.007, and 0.126 ± 0.005, respectively, $p<0.05$). The triploid line DBY09, was not significantly different from any of the 2006-year class (0.113 ± 0.009, $p>0.05$) (Figure 3.13).

By 29 months, XB06 was the only 2006-year class line in which triploidy had an effect on meat yield (Figure 3.14). Diploid XB06 had greater meat yield as a diploid than as triploid (0.0.120 ± 0.006 and 0.104 ± 0.005, respectively, $p<0.05$). Diploid DBY09 had smaller meat yield than both LGT09 and XB06, both of which had similar meat yields (0.100 ± 0.004, 0.113 ± 0.013, and 0.120 ± 0.006, respectively, $p<0.05$). There were no differences between triploid 2006-year class lines (Figure 3.14).

Superlines

There were no significant differences in diploid:triploid comparisons of the Superlines by 17 months (Figure 3.13). The diploid Superline SL-XB had the lowest meat yield, while hANA, Lola, and SL-DBY all had equivalent meat yields (hANA: 0.124 ± 0.006, Lola: 0.126 ± 0.007, SL-DBY: 0.131 ± 0.007, and SL-XB: 0.110 ± 0.008). There were no significant differences in meat yield of the triploid Superlines (Figure 3.13).

By 29 months, diploid Superlines SL-DBY and SL-XB were the only Superlines in which ploidy had an effect on meat yield (Figure 3.14). Diploid SL-DBY and SL-XB had meat yields larger than their triploid counter parts (SL-DBY: diploid 0.116 ± 0.008 and triploid 0.096 ± 0.005, SL-XB: diploid 0.102 ± 0.005 and triploid 0.097 ± 0.004, $p<0.05$). There were no differences between triploid Superlines (Figure 3.14).

4. DISCUSSION

This study examined the effect of the diploid female on triploids created from tetraploid × diploid crosses. The major objective of this chapter was to compare field performance between successive generations of selected lines. To achieve this, the selected lines from the 2006-year class were compared with the 2008 Superlines as both diploids and triploids. This comparison is not a comparison of truly successive generations. The comparison made in this chapter is one of a generation of composite lines (Superlines) with representatives of their founder populations (lines from the 2006-year class). In Chapter 2, wild triploids (+SS) were compared to selected triploids (SSS) and differences were attributed largely to the possible differences in heterozygosity as they manifest across different environments. In this chapter, the comparison is more subtle, i.e., between two selectively bred sources: SSS_{2006} *versus* $SSS_{Superline}$, and focuses on additive gains that may have obtained from selective breeding, one generation to the next.

Growth metrics included shell height, whole weight, tissue weight, and a derived measure, meat yield. The results show that, as in Chapter 2, these metrics are strongly influenced by the environment. For example, both the 2006-year class lines and Superlines had the lightest wet tissue weights, as diploids and triploids, in the low salinity environment. In contrast to the results of Chapter 2, the genetic contribution of the

diploid parent seems to play a minor role. The differences between diploids from the 2006-year class and Superlines were negligible for all measures.

The lack of differences between 2006-year class and Superline triploids was not an unexpected outcome. Not only is there only a one generational difference from the diploid parent – itself contributing only 1/3 of the genes to a triploid – but the genetic architecture of the two diploid generations is fundamentally different. The difference in genetic architecture stems from the fact that 25 selectively bred lines were collapsed into the four Superlines. This line consolidation occurred as a result of shifting the breeding strategy at ABC from one based primarily on increasing disease resistance to regionally based selection for growth traits.

Three base populations (DBY, XB, and Louisiana origin) comprising 25 separate lines and selected for disease resistance (Dermo and MSX diseases) were consolidated into Superlines. The Superlines (SL) were created by crossing over 100 individuals with pair-matings from the previous lines, consolidating all XB-derivatives into a XB line; all DBY-derivatives into a DBY line, etc. Consolidating the lines based on the three base populations through such a large number of crosses served two purposes: 1) it simplified the breeding scheme with fewer lines and 2) it widened the genetic diversity before the start of selection for growth. While each of the 25 lines used to produce the Superlines originated from the same three base populations, they were all closed populations and isolated from each other. One consequence of the gene flow caused by mixing distinct populations, such as these 25 lines, is a change in allelic frequency due to linkage (gametic) disequilibrium.

Linkage disequilibrium is derived from the idea that individuals contribute gametes to the next generation rather than genotypes. Linkage disequilibrium occurs when alleles at different loci are not distributed independently but are linked (Lewontin and Kojima, 1960). Selection, non-random mating, and population mixing can all lead to linkage disequilibrium. While the new gene combinations in mixed population have not evolved together (or been selected together), the resulting new population can result in allelic associations that are both good and bad (positive and negative heterosis) that presumably would manifest as higher phenotypic variation within a line. Eventually, equilibrium (i.e., expected allelic frequencies) is re-established through random matings and is dependent on recombination among these loci. The Superlines are a mixture of many different lines each, so it is likely that the Superlines are likely exhibiting linkage disequilibrium.

Cumulative gain in performance traits through successive generations of selectively bred lines is the driving force of the ABC breeding program. Each successive generation increases the frequency of optimal alleles providing additive gains. Cumulative improvements in triploids may obtain either through additive gains or through an increase in the dosage of beneficial effects from those optimal alleles as a result of having a third set of chromosomes, known as the additive dosage effect.

In a study of the Sydney rock oyster (*S. glomerata*) the additive dosage effect was observed in triploids through a comparison of diploid and chemically induced triploid progeny of a third generation breeding line and an unselected control group (Hand et al., 2004). The relative improvements of the diploid lines over control groups were maintained when triploidy was induced. In this Chapter, however, diploid lines from the

2006-year class and diploid Superlines had similar growth, indicating the effect of additive gains through selection did not increase when the Superlines were produced. But they did not falter either. As triploids, the 2006-year class lines generally had greater triploid advantages for all growth metrics suggesting that any linkage disequilibrium may be negatively affecting triploid advantage.

4.1 Choptank River

Throughout this study diploid and triploid performance was similar in the Choptank River for all growth metrics measured (shell height, whole wet weight, and wet tissue weight) for both the 2006-year class lines and Superlines (Table 3.5). As discussed in detail in Chapter 2, it appears that low salinity is the major influence on growth in the Choptank River. The average salinity in the Choptank River during the study period was 9, ranging from 6 ppt to 13 ppt (Figure 2.5) and is below the accepted optimum salinity range for oysters (approximately 14 – 28 ppt) (Galstoff 1964; Loosanoff 1965). Low salinity negatively affects many aspects of oyster biology by reducing valve activity (Loosanoff, 1952; Galtsoff, 1964), reduce feeding efficiency (Loosanoff, 1952), reduce respiration regulation (Shumway and Koehn, 1982), depress gametogenesis (Butler, 1949; Loosanoff, 1952; Calabrese and Davis, 1970), and growth (Loosanoff, 1952; Chanley, 1958; Davis, 1994). Most studies that have investigated the effect of salinity on oysters focused on acute fluctuations in salinity, however, some of these effects last even after oysters have generally acclimated to the salinity change. All of the oysters in this study were spawned and reared in the ABC Gloucester Point, VA, USA hatchery that experiences a salinity range of 13-25 ppt. Juvenile oysters spawned and reared at the salinity at the ABC hatchery certainly must have experienced an acute shock when

transferred to the Choptank River at a lower salinity. This acute salinity shock is reasoned to be the cause of an apparent lack of growth of oysters in this estuary until early summer of 2011.

All oysters in the Choptank suffered suppressed growth. Neither diploids nor triploids reached harvest size (76mm) in this site. The only metric that truly stood out was wet tissue weight and the diploid lines surpassed the triploid lines (Figure 3.11). The triploid selected lines had a greater disadvantage from the stress of low salinity than did the diploids, and seemingly a negative triploid advantage (triploid disadvantage).

Changes in meat yield over time provide an insight into overall condition and because meat yield is highly dependent on changes in condition due to reproduction, interesting aspects of the relationship between diploid and triploid oysters are revealed through changes in meat yield through reproductive periods. Overall, in all groups at all sites, meat yield declined over the course of the study (Figure 3.12).

The diploid selected lines (2006-year class and Superlines) showed a pattern of greater tissue growth than shell growth prior to the second spawning period, as evidenced by increases in meat yield, then decreased, presumably due to loss of tissue mass from spawning, to a meat yield similar to the triploids. Meat yield of triploids increased, indicating the triploids are undergoing some gonadogenesis, but not to the same level as the diploids, which is expected due to the limited gametogenesis in triploids. The increase in meat yield of the diploid Superlines leading up to spawning was not observed in the two groups of wild stocks from Chapter 2.

In Choptank River, two lines of Louisiana origin had superior growth performance for all parameters. The 2006-year class line OBOY09 was the largest

triploid of the 2006-year class lines. The Superline Lola was the largest diploid and triploid line of the Superlines. The Superline Lola was founded from Louisiana based lines selected for performance in low salinity environments, of which OBOY09 is one representative line. The superior performance of the diploid Lola Superline confirms that selection of Lola for growth in low salinity environments has succeeded. Differences in salinity tolerance and mechanisms occur in *C. virginica* between populations originating in the Chesapeake Bay and those along the Atlantic coast of the U.S.A. (Pierce et al., 1992). Difference in magnitude of tolerance and mechanisms were suggested to be largely genetic as the populations were geographically isolated. As triploids, the superior performance of the OBOY09 and Lola lines indicate that selection for low salinity environments is maintainable across a generation and transferable to the triploid construct. It is notable that the relative tolerance to low salinity obtained from selection in these Louisiana-based lines manifests in the triploid construct because the diploid parent ($4n \times 2n$) is providing only 1/3 of the total genetic material of the triploid. This appears to be sufficient for some adaption of the triploid for lower salinity.

4.2 Rappahannock River

For shell height in the Rappahannock River, there was no triploid advantage for the Superlines, but there was for two lines from the 2006-year class: DBY09 and LGT09 (Figure 3.5). The triploid advantage for DBY09 and LGT09 was small though (14% and 15%, respectively, Table 3.5). The Superlines, along with the 2006-year class lines, show a triploid advantage for whole wet weight and tissue weight. The Rappahannock River is characterized as a 'good' growing site because it falls within the optimum salinity range for oysters of 14 – 28 ppt and historically has had light disease pressure

(Dermo). It appears that, for shell height, the selective breeding of the diploid lines at ABC has enhanced growth performance sufficiently that in an environment like the Rappahannock River, diploids are performing comparatively with triploids.

For whole weight and wet tissue weight, the triploid advantage of the 2006-year class lines in the Rappahannock River was roughly twice as great, on average, as the Superlines (2006-lines – 35% and Superlines – 13%) and wet tissue weight (2006-lines – 25% and Superlines – 12%). Chapter 1 showed that SSS triploids consistently have a lower triploid advantage than triploids made from wild groups (SS+) suggesting that heterosis and/or sterility may be playing a significant role in the triploid advantage. Heterosis is the increase in average performance above the mid-parent value, increases with heterozygosity, and generally occurs when two inbred groups are crossed (Griffing, 1990; Hedgecock et al., 1996; Hawkins et al., 2000).

The greater triploid advantages observed for the 2006-year class lines than the Superlines may be attributable to heterosis. Additive gains are not likely to play a major role in the triploid advantage, at least across one generation, because of the comparable diploid performance across the generations and sterility is a common feature among the triploids. The four lines from the 2006-year class used in this study have undergone several generations of selection on distinct populations of oysters, presumably reducing heterozygosity while increasing the likelihood of obtaining beneficial alleles. The Superlines, by contrast, are hybrids of 9-11 pre-established breeding lines each (some of which were hybrids) that likely increased the heterozygosity of the Superlines relative to the four representative 2006-year class lines. The diploid performance was similar because the Superlines became an average of the founder populations. The tetraploid

used in the triploid cross ($4n \times 2n$) is also a hybrid of the same populations the Superlines were derived from (DBY, XB, and LA germ plasm). Heterosis can occur when the inbred groups are crossed. The lines from the 2006-year class are more inbred than the Superlines because the 2006-year class lines were closed populations previously to the consolidation into Superlines. It is possible therefore, that the lines from the 2006-year class may be obtaining more advantage from heterosis as a result of being crossed with the tetraploid than the Superlines. The rationale for possible heterosis in the 2006-year class lines stems from the fact that the Superlines are a hybrid of the same base populations as tetraploid, as evidenced by the greater triploid advantage observed for the 2006-year class compared to the Superlines.

4.3 York River

For shell height, selected lines made into triploids (SSS) showed no significant effect of an extra set of chromosomes unless exposed to disease pressure, i.e., in the York River (Figure 3.3). In the York River, Dermo and MSX are endemic and it is here that the greatest triploid advantages were observed for both groups of selected lines.

Growth is inhibited by the two diseases likely to be encountered in the York River: Dermo and MSX (disease analysis is presented in Chapter 4). Shell deposition rates are lower in oysters with Dermo infections than those without (Menzel and Hopkins, 1955) and oysters with MSX infections typically die within several weeks of infection, but some oysters that may be more tolerant show signs of reduced growth (Barber et al., 1988a). While the 2006-year class lines and the Superlines have disease resistance from selective breeding, it is clear from the observed triploid advantage of each group in the York River that the tetraploid parent in the triploid cross ($4n \times 2n$) has

provided addition benefits for growth, evidenced by shell height, whole wet weight, and tissue weight. As both groups of selected lines are disease resistant, this benefit may be increased overall fitness that is often associated with increased heterozygosity. Sterility, however, cannot be ruled out as providing a benefit to triploid growth performance, especially in the face of disease. The increased triploid advantages observed in the York River over the advantages observed in the Rappahannock River, where there is no disease, indicate that sterility is providing a significant advantage in this stressful environment. The triploid advantage therefore in the York River is likely because of a combination of increased heterozygosity and energy partitioning caused by triploid sterility, though to what degree each of these factors influence the triploid advantage is unclear without examining the physiological differences of diploid and triploid oysters.

Meat yield in the York River, while declining overall, was observed to fluctuate up and down across time. This pattern of meat yield change across time in the York River is different from either the Choptank or Rappahannock Rivers (Figure 3.12) and is largely attributed to the tumbling the oyster received in this environment. Tumbling affects growth of the oyster in two ways: chipping new shell growth and reducing time spent with the shell open and actively pumping. The York River, relative to the Choptank and Rappahannock River, is a high-energy site with significant wave action and because of this the oysters are frequently tumbled within the grow-out cages.

4.4 Conclusions

One of the objectives of this study was to compare field performance among several generations of selected lines to ascertain if gains made from selection in diploid lines were realized in triploid crosses. This was attempted by comparing selected 2006-

year class lines with the 2008 Superlines. It was shown that relative performance of diploids is generally maintained in their triploid counterparts, but due to the similar performance of the diploids from these two generations of selected lines, the degree to which additive gains across generations manifest in triploids is probably negligible. Though large differences across the generation gap were not observed, the relative rankings of lines as diploids were generally maintained when they were triploid suggesting that gains through selection for regional adaptation can be maintained across ploidy changes. Similar to the comparisons of the wild stocks and Superlines described in Chapter 2, the results presented here show that growth is heavily influenced by environment.

While diploid selected lines can perform as well as triploids in some environments (e.g., Choptank and Rappahannock Rivers), selected triploids are still superior to even selected diploids in others (e.g., York River). Selection for specific environments (mainly based on salinity) is at the core of ABC's diploid breeding efforts. It follows that because many of the advancements made through selection are maintained in triploids, further efforts can be made to improve performance, especially in lower salinity environments. Tetraploid lines are being developed at ABC to explore additional routes of triploid improvement. The Superlines are being transformed into tetraploid lines via chemical induction to establish new tetraploid populations founded on the years of additive gains achieved through selective breeding of the diploid lines. More importantly, and in line with ABC's breeding efforts, these future tetraploid lines will be based from diploid Superlines that have experience selection for specific environments.

A major drawback in this study was that at the time of spawning, the Superlines only had one generation available for study. As a result of the availability of only one generation of Superlines, four comparative lines from the 2006-year class were chosen as representatives of the founder populations of the Superlines. At the conclusion of this study, the Superlines are now established and further study of how growth selection is realized in triploids can be made with consecutive generations of Superlines in place of four representatives of the Superline founder populations.

		Diploid		Tetraploid
Stock	Type	No. dams	No. of sires	No of sires
LGT09	'06	12	26	11
OBOY09	'06	10	17	10
DBY09	'06	10	10	11
XB06	'06	29	21	10
hANA	SL	10	9	10
Lola	SL	15	11	11
SL-DBY	SL	10	10	11
SL-XB	SL	10	10	11

Table 3.1: Number of *C. virginica* broodstock used per line (2006-year class and Superlines) to generate diploid and triploid offspring. For diploids, diploid dams and sires were used; for triploids, diploid dams and one set of 11 tetraploid sires (pooled sperm). '06 = 2006-year class lines; SL = Superlines.

	Diploid			Triploid		
Stock	**Shell height (mm)**		**95%CI**	**Shell height (mm)**		**95%CI**
Choptank River						
LGT09	21.2	±	1.2	19.5	±	0.9
OBOY09	NA	±	NA	17.5	±	1.0
DBY09	19.2	±	1.1	20.2	±	1.2
XB06	NA	±	NA	NA	±	NA
hANA	20.9	±	0.9	20.9	±	0.9
Lola	20.4	±	1.1	22.0	±	0.8
SL-DBY	16.2	±	0.9	19.6	±	0.9
SL-DBY	18.6	±	0.9	19.6	±	1.1
Rappahannock River						
LGT09	25.2	±	1.4	23.8	±	1.1
OBOY09	21.7	±	1.0	24.4	±	1.1
DBY09	25.5	±	1.1	21.8	±	0.8
XB06	NA	±	NA	NA	±	NA
hANA	23.7	±	1.0	24.4	±	1.2
Lola	24.4	±	1.1	27.2	±	1.2
SL-DBY	22.6	±	0.9	25.3	±	1.1
SL-XB	22.3	±	1.1	25.5	±	1.2
York River						
LGT09	19.0	±	1.2	22.5	±	0.7
OBOY09	17.5	±	1.0	20.1	±	1.0
DBY09	21.0	±	1.0	21.7	±	1.1
XB06	26.6	±	1.6	22.6	±	1.3
hANA	19.4	±	1.1	24.3	±	1.1
Lola	18.8	±	1.0	23.3	±	0.7
SL-DBY	17.5	±	0.9	22.1	±	0.8
SL-XB	19.2	±	0.7	23.0	±	0.8

Table 3.2: Initial shell heights (mean ± 95%CI) of diploids and triploids from the 2006-year class lines and Superlines at the deployment into the experimental design (April 2011), after the field nursery period, in the Choptank, Rappahannock, and York Rivers.

Source	Growth December 2011				Growth December 2012			
	df	MS	F	P	*df*	MS	F	P
Shell height								
Site	2	65152	906.77	<0.001	2	33329	227.93	<0.001
Ploidy	1	2211	30.77	<0.001	1	21553	147.40	<0.001
Stock	8	1366	19.01	<0.001	8	2693	18.42	<0.001
Site × Ploidy	2	5022	69.89	<0.001	2	3444	23.56	<0.001
Site × Stock	15	490	6.82	<0.001	15	747	5.11	<0.001
Ploidy × Stock	8	583	8.12	<0.001	8	525	3.59	<0.001
Error	2513	72			2452	146		
Whole wet weight								
Site	2	81353	1060.03	<0.001	2	520670	753.56	<0.001
Ploidy	1	9426	122.82	<0.001	1	159802	231.28	<0.001
Stock	8	1265	16.48	<0.001	8	11418	16.53	<0.001
Site × Ploidy	2	5197	67.721	<0.001	2	43255	62.60	<0.001
Site × Stock	15	580	7.55	<0.001	15	2711	3.92	<0.001
Ploidy × Stock	8	400	5.21	<0.001	8	2364	3.42	0.001
Error	2513	77			2452	691		
Wet tissue weight								
Site	2	2197.9	903.12	<0.001	2	3100	240.60	<0.001
Ploidy	1	17.2	7.06	<0.001	1	768.7	59.66	<0.001
Genotype	8	28.5	11.72	<0.001	8	176.3	13.68	<0.001
Site × Ploidy	2	100.6	41.33	<0.001	2	528.3	41.00	<0.001
Site × Stock	15	12.5	5.13	<0.001	15	39.3	3.05	<0.001
Ploidy × Stock	8	18.7	7.66	<0.001	8	85.4	6.63	<0.001
Error	2463	2.4			2452	12.9		
Meat yield								
Site	2	1.79	1404.80	<0.001	2	0.38	223.41	<0.001
Ploidy	1	0.13	208.11	<0.001	1	0.03	38.08	<0.001
Stock	8	0.07	14.37	<0.001	8	0.12	17.43	<0.001
Site × Ploidy	2	0.05	42.55	<0.001	2	0.02	11.64	<0.001
Site × Stock	15	0.04	3.90	<0.001	15	0.04	3.07	<0.001
Ploidy × Stock	8	0.06	11.81	<0.001	8	0.03	4.81	<0.001
Error	2463	1.57			2452	2.09		

Table 3.3: Analysis of variance for growth traits (shell height, whole wet weight, wet tissue weight, and meat yield) at 17 months (December 2011) and 29 months (December 2012).

	Diploid			Triploid		
Stock	**Shell height (mm)**		**95%CI**	**Shell height (mm)**		**95%CI**
Choptank River						
LGT09	21.2	±	1.2	19.5	±	0.9
OBOY09	NA	±	NA	17.5	±	1.0
DBY09	19.2	±	1.1	20.2	±	1.2
XB06	NA	±	NA	NA	±	NA
hANA	20.9	±	0.9	20.9	±	0.9
LOLA	20.4	±	1.1	22.0	±	0.8
DEBY	16.2	±	0.9	19.6	±	0.9
XB	18.6	±	0.9	19.6	±	1.1
Rappahannock River						
LGT09	25.2	±	1.4	23.8	±	1.1
OBOY09	21.7	±	1.0	24.4	±	1.1
DBY09	25.5	±	1.1	21.8	±	0.8
XB06	NA	±	NA	NA	±	NA
hANA	23.7	±	1.0	24.4	±	1.2
Lola	24.4	±	1.1	27.2	±	1.2
SL-DBY	22.6	±	0.9	25.3	±	1.1
SL-XB	22.3	±	1.1	25.5	±	1.2
York River						
LGT09	19.0	±	1.2	22.5	±	0.7
OBOY09	17.5	±	1.0	20.1	±	1.0
DBY09	21.0	±	1.0	21.7	±	1.1
XB06	26.6	±	1.6	22.6	±	1.3
hANA	19.4	±	1.1	24.3	±	1.1
Lola	18.8	±	1.0	23.3	±	0.7
SL-DBY	17.5	±	0.9	22.1	±	0.8
SL-XB	19.2	±	0.7	23.0	±	0.8

Table 3.4: Initial whole wet weights (mean ± 95%CI) of diploids and triploids from the 2006-year class lines and Superlines at the deployment into the experimental design (April 2011), after the field nursery period, in the Choptank, Rappahannock, and York Rivers.

		Shell height			Whole wet weight			Wet tissue weight		
		Experimental site			Experimental site			Experimental site		
Group	**Line**	**MD**	**RR**	**YR**	**MD**	**RR**	**YR**	**MD**	**RR**	**YR**
2006-year class lines	LGT09	3	*15	*20	3	*35	*53	(13)	15	*43
	OBOY09	ND	4	dead	ND	12	dead	ND	*25	dead
	DBY09	2	*14	11	(6)	*59	*44	*(26)	*35	*42
	XB06	ND	ND	17	ND	ND	*55	ND	ND	*36
Superlines	hANA	8	2	*15	14	9	*32	4	8	23
	Lola	4	(1)	*12	9	11	*20	(6)	12	11
	SL-DBY	4	3	9	(5)	10	*42	*(25)	16	22
	SL-XB	6	6	16	(12)	*23	*33	(27)	10	15

Table 3.5: Estimated triploid advantage (%) at 29 months (December 2012) of wild stocks and Superlines in the Choptank, Rappahannock, and York Rivers. Parentheses indicate negative values. '*' Indicates the difference between diploid and triploid stocks/lines are significant at the alpha = 0.05 level. 'ND' signifies the stock or line was not deployed in a given site.

DIPLOID

♂ \ ♀	2006-year class lines				F1 Superlines			
	LGT09	OBOY 09	DBY09	XB06	hANA	Lola	SL-DBY	SL-XB
LGT09	2n							
OBOY09		2n						
DBY09			2n					
XB06				2n				
hANA					2n			
Lola						2n		
SL-DBY							2n	
SL-XB								2n

TETRAPLOID

4B	3n	3n	3n	3n	3n	3n	3n	3n
	2006-year class lines				F1 Superlines			

Figure 3.1: Mating design for diploid and triploid crosses of *C. virginica*. Solid boxes indicate which crosses were made. Aliquots of pooled eggs were split in half for diploid and triploid crosses. Diploids were produced from sperm of each stock or line and triploids were produced from the other half of eggs fertilized with pooled sperm from tetraploid males of family 4B. For wild stock designation, see Table 3.1 (WIC, MBY, RAP, CHES, and PATX).

