ABSTRACT

COUCH, CHARLENE REESE. Microsatellite DNA marker-assisted selective breeding of striped bass. (Under the direction of Dr. Craig. V. Sullivan).

Although the hybrid striped bass (HSB; female white bass, *Morone chrysops* x male striped bass, *M. saxatilis*) supports the fourth most valuable form of finfish aquaculture in the United States, neither parental species has been genetically improved. Expansion of the HSB industry is limited by culture inefficiencies associated with reliance on wild broodstock for annual fingerling production. Domestication and selective breeding are expected to increase production efficiency and to promote market expansion. Resource limitations currently prohibit the individual rearing of multiple larval families for striped bass performance testing, necessitating a breeding program that is based on communal rearing of progeny groups with molecular markers as genetic tags for offspring identification. This dissertation research addresses fundamental questions relevant to selective breeding of the male parental species of the HSB, the striped bass, including:

(1) Evaluation of genetic variation within a captive striped bass broodstock population;

(2) Examination of the feasibility of communal rearing protocols based on microsatellite markers for progeny identification during performance evaluations of striped bass;

(3) Assessment of paternal variation in performance traits of striped bass at both research and commercial scale throughout the HSB production cycle.

Examination of three captive striped bass broodstock strains using three highly variable microsatellite markers revealed that the broodstock population contains moderately high

genetic diversity, with an average allelic richness of 13.7 alleles per locus and an average observed heterozygosity of 0.84. Crosses among the three differentiated strains should provide a valuable starting point for establishing a highly variable base population for selective breeding.

Twenty-four experimental families were produced from captive, genotyped broodstock for communal evaluations of progeny survival and performance. Parentage was determined by microsatellite genotyping at six loci and more than 99% of progeny were attributable to a single sire-dam pair at each production phase and in all rearing environments. Application of large-scale communal rearing trials based on microsatellite markers for progeny identification should be a viable approach in a selective breeding program for striped bass.

There was limited evidence of family effects on early growth or survival to 35 days of age; however, significant paternal effects on growth performance, body shape, and carcass traits were detected at later culture stages and variation in antimicrobial peptide activity, a measure of innate disease resistance, differed by strain within the research ponds. In general, progeny of domesticated Santee:Chesapeake sires out-performed those of other strains both at research-scale and in the commercial tank. In research ponds, performance of fish as yearlings (Phase II) allowed prediction of performance at Phase III (18-20 months of age). Performance in research ponds also was predictive of Phase III performance in the commercial tank. Results from performance evaluations provide evidence of genetic variation in economically important traits which may be exploited for selective breeding of striped bass.

This research provides fundamental information needed to accelerate selective breeding and to increase production efficiency for the hybrid striped bass industry.

MICROSATELLITE DNA MARKER-ASSISTED SELECTIVE BREEDING OF STRIPED BASS

by
CHARLENE REESE COUCH

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North Carolina State University
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DEDICATION

This work is dedicated to the good people of the United States hybrid striped bass industry

and to the memory of my grandmother, Callie Ensley Reese.

BIOGRAPHY

Charlene Reese Couch was born on June 28, 1967 in Gastonia, NC and raised on a small farm in Kings Mountain, NC by her parents, Robert and Joyce Ledford. Charlene graduated from Kings Mountain Senior High School and pursued a Bachelor of Science degree in Biology at the University of North Carolina-Wilmington where she was first exposed to scientific research when offered an opportunity to study mammalian zoogeography on North Carolina's barrier islands. Shortly after graduation in 1989, Charlene married Paul Couch and moved to Bethlehem, PA in order for Paul to pursue seminary education. During this time, she became interested in genetics and returned to school in 1995 in Richmond, VA, obtaining a Master of Science in Biology in 1998 from Virginia Commonwealth University with an emphasis in population genetics. After applying to the graduate program at North Carolina State University, Charlene was offered a position in Dr. Craig Sullivan's laboratory in the Department of Zoology where she was able to integrate her interests in molecular biology and field work by using microsatellite markers to support development of a striped bass breeding program. After completion of her doctoral education, Charlene will further her training in quantitative and population genetics through a postdoctoral position in the laboratory of Dr. Trudy Mackay in the Department of Genetics. It is Charlene's hope that the sum of these experiences and training will enable her to develop a career in which she can use molecular methods to address issues relevant to local and regional agriculture, preferably in the area of aquaculture genetics.

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INTRODUCTION

The striped bass, *Morone saxatilis*, is native to the Atlantic coast of North America, occurring from the St. Lawrence River in Canada, south to the St. Johns River in Florida, and disjunctly west into the Gulf of Mexico to Louisiana (Raney and Wolcott 1955). These top predators are highly prized as foodfish and gamefish and have been widely stocked into reservoirs throughout the United States and in rivers along the Atlantic coast, in tributaries of the Gulf of Mexico, and along the California coast. Spawning grounds for striped bass have been identified in the Miramichi and Shubenacadie Rivers in maritime Canada (Robinson and Courtenay 1999; Robinson et al. 2004), the Hudson River and the Delaware River (Waldman and Wirgin 1994), various tributaries of Chesapeake Bay (Laughlin and Turner), the Roanoke River (Trent and Hassler 1968), the Santee-Cooper river system (Bulak et al. 1993; Diaz et al. 1998), the St Johns River and the Apalachicola-Chattahoochee-Flint River system (Wirgin et al. 1991). Populations from Maine to southern North Carolina, including the Hudson, Chesapeake and likely the Roanoke (Berggren and Lieberman 1978; Waldman and Fabrizio 1994), are anadromous and spawn in brackish to freshwater estuaries in spring. Juveniles of these groups mature inshore and the adults engage in seasonal migrations along the Atlantic Coast, comprising the Atlantic mixed stock fishery (Berggren and Liebermann 1978; Waldman et al 1988; Wirgin et al. 1993). Differentiation among spawning stocks suggests natal fidelity of migrating striped bass to their rivers of origin (Chapman 1987). Populations at the extreme ends of the range, from Maine north to the St. Lawrence River in Canada (Raney and Wolcott 1955) and from southern North Carolina south to Florida are non-migratory and are considered to be estuarine or riverine stocks (Setzler et al. 1980).

Since colonial times, the striped bass has supported large commercial and recreational fisheries (Pearson 1938), but overharvest and habitat degradation resulted in several severe population declines, most recently in the mid-1970s (Koo 1970; Richards and Rago 1999). During the mid-1980s, in response to the market void created by the striped bass population crashes and regulatory limitations on the fisheries, commercial aquaculture of a hybrid striped bass (hybrid striped bass; striped bass X white bass, and reciprocal cross) began. The hybrids were hardier under culture conditions than either of their parental species, apparently due to broader tolerances for temperature and dissolved oxygen and to increased tolerance of stress (Smith et al. 1985; Hallerman 1994; Noga et al. 1994). Hybrid striped bass initially were produced in earthen ponds in North Carolina but have since been successfully reared as foodfish in intensive tank and raceway systems, as well as in both freshwater and marine cages, throughout the United States (J. Carlberg, *personal communication*). Production of hybrid striped bass is currently the fourth most valuable form of finfish aquaculture in the United States (Pritchard 2005). Although the hybrids are fertile, direct perpetuation of favorable traits in hybrid lines is not possible as the F_2 offspring exhibit lower hatchability, decreased larval viability, decreased growth (Smith and Jenkins 1984) and increased incidence of deformities (Bosworth et al. 1997) compared to the F_1 hybrids. The hybrid striped bass farming industry remains largely dependent on wild broodfish for annual fingerling production.

Following its development in the mid-1980's, the hybrid striped bass industry grew rapidly, expanding by more than 600% between 1988 and 1993 (Figure 1). Production has since plateaued and remains static at approximately 12 million pounds per year. High costs of production dictate that hybrid striped bass be marketed to gourmet restaurant and live fish

markets in the United States and abroad where higher price points are acceptable. Private and government marketing experts estimate that U.S. hybrid striped bass production could rapidly increase five-fold to 50 million pounds and $100 million in annual revenue if market prices could be decreased only moderately. Production costs have risen more than 7% per year but the market price has increased only 0.2% per year since 1995 (J. Carlberg, *personal communication*). A decrease in production cost of hybrid striped bass would allow sales of hybrid striped bass directly to U.S. consumers in traditional retail markets, much like catfish is marketed today. It is well recognized in the hybrid striped bass industry that genetic improvement is necessary for realizing sustainable production and promoting industry expansion. Economists at Kent SeaTech Corporation predict that production costs for hybrid striped bass could be reduced by ~12% if selective breeding can yield just 20% faster growth rates. Gains in growth rates of this magnitude have been achieved through only 1-2 generations of selective breeding of other aquaculture species, including salmon (Gjedrem 1979, Hershberger et al. 1990), trout (Kincaid et al. 1977), and catfish (Dunham 1987). Most finfish for which selection trials have been made appear to respond with gains of 10-20% per generation in traits such as growth rate (Refstie et al. 1997), as do shellfish (Calvo et al. 2003). Domestication, without directed selection, has resulted in 3-6% improvement in catfish growth per generation (Dunham and Smitherman 1983). Knibb (2000) estimates that even modest genetic gains of ~10% by breeding could double profits for marine aquaculture companies, and these genetic gains will compound for each generation of selection. Selection has proved successful for the Atlantic salmon industry, where a selective breeding program produces a cost benefit ratio of 1:15 (Gjedrem 1997). For poultry, it has been estimated that nearly all of the 300-400% improvement in growth between 1957 and 1991 was due to

selective breeding and that very little was due to improved husbandry techniques (Havenstein et al. 1994). Because the hybrid striped bass industry mainly utilizes wild broodfish for spawning, there is much untapped genetic potential for development of a superior cultivar and the potential economic returns from domestication and selective breeding may be equally dramatic.

Research has revealed significant performance differences among geographic stocks and families of captive striped bass (Brown et al. 1998; Woods et al. 1999, Jacobs et al. 1999) and white bass (White 2000; Kohler et al. 2001) and among geographic strains of wild striped bass (Conover et al. 1997; Secor et al. 2000). With high reproductive output and variation within and among stocks and strain of white and striped bass, it is expected that these fish will respond strongly to selection (Hallerman and Beckmann 1988; Poompuang and Hallerman 1997; Garber and Sullivan 2006). Hybrid striped bass growers, along with university and government scientists, have recently initiated a National Program of Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry (hereafter, National Breeding Program) that is committed to development of domesticated and selectively improved *Morone* broodstocks. Domestication and selective breeding of these economically important species will greatly reduce or eliminate dependence on unreliable methods of wild capture as well as alleviate pressure on wild stocks. Access to reliable sources of broodstock, application of efficient selective breeding designs, and successful and predictable control of reproduction of captive fish will enable hybrid growers to maximize the quality and quantity of fingerlings and foodfish for production. Domestication and selective improvement of fish that are adapted to prevailing culture conditions is vital for

sustaining and improving growth of the hybrid striped bass industry and for enabling this industry to better compete with producers of other wild and farmed food fish.

The commercial production cycle of hybrid striped bass begins with acquisition of broodstock for spawning. Seedstock producers are largely dependent upon the annual capture of wild white bass and striped bass broodstock directly from their spawning grounds. Gravid female white bass are induced to ovulate with injections of human chorionic gonadotropin (hCG; Kohler 1997) and then the eggs are manually stripped from females and fertilized *in vitro* with milt from striped bass males. Fertilized eggs are incubated in McDonald hatching jars for approximately 48 hours (Rees and Harrell 1990) and newly hatched fry swim up from the jars into large aquaria where they are incubated for 2 to 5 days until their mouth parts have developed and exogenous feeding can begin (Humphries and Cumming 1973). The majority of fry produced by the hybrid striped bass industry are stocked into outdoor earthen ponds which have been fertilized and prepared in order to stimulate phytoplankton and zooplankton blooms. Small zooplankton such as rotifers, copepods and cladocerans serve as the source of food for the young larvae for the first few weeks of life (Geiger 1983a,b, 1985). In intensive fry culture, larvae are stocked into tanks and zooplankton species are supplied as a food source until the fry can be trained to accept artificial feeds. Prepared feeds are introduced to pond-reared fry at 22 to 27 days of age (Ludwig 1994; Hodson 1995). The fish are fed a high protein starter diet several times per day until their harvest at 30-45 days after stocking. At the end of this Phase I rearing period, when fingerlings are approximately 1 gram or more in weight, the fish are seined from the ponds and graded to remove any extraordinarily large individuals, which may become cannibalistic (Parker and Geiger 1984; Hodson 1995). Cannibalism appears to be only a minor problem in pond culture but can

cause substantial mortality in intensive culture (Kerby et al. 1988). Fingerlings are generally maintained in raceway or tank systems until they have been trained to accept artificial feeds, after which time they are sold to producers for stocking into outdoor ponds or into commercial tank or raceway systems for production of Phase II fingerlings (90-225g) and subsequently for production of Phase III food fish (568-681g) (Hodson 1995).

Because the hybrid is a terminal cross, simultaneous improvement of the two parental species of the hybrid will be necessary in the National Breeding Program. Efforts toward domestication of both striped bass and white bass are underway at several university and government facilities. The majority of hybrid striped bass culture relies on production of the sunshine bass, a cross between a white bass female and a striped bass male. This cross has been reported to have better growth performance and stress tolerance than the white bass, palmetto bass (female striped bass x male white bass) or striped bass (Noga et al. 1994; Rudacille and Kohler 2000; Myers and Kohler 2000); however, contradictory growth performance has been demonstrated by several investigators (Smith et al. 1985; Bosworth et al. 1997; Jenkins et al. 1998) for whom the palmetto bass and/or the striped bass showed better performance characteristics. A definitive study to evaluate these crosses in a well-replicated experiment remains to be completed, and thorough evaluation of the performance of domesticated striped bass in comparison to the hybrid crosses is lacking. In any case, sunshine bass presently constitute the bulk of hybrid striped bass production because the fingerlings are much more readily available to fingerling and foodfish producers (Harrell 1997) owing to the fact that female white bass are smaller and are more easily housed, handled and spawned than are striped bass females (Smith et al. 1996), particularly in a commercial setting. Fingerling producers typically spawn multiple white bass females and

then fertilize the eggs *in vitro* with striped bass sperm. Therefore, for a hybrid striped bass breeding program, distribution of improved lines to fingerling producers and preservation of various genetic lineages will be best accomplished by distributing gametes from the male parent, the striped bass. Techniques have been developed for both short term storage and long term cryopreservation of striped bass semen (Kerby et al. 1985; Jenkins-Keeran et al. 2001; Jenkins-Keeran and Woods, 2002a,b; He and Woods 2003; Thirumala et al. 2006). In addition to the relative ease of preserving and distributing striped bass male gametes, striped bass males become reproductively mature at an earlier age than do females (2-3 years rather than 4-5 years) (Sullivan et al. 1997), and this shorter generation time should prove beneficial in a breeding program since improved lines of males will be available for reproduction more quickly than would females of their year class. Because distribution of male gametes is easily accomplished and because rapid gains in a program of selective breeding are most likely to be made using the male striped bass, efforts at North Carolina State University (NCSU) have focused on selective improvement of the striped bass parent. Additionally, several authors have suggested that striped bass as a cultivar may be superior to the current hybrid striped bass product (Smith et al. 1985; Jenkins et al. 1988) and improved striped bass may be desirable in some market sectors (Garber and Sullivan 2006). Use of selectively improved striped bass would eliminate the need for maintaining two parental species and would reduce environmental concerns about genetic contamination from hybrid striped bass escaping into the wild. Selective improvement of striped bass will support the primary goals of the National Breeding Program.

The largest and most genetically diverse collection of captive striped bass broodstock is held at the NCSU Pamlico Aquaculture Field Laboratory (PAFL) in Aurora, North

Carolina. The broodstock group consists of a number of wild-caught strains collected throughout the geographic range of the species, as well as several captive-bred lines produced at PAFL or obtained from the University of Maryland or from commercial or government researchers. Despite challenges associated with spawning striped bass in captivity, recent improvements in broodstock husbandry techniques including improved methods for detection of the onset and course of reproductive maturation (Weber et al. 2000), staging of biopsied oocytes to identify female candidate spawners (Rees and Harrell 1990; Sullivan et al. 1997), induction of gonadal maturation using implanted pellets that chronically release synthetic hormones (Woods and Sullivan 1992, 1993; Hodson and Sullivan 1993), and maturation of broodstock out-of-season by photothermal conditioning (Blythe et al. 1994; Smith and Jenkins 1988; Clark et al. 2005) have permitted researchers to successfully close the reproductive cycle of striped bass. These advances set the stage for genetic improvement of the fish in a program of domestication and selective breeding. Using these new technologies, both striped bass and white bass have been reared through several successive filial generations of domestication in captivity (Woods et al. 1992; Woods and Sullivan 1993; Hopper 1999). NCSU researchers have repeatedly verified that domesticated striped and white bass broodstock can be used to reliably produce hybrid striped bass fingerlings with rates of fecundity, fertility, hatching and fry survival equal to or greater than values observed for fully mature wild broodfish (Hodson et al. 2000). Such necessary advances in control of reproduction make possible the selective breeding of these fish as it is now technically feasible to generate numerous even-aged families for evaluation of the genetic variation underlying economically important performance traits.

Genotypic components of progeny performance traits have not been assessed for captive striped bass during the commercial production cycle. This deficiency is due primarily to the daunting logistical difficulty of maintaining numerous progeny groups in separate rearing units until they are large enough to be physically tagged, a size reached well after the Phase I rearing period. Not only is synchronous spawning of multiple striped bass families difficult, rearing of numerous replicated families separately in outdoor ponds requires many more ponds than are currently available to the National Breeding Program. Due to these resource limitations, the use of communal pond rearing techniques offers promise for selective breeding of striped bass and hybrid striped bass because multiple families can be stocked into the same pond or tank environment and reared under identical environmental conditions. Communal rearing techniques can thereby reduce the number of rearing units necessary for production of many families and increase the number of families or groups that can be compared (Moav and Wohlfarth 1974; McGinty 1987; Macbeth 2005). Additionally, by rearing all families in the same environment, the environmental component of phenotypic variation among families can be largely minimized, unmasking additive genetic contributions to growth and other commercially important performance traits with greatly reduced tank or pond effects.