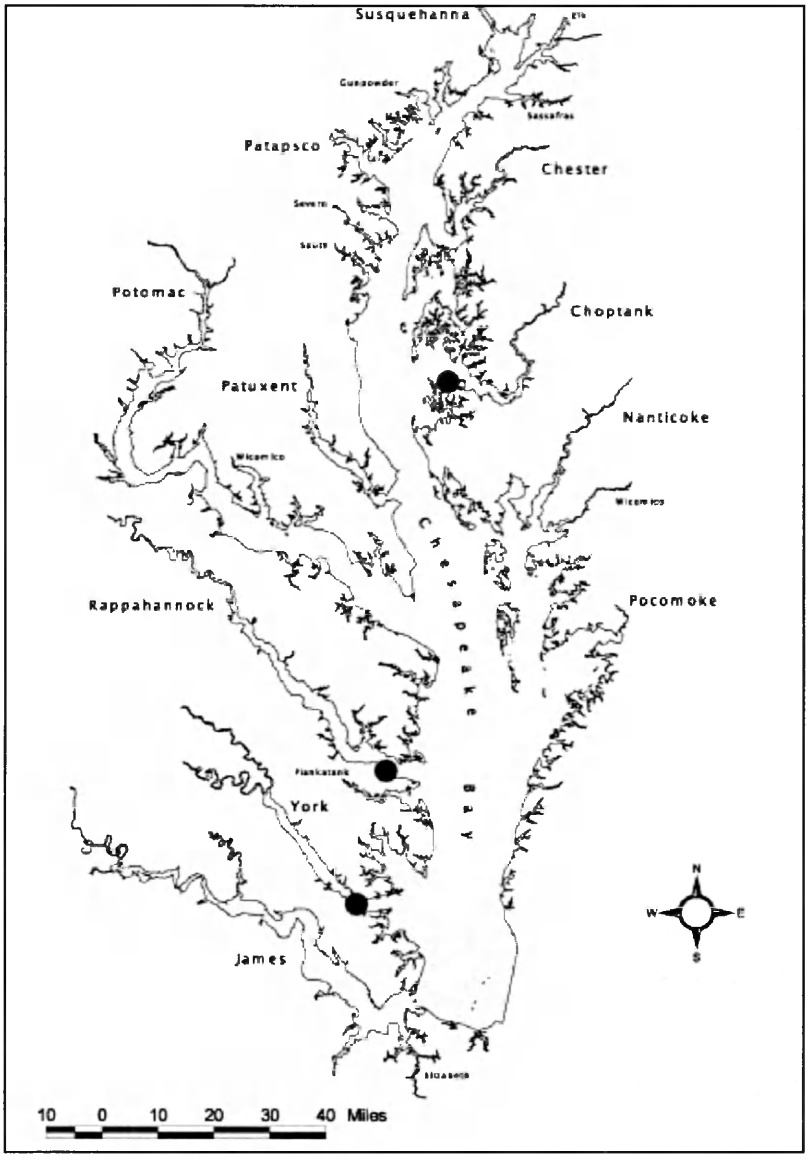

Figure 3.2: Map of the experimental grow-out sites in the Chesapeake Bay. Oysters were grown in three estuaries: York River, VA, Rappahannock River, VA, and Choptank River, MD. Specific experimental site locations are marked with black circles.

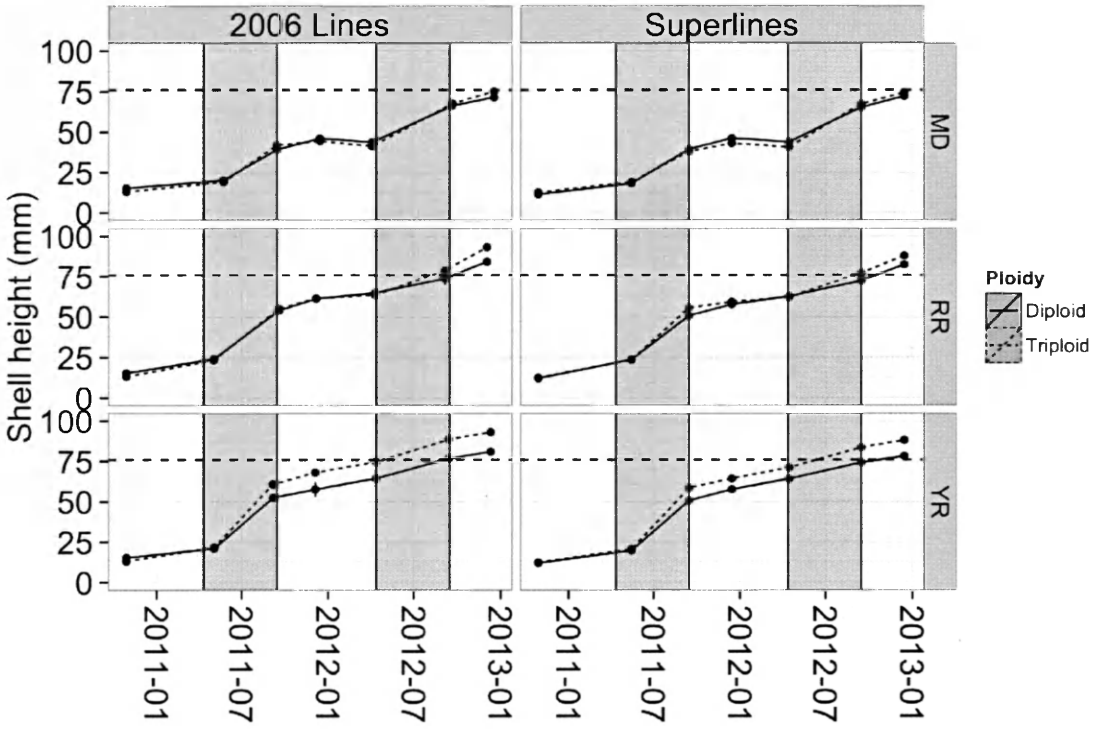

Figure 3.3: Average shell height (mm ± SEM) growth curves of diploids (solid line) and triploids (dashed line) of the 2006-year class lines (2006 Lines) and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Growth curves begin with measurements taken from animals at the deployment to field nurseries at the final grow-out sites. Dashed lines indicate harvest size (shell height ≥76mm). Shaded area indicates typical reproductive period in Chesapeake Bay.

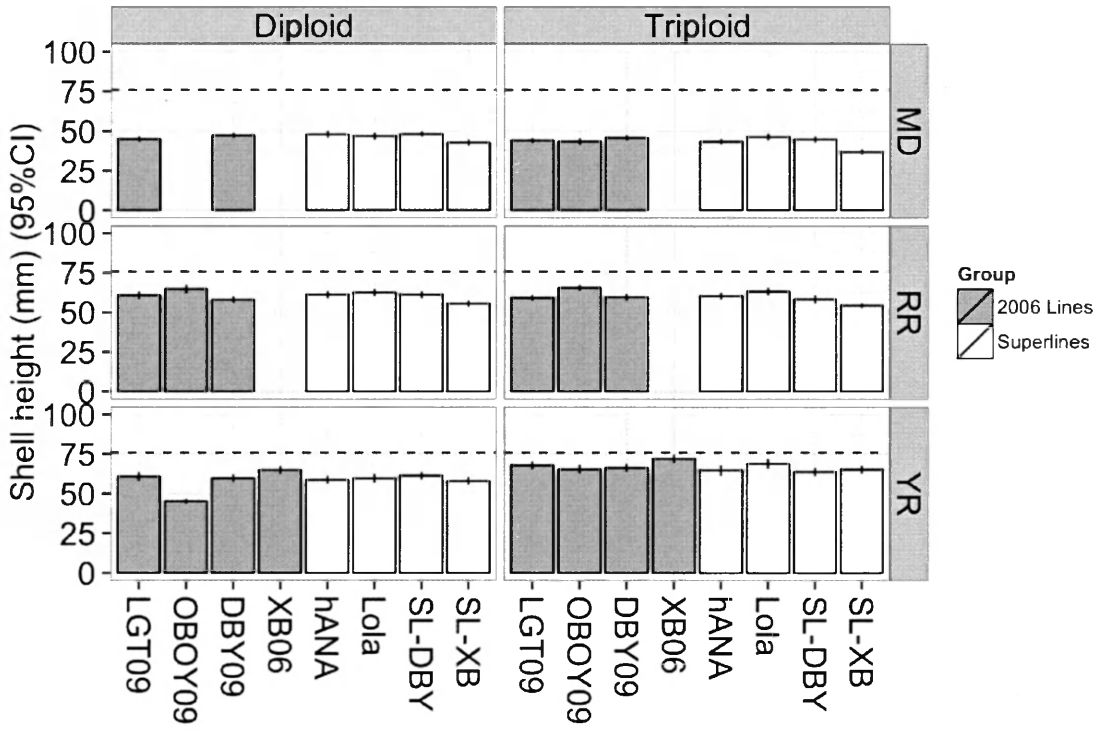

Figure 3.4: Shell height (mean ± 95%CI) of diploid and triploid 2006-year class lines and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months). Dashed lines indicate harvest size (shell height ≥76mm).

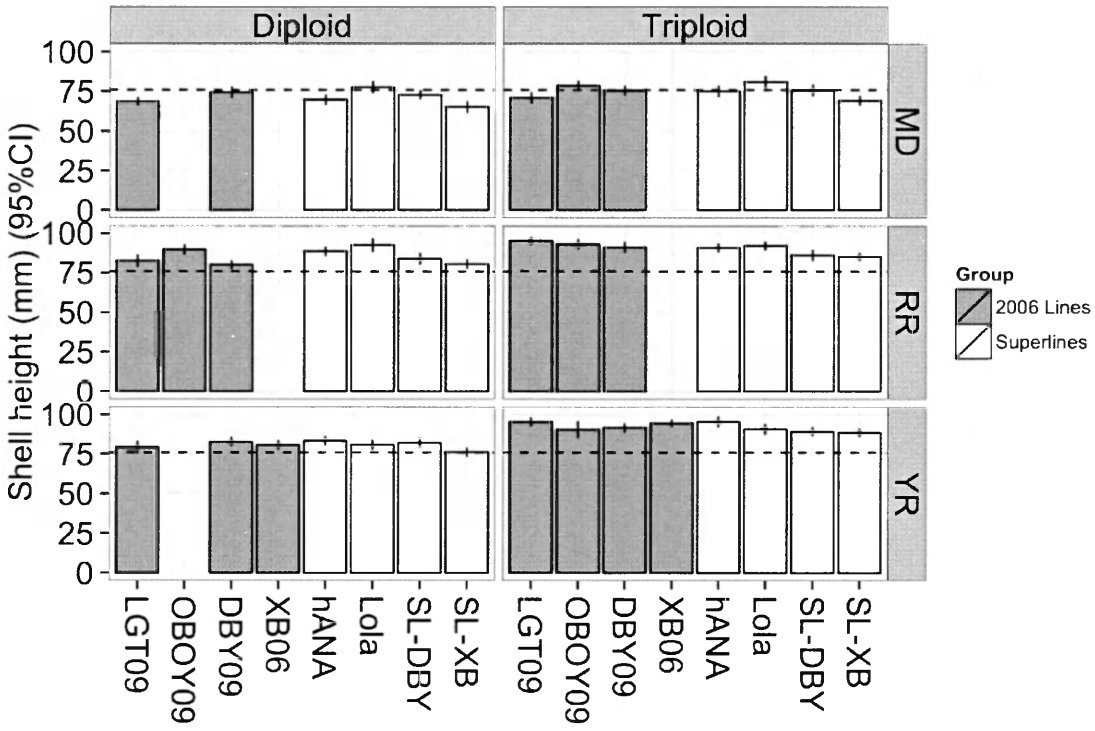

Figure 3.5: Shell height (mean ± 95%CI) of diploid and triploid 2006-year class lines and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months). Dashed lines indicate harvest size (shell height ≥76mm).

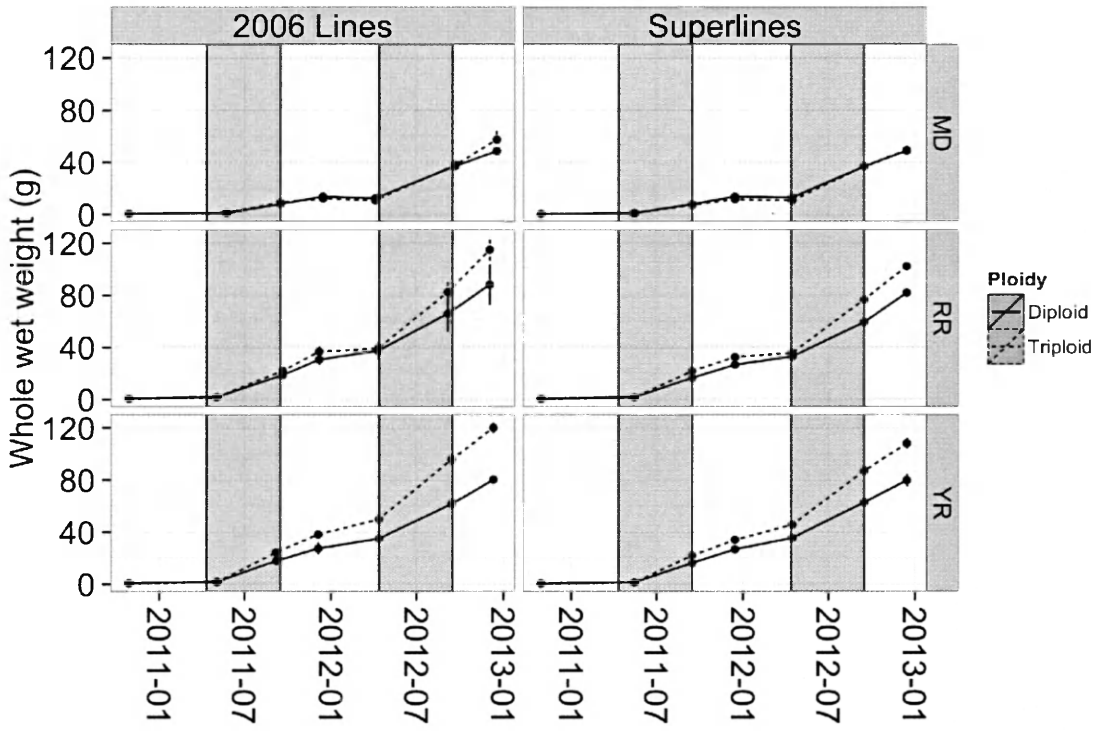

Figure 3.6: Average whole weight (g ± SEM) growth curves of diploids (solid line) and triploids (dashed line) of the 2006-year class lines (2006 Lines) and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Growth curves begin with measurements taken from animals at the deployment to field nurseries at the final grow-out sites. Shaded area indicates typical reproductive period in Chesapeake Bay.

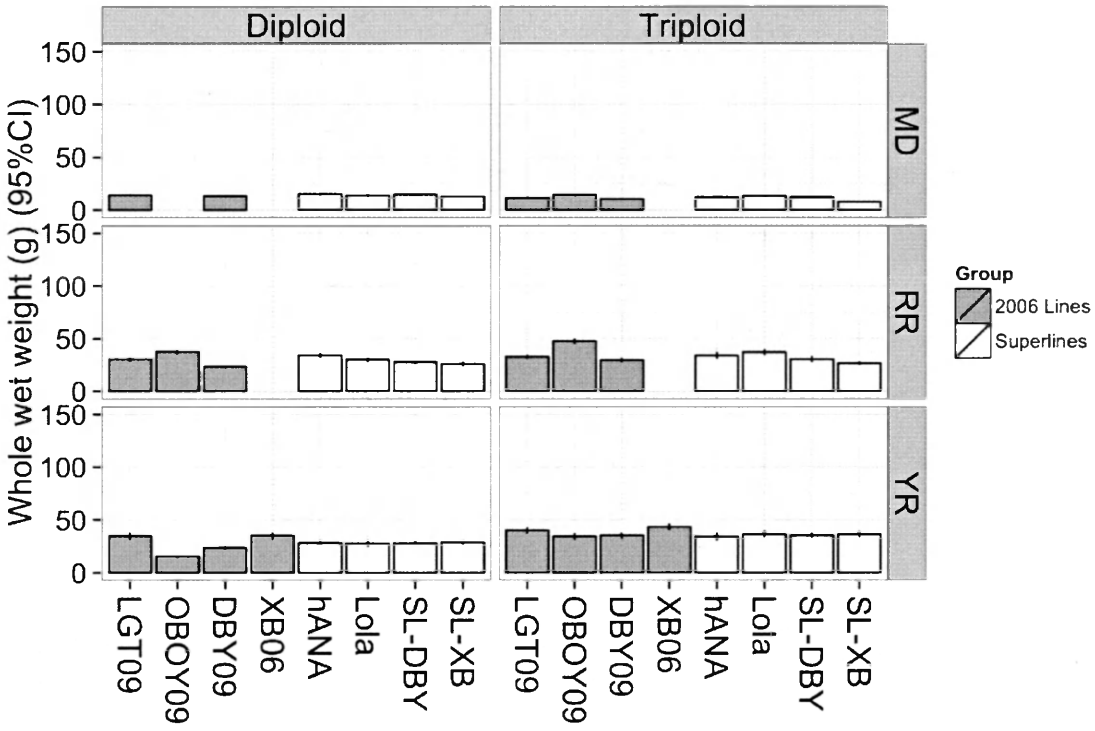

Figure 3.7: Whole wet weight (mean ± 95%CI) of diploid and triploid 2006-year class lines and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months).

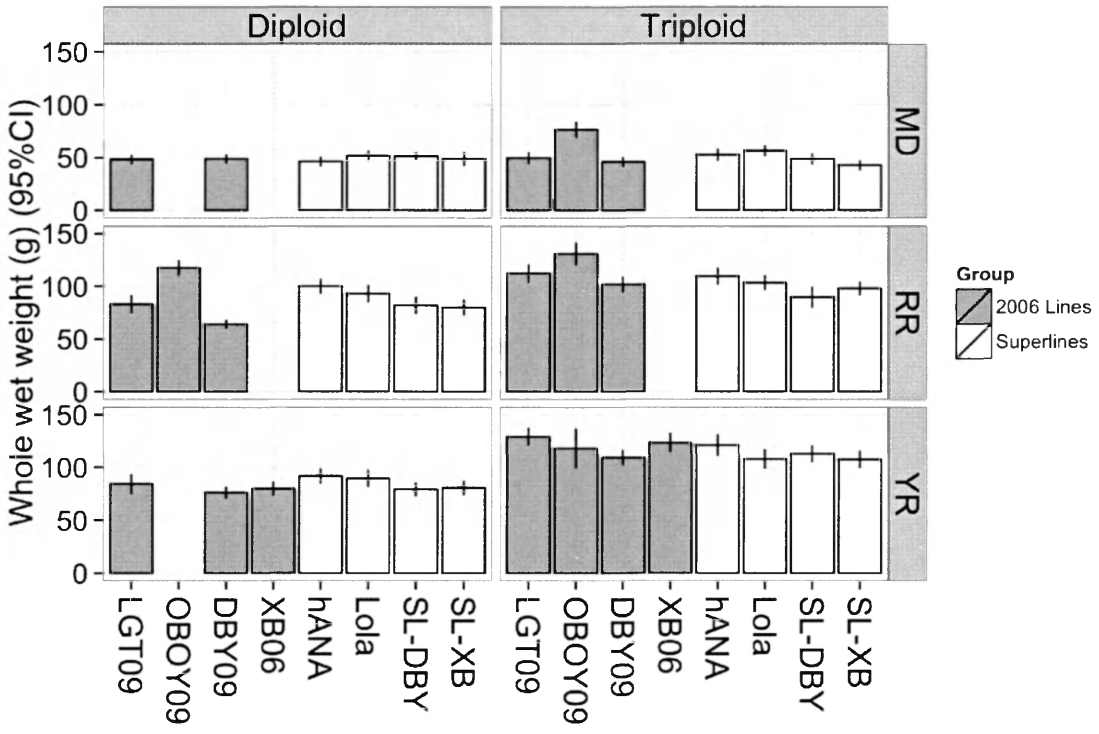

Figure 3.8: Whole wet weight (mean ± 95%CI) of diploid and triploid 2006-year class lines (2006 Lines) and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months).

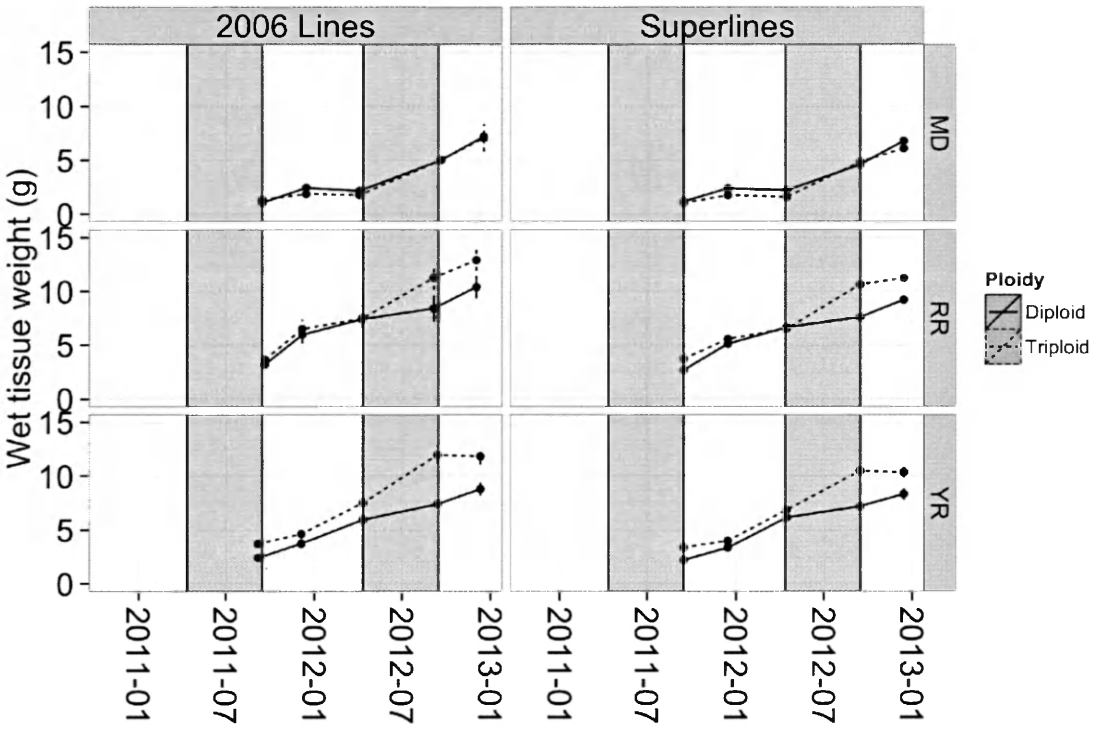

Figure 3.9: Average wet tissue weight (g ± SEM) growth curves of diploids (solid line) and triploids (dashed line) of the 2006-year class lines (2006 Lines) and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Growth curves begin with measurements taken in September 2011. Shaded area indicates typical reproductive period in Chesapeake Bay.

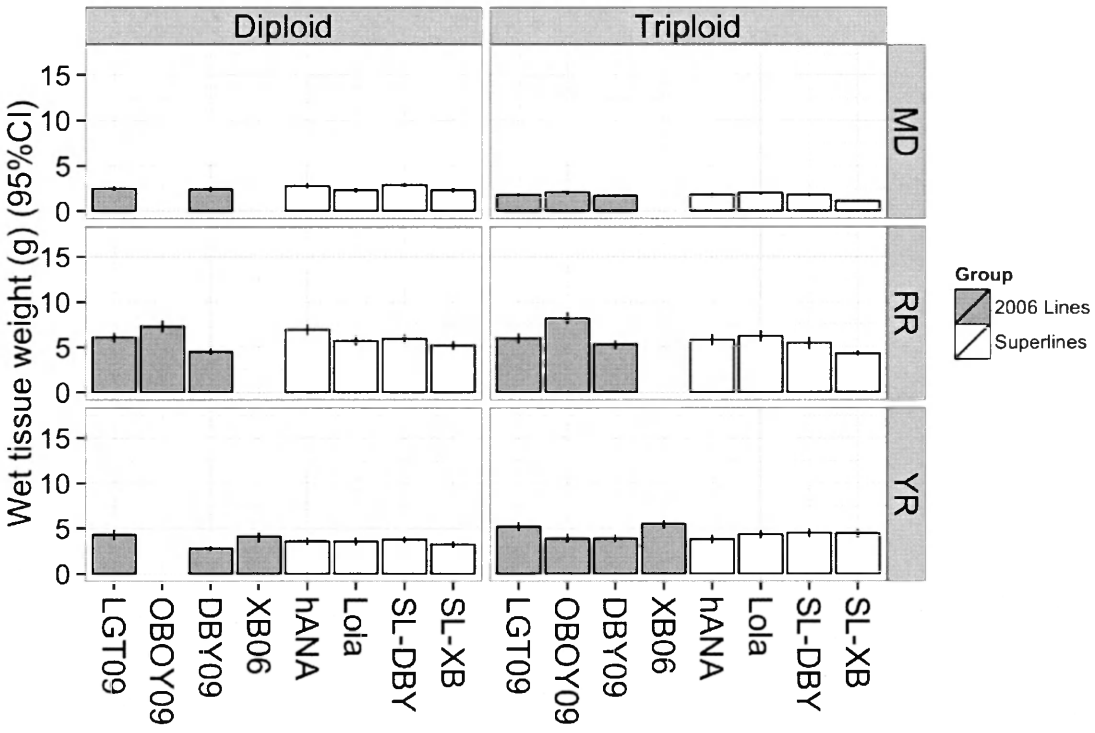

Figure 3.10: Wet tissue weight (mean ± 95%CI) of diploid and triploid 2006-year class lines (2006 Lines) and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months).

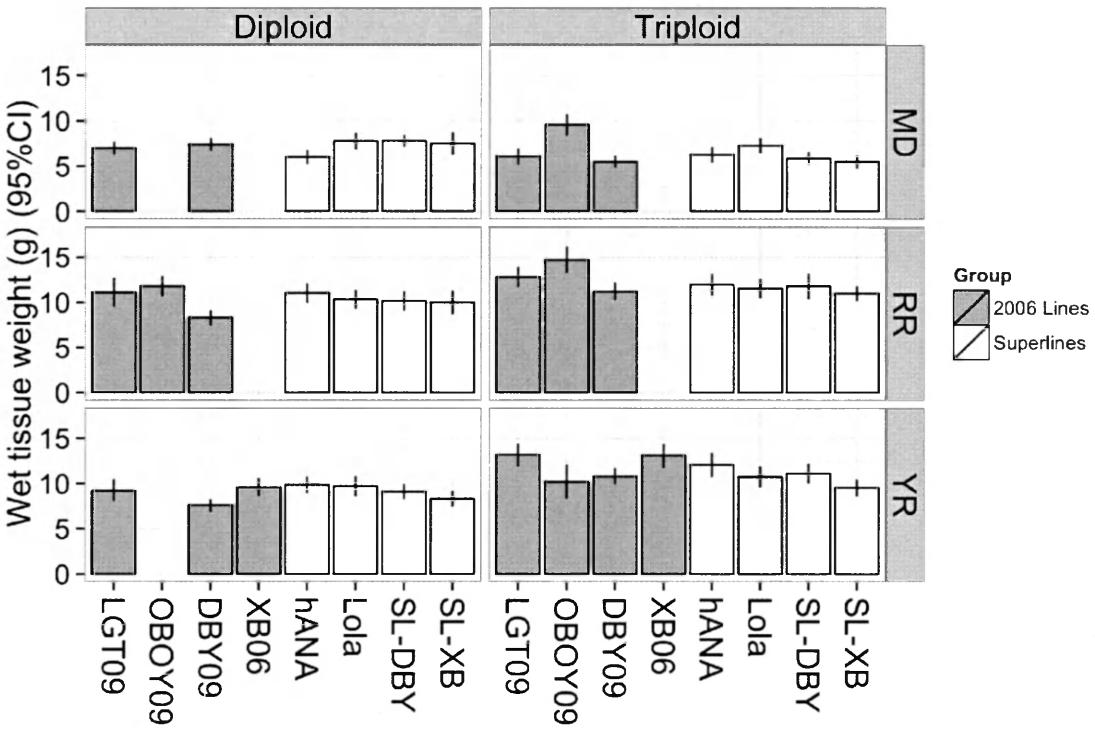

Figure 3.11: Wet tissue weight (mean ± 95%CI) of diploid and triploid 2006-year class lines and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months).

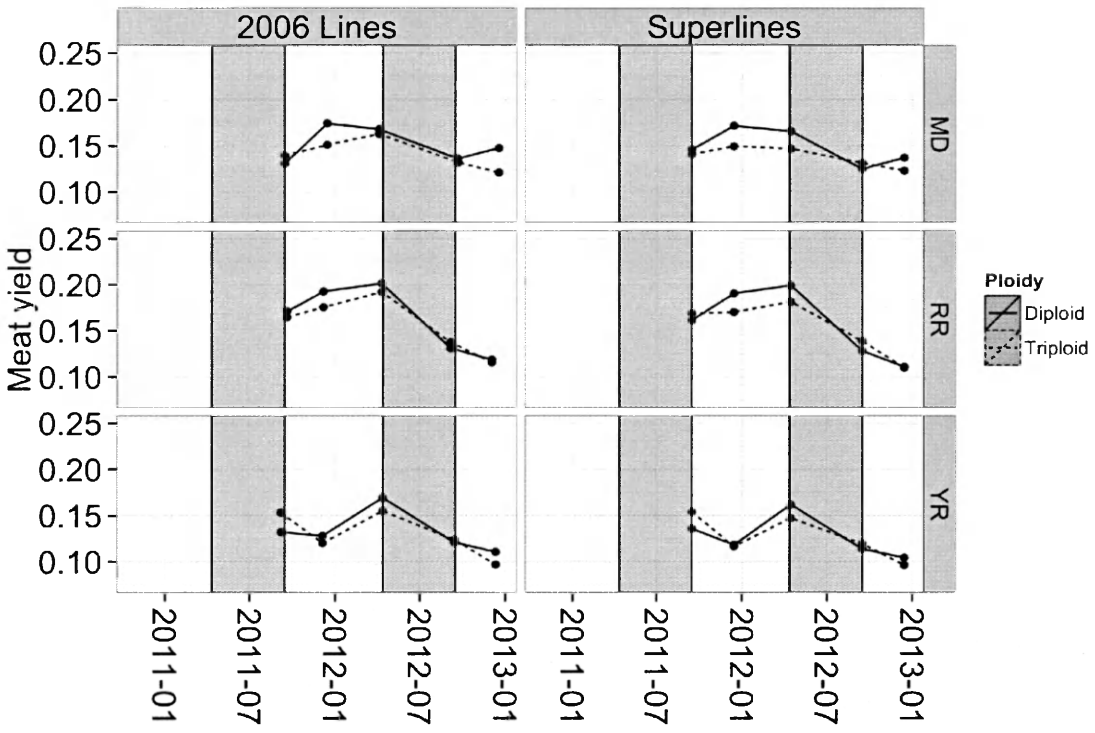

Figure 3.12: Meat yield curves of diploids (solid line) and triploids (dashed line) of the 2006-year class lines (2006 Lines) and the Superlines at the three grow-out sites: Choptank River (MD), Rappahannock River (RR), and York River (YR). Curves begin at September 2011 when wet tissue weight was first measured. Shaded area indicates typical reproductive period in Chesapeake Bay.

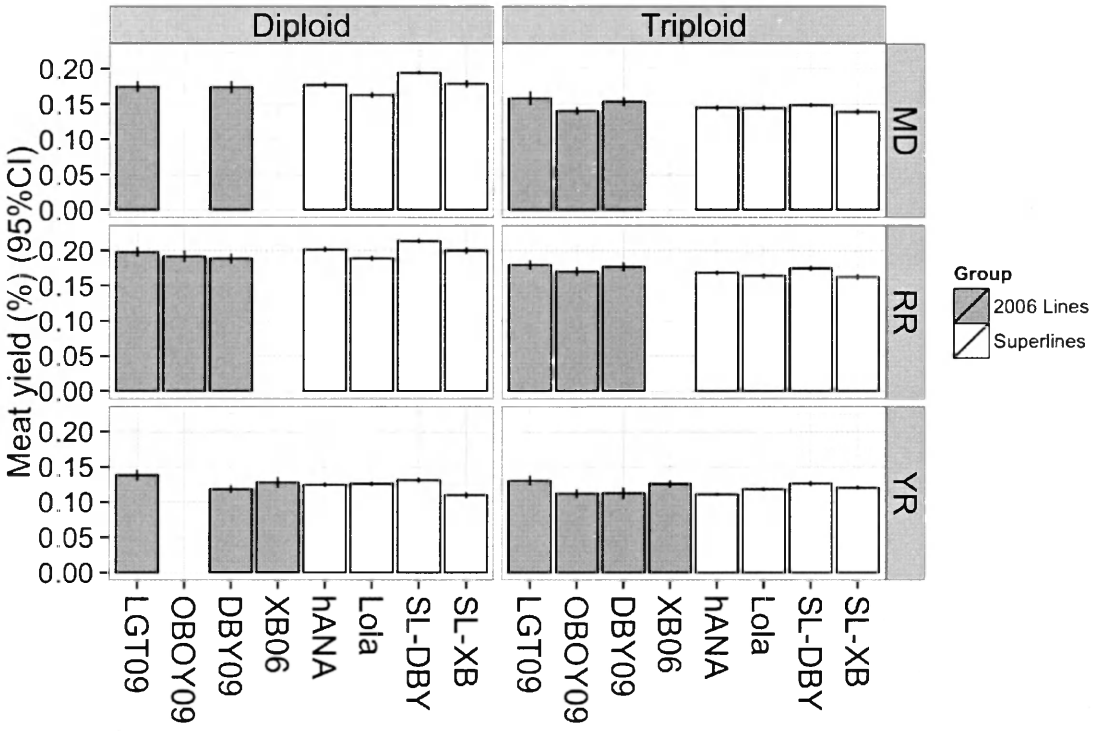

Figure 3.13: Meat yield (mean ± 95%CI) of diploid and triploid 2006-year class lines (2006 Lines) and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2011 (17 months).

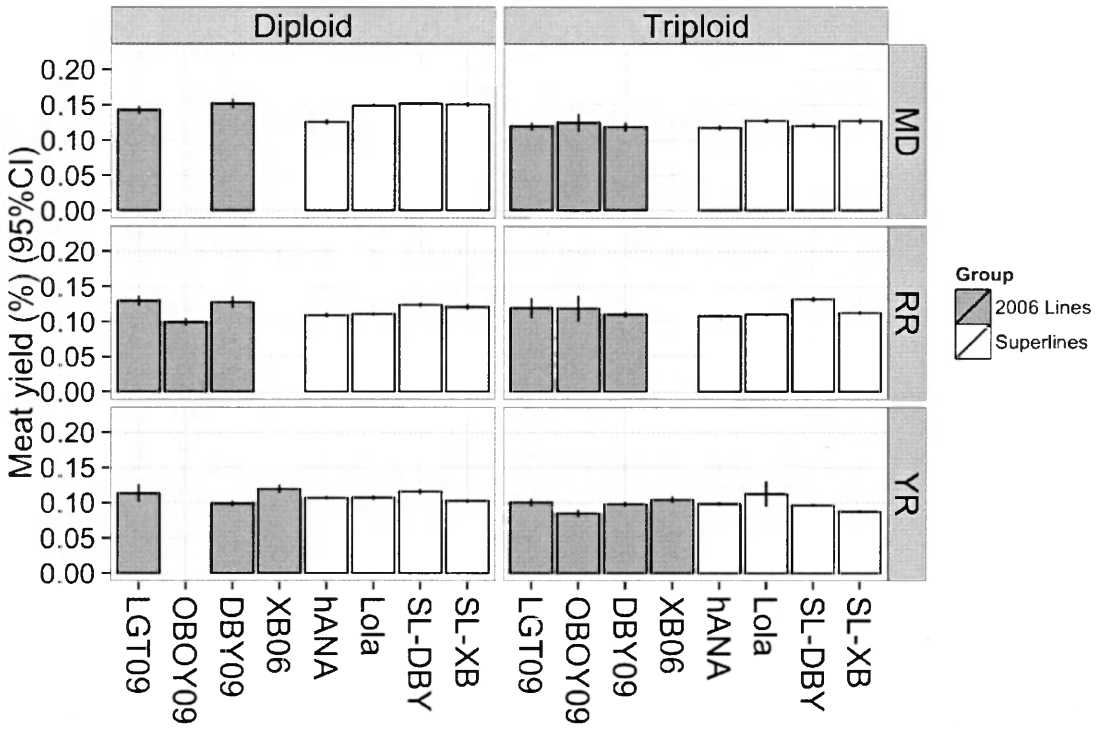

Figure 3.14: Meat yield (mean ± 95%CI) of diploid and triploid 2006-year class lines (2006 Lines) and Superlines in the Choptank River (MD), Rappahannock River (RR), and York River (YR) in December 2012 (29 months).

Chapter Four: Triploid advantages for survival and disease resistance in *C. virginica* produced from wild stocks and breeding lines

1. INTRODUCTION

Triploid oysters can have faster growth than diploids and greater survival in certain environments, described as the 'triploid advantage' in the previous Chapters. The results of Chapters 2 and 3 show that growth metrics (shell height, whole wet weight, to wet tissue weight) are influenced strongly by both the genetic contribution of the diploid parent and the environment. The general conclusion is that there is an advantage to being triploid, but not everywhere. Notably, the triploid advantage for growth was greatest in the York River (Chapters 2 and 3) where Dermo (caused by the protozoan parasite *Perkinsus marinus*) and MSX disease (caused by another protozoan parasite *Haplosporidium nelsoni*) were found during this study.

Dermo disease has an infection pattern that generally begins through ingested parasites crossing the epithelium of the stomach or intestines (Mackin 1951a; Perkins, 1988) during the warmer months from May to October and with prevalence peaking around September and October (Andrews and Hewatt, 1957). Prevalence declines through the winter and increases in the following spring (Andrews, 1988; Bushek, 1994; Ragone Calvo and Burreson, 1994). It is during the second year of infection that the disease generally reaches lethal infection intensities. Prior to death oysters infected with Dermo disease show signs of extensive tissue lysis (Mackin, 1951b) and reduced shell and soft tissue growth that is correlated with infection intensity (Ray et al., 1953; Menzel and Hopkins, 1955). Newell et al. (1994) measured metabolic function of oysters

infected with *P. marinus* and found no relationship between infection intensity and metabolic rates. These authors suggest the effects of Dermo disease on oysters are caused by competition with the host for nutrients rather than inhibiting physiological functions. Competition for host resources has been supported by observed depletion of energy reserves as well as the replacement of storage cells with haemocytes as infections advance (Mackin, 1962).

Resistance to Dermo disease was first reported by Andrews (1954) but it was not until later that heritability of Dermo disease resistance was demonstrated through controlled factorial crosses of geographically separate diploid oyster stocks (Bushek, 1994). Even with the clear demonstration of the heritability of Dermo resistance the expression of this resistance can be confounded by other factors such as additional disease pressures (e.g. MSX) and oyster condition prior to infection (because of the host-parasite competition for nutrients). The evidence of a triploid advantage for disease resistance in *C. virginica* is unclear. Chemically induced triploids were shown to be equally susceptible to Dermo infections as diploids (Meyers et al., 1991; Barber and Mann, 1991). Meyers et al. (1991) did not observe a difference in survivorship of diploid and chemically induced triploid oysters as all oysters had died by the end of the first year. Barber and Mann (1991) did not detail survival data but report similar survival between diploids and triploids. Another study documented mated triploids having greater survival than diploids but no difference in the prevalence of Dermo and MSX infections (Dégremont et al., 2012). In the York River, VA, where Dermo (and MSX) are enzootic, mated triploids ($4n \times 2n$) produced from disease resistant broodstock were shown to have as high as 20% greater survival than diploids (Dégremont et al. 2012). While enhanced

Dermo disease resistance of triploids is undemonstrated, survival of triploid *C. virginica* is generally greater than that of diploids when faced with Dermo pressure (Dégremont et al., 2012). Improved survival of triploids may be a physiological advantage unrelated to genetic origin and may be a result of improved general health through the reproductive season when *P. marinus* is competing with the host for nutrients and environmental stress may be high (Nell, 2001; Harding, 2007; Piferrer et al., 2009; ABC, 2010).

The triploid advantage on growth, when it occurs, may serve as remediation for disease-based mortality by "outrunning" the disease. By harvesting oysters before the second season of infection, the disease can largely be avoided (i.e., oyster can 'outrun' the disease) (Barber and Mann, 1991). Such a strategy is not feasible for diseases that result in acute mortality events like MSX, which causes oysters to die within a month or two of infection (Haskin et al., 1965; Andrews, 1966).

Growth-based refuge was shown to work against the bacterial juvenile oyster disease (JOD), caused by *Roseovarius crassostreae*, with *Crassostrea virginica* (Barber et al., 1998; Davis and Barber, 1999). These studies observed reduced JOD mortality in groups that had faster growth rates in several sites in Maine and Massachusetts. Davis and Barber (1999) suggest that, at least partially, the refuge from faster growth may be a result of inadvertent selection for more robust oysters. Outrunning Dermo is also a rationale for producing faster growing oysters in the Chesapeake, and largely works for half-shell oysters because they reach harvest size before disease related mortality can occur. Outrunning disease may not work for spat-on-shell as the growing times can be longer – up to 2.5 years.

MSX disease is caused by the spore-forming protozoan *Haplosporidium nelsoni*. The mode of transmission to oysters of this parasite is unknown and several controlled transmission attempts have not succeeded (Canzonier, 1968; Sprague et al., 1969; Andrews, 1979). Early infections can be found in gill and palp epithelia and parasites multiply between these cells until they achieve penetration into underlying tissues (Haskin et al., 1965). Once the epithelial barrier is breached susceptible oysters typically die within a month or two (Haskin et al., 1965; Andrews, 1966). Growth of MSX infected oysters stops several weeks before death occurs (Andrews, 1966) and because oysters succumb to MSX disease rapidly losses in soft tissue condition are often absent (Ford et al., 1988). Clearance rates of infected oysters were lower than uninfected oysters (Newell, 1985). Similar to Dermo disease, oysters infected with MSX show decreased energy reserves (Barber et al., 1988b) and repressed reproductive efforts (Ford and Figueras, 1988). The cause of death from MSX is not completely understood because susceptible oysters appear in good condition when they die and resistant oysters show signs of tissue damage and condition loss.

Natural resistance to MSX disease developed rapidly in native Delaware Bay oyster populations (Haskin and Ford, 1979). Heritability of MSX resistance is high and several strains were developed that are resistant to MSX disease (Andrews, 1968; Haskin and Ford, 1979). Resistance of triploids is unclear. Chemically induced triploid *C. virginica* were observed to have higher MSX infection prevalence than comparative diploids, but higher survival rates (Matthiessen and Davis, 1992). Higher infections coupled with lower mortality suggests that triploids may be more resistant to the negative effects of MSX, at least in this instance. Another study documented mated triploids ($4n \times$

$2n$) having greater survival on average than diploids with the heaviest MSX infections in a diploid line in the York River, VA, where MSX is enzootic (Dégremont et al. 2012).

The evidence for direct improved disease resistance of triploid oysters is unclear. For the Pacific oyster, *C. gigas*, one study has shown triploids to have higher summer mortality than diploids, which is caused by stress and depletion of energy reserves (Cheney et al., 2000). Several other studies have contradicted this finding by reporting triploid *C. gigas* to be less susceptible to summer mortality (Gagnaire et al., 2006; Boudry et al., 2008; Dégremont et al., 2010). Investigations were carried out to identify the source of higher survival of triploid *C. gigas* in the face of summer mortality. Several explanations proffered were in increased granulocyte percentages, phagocytosis activity, and percentages of cells containing hydrolytic enzymes of haemocytes, which are part of the internal defense mechanisms of oysters (Sami et al., 1991; Gagnaire et al., 2006; Duchemin et al., 2007). All of these studies indicated seasonal variation in immune status, but Duchemin et al. (2007) showed that seasonal variation in haemocyte cellular integrity and immunocompetency were lower for triploids than diploids of *C. gigas*. As with *C. gigas*, the evidence of a triploid advantage for disease resistance in *C. virginica* is unclear.

Relevant to triploid disease resistance in this study is how chromosomes are inherited in mated triploids ($4n \times 2n$). One of the main objectives of selective breeding in the Chesapeake Bay was to employ selective breeding to address the endemic problems of MSX and Dermo disease. Thus domesticated disease-resistant lines are at the core of oyster breeding in this region. To create broodstock populations for triploid crosses, tetraploids were made from these disease resistant lines. These tetraploids with four sets

of chromosomes selected for disease resistance (SSSS) are then bred with either selected diploids (SS) or wild-type diploids (++) producing selected triploids (SSS) and a hybrid triploid (SS+). This biases tests of the effect of disease susceptibility of the diploid parent in triploid crosses ($4n \times 2n$). Triploids from wild-type diploid females will have two of their three sets of chromosomes from disease resistant tetraploids and theoretically have a survival advantage over their diploid counterparts under disease pressures as a result. This does not necessarily mean these triploids from wild-type diploids will have the same survival rates as triploids from crosses of disease resistant diploids and disease resistant tetraploids. Any difference between these two types of triploids will inform as to whether or not (given the current stocks of disease resistant tetraploids) the diploid parent contributes to any increase in resistance to diseases or not.

The counterpart to disease resistance is survival to harvest. Selectively bred diploid and triploid lines were shown to have similar growth patterns (Chapters 2 and 3). Given this similar performance of selected diploid and triploid lines, survival may be the determining factor in whether or not the triploid advantage is observed for a given line in a certain environment. This study will further guide breeding efforts by answering several questions in regard to survival and disease resistance of triploids – do wild-type triploids survive as well as selected triploids and do wild-type triploids show similar resistance to disease pressure?

2. MATERIALS AND METHODS

2.1 Crosses

Diploid and triploid oysters were produced at the ABC Oyster Hatchery, Gloucester Point, VA through June and July 2010. Broodstock used to produce these oysters were collected from five wild populations in the Chesapeake Bay as well as from two groups of selectively bred disease resistant (DR) lines – 2006-year class lines and Superlines – from ABC's breeding program.

Of the five wild stocks used, three were collected from different estuaries in Virginia, which range in environmental conditions. The Great Wicomico River (WIC) has low salinity (range 10-15 ppt) and only sporadically intense disease pressure, therefore oysters from this location show higher susceptibility to disease pressures, both MSX and Dermo (Southworth et al., 2010; ABC, unpublished data). The Rappahannock River (RAP) has moderate salinity (range 13-20 ppt) and disease pressure from both parasites and, in addition, is a common source of broodstock used in commercial hatcheries, allowing for a comparison between a commonly used industry product (diploids and triploids generated from Rappahannock brood) and selected ABC lines. Wild oysters from Mobjack Bay (MBY) are the standard control used within ABC's breeding program because of their higher salinity origin and frequent disease exposure from both MSX and Dermo. The remaining two wild stocks were collected from estuaries in Maryland: Chester River (CHES) and Patuxent River (PATX). These

estuaries were chosen because the Patuxent River exhibits constant Dermo pressure (Albright et al., 2007; McCollough et al., 2007) while oysters in the Chester do not (Abbe et al., 2010).

Four lines from ABC's 2006-year class lines were used: LGT, OBOY, DBY, and XB. The LGT line was derived from wild oysters in Grande Terre, LA in 2000 and selected by ABC for disease resistance since then for four generations. OBOY was introduced into ABC's breeding program in 2002 as an F_3 generation derived from wild oysters in Oyster Bayou, LA and subsequently selected for Dermo resistance by Dr. Jerome LaPeyre's program at Louisiana State University. XB was developed in Delaware Bay, NJ at Rutgers University by S. Allen from a consolidation of many lines produced by Ford and Haskin (1987) prior to 1988. They were brought to Chesapeake Bay in 1998 and propagated within ABC (Dégremont et al., 2006). Due to limited availability of the 2006-year classes of LGT, OBOY, and XB, 2009-year classes of these three lines were used. These were propagated from the 2006-year class of the corresponding line via random pooled spawns through an effort to preserve the germ plasm of these lines. The DBY line was developed from wild oysters from Delaware Bay, NJ that were collected in 1987 and selected for Dermo and MSX resistance for four generations in the York River, VA (Ragone Calvo et al., 2003). Subsequent selection and generations were produced by ABC's breeding program. The 2006-DBY year class is an F_7 generation.

The four Superlines are Lola, hANA, SL-DBY, and SL-XB. LOLA was produced using progenitors from Louisiana, known to be Dermo resistant (Ragone Calvo et al., 2003; ABC breeding manual), and selected in Virginia for MSX resistance for

three generations. Since 2007, however, this line had been further selected for low salinity tolerance in a mesohaline site (Yeocomico River, VA). hANA was also developed using progenitors from Louisiana, however, since 2007 these animals were selected for increased MSX disease resistance in a polyhaline site (York River, VA). The SL-DBY line was developed from wild oysters from Delaware Bay, NJ that were collected in 1987 (Ragone Calvo et al., 2003). SL-XB was created in Delaware Bay, NJ then transferred to Chesapeake Bay for selection under ABC's breeding program beginning in 1998 (Ford and Haskin, 1987; Dégremont et al., 2006). A detailed pedigree of the Superlines can be found in ABC's breeding manual (ABC, 2010).

Broodstock were conditioned in a flow-through system at ABC's conditioning facility, the Kauffman Aquaculture Center (KAC) on Locklies Creek, VA. In the flow-through system, water temperature was held constant at 23°C. Broodstock were batch fed a cultured-algae cocktail containing *Isochrysis* sp., *Tetraselmis chui*, and *Chaetoceros muelleri*. When all stocks had conditioned, they were transferred to ABC's research hatchery in Gloucester Point, VA for spawning and larval rearing.

Eggs obtained from at least 10 dams per stock (wild) or line (selected) were stripped from gonad tissue and pooled in plastic beakers. The pools of eggs were then divided into two groups containing 3×10^6 eggs each, one for diploids and one for triploids. To produce diploids, one group of eggs was fertilized with sperm pooled from at least 10 sires of the same stock/line when available. To produce triploids, the remaining groups of eggs were fertilized with sperm pooled from 11 sires from a single tetraploid family following the methods of Guo et al. (1996). The number of dams and sires from each group are outlined in Table 4.1. Several crosses were made with reduced

dam and sire numbers due to the unavailability of enough ripe broodstock. This produced 26 groups: 13 diploid and 13 triploid (Figure 4.1)

2.2 Larval Rearing

Larvae were reared through settlement following the ABC protocol adapted from Helm et al. (2004) in 60L flat-bottom larvae tanks, consisting of daily batch feeding of microalgae and complete water exchanges three times a week (Monday, Wednesday, and Friday). Larval tank densities were adjusted based on age (in days) post-fertilization, such that, on days two, seven, and 14, the densities were adjusted to 10-larvae·mL^{-1}, 5-larvae·mL^{-1}, 2.5-larvae·mL^{-1}, respectively. Eyed-larvae were collected on 212µm for diploids or 250µm nylon screen for triploids. Competent eyed-larvae were transferred to $16cm^2$ downwellers for settlement. After two weeks in this downwelling system, the spat were moved into a flow-through upweller based nursery until field deployment.

2.2.1 Ploidy Determination

Ploidy was determined at various stages of rearing by flow cytometry to confirm the success of triploid crosses (Allen, 1983). Prior to pooling sperm from the 11 tetraploid sires, sperm from each individual were confirmed 100% di-haploid by analyzing gametes dissected from gonad tissue. Ploidy was analyzed again at the prodissoconch I larval stage on larvae collected on a 48µm nylon screen 48hrs post-fertilization by sampling 2000 larvae and prior to field deployment by sampling 50 spat from each group. At each of these sampling points all groups were confirmed 100% diploid or triploid.

2.3 Experimental Sites and Design

Oysters were deployed at three sites in the Chesapeake in November 2010. In the Virginia portion of the Chesapeake Bay, the two sites were the York River (13-25 ppt) and the Rappahannock River (13-20 ppt). The York River site is opposite VIMS on a private lease operated by Tommy Leggett of Chessie Seafood Company. The grow-out location in the Rappahanock River is on a lease owned by the Rappahannock River Oysters, LLC in Topping, VA. These sites were chosen in order to perform this experiment under environmental conditions of commercial operations. In the Maryland portion of the Chesapeake Bay, oysters were deployed in the Choptank River (5-12 ppt) adjacent to the University of Maryland Horn Point Environmental Laboratory (Figure 4.2).

Stocks were deployed in off-bottom cages at each of the Chesapeake Bay sites for evaluating survival. The off-bottom cages were designed and manufactured by the Chesapeake Bay Oyster Company. A single cage can hold three full-sized oyster grow-out bags. Off-bottom cages were chosen because it is the most common method of commercial culture in the Chesapeake Bay. Due to low survival in the field nursery, the diploid and triploid wild RAP group was not deployed to the Choptank River site.

2.3.1 Sampling

2.3.1.1 Mortality

A total of 300 oysters from each group were divided into six replicates of 50 oysters each. Each replicate was contained in polyethylene mesh socks (60cm in length; expanded sock mesh diameter approx. 2cm). Each replicate (thirteen diploid groups and thirteen triploid groups with six replicates each: 156 total) was randomly assigned to an

oyster grow-out bag totaling six replicates per bag. Bags were randomly assigned to one of two blocks based on ploidy (diploid and triploid).

Mortality was estimated by counting the number of live oysters within each replicate every April/May, August/September, and December of 2011 and 2012. The cumulative mortality rate was calculated from the ratio of number of oysters alive at a sampling event to the number of oysters alive at deployment. Dead oysters were discarded at each sampling point.