Communal stocking of common carp in ponds (Moav and Wohlfarth 1974) and cages (Wohlfarth and Moav 1991) has proven that mixed (as compared to separate) rearing is a valuable and efficient method for performance testing of numerous family groups of fish. This principle also has been demonstrated in catfish (Dunham et al. 1982) and tilapia (McGinty 1983, 1987). Although competition among families may exist in mixed groups, consistent ranking of phenotypic trait means in both separate and communal rearing

experiments was observed in the above studies of carp, catfish and tilapia. Similarly, although not a stated goal of their study, Jacobs et al. (1999) reared several strains of striped bass in two intensive culture facilities with one facility utilizing separate rearing and the other utilizing communal rearing. Culture conditions were slightly different between the two facilities but rank order of growth performance did not differ among strains, providing some evidence that communal rearing of striped bass can produce results consistent with separate rearing. Communal rearing techniques have been utilized for performance evaluations of catfish (Dunham et al. 1982; Bosworth et al. 1998), various races of carp (Wohlfarth and Moav 1990), coho salmon (Hershberger et al. 1990), African catfish (Volckaert and Hellemans 1999), rainbow trout (Iwamoto et al. 1986; Herbinger et al. 1995, 1997), European sea bass (Garcia de Leon et al. 1998), Atlantic salmon (O'Reilly et al. 1998; Obedzinski and Letcher 2004) and brown trout (Glover et al. 2004). Use of communal rearing techniques for striped bass is expected to permit efficient and meaningful comparisons of performance among fish from numerous families during Phase I fingerling production. Evaluation of communal rearing techniques and data from family performance comparisons should provide valuable information for development of a selective breeding program for striped bass.

For communal evaluation of Phase I phenotypic traits in striped bass, families of larvae must be mixed and stocked into ponds only a few days after hatching, when they are much too small to be physically tagged for individual identification. The challenge of individually identifying offspring after communal rearing can be resolved through the use of highly variable molecular markers as innate genetic tags. By genotyping all parents and sampled offspring with polymorphic molecular markers, such as microsatellite markers,

parentage of each communally reared individual can be determined. This technique has been

successfully applied in a number of communal rearing strategies for aquacultured species

(e.g., Herbinger et al. 1995; Garcia de Leon et al. 1998; O'Reilly et al. 1998). Microsatellites

are short (1-6 base pairs), tandemly repeated DNA sequences (Queller et al. 1993). Generally

considered selectively neutral, microsatellite repeat regions are fairly evenly distributed

throughout the animal genome (Tautz and Renz 1984). Due to high mutation rates (on the

order of 10^{-4} per generation; Edwards et al. 1992; Weber and Wong 1993; O'Reilly et al.

1998; Norris et al. 2000), microsatellite markers are often highly polymorphic. These loci can

be amplified from DNA from very small tissue samples by using the polymerase chain

reaction (PCR) (Queller et al. 1993). Microsatellite markers have proven useful in assessment

of population genetic variation and stock identification (Ruzzante et al. 1996b; Chapman et

al. 1999), for analysis of kinship and parentage (DeWoody et al. 1998; O'Reilly et al. 1998),

and for selective breeding (Waldbieser and Wolters 1999). Conservation of PCR priming

sites, sequences adjacent to the microsatellite repeat region, often serves to expedite studies

of organisms for which microsatellites have not been sequenced. Microsatellite markers

developed for striped bass have been shown to be polymorphic in both white bass and hybrid

striped bass (Couch et al. 2006), and those developed for the closely related European

seabass (*Dicentrarchus labrax*) have proven polymorphic in striped bass, white bass, white

perch and hybrid striped bass (Han et al. 2000; C.R. Couch, A.F. Garber and C.V. Sullivan,

unpublished data). As such, microsatellite markers should be useful for individual

identification and estimation of genetic variation in a program of selective breeding for these

species.

The dissertation research described in the following chapters is directed at examination of genetic variation within and among captive strains of striped bass broodstock and at understanding potential genetic influences on survival and performance of communally reared striped bass. These experiments provide necessary basic information for selective improvement of striped bass. This information will be a critical first step toward initiating long-term selective breeding efforts to genetically improve and further domesticate *Morone* species for the hybrid striped bass industry.

ORGANIZATION OF THE THESIS

The six chapters of the dissertation outline the need for selective breeding for *Morone* species and provide substantial information to support the goals of the National Program of Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry.

Chapter 1—Chapter 1 provides an introduction to the rationale for the dissertation research and introduces information regarding the biology and distribution of wild striped bass, an overview of the production cycle of the hybrid striped bass, and the need for domestication and selective genetic improvement of the parent species of the hybrid to enable more efficient and sustainable aquaculture production of these fish. The organization of the dissertation is described.

Chapter 2—In Chapter 2, genetic variation within and among three captive striped bass broodstock strains available to the National Breeding Program is examined. Three

microsatellite DNA were utilized to estimate the genetic variability of the strains for a program of domestication and selective improvement.

Chapter 3— Chapter 3 addresses the feasibility of using microsatellite markers for progeny identification in order to support communal rearing protocols for performance evaluations of striped bass. Utilizing the genotyped striped bass broodstock described in Chapter 2 as parents, twenty-four experimental families were produced for examination of genetic variation in growth-related phenotypic traits and in survival in communally reared families of striped bass.

Chapter 4—In Chapter 4, variation in growth performance is evaluated for six families of striped bass during Phase II rearing in research ponds at PAFL and in a commercial production tank at Kent SeaTech Corporation. A microsatellite marker-assisted communal rearing approach is utilized to examine paternal effects on progeny growth of fingerlings.

Chapter 5—In Chapter 5 of the dissertation, performance traits are examined for eighteen half-sibling families reared in order to evaluate the genetic basis of commercially important performance characteristics for Phase III, or market-size, striped bass. All families were evaluated in research ponds and six of the families also were reared in a commercial tank production system. Six microsatellite markers were utilized as innate genetic tags for parentage assessment.

Chapter 6—A summary of the major findings of the dissertation research and a description of relevant areas of future investigation are described in Chapter 6.

The four research chapters (Chapters 2-5) are prepared in general manuscript format and therefore contain some necessary repetition of information. Tables and figures are numbered in sequential order throughout the thesis and are inserted at the end of each chapter in which they are referenced. In a few cases, tables and figures presented in earlier chapters are referenced in later chapters but these figures and tables are not duplicated for each use. A single list of literature citations follows Chapter 6 and appendices are sequentially numbered and grouped together at the end of the dissertation.
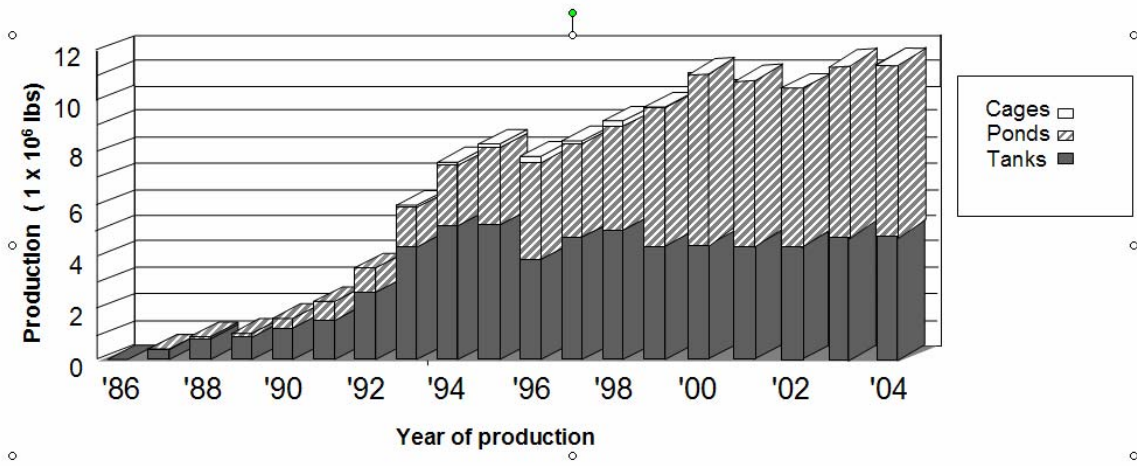
Figure 1. Trends in U.S. hybrid striped bass production (million pounds) through 2004. Data are from state agriculture extension programs and a survey of 27 major producers conducted by Kent SeaTech Corporation.

CHAPTER 2

GENETIC ASSESSMENT OF A CAPTIVE STRIPED BASS (*MORONE SAXATILIS*) BROODSTOCK

ABSTRACT

Genetic variation provides the raw material for evolution and is necessary for selective breeding. In a program of selective breeding, a genetically variable founder population can capture the scope of phenotypic variation which is available for selective improvement of the species or strain. Maintenance of genetic variation during production of the broodstock population and during subsequent generations of captive breeding and selection is critical for avoidance of inbreeding and resultant inbreeding depression of fitness and performance traits. In the present study, three captive striped bass broodstock strains, one wild (Roanoke F_0), and two domestic (Chesapeake F_2 and Santee:Chesapeake F_1), were genotyped at three microsatellite DNA loci in order to estimate the genetic variability of the strains for a program of domestication and selective improvement. All microsatellite loci were highly polymorphic in the striped bass broodstock population. A total of 54 alleles was observed in the population and average allelic richness was 13.7 alleles per locus. Within broodstock strains, allelic diversity was generally high with the greatest average number of alleles per locus observed in the wild Roanoke strain (15 alleles), and fewer alleles observed within domesticated strains (10.3 alleles) or Santee:Chesapeake strains (8.9 alleles). Unique alleles were detected in all strains and one domesticated lineage was distinguished by the presence of a high frequency unique allele. Average observed heterozygosity (H_O) over all loci was 0.84, with H_O of 0.86 for the wild strain and 0.87 and 0.79 for the domesticated strains. Only the Chesapeake strain failed to conform to Hardy-Weinberg expectations, possibly due to the breeding history of this group. The inbreeding coefficient, F_{IS}, was not significantly different from zero for either the Roanoke or the Santee:Chesapeake strains but was less than zero for the Chesapeake strain. Significant differences in genic and genotypic distributions were

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present for all strain pairs. Pairwise F_{ST} values among the strains ranged from 0.031 to 0.07

and revealed low levels of differentiation between the Roanoke and Chesapeake groups and

moderate differentiation between the Santee:Chesapeake group and the other two strains.

Overall, the broodstock population appears to have moderately high genetic diversity and

crosses among the differentiated strains may prove a valuable starting point for establishing a

highly variable base population for selective breeding activities.

INTRODUCTION

Striped bass, *Morone saxatilis*, are native to the Atlantic coast of North America and

occur from the St. Lawrence River in Canada, south to the St. Johns River in Florida, and

disjunctly west into the Gulf of Mexico to Louisiana (Raney and Wolcott 1955). These top

predators are highly prized as food and gamefish and have been widely stocked in freshwater

reservoirs and rivers throughout the United States. Populations from Maine to Cape Hatteras,

including the Hudson River, Chesapeake Bay and the Roanoke River (Berggren and

Lieberman 1978; Wirgin and Fabrizio 1994), are anadromous and spawn in brackish to

freshwater estuaries in spring with juveniles maturing in estuaries and adults engaging in

seasonal migrations along the Atlantic Coast. Together, these groups comprise the Atlantic

mixed stock fishery (Berggren and Liebermann 1978; Waldman et al 1988; Wirgin et al.

1993). Populations at the extreme ends of the range, from Maine north to the St. Lawrence

River in Canada (Raney and Wolcott 1955) and those from southern North Carolina south to

Florida are non-migratory and are considered to be estuarine or riverine stocks (Setzler et al.

1980). Genetic differentiation among spawning stocks suggests natal fidelity of striped bass

to their rivers of origin (Chapman 1987; Wirgin et al. 1997a,b).

Since colonial times, striped bass have supported large commercial and recreational fisheries (Pearson 1938), but overharvest and habitat degradation has resulted in a number of severe population declines (Koo 1970; Richards and Rago 1999), most recently in the mid-1970s. Coastal harvest restrictions were imposed under the Atlantic States Marine Fisheries Commission management plan and improved management has allowed many striped bass populations to rebound (Richards and Rago 1999). During the mid-1980s, in response to the market void created by the striped bass population crashes and regulatory limitations on the fisheries, commercial aquaculture of a hybrid striped bass (striped bass X white bass, *M. chrysops*, and reciprocal cross) began. These hybrids appeared to be hardier under culture conditions than either of their parental species, perhaps due to broader tolerances for temperature and dissolved oxygen and to increased tolerance for crowding (Bonn et al. 1976; Kerby and Harrell 1990; Hallerman 1994). Hybrid striped bass initially were produced in earthen ponds but have since been successfully reared as foodfish in intensive tank and raceway systems, as well as in both freshwater and marine cages. At present, production of hybrid striped bass is the fourth most valuable form of finfish aquaculture in the United States (Pritchard 2005). Although the hybrid striped bass is fertile, direct perpetuation of favorable traits in hybrid lines is not possible as the F_2 offspring exhibit lower hatchability, decreased larval viability and decreased growth (Smith and Jenkins 1984) when compared to the F_1 hybrids. The hybrid striped bass industry remains largely dependent on wild striped bass and white bass broodfish for annual fingerling production, resulting in production inefficiencies that limit expansion of the industry.

Domestication of this economically important species will greatly reduce or eliminate dependence on unreliable methods of wild capture as well as reducing pressure on wild

stocks. Domestication is necessary for growth and maintenance of the hybrid bass industry

and to fully exploit the genetic potential of different stocks. Access to reliable sources of

broodstock, application of efficient selective breeding designs, and successful and predictable

control of the reproduction of captive fish will enable hybrid growers to maximize the quality

and quantity of fry and of food fish produced. Selective breeding of high performance fish

that are well adapted to commercial culture conditions is vital for sustaining and improving

growth of the hybrid striped bass industry and will enable the industry to better compete with

other producers of wild and cultured food fish. In contrast to other domestic livestock,

limited effort has been made to establish domesticated aquaculture species. Breeding

programs for Atlantic salmon, tilapia, channel catfish, and rainbow trout have been initiated

for domestication and genetic improvement, but work with *Morone* species has barely begun.

For any domestication or selective breeding program, control of the reproductive

cycle is necessary. Artificial culture of striped bass began in the late 1800s in North Carolina

with collection of adult striped bass from the spawning grounds on the Roanoke River

(Worth 1884; Harrell 1997). Gravid females and ripe males were manually stripped of

gametes and the fertilized eggs were incubated in hatching jars to produce fry for stocking

into the river. Not until the mid 1960s was stimulation with exogenous hormones employed

to induce ovulation in females (Stevens et al. 1965). Research conducted on temperate basses

at the North Carolina State University Pamlico Aquaculture Field Laboratory (PAFL) has led

to development of a viable hybrid striped bass farming industry by providing methods for

controlled propagation of captive broodstock (Sullivan et al.1997; Hodson et al. 2000). The

white bass parent of the hybrid striped bass is readily spawned in captivity using injected

human chorionic gonadotropin (hCG) to stimulate final maturation and ovulation (Kohler et

al. 1994). Owing to its small size, the white bass is easily handled and may be retained in freshwater ponds or tanks after spawning. The striped bass poses a greater challenge for captive spawning due to its larger size and lower tolerance of handling and stress (Sullivan et al. 1997). Female fish can be difficult to spawn and often wild striped bass broodfish are not retained after spawning by commercial producers or they do not survive the spawning season.

Despite challenges to spawning striped bass in captivity, recent improvements in broodstock husbandry techniques including improved methods for detection of the onset and course of reproductive maturation (Weber et al. 2000), staging of biopsied oocytes to identify candidate female spawners (Rees and Harrell 1990; Sullivan et al. 1997), induction of gonadal maturation using implanted pellets that chronically release synthetic hormones (Woods and Sullivan 1992, 1993; Hodson and Sullivan 1993), and maturation of broodstock out-of-season by photothermal conditioning (Blythe et al. 1994; Smith and Jenkins 1988; Clark et al. 2005) have permitted researchers to successfully close the reproductive cycle of striped bass. These advances set the stage for genetic improvement of the fish in a modern program of domestication and selective breeding. Using these new technologies, striped bass and white bass have been reared through several successive filial generations of domestication in captivity (Woods et al. 1992; Woods and Sullivan 1993; Hopper 1999). The NCSU researchers have repeatedly verified that domesticated striped and white bass broodstock can be used to reliably produce hybrid striped bass fingerlings with rates of fecundity, fertility, hatching and fry survival equal to or greater than values observed for fully mature wild broodfish (Hodson et al. 2000). These discoveries provide the precise control of reproduction necessary for genetic improvement of the fish through a program of

domestication and selective breeding. Development of captive strains to produce an improved cultivar is critical to the viability and continued growth of the United States hybrid striped bass farming industry.

A newly established National Program of Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry is committed to development of domesticated *Morone* broodstocks, selective breeding of the fish for improved performance, and improvement of germplasm cryopreservation techniques to protect valuable domesticated lineages and facilitate rapid transfer of gametes to commercial fingerling producers. Efforts toward domestication of both parent species are now underway at several university and government facilities. One long term goal of the selective breeding program is improvement of performance traits of the male parent of the hybrid striped bass, the striped bass, because the male is more easily spawned and housed than the female and because dissemination of improved gametes can easily be accomplished by cryopreservation of semen for distribution to producers. The largest and most genetically diverse collection of captive striped bass broodstock is held at the North Carolina State University Pamlico Aquaculture Field Laboratory (PAFL) in Aurora, North Carolina. The broodstock group consists of a number of wild-caught strains collected throughout the geographic range of the species as well as several captive-bred lines produced at PAFL or obtained from the University of Maryland or from other commercial or government researchers. As is often the case in cultured fish populations, pedigree information is insufficient to resolve the number of founder individuals for many of these groups and the genetic constitution of the strains is, in part, unknown. Use of molecular marker technologies with statistical estimators of parentage (e.g., Queller and Goodnight 1989; DeWoody et al. 1998; Cunningham et al. 2001) can allow reconstruction of

breeding histories in the absence of pedigree information and may permit estimations of genetic diversity and inbreeding.

Although genetic differentiation has been detected among several geographic strains of striped bass (Wirgin et al. 1991; Wirgin and Maceda 1999; Diaz et al. 1997; Wirgin et al. 1997a,b; Roy et al. 2000), low genetic variation appears to characterize this species (Grove et al. 1976; Sidell et al. 1980; Waldman et al. 1988). Striped bass exhibit unusually low genetic diversity as compared to other marine and anadromous fishes (DeWoody and Avise 2000). This low genetic variability has limited the number of informative molecular markers available for striped bass. Use of highly polymorphic markers, such as microsatellite DNA markers, will be necessary to discriminate among populations and stocks and for pedigree tracking in a program of selective breeding. Microsatellites are short (1-6 base pairs), tandemly repeated DNA sequences that are fairly evenly distributed throughout the animal genome (Tautz and Renz 1984). Microsatellites are co-dominantly inherited and are easily amplified from very small amounts of tissue using the polymerase chain reaction (PCR). Due to high mutation rates, on the order of 10^{-4} per generation (Edwards et al. 1992; Weber and Wong 1993), microsatellite markers are often extremely polymorphic and have proven useful in fishes for assessing population genetic variation and stock identification (Ruzzante et al. 1996; Chapman et al. 1999), analysis of kinship, parentage and assessment of inbreeding (Wolfus et al. 1997; DeWoody et al. 1998; O'Reilly et al. 1998; Villaneuva et al. 2002; Rodgveller et al. 2005), linkage mapping (Jackson et al. 1998; Kocher et al. 1998; Waldbieser et al. 2001; Nichols et al. 2003a,b; Gilbey et al. 2004), and analyses of quantitative trait loci (QTL) (Jackson et al. 1998; Danzmann et al. 1999; Sakamoto et al. 1999; Ozaki et al. 2001; Moen et al. 2004; Leder et al. 2006). The high variability and proven

utility of microsatellite markers in other species make them appealing as DNA markers for assessing captive *Morone* populations and for parentage analysis in captive-bred fish. At the time of this study, only eight published microsatellite sequences and several unpublished sequences were available for striped bass; of these, three proved highly polymorphic (>6 alleles per locus) in our populations and amplified reliably.