2.3.1.2 Disease Sampling

P. marinus diagnosis was performed following Ray's fluid thioglycollate culture method (Ray, 1966). Eight oysters were haphazardly selected from each replicate (16 oysters total per stock/line) in the Fall 2011 and 2012. Approximately 1cm^2 pieces of mantle, gill, and rectal tissue from each oyster were placed in a test tube containing 9.5ml sterile thioglycollate media. Each test tube was then inoculated with 0.5ml penicillin-streptomycin solution and 50µl of nystatin and then incubated in the dark at 25°C for 5-7 days. After the incubation period, tissue samples were removed from the test tubes containing culture media and placed on glass microscope slides. Several drops of Lugol's iodine solution were added and then the tissue samples were macerated with a scalpel blade. Slides were covered with glass coverslips for examination under compound microscopes at 40× magnification. *P. marinus* infection intensities were assigned from a code modified after Quick and Mackin (1971) ranging from 0.5 (a rare infection) to 5 (heavily infected) (Table 2.2).

In the Choptank River and Rappahannock River, where disease exposure is rare, a sentinel group comprised of oysters from the diploid group CHES (spawned from wild

oysters from the Chester River) was established to monitor any disease pressure that may arise. This stock was chosen because of its naiveté to the two diseases of interest (Dermo and MSX). At each site, a separate cage was stocked with the sentinel group to limit the potential for contagion from nearby oysters. Sentinel sampling (n = 15) took place in July, August, and September in 2011 and 2012 at the Choptank River and Rappahannock River to obtain indication of overall disease presence and intensity. Evidence warranting a full-scale sampling in October was established as infection of weighted prevalence of 3 or more on the Mackin scale (1-5). In October of 2011 and 2012 all groups in the York River were to be sampled regardless of any indication of the sentinels due to the persistent presence of Dermo in this estuary.

For MSX in the York River, 15 oysters from each group were sampled in May of 2012 and processed according to paraffin histopathological technique of Ford and Haskin (1982). A transverse tissue section containing digestive (tissue name), mantle, gill, and was dissected and fixed in Davidson's fixative (formalin, 95% ethanol, glacial acetic acid), dehydrated, cleared, and embedded in paraffin. 5-µm sections were stained with Harris's hematoxylin and eosin Y. Oysters were then examined for MSX infection intensity and rated as having no infection, rare, light, moderate, or heavy infections.

2.3.1.3 Environmental Parameters

In the Choptank, Rappahannock and York Rivers, average daily temperatures were estimated from hourly temperature measurements using submersible temperature data loggers. Individual and cumulative day degrees were calculated from average daily temperatures. Salinity data for the Rappahannock and York Rivers were taken from long-term monitoring stations from the Virginia Estuarine and Coastal Observing

System. The University of Maryland Center for Environmental Science's Oyster Hatchery provided salinity data for the Choptank River. These data are reported in Chapter 2.

2.4 Analyses

Differences in *P. marinus* prevalence and intensity between stocks/lines were analyzed with chi-square contingency tables using stocks/lines as row variable and number of infected and intensity level as columns. *P. marinus* infections were analyzed in October 2011 and 2012 in the York River. *H. nelsoni* infections were analyzed in May 2012. Prevalence was calculated as:

Prevalence = number of infected oysters / total number of oyster sampled (1)

Cumulative mortality was analyzed two ways. The first analysis was at 17 and 29 months with Chi-square contingency tables using stocks/lines as row variables and number dead as column variables. Confidence intervals of 95% were generated to infer statistical significance among stocks and lines. The second method was a 'time-response' analysis. Cumulative mortality density functions (S) using nonparametric product-limit estimators described by Newman and Dixon (1996). Mortality is initially zero and increases over time (T) (Equation 4).

$$S(t_i) = \prod_{j=1}^{i} \left(1 - \frac{d_j}{n_j}\right) \quad (4)$$

where n_j is the number of individuals alive before t_j, and d_j is the number of individuals that have died before t_j. The variance of the product-limit $S(t_i)$ is estimated by Greenwood's formula (Equation 5).

$$\sigma^2 = \sum_{j=1}^{i} \frac{d_j}{n_j(n_j - d_j)} \quad (5)$$

where $s_j = n_j - d_j$. If, prior to the end of the time in the field, no individuals were censored, then Equation 5 becomes Equation 6

$$\sigma^2(t_i) = \frac{s(t_i)[1-s(t_i)]}{N} \qquad (6)$$

where N is the total number of individuals. Dixon and Newman (1991) detail the SAS software code. These procedures use the Wilcoxon test for equivalence of mortality curves. The analysis was generated using SAS software, Version 9.3 of the SAS System for Windows.

3. RESULTS

3.1 Mortality

Significant differences in cumulative mortalities were found among stocks and lines at each site ($p<0.05$). By December 2011 (17 months post-spawn), cumulative mortality across all sites ranged from 6.3±6.4% to 77.7±10.2% among diploid stocks/lines and from 3.7±3.2% to 34.3±29.6% among triploid stocks/lines among all sites. By the end of the study, in December 2012 (29 months post-spawn), the greatest average mortality for diploids was observed in the York River (69.6±8.0%) followed by the Rappahannock River (44.9±5.3%). Diploid stocks/lines in the Choptank River had an average mortality rate of 37.4±8.8%. The highest average cumulative mortality for triploid stocks/lines was observed in the Choptank River (43.2±7.4%), followed by the York River (40.0±6.7%), with the lowest mortality in the Rappahannock River (34.7±4.7%).

3.1.1 Choptank River, MD

Wild Virginia Stocks

By 17 months, triploidy had affected mortality in only one stock, WIC (Figure 4.3). Diploid WIC had lower cumulative mortality than its triploid counterpart (diploid: 6.7±3.1% and triploid 30.5±6.6%). Diploid and triploid MBY stocks were not deployed to the Choptank River because of survival limitations in the nursery. There were no

differences among cumulative mortalities of individual diploid or triploid wild Virginia stocks by 17 months in the Choptank River (Figure 4.3).

By 29 months in the Choptank River, the effect of triploidy in the WIC stock was still present and had a triploid disadvantage of (-140%) (Figure 4.4, Table 4.6). Diploid WIC had lower cumulative mortality than its triploid counterpart (diploid: 20.0±16.0% and triploid 48.0±7.5%, $p<0.05$). There were no differences among cumulative mortalities of individual diploid wild Virginia stocks or among the individual triploid wild Virginia stocks by the end of the study (Figure 4.4).

Wild Maryland Stocks

By 17 months, there was no triploid advantage for cumulative mortality in the wild Maryland stocks (Figure 4.3). There were also no differences among cumulative mortalities of individual diploid wild Maryland stocks. Of the individual triploid stocks, CHES had a higher cumulative mortality than PATX, but this difference was not significant (22.3±5.6% and 10.0±6.7%, respectively, $p>0.05$) (Figure 4.3).

By 29 months the differences in the wild Maryland stocks were similar to those at 17 months (Figure 4.4). There was no significant effect of triploidy on cumulative mortality in the wild Maryland stocks despite CHES having a triploid disadvantage of (-67%) and an advantage for PATX of 29% (Table 4.6). There were also no differences among cumulative mortalities of individual diploid wild Maryland stocks. Of the individual triploid stocks, CHES had a higher cumulative mortality than PATX, but this difference was not significant (35.7±14.8% and 16.0±5.3%, respectively, $p>0.05$) (Figure 4.4).

2006-Year Class

By 17 months, there was no effect of triploidy on cumulative mortality in the 2006-year class lines (Figure 4.3). Diploid lines OBOY09 and XB06 and triploid XB06 were not deployed in the Choptank River due to survival limitations in the nursery. There were no differences among cumulative mortalities of individual diploid and triploid 2006-year class lines by 17 months in the Choptank River (Figure 4.3).

By 29 months, there were no differences in diploid:triploid comparisons or among individual diploid or triploid lines (Figure 4.4). DBY06 had a triploid advantage of 1% and LGT09 had a 17% triploid advantage, though the not significant (Table 4.6).

Superlines

By 17 months, there was no triploid effect on cumulative mortality in the Superlines (Figure 4.3). Diploid SL-XB had greater cumulative mortality than the diploid Superlines hANA, Lola, and SL-DBY, though this difference was only significant ($p<0.05$) for hANA and SL-DBY (34.3±8.8%, 12.7±4.3%, 21.0±7.8%, and 17.0±5.9%, respectively). There were no differences among cumulative mortalities of individual triploid Superlines by 17 months in the Choptank River (Figure 4.3).

By 29 months, triploid hANA was the only Superline to have greater cumulative mortality than its diploid counterpart, though this difference was not significant (62.8±5.1% and 44.4±16.1%, respectively, $p>0.05$), and equate to a triploid disadvantage of (-41%) (Figure 4.4, Table 4.6). There were no significant differences among the cumulative mortalities of the individual diploid Superlines ($p>0.05$). From the triploids however, hANA had significantly greater mortality than Lola and SL-DBY (62.8±5.1%, 40.0±10.2%, and 34.0±8.8%, respectively, $p<0.05$). Triploid SL-XB had significantly greater mortality than SL-DBY (54.0±7.4% and 34.0±8.8%, respectively, $p<0.05$). The

cumulative mortalities of triploid lines Lola and SL-DBY were not significantly different (Figure 4.4).

Mortality

In the Choptank River, of the wild Virginia stocks, triploid WIC had a higher probability of mortality over the entire duration of the study than diploid WIC. There was no difference in predicted probability of death at any time for diploid and triploid RAP stocks (Figure 4.5). Both diploid wild Maryland stocks had higher probabilities of death over time than their triploid counterparts. Diploid and triploid CHES stocks had higher probabilities of mortality than diploid and triploid PATX stocks (Figure 4.6). From the 2006-year class lines, diploid:triploid comparisons are only available for DBY06 and LGT09. Both of these showed no difference in probabilities of mortality and were also not different from each other (Figure 4.7). Overall among the Superlines, similar patterns of predicted mortality occur: rise in mortality probability to 50-75%. Triploid hANA had a higher probability of mortality than its diploid counter part while triploid SL-XB had a lower probability of mortality than its diploid counterpart (Figure 4.8).

3.1.2 Rappahannock River, VA

Wild Virginia Stocks

By 17 months, triploidy had affected mortality in only one stock, RAP (Figure 4.3). Diploid RAP had lower cumulative mortality than its triploid counterpart (diploid: 6.3±5.1% and triploid 22.0±5.8%, $p<0.05$). Diploid WIC and MBY had similar cumulative mortalities and were both significantly higher than RAP (15.7±3.3%, 12.7±1.7%, and 6.3±5.1%, respectively, $p<0.05$). There were no differences among

cumulative mortalities of individual triploid wild Virginia stocks by 17 months (Figure 4.3).

By 29 months, WIC and MBY had triploid advantages of 46%, 39%, respectively, while RAP had a triploid disadvantage of (-7%), but differences in diploid:triploid comparisons were not significant (Table 4.6). There were no differences among cumulative mortalities of individual diploid or triploid wild Virginia stocks by the end of the study (Figure 4.4).

Wild Maryland Stocks

By 17 months, the effect of triploidy was greater mortality in the triploid CHES stock than in the diploid CHES stock (33.3±4.9% and 14.0±7.2%, respectively, $p<0.05$) (Figure 4.3). Triploid PATX had lower mortality than diploid PATX, but this difference was not significant (12.0±3.4% and 42.7±24.9%, respectively, $p>0.05$). There were no differences among cumulative mortalities of individual diploid wild Maryland stocks. Of the individual triploid stocks, CHES had a higher cumulative mortality than PATX (33.3±4.9% and 12.0±3.4%, respectively, $p<0.05$) (Figure 4.3).

By 29 months, there was no effect of triploidy on cumulative mortality in the wild Maryland stocks (Figure 4.4) despite a triploid disadvantage of CHES (-13%) and a triploid advantage for PATX of 55% (Table 4.6). Among the individual diploid stocks, PATX had greater mortality than CHES, but this difference was not significant (56.3±18.6% and 39.0±15.1%, respectively, $p>0.05$). Of the individual triploid stocks, CHES had a higher cumulative mortality than PATX, but this difference was also not significant (44.0±10.5% and 25.3±8.2%, respectively, $p>0.05$) (Figure 4.4).

2006-Year Class

By 17 months, there was no effect of triploidy on cumulative mortality in the 2006-year class lines. There were no differences among cumulative mortalities of individual diploid and triploid 2006-year class lines by 17 months in the Choptank River (Figure 4.3).

By 29 months, DBY06 was the only line to have a significant triploid advantage (47%, Table 4.6). Diploid DBY06 had greater mortality than its triploid counterpart (59.7±8.4% and 31.3±9.9%, respectively, $p<0.05$). There were no differences among the individual diploid or triploid 2006-year class lines in the Rappahannock River (Figure 4.4).

Superlines

By 17 months, there was no effect of triploidy on cumulative mortality in the Superlines (Figure 4.3). Diploids SL-XB and SL-DBY had the highest cumulative mortalities of the diploid lines, but these differences were not significant (hANA: 13.7±8.9%; Lola: 15.3±10.0%; SL-DBY: 26.3±14.1%; SL-XB: 27.0±9.1%, $p>0.05$). Triploids hANA and SL-DBY had the highest cumulative mortalities of the triploid lines, but these differences were not significant (hANA: 32.3±15.2%; Lola: 16.0±4.5%; SL-DBY: 34.3±23.7%; SL-XB: 27.0±4.7%, $p>0.05$) (Figure 4.3).

By 29 months, there were no differences among any diploid:triploid comparisons (Figure 4.4). Even though the diploid:triploid comparisons did not yield differences, triploid advantages were estimated to range from (-7%) to 20% (Table 4.6). The only difference among the cumulative mortalities of the individual diploid Superlines was that SL-XB had greater mortality than Lola (53.7±12.0% and 27.7±9.8%, respectively, $p<0.05$). Similar to the diploid Superlines, the only difference among the cumulative

mortalities of the individual triploid Superlines was that SL-XB had greater mortality than Lola (42.7±2.4% and 29.7±9.8%, respectively, $p<0.05$) (Figure 4.4).

Mortality

In the Rappahannock River, of the wild Virginia stocks, triploid RAP had a higher probability of mortality over the entire duration of the study than diploid RAP. There was no difference in predicted probability of death at any time for diploid and triploid WIC or MBY stocks. Probability of mortality of these stocks gradual increased to between 35-50% by the end of the study (Figure 4.5). Diploid wild Maryland stock CHES had lower probabilities of death over time than their triploid counterparts. Diploid PATX had a higher probability of mortality throughout the study than its triploid counterpart (Figure 4.6). From the 2006-year class lines, the only diploid:triploid comparison that indicated an effect of triploidy was that of the diploid and triploid DBY06 lines. Diploid DBY06 continuously had a higher probability of mortality than its triploid counterpart (Figure 4.7). Triploid hANA had a higher probability of mortality than its diploid counter part. There were no other effects of triploidy in the Superlines (Figure 4.8).

3.1.3 York River, VA

Wild Virginia Stocks

By 17 months, triploidy had affected mortality in only one stock, WIC (Figure 4.3). Diploid WIC had greater cumulative mortality than its triploid counterpart (diploid: 48.7±5.5% and triploid 10.0±3.4%, $p<0.05$). Diploid WIC and RAP had similar cumulative mortalities and were both significantly higher than MBY (48.7±5.5%, 31.0±10.3%, and 15.3±5.8%, respectively, $p<0.05$). There were no differences among

cumulative mortalities of individual triploid wild Virginia stocks by 17 months (Figure 4.3).

By 29 months, the triploid advantage had manifested in two stocks, WIC and RAP (Figure 4.4). Both WIC and RAP had higher mortalities as diploids than triploids (WIC: diploid 77.5±9.1% and triploid 46.8±8.3%; RAP: diploid 60.4±5.0% and triploid 40.0±2.3%, $p<0.05$) equating to a triploid advantage of 40% and 34%, respectively (Figure 4.6). Diploid MBY and RAP had similar cumulative mortalities and were both significantly lower than WIC (51.6±10.5%, 60.4±5.0%, and 77.5±9.1%, respectively, $p<0.05$). There were no differences among cumulative mortalities of individual triploid wild Virginia stocks by the end of the study (Figure 4.4).

Wild Maryland Stocks

By 17 months, the effect of triploidy was greater mortality in the diploid PATX stock than in the triploid PATX stock (70.7±2.6% and 13.7±6.0%, respectively, $p<0.05$) (Figure 4.3). Diploid CHES had greater mortality than triploid CHES, but this difference was not significant (44.7±19.1% and 22.0±13.5%, respectively, $p>0.05$). Diploid PATX had higher mortality by 17 months than CHES (70.7±2.6% and 44.7±19.1%, respectively, $p<0.05$). There were no differences among the individual triploid stocks ($p>0.05$) (Figure 4.3).

By 29 months, a triploid advantage for cumulative mortality was present in both wild Maryland stocks (Figure 4.4). Diploids CHES and PATX both had significantly higher mortalities than their triploid counter parts (CHES: diploid 79.5±7.8% and triploid 52.5±7.5%; PATX: diploid 91.5±3.8% and triploid 44.0±5.8%, $p<0.05$) resulting in triploid advantages of 35% for CHES and 52% for PATX (Table 4.6). Among the

individual diploid or among the individual triploid stocks there were no significant differences in cumulative mortality ($p>0.05$) (Figure 4.4).

2006-Year Class

By 17 months, all four of the diploid 2006-year class lines had greater cumulative mortalities than their triploid counterparts (DBY06: diploid 33.7±11.1% and triploid 7.0±3.9%; XB09: diploid 34.3±5.8% and triploid 12.7±5.9%; OBOY09: diploid 77.7±8.2% and triploid 17.7±5.2%; LGT09: diploid 41.3±13.6% and triploid 14.0±3.8%, $p<0.05$). Among the individual diploid lines, OBOY09 had higher mortality than the remaining 2006-year class lines ($p<0.05$). Among cumulative mortalities of individual triploid 2006-year class lines, the only significant difference was OBOY09 having higher mortality than DBY06 ($p<0.05$) (Figure 4.3).

By 29 months, all but LGT09 had greater cumulative mortalities than their triploid counterparts (DBY06: diploid 50.4±11.1% and triploid 22.3±3.6%; XB09: diploid 59.0±7.2% and triploid 29.2±6.1%; OBOY09: diploid 94.0±2.8% triploid 68.8±5.8%, $p<0.05$). Triploid advantages for cumulative mortality in the 2006-year class lines ranged from 27% to 56% (DBY06 – 56%, XB09 – 51%, OBOY09 – 27%, and LGT09 – 45%, Table 4.6). Diploid OBOY09 had greater mortality than all of the 2006-year class diploid lines (OBOY09: 94.0±2.8%, DBY06: 50.4±11.1%, XB09: 59.0±7.2%, LGT09: 64.0±21.0%, $p<0.05$). Among individual triploid lines, OBOY09 had greater mortality than all of the 2006-year class diploid lines (OBOY09: 68.8±5.8%, DBY06: 22.3±3.6%, XB09: 29.2±6.1%, LGT09: 35.0±7.9%, $p<0.05$). There were no other differences among individual diploid or among individual triploid lines (Figure 4.4).

Superlines

By 17 months, all four of the diploid Superlines had greater cumulative mortalities than their triploid counterparts (hANA: diploid 44.7±14.4% and triploid 10.3±4.0%; Lola: diploid 46.7±14.4% and triploid 13.0±4.9%; SL-DBY: diploid 35.7±9.3% and triploid 3.7±2.6%; SL-XB: diploid 62.0±10.0% and triploid 15.0±4.7%, $p<0.05$). The only difference among individual diploid Superlines was that SL-XB had a higher mortality than SL-DBY (62.0±10.0% and 35.7±9.3%, respectively, $p<0.05$). Triploids hANA, Lola, and SL-XB all had significantly higher mortalities than SL-DBY (10.3±4.0%, 13.0±4.9%, 15.0±4.7%, and 3.7±2.6%, respectively, $p<0.05$) (Figure 4.3).

By 29 months, all four of the diploid Superlines had greater cumulative mortalities than their triploid counterparts (hANA: diploid 72.5±15.4% and triploid 36.0±4.7%; Lola: diploid 73.6±11.3% and triploid 41.2±11.5%; SL-DBY: diploid 51.3±4.0% and triploid 24.0±7.6%; SL-XB: diploid 79.3±6.0% and triploid 44.0±6.1%, $p<0.05$). The triploid advantage for mortality was 50% for hANA, 44% for Lola, 53% for SL-DBY, and 45% for SL-XB (Table 4.6). Diploids hANA, Lola, and SL-XB all had significantly higher mortalities than SL-DBY (72.5±15.4%, 73.6±11.3%, 79.3±6.0%, and 51.3±4.0%, respectively, $p<0.05$). Both triploid Superlines hANA and SL-XB had significantly greater mortalities than SL-DBY (36.0±4.7%, 44.0±6.1%, and 24.0±7.6%, respectively, $p<0.05$) (Figure 4.4).

Mortality functions

In the York River, all stocks and lines had higher probabilities of mortality as diploids than as triploids. The probability of mortality among triploid stocks/lines was highest in the wild Maryland stocks (mean 94%, Figure 4.5). The wild Virginia triploid stocks had the next highest probability of mortality (mean 68%, Figure 4.6), followed by

the 2006-year class lines (mean 39%, Figure 4.7), and the triploid Superlines had the lowest estimated probabilities of mortality (mean 31%, Figure 4.8).

3.2 Disease

3.2.1 Perkinsus marinus

3.2.1.1 York River, VA

Wild Virginia Stocks

In the first year (October 2011), *P. marinus* infections were found in all three of the diploid wild Virginia stocks (Table 4.3, Figure 4.9). Prevalence of *P. marinus* infections in the diploid stocks ranged from 6.25-12.50%, with diploid RAP having the highest infection rate. Infection intensity in the diploid stocks was rare for WIC and RAP and light for MBY (intensity descriptions are described in Table 4.2). The triploid WIC stock was the only triploid wild Virginia stock to show any indication of *P. marinus* infection. Infection prevalence in the triploid WIC stock was 6.25% with an infection intensity rated as light (Table 4.3, Figure 4.9).

By the second year (November 2012), the prevalence of *P. marinus* infections increased to 80% for the diploid RAP stock, 87.5% for the diploid WIC stock, and 93.75% for the diploid MBY stock (Table 4.4, Figure 4.9). Infection intensity in the diploid WIC stock ranged from very light to moderate, rare to very heavy in the diploid MBY stock, and from rare to moderate-to-heavy in the diploid RAP stock. By November 2012 all three triploid stocks had *P. marinus* infections. Infection prevalence for triploid stocks WIC and MBY was 31.25% and 50% for RAP. The highest infection intensity in all three of the triploid wild Virginia stocks was rated as light (Table 4.4, Figure 4.9).

Wild Maryland Stocks

Both wild Maryland stocks (CHES and PATX) had *P. marinus* infections in the first year (at October 2011) (Table 4.3, Figure 4.9). Prevalence of *P. marinus* infections in the diploid stocks was 50% for the CHES stock and 75% for the PATX stock. Infection intensity in the diploid CHES stock ranged from rare to heavy and for the PATX diploid stock from very light to heavy. The triploid CHES stock was the only triploid wild Maryland stock to show any indication of *P. marinus* infection. Infection prevalence in the triploid CHES stock was 6.25% with an infection intensity rated as light (Table 4.3, Figure 4.9).

By November 2012, the prevalence of *P. marinus* infections increased to 100% for the diploid CHES stock (Table 4.4, Figure 4.9). The diploid PATX growth replicates from which samples are drawn for disease analysis did not survive to November 2012. Infection intensity in the diploid CHES stock ranged from light to very light. By November 2012, both triploid stocks had *P. marinus* infections. Infection prevalence for triploid stock CHES was 50% and 14.29% for triploid PATX. Infection intensity ranged from very light to light-to-moderate in the triploid CHES stock and from very light to light in the triploid PATX stock (Table 4.4, Figure 4.9).

2006-Year Class

By October 2011, all of the 2006-year class lines, except DBY06, had *P. marinus* infections (Table 4.3, Figure 4.9). Prevalence of *P. marinus* infections in the diploid stocks was 18.75% for XB09, 6.25% for OBOY09, and 12.5% for LGT09. Infection intensity for XB09 ranged from rare to light-to-moderate, OBOY09 infection intensity was rated as light, and LGT09 ranged from very light to moderate. None of the triploid

2006-year class lines presented any indication of *P. marinus* infections (Table 4.3, Figure 4.9).

By November 2012, the prevalence of *P. marinus* infections increased in all 2006-year class lines (Table 4.4, Figure 4.9). The diploid XB09 growth replicates that samples were taken from for disease analysis did not survive to November 2012. Prevalence of infection in the diploid DBY06 line was 81.25%, 68.75% in the OBOY09 line, and to 100% for the LGT09 line. Infection intensity in the diploid 2006-year class lines ranged from rare to moderate. By November 2012, all four of the 2006-year class lines had *P. marinus* infections. Of the triploid lines, infection prevalence was 25% for DBY06, 37.5% for XB09, 25% for OBOY09, and 18.75% for LGT09. Infection intensity ranged from rare to light for both DBY06 and XB09, rare to very light for LGT09, and rare for OBOY09 (Table 4.4, Figure 4.9).

Superlines

By October 2011, all four Superlines had *P. marinus* infections. Prevalence of *P. marinus* infections in the diploid Superlines ranged from 18.75-37.50% (hANA: 37.5%, Lola: 18.75%, SL-DBY: 25%, and SL-XB: 18.75%) (Table 4.3, Figure 4.9). Infection intensity in the diploid Superline hANA ranged from rare to heavy, in Lola from rare to moderate-to-heavy, in SL-DBY from rare to light, and in SL-XB from very light to heavy. The triploid line SL-DBY was the only triploid Superline to show any indication of *P. marinus* infection. Infection prevalence in this line was 6.25% with an infection intensity rated as heavy (Table 4.3, Figure 4.9).

By November 2012, the prevalence of *P. marinus* infections remained at 37.5% in the diploid hANA line, but increased for the remaining three Superlines (Table 4.4,

Figure 4.9). The diploid line Lola had an infection prevalence of 100%, 50% for SL-DBY, and 87.5% for SL-XB. Infection intensity in the diploid line hANA ranged from rare to moderate. Both diploid lines Lola and SL-XB had infection intensities ranging from very light to moderate-to-heavy. The infection intensity of SL-DBY ranged from rare to moderate-to-heavy. All four triploid Superlines had *P. marinus* infections by November 2012. Infection prevalence for triploid lines ranged from 6.67-37.5% (hANA: 37.5%, Lola: 37.5%, SL-DBY: 6.67%, and SL-XB: 18.75%). Infection intensity for the triploid hANA line ranged from rare to moderate, for the Lola line rare to light, rare for the SL-DBY line, and the SL-XB line ranged from rare to light (Table 4.4, Figure 4.9).

3.2.2 MSX

3.2.2.1 York River, VA

Wild Virginia Stocks

Two of the wild Virginia stocks had *H. nelsoni* infections (WIC and MBY). Prevalence of *H. nelsoni* infections in the diploid stocks was 13.33% for the WIC stock and 6.67% for the MBY stock. Infection intensity in the diploid WIC stock ranged from light to heavy and for the MBY diploid stock at rare. The triploid wild Virginia stocks had no *H. nelsoni* infections (Table 4.5, Figure 4.10).

Wild Maryland Stocks

There was no *H. nelsoni* in either of the wild Maryland stocks (CHES or PATX). The triploid CHES stock was the only triploid wild Maryland stock to show any indication of *H. nelsoni* infection. Infection prevalence in the triploid CHES stock was 12.5% with an infection intensity rated as light (Table 4.5, Figure 4.10).

2006-Year Class

None of the diploid lines of the 2006-year class had *H. nelsoni* infections. Diploid OBOY09 was not sampled for *H. nelsoni* infections because of low survival. The triploid 2006-year class line XB09 had no infection. The remaining three lines had *H. nelsoni* infections with a prevalence of 6.67% each. The infection intensity of DBY06 was rated as moderate and for both XB09 and LGT09, light (Table 4.5, Figure 4.10).

Superlines

SL-XB was the only diploid Superline to show a *H. nelsoni* infection. Prevalence of this infection was estimated to be 6.67% with the infection intensity rated as light. Triploid Superlines hANA and SL-XB had *H. nelsoni* infections. Infection prevalence for triploid hANA was 10% and 6.67% for triploid SL-XB. Infection intensity for hANA was rated as light and rare for SL-XB (Table 4.5, Figure 4.10).

4. DISCUSSION

This study examined the effect of the diploid female on triploids created from tetraploid × diploid crosses. Five populations of wild oysters were used for selected tetraploid × wild-type diploid crosses and eight lines of selected oysters were used to for selected tetraploid × selected diploid crosses. Metrics for this Chapter were restricted to mortality and Dermo and MSX disease prevalence and intensity. The results show that *i*) mortality was influenced heavily by both the genetic contribution of the diploid parent and the environment and *ii*) triploidy has a positive effect on Dermo resistance.

The tetraploid broodstock used in the triploid crosses were produced from disease resistant lines. Tetraploids, possessing four sets of chromosomes and, in this case, chromosomes selected for disease resistance (SSSS), were bred with either selected diploids (SS) or wild-type diploids (++) producing selected triploids (SSS) or hybrid triploids (SS+). The genetic origin of the tetraploid parent biases of the test for disease susceptibility in the diploid parent of triploid crosses ($4n \times 2n$) because triploids from wild-type diploid females have two of their three sets of chromosomes from disease resistant tetraploids. The +++ genotype could not be tested.

4.1 Choptank River

There was no triploid advantage for survival in the Choptank River. On the contrary, there seems to be a triploid disadvantage, at least for the following crosses: WIC (-140%), RAP (-62%), CHES (-67%), and hANA (-41%) (Table 4.6). In this study,

low salinity was a stress that likely influenced survival, as well as growth, in the Choptank River. The average salinity in the Choptank River during the study period was 9 ppt and ranged from 6 ppt to 13 ppt (Figure 2.5), a range commonly accepted as below optimum for oysters (i.e., 14 – 28 ppt) (Galstoff 1964; Loosanoff 1965). Low salinity negatively affects many aspects of oyster biology by reducing valve activity (Loosanoff, 1952; Galtsoff, 1964), reduce feeding efficiency (Loosanoff, 1952), reduce respiration regulation (Shumway and Koehn, 1982), depress gametogenesis (Butler, 1949; Loosanoff, 1952; Calabrese and Davis, 1970), growth (Loosanoff, 1952; Chanley, 1958; Davis, 1994), and even survival (Butler, 1952, 1954). Most studies that have investigated the effect of salinity on oysters focused on acute fluctuations in salinity, however, some of these effects last even after oysters have generally acclimated to the salinity change affecting the general health and condition of oysters after initial salinity fluctuations.

Oysters in this study were spawned and reared in the ABC Gloucester Point hatchery that experiences a salinity range (13-25 ppt), higher than that at the Choptank River site. Juvenile oysters spawned and reared at the higher salinity at the ABC hatchery certainly must have experienced an acute shock when transferred to the Choptank. Significant mortality was not observed after transfer of juveniles, but the overall effect may have put oysters in the Choptank at a disadvantage relative to their cohorts at the other experimental sites.

In the low salinity environment of the Choptank River the diploid wild stocks, both from Virginia and Maryland, had the lowest cumulative mortalities by the end of the study and, consequently, the greatest triploid disadvantage (Figure 4.4, Table 4.6). In contrast to the wild stocks, the 2006-year class lines and the Superlines had similar

mortalities whether they were diploid and triploid. It is possible that the poor survival of these eight selectively bred lines, compared to wild stocks, was due to adaptation to mesohaline conditions through artificial selection, with the exception of Superline Lola. Lola, a low salinity line, had the lowest mortality of diploid (2n Lola – 40%) and second lowest of the triploid (3n Lola – 40%) selected lines (behind SL-DBY – 34%) at the end of the study. Poor survival due to mesohaline adaptation is supported by the contrasting survival of diploid and triploid wild stocks. Triploid wild stocks have two chromosome sets that are adapted for mesohaline environments (SS+). It was after this addition of selectively bred genetic material that oysters become disadvantaged for survival.

The triploid stock with the lowest cumulative mortality originated from a wild population in Maryland (PATX). The superior performance of a stock obtained from lower salinity waters suggests some inherent ability to survive the stress of low salinity in this population compared to oysters native to higher salinity (e.g., wild Virginia stocks) and those selected for several generations in higher salinity environments did not survival as well. Contrary to the triploid advantage experienced by the PATX stock (29%), the CHES stock, also obtained from lower salinity waters, experienced a triploid disadvantage (-67%). CHES and PATX have similar mortality rates as diploids (21% and 22%, respectively) but differed drastically as triploids. Due to the contrary triploid advantages, it becomes difficult to surmise that one dose of low salinity genes (from the wild-type parent) would be enough to compensate any low salinity stress. The PATX stock did poorly at other sites as well.

4.2 Rappahannock River

The Rappahannock River is characterized as a 'good' growing site because it falls within the optimum salinity range for oysters of 14 – 28 ppt and during this study had no disease pressure. During the course of this study, however, no disease (Dermo or MSX) was detected in the Rappahannock River study site and only DBY06 had triploid advantage for mortality (+47%). With the absence of disease and other environmental stresses, like low salinity, it is notable that the mortality rates of selected triploids were not significantly different from their diploid counterparts (with the exception of DBY06). It appears from this observation that with the lack of significant stressors (low salinity or disease) at the Rappahannock River, triploidy provides little advantage for survival. The absence of a triploid advantage for mortality in environments with negligible disease pressure has been observed by other investigators confirming triploid provide little advantage for survival when there are few or no major external sources of mortality (Dégremont et al., 2012, Matthiessen and Davis, 1992).

4.3 York River

In the York River, where salinity and disease pressure are high, mortality was greater in diploids than triploids for all stocks/lines. All but two stocks (wild VA stock MBY and 2006-year class line LGT09) had a triploid advantage for survival in the York River. Mortality was, in part, influenced by Dermo disease, evident from the significantly greater prevalence and intensity observed in diploids than triploids (Figure 4.9) and a lack of observed MSX infections (Figure 4.10). The impacts of *P. marinus* were likely not the only factor in oyster mortality because infection intensities observed were not heavy enough to lead to significant mortality that could be attributed solely to Dermo.

One of more remarkable differences between diploids and triploids in all crosses is the lack of Dermo infections in triploids compared to diploids. The two wild Maryland stocks had the greatest infection intensity and prevalence as diploids but as triploids had similar infections as the other triploid stocks/lines (Figure 4.9; Table 4.4). Attempting to partition the source of disease resistance in this study is confounded by the source population of the tetraploids used in triploid crosses ($4n \times 2n$), which originated from domesticated diploid lines that were bred for increased survival in the presence of disease stress. As a result, all triploids, regardless of the origin of the diploid parent (wild-type or selectively bred), possess at least two sets of chromosomes from a selectively bred line.

All triploid stocks and lines had a lower prevalence of Dermo disease than their diploid counterparts by December 2012 (Figure 4.4). The advantage of triploids on Dermo disease resistance in the literature is not consistent with the findings of this study. One study, using three disease resistant lines at three sites in the Chesapeake Bay, observed that the differences in Dermo disease infections were driven by line. There were no significant differences in infection rates between diploid and triploid oysters (Dégremont et al., 2012). In another study, difference between diploid and triploid oysters were not found after exposure to infective Dermo cells. 100% of diploids and 98% of triploid died after 150 days (Meyers et al., 1991). The observations of the present study show a clear advantage of triploidy on Dermo disease. At the end of the first year in the field (December 2011), most diploid groups had Dermo infections though the infection prevalence observed in the diploid groups was low. In the triploid stocks/lines, Dermo prevalence was low and only two wild stocks (WIC and CHES) and one Superline (SL-DBY) had any infections. By December 2012, all stocks/lines as diploid and triploid

showed the presence of Dermo disease but the prevalence was notably less in triploids (7-50%) than diploids (38-100%). The average prevalence of the wild Virginia stocks was 87% for diploids compared to 38% for triploids. The wild Maryland diploids, being naïve to disease pressure, had a prevalence of 100% compared to 32% for triploids. The selected groups, like both wild groups, saw a triploid advantage for resistance to Dermo disease with diploids having greater prevalence than triploids (2006-lines: diploid – 83% vs. triploid – 27% and Superlines: diploid – 69% vs. triploid – 25%). One striking detail about Dermo prevalence as it relates to the triploid advantage is that the prevalence among all triploid groups are similar (wild VA – 38%, wild MD – 32%, 2006-lines – 27%, and Superlines – 25%). Given the triploids groups had similar infection prevalence, it appears that triploidy affects Dermo resistance positively regardless of the diploid parent's genetic origin. Sources of the advantage may be results of physiological changes due to triploid sterility rather than resistance *per se*.

Resistance of triploids to Dermo infections may be a result of increased energetic reserves from a lack of spawning activity. Tissue lysis has often been attributed as a major cause of death for oysters with Dermo disease (Mackin, 1951b; Ray, 1954; Perkins, 1976), but given the documented differences in energy reserves of diploid and triploid oysters (Allen and Downing, 1986) the likely effects of Dermo on energy demand in oysters cannot be overlooked. Choi et al. (1989) investigated the energy demand of Dermo at varying infection intensities and determined that the depletion of energy from the host was often sufficient to account for the deleterious effects of Dermo disease, such as, reduced somatic and gametic growth. The nutrient depletion hypothesis was reinforced by Newell et al. (1994) when the authors could not find Dermo induced

changes in metabolic rates of host oysters indicating that oysters were not regulating metabolic activity to compensate for nutrient loss to parasites. Nutrient depletion as a source of deleterious Dermo effects fits soundly with the observations of this study. If triploid oysters maintain greater energy reserves as a consequence of triploid sterility, it follows that they would have a greater energy supply that would need to be competed for before Dermo would cause any negative effects from nutrient depletion.

Chemically induced triploids are reported to have lower mortality and higher condition index at comparable MSX infections indicating that triploids may have a greater ability to tolerate the deleterious effects of MSX than diploids but may not necessarily be more resistant (Mattiessen and Davis, 1992). Another study, using mated triploids ($4n$ x $2n$), found a mix of lower and comparable incidence rates of MSX from three triploid lines compared to three diploid lines (Dégremont et al., 2012).

In this study, MSX infections were low in the York River in 2012 and because of this, few conclusions can be drawn about MSX infection for ploidy comparison. Wild Virginia stocks WIC and MBY and Superline XB were the only diploid stocks/lines to show MSX infections and while several triploid wild stocks and selected lines had MSX infections the intensities were low and as a result it is unlikely that mortality could be caused by MSX infections in this study.

4.4 Conclusions

Overall, it appears that there is a triploid advantage for resistance to Dermo disease that may be metabolically mediated. For MSX however, no conclusions can be drawn because of the light disease loads observed in this study, even in "susceptible" diploids. These results provide growers valuable information regarding broodstock

selection when disease is of concern. When considering oyster culture under Dermo pressure, the differences among triploid lines produced from various diploid females are minimal making broodstock selection simple: use any diploid.

In an environment with no disease pressure and otherwise suitable growing conditions (e.g., Rappahannock River), triploidy significantly decreased mortality for only one line (2006-year class line DBY06). In fact, mortality rates of wild-mated triploids were, on average, comparable to that of mated triploids from selected diploid lines (wild VA stocks – 30%; wild MD stocks – 35%; 2006-lines – 32%; Superlines – 40%). Conversely, in a 'poor' growing environment (Choptank River) triploidy actually produced a disadvantage for survival.

Environment by genotype interactions play a significant role is oyster performance. Examining the changes in the relationship between diploid and triploid mortality rates of a given stock/line across environments illustrate the effect of E×G on mortality rates (Figures 4.5-4.8). For example, wild Virginia stock WIC is estimated to have significantly greater mortality in triploids than diploids in the low salinity environment indicating that triploidy in the WIC stock is disadvantageous for survival in this setting. In the Rappahannock River, where oysters experienced little stress from salinity or disease, there was little difference in the predicted probability of mortality in diploid and triploid WIC stocks. Finally, under disease pressure in the York River, triploids were estimated to have lower mortality rates through time than diploids. An effect of triploidy that ranges from disadvantageous to advantageous follows the notion that triploidy may be thought of as a tool useful in some applications and not in others.

For low salinity, it appears that triploidy may not be the proper tool but for survival under disease pressure, it is.

		Diploid		Tetraploid
Stock	Type	No. dams	No. of sires	No of sires
WIC	++	10	8	11
MBY	++	6	12	10
RAP	++	10	7	11
CHES	++	10	6	10
PATX	++	10	11	11
LGT09	'06	12	26	11
OBOY09	'06	10	17	10
DBY09	'06	10	10	11
XB06	'06	29	21	10
hANA	SL	10	9	10
Lola	SL	15	11	11
SL-DBY	SL	10	10	11
SL-XB	SL	10	10	11

Table 4.1: Number of *C. virginica* broodstock used per stock (wild) or line (selected) to generate diploid and triploid offspring. For diploids, diploid dams and sires were used; for triploids, diploid dams and one set of 11 tetraploid sires (pooled sperm). ++ = wild stock; '06 = 2006-year class lines; SL = selected Superlines; Rivers systems for wild stocks: WIC = Wicomico, Virginia; MBY = Mobjack Bay, Virginia; RAP = Rappahanock, Virginia; CHES = Chester, Maryland; PATX = Patuxent, Maryland.

Disease Intensity		Numeric Score	Characteristic
Negative	N	0	No parasites found
Rare	R	0.5	1-2 cells found in entire preparation
Very light	VL	1	3-10 cells found in entire preparation
Light	L	1	11-100 cells present in entire preparation
Light-to-moderate	LM	2	Localized concentrations of >25 cells or 1-2 cells in each field of view at 100X magnification
Moderate	M	3	>3 cells in each field of view at 100X magnification
Moderate-to-heavy	MH	4	Parasites present in large numbers. Less than half of preparation showing macroscopic blue reaction
Heavy	H	5	Majority of tissue appears green-blue macroscopically
Very heavy	VH	5	Entire tissue preparation appears blue-black macroscopically

Table 4.2: *Perkinsus marinus* infection intensity ratings and descriptions modified from Quick and Mackin (1971) for used with RFTM assays. Numeric scores listed are those used to calculate weighted prevalences.

Diploids											
				Intensity level (%)							
Group	Stock	n	% infected	R	VL	L	LM	M	MH	H	VH
VA++	WIC	16	6.25	6.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	MBY	16	6.25	0.00	0.00	6.25	0.00	0.00	0.00	0.00	0.00
	RAP	16	12.50	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MD++	CHES	16	50.00	6.25	6.25	12.50	0.00	6.25	6.25	12.50	0.00
	PATX	16	75.00	0.00	12.50	25.00	12.50	6.25	6.25	12.50	0.00
2006 Lines	DBY06	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	XB09	16	18.75	12.50	0.00	0.00	6.25	0.00	0.00	0.00	0.00
	OBOY09	16	6.25	0.00	0.00	6.25	0.00	0.00	0.00	0.00	0.00
	LGT09	16	12.50	0.00	6.25	0.00	0.00	6.25	0.00	0.00	0.00
Superlines	hANA	16	37.50	12.50	6.25	6.25	0.00	6.25	0.00	6.25	0.00
	Lola	16	18.75	6.25	6.25	0.00	6.25	0.00	0.00	0.00	0.00
	SL-DBY	16	25.00	6.25	0.00	18.75	0.00	0.00	0.00	0.00	0.00
	SL-XB	16	18.75	0.00	6.25	0.00	0.00	6.25	0.00	6.25	0.00

Triploids											
				Intensity level (%)							
Group	Stock	n	% infected	R	VL	L	LM	M	MH	H	VH
VA++	WIC	16	6.25	0.00	0.00	6.25	0.00	0.00	0.00	0.00	0.00
	MBY	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	RAP	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MD++	CHES	16	6.25	0.00	0.00	6.25	0.00	0.00	0.00	0.00	0.00
	PATX	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2006 Lines	DBY06	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	XB09	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	OBOY09	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	LGT09	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Superlines	hANA	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Lola	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SL-DBY	16	6.25	0.00	0.00	0.00	0.00	0.00	0.00	6.25	0.00
	SL-XB	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.3: *Perkinsus marinus* infections (n = number of oysters tested) in the York River, VA in October 2011 of diploid and triploid Wild Virginia Stocks (VA++), Wild Maryland Stocks (MD++), 2006-Year class lines (2006 Lines), and selected Superlines (Superlines). Percent infected and intensity level (%) are based on the sample size (n). Intensity levels were recorded as Rare (R), Very light (VL), Light (L), Light-to-moderate (LM), Moderate (M), Moderate-to-heavy (MH), Heavy (H), or Very heavy (VH). Descriptions of intensity levels are given in Table 4.2.

Table 4.4

Diploids

Group	Stock	n	% infected	Intensity level (%)							
				R	VL	L	LM	M	MH	H	VH
VA++	WIC	8	87.50	0.00	37.50	12.50	25.00	12.50	0.00	0.00	0.00
	MBY	16	93.75	18.75	12.50	12.50	12.50	12.50	12.50	6.25	6.25
	RAP	15	80.00	6.67	20.00	33.33	6.67	6.67	6.67	0.00	0.00
MD++	CHES	16	100.00	0.00	0.00	12.50	12.50	37.50	18.75	6.25	12.50
	PATX	-	-	-	-	-	-	-	-	-	-
2006 Lines	DBY06	16	81.25	18.75	6.25	18.75	6.25	31.25	0.00	0.00	0.00
	XB09	-	-	-	-	-	-	-	-	-	-
	OBOY09	16	68.75	12.50	6.25	12.50	18.75	18.75	0.00	0.00	0.00
	LGT09	16	100.00	6.25	6.25	12.50	0.00	18.75	0.00	0.00	0.00
Superlines	hANA	16	37.50	18.75	6.25	6.25	0.00	6.25	0.00	0.00	0.00
	Lola	7	100.00	0.00	42.86	28.57	14.29	0.00	14.29	0.00	0.00
	SL-DBY	16	50.00	12.50	18.75	6.25	6.25	0.00	6.25	0.00	0.00
	SL-XB	8	87.50	0.00	25.00	12.50	25.00	0.00	25.00	0.00	0.00

Triploids

Group	Stock	n	% infected	Intensity level (%)							
				R	VL	L	LM	M	MH	H	VH
VA++	WIC	16	31.25	0.00	6.25	25.00	0.00	0.00	0.00	0.00	0.00
	MBY	16	31.25	6.25	6.25	18.75	0.00	0.00	0.00	0.00	0.00
	RAP	8	50.00	0.00	25.00	12.50	0.00	0.00	12.50	0.00	0.00
MD++	CHES	16	50.00	0.00	18.75	12.50	18.75	0.00	0.00	0.00	0.00
	PATX	14	14.29	0.00	7.14	7.14	0.00	0.00	0.00	0.00	0.00
2006 Lines	DBY06	16	25.00	6.25	0.00	18.75	0.00	0.00	0.00	0.00	0.00
	XB09	8	75.00	12.50	25.00	37.50	0.00	0.00	0.00	0.00	0.00
	OBOY09	16	25.00	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	LGT09	16	18.75	6.25	12.50	0.00	0.00	0.00	0.00	0.00	0.00
Superlines	hANA	16	37.50	18.75	6.25	6.25	0.00	6.25	0.00	0.00	0.00
	Lola	16	37.50	6.25	18.75	12.50	0.00	0.00	0.00	0.00	0.00
	SL-DBY	15	6.67	6.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SL-XB	16	18.75	6.25	6.25	6.25	0.00	0.00	0.00	0.00	0.00

Table 4.4: *Perkinsus marinus* infections (n = number of oysters tested) in the York River, VA in November 2012 of diploid and triploid Wild Virginia Stocks (VA++), Wild Maryland Stocks (MD++), 2006-Year class lines (2006 Lines), and selected Superlines (Superlines). Percent infected and intensity level (%) are based on the sample size (n). Intensity levels were recorded as Rare (R), Very light (VL), Light (L), Light-to-moderate (LM), Moderate (M), Moderate-to-heavy (MH), Heavy (H), or Very heavy (VH). Descriptions of intensity levels are given in Table 4.2.

Diploids							
				Intensity level (%)			
Group	Stock	n	% infected	R	L	M	H
VA++	WIC	15	13.33	0.00	6.67	0.00	6.67
	MBY	15	6.67	6.67	0.00	0.00	0.00
	RAP	8	0.00	0.00	0.00	0.00	0.00
MD++	CHES	15	0.00	0.00	0.00	0.00	0.00
	PATX	15	0.00	0.00	0.00	0.00	0.00
2006 Lines	DBY06	15	0.00	0.00	0.00	0.00	0.00
	XB09	15	0.00	0.00	0.00	0.00	0.00
	OBOY09	-	-	-	-	-	-
	LGT09	15	0.00	0.00	0.00	0.00	0.00
Superlines	hANA	15	0.00	0.00	0.00	0.00	0.00
	Lola	14	0.00	0.00	0.00	0.00	0.00
	SL-DBY	15	0.00	0.00	0.00	0.00	0.00
	SL-XB	15	6.67	0.00	6.67	0.00	0.00

Triploids							
				Intensity level (%)			
Group	Stock	n	% infected	R	L	M	H
VA++	WIC	15	0.00	0.00	0.00	0.00	0.00
	MBY	15	0.00	0.00	0.00	0.00	0.00
	RAP	15	0.00	0.00	0.00	0.00	0.00
MD++	CHES	16	12.50	0.00	12.50	0.00	0.00
	PATX	15	0.00	0.00	0.00	0.00	0.00
2006 Lines	DBY06	15	6.67	0.00	0.00	6.67	0.00
	XB09	15	0.00	0.00	0.00	0.00	0.00
	OBOY09	15	6.67	0.00	6.67	0.00	0.00
	LGT09	15	6.67	0.00	6.67	0.00	0.00
Superlines	hANA	10	10.00	0.00	10.00	0.00	0.00
	Lola	16	0.00	0.00	0.00	0.00	0.00
	SL-DBY	15	0.00	0.00	0.00	0.00	0.00
	SL-XB	15	6.67	6.67	0.00	0.00	0.00

Table 4.5: *Haplosporidium nelsoni* infections (n = number of oysters tested) in the York River, VA in May 2012 of diploid and triploid Wild Virginia Stocks (VA++), Wild Maryland Stocks (MD++), 2006-Year class lines (2006 Lines), and selected Superlines (Superlines). Percent infected and intensity level (%) are based on the sample size (n). Intensity levels were recorded as Rare (R), Very light (VL), Light (L), Light-to-moderate (LM), Moderate (M), Moderate-to-heavy (MH), Heavy (H), or Very heavy (VH). Descriptions of intensity levels are given in Table 4.2.

		Mortality		
		Experimental site		
Group	**Line**	**MD**	**RR**	**YR**
Wild VA	WIC	*(140)	46	*40
	MBY	ND	39	30
	RAP	(62)	(7)	*34
Wild MD	CHES	(67)	(13)	*34
	PATX	29	55	*52
2006-year class lines	DBY06	1	*47	*56
	XB09	ND	ND	*51
	OBOY09	No 2n	10	*27
	LGT09	17	42	45
Superlines	hANA	*(41)	(2)	*50
	Lola	0	(7)	*44
	SL-DBY	22	3	*53
	SL-XB	4	20	*45

Table 4.6: Estimated triploid advantage (%) at 29 months (December 2012) of wild stocks, 2006-year class lines, and Superlines in the Choptank (MD), Rappahannock (RR), and York Rivers (YR). Parentheses indicate negative values. '*' Indicates the difference between diploid and triploid stocks/lines are significant at the alpha = 0.05 level. 'ND' signifies the stock or line was not deployed in a given site.

DIPLOID

♀ / ♂	VA++			MD++		2006-year class lines				F1 Superlines			
	WIC	MBY	RAP	CHES	PATX	LGT09	OBOY 09	DBY09	XB06	hANA	Lola	SL-DBY	SL-XB
WIC	2n												
MBY		2n											
RAP			2n										
CHES				2n									
PATX					2n								
LGT09						2n							
OBOY09							2n						
DBY09								2n					
XB06									2n				
hANA										2n			
Lola											2n		
SL-DBY												2n	
SL-XB													2n

TETRAPLOID

4B	3n	3n	3n	3n	3n	3n	3n	3n	3n	3n	3n	3n	3n
	VA++			MD++		2006-year class lines				F1 Superlines			

Figure 4.1: Mating design for diploid and triploid crosses of *C. virginica*. Solid boxes indicate which crosses were made. Aliquots of pooled eggs were split in half for diploid and triploid crosses. Diploids were produced from sperm of each stock or line and triploids were produced from the other half of eggs fertilized with pooled sperm from tetraploid males of family 4B. For wild stock designation, see Table 4.1 (WIC, MBY, RAP, CHES, and PATX).