To assess the genetic variation of NCSU captive broodstock strains and establish a baseline for evaluating inbreeding in future generations, we genotyped three of the primary captive striped bass broodstock strains, one wild and two domestic strains, using three variable microsatellite DNA markers. This information will provide valuable information for assessing genetic variation in the captive striped bass broodstock and subsequent generations of captive-bred fish in a program of domestication and selective breeding.

<center>MATERIALS AND METHODS</center>

Broodstock Strains--The sampled broodstock represented the three most numerous striped bass broodstock groups available at PAFL. These included 46 striped bass from a wild Roanoke River-Albemarle Sound lineage held in captivity for two years (R:F_0-97), 35 fish from an F_2 Chesapeake Bay lineage (C:F_2-91), and 39 fish from an F_1 Santee River x F_2 Chesapeake Bay lineage (SC:F_1-94) (Table 1). For each strain, roughly equivalent numbers of fish of each gender were genotyped. Fish from the R: F_0-97 strain were captured by hook and line in 1997 from Albemarle Sound near Manteo, NC. Angled fish were estimated to be 4-5 years of age and are likely part of the Roanoke River spawning stock. The SC:F_1-94 line was propagated at PAFL by tank spawning (Salek et al. 2001) eight Chesapeake F_2-1989 males from the Crane Aquaculture Facility (production methods in Woods and Sullivan

<center>25</center>

1993; Woods et al. 1992) with four Santee River F_1-1991 females produced by the South

Carolina Division of Wildlife and Marine Resources hatchery in Bonneau, South Carolina.

The C:F_2-1991 group is composed of a small number of F_2 Chesapeake Bay fish of mixed-

stock origin that were generated by mating one female with several males (Woods et al.

1999). The strain was previously evaluated by Woods et al. (1999) in a comparison of

domestic and wild striped bass lines and by Woods et al. (1995) for the absence of

introgressed white bass alleles. As with most aquaculture broodstock populations, the

number of parents used to produce the Santee River parents of the SC:F_1-94 fish, the

Chesapeake parents of the SC:F_1-1994 fish and the Chesapeake parents of the C:F_2-1991 is

unknown, as is the relative contribution of each parent to the current broodstock strains.

DNA Sampling and Extraction--DNA samples were collected in November 1999

from 120 striped bass held at the Pamlico Aquaculture Field Laboratory (PAFL) in Aurora,

North Carolina. One to three milliliters of whole blood was collected from each fish and

mixed with 50µl 0.34-M dipotassium EDTA as an anticoagulant (after Waldbieser and

Wolters 1999). Blood samples were stored in duplicate freezers at -80° C. Genomic DNA

was extracted from 1µl whole blood using a phenol: chloroform extraction procedure

modified from Saghai-Maroof et al. (1984). DNA was solubilized in 100µl 1X TE (10 mM

Tris, 0.1 mM EDTA, pH 8.0) buffer, and stored at -20° C.

Microsatellite Genotyping--Three variable microsatellite were utilized for

genotyping, *SB91* and *SB113* (Roy et al. 2000) and *SB108* (Wirgin, *pers. comm.*). Primer

sequences and annealing temperatures for these three marker loci are shown in Table 2. PCR

amplification of *SB91* and *SB108* was carried out in multiplex 15µl reactions consisting of

1µl diluted (1:20 in sterile water) template DNA (~10ng/µl), 2mM $MgCl_2$, 48.25µM of each

dNTP (Promega Corporation, Madison, WI), 1.5μl 10X reaction buffer (QIAGEN Inc., Valencia, CA), 0.48μM forward primer (Integrated DNA Technologies, Coralville, Iowa), 0.50μM reverse primer (Applied Biosystems, Foster City, CA), and 0.48 U *Taq* DNA polymerase (HotStar *Taq*, QIAGEN Inc.). Negative and positive controls were included in each PCR run and on all electrophoresis gels. Reverse primers were 5'fluorescently labeled with NED[TM] or 6FAM[TM] (Applied Biosystems) fluorophores for detection on ABI 377 automated DNA sequencers. Thermal cycling parameters for the two multiplexed loci included initial denaturation at 95 ºC for 15 minutes, 30 cycles each of denaturation at 94 ºC for 30 seconds, annealing temperature for 20 seconds, and elongation at 72ºC for 30 seconds, and final elongation at 72 ºC for 5 minutes. Amplification of *SB113* was carried out in the laboratory of Dr. Bert Ely, University of South Carolina, using similar reaction conditions and a HEX[TM]-labeled reverse primer. Genescan[TM]-350 ROX[TM] internal size standard (Applied Biosystems) was added to each sample to ensure accurate and consistent scoring of alleles. Deionized formamide (Sigma-Aldrich, St Louis, MO) was mixed with amplification products for chemical denaturation and samples were heat denatured at 95ºC for 5 minutes. Denatured samples were held on ice prior to transfer into 36 cm Long Ranger acrylamide gels (Cambrex Corporation, East Rutherford, NJ) with 48-well membrane combs (The Gel Company, San Francisco, CA). Electrophoresis was performed on ABI 377 automated DNA sequencers (Applied Biosystems) at the NCSU Genome Research Laboratory. Data were collected and allele sizes determined using GeneMapper® software v3.0 (*SB91* and *SB108*) or GeneScan® and Genotyper® v2.0 software (Applied Biosystems) for *SB113*.

Statistical Analyses--Calculation of the total number of alleles (*A*), allele and genotype frequencies, observed (H_O) and expected (H_E) heterozygosities, tests for

conformance to Hardy-Weinberg equilibrium (HWE), and tests of strain differentiation and genotypic linkage disequilibrium were conducted using GENEPOP version 3.4 (http://wbiomed.curtin.edu.au/genepop/) (Raymond and Rousset 1995a). Allelic richness (A_R), a measure of the number of alleles per locus in each strain adjusted for sample size, was made using FSTAT version 2.9.3.2 (Goudet, 1995) software. Hardy-Weinberg exact tests were used to test the null hypothesis of the random union of gametes in the striped bass broodstock population by locus and over all loci. Exact P-values were estimated for the tests using a Markov chain method (Guo and Thompson 1992) in GENEPOP v3.4. Deviations from HWE were examined for heterozygote excess or heterozygote deficiency with multi-samples versions of the exact test by locus and strain. Significant deviations indicated non-random union of gametes. Genic differentiation, or the random distribution of alleles across strains in the populations, was tested by calculating unbiased estimates of P-values of the probability test (Raymond and Rousset, 1995b) for each microsatellite locus and for all pairwise comparisons between strains. Fisher's exact test was used to test genic differentiation over all loci and strains. Similarly, genotypic differentiation was tested by calculating unbiased estimates of the p-values of a log-likelihood based exact test (Goudet et al. 1996) by locus and between strain pairs, and globally by Fisher's test across all loci and strains. Pairwise tests for linkage disequilibrium between loci were tested within each strain and over all strains. All Markov chains consisted of 2000 dememorization steps, 1000 batches, and 2000 iterations. In each instance where multiple independent tests were performed, significance levels (α) were determined by sequential Bonferroni correction (Rice, 1989).

F-statistics (Wright, 1943; 1951; 1965; Weir and Cockerham, 1984) were calculated using FSTAT software version 2.9.3.2 (Goudet, 1995) to examine the genetic structuring of the broodstock population. F_{IS}, or the coefficient of inbreeding, was calculated for each strain and locus and over all strains and loci. F_{IS} values significantly larger than zero indicate inbreeding. F_{ST}, or genetic divergence among the broodstock strains, measured as the overall reduction in heterozygosity in subpopulations (strains) compared to the total broodstock population, was calculated for each strain pair and across all strains, incorporating information about strain sample sizes and total sample size (Cockerham, 1973; Weir and Cockerham, 1984). F_{ST} values can range from 0, representing no genetic divergence, to 1.0, indicating fixation of alternate alleles in different subpopulations. Values within the range of 0-0.05 indicate little genetic differentiation, and values from 0.05 to 0.15 indicate moderate differentiation (Hartl and Clark 1997). The probability that an F_{IS} or F_{ST} value was significantly different than zero was tested in FSTAT using 200 randomizations. Significance values for pairwise F_{ST} differences between population pairs were calculated by permutation tests.

Estimates of genetic distance using the proportion of shared alleles, (1-P_{SA}; Bowcock et al. 1994) were calculated between each population pair and individually using MICROSAT2 (Minch 1997; http://hpgl.stanford.edu/projects/microsat/programs). To investigate sub-structuring within strains which may have caused deviations from HWE, distance measures also were calculated between all pairs of individuals. Distance matrices were generated for each strain and used to construct a neighbor-joining tree with the NEIGHBOR algorithm in PHYLIP software version 3.6 (Felsenstein 1993; http://evolution.genetics.washington.edu/phylip.html). The resulting tree files were

visualized with TREEVIEW software version 1.6.6 (Page 1996;

http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

<center>RESULTS</center>

Overall genetic diversity-- Measures of genetic diversity, including observed numbers

of alleles (A), allele richness (A_R), observed (H_O) and expected (H_E) heterozygosities, and

inbreeding coefficients (F_{IS}) for each locus and strain and averaged over all loci are detailed

in Table 3. All microsatellite loci were highly polymorphic in the striped bass broodstock

population. A total of 54 alleles was observed in the population, with a range of 10-28 alleles

per locus and a mean of 18 alleles per locus. Average allelic richness, adjusted for

differences in sample sizes among strains, was 13.7 alleles per locus. Overall observed

heterozygosities (H_O) by locus ranged from 0.77 to 0.93 and the average H_O over all loci was

0.84. Significant deviation from Hardy-Weinberg expectation was observed in the striped

bass broodstock population and appeared to result from significant overall deviations from

HWE at *SB113* ($P<0.0167$), but no significant heterozygote excess or deficit was detected for

the locus. F_{IS} values for the broodstock population were not significantly different from zero

for any locus or over all loci, indicating an absence of detectable inbreeding within the

population. *F*-statistics for the broodstock population are presented by locus in Table 4.

Average overall F_{ST} was 0.057, indicating moderate genetic differentiation. Approximately

6% of divergence was attributable to genetic differences among strains while the remaining

94% was due to within-strain differences. The overall total inbreeding across all loci (F_{IT})

was 0.036.

<center>30</center>

Genetic diversity by strain-- Within broodstock strains, allelic diversity was generally high with the greatest average number of alleles per locus observed in the Roanoke strain (16 alleles), and with fewer alleles observed within the Chesapeake (10.3 alleles) and the Santee:Chesapeake groups (9 alleles). Allelic richness for each strain was similar, with an average of 15, 10.3, and 8.9 alleles per locus, respectively, for the three strains (Table 3). Although all strains had overlapping allelic distributions for the three loci, unique (private) alleles were observed in each strain (Table 5). The wild Roanoke strain had the greatest number of unique alleles (12) while the Chesapeake strain had four unique alleles. Only two unique alleles were observed for the Santee:Chesapeake strain; however, one of these alleles was present at high frequency (0.276) in the strain. Average H_O was generally high, with 0.86 for the Roanoke strain, 0.87 for the Chesapeake strain and 0.79 for the Santee:Chesapeake strain (Table 3). There was no difference in the number of alleles, allelic richness or H_O among strains. The inbreeding coefficient, F_{IS}, was not significantly different from zero for either the Roanoke or the Santee:Chesapeake strains, indicating no evidence of inbreeding within those strains. For the Chesapeake strain, however, F_{IS} was significantly less than zero and may represent an excess of heterozygotes in this strain.

Probability tests revealed significant differences in both genic and genotypic distributions overall ($X^2=\infty$, $P<0.0000$) and for all strain pairs. Pairwise genic differences by locus, adjusted for the number of loci and strains examined, were observed between all strain pairs for the *SB113* locus ($P<0.0056$), between the Roanoke group and both other strains for the *SB108* locus and between the Santee:Chesapeake group and both other strains at *SB91* (Table 6); pairwise genotypic differences by locus showed an identical pattern (data not shown). Pairwise F_{ST} values among the strains ranged from 0.031 to 0.074 (Table 7),

revealing lower levels of differentiation between the Roanoke and Chesapeake groups and moderate differentiation between the Santee:Chesapeake group and the other two strains. Significant genetic divergence (P<0.0167) was observed between all strain pairs. Estimates of genetic distance using Bowcock et al.'s (1994) algorithm for the proportion of shared alleles (1-P_{SA}) between population pairs were lowest (0.387) between the Roanoke and Chesapeake strains and highest (0.485) between the Chesapeake and Santee:Chesapeake strains (Table 7). Average genetic distances were greater within the strains than among the strains.

For each strain over all loci, only the Chesapeake group failed to conform to Hardy-Weinberg expectations (X^2=21.38; P=0.0016). No significant heterozygote deficit was detected for any locus or strain; however, an excess of heterozygotes was observed within the Chesapeake strain (P=0.0110). To investigate the presence of sub-structuring within the Chesapeake group that might be the source of the deviation from HWE, distance matrices were used to construct Neighbor-joining trees (Saitou and Nei 1987) based on the proportion of shared alleles among individuals within the strain (1-P_{SA}) and revealed evidence of three subgroups within the Chesapeake strain (Figure 2). Analysis of the Chesapeake strain subgroups for conformity to HWE revealed no significant deviation from HWE by group, locus, or over all groups and loci. Genotypic linkage disequilibrium was detected only in the Chesapeake strain between *SB91* and *SB108* (P<0.0056); however, when the three Chesapeake subgroups were examined, no linkage disequilibrium was present for any locus pair.

Measures of population genetic variation include allelic diversity and heterozygosity. For the three captive striped bass broodstock strains, overall measures of genetic diversity were high, with an average of 18 alleles per locus, average allelic richness of 13.7 alleles per locus, and an average observed heterozygosity of 84%. The overall measure of inbreeding, F_{IS}, was not different from zero, indicating random mating within the broodstock population with no evidence of inbreeding. These are the first estimations of genetic diversity for any captive striped bass broodstock population and represent the requisite initial steps toward a program of DNA marker-assisted domestication and selective breeding of striped bass. Our results are comparable to published measures of genetic variation at microsatellite loci for other anadromous and marine fishes (DeWoody and Avise 2000) and also to results from previous investigations with wild striped bass. Using the same three microsatellite loci as in the present study, Robinson et al. (2004) observed very similar H_O and allelic diversity by locus, with the lowest heterozygosity and number of alleles at *SB91* and the highest at *SB113*. These authors examined populations from three rivers within the Gulf of St. Lawrence, where native stocks had suffered severe historic population declines, as well as populations in the Shubenacadie River and the Hudson River; the higher diversity found in the wild Hudson River population was most similar to our observations with captive striped bass. Wirgin et al. (2005) observed 14 alleles in Santee Cooper River and Florida populations when using the hypervariable locus *SB113*, very near to our estimates of allelic richness for the two domesticated strains. The results with our captive broodstock also are within the range of those detected by researchers using other highly polymorphic molecular markers. Brown et al (2005) observed heterozygosities of 26%-89% for populations within tributaries

of Chesapeake Bay and Laughlin and Turner (1996) detected an average heterozygosity of 76% for populations within the lower Chesapeake Bay, similar to the magnitude of heterozygosity observed in our captive broodfish population. Similarly, Leclerc et al. (1996) discriminated among rivers within Chesapeake Bay and saw heterozygosities of 30-64%, and Diaz et al. (2000) observed similar levels of heterozygosity in striped bass from the Santee-Cooper river system.

Significant differences in allelic and genotypic distributions were present among the captive striped bass broodstock strains, indicative of genetic differentiation among the strains. Likewise, significant differences among strains were observed for F_{ST} values. The pattern of estimates of genetic distance based on allele sharing (1-P_{SA}) among strain pairs was similar to that of F_{ST}, with the Santee:Chesapeake and Chesapeake strains being the most different from one another. Average F_{ST} overall was 0.057, with approximately 6% of divergence attributable to genetic differences among the strains while 94% was due to differences within the strains. Our estimate is within the range of F_{ST} estimates for wild striped bass populations observed by Robinson et al. (2004), where F_{ST} was low among closely related southern Gulf of St. Lawrence tributaries (0.0022-0.0111) and higher for comparisons of southern Gulf of St. Lawrence populations with those from the Shubenacadie (0.1144) or Hudson Rivers (0.2075). Similar estimates were made by Brown et al. (2005) with microsatellite markers for populations within Chesapeake Bay, where θ (F_{ST}) was estimated to be 0.0004-0.0313 among tributaries of the Bay; however, these groups did not evidence population subdivision, unlike our broodstock strains. Laughlin and Turner (1996) observed an average F_{ST} of 0.075 in Chesapeake Bay. Observations by Diaz et al (1998) were much lower (0.001-0.008) among striped bass in the Santee, Congaree and Wateree Rivers in

South Carolina, likely due to hatchery supplementation (stocking), low recruitment and high mortality. Our observations also are similar to those made for captive rainbow trout strains (Hershberger 1992), where greater differentiation was observed within the groups rather than among strains, perhaps due to the breeding history of the strains. Silverstein et al (2004) saw F_{ST} of 0.089 among rainbow trout broodfish strains which had been isolated from each other and bred in captivity for thirty to seventy years. Differences among the captive striped bass broodstock groups may be caused by genetic differences inherent in the progenitor populations for each strain or from genetic drift due to the limited numbers of spawning individuals used to produce a strain. Loss of alleles may be exacerbated by the unintentional effects of selection due to rearing of the animals in captivity. Although differences in allelic distributions and genotypic distributions were evident, evaluation of the striped bass broodstock with additional loci should indicate with more certainty whether the strains differ significantly from one another in the numbers of alleles per locus or in heterozygosity.

A significant deviation from HWE observed in the captive striped bass broodstock population appears to be associated with the *SB113* locus. There was no detectable evidence of heterozygote deficiency at this locus, as might be the case if null alleles were present, and there was no evidence of a heterozygote excess. This deviation may be due to pooling of the three strains for the overall analysis, since random mating does not occur among strains and allele frequencies differed significantly between strain pairs, particularly at *SB113*. Deviations from HWE also may be due to structuring within the strains or to the limited numbers of fish sampled. Although significant linkage disequilibrium was indicated for the *SB91* and *SB108* locus pair, LD was evident only within the Chesapeake population and likely is a result of the sub-structuring of this strain due to its breeding history.

The significant excess of heterozygotes and the presence of genotypic linkage disequilibrium between two loci within the Chesapeake population may be the result of the limited number of parents used to produce the Chesapeake F_2-1991 strain (Table 1). Such bottlenecking would have resulted in chance reductions in allele numbers, but since the broodstock strain was sampled only one generation after the presumed bottleneck, loss of heterozygosity may not yet be evident (Piry et al. 1999). The possibility of family subdivision within the Chesapeake group as seen in the Neighbor-joining tree also may be due to the breeding history of the strain. The presence of a few distinct groups as the result of non-random mating may have caused deviations from HWE that were observed when the strain was considered as a whole. No heterozygote deficits were observed but a significant heterozygote excess was seen in the Chesapeake strain and may represent evidence of recent population bottlenecking during captive breeding. Methods of detection of recent bottleneck events have been formulated (Luikart and Cornuet 1997; Cornuet and Luikart 1996; Luikart et al. 1998; Piry et al. 1999) but require a minimum of five loci for reliable detection of past bottlenecks.

Although significant linkage disequilibrium was indicated for the *SB9*1 and *SB108* locus pair, LD was evident only within the Chesapeake population and probably is a result of the sub-structuring of this strain. Linkage disequilibrium may have been caused by mating between relatives (inbreeding) during captive production of the strain (Hartl and Clark 1997). Linkage disequilibrium was not detected when the strain was re-examined with the three separate subgroups. When these same three loci were used to evaluate wild, presumably randomly mating, striped bass populations, no linkage disequilibrium was observed among the marker loci (Robinson et al. 2004).

High levels of heterozygosity within the broodstock strains suggest that two generations of captive breeding for the Chesapeake and Santee:Chesapeake strains have not resulted in an appreciable loss of genetic diversity. However, when compared to the wild Roanoke population, allele richness does appear to be lower in the two domesticated groups (Table 2). The diversity of the wild founder populations of the domesticated Chesapeake and Santee:Chesapeake strains is unknown. The diversity of these groups was likely limited by the number of founder individuals which were brought into captivity and which spawned successfully; however, wild progenitors from Chesapeake Bay and the Santee-Cooper river system would potentially mirror the relative variation of the wild populations from which those fish were drawn. Santee-Cooper river system populations may have inherently low levels of variation, as observed by Diaz et al. (1998) and Wirgin et al. (2005), due to years of hatchery supplementation to restore diminished population sizes, but Chesapeake populations are expected to be fairly diverse (Wirgin et al. 2005; Brown et al. 2005). The Roanoke River-Albemarle Sound population appears to be one of the more highly diverse populations of striped bass (Stellwag and Payne 1994). Populations which experience bottlenecks, or significant contractions in population size, as is often the case in captive bred populations, can lose allelic diversity rather rapidly since rare alleles may be lost by chance when the number of breeding individuals is restricted (Tave 1993; Hara and Sekino 2003). Heterozygosity levels are not reduced as quickly as allelic diversity, however, and may be evidenced as heterozygote excesses within the first few generations after a bottleneck event (Nei 1975; Luikart and Cornuet 1997). This trend may be the case in our domesticated populations which had quite high heterozygosity levels but far fewer alleles (Chesapeake, 31 alleles, H_O=0.87; Santee:Chesapeake, 27 alleles, H_O=0.79) than the wild Roanoke population

(58 alleles, H_O=0.86). The captive-bred Chesapeake and Santee:Chesapeake populations also had substantially fewer unique alleles per strain (4 and 2 alleles, respectively) than did the wild Roanoke strain (12 alleles).

Propagating and maintaining striped bass broodstock strains separately for domestication and selective breeding should permit improvement of economically important traits for commercial culture. However, limitations inherent in hatchery production of striped bass, including the housing and spawning of adequate numbers of these large fish, as well as the effects of unintentional selection due to captive rearing, may reduce the genetic diversity of broodfish populations over time (Allendorf and Ryman 1987; Doyle and Herbinger1995). Like many marine fishes, female striped bass have tremendous fecundity (230,000 eggs per kilogram body weight; Secor et al. 1992) and a single individual is capable of producing sufficient numbers of offspring for an entire captive-bred year class. Additionally, eggs from a single female often may be fertilized by more than one male to ensure fertilization and families often are pooled for larval rearing to conserve hatchery and larval rearing space; therefore, exact contributions of each parental pair often are unknown. Substantial differences in contribution among parents are possible and have been observed for striped bass (see Chapter 3) and hybrid striped bass (A.F. Garber and C.V. Sullivan, *unpublished data*) and variation in parental contribution also may influence the rate of inbreeding.

Maintenance of adequate genetic diversity is necessary to promote fitness of captive broodstock strains and to provide raw material for selective improvement. Achievement of this goal should be possible if sufficiently large numbers of broodstock are utilized and genetic variation is monitored in successive generations. Measures of genetic diversity, such as heterozygosity, have been correlated with population fitness (Reed and Frankham 2003;

Shikano and Taniguchi 2003); however, assessment of heterozygosity levels may not be a sensitive means for detecting very short-term bottlenecks (Hedgecock and Sly 1990; Perez-Enriquez et al. 1999), so close monitoring of reductions in allelic diversity is critical. The probability of losing low frequency alleles increases with decreasing effective population size (N_e; the average number of individuals that contribute to the subsequent generation) (Tave 1993) and losses of allelic diversity have been associated with decreased fitness in captive Senegal sole broodstock (Porta et al. 2006) and in Atlantic salmon (Primmer et al. 2003). Restriction of N_e, to 20 fish per generation in the Donaldson strain of rainbow trout has resulted in 40-60% inbreeding within about 40 years. The restricted number of parents is likely is the cause of the reduced allelic diversity observed in this strain and these reductions in diversity have been associated with decreased hatching rates (Hershberger 1985).

By utilizing an appropriate number of parents for production of subsequent generations and by tracking pedigrees using physical or genetic tags to prevent breeding among close relatives, broodstock managers may control the genetic health of striped bass broodstock populations. The number of parents necessary for maintaining genetic diversity of striped bass broodstock in a program of captive breeding is based on the level at which the negative effects of inbreeding can occur and the number of generations over which inbreeding effects will accumulate (Tave 1993). In general, inbreeding increases the frequency of homozygous individuals. Based on the relationship between effective population size (N_e) and inbreeding (F), where $\Delta F = 1/(2N_e)$ (Falconer and Mackay 1996), an effective population size of 10 fish is expected to cause 5% inbreeding per generation. Reductions in genetic variation may result in inbreeding depression, or reductions in fitness traits such as survival, fertility, fecundity or physiological parameters; however, the level of

inbreeding at which detrimental effects on fitness or other traits occur is unknown for striped

bass, as is the case for most aquaculture species. Investigations with rainbow trout (Kincaid

1976) suggest that when inbreeding reaches the level of 12.5%, effects such as decreased

weight of juveniles may begin to occur. Gjerde et al. (1983) observed significant effects of

inbreeding (>10%) on fitness traits such as eyed-egg survival, and Aulstad et al. (1972)

observed 10.6% inbreeding depression with inbreeding of 25%. Similarly, susceptibility of

Chinook salmon to disease increases with inbreeding of 25% (Arkush et al. 2002). Average

inbreeding of less than 9% resulted in fairly low levels of inbreeding depression (0.2% per

1% increase in inbreeding) for growth in farmed rainbow trout, considered to be an

acceptable level of inbreeding (Bentsen and Olesen 2002). It appears clear that, since

inbreeding depression can be substantial, matings among close relatives should be avoided in

a program of captive breeding for striped bass. Tave (1993) recommends using 5%

inbreeding as a conservative value and 10% as a liberal value of the permissible level of

inbreeding for any species for which such information is unknown.

Effective population size (N_e), or the average number of individuals that contribute to

the subsequent generation, can be calculated from the formula $N_e = 4N_mN_f/(N_m + N_f)$ (Hartl

and Clark 1997). As can be determined from the above formula, use of equal numbers of

male and female parents can maximize N_e, as can equivalent contribution from the parents.

Bentsen and Olesen (2002) demonstrated that by selecting 20 or more pairs for random

mating, a rate of inbreeding below 4% per generation could be maintained during selection.

These authors recommended use of 50 or more pairs to maximize the rate of selection while

minimizing the rate of inbreeding; this number of breeders restricted the rate of inbreeding to

~1% per generation. Similarly, Meuwissen and Woolliams (1994) suggested that N_e of 31 to

250 individuals is necessary to prevent declines in fitness due to inbreeding in livestock. Similar numbers of parents, in a hierarchical mating design in Atlantic salmon, maintained inbreeding depression for growth at less than 5% (Rye and Mao 1998). Kincaid (1976) suggested use of 200 parents per generation to minimize inbreeding and Ryman and Ståhl (1980) suggested 60 per generation. Similarly, Allendorf and Ryman (1987) recommended use of 200 breeders per generation. The generation time for domestic striped bass may be as low as two years for males and four to five years for females (Sullivan et al. 1997). Assuming that inbreeding accumulation of 5% can be tolerated over 15 generations, we can use the formula $F=1/(2 N_e)$ to calculate that striped bass breeders should strive for an N_e of 150 fish. This practice should limit inbreeding and impacts of inbreeding depression for the next 45-53 years of a breeding program. If higher levels of inbreeding can be tolerated, say 10%, an N_e of only 75 fish would be necessary. Although these numbers are higher than have been routinely used in hatchery production, the improved control of striped bass reproduction, particularly with the use of tank spawning techniques (Salek et al. 2001; C.V. Sullivan, *unpublished data*), combined with our ability to trace parentage of mixed progeny groups of fry using microsatellite DNA markers, should make these goals quite achievable.

The exact effective population sizes for the captive striped bass strains evaluated here are unknown and can only be approximated from historic records. However, in the absence of precise breeding records, it may be possible to estimate the number of breeders using molecular data. Estimates of N_e can be made from linkage associations among loci (Hill 1981; Waples 1991) or from the magnitude of heterozygote excess in a population (Robertson 1965; Pudovkin et al. 1996). The former method requires knowledge of linkage relationships, sample sizes of approximately 90 individuals and data from more than 6 loci

(Bartley et al. 1992). Estimates of N_e from the magnitude of heterozygote excess in a population have been proposed by Pudovkin et al. (1996) and the bias and precision of this method was explored by Luikart and Cornuet (1999). The heterozygote-excess method is based on the expectation that chance allele frequency differences between small numbers of male and female parents may result in an excess of heterozygotes in their offspring (Cornuet and Luikart 1996). Although Luikart and Cornuet (1999) determined that this method may require as many as 10 loci and 30 progeny for precise estimation of N_e, their examination of a microsatellite dataset from bull trout with 4 loci and only 14 progeny estimated an N_e that was similar in magnitude to the known number of parents for this group. Applying this method to our striped bass broodstock strains using Peel and colleagues' (2004) NeEstimator version 1.3 software gave an estimated of N_e of 10.6 for the Chesapeake strain, 19 for the Santee:Chesapeake strain, and 71.7 for the Roanoke strain. These values are ranked similarly to the genetic distances calculated for the strains and, as expected from historic records, the lowest N_e occurs in the Chesapeake strain. Although the demographic history of the Chesapeake and Santee:Chesapeake strains is not known in detail, N_e can be affected not only be numbers of individuals utilized but also by unequal sex ratios. Based on the known difficulty of spawning striped bass females and the comparative ease of spawning the males, estimates of low N_e for the two captive-bred strains is likely the result of using limited numbers of spawning individuals and skewed sex ratios used to produce the current strains. The two domestic broodstock strains (Chesapeake and Santee:Chesapeake) are known to have been produced from a limited number of parents (probably 8 or fewer parents for the Chesapeake strain and ≤ 12 for the Santee:Chesapeake strain) with more male than female parents used. Prior generations also may have been restricted due to difficulties associated

with early efforts at captive spawning and rearing of striped bass. Together, these methods may have resulted in reductions in population size that resulted in lower allelic variation in these strains as compared to the wild strain.

Summary--This snapshot of the genetic diversity of three primary captive striped bass strains will provide a valuable starting point for initiation of selective breeding activities for striped bass and for maintaining diversity over subsequent generations of captive breeding. The differences in strain allele frequency distributions and the presence of unique alleles observed in each strain may serve to facilitate identification of strain origins and determination of relationships among strains and families within the captive broodstock population (Waldbieser and Wolters 1999) and tracking of introgression among strains (Wirgin et al. 2005). Genetic variation evident within the captive striped bass strains should be exploitable in a selective breeding program for improvement of commercially valuable phenotypes such as growth, feed conversion efficiency (kilograms of food per kilogram of growth), disease resistance and carcass traits. Using an adequate number of parents, crosses among the distinct broodstock strains may increase the genetic diversity available for selection, providing a genetically heterogeneous founder population from which to initiate directed selection.

Use of microsatellite loci for differentiation among strains and for tracking pedigrees within the broodstock population should control the incidence of inbreeding and minimize coancestry (Caballero and Toro 2000; Doyle et al. 2001) within future generations. Combined with use of appropriate numbers of parents, these methods should permit breeders to control deleterious effects of inbreeding. The ability to genotype broodstock will allow hatchery managers to select and retain a limited number of genetically variable individuals,

permitting genetic variation to be maintained at a maximum level despite limitations in hatchery space and captive rearing resources. Passive integrated transponder tags (PIT tags) already are in use at PAFL to identify individual adult striped bass broodstock. Combined with the application of highly variable microsatellite markers to track pedigrees in communally reared groups of offspring, these tools should help control the incidence of matings between close relatives and limit inbreeding in subsequent generations of striped bass broodstock. Physical and molecular tagging methods also will permit more accurate calculations of N_e since genotyping of parents can allow hatchery managers to ascertain the number and relative contribution of each broodstock individual to subsequent generations (Waldbieser and Wolters 1999; Doyle and Herbinger 1995; Villanueva et al. 2002), even when larvae must be pooled for communal rearing. Access to hundreds of new microsatellite markers for striped bass (Rexroad et al. 2006; Couch et al. 2006) and implementation of high-throughput fluorescent genotyping methods should enable careful tracking and management of captive striped bass genetic resources.

Dominique Donato assisted with DNA extractions, and Wade Gereats, Michael Hopper,

Andrew McGinty, Naoshi Hiramatsu, and Kaori Hiramatsu aided with sampling of broodfish.

Table 1. Origin of sampled striped bass broodstock strains from Pamlico Aquaculture Field Laboratory.

Strain	Generation	Year of origin	Place of origin	Numbers of fish spawned per generation
Roanoke	F_0	1992-1993 year class; captured in 1997	Albemarle Sound near Mann's Harbor Bridge, Manteo, North Carolina	Captured 54-56 fish
Chesapeake	F_2	1991	Mixed stocks of Chesapeake Bay fish; spawned at Crane Aquaculture Facility, University of Maryland	1 female and several males
Santee:Chesapeake	$F_{1.5}$	1994	Female parents were F_1 fish spawned in captivity from wild Santee-Cooper river system striped bass by the South Carolina Division of Wildlife and Marine Resources in Bonneau, South Carolina. Male parents were Chesapeake F_2-1989 fish produced from F_1 Chesapeake Bay mixed stocks at Crane Aquaculture Facility, University of Maryland. These males may have been the progeny of one female and four males. The Santee:Chesapeake F_1-1994 fish were spawned at Pamlico Aquaculture Field Laboratory, Aurora, NC	4 females, 8 males

Table 2. Microsatellite loci utilized in broodstock genetic analysis.

Locus	Primer sequences (5'-3')	GenBank Accession No.	T_A (°C)	Source
SB 91	F -- AGACACCAGATAAGGAGA R -- TAGATTCACACAAGGTGC	AF200743	47	Roy et al. 2000
SB 108	F -- ACTCTCGTATCGAACCAT R -- CTGGTCAAGCCTTTACTG	---	47	Wirgin (pers. comm.)
SB113	F -- GATCGCGGTTATTACAGT R -- GACTATCTCCCCTGAAAT	AF177512	46	Roy et al. 2000

Table 3. Summary of genetic diversity measures for striped bass broodstock strains by locus and over all loci. Measures include observed number of alleles (A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, and inbreeding coefficient (F_{IS}).

Locus	Roanoke (*N*=46)				Chesapeake (*N*=35)				Santee:Chesapeake (*N*=39)				Overall (*N*=120)			
	A	A_R	H_O/H_E	F_{IS}	A	A_R	H_O/H_E	F_{IS}	A	A_R	H_O/H_E	F_{IS}	A	A_R	H_O/H_E	F_{IS}
SB91	10	9.2	0.74/0.79	0.062	7	7.0	0.80/0.72	-0.106	6	5.9	0.76/0.71	-0.076	10	8.0	0.77/0.74	-0.028
SB108	14	13.4	0.89/0.87	-0.022	11	11.0	0.83/0.82	-0.016	8	8.0	0.74/0.79	0.067	16	11.9	0.82/0.83	0.007
SB113	24	22.4	0.96/0.95	-0.006	13	13.0	0.97/0.89	-0.094	13	12.7	0.87/0.83	-0.048	28	21.1	0.93/0.89	-0.045
Avg.	16	15	0.86/0.87	0.009	10.3	10.3	0.87/0.81	-0.071	9	8.9	0.79/0.78	-0.017	18	13.7	0.84/0.82	-0.022

Table 4. F-statistics for three striped bass broodstock populations.

Locus	F_{IS}	F_{ST}	F_{IT}
SB91	-0.028	0.063	0.037
SB108	0.007	0.038	0.044
SB113	-0.044	0.068	0.026
Avg.	-0.022	0.057	0.036

Table 5. Unique (private) alleles and allele frequencies observed in three striped bass broodstock strains.

Locus	Allele (bp)	Frequency	Strain
SB91	137	0.011	Atlantic
	144	0.011	Atlantic
SB108	178	0.011	Atlantic
	193	0.014	Chesapeake
	201	0.022	Atlantic
	207	0.043	Atlantic
	209	0.014	Chesapeake
	217	0.011	Atlantic
SB113	190	0.276	Santee:Chesapeake
	192	0.013	Santee:Chesapeake
	202	0.033	Atlantic
	212	0.011	Atlantic
	214	0.014	Chesapeake
	220	0.014	Chesapeake
	224	0.033	Atlantic
	228	0.022	Atlantic
	260	0.044	Atlantic
	262	0.011	Atlantic

Table 6. Pairwise genic differentiation among three striped bass strains.

Locus	Populations[1]	Probability	Standard Error
SB91	R - C	0.3406	0.0024
SB91	R - S	0.0000***	0.0000
SB91	C - S	0.0000***	0.0000
SB108	R - C	0.0000***	0.0000
SB108	R - S	0.0000***	0.0000
SB108	C - S	0.1529	0.0020
SB113	R - C	0.0000***	0.0000
SB113	R - S	0.0000***	0.0000
SB113	C - S	0.0000***	0.0000

*** Significant at $P<0.0056$.

[1]Striped bass strain abbreviations are Roanoke (R), Chesapeake (C) and Santee:Chesapeake (S).

Table 7. Genetic differentiation (F_{ST}; above diagonal) and genetic distance (1-P_{SA}; below diagonal) for three broodstock strains.