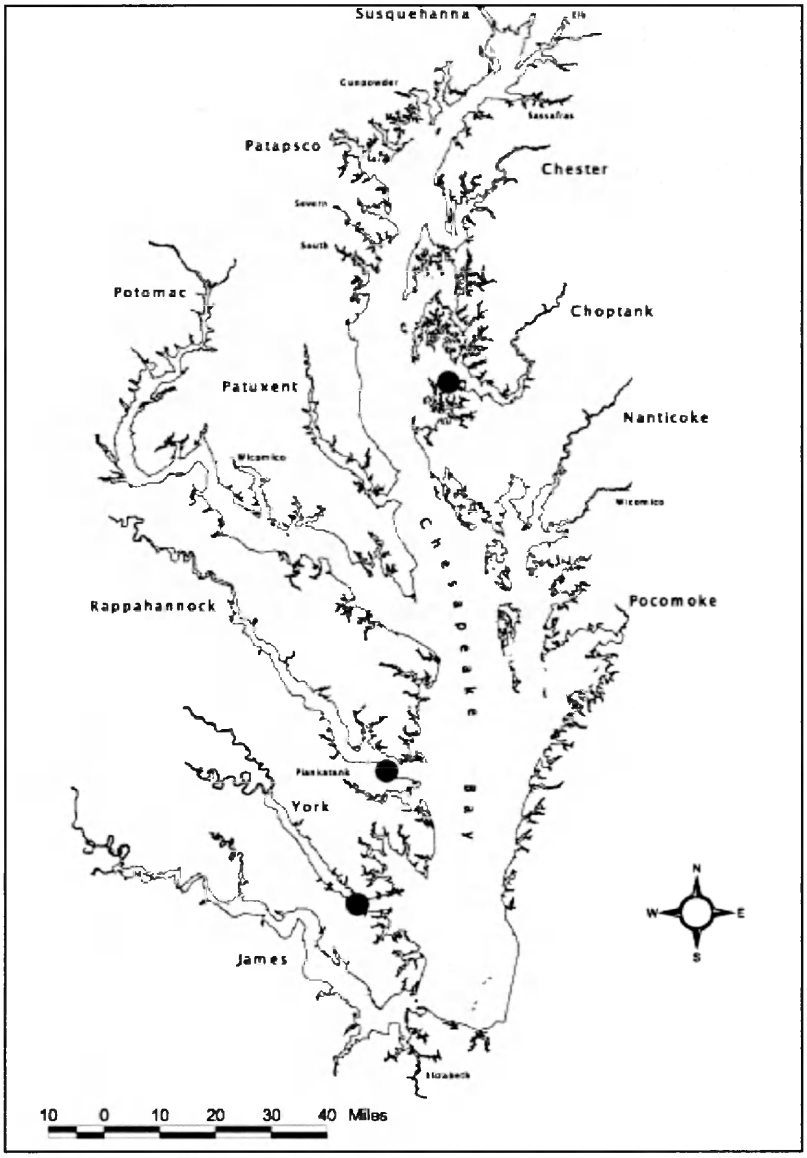

Figure 4.2: Map of the experimental grow-out sites in the Chesapeake Bay. Oysters were grown in three estuaries: York River, VA, Rappahannock River, VA, and Choptank River, MD. Specific experimental site locations are marked with black circles.

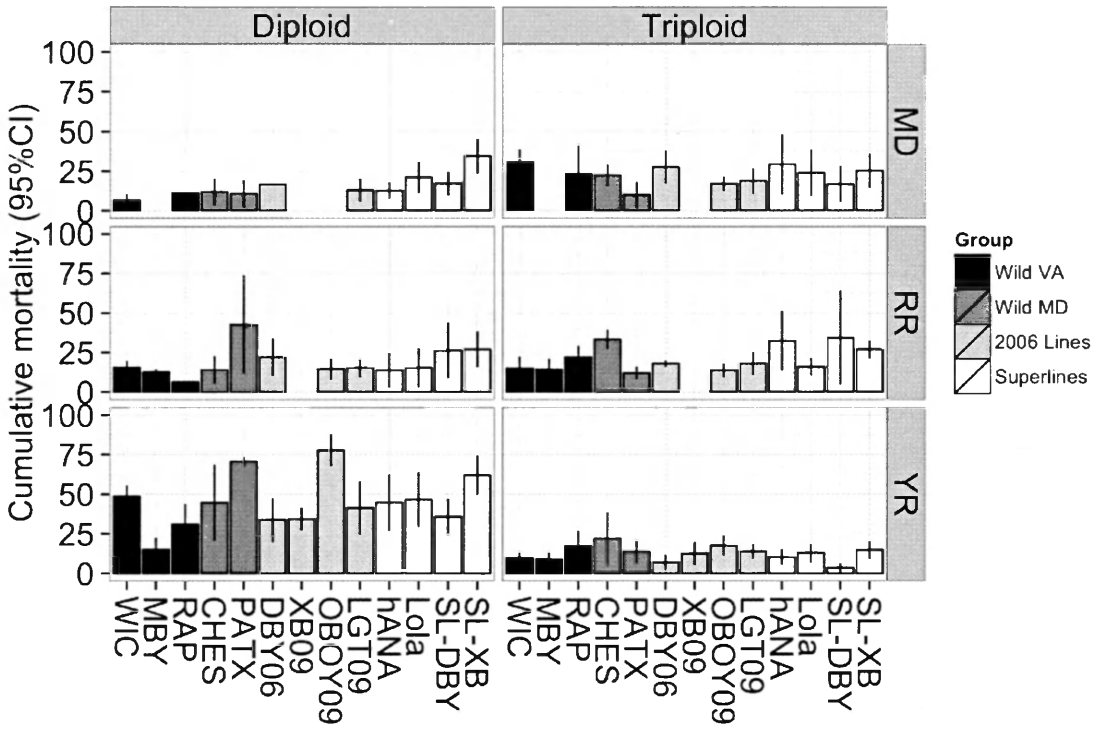

Figure 4.3: Cumulative mortality (mean ± SEM) of diploid and triploid wild stocks and selected lines in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers in December 2011 (17 months). Wild Virginia stocks are indicated with solid black bars, wild Maryland stocks with dark grey, 2006-year class lines with light grey bars, and the Superlines with white. For wild stock designation, see Table 4.1 (WIC, MBY, RAP, CHES, and PATX).

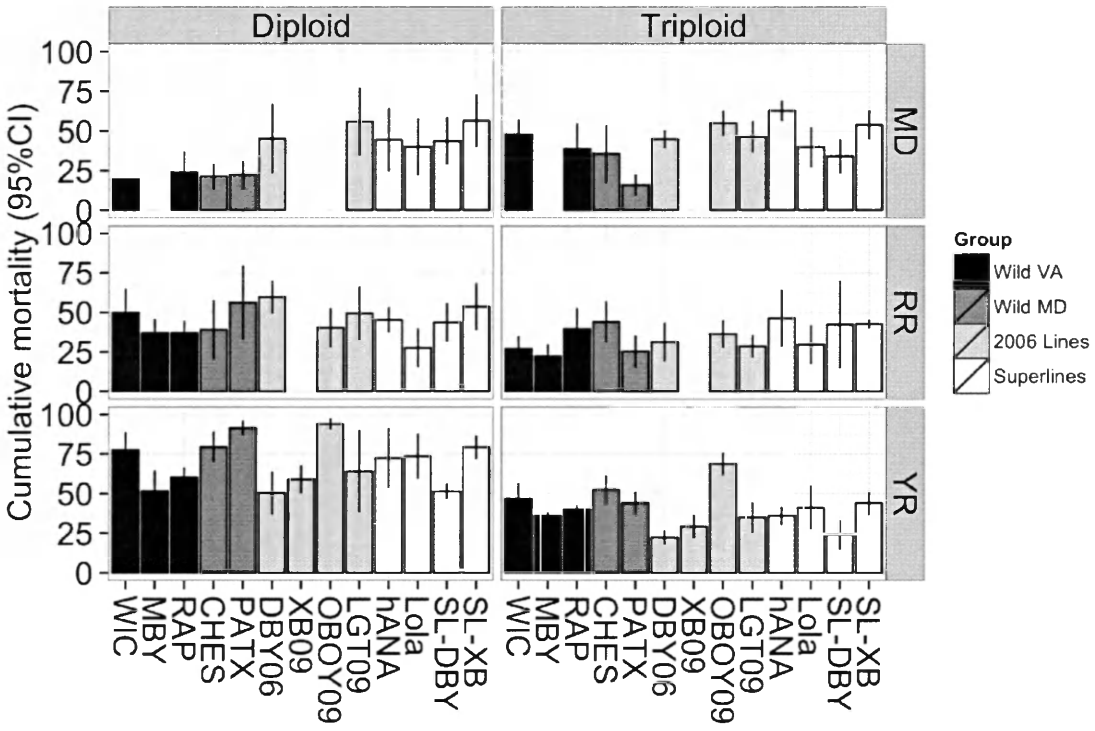

Figure 4.4: Cumulative mortality (mean ± SEM) of diploid and triploid wild stocks and selected lines in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers in December 2012 (29 months). Wild Virginia stocks are indicated with solid black bars, wild Maryland stocks with dark grey, 2006-year class lines with light grey bars, and the Superlines with white. For wild stock designation, see Table 4.1 (WIC, MBY, RAP, CHES, and PATX).

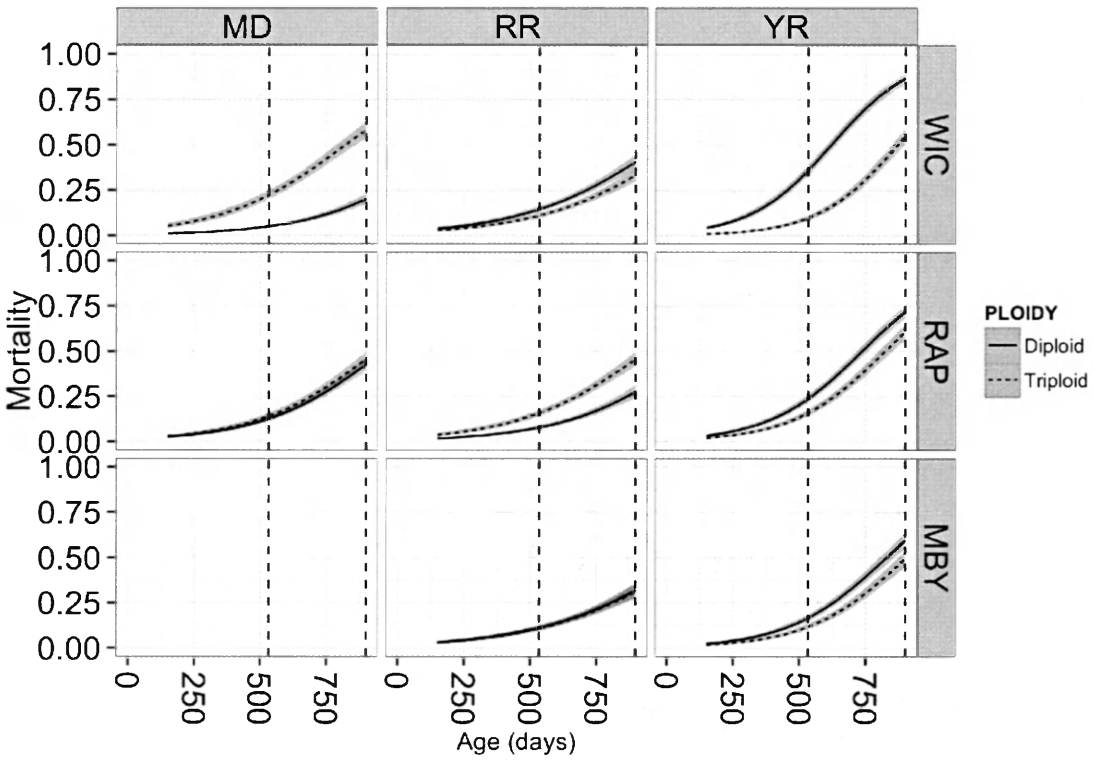

Figure 4.5: Mortality functions of diploid and triploid wild Virginia stocks in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Grey error bars indicate 95% confidence intervals. Mortality data was collected beginning in September 2011 ending in December 2012. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

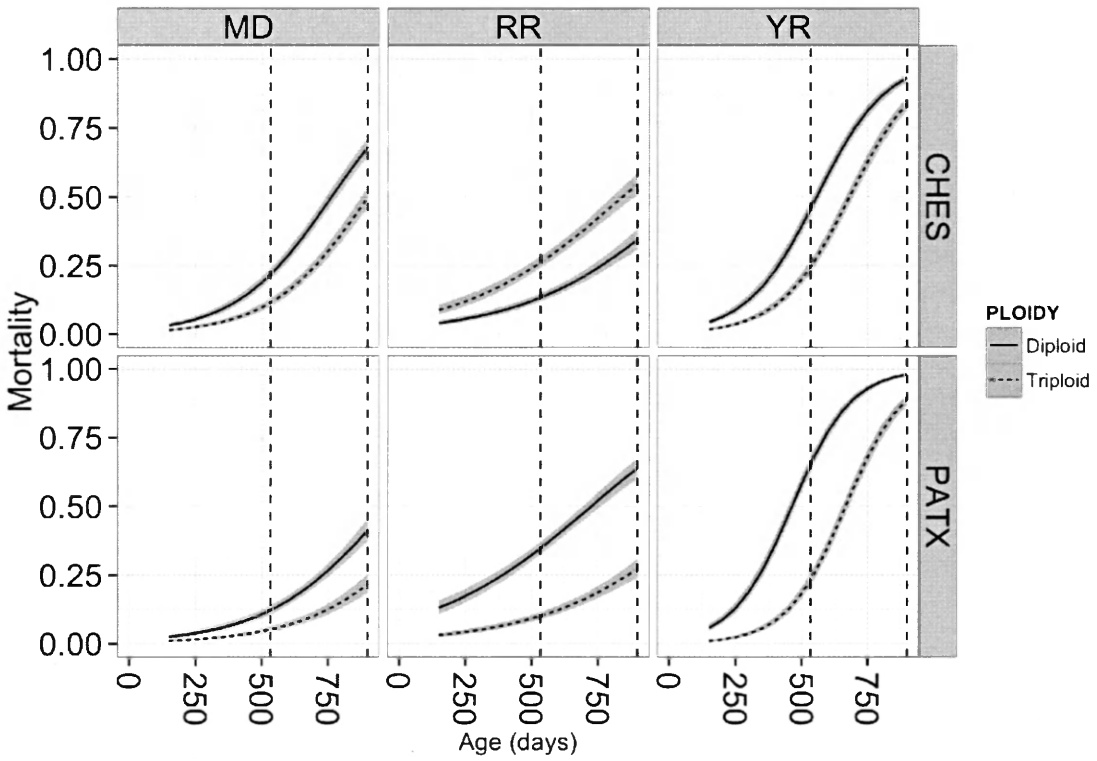

Figure 4.6: Mortality functions of diploid and triploid wild Maryland stocks in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Grey error bars indicate 95% confidence intervals. Solid lines indicate diploids and dashed lines triploids. Mortality data was collected beginning in September 2011 ending in December 2012. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

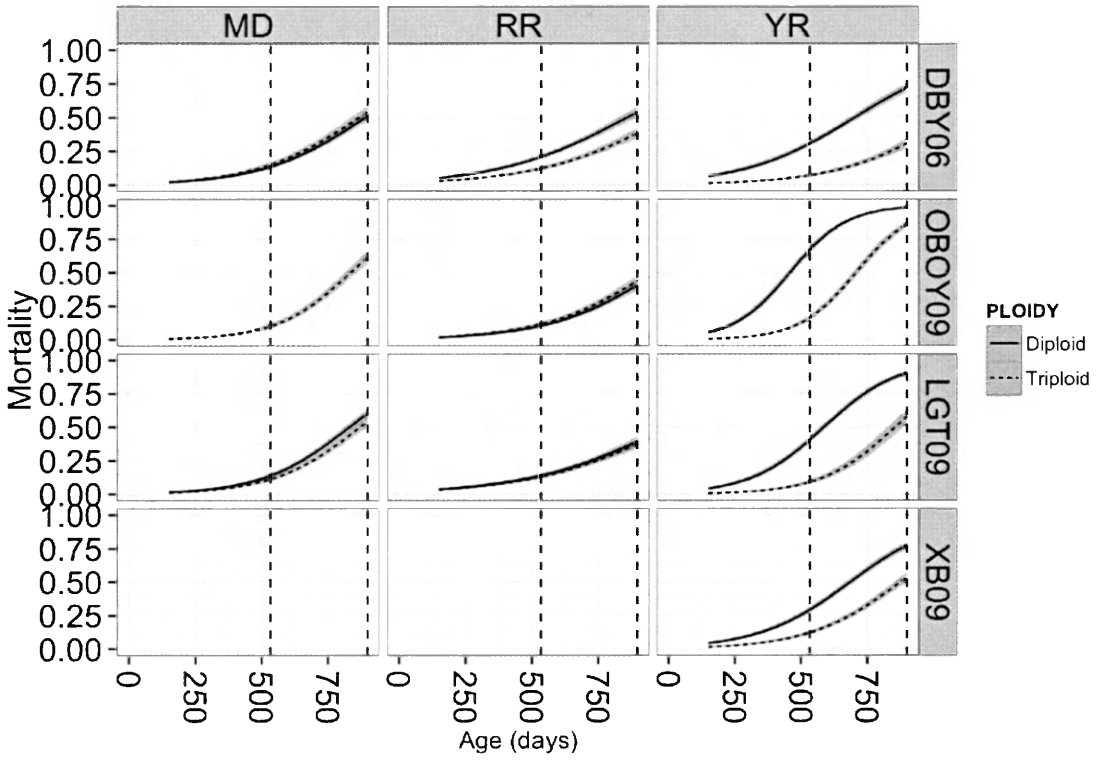

Figure 4.7: Mortality functions of diploid and triploid 2006-year class lines in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Grey error bars indicate 95% confidence intervals. Mortality data was collected beginning in September 2011 ending in December 2012. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

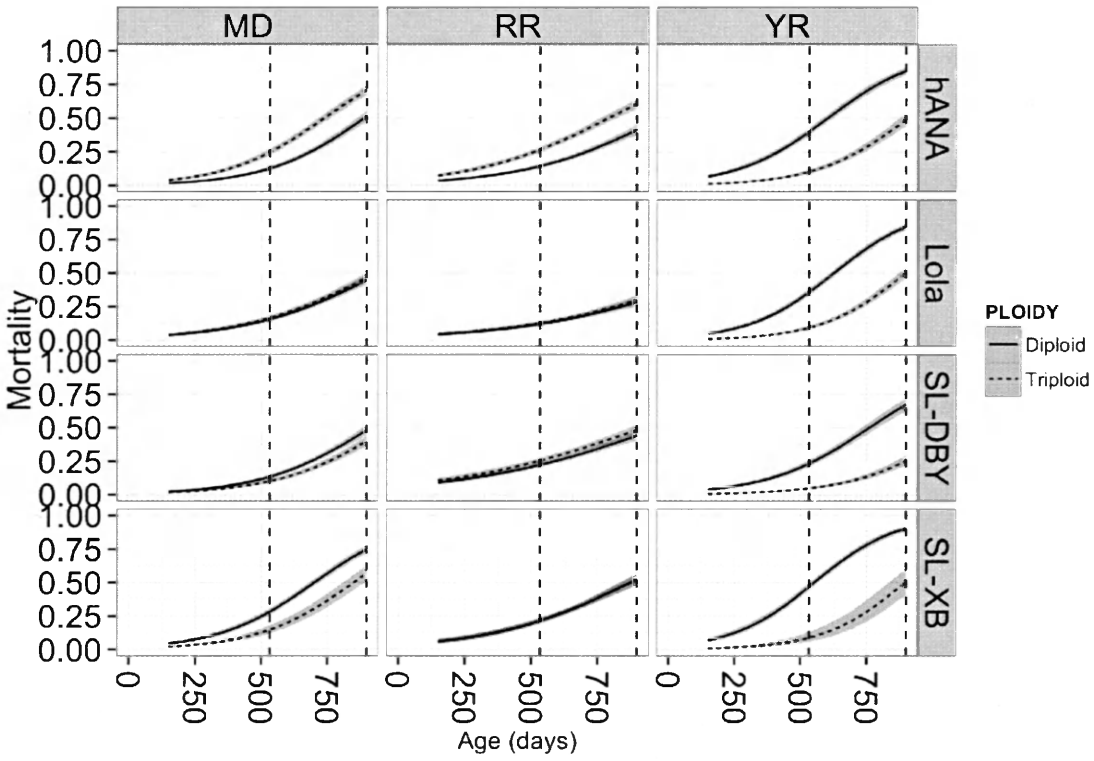

Figure 4.8: Mortality functions of diploid and triploid Superlines in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Grey error bars indicate 95% confidence intervals. Mortality data was collected beginning in September 2011 ending in December 2012. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

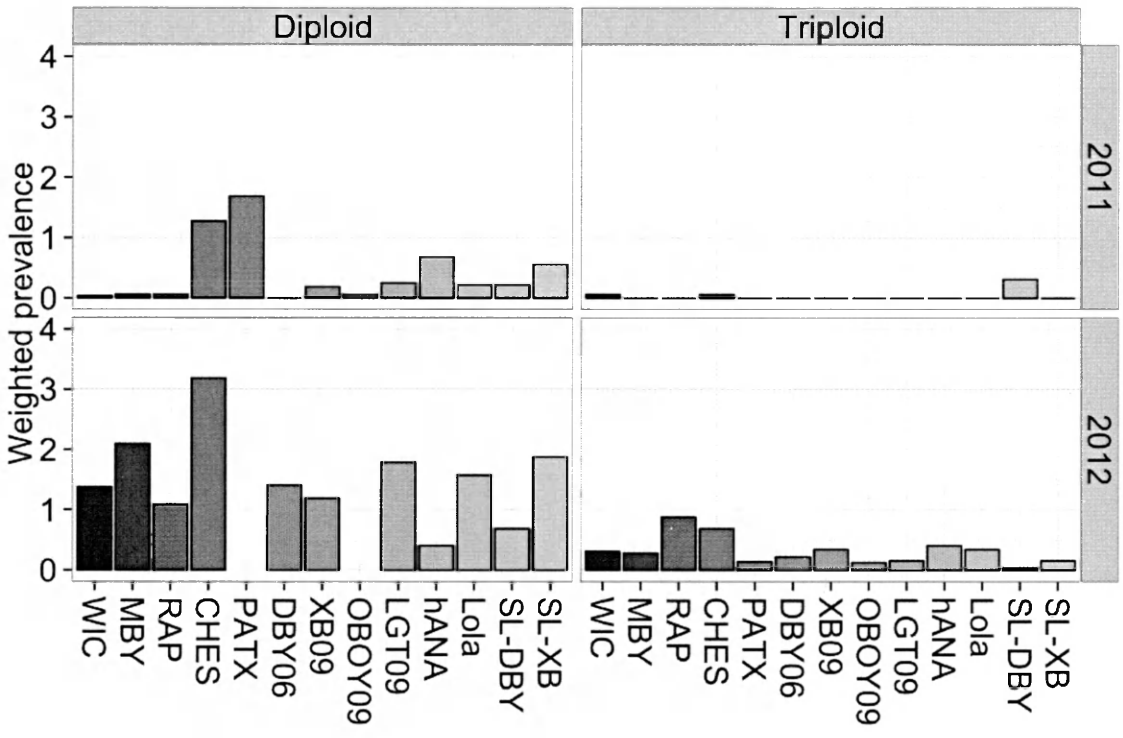

Figure 4.9: Weighted prevalence of *Perkinsus marinus* at the York River in October 2011 (2011) and November 2012 (2012) of diploid and triploid wild stocks (from Virginia and Maryland) and selected lines (2006-year class and Superlines).

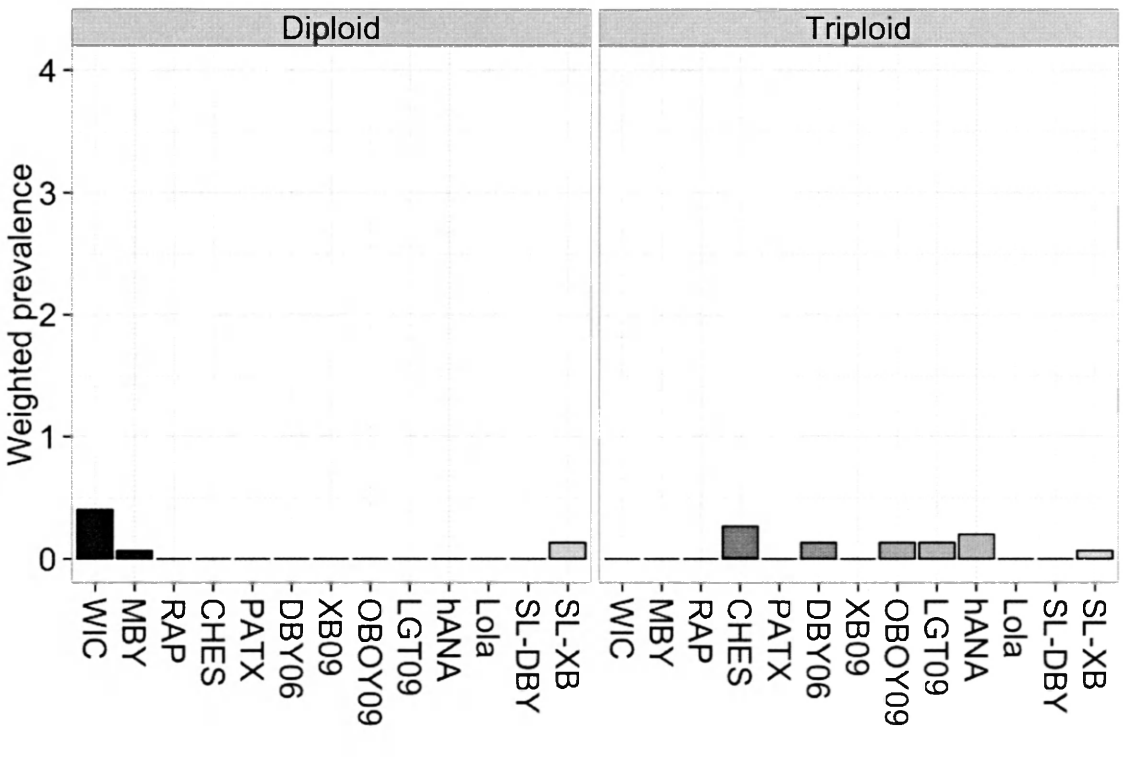

Figure 4.10: Weighted prevalence of *Haplosporidium nelsoni* at the York River in May 2012 of diploid and triploid wild stocks (from Virginia and Maryland) and selected lines (2006-year class and Superlines).