	Roanoke	Chesapeake	Santee:Chesapeake
Roanoke	{0.791}	0.031**	0.067**
Chesapeake	0.387 (0.108)	{0.692}	0.074**
Santee:Chesapeake	0.460 (0.082)	0.485 (0.188)	{0.659}

** Indicates F_{ST} values significant at $P<0.01667$.

Values above the diagonal are pairwise F_{ST} values. Values below the diagonal are 1-P_{SA} genetic distances with standard errors in parentheses. Values in boldface type along the diagonal are the average within strain pairwise genetic distances.
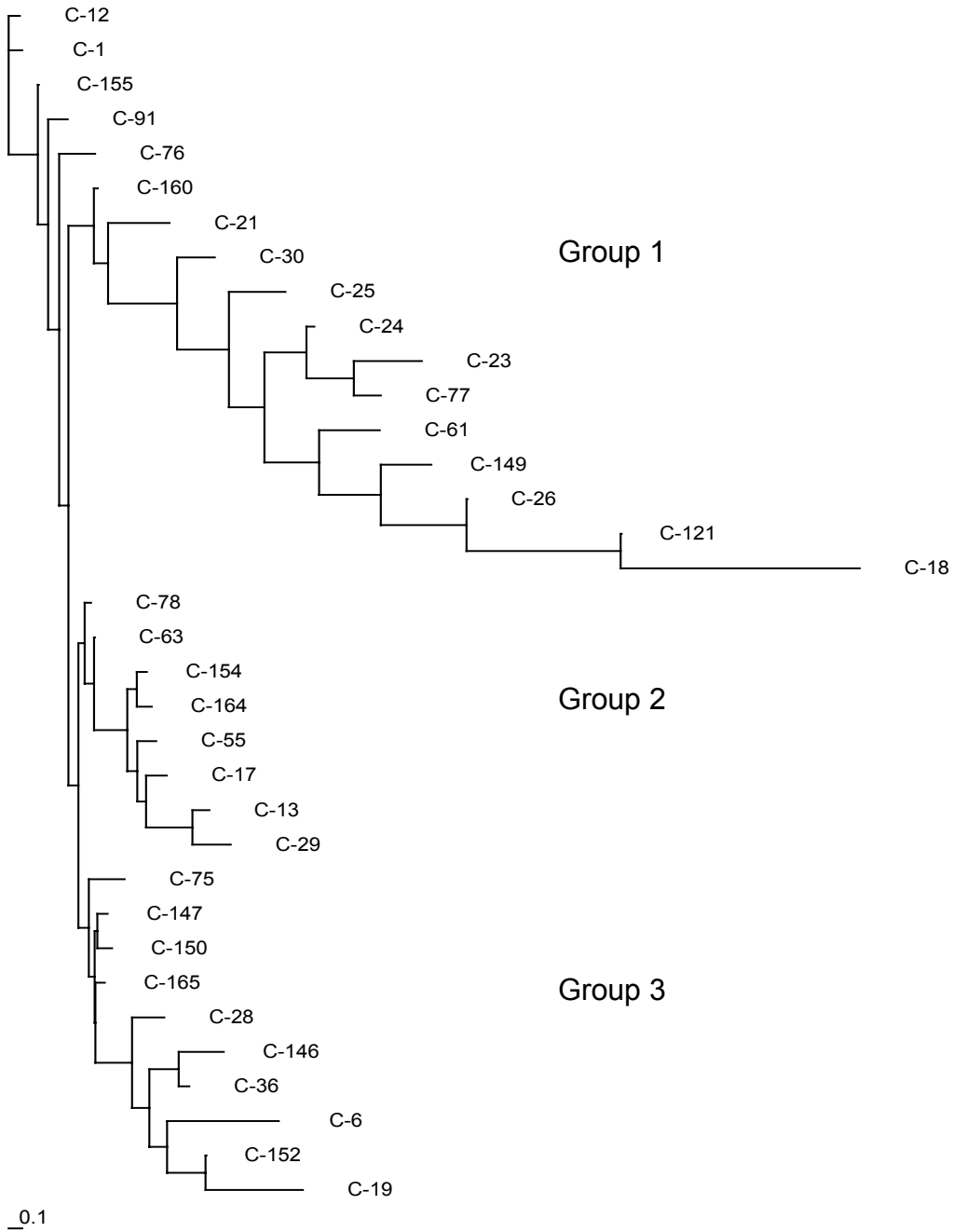
Figure 2. Neighbor-joining tree constructed from the distance matrix of the transformed proportion of shared alleles [-ln (1-PSA)] for the Chesapeake strain. The three groupings (Groups 1-3) within the Chesapeake strain were analyzed as separate units after tests of HWE showed deviation within the strain.

CHAPTER 3

COMMUNAL REARING OF STRIPED BASS LARVAE FOR EVALUATION OF PHASE I FINGERLING

SURVIVAL AND GROWTH PERFORMANCE

ABSTRACT

Aquaculture production of striped bass and hybrid striped bass is the fourth most valuable form of finfish aquaculture in the United States. Despite their importance for commercial culture, these species have not been genetically improved and the hybrid striped bass industry remains dependent on capture of wild fish as broodstock for annual fingerling production. To support the goals of the newly established National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry, paternal genetic variation in growth performance and survival was evaluated in captive striped bass during the first phase of commercial culture, Phase I rearing. Twenty-four experimental crosses were generated from domesticated and wild strains of striped bass and the resulting progeny were stocked by dam in 6-family groups into outdoor pond mesocosms. Larvae were stocked at 4-6 days post hatch, when they were far too small for physical tagging, and they were reared together until harvest at 34-40 days after hatching. Parentage of the pooled progeny was determined by microsatellite genotyping. More than 99% of progeny could be attributed to specific sire-dam pairs using only six microsatellite markers. Although variation in growth was observed in the striped bass during Phase I fingerling production, there was limited evidence from these experiments for any effect of sire or strain on early growth. Examination of additional families in a more highly replicated design may allow for detection of genetic influences on growth in these early life stages. However, it is possible that random environmental effects within small aquaculture ponds may swamp any genetic influences during this early rearing period. The high degree of success in parentage determination for individuals co-stocked as larvae for rearing in a common environment illustrates the feasibility of a microsatellite-

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based approach for performance evaluations and pedigree tracking in the National Breeding Program.

INTRODUCTION

Since its inception in North Carolina in 1986, aquaculture production of striped bass and hybrid striped bass (hybrid striped bass; female white bass, *Morone chrysops* x male striped bass, *M. saxatilis*) has become the fourth most valuable form of finfish aquaculture in the United States (Pritchard 2005). Commercial production of hybrid striped bass increased rapidly from 350,000 pounds in 1989 to just over 12 million pounds in 2005, with an average value to the farmer of about $ 2.70 per pound (J. Carlberg, President-Kent SeaTech Corporation, *personal communication*). However, growth of the hybrid striped bass farming industry has recently slowed (Figure 1) due to competition from foreign products, seasonally limited availability of fingerling seedstock, and high production costs. Despite their value to U.S. aquaculture, neither the hybrid striped bass nor its white bass or striped bass parents have been genetically improved (Harrell and Webster 1997). Nearly all hybrid striped bass are produced from wild parents, but growers and researchers recognize that continued reliance on wild broodfish will not support industry growth or increased production efficiency (Woods 2001). Instead, expansion of hybrid striped bass farming will require full domestication and genetic improvement of these fishes. Leading producers of hybrid striped bass, as well as federal scientists and several university researchers, have recently joined with North Carolina State University (NCSU) to establish a National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry (hereafter referred

to as the National Breeding Program). Captive striped bass broodstocks are under domestication at NCSU and the University of Maryland and some domesticated fish are being held on several commercial farms.

Commercial production of hybrid striped bass begins with acquisition of broodstock for hatchery production of larvae. Seedstock producers remain largely dependent on the annual capture of wild white bass and wild striped bass broodfish from their spawning grounds. Gravid female white bass are induced to ovulate with injections of human chorionic gonadotropin (hCG; Kohler 1997). Eggs are manually stripped from females and fertilized *in vitro* with milt from striped bass males and fertilized eggs are incubated in McDonald hatching jars for approximately 48 hours (Rees and Harrell 1990). Newly hatched fry swim up from the jars into large aquaria where they are incubated 2 to 5 days then fry are stocked into outdoor earthen ponds which have been fertilized and prepared in order to stimulate zooplankton production. Small zooplankton such as rotifers, copepods and cladocerans serve as food for the young larvae during their first few weeks of life (Geiger 1983a,b, 1985). At the end of this Phase I rearing period, when fingerlings are approximately 1 gram or more in weight, the fish are seined from the ponds and graded to remove any unusually large individuals, which may become cannibalistic (Parker and Geiger 1984; Hodson 1995). Fingerlings are trained to accept artificial feeds and then are sold to producers for stocking into outdoor ponds or into commercial tank or raceway systems for production of Phase II fingerlings (90-225g body weight) and subsequently for production of Phase III food fish for market (568-681g body weight) (Hodson 1995).

In contrast to other livestock species, only limited efforts have been made to domesticate and selectively improve cultured fishes. Successful breeding programs have

been instituted for channel catfish (Smitherman and Dunham et al. 1985), Atlantic salmon (Gjedrem 1979), rainbow trout (Gjerde 1986), and tilapia (Eknath et al. 1993), but similar work on *Morone* species has only just begun. It has been heretofore impossible to evaluate genetic contributions to phenotypic traits during early life stages of *Morone* species because of the technical challenges associated with the ability to simultaneously spawn multiple females and reliably generate large numbers of genetically diverse, even-aged families for testing. Scientists have now closed the reproductive cycle of striped bass (Woods et al. 1990, 1992; Woods and Sullivan 1993; Hodson and Sullivan 1993; Blythe et al. 1994; Sullivan et al. 1997; Clark et al. 2005) and have produced several generations of domesticated striped bass (Woods et al. 1992, 1995, 1999; Hodson et al. 2000) and white bass (Kohler et al. 1994; Smith et al. 1996; Kohler 1997; Hopper 1999; Hodson et al. 2000). The largest and most diverse repository of these species is located at the NCSU Pamlico Aquaculture Field Laboratory (PAFL) in Aurora, North Carolina. Scientists at NCSU also have verified that the domesticated broodstock can reliably produce quality offspring at rates of fecundity, fertility, hatching and survival equivalent to progeny of wild broodfish captured directly from their spawning grounds (Sullivan et al. 1997; Hodson et al. 2000). Such necessary advances make possible the selective breeding of these fish as it is now technically feasible to reliably spawn striped bass and to generate numerous families for evaluation of genetic components of performance. Performance evaluations will allow investigation of variation in phenotypic traits that are important in hybrid striped bass production, a critical first step in developing a long-term selective breeding program to genetically improve and further domesticate *Morone* species.

Production of multiple even-aged families of striped bass will enable evaluation of genotypic components of progeny performance traits. The remaining challenge for these investigations in *Morone* is the daunting logistical difficulty of maintaining numerous progeny groups in separate tank or pond rearing units until they are large enough to be physically tagged, a size reached well after Phase I rearing. Maintenance of multiple families in separate tanks for intensive indoor culture is both expensive and labor intensive for marine species such as striped bass or hybrid striped bass since larvae must be provided with live prey as a food source until weaning. Additionally, the culture of microalgae is necessary for feeding cultured rotifers and other zooplankton as food for larval hybrid striped bass. Carefully metered delivery of zooplankton feed, removal of surface oil film from the rearing tanks to facilitate swim bladder inflation, proper selection of tank color and precise control of water quality all are requisite for fry survival in intensive culture. Although these larviculture techniques are being improved (Martin-Robichaud and Peterson 1998; Ludwig 1994; Denson and Smith 1997) and have shown survival rates in excess of 50% at laboratory scale (Ludwig 1993; Denson and Smith 1997; Ludwig 2003), large scale intensive rearing systems and commercial protocols for *Morone* larviculture are presently unavailable. Similarly, rearing numerous replicate families separately in outdoor ponds requires many more ponds than are currently available to the National Breeding Program. Due to these resource limitations, the use of communal pond rearing techniques offers promise for selective breeding of striped bass and hybrid striped bass because multiple families can be stocked into the same pond or tank environment and compared under identical environmental conditions. Communal rearing techniques can reduce the number of rearing units necessary for production of many families while increasing the number of families or groups that can be compared (Moav and

Wohlfarth 1974; McGinty 1987; Macbeth 2005). Additionally, by rearing all families in the same environment, the environmental component of phenotypic variation among families can be largely minimized, unmasking additive genetic contributions to growth and other commercially important performance traits.

Communal stocking of common carp in ponds (Moav and Wohlfarth 1974) and cages (Wohlfarth and Moav 1991) has proven that mixed (as compared to separate) rearing is a valuable and efficient method for performance testing of numerous family groups of fish. This principle also has been demonstrated in catfish (Dunham et al. 1982) and tilapia (McGinty 1983, 1987). Competition among families could potentially occur among mixed family groups; however, consistent ranking of phenotypic trait means in both separate and communal rearing experiments was observed in the studies of carp, catfish and tilapia cited above. Although not a stated goal of their study, Jacobs et al. (1999) reared several strains of striped bass in two intensive culture facilities, with one facility utilizing separate rearing and the other utilizing communal rearing. Culture conditions were slightly different between the two facilities but the rank order of growth performance did not differ among strains between the two facilities, providing evidence that communal rearing of striped bass might produce results consistent with those obtained from separate rearing. Communal rearing techniques have been utilized for performance evaluations of catfish (Dunham et al. 1982; Bosworth et al. 1998), various races of carp (Wohlfarth and Moav 1990), coho salmon (Hershberger et al. 1990), African catfish (Volckaert and Hellemans 1999), rainbow trout (Iwamoto et al. 1986; Herbinger et al. 1995, 1997), European sea bass (Garcia de Leon et al. 1998), Atlantic salmon (O'Reilly et al. 1998; Obedzinski and Letcher 2004) and brown trout (Glover et al. 2004). Use of communal rearing techniques for striped bass is expected to permit meaningful

comparisons of performance among fish from numerous families during Phase I fingerling production. Evaluation of communal rearing techniques and data from family performance comparisons should provide valuable information for development of a selective breeding program for striped bass.

For communal evaluation of Phase I phenotypic traits in striped bass, families of larvae must be mixed and stocked into ponds only a few days after hatching, when they are much too small to be physically tagged for individual identification. The challenge of individually identifying offspring after communal rearing can be resolved through the use of highly variable molecular markers as innate genetic tags. By genotyping all parents and sampled offspring with polymorphic molecular markers, the parentage of each communally reared offspring can be determined. This technique has been successfully applied in a number of communal rearing strategies for aquacultured species (e.g., Herbinger et al. 1995; Garcia de Leon et al. 1998; O'Reilly et al. 1998). Microsatellites are short (1-6 base pairs), tandemly repeated DNA sequences (Queller et al. 1993). Generally considered selectively neutral, microsatellite repeat regions are fairly evenly distributed throughout the animal genome (Tautz and Renz 1984). Due to high mutation rates (on the order of 10^{-4} per generation; Edwards et al. 1992; Weber and Wong 1993; O'Reilly et al. 1998; Norris et al. 2000), microsatellite markers are often highly polymorphic. These loci can be amplified from DNA from very small tissue samples by using the polymerase chain reaction (PCR) (Queller et al. 1993). Microsatellite markers have proven useful in assessment of population genetic variation and stock identification (Ruzzante et al. 1996b; Chapman et al. 1999), for analysis of kinship and parentage (DeWoody et al. 1998; O'Reilly et al. 1998), and for selective breeding (Waldbieser and Wolters 1999). Conservation of PCR priming sites, sequences

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adjacent to the microsatellite repeat region, often serves to expedite studies of organisms for which microsatellites have not been sequenced. Microsatellite markers developed for striped bass have been shown to be polymorphic in white bass and hybrid striped bass (Couch et al. 2006), and those developed for the closely related European seabass (*Dicentrarchus labrax*) have proven polymorphic in striped bass, white bass, white perch and hybrid striped bass (Han et al. 2000; C.R. Couch, A.F. Garber and C.V. Sullivan, *unpublished data*). As such, microsatellite markers should be useful for individual identification and estimation of genetic variation in a program of selective breeding for these species.

A measurable relationship between genetics and larval growth or survival will permit selection of top-performing progeny or families for breeding in order to amplify these traits in subsequent generations. To date, the role and relative importance of genetic background in Phase I performance of striped bass is unknown. Aquacultured populations of striped bass exhibit variation in larval survivorship (Geiger and Parker 1985; Zastrow et al. 1989; Bosworth et al. 1997) and growth (Zastrow et al. 1989; Bosworth et al. 1997). Tremendous hatchery selection for larval survival occurs early in life, with most mortality due to inferior larvae occurring in the first 5 days after hatching (Rees and Harrell 1990), followed by an average survival of only 20-40% in the Phase I fingerling production ponds (Kerby et al. 1988; Smith et al. 1996). Very early larval survival is likely a reproductive fitness trait that may not be greatly influenced by the additive genetic variation that can be exploited by selective breeding (Falconer and Mackay 1996). However, characters associated with Phase I survival, such as early growth, may possess phenotypic variation which can be utilized in selective breeding. Differences in survival that are related to dam (female parent) are known for many species, including rainbow trout (Springate et al. 1984; Herbinger et al. 1995;

Nagler et al. 2000), chinook salmon (Heath et al. 1999) and cod (Gjerde et al. 2004), and appear to be due to maternal effects associated with egg size and egg quality. Maternal effects also are known for striped bass, with female weight being associated with egg size, egg composition, and larval size (Houde 1987; Monteleone and Houde 1990). Size at first feeding in striped bass may indicate increased survival through the early life stages (Rose and Cowan 1993). Additionally, striped bass egg characteristics (size, density, oil globule size, fatty acid content) vary among watersheds and are likely due to local genetic adaptations (Bergey et al. 2003). The contribution of sire to early survival in striped bass is unknown. Paternal differences in early survival of progeny were not detected for African catfish (Volckaert and Hellemans 1999) or for survival to one year in rainbow trout (Herbinger et al. 1995). However, differences in survival to 40 days have been observed among families of the European seabass, a close relative of the striped bass. In this species, survival was strongly influenced by both the male and the female parents (Saillant et al. 2001a). Evaluation of survival of striped bass during Phase I rearing merits investigation since a genetically-based predisposition to survive would be of great value to commercial hatcheries. By selecting combinations of individuals or strains that yield high progeny survival, growers could limit numbers of larvae produced and maintained during critical fry production periods by minimizing losses, thereby reducing costs while ensuring an adequate supply of fingerlings for growout. Evidence for genetic determinants of Phase I survival would encourage growers to reproduce broodstock with the inherent ability to produce maximally viable offspring, effectively reducing time and resources required for hatchery operations. Increased survival during the early life stages also would facilitate performance evaluation of multiple families in a selective breeding program.

In addition to larval viability, growth performance in early life stages may have genetic underpinnings and might function as a predictor of the future performance of individuals or families of fish. Relative size differences among striped bass larvae do appear to be maintained later in life (Monteleone and Houde 1990; Bosworth et al. 1997). Phenotypic differences in measures of performance that are related to genetic background are most likely based upon additive genetic variation. Differences in growth in body length have been described for wild juvenile striped bass larvae (Brown et al. 1998) and juveniles (Conover et al. 1997) from different latitudes, and these differences could be a reflection of genetic differentiation between geographic lineages of striped bass. The evidence of phenotypic variation in growth of striped bass during Phase I rearing may indicate that such traits can reasonably be selected for in a breeding program, but this remains to be confirmed. The role of genetic factors in determining early growth in length or body weight has not been described for striped bass and no estimates of heritability are available for these traits.

The aquaculture setting, under which environmental conditions may be closely controlled, provides a venue for investigating genetic determinants of larval survival and performance. Control of multiple environmental conditions, including those that influence reproductive health and readiness of broodstock, managing the numbers and genetic constitution of fry entering Phase I rearing, and optimizing the environmental conditions to which developing larvae are exposed should enable detection of any genetic variation underlying larval success (survival and growth). In addition to environmental factors, much of the variation in larval success seen in the wild appears to be related to natural trophic interactions (Fogarty et al. 1991; Cowan et al. 1993). By managing zooplankton food sources (Geiger 1983b; Geiger and Turner 1990; Ludwig 1999; Ludwig 2000) and invertebrate

predation (Geiger and Parker 1985; Brewer and Rees 1990) in pond mesocosms, much variation due to trophic factors may be reduced, permitting genetic influences on larval success to be revealed. Additionally, the ability to determine the parentage of larvae and Phase I fingerlings based on microsatellite genotypes should both permit and simplify our experimental design by alleviating confounding effects of replicate treatments and environments on phenotypic and genetic comparisons.

Objectives and Hypotheses-- To support the goals of the National Breeding Program, we evaluated the feasibility of a communal rearing approach for assessing paternally based variation in the survival and growth of striped bass larvae. A series of experimental crosses was conducted within and among domesticated and wild strains of striped bass to evaluate larval survival and performance (growth). A communal rearing approach was utilized in which offspring from various experimental crosses were co-stocked into outdoor pond mesocosms. Using microsatellite genotypes as molecular tags, larvae from multiple families were reared together from 4-6 days after hatching until they were harvested as Phase I fingerlings at 34-40 days after hatching. These methods permitted evaluation of changes in microsatellite allele frequencies (from ~5 dph to ~40 dph) and assessment of genetic variation in larval survival and growth rate by sire and strain.

The two main objectives of the study were (1) to investigate the feasibility of microsatellite marker genotyping in a communal rearing program for parentage identification and pedigree management for striped bass, and (2) to evaluate paternally-based differences in survival rate and growth performance among families of striped bass larvae.The following hypotheses were investigated:

1) There are significant differences in Phase I survival of larval families reared in outdoor aquaculture ponds; *i.e.*, larvae with certain paternal genetic backgrounds (sires) survive better during early life stages;

2) There are significant changes in allele and genotype frequencies in populations of pond-reared striped bass larvae due to differential survival of certain genotypes;

3) There are significant differences in performance (length and weight, proxies for growth rate; shape, or condition factor; and incidence of external deformities) among paternal families of striped bass during Phase I rearing.

METHODS

Broodstock Genotyping—Prior to production of experimental families, PAFL striped bass broodstock ($N=120$) were genotyped at three polymorphic microsatellite primer loci (see Methods, Chapter 2) in order to evaluate the genetic variability of the broodstock strains. Broodstock were highly variable at all the three loci, in most cases facilitating the use of males with unique alleles for at least one microsatellite locus in experimental crosses so that the parentage of communally reared progeny could be unambiguously determined.

Phase I Experimental Crosses—Experimental crosses among striped bass broodstock were conducted in April 2001 to produce even-aged half-sibling striped bass families for evaluation of survival and performance. Crosses were made using captive wild and domesticated broodstock held at the NCSU Pamlico Aquaculture Field Laboratory in Aurora, NC. All experiments were conducted in accordance with the 1996 *Guide for Care and Use of Laboratory Animals* published by the National Research Council under a protocol approved

by the NCSU Institutional Animal Care and Use Committee. Broodstock used to produce the experimental families included striped bass of wild Roanoke River-Albemarle Sound origin (R:F_0-1997; captured wild as adults in 1997, $\geq$ 8 years of age), F_2-generation Chesapeake Bay (C:F_2-1991) striped bass (10 years of age), and F_1-generation Santee x F_2-generation Chesapeake fish (SC:F_1-94; 7 years of age) (Table 1). All fish were individually identified by the unique numerical codes of passive integrated transponder (PIT) tags (Destron IDI, Boulder, CO) implanted into the lateral musculature.

Broodstock were held in outdoor flow-through tanks (7.3 m diameter x 0.92 m deep) under ambient photoperiod as previously described (Hodson and Sullivan 1993; Hodson et al. 2000; Kennedy 2002). Fish were evaluated for spawning potential by manual expression of milt from males or by ovarian biopsy of females. Biopsied oocytes were placed in ice-cold 320 mOsm Cortland's physiological saline solution (Wolf 1963) and examined under a stereomicroscope at 40X magnification. Oocyte maturity was staged based on oocyte diameter and the degree of lipid droplet coalescence and ooplasm clarity (Rees and Harrell 1990). If judged eligible for spawning, females and males received a pelleted intramuscular implant of GnRHa (synthetic analogue of human gonadotropin-releasing hormone) in a cholesterol/cellulose matrix to induce oocyte maturation and spawning (Hodson and Sullivan 1993). Males received an average of 41μg/kg GnRHa and females received an average of 39μg/kg GnRHa. Candidate spawners were then transferred to indoor 2.97 m diameter tanks and the females were evaluated periodically by ovarian biopsy as described above for oocyte development and to predict the time of ovulation. Female striped bass that did not ovulate within 48 hours were injected with 330 IU/kg of human chorionic gonadotropin to induce final maturation and ovulation.

Immediately upon detection of ovulation, eggs were manually stripped from the females for *in vitro* fertilization. In three cases, females did not ovulate fully and eggs were retrieved after surgical ovariectomy. Eggs from each female were divided into three aliquots of equal volume and each aliquot was fertilized with measured, equivalent volumes of semen from a pair of males (1-3ml semen per male). Six males were used to fertilize eggs from each dam, two each from the three broodstock strains. Sperm motility for each male was verified immediately prior to each spawning event using 40X microscopic magnification and fresh water for sperm activation. Fertilized eggs from each dam were incubated separately by sire in standard McDonald hatching jars. Percent fertility was estimated at 24-36 hours after spawning and eggs from each cross were allowed to hatch into separate 87.5 liter aquaria. At 2-3 dph, numbers of larvae were estimated by volumetric sampling of the aquaria (Smith and Whitehurst 1990). Water flow and aeration were briefly stopped and then water in the aquarium was mixed gently to evenly distribute larvae. Samples of larvae were collected by making repeated stabs of the water column with a 0.5 cm diameter glass tube and collecting the sampled water into a 100 ml graduated cylinder. The sample was transferred into a white cup, diluted with hatchery water to reduce the fry density, and then slowly poured into a pail of water. Individual fry were counted as they passed over the lip of the cup. The process was repeated two or more times per aquarium and the average number of fry per ml was used to estimate the number of fry in the original tank volume. These estimates were used to pool equivalent numbers of larvae from each pair of sires by dam to produce 6 even-aged half-sibling sire families of equal size per dam.

Phase I Rearing Trials—Five to fourteen days prior to stocking, Phase I fingerling production ponds were filled with well water and fertilized with cottonseed meal and

phosphoric acid to ensure proper zooplankton blooms (Geiger and Turner 1990; Hodson 1995). Immediately prior to pond stocking (6-8 days post fertilization), samples of approximately 100-200 larvae were collected from each pooled group of larvae. Larvae were anesthetized with a lethal dose of tricaine methane sulphonate (MS-222; Sigma-Aldrich Co., St. Louis, MO) and preserved in 70% ethanol for subsequent genetic evaluation of larval microsatellite allele frequencies and to determine the percent contribution of each male. Fry were stocked by dam at a rate of ~200-300,000 per hectare (ha) into 0.1 ha research ponds at the Pamlico Aquaculture Field Laboratory (Table 10). Fingerlings from one dam (2E55) also were stocked at a rate of ~354,000 per hectare into a single 1.6 ha commercial Phase I fingerling production pond at Keo Fish Farms in Keo, Arkansas. Water quality and zooplankton blooms and predatory aquatic insects in ponds were controlled using standard pond management practices (Bonn et al. 1976; Geiger 1983a; Geiger and Turner 1990; Hodson 1995). A high-protein prepared feed was introduced at approximately three weeks after stocking and was offered three times per day at a rate of 1.7 kg/hectare/day until the ponds were harvested.

At 34-44 days after stocking, fingerlings were harvested from the ponds by repeated seining. For all research ponds, overall percent survival was estimated by weighing three samples of fingerlings from each pond and counting the number of fingerlings in each sample. The total weight of the remaining fingerlings was determined and the total number of fingerlings recovered from the pond was estimated from the average number of fish per kg in the samples. The single exception to this method occurred with the 512C group which were few in number and were individually hand counted. After harvest, fingerlings were graded by size into two categories using conventional floating bar graders to produce more uniformly

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sized groups for subsequent rearing and to reduce the likelihood of cannibalism during feed training (Parker and Geiger 1984). A sub-sample of fingerling progeny from dams 152D and 5F4B was collected from both size categories (approximately 200 per dam). Corresponding samples for dams 2E55 and 512C were collected prior to grading. All sampled fingerlings were euthanized with an overdose of MS-222 prior to taking phenotypic measurements and were then individually preserved in 70% ethanol for subsequent genetic analysis. Fingerling progeny of dam 2E55 from the Keo Fish Farms (commercial) pond were harvested by seining but were not graded and the overall fingerling survival estimate was provided by the Keo farm manager, M. Clark. A sample of 1000 fish from the Keo Fish Farms pond was shipped live by airfreight to NCSU for phenotypic measurements and genetic analyses. Two hundred and fifty of these fingerlings were weighed, measured and preserved individually in 70% ethanol. Phenotypic data collected for all sampled fingerlings included body weight (g), total length (mm), Fulton-type condition factor [(weight/length^3)*100,000] and incidence of external deformities (e.g., scoliosis, jaw or opercular malformations, and eye deformities).

DNA Extraction—DNA was isolated from whole, ethanol preserved larvae or from approximately 4 mg ethanol-preserved fingerling muscle tissue using the PUREGENE® DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN). Extractions were performed according to the manufacturer's directions with omission of the RNase A step. DNA was rehydrated in 0.1X TE buffer and stored at -20 °C. Genomic DNA was extracted from broodstock blood samples (see Methods, Chapter 2) using a phenol: chloroform extraction procedure modified from Saghai-Maroof et al. (1984). Broodstock DNA was solubilized in 100µl 1X TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) buffer and diluted 1:20 in sterile water before use.

Microsatellite Genotyping—At the time of spawning, only three reliable and highly polymorphic microsatellite loci were used for genotyping striped bass in our laboratory (Methods, Chapter 2). During fish rearing trials, additional microsatellite markers were published (Brown et al. 2003) or were developed in our laboratory (Couch et al. 2006; Rexroad et al. 2006). The six markers used for parentage assignment are listed in Table 9. These markers were selected in order to maximize the success of parentage assignment for communally reared striped bass. The markers were optimized for multiplex PCR so that only two reactions were necessary to amplify all six loci. Primers were labeled with fluorescent tags so that all loci could be pooled for fluorescent genotyping.

PCR amplification of microsatellite loci was carried out in 12μl reactions in 96-well PCR plates. Two multiplexed sets of three microsatellite loci were amplified then pooled for fluorescent genotyping. PCR reactions consisted of 1.0μl of template DNA (~10 ng/μl), 2mM $MgCl_2$, 48.25μM of each dNTP (Promega Corporation, Madison, WI), 1.2μl 10X reaction buffer (QIAGEN Inc., Valencia, CA), 0.48μM forward primer (Integrated DNA Technologies, Coralville, IA), 0.50μM reverse primer (Applied Biosystems), and 0.48U *Taq* DNA polymerase (HotStar *Taq*, QIAGEN Inc.). Sterile deionized water was used as a negative control for each 96-well plate of PCR to ensure that reagents were not contaminated. A positive control, consisting of a parent DNA sample of known quality, was used on each plate to verify offspring DNA quality and concentration. Reverse primers were fluorescently labeled with PET™, NED™, VIC® (Applied Biosystems), or 6-FAM™ (Integrated DNA Technologies, Coralville, Iowa) fluorescent dyes to permit visualization of allele sizes using an ABI 3700 (Applied Biosystems) automated DNA sequencer in NCSU's Genome Research Laboratory. Thermal cycling parameters for Multiplex 1 (*SB91*, *SB108*

and *SB6*) included an initial denaturation step of 15 minutes at 95 °C followed by 30 cycles each of denaturation at 94 °C for 30 seconds, annealing at 49 °C for 30 seconds, and elongation at 72 °C for 50 seconds. One cycle of further elongation was done at 72ºC for 6 minutes. Cycling parameters for Multiplex 2 (*MSM 1067, AT150-2#4 and AG25-1#1*) were the same as for Multiplex 1 with the exception of a 58 °C annealing temperature and a final denaturation of 72 °C for 5 minutes. Double-stranded PCR products were purified by gel filtration with a Performa DTR 96-Well Short Plate (Edge Biosystems, Gaithersburg, MD) to remove salts and unincorporated primers. An internal fluorescent size standard, GeneScan™ -500 LIZ (0.5µl; Applied Biosystems), was added to each 0.6µl sample of clean PCR product for accurate and consistent scoring of alleles. Samples were chemically denatured by addition of 9.0µl Hi-Di Formamide (Applied Biosystems) and heat denatured at 95 °C for 5 minutes. Electrophoresis data were collected and allele sizes were determined using ABI PRISM Genemapper version 3.0 software.

Statistical analyses—

Assignment of parentage—Parentage assignment of offspring was based on genotypes for a minimum of five microsatellite loci. Individual samples that failed to amplify were re-extracted when possible and re-genotyped; those for which genotypes could not be determined for at least five loci were removed from subsequent analysis. Parentage of progeny was assigned using probability tests in PROBMAX2 version 1.2 software (Danzmann 1997). The genotypes of any unassigned progeny (probability <1.0 of belonging to a single parental pair) were evaluated for allele calling or data entry errors and then re-genotyped if necessary and possible. Progeny for which unambiguous parentage assignment

could not be made (e.g., progeny assigned to more than one sire) were excluded from further analyses.

Paternal and strain effects on survival—Overall survival by dam was calculated from the initial and final estimated counts for each pond. After progeny were assigned to parental pairs, survival by sire was estimated for each pond of half-sibling families. The proportion of each sire in the initial larval populations or in the final Phase I populations were estimated from fry counts or fingerling counts as described above (page 68-70) using the formula $\hat{N}^* = N\bar{y}$, where $\hat{N}^*$ represents the estimated number of fish in the population (at the start or end of PhaseI), N is the number of sample units of volume in the population, $\bar{y}$ is the average number of fish sampled per unit of volume ($\bar{y} = \sum_{j=1}^{n} y_i / n$), and n is the number of units of volume sampled. Estimated survival by sire was calculated from the formula $\hat{S}_i = \dfrac{\hat{N}_2^* \hat{p}_{2i}}{\hat{N}_1^* \hat{p}_{1i}}$ (K.H. Pollock, *personal communication*), where $\hat{N}_2^*$ is the estimated total number of fingerlings recovered for a pond, $\hat{p}_{2i}$ is the estimated proportion of the total recovered by sire family (i=sire 1…6), $\hat{N}_1^*$ is the estimated total number of larvae stocked, and $\hat{p}_{1i}$ is the estimated proportion of larvae stocked by sire family. A Taylor series expansion (Seber 1982) was used to calculate the variances of survival estimates for each sire family in order to calculate standard errors of each survival estimate, with

$$Var(\hat{S}_i) = [\hat{S}_i]^2 * \left[\frac{Var(\hat{N}_2^*)}{(\hat{N}_2^*)^2} + \frac{Var(\hat{p}_{2i})}{(\hat{p}_{2i})^2} + \frac{Var(\hat{N}_1^*)}{(\hat{N}_1^*)^2} + \frac{Var(\hat{p}_{1i})}{(\hat{p}_{1i})^2} \right]$$. Differences in

estimated survival among sire families were examined by Z-tests using a formula modified

from Williams et al. (2002) to test the hypothesis of no difference in survival between a pair

of sires, where the statistic, $Z = \dfrac{\hat{S}_1 - \hat{S}_2}{\sqrt{Var(\hat{S}_1) + Var(\hat{S}_2) - 2\,cov(\hat{S}_1, \hat{S}_2)}}$. The covariance term

was removed from the calculation of Z as it was near zero for all comparisons. Differences in

survival between pairs of strains also were compared by Z-tests.

Proportional contribution by strain and sire—Proportional contribution of each strain

and sire to the initial larval populations were estimated from genotyped proportions of the

sample. Chi-square (Zar 1984) was used to test the null hypothesis that contributions were

equal (expected proportions were 0.1667 for sire, 0.3333 for strain). Where the null

hypothesis was rejected, Z-tests were used to test for differences between the proportional

contributions of individual pairs, with sequential Bonferroni correction for multiple tests

(Rice 1989).

Paternal variation in body weight and total length—Phenotypic trait measures were

evaluated for normal distribution by PROC UNIVARIATE in SAS version 9.1 software

(Cary, NC). Results from the Kolmorogov-Smirnov goodness-of-fit test for normal

distribution, as well as histograms and normal probability plots, were examined for dam

families by pond, and in the case of dams 152D and 5F4B, by grade within pond. Least

squares means were calculated for all phenotypic traits, and overall differences in means for

body weight, total length and condition factor among sire families were tested with analysis

of variance (ANOVA) using PROC GLM with sire as a fixed effect. Single factor ANOVA was used to examine trait means by sire within ponds. For dams 152D and 5F4B, where fingerlings were first graded into two size categories (or grades) and unequal proportions were sampled by grade, phenotypic measures were adjusted using a WEIGHT statement. Two replicate ponds were used to rear the offspring of dam 152D and sire family means were compared using the fixed effects of sire and pond with the mean square for sire*pond as the denominator for the F-tests. For dams 512C, 2E55, and 5F4B, progeny were reared in single ponds without replication of the experiment. The lack of pond replication for these dams provides no means for estimating the variation in phenotypic measures among the sire families within each dam due to the effects of pond. However, because all sire families compared for each dam were reared in the same pond, the error mean square for the experiment was used as the denominator for the F-tests. This method of analysis is considered a form of pseudoreplication (Hurlbert 1984) since fish are treated as the experimental unit, rather than pond, with measures on each fish constituting sub-sampling within each pond, and there is an increased risk of Type I error for these analyses. However, all progeny of each dam were reared under identical conditions within the same pond and sire families that are compared within each dam should share the same environmental variation in phenotype. Dam families also were evaluated as described above for differences in phenotypic means by strain of the sire (Chesapeake, Roanoke or Santee:Chesapeake) in PROC GLM. In cases where significant overall differences were indicated by ANOVA ($P \leq 0.05$), pairwise differences among sire family means were investigated with the Tukey-Kramer multiple comparison procedure in SAS 9.1. All phenotypic trait means are presented as least squares means plus or minus the standard error (SE) of the mean.