Chapter Five: Summary

Summary

When it occurs, the effect of triploidy is often an advantage characterized by faster growth and increased survival compared to diploid oysters grown in similar conditions. In some cases, however, the effect of triploidy can manifest as a disadvantage. Obviously, the value of triploid oysters in the Chesapeake Bay had obtained from the triploid advantage. The triploid advantage was hypothesized to be the result of genetic effects, physiological changes, or a combination of both. The causative genetic mechanisms at play may include additive genetic effects and heterosis while the physiological changes result from reduced gametogenesis. This study provided comparisons of growth rates and survival rates among diploid and triploid *C. virginica.* It also compared these parameters among triploid groups produced from various genotypes, ranging from wild to highly selected lines.

The various genotypes used in this study consisted of wild stocks from the Virginia portion of the Chesapeake Bay as well as wild stocks from the Maryland portion, four lines from ABC's 2006-year class of selected lines, and four of ABC's Superlines. In addition to testing for the effect of various genotypes on the triploid advantage, three experimental sites ranging in salinity and disease pressure (Choptank River – low salinity and no disease pressure; Rappahannock River – moderate salinity and occasional disease pressure; York River – higher salinity and consistent disease

pressure) were chosen to investigate the influence of the environment on the triploid advantage.

Wild stocks vs. Superlines (SS+ vs. SSS)

The first objective of this study was to compare field performance among wild and selected lines both as diploids and as triploids. Several aspects of these comparisons were of particular interest to further our knowledge of the triploid advantage. The relative performance of diploids and their triploid counterparts provided evidence that the genomic contribution of the diploid parent in a tetraploid × diploid cross is significant (i.e., in some cases triploids from wild type diploids did not perform as well as triploids from selected diploids). The differences in relative performance across the study sites showed that the effect of the environment is also significant.

There was a distinct lack of triploid advantage for growth parameters under low salinity in the Choptank River. The triploid advantage increased with salinity and the addition of disease pressure, which illustrated the importance of the environment. For growth, triploid advantage was adversely affected by the stress of low salinity regardless of diploid genotype. For example, triploids in this environment did not reach harvest size (76mm) by the end of the study, which was not the case in the higher salinity sites (York and Rappahannock Rivers). For survival, triploidy provided a significant disadvantage for at least two of the genotypes under low salinity stress (wild Virginia stock WIC (-140%) and Superline hANA (-40%)). As salinity increased so did the triploid advantage for survival and was typically greater for the more disadvantaged groups (triploids from wild-type diploid females).

The Rappahannock River site was an environment conducive to oyster growth because of the lack of disease pressure during this study and salinity falling in the physiological optimum range for oysters (14 - 28 ppt (Galstoff 1964; Loosanoff 1965)). Essentially, this site offered oysters the opportunity to express genetic potential with minimal environmental stress. The results showed that breeding efforts could improve diploids to perform as well as triploids as evidenced by the Superlines. Growth of triploid wild stocks varied but several triploid wild stocks were similar to the triploid Superlines indicating that in an environment such as the Rappahannock River, the genotype of the diploid parent has little influence the triploid performance. Conversely, the double set of chromosomes from the tetraploid is more influential than the single set from the diploid female. Overall triploid advantages were lower in the Rappahannock River than in the York River suggesting that the greatest value of triploidy is not the ability to outperform diploids regardless of environmental factors, but rather the ability to outperform diploids under specific stresses (e.g., disease pressure).

In the York River the triploid advantage was greatest for the most disadvantaged groups (wild Maryland stocks). The difference in the triploid advantage across groups under disease pressure (the tetraploid parent is from a disease resistant origin) informs us about the role of disease resistance in the tetraploid parent. For example, the wild Maryland triploid CHES, a highly susceptible stock, has similar wet tissue weights under disease pressure as the triploid Superlines. All wild stocks had greater triploid advantages under disease pressure, which suggests the possibility of heterosis through increased heterozygosity from using wild-type diploids over selected diploids. The increased performance of all of the triploid groups in the presence of disease may also be

due to a shift in energy expenditure in lieu of gametogenesis due to triploid sterility, which were not measured in this study.

Another unique comparison was between wild stocks originating in Maryland with both wild and selected Virginia stocks at each site, and in particular the Maryland study site. With few studies done to examine the potential of appropriate Virginia stocks in Maryland, and vice versa, this study provided valuable information for use of wild stocks in private aquaculture operations. In the Choptank River, wild diploids originating in Maryland and Virginia performed similarly for shell height, whole wet weight, and wet tissue weight. In this low salinity environment the diploid low-salinity Superline Lola outperformed both diploid wild Maryland stocks for shell height. For wet tissue weight, Superlines Lola, SL-DBY, and SL-XB all outperformed wild Maryland diploid stocks. As triploids, there were no differences in growth parameters of any of the wild stocks (either from Virginia or Maryland) and the Superlines with one exception: triploid Superline Lola had greater wet tissue weight than triploid wild Maryland stock PATX. The lack of differences in the triploid stocks/lines is another manifestation of the adverse effect of low salinity on triploid performance. Survival of the wild Maryland stocks in the Choptank River was significantly lower than all selected lines as diploids and comparable to the diploid wild Virginia stocks. As salinity increased so did the disadvantage of the wild Maryland stocks relative to wild Virginia stocks and the selected lines. In both the Rappahannock and York Rivers, as diploids and triploids, the selected lines outperformed the wild Maryland stocks. It was only in the York River, under disease pressure, that the diploid wild Maryland stocks performed worse than wild Virginia stocks and only for whole wet weight and wet tissue weight. Survival of the

diploid and triploid wild Maryland stocks in the Rappahannock River was comparable to the wild Virginia stocks and Superlines as there were no significant stresses in this river system (e.g. disease or low salinity). Survival of wild diploid Maryland stock in the York River was low, but as triploids the wild Maryland stocks were similar to both wild Virginia stocks and Superlines for survival.

High growth and survival rates are desired characteristics in oysters for aquaculture. Utilizing growth metric statistics and survival rates to compare relative performances over time of stocks/lines in multiple environments can be difficult, especially when these metrics are considered together in an attempt to form an overall understanding of the potential of a certain stock/line. In an attempt to simplify the overall picture of the 'quality' of the stocks and lines, I derived a new metric from shell height measurements and survival rates to estimate the probability of being both alive and harvest size (76mm) at a given time. This was done by combining the 'time-response' analysis of mortality from Chapter 4 with the results from a 'time-response' analysis of oysters growing to harvest size (76mm) following the same procedure as for mortality. The derived parameter of the probability of being both alive and harvest size, hereafter called "harvestability," was useful for visualizing how the genotype and environment act in concert to produce a triploid advantage (or lack of one). The environmental effect on the triploid advantage is clear from comparisons across sites for a given stock or line and is similar among the wild Virginia stocks (Figure 5.1), similar among the wild Maryland stocks (Figure 5.2), or similar among the Superlines (Figure 5.3). At low salinity, harvestability shows a disadvantage for triploids. In the Rappahannock River, there is

minimal effect and under high disease pressure, there is significant effect on harvestability.

Comparisons between stocks and lines within a site provide clear differences in the overall utility of a genotype relative to alternative choices. For example, within the York River, triploid Superline hANA had the greatest shell height by the end of the study, but when shell height is considered with survival hANA is no longer the best performing triploid Superline (SL-DBY had the greatest estimated probability of being both harvest size and alive by the end of the study, Figure 5.4). It is not uncommon for oyster aquaculturists to harvest a cohort multiple times. If multiple harvests were important, triploid Superlines hANA and Lola would be better choices in the York River as they have higher probabilities of being both harvest size and alive earlier than the other Superlines. This more holistic view of harvestability allows for more appropriate broodstock choices for a variety of culture methods and goals.

2006-year class lines (SSS) vs. Superlines (SSS)

The second objective was to compare field performance among several generations of selected lines. It was of great interest to examine whether gains achieved through selection across generations carry in their triploid counterpart and to quantify these gains to determine how they translate from diploid to triploid. To achieve this, the selected 2006-year class lines were compared with the 2008 Superlines. This comparison, however, is not a direct comparison of two consecutive generations of selected lines.

In 2008, ABC's breeding program changed its strategy from one based primarily on increasing disease resistance to regionally based selection for growth traits that

included a major line consolidation from 25 lines to 4 Superlines. As a result of this line consolidation, the Superlines are not truly successive generations for the four 2006-year class lines in this study, but rather composites of lines (including the four 2006-year class lines here) based on three main base populations (DBY, XB, and, Louisiana). The four lines from the 2006-year class were chosen as representatives of those base populations.

The results show that, as with the wild stock and Superline comparison, growth metrics were strongly influenced by the environment. The genetic contribution of the diploid parent seems to play a minor role in the 2006-year class line—Superline comparison. The differences between diploids from the 2006-year class and Superlines were negligible for all growth measures within each site. A consequence of gene flow caused by mixing distinct populations is a change in allelic frequency due to linkage disequilibrium. Each Superline is a mixture of many different lines, so it is likely that the Superlines are exhibiting linkage disequilibrium to some degree.

Harvestability plots show how the performance of selected lines changed from the 2006-year class to the Superlines (Figures 5.3 and 5.4). In the Choptank River all selected lines performed equally poorly. In the Rappahannock and York Rivers, where triploid advantages begin to manifest, there are subtle differences. In the Rappahannock River, triploids of the 2006-year class DBY06 have greater probability of being harvest size and alive than diploid DBY06. The Superline SL-DBY, however, shows no difference in diploids and triploids. The remaining lines are generally similar. In the York River, it is interesting that triploid DBY06 and SL-DBY both have the highest harvestability at the end of the study, but by December 2011 triploid DBY06 a much higher harvestability index than the Superline SL-DBY.

Disease

The fourth objective was to compare disease prevalence and intensities among triploids made from susceptible diploids and those from diploids selected for disease resistance. Triploid oysters typically survive better than diploid oysters under Dermo disease pressure.

The results showed that triploidy had a positive effect on Dermo resistance. All triploid stocks and lines had a lower prevalence of Dermo disease than their diploid counterparts by the end of the study. One striking detail about Dermo infection as it relates to the triploid advantage is that the prevalence among all triploid groups were similar (wild VA – 38%, wild MD – 32%, 2006-lines – 27%, and Superlines – 25%). It appears that triploidy promoted Dermo resistance regardless of the genetic origin of the diploid parent. The advantage may come from physiological changes due to triploid sterility rather than resistance *per se*. For MSX however, no conclusions can be drawn because of the light disease loads observed in this study, even in "susceptible" diploids.

Conclusion

The triploid effect on growth and survival ranged from positive to negative depending on environmental factors. This environment by genotype effect suggests that triploidy should be thought of as a tool useful in some applications but not in others. For low salinity, it appears that triploidy may not be the proper tool but for oysters grown under disease pressure, it certainly is. The effect of triploidy appears to be caused by both genetic effects and physiological changes, with the environment influencing the expression of each.

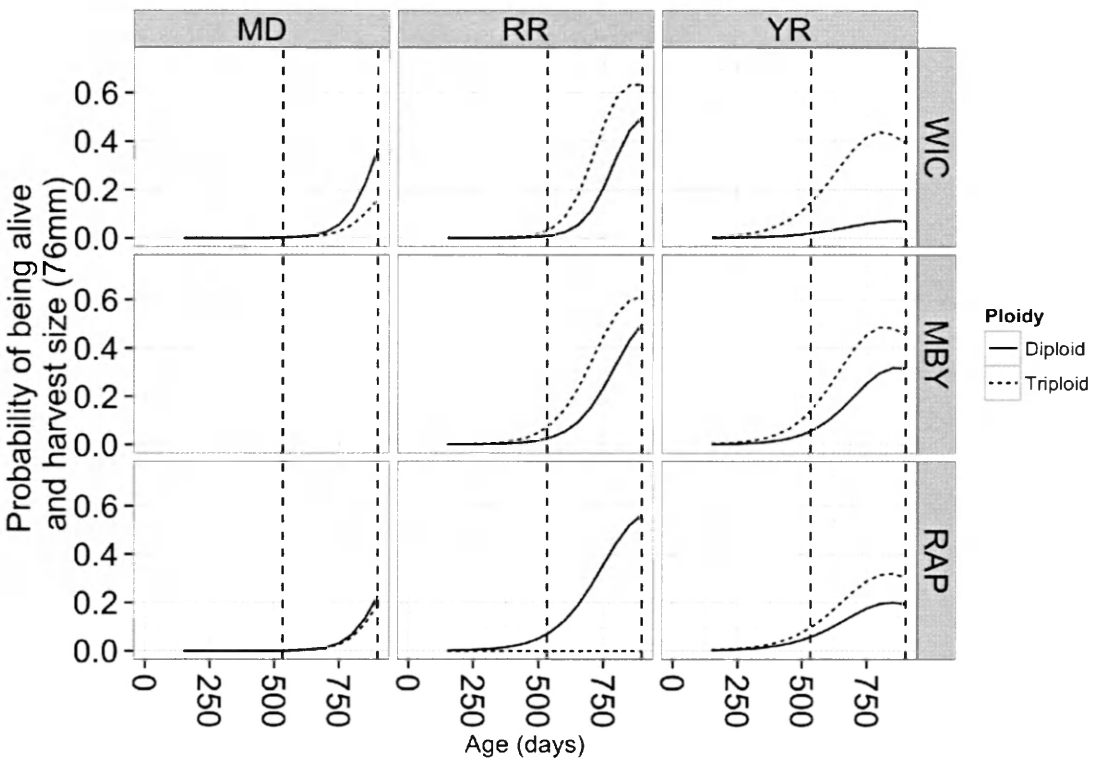

Figure 5.1: Distributions of the estimated probability of survival and being harvest size (76mm) for diploid and triploid wild Virginia stocks in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

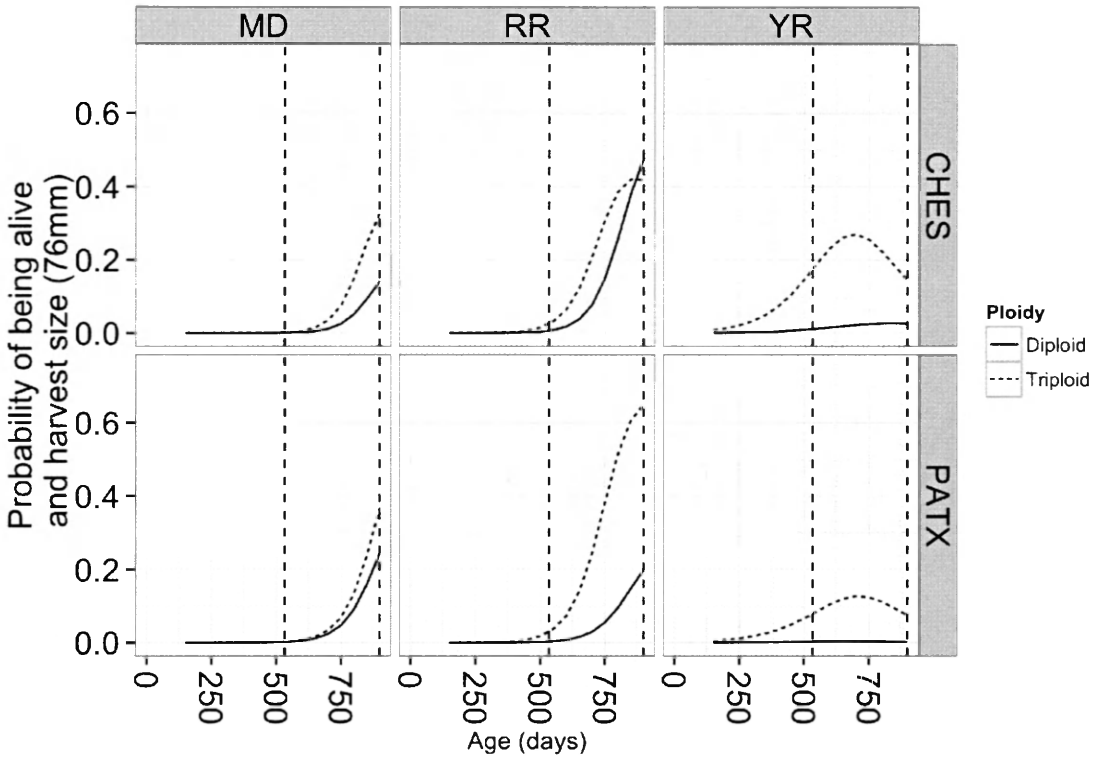

Figure 5.2: Distributions of the estimated probability of survival and being harvest size (76mm) for diploid and triploid wild Maryland stocks in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

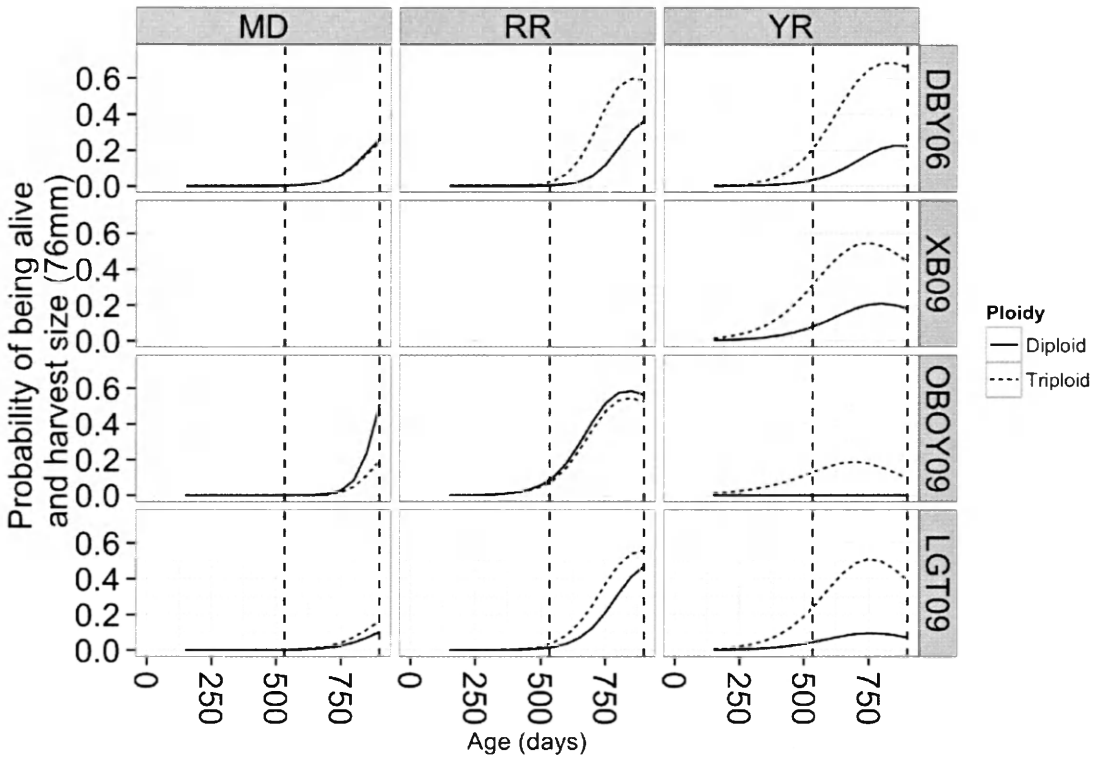

Figure 5.3: Distributions of the estimated probability of survival and being harvest size (76mm) for diploid and triploid 2006-year class lines in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

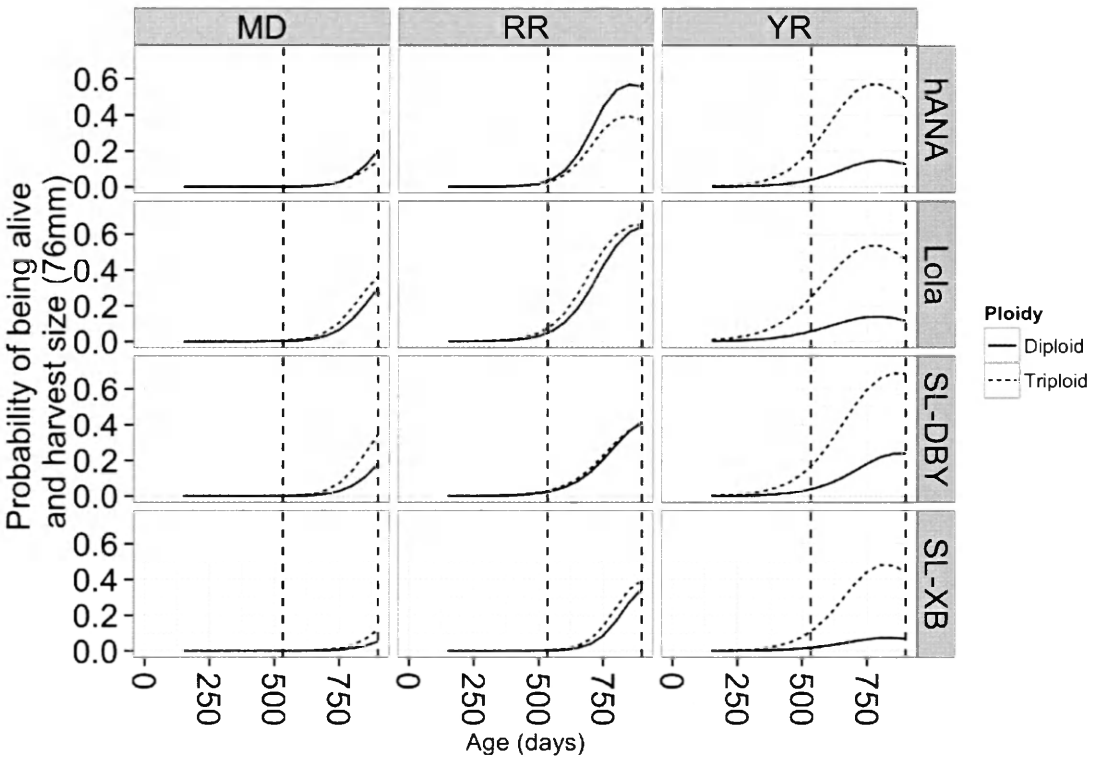

Figure 5.4: Distributions of the estimated probability of survival and being harvest size (76mm) for diploid and triploid Superlines in the Choptank (MD), Rappahannock (RR), and York (YR) Rivers. Solid lines indicate diploids and dashed lines triploids. Dashed vertical lines indicate December 2011 (17 months) and 2012 (29 months).

Literature Cited

Abbe, G. R., McCollough, C. B., Barker, L. S., and C. F. Dungan. 2010. Performance of disease-tolerant strains of Eastern oyster (*Crassostrea virginica*) in the Patuxent River, Maryland, 2003-2007. Journal of Shellfish Research 29:161-175.

ABC. 2010. Breeding Manual. http://www.vims/edu/research/units/centerspartners/abc_migrate/_docs/oyster_breeding_program.pdf

Albright, B. W., Abbe, G. R., McCollough, C. B., Barker, L. S., and C. F. Dungan. 2007. Growth and mortality of dermo-disease-free juvenile oysters (*Crassostrea virginica*) at three salinity regimes in an enzootic area of Chesapeake Bay. Journal of Shellfish Research 26:451-463.

Allen, S. K., Jr. 1983. Flow cytometry: Assaying experimental polyploid fish and shellfish. Aquaculture 33:317-328.

Allen, S. K., Jr. 2005. Stalemate over the new oyster: The evolution of commercial trials with *C. ariakensis* and prognosis for the industry. Marine Resource Bulletin 37(2):2-16.

Allen, S. K., Jr. and D. Bushek. 1992. Large scale production of triploid *Crassostrea virginica* (Gmelin) using 'stripped' gametes. Aquaculture 103:241-251.

Allen, S. K., Jr. and S. L. Downing. 1986. Performance of triploid Pacific oysters, *Crassostrea gigas*. I. Survival, growth, glycogen content, and sexual maturation in yearlings. Journal of Experimental Marine Biology and Ecology 102:197-208.

Allen, S. K., Jr. and S. L. Downing. 1987. Induced triploidy in the Pacific oyster, *Crassostrea gigas*: Optimal treatments with cytochalasin B depend on temperature. Aquaculture 61:1-15.

Allen, S. K., Jr., Downing, S.L., and K. K. Chew. 1989. Hatchery Manual for Producing Triploid Oysters. University of Washing Press, Seattle, Washington.

Allen, S. K., Jr. and S. L. Downing. 1990. Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. Canadian Journal of Fisheries and Aquatic Sciences 47:1213-1222.

Allen, S. K., Jr., Erskine, A. J., Walker, E. J., and G. A. DeBrosse. 2005. Production of tetraploid Suminoe oysters *C. ariakensis*. Aquaculture 247:3.

Andrews, J. D. 1966. Oyster mortality studies in Virginia. IV. Epizootiology of MSX, a protistan parasite of oysters. Ecology 47:19-31.

Andrews, J. D. 1979. Oyster diseases in Chesapeake Bay. Marine Fisheries Review 41(1-2):45-53.

Andrews, J. D. 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. Pp47-63 in W. S. Fisher (ed). Disease Processes in Marine Bivalve Molluscs. American Fisheries Society Special Publication 18. Bethesda, Maryland.

Andrews, J. D. 1996. History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950-1984. Journal of Shellfish Research 15:13-16.

Andrews, J. D. and W. F. Hewatt. 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters in Chesapeake Bay. Ecological Monographs 27:1-26.

AOAC (Association of Official Analytical Chemists). 2007. Official methods of analysis of AOAC International. 18th revised edition. Gaithersburg, MD, US.

Auger, D. L., Gray, A. D., Ream, T. S., Kato, A., Coe, E. H., and J. A. Birchler. 2005. Nonadditive gene expression in diploid and triploid hybrids of Maize. Genetics 169:389-397.

Barber, B. J., Ford, S. E., and H. H. Haskin. 1988a. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition index and relative fecundity. Journal of Shellfish Research 7:25-32.

Barber, B. J., Ford, S. E., and H. H. Haskin. 1988b. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. II. Tissue biochemical composition. Comparative Biochemical Physiology 91:603-608.

Barber, B. J. and R. Mann. 1991. Sterile triploid *Crassostrea virginica* (Gmelin, 1791) grow faster than diploids but are equally susceptible to *Perkinsus marinus*. Journal of Shellfish Research 10:445-450.