Paternal variation in incidence of deformities— Differences among sire families in the incidence of deformities (using presence/absence data) were tested within each dam by Chi-square analysis of 2 x 6 contingency tables (2 x 5 for the 512C dam) with the PROC FREQ option in SAS. Differences were tested by pond for the 152D group. The null hypotheses were that the proportion of deformities does not differ among the sires and the proportion of deformities is not different between the 152D ponds. For contingency tables with small sample sizes or those with cells containing small (<5) numbers of observations, Fisher's exact test was used to evaluate the tables. The level of significance was $\alpha=0.05$ for all tests of contingency tables.

Population differentiation—Allele frequencies of larvae and fingerlings by pond and by sire were determined using GENEPOP version 3.4 software (http://wbiomed.curtin.edu.au/genepop/) (Raymond and Rousset 1995). Genic differentiation between larval and fingerling populations was tested by calculating unbiased estimates of P-values of the probability test (Raymond and Rousset, 1995a) by locus; Fisher's exact test was used to test differentiation across all loci and populations. Similarly, genotypic differentiation was tested by calculating unbiased estimates of the P-values of a log-likelihood based exact test (Goudet et al. 1996) by locus and globally by Fisher's test applied across loci and populations. Exact P-values were estimated using a Markov chain method (Guo and Thompson 1992) and all Markov chains consisted of 2000 dememorization steps, 1000 batches, and 2000 iterations to produce standard errors <0.001. Significance levels for multiple independent tests of allelic or genotypic differentiation were adjusted using sequential Bonferroni correction (Rice 1989). For all dams, the allelic and genotypic distributions were evaluated and compared between the larval population at the time of

stocking and the fingerling population at the time of harvest. For the 152D family, reared in two replicate ponds, comparisons also were made between the two ponds at the end of Phase I. For both the 152D and 5F4B families, allele and genotype frequencies were compared for the two size grade categories within each dam. For the 2E55 family, the same group of pooled larvae was subdivided to stock both the research and commercial ponds; however, the ponds were stocked several days apart, so allele and genotype frequencies were compared between these two stocking groups to determine if any changes in allele or genotype frequencies had occurred in the two days between stocking events.

Mendelian inheritance of microsatellite alleles—The Mendelian inheritance of alleles at the six marker loci was evaluated with the log-likelihood ratio test (*G*-test; Zar 1984) using expected 1:1 genotypic ratios for co-dominant markers. This allowed confirmation that the microsatellite marker alleles segregated in expected Mendelian ratios and that genotype determinations were not affected by the presence of null, or non-amplifying, alleles at any locus (Callen et al.1993; Pemberton et al. 1995; Dakin and Avise 2004). Distortion of Mendelian segregation also may be caused by selection against certain genotypes, competition among gametes at fertilization, or simply by mis-scoring of markers. Markers which evidenced segregation distortion were evaluated for allele scoring or data entry errors.

RESULTS

Production of experimental families—Of 13 female striped bass identified as candidate spawners for the first spawning trial (Spawning Run #1, 15-17 April 2001), 11 fish

were successfully spawned by *in vitro* fertilization techniques. However, due to low fertility, eggs from eight of these fish were discarded 36 hours after fertilization. The remaining three fish produced adequate numbers of fry for stocking into outdoor PAFL research ponds (dams 152D, 5F4B, 735A). For the second spawning trial (Spawning Run #2, 22-26 April 2001), 13 candidate female spawners were selected and nine of these were spawned, but only three dams produced enough fry for stocking into research ponds (dams 2E55, 512C, 6A1D). As noted, fry from dam 2E55 dam also were stocked into a commercial pond. Data on numbers of fry stocked, numbers of fingerlings recovered and estimated survival by dam are presented in Table 10. Data are not shown for dams 735A and 6A1D as no fingerlings were recovered at the end of Phase I. In the research-scale ponds at PAFL, cumulative survival ranged from 0% to 22.7%, and the average survival over all six dams (total of 7 ponds) was 8.2%. Survival in the commercial pond at Keo Fish Farms was estimated by Keo personnel at 17.8%.

Genotyping and parentage assignment—Numbers of larvae and fingerlings sampled for genotyping and proportions by sire are shown in Table 11. Over 99.6% of the 2,219 sampled fish were successfully genotyped at five or more loci with only 9 fish excluded due to incomplete genotypes. Of the fish successfully genotyped, 99.5% (2,199) of these were attributable to specific parental pairs. Unique alleles were observed at five or more loci in all dam families (Table 12), facilitating unequivocal assignment of communally-reared progeny to specific parental pairs. All alleles observed in the parents were detected in the progeny with the exception of two unique alleles from sire 5A46 mated with dam 512C; however, no progeny of this sire were identified in either the larval or the fingerling population, suggesting that this sire failed to contribute any offspring. All other sires contributed

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progeny. Mendelian inheritance of the six microsatellite loci was tested using data from the two largest sire families sampled in the Phase I offspring. These were the progeny of dam 2E55 x sire 292B and the progeny of dam 2E55 x sire 2A20. No deviation from expected genotypic ratios was observed in these families (Table 13).

Proportional contribution to larval populations—Chi-square analysis revealed that proportional contribution varied by sire within the sampled larval populations for each pond ($P<0.001$) and Z-tests indicated pairwise differences in contribution among sires (Appendix Table I). However, proportional contribution by strain was not different than expected. Although measured aliquots of eggs from a dam were fertilized by equivalent amounts of milt from each sire, each aliquot was simultaneously fertilized by one pair of males from each strain (for a total of six males and three sire broodstock strains per dam). Larvae from the pairs were then pooled by dam at 6-8 days post hatch using volumetrically estimated larval numbers to produce the groups of six half-sibling families per dam for communal rearing. Therefore, individual estimates of sire contribution to the total larval population could not be made in the hatchery but were instead estimated from genotypic proportions observed in the pooled sample of larvae on the day of pond stocking. Sperm competition between males in a pair, differences in sperm viability or chance factors related to pooling of groups could account for the observed differences in sire contribution. Within each dam, proportional contribution varied greatly (Figures 3-5), with the largest differences within the 512C dam family where sire contribution ranged from zero to more than 35%. Significant differences in contribution between sire pairs were noted in each pond.

Survival by sire and strain—Estimated survival by sire family for each dam is illustrated in Figures 6-8. Pairwise Z-tests for differences in survival are reported in

Appendix Table II. Survival varied greatly among sires within a pond, ranging from near zero in several cases to as high as 0.42 (±0.18) for sire 631D mated with dam 5F4B. Significant differences among sire pairs were present only within the 152D dam family after sequential Bonferroni correction. Although some sires were used for more than one cross, no clear pattern could be observed for a given sire with the exception of the relatively high survival of progeny of sire 3F11 across three different dams (dams 512C, 152D and 5F4B). Replicate ponds for dam 152D also gave some evidence of a repeatable pattern for sire family survival, with sires 3F11 and 2A20 surviving in greater numbers than progeny of sire 5C5D. Very generally, the best surviving progeny in each pond were sired by Chesapeake or Santee:Chesapeake sires; however, there was no significant difference for any pairwise comparison between strains except in the 152D ponds where Chesapeake sire families had higher survival than the Santee:Chesapeake sire family 5C5D (A5) and where Santee:Chesapeake sire family 3F11 had greater survival than progeny of Santee:Chesapeake sire 5C5D or Chesapeake sire family 631D (A7).

Sire-based phenotypic differences—Performance traits of body weight, total length, and condition factor, as well as incidence of external deformities, were evaluated for all sires and strains within each dam after testing for normal distribution of the data. All data were normally distributed (data not shown). Least squares means for all traits by dam are presented in Tables 15 and 16 by sire and strain, respectively. Results for comparisons of trait means among the sire families are detailed below by dam.

Dam 2E55—For the progeny of dam 2E55 reared in the research pond, overall differences in mean body weight, $F_{(5,120)}=4.02$, $P=0.0021$ and mean total length, $F_{(5,120)}=4.50$, $P=0.0009$ were detected among sire families. Body weights of the sire families

ranged from 0.41g ± 0.14 to 0.81g ± 0.03. For body weight, no pairwise differences between

sire families were evident with the Tukey multiple comparisons method; however, for total

length, progeny of Chesapeake sire 2A20 were larger than progeny of Santee:Chesapeake

sire 292B and Roanoke sire 4664 (Figure 9). Total lengths ranged from 3.50cm ± 0.26 to

4.29cm ± 0.06. No differences were observed for mean condition factor. Incidence of

deformities was 3% and there was no association of deformities with sire. Overall differences

were detected for both weight by strain of sire, $F_{(2,125)}$=3.36, P=0.0378 and total length by

strain, $F_{(2,124)}$=3.79, P=0.0253, with Chesapeake progeny being heavier than Roanoke

progeny and greater in length than either the Roanoke or the Santee:Chesapeake strains

(Table 16).

For the 2E55 dam's progeny which were stocked into the commercial pond, overall

differences in mean body weight, $F_{(5,242)}$=4.13, P=0.0013, and total length, $F_{(5,242)}$=5.27,

P<0.0001, also were observed among sire families. Body weight ranged from 0.35g ± 0.01 to

0.41g ± 0.01. Body weights of the progeny of Chesapeake sires 2A20 and 631D were larger

than those of Roanoke sire 7213. Total length ranged from 3.20cm ± 0.05 to 3.39cm ± 0.03.

Progeny of Chesapeake sire 631D were greater in length than those of Santee:Chesapeake

sire 292B or Roanoke sires 4664 and 7213. Those of Chesapeake sire 2A20 were greater in

length than those of Roanoke sire 7213 (Figure 10). No differences were observed for mean

condition factor by sire. No deformities were recorded for any of the sampled fish from this

pond. Overall differences were detected for both weight and length by strain of sire

($F_{(2,245)}$=8.85, P=0.0002 and $F_{(2,245)}$=10.74, P<0.0001, respectively). Progeny of the

Chesapeake strain sires had heavier mean body weight than Roanoke progeny, and

Chesapeake progeny had greater total length than those of either of the other two sire strains (Table 16).

Dam 512C—For dam 512C, overall differences in mean body weights among sire families were detected, $F_{(4,118)}=2.79$, $P=0.0294$, but no pairwise differences were found between the sire families (Figure 11). Body weight ranged from 0.28g ± 0.06 to 0.47g ± 0.03. Total length ranged from 3.06cm ± 0.18 to 3.63cm ± 0.09. Sire families also differed in mean total length, $F_{(4,116)}=3.58$, $P=0.0087$, with progeny from Chesapeake sire 5A46 being significantly greater in mean length than those of Roanoke sire 2130 (Figure 11). No differences were detected among sire families for condition factor. There was no difference in trait values among the three sire strains (Table 16). One Chesapeake sire (5709) failed to contribute any progeny. Incidence of deformities was 16% but there were no differences in deformities among the sire families.

Dam 152D—For dam 152D, fish from two replicate ponds were sampled after the fingerlings had been graded into two size categories. In pond A5, the large size category represented 38.8% of the total number of fingerlings recovered. In pond A7, the large size category represented 61.2% of the total. Over both ponds, the proportion of the large size category was 52.1%. Evaluation of sire family means by ANOVA revealed no differences in body weight, total length or condition factor by sire family (Figure 12). Body weights ranged from 1.24g ± 0.10 to 1.44g ± 0.07. Total lengths ranged from 4.78cm ± 0.09 to 5.05cm ± 0.10. Condition factor ranged from 1.03 ± 0.03 to 1.06cm ± 0.02. There was no effect of pond on body weight or length, but there was an effect of pond on condition factor, $F_{(1,5)}=20.54$, $P<0.001$. No effect of sire strain was detected for any trait (Table 16). Overall incidence of deformities in the 152D group was 25%, with deformities recorded for 37% of

the progeny from pond A5 and 15% of the progeny from pond A7. For incidence of deformity, significant overall differences were observed for sire, $X^2_{(5, N=401)} = 26.47$, $P<0.0001$. This appeared to be due to the high number of deformities for progeny of sire 121 (43% as opposed to 3-26% for the other sires). Pond A5 showed a significant effect of sire, $X^2_{(5, N=187)} = 23.40$, $P=0.0003$ but there was no effect of sire within pond A7.

Dam 5F4B—For dam 5F4B, progeny in the large size category represented 43.8% of the total fingerlings recovered. No effect of sire was present for weight or length, but there was an effect of sire for condition factor, $F_{(5,210)} = 3.28$, $P=0.0071$. Weights ranged from 1.31g ± 0.10 to 1.57g ± 0.14. Total length ranged from 4.92cm ± 0.12 to 5.20cm ± 01.7. No differences were detected among any pairs of sire families for condition factor (Figure 13). No differences were evident for any trait by sire strain (Table 16). Deformities were observed in 46% of the progeny. There was no effect of sire on the incidence of deformities.

Population differentiation—Genotype frequency distributions were compared between the larval and fingerling pond populations to detect any difference in genetic composition. Pairwise comparisons of genotypic frequencies among larval and fingerling populations revealed significant differences ($P<0.00417$) between 2E55 larvae stocked at research scale and the fingerling population 34 days later, and between larvae stocked at Keo Fish Farms and the fingerling population 37 days later. The 2E55 dam family of pooled larvae, subdivided to stock both the research pond at PAFL and the commercial pond at Keo Fish Farms, exhibited no differences in genotype frequency distributions between the two subdivisions despite the two day difference in pond stocking dates. The research pond fingerling population evidenced loss of two genotypes, both at *SB108*; this may be due to inadequate sampling from the fingerling population ($N=126$) or to random losses of some

genotypes associated with low survival in this pond. No alleles were lost from either 2E55 pond.

For the 512C dam, genotype frequencies differed between the initial larval population stocked and the fingerling population sampled 35 days later (P<0.00417). Two genotypes were lost, one at *SB6* and the other at *AT150-2#4*. This may be due to insufficient sampling (N=105) or to low pond survival. No alleles were lost from this pond.

Larval and fingerling genotype frequencies did not differ for the 5F4B dam family. Genotype frequencies also did not differ between the 5F4B larvae compared to the two size categories of fingerlings, or between the two size categories. No alleles or genotypes were lost from this pond during the Phase I rearing period.

Family 152D was stocked into two research-scale ponds. Genotypic distributions differed between larval and fingerling populations in both ponds (P<0.00417). The two final fingerling populations (pond A5 compared to A7) also were significantly different from each other (P<0.00417). No alleles or genotypes were lost during the Phase I rearing period from either pond.

Within ponds for dam 152D, the small and large size categories differed significantly from each other in allele and genotype frequencies. In pond A5 this was due to differences in allele and genotype frequency distributions only at locus *AT150-2#4*, but in pond A7 all loci differed between the two groups. For both size categories in the A5 pond, losses of genotypes were observed at *AT150-2#4,* three in the small size category and five in the large size category. Five genotypes also were lost at this locus in the pond A7 small size category and three in the large size category but there were no losses of alleles in any grade. Losses of

genotypes may be due to sampling error or possibly to the action of selection on the *AT150-2#4* locus, but there is inadequate information available to evaluate the latter hypothesis.

<div align="center">DISCUSSION</div>

Efficacy of microsatellite genotyping for communal rearing of striped bass—This study successfully demonstrates the utility of microsatellite markers for parentage identification in communally reared families of striped bass. The results show that progeny groups composed of one female and six males, reared in the same pond from 4-6 days after hatching to harvest as fingerlings more than 34 days later, could be unambiguously attributed to specific parental pairs using only 5-6 microsatellite loci for genotyping. These markers were intermediate in degree of polymorphism in the striped bass broodstock (see Results, Chapter 2), with an average of 7.8 alleles per locus and a range of 4-13 alleles per locus. One hundred percent of the progeny from dams 512C and 5F4B were attributable to a specific sire. For dam 2E55, there were 10 instances (out of a total of 773 progeny) where parentage could not be unequivocally determined and progeny were assigned to two possible sires, both from the Roanoke strain. In the single instance where one larva from dam 152D could not be assigned, that individual also was attributable to two Roanoke sires. The Roanoke strain was the most variable of the PAFL broodstock strains (see Results, Chapter 2) and failure to assign progeny from Roanoke sires was unexpected; however, incomplete genotypes (missing one allele) or shared alleles between these sires and the dam accounted for the ambiguity in parentage assignment. A remarkably high percentage of assignment success was

observed in this study with more than 99% of the 2,199 sampled progeny attributed to specific parental pairs.

Use of microsatellite markers to assign mixed larval progeny to their original parental pair during communal rearing trials has previously been demonstrated in various fish species. In African catfish (*Clarias gariepinus*), Volckaert and Hellemans (1999) were able to assign 85-91% of the progeny of a 4 x 4 factorial cross (16 families) using 9 markers. Garcia de Leon et al. (1998) utilized microsatellite markers for communally reared European sea bass families, effectively assigning 96% of the progeny of six parents to their single parental pair by using only two highly variable markers. In their study, no sires shared alleles nor did any dams share alleles, facilitating unambiguous assignment of the progeny. In an evaluation of Atlantic salmon progeny from a 12 x 12 cross, O'Reilly et al. (1998) established parentage of more than 81% of communally reared salmon using four microsatellite markers, each of which had 8-14 alleles per locus in their population. Perez-Enriquez et al. (1999) utilized four highly variable microsatellite markers as genetic tags and was able to determine parentage for 73% of the progeny of 248 red sea bream broodstock reared for stock enhancement of wild populations. This first application of microsatellite genotyping for parentage determination in striped bass has proven similarly successful to these published studies and should be a valuable tool for progeny identification and pedigree tracking in the striped bass breeding program.

Survival—Recovery of hybrid striped bass or striped bass fingerlings from ponds at the end of Phase I can be highly variable, ranging from 0% to more than 50%. Forty-five percent survival is considered quite successful in hybrid striped bass Phase I fingerling production and 15-20% survival is considered typical in commercial ponds (Hodson 1995).

In the present study, overall survival ranged from 0% to more than 22% in the small research-scale ponds, with an average of approximately 8% over all the research ponds. Survival in the larger commercial pond was estimated at approximately 17%. Differences in survival were apparent among sire families in most ponds, but with the exception of the generally high survival of progeny from Santee:Chesapeake sire 3F11 and the generally low survival of progeny from Roanoke sire 3B62, there was limited evidence for paternal effects on survival. Although there was a significant effect of sire strain only in one pond, the better surviving sire families in each pond nearly always originated from the domesticated Chesapeake or Santee:Chesapeake strains. This information may suggest that domestication confers some improvement in survivability during Phase I culture; however, this conclusion cannot be stated with any certainty without further examination of additional families in a more highly replicated and environmentally controlled setting.