Barber, B. J. 1996. Gametogenesis of eastern oysters, *Crassostrea virginica* (Gmelin, 1791) and Pacific oysters, *Crassostrea gigas* (Thunberg, 1793) in disease endemic lower Chesapeake Bay. Journal of Shellfish Research 15:285-290.

Barber, B. J., Davis, C. V., and M. A. Crosby. 1998. Cultured oysters, *Crassostrea virginica*, genetically selected for fast growth in the Damariscotta River, Maine, are resistant to mortality caused by juvenile oyster disease (JOD). Journal of Shellfish Research 17:1171-1175.

Bayne, B. L. 1975. Reproduction in bivalve molluscs under environmental stress. F. J. Vernberg, ed. Physiological Ecology of Estuarine Organisms. Columbia, SC. University of South Carolina Press. p.259-277.

Bayne, B. L. 1976. Aspects of reproduction in bivalve molluscs under environmental stress. M. L. Wiley, ed. Estuarine Processes. New York, NY. Academic Press. p.432-448.

Bayne, B. L. and R. C. Newell. 1983. Physiological energetics of marine molluscs. In: The Mollusca. Vol. 4(1) Saleuddin, A.S.M., Wilbur, K.M. (eds.) Academic Press, New York., pp. 407-515.

Benomar, S., Costil, K., El Filali, F., Mathieu, M., and A. Moukrim. 2010. Annual dynamics of glycogen, lipids and proteins during the sexual cycle of *Perna perna* (Mollusca: Bivalvia) from south-western Morocco. Journal of the Marine Biological Association of the United Kingdom 90:335-346.

Breuer, J. P. 1962. An ecological survey of the lower Laguna Madre of Texas, 1953-1959. Publications of the Institute of Marine Science, 8:153-183.

Brown, M. R. 2011. Rapid compositional analysis of oysters using visible-near infrared reflectance spectroscopy. Aquaculture 317:233-239.

Brown, M. R., Kube, P. D., O'Connor, S., Cunningham, M., and H. King. 2012. Application of Near-Infrared Reflectance Spectroscopy for the Rapid Chemical Analysis of Sydney Rock Oyster (*Saccostrea glomerata*) and Pacific Oyster (*Crassostrea gigas*). Journal of Shellfish Review 31:1051-1060.

Burklew, M. 1971. Extraction of Glycogen from Oysters. Florida Department of Natural Resources. In: Leaflet Series: Volume VI – Chemistry. Part 2 (Biochemistry) No. 4.

Burreson, E. M. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: I. Susceptibility of native and MSX-resistant stocks. Journal of Shellfish Research 10:417-423.

Burreson, E. M. and R. L. Calvo. 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. Journal of Shellfish Research Special Publication. Journal of Shellfish Research 15:17-34.

Bushek, D. 1994. Dermo disease in the American oyster: Genetics of host-parasite interactions. Ph.D. Dissertation. Rutgers University, New Brunswick, NJ. 189pp.

Butler, P. A. 1949. Gametogenesis in the oyster under conditions of depressed salinity. Biological Bulletin 96:263-269.

Bulter, P. A. 1952. Growth and mortality rates in sibling and unrelated oyster populations. Proceedings of the Gulf and Caribbean Fisheries Institute 4:71.

Calabrese, A. and H. C. Davis. 1970. Tolerances and requirements of embryos and larvae of bivalve mollusks. Helgoländer wissenschaftliche Meeresuntersuchungen 20:553-564.

Calderwood, H. and A. Armstrong. 1941. Determination of glycogen in oysters. Journal of the Association of Official Agricultural Chemists 24:154-165.

Calvo, G. W., Luckenback, M. W., Allen, S. K., Jr., and E. M. Burreson. 2001. A comparative field study of *Crassostrea ariakensis* (Fujita, 1913) and *Crassostrea virginica* (Gmelin, 1791) in relation to the salinity in Virginia. Journal of Shellfish Research 20:221-229.

Canzonier, W. J. 1968. Present status of attempts to transmit *Minchinia nelson* under controlled conditions (Abstract). Proceedings of the National Shellfisheries Assocoiation 58:1.

Carriker, M. R. and P. M. Gaffney. 1996. A catalogue of selected species of living oysters (*Ostreacea*) of the world. In: The Eastern Oyster *Crassostrea virginica* (Eds. Kennedy, V. S., Newell, R. I. E., and A. F. Eble), pp1-18. Maryland Sea Grant, College Park, Maryland

Chávez-Villalba, J., Villelas-Ávila, R., and C. Cáceres-Martínez. 2007. Reproduction, condition and mortality of the Pacific oyster *Crassostrea gigas* (Thunberg) in Sonora, México. Aquaculture Research 38:268-278.

Chanley, P. E. 1958. Survival of some juvenile bivalves in water of low salinity. Proceedings of the National Shellfisheries Association 48:52-65.

Cheney, D. P., MacDonald, B. F., and R. A. Elston. 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. Journal of Shellfish Research 19:353-359.

Choi, K-S., Wilson, E. A., Lewis, D. H., Powell, E. N., and S. M. Ray. 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the

thioglycollate method. Journal of Shellfish Research 8:125-131.

Crosby, M. P. and L. D. Gale. 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. Journal of Shellfish Research 9:233-237.

Davis, J. P. 1994. Studies on the influence of ambient temperature and food supply on growth rate, carbohydrate content and reproductive output in diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Dissertation, University of Washington.

Davis, C. V. and B. J. Barber. 1999. Growth and survival of selected lines of Eastern oyster, *Crassostrea virginica* (Gmelin) affected by juvenile oyster disease. Aquaculture 178:253-271.

Dégremont, L., Allen, S. K., Jr., Burreson, E. M., and G. DeBrosse. 2006. Survival and growth of *Crassostrea virginica* lines in Chesapeake and Delaware Bays. Journal of Shellfish Research 15:722.

Dégremont, L., Garcia, C., Frank-Lawale, A., and S. K. Allen, Jr. 2012. Triploid oysters in the Chesapeake Bay: Comparison of diploid and triploid *Crassostrea virginica*. Journal of Shellfish Research 31:21-31.

Desrosiers, R. R., Gerard, A., Peignon, J-M., Naciri, Y., Dufresne, L., Morasse, J., Ledu, C., Phelipot, P., Guerrier, P., and F. Dube. 1993. A novel method to produce triploids in bivalve molluscs by the use of 6-dimethylaminopurine. Journal of Experimental Marine Biology and Ecology 170:29-43.

Dittman, D. E., Ford, S. E., and D. K. Padilla. 2001. Effects of *Perkinsus marinus* on reproduction and condition of the eastern oyster, *Crassostrea virginica*, depend on timing. Journal of Shellfish Research 20:1025-1034.

Dridi, S., Romdhane, M. S., and M. Elcafsi. 2007. Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas*, in relation to the gametogenic cycle and environmental conditions on the Bizert lagoon, Tunisia. Aquaculture 263:238-248.

Duchemin, M. B., Fournier, M., and M. Auffret. 2007. Seasonal variations of immune paramters in diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Aquaculture 264:73-81.

Eudeline, B., Allen, S. K., Jr., and X. Guo. 2000a. Optimization of tetraploid induction in Pacific oysters, *Crassostrea gigas*, using first polar body as a natural indicator. Aquaculture 187:73-84.

Eudeline, B., Allen, S. K., Jr., and X. Guo. 2000b. Delayed meiosis and polar body release in eggs of triploid Pacific oysters, *Crassostrea gigas*, in relation to tetraploid production. Journal of Experimental Marine Biology and Ecology 248:151-161.

Ford, S. E. 1985. Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, and Mackin) in oysters. Journal of Shellfish Research 5:85-90.

Ford, S. E. and A. J. Figueras. 1988. Effects of sublethal infection by the parasite *Haplosporidium nelsoni* (MSX) on gametogenesis, spawning, and sex ratios of oysters in Delaware Bay, USA. Diseases of Aquatic Organisms 4:121-133.

Ford, S. E. and H. H. Haskin. 1982. History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. Journal of Invertebrate Pathology 40:118-141.

Ford, S. E. and H. H. Haskin. 1987. Infection and mortality patterns in stocks of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). Journal of Parasitology 73:368-376.

Ford, S. E., Wargo, R. N., and L. M. Ragone. 1988. Metabolic condition and infection levels preceding death in oysters exposed to *Haplosporidium nelsoni* (MSX), with an hypothesis about cause of death. Abstracts of the 3rd International Colloquium on Pathology in Marine Aquaculture, Gloucester Point, Virginia p.41-42.

Gabbot, P. A. 1976. Energy metabolism. B. L. Bayne, ed. Marine Mussels. Cambridge: Cambridge University Press. p.293-355.

Gabbott, P. A. 1983. Developmental and seasonal metabolic activities in marine molluscs. In, *The Mollusca, Volume 2*, edited by P. W. Hochachka, Academic Press, New York, 165-217 pp.

Gagnaire, B., Soletchnik, P., Madec, P., Geairon, P., Le Moine, O., and T. Renault. 2006. Diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), reared at two heights above sediment in Marennes-Oleron Basin, France: Difference in mortality, sexual maturation and hemocyte parameters. Aquaculture 254:606-616.

Galtsoff, P. S. 1964. The American oyster, *Crassostrea virginica* Gmelin. Fishery Bulletin 64:1-480 pp.

Glancy, J. B. 1965. Method of raising shellfish seed in a simulated habitat. U.S. Patent No. 3,196,833, July 27, 1965.

Goulletquer, P., Heral, M., and B. J. Rothschild. 1994. Causes of decline of oyster

production (*Crassostrea virginica*) in the Maryland portion of the Chesapeake Bay: A literature study. Haliotis 23:87-112.

Goulletquer, P., Joly, J. P., Gérard, A., Le Gagneur, E., Moriceau, J., Peignon, J. M., Heurtebise, S., and P. Phelipot. 1996. Performance of triploid Pacific Oysters *Crassostrea gigas* (Thunberg) reared in high carrying capacity ecosystem: survival, growth and proximate biochemical composition. Haliotis 25:1-12.

Grabowski, J. H., Peterson, C. H., Power, S. P., Gaskill, D., and H. C. Summerson. 2004. Growth and survivorship of non-native (*Crassostrea gigas* and *Crassostrea ariakensis*) versus native oysters (*Crassostrea virginica*). Journal of Shellfish Research 23:781-793.

Griffing, B. 1990. Use of a Controlled-Nutrient Experiment to Test Heterosis Hypotheses. Genetics 126:753-767.

Guo, X. and S. K. Allen, Jr. 1994. Viable Tetraploids in the Pacific Oyster (*Crassostrea gigas*Thunberg) Produced by Inhibiting Polar Body I in Eggs from Triploids. Molecular Marine Biology and Biotechnology 3:42-50.

Guo, X., Cooper, K., Hershberger, W. K., and K. K. Chew. 1992. Genetic consequences of blocking polar body I with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*: I. Ploidy of resultant embryos. Biological Bulletin 183:381-386.

Guo, X., DeBrosse, G. A., and S. K. Allen, Jr. 1996. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142:149-161.

Guo, X., Wang, J., Landau, B. J., Li, L., DeBrosse, G. A., and K. D. Krista. 2002. The successful production of tetraploid eastern oyster, *Crassostrea virginica* Gmelin. Journal of Shellfish Research 21:380-381.

Guo, X., Wang, Y., Xu, Z., and H. Yang. 2009. Chromosome set manipulation in shellfish. Pages 165-195 in G. Burnell and G. Allan (eds.) New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management. Woodhead Publishing. 1232 pp.

Hand, R. E., Nell, J. A., Smith, I. R., and G. B. Maquire. 1998. Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley). Journal of Shellfish Research 17:1115-1127.

Hand, R. E., Nell, J. A., and P. A. Thompson. 2004. Studies on triploid oysters in Australia. XIII. Performance of diploid and triploid Sydney rock oyster,

Saccostrea glomerata (Gould, 1850), progeny from a third generation breeding line. Aquaculture 233:93-107.

Harding, J. M. 2007. Comparison of growth rates between diploid DEBY eastern oysters, (*Crassostrea virginica*, Gmelin 1791), triploid eastern oysters, and triploid Suminoe oysters (*C. ariakensis*, Fugita 1913). Journal of Shellfish Research 26:961-972.

Haskin, H. H., Canzonier, W. J., and J. L. Myhre. 1965. The history of MSX on Delaware Bay oyster grounds, 1957-65 (Abstract). American Malacological Union Bulletin 32:20-21.

Haskin, H. H. and S. E. Ford. 1979. Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. Marine Fisheries Review 41:54-63.

Hawkins, A. J. S., Widdows, J., and B. L. Bayne. 1989. The relevance of whole body protein metabolism to measured costs of maintenance and growth in *Mytilus edulis*. Physiological Zoology 62:745-763.

Hawkins, A. J. S., Magoulas, A., Héral, M., Bougrier, S., Naciri-Graven, Y., Day, A. J., and G. Kotoulas. 2000. Separate effects of triploidy, parentage and genomic diversity upon feeding behavior, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. Genetic Research 76:273-284.

Hedgecock, D. and J. P. Davis. 2007. Heterosis for yield and crossbreeding of the Pacific oyster Crassostrea gigas. Aquaculture 272:17-29.

Hedgecock, D., Cooper, K., and W. Hershberger. 1991. Genetic and environmental components of variance in harvest body size among pedigreed Pacific oysters *Crassostrea gigas* from controlled crosses. Journal of Shellfish Research 10(2):516.

Hedgecock, D., McGoldrick, D. J., and B. L. Bayne. 1995. Hybrid vigor in Pacific oysters: an experimental approach using crosses among inbred lines. Aquaculture 137:285-298.

Hedgecock, D., McGoldrick, D. J., Manahan, D. T., Vavra, J., Appelmans, N., and B. L. Bayne. 1996. Quantitative and molecular genetic analyses of heterosis in bivalve molluscs. Journal of Experimental Marine Biology and Ecology 203:49-59.

Helm, M. M., Bourne, N., and A. Lovatelli (comp./ed.). 2004. Hatchery culture of bivalves. A practical manual. *FAO Fisheries Technical Paper*. No. 471. Rome, FAO. 177p.

Hudson, K. L., Kozlowski, A., Erskine, A. J., and S. K. Allen, Jr. 2005. Comparative field trials of triploid *Crassostrea ariakensis* with *C. virginica* at eight field sites in the Chesapeake Bay: Growth, mortality, condition, reversion and gametogenesis. Journal of Shellfish Research 24:658-659.

Isaksson, T. and T. Næs. 1988. The effect of multiplicative scatter correction (MSC) and linearity improvement in NIR spectroscopy. Applied Spectroscopy 42:1273-1284.

Jimare Benito, M. T., Bosch Ojeda, C., and F. Sanchez Rojas. 2008. Process analytical chemistry: Applications of near infrared spectrometry in environmental and food analysis: An overview. Applied Spectroscopy Reviews 43:452-484.

Johnson, R. M., Shrimpton, J. M., Cho, G. K., and D. D. Heath. 2007. Dosage effects on heritability and maternal effects in diploid and triploid Chinook salmon. Heredity 98:303-310.

Kennedy, V. S. 1996. The ecological role of the eastern oyster, *Crassostrea virginica*, with remarks on disease. Journal of Shellfish Research 15:177-183.

Kesarcodi-Watson, A., Lucas, J. S., and D. W. Klumpp. 2001. Comparative feeding and physiological energetics of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* I. Effects of oyster size. Aquaculture 203:177–193.

Kingsley-Smith, P. R., Harwell, H. D., Kellogg, M. L., Allen, S. M., Allen, S.K., Jr., Meritt, D. W., Paynter, K. T., and M. W. Luckenback. 2009. Survival and Growth of Triploid *Crassostrea virginica* (Gmelin, 1791) and *C. ariakensis* (Fujita, 1913) in Bottom Environments of Chesapeake Bay: Implications for an Introduction. Journal of Shellfish Research 28:169-184.

Kirby, M. X. 2004. Fishing down the coast: historical expansion and collapse of oyster fisheries along continental margins. Proceedings of the National Academy of Sciences of the United States of America 101:13096-13099.

Lannan, J. E. 1980. Broodstock management of *Crassostrea gigas*: I. Genetic variation in survival in the larval rearing system. Aquaculture 21:323-336.

Loosanoff, V. L. 1965. Gonad development and discharge of spawn in oysters of Long Island Sound. Biological Bulletin 129:546-561.

MacKenzie, C. L., Jr. 2007. Causes underlying the historical decline in Eastern oyster (*Crassostrea virginica* Gmelin, 1791) landings. Journal of Shellfish Research 26:927-938.

Mackin, J. G. 1951a. Incidence of infection of oysters by *Dermocystidium marinum* in the Barataria Bay area of Louisiana. Proceedings of the National Shellfisheries

Association 1951:22-35.

Mackin, J. G. 1951b. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* (Mackin, Owen, and Collier). Bulletin of Marine Science of the Gulf and Caribbean 1:72-87.

Mackin, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publications of the Institute of Marine Science, University of Texas 7:1-131.

Mann, R. 1978. A comparison of morphometric, biochemical, and physiological indicies of condition in marine bivalve molluscs. Pages 484-497 in J. H. Thorp and J. W. Gibbons (eds.) Early and Environmental Stress in Aquatic Systems. United States Department of Energy. Symposium Series (771114). Woods Hole Oceanographic Institute, Massachusetts.

Mann, R. and E. N. Powell. 2007. Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. Journal of Shellfish Research 26:905-917.

Matthiessen, G. C. and J. P. Davis. 1992. Observations on growth rate and resistance to MSX (*Haplosporidium nelsoni*) among diploid and triploid eastern oysters (*Crassostrea virginica* (Gmelin, 1797)) in New England. Journal of Shellfish Research 11:449-454.

McCollough, C. B., Albright, B. W., Abbe, G. R., Barker, L. S., and C. F. Dungan. 2007. Aquisistion and progression of *Perkinsus marinus* infections by specific-pathogen-free juvenile oysters (*Crassostrea virginica* Gmelin) in a mesohaline Chesapeake Bay tributary. Journal of Shellfish Research 26:465-477.

McCombie, H., Ledu, C., Pascal, P., Lapègue, S., Boudry, P., and A. Gérard. 2005. A complementary method for production of tetraploid *Crassostrea gigas* using crosses between diploids and tetraploids with cytochalasin B treatments. Marine Biotechnology 7:318-330.

MdDNR (Maryland Department of Natural Resources). 2010. http://dnr.maryland.gov/fisheries/oysters/mtgs/120909/oac_presentation_december-9_2009.pdf

Menzel, R. W. and S. H. Hopkins. 1955. Growth of oysters parasitized by the fungus *Dermocystidium marinum* and by the trematode *Bucephalus cuculus*. Journal of Invertebrate Pathology 41:333-342.

Meyers, J. A., Burreson, E. M., Barber, B. J., and R. Mann. 1991. Susceptibility of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg, 1793) and

Eastern oysters, *Crassostrea virginica* (Gmelin, 1791) to *Perkinsus marinus*. Journal of Shellfish Research 10:433-437.

Mitton, J. B. and M. C. Grant. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annual Review of Ecology and Systematics 15:479-499.

Moukrim, A., Id Halla M., Kaaya A., Bouhaimi A., Benomar S., and M. Mathieu. 2008. Pattern of reserve storage of the two mussel species (*Perna perna* and *Mytilus galloprovincialis*) living on Moroccan coasts: Annual variation and effect of pollution. Iberus 26:17–28.

Murray, T. J. and K. Hudson. 2013. Virginia Shellfish Aquaculture Situation and Outlook Report: Results of 2012 Virginia shellfish aquaculture crop reporting survey. VSG-13-02, VIMS Marine Resource Report No. 2013-02. 20 pp.

Nell, J. A. 2001. Farming triploid oysters. Aquaculture 210:69-88.

Newell, R. I. E. 1985. Physiological effects of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, & Mackin) on the American oyster *Crassostrea virginica* (Gmelin). Journal of Shellfish Research 5:91-95.

Newell, R. I. E., Paynter, K. T., and E. M. Burreson. 1994. Physiological effects of protozoan parasitism on the eastern oyster *Crassostrea virginica*: feeding and metabolism (Abstract). Journal of Shellfish Research 13:294.

Normand, J., Ernande, B., Haure, J., McCombie, H., and P. Boudry. 2009. Reproductive effort and growth in *Crassostrea gigas*: Comparison of young diploid and triploid oysters issued from natural crosses or chemical induction. Aquatic Biology 7:229-241.

Paolisso, M., Herman, S., and N. Dery. 2005. Cultural Analysis for EIS on Oyster Restoration Alternatives, Including *Crassostrea ariakensis*. MD DNR Report.

Paynter, K. T. and E. M. Burreson. 1991. Effects of Perkinsus marinus infection in the Eastern oyster, Crassostrea virginica: II. Disease development and impact on growth rate at different salinities. Journal of Shellfish Research 10:425-431.

Paynter, K. T., Goodwin, J. D., Chen, M. E., Ward, N. J., Sherman, M. W., Meritt, D. W., and Allen, S. K., Jr. 2008. *Crassostrea ariakensis* in Chesapeake Bay: Growth, disease and mortality in shallow water environments. Journal of Shellfish Research 27:509-515.

Perkins, F. O. 1976. *Dermocystidium marinum* infection in oysters. Marine Fisheries Review 38(10):19-21.

Perkins, F. O. 1988. Structure of protistan parasites found in bivalve molluscs. pp93-111 in W. S. Fisher (ed). Disease Processes in Marine Bivalve Molluscs. American Fisheries Society Special Publication 18. Bethesda, Maryland.

Pianka, E. R. 1970. On r and K selection. American Naturalist 104:592-597.

Piferrer, F., Beaumont, A., Falguière, J., Flajšhans, M., Haffray, P., and L. Colombo. 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293:125-156.

Pinheiro, J., Bates, D., DebRoy, S., and D. Sarkar. R Development Core Team. 2013. nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-108.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Racotta, I. S., Palacios, E., Ibarra, A. M., Ramirez, J. L., Arcos, F., and O. Arjona. 2008. Comparative biochemical composition of ploidy groups of the lion-paw scallop (*Nodipecten subnodosus* Sowerby) supports the physiological hypothesis for the lack of advantage in triploid mollusc's growth in food-rich environments. Marine Biology 153:1245-1256.

Ragone Calvo, L. M. and E. M. Burreson. 1994. Characterization of overwintering infections of *Perkinsus marinus* (Apicomplexa) in Chesapeake Bay oysters. Journal of Shellfish Research 13:123-130.

Ragone Calvo, L. M., Calvo, G. W., and E. M. Burreson. 2003. Dual disease resistance in a selectively bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake Bay. Aquaculture 220:69-87.

Rainer, J. S. and R. Mann. 1992. A comparison of methods for calculating condition index in Eastern oysters, *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research 11:55-58.

Ray, S. M., Mackin, J. G., and J. L. Boswell. 1953. Quantitative measurements of the effect on oysters of disease caused by *Dermocystidium marinum*. Bulletin of Marine Science of the Gulf and Caribbean 3:6-33.

Ray, S. M. 1954. Biologican studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Institute Pamphlet Special Issue, November 1954.

Ray, S. M. 1966. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. Proceedings of the National Shellfisheries Association 54:55-69.

Sami, S., Shpigel, M., and M. Faisal. 1991. Comparison between the host defense mechanism of diploid and triploid oysters *Crassostrea gigas*: effect of thermal stress on hemocyte surface Concanavalin A-binding sites. Zeitschfirt fuer Angewandte Zoologie 78:69-78.

Seifter, S., Dayton, S., Novio, B., and E. Muntmi. 1950. The estimation of glycogen with the anthrone reagent. Archives of Biochemistry and Biophysics 25:191-200.

Shumway, S. E. and R. K. Koehn. 1982. Oxygen consumption in the American oyster *Crassostrea virginica*. Marine Ecology Progress Series 9:59-68.

Southworth, M., Harding, J. M., Wesson, J. A., and R. Mann. 2010. Oyster (*Crassostrea virginica*, Gmelin 1791) population dynamics on public reefs in the Great Wicomico River, Virginia, USA. Journal of Shellfish Research 29:271-290.

Sprague, V. A., Dunnington, E. A., and E. Drobeck. 1969. Decrease in incidence of *Minchinia nelsoni* in oysters accompanying reduction in salinity in the laboratory. Proceedings of the National Shellfisheries Association 59:23-26.

Stanley, J. G., Allen, S. K., Jr., and H. Hidu. 1981. Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture 23:1-10.

Stanley, J. G., Hidu, H., and S. K. Allen, Jr. 1984. Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. Aquaculture 37:147-155.

Steiner, J., Termonia, Y., and J. Deltour. 1972. Comments on smoothing and differentiation of data by simplified least-square procedure. Analytical Chemistry 44:1906-1909.

Tarnowski, M. (ed.). 2010. Maryland Oyster Population Status Report: 2008 Fall Survey. MDNR Publ. No. 17-4222010-448.

VMRC. 1996. 1996 Annual commercial fisheries statistics summary report. The Plans and Statistics Department of the Fisheries Management Division.

VMRC. 2008. 2008 Annual commercial fisheries statistics summary report. The Plans and Statistics Department of the Fisheries Management Division. 5 pp.

Walne, P. R. 1970. The seasonal variation of meat and glycogen content of seven populations of oysters, *Ostrea edulis* L., and a review of the literature. Fisheries

Investigations London 26:1-35.

Walne, P. R. and R. Mann. 1975. Growth and chemical composition in *Ostrea edulis* and *Crassostrea gigas*. Pages 587-607 in H. Barnes (ed.), Proceedings of the Ninth European Marine Biology Symposium. Aberdeen University Press, Aberdeen, Scotland.

Wang, Y., Guo, X., DeBrosse, G., and S. Ford. 2005. Superior growth in natural triploid eastern oyster produced by diploid x tetraploid crosses. Journal of Shellfish Research 24:1274.

Wang, Y., DeBrosse, G., Karne, R., Bagnall, P., Blake, J., Ford, S., and D. Bushek. 2006. Superior growth of triploid eastern oyster depends on culture environment. Journal of Shellfish Research 25:675.

Wells, W. F. 1933. Method of shellfish culture. Patent No. 1,933,950, Nov. 14, 1933.

Widdows, J. and A. J. S. Hawkins. 1989. Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding and growth components. Physiological Zoology 62:764-784.

Williams, P. 2008. Near-infrared technology: getting the best out of light. Nanaimo, BC: PDK Projects. 163pp.

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