Unpredictable variation in environmental factors may be so great as to mask any underlying genetic effects on a fitness trait such as survival rate. Large random effects on genetic variation have been noted in wild populations where climatic factors influence food availability and larval transport (Hedgecock 1994a). In natural populations of many marine fishes, the combination of high fecundity and environmentally-determined variation in larval survivorship can dramatically influence reproductive success and subsequent recruitment (Turner et al. 1999). Climatic and oceanographic factors affecting adult reproduction may determine reproductive success even before fertilization occurs, and timing and location of spawning and chance environmental factors may affect larval viability. Hedgecock's (1994a) "sweepstakes" hypothesis describes the chance matching of spawning events to environmental conditions that are conducive to fertilization and larval survival, whereby

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larvae from a limited subset of spawning individuals may come to dominate a given year class. Genetic variation in cohort success due to environmental variation has been described in cod (Ruzzante et al. 1996), herring and capelin (Lambert 1984), red drum (Turner et al. 1998) and striped bass (Chapman 1990). "Chaotic genetic patchiness" (Larson and Julian 1999; Hedgecock 1994a) or the small scale, random genetic differences in marine populations, has been attributed to both chance ("sweepstakes") factors and to natural selection acting on larval populations. Spatial and temporal genetic variation is seen in numerous marine organisms, from mollusks to fishes, and evidence exists to support both chance (Julian 1996) and selection (Hedgecock 1994a; Li and Hedgecock 1998) as proximal causes of this variation and important determinants of recruitment. This means that not only does variation in early life history determine population structure, but also that there may be genetic components to variation acting in concert with stochastic environmental factors.

Unpredictable environmental factors appear to play a large role in early survival and recruitment of wild striped bass (Cowan et al 1993; Rutherford et al. 1997). It is unclear whether survival and recruitment are mainly determined stochastically or by innate genetic factors that promote survival. Letcher et al. (1996) modeled larval and juvenile survival for fishes and determined that both extrinsic factors, such as predation and prey size, and intrinsic factors, such as growth and susceptibility to starvation, play substantial roles in early survivorship. Ludwig (2003) found that hybrid striped bass fed the greatest amount of food also had the highest survival rate. It is generally suggested that early survival has low heritability in fishes but that early growth has low to moderate heritability (Robison and Luempert 1984). In salmonids, heritabilities for survival during early life stages is low, on the order of 0.04 ± 0.01 to 0.08 ± 0.02 in Atlantic salmon to 0.05 ± 0.02 to 0.09 ± 0.03 in

rainbow trout (Rye et al. 1990). Similar estimates of heritability also have been calculated in Atlantic cod (0.00 ± 0.14) (Gjerde et al. 2004). However, evidence exists for genetic effects in early survival for European sea bass. Significant family effects on survival (due both to male and female effects) were observed during a 40-day tank rearing experiment (Garcia de Leon et al. 1998) and size at first feeding was correlated with survival in later larval and nursery stages. Rose and Cowan (1993) modeled the population dynamics of striped bass and determined that larger size at first feeding is related to improved survival to age one and that growth in the 15 days immediately after first feeding was greater in larvae that survive to age one than in those that fail to survive. In their simulations, early mortality appeared dependent on larval size at the earliest stages but mortality became random in older juvenile fish.

Maternal effects on early survival, rather than genetic effects, appear to be the case for many fish species. Egg sizes vary significantly among many marine fish species (Chambers and Leggett 1996) and this variation is thought to be due to environmental influences on the female during the period of oocyte growth, particularly diet and photothermal conditions. The size of the egg may have significant effects on larval survival and growth since at fertilization the egg provides not only genetic material but also an extranuclear yolk supply for the embryo. In striped bass, Cowan et al. (1993) used computer models to determine that year class strength in wild populations is largely related to maternal effects on survival and fitness. This result also has been observed in natural populations. Zastrow et al. (1989) reported that progeny of female striped bass from Chesapeake Bay showed variation in hatching success, mouth size, and body weight at 5 days post hatch (dph) with all traits related to female body size. These traits, as well as hatchability, appear to be due to larger egg weights in the larger females and to the higher protein and lipid content of

those eggs. Monteleone and Houde (1990) reported that larvae produced from smaller females tended to be smaller in size than those from large females. The larger larvae also grew faster in length and these relative size differences were maintained from 5 days post hatch to 25 days post hatch, although no differences in survival were detected.

However, conflicting evidence from Secor et al. (1990) indicated that female size was not related to egg weight and also that larvae with larger initial sizes lost this size advantage after only a few days. As with many life history traits, larval survival traits generally have low heritability (near zero) (Funk et al. 2005; Kruuk et al. 2000). Size at hatching is directly related to egg size in many fishes. In rainbow trout, larger eggs produce larger fry, but this difference does not persist beyond the first four weeks of life (Springate and Bromage 1985) and is no longer apparent at one year of age (Herbinger et al. 1995). In Atlantic cod, size at hatching is related to maternal effects on egg size (Clemmesen et al. 2003); however, these authors reported that environmental factors related to food availability may have a greater influence on the growth and condition of the larvae than do maternal factors. In Baltic cod, early survival (0-4 days), standard length at hatching, and survival to 5 days is directly related to maternity (Trippell et al. 2005). These authors also found significant effects of sire on early life history traits, most notably as an interaction effect during the 5-9 days after fertilization. Rideout et al. (2004) observed significant paternal effects on hatching success, body length and other morphological traits in haddock.

Disappearance of maternally induced size differences and compensation in growth of smaller fish has been observed by 60 days of age in rainbow trout (Springate and Bromage 1985), more than 25 dph in catfish (Reagan and Conley 1977) and more than 22 dph tilapia (Rana 1985). It is unknown whether these maternally derived effects persist beyond 25 days

after hatching in striped bass; however, in laboratory populations, survival did not vary between the progeny of large and small females (Monteleone and Houde 1990). The relatively small size of the striped bass egg as compared to the salmonid egg may reduce the duration of maternal effects on larval striped bass. The effect of female on larval survival of striped bass remains unclear and should be investigated in greater detail with females held under similar environmental conditions and with replicated larval rearing trials.

Survival during the Phase I rearing period for hybrid striped bass appears to be dependent on a number of non-genetic factors since survival can vary greatly by pond due to challenges associated with developing and maintaining an adequate zooplankton bloom throughout the first few weeks of larval rearing. Additional variables include predatory aquatic insect larvae and water quality variables such as pond water temperature, pH, and dissolved oxygen. These factors can be even more unstable in small ponds and have a large impact on larval striped bass viability (Davies 1970). Although we attempted to control environmental variables as much as possible by using commercial hybrid striped bass aquaculture production techniques and by communally rearing even-aged larvae pooled by dam, differences in survival were not apparent. These results may be due to the limited numbers of families tested or to the low replication of most dam groups. It is also possible that the magnitude of genetic versus environmental factors in striped bass survival may only be apparent under more highly controlled and replicated experimental conditions such as may be achieved using intensive larviculture techniques applied in indoor tank systems. In such systems, Ludwig (2003) has reported high Phase I survival rates (in excess of 50%) and, therefore, these smaller experimental systems may be useful for producing replicated communal groups.

Population differentiation—For all dam families except 5F4B, larval and fingerling populations differed in allelic and genotypic composition; however, sire-based differences in survival that might underlie this differentiation were not detectable, as described above. Although the replicate 152D ponds revealed roughly similar patterns in survival of the progeny from some sires, these two ponds were significantly different from one another in terms of allelic and genotypic constitution. Genotypes were lost at several loci in two ponds, the 2E55 research pond and the 512C pond. These ponds both had very low survival rates and quite small fingerling sample sizes, so losses of genotypes likely are attributable to random losses of individuals bearing less common genotypes or to insufficient sampling. No genotypes were lost in any other ponds and, importantly, no alleles were lost at any of the six loci during the Phase I rearing period. This information carries important implications for conservation of genetic variation in a program of selective breeding for striped bass.

Based on the role of chance environmental influences on survival in wild fish populations as described above, it is certainly plausible that a few families may dominate a given aquaculture year class, especially if individual families are reared in separate ponds with low or no survival in some ponds, or if larvae from different dams or of different ages are combined for rearing. However, in this study, even-aged half-sibling groups from a single dam were stocked into the same pond, exposing all families to the same environmental variables of food availability, predation, water quality and maternal environment. Although survival differed significantly among several sires for dams 2E55, 152D and 5F4B, there was no clear pattern of survival associated with any sire, except perhaps Santee:Chesapeake sire 3F11, and there was no net loss of alleles due to very low survival of any contributing sire, even in ponds where the overall survival rate was less than 5%. In the absence of genetic

effects on survival, the fact that there was no net loss of alleles would be expected given the use of neutral microsatellite loci.

The successful retention of allelic variation during the Phase I rearing period, where survival is the lowest and most variable of any of the culture phases, implies that the spawning and husbandry techniques currently utilized by the striped bass breeding program should be adequate for production of genetically variable striped bass broodstock as long as the contribution of each parent can be confirmed in order to maximize the effective population size and if pedigree relationships can be identified by genotyping prior to breeding in order to limit the risk of crossing close relatives. Except in the single case where there was no contribution by one sire, all sire families were represented at the end of Phase I in all ponds that produced Phase I fingerlings. Although sire proportions differed in the initial larval populations for each dam, there were no differences in initial contribution by sire strain and there was no difference in final survival by sire strain. The pooling regime utilized in the hatchery, where fry were counted volumetrically and pooled in roughly equivalent numbers by strain, resulted in equal representation by strain in both larval and fingerling populations. If adequate hatchery space is available for separate egg and larval incubation by sire, use of a similar method for pooling by sire should produce relatively even contribution by each family and maximize the effective population size of each new broodstock year class. In any event, the breeding program should be able to utilize a careful pooling regime in the hatchery to assure similar contribution of parental pairs or families and should be able to recover those alleles at the end of the Phase I period, even in instances where overall pond survival is quite low.

Growth and deformities by sire—The evaluation of body weight and total length means for experimental striped bass families revealed significant overall differences within all dam groups except for the replicated 152D group. In all cases where significant pairwise differences were present between sires, the top-performing sire originated from the Chesapeake strain. Examination of growth performance by sire strain revealed differences in weight and length only for the 2E55 dam; however, these differences were similar in both the commercial pond and the research pond where the fastest growing progeny of the 2E55 dam were sired by the two Chesapeake males (631D and 2A20). Although these same two Chesapeake sires also were crossed with the 152D and 5F4B dams, no differences were found in length or weight by sire strain for either of these two dams. It is possible that with greater replication of the 2E55 and 512C groups, and the use of pond as the unit of replication rather than fish, resulting in an estimate of random variation between ponds of these pooled offspring, the differences observed among the sires might not have been detected. The effects of specific crosses between individual dams and sires or among strains also cannot be discounted. In general, although variation in growth of progeny was observed in striped bass populations during Phase I production, there was limited evidence from these experiments that the effects of sire or sire strain influence early growth. Differences in body shape, as measured by condition factor were apparent only for one dam. Similarly, effects of parent on condition factor were not present in European sea bass in early life stages (Garcia de Leon et al. 1998). Although body shape is important in market-sized fish, Phase I fingerlings are generally sold by weight, so this trait, although easily calculated from length and weight data, may not be important for consideration at this stage of life. There also appears to be no effect of sire on incidence of deformities in striped bass as only one pond

showed differences in deformity by sire (dam 152D, pond A5). Malformations of the jaw, spine and eye may be the result of environmental factors present early in development rather than any innate genetic control. No abnormalities were seen in progeny of the 2E55 dam that were reared in the commercial pond; however, progeny of this dam only evidenced 3% deformities while 15-46% of the progeny of the other three dams showed deformities in research-scale ponds. Uncontrolled variation in husbandry conditions in the hatchery or the highly variable environment of the small research ponds may have been the source of these deformities. Similar conclusions were reached by Garcia de Leon et al. (1998) for seabass, where there was no effect either of sire or dam on the incidence of physical abnormalities in young sea bass and where 11-39% deformities were reported. Fin and mouth deformities also do not appear to be under genetic control for two-summer-old common carp (Kocour et al. 2006). In these fish, there may be some early influence of dam on mouth deformities that is due to maternal effects rather than to additive genetic variation. Vertebral deformities in catfish also appear to have no genetic basis (Dunham and Smitherman 1991). These authors suggest that environmental factors such as water quality and temperature may influence the occurrence of these traits in larval fish.

Genetic effects on larval fish growth have been reported by several authors, and growth in several fish species during early life does appear to have a significant genetic component. In wild grayling (*Thymallus thymallus*) populations, early life history traits show substantial additive genetic variance, influenced by both the dam and by the sire (Haugen and Vøllestad 2000). These authors speculate that this genetic variation may be maintained by environmental heterogeneity and overlapping generations in these fish. Brown et al. (1998) observed differences in larval length among different geographic populations of striped bass

reared in captivity. Larvae from northern populations (New York and Maryland) grew faster than those from southern populations (South Carolina and Florida), possibly due to genetic differences related to selective pressures of the shorter growing season in northern climates. The influence of maternal effects (egg size, yolk and oil droplet volume) on larval growth was not statistically significant for their study and the differences in larval growth appeared to be genetic in origin. However, additive genetic variation is generally low for juvenile body weight in rainbow trout, salmon, carp and tilapia (Gjedrem 1983). Examination of early growth in European seabass revealed no sire effect for total length at 11 or 40 days, although female effects were apparent, and larval length was correlated with survival to 40 days. Dam effects disappeared and sire effects became significant in European seabass later in life, at 116 days (Garcia de Leon et al. 1998). Saillant et al. (2001a) reported that length of 8-day-old sea bass larvae is mainly under influence of the dam but saw no relationship of egg size to larval growth. However, a significant effect of sire on hatching success was reported by these authors. African catfish also showed no effects of sire on length and weight until 9 months of age (Volckaert and Hellemans 1999) and Doupe and Lymbery (2005a,b) found that maternal and environmental effects, rather than sire, accounted for much of black bream growth to 75 days of age but that sire effects become important in later growing stages. An effect of sire can be detected in juvenile cod where heritability for body weight is 0.29 ± 0.27 to 0.56 ± 0.26 (Gjerde et al. 2004), but these effects were recorded for 6 month old fish and the paternal influences may appear after the early growth period that would correspond to Phase I rearing in striped bass.

Differences in allelic and genotypic make up between duplicate ponds of the same communally reared group (152D) at the end of the Phase I production cycle indicate that the

random effects of environmental variation may have swamped any genetic influences during this phase or that any genetic differences are simply very small. In the replicated portion of the study (dam 152D), survival showed some pattern among sires although ponds were different in allelic and genotypic frequencies at the end of Phase I. There were no differences in length or weight by sire family for this dam although effects of sire were observed for other dams. The influence of genetic factors on early striped bass survival and growth should be investigated in greater detail with additional communally reared families and with greater numbers of replicate rearing units. Use of larviculture techniques to produce the Phase I families in indoor, easily replicated and controlled tank systems may offer the greatest chance of success in teasing apart the genetic and environmental factors that influence Phase I striped bass performance. Such a controlled experiment may allow development of a model that incorporates the effect of both innate genetic factors and abiotic environmental conditions on larval survival and growth.

Application of molecular markers for selective breeding of SB to support the hybrid striped bass industry— At the initiation of this study, the number of available variable microsatellite markers was very limited for striped bass, due in part to the limited genetic variation in this species (Grove et al. 1976; Waldman 1998) and in part to restrictions imposed by marker development technologies. However, by employing microsatellite enrichment methods to enhance the numbers of microsatellite loci identified, our laboratory has recently developed 498 new markers (Couch et al. 2006, Appendix III; Rexroad et al. 2006), bringing the total available for striped bass to more than 500 markers. Utilization of the most variable of these markers for progeny identification should reduce the number of markers necessary for unambiguous parentage assignment. Optimization of multiplex PCR

for amplifying these loci should dramatically reduce the costs of reagents and labor associated with genotyping multiple loci (Olsen et al. 1996). Villanueva et al. (2002) used computer simulation to determine the number of markers necessary for parentage assignment in salmon and found that 6 loci with 10 equally frequent alleles was sufficient to assign 99% or more of the offspring of 200 parents. Use of the most highly variable new striped bass markers, of which more than 36 loci had $\geq$10 alleles per locus, should greatly increase the number of families which can be communally reared for performance testing and increase the effective population size that could be maintained for broodstock production. In programs where pedigree information may be incomplete, as is the case for striped bass, microsatellite genotyping also will allow calculation of relatedness among individuals and testing of the likelihood of pedigree relationships (Queller and Goodnight 1989; Norris et al. 2000), providing information that will reduce the risk of inbreeding of closely related un-pedigreed fish.

Proportional contribution by sire strain to the initial larval populations did not differ within each dam. However, proportional contribution by sire was different in all dam families and in each pond and many sire pairs differed in their contribution to the larval groups, with one sire failing to contribute any progeny despite verification of sperm motility immediately prior to fertilization of the eggs. Detection of each parent's contribution to the subsequent generation is necessary for an effective selective breeding program for any livestock species. For highly fecund fishes such as striped bass, where a single female can easily produce hundreds of thousands to millions of eggs in a given spawning season, tracking of parental contribution is critical since unequal contribution by parental pairs will reduce the effective population size of a broodstock population and reductions in genetic

variation can accumulate rapidly. As with most aquaculture breeding programs, facilities for production of numerous larval families of striped bass or hybrid striped bass are limited. Broodstock are often tank spawned in small groups where relative sire contribution is not known, or, if strip spawned for *in vitro* fertilization, multiple sires are used to fertilize each female's eggs to guarantee fertilization success. Due to space limitations, groups of larval families are generally pooled in the hatchery during incubation of eggs, and fry from various dams and sires may be combined for subsequent pond culture. The demonstrated success in determining proportional contribution of each parental pair by microsatellite marker genotyping indicates that use of microsatellite genotyping will serve as an excellent means of individual or family identification for tracking pedigrees and for alleviating inbreeding in the striped bass broodstock population.

The absence of detectable paternal variation in Phase I survival or growth indicates that, at least in the short term, performance testing of striped bass families in ponds may be unnecessary during the Phase I period for the breeding program. This would eliminate the requirement for replicated ponds needed for performance testing. Two options are available for production of fingerlings for Phase II progeny testing. In the first option, fingerlings could be separately reared in ponds as single parent crosses and then be physically tagged at the start of Phase II production. This would facilitate common garden performance testing during the later culture Phases and at the same time eliminate the costs and time necessary for identification by genotyping. Implantation with coded wire tags, tattooing, or subcutaneous injection with various colors and patterns of fluorescent elastomer could be used for individual identification of progeny at later life stages without the need for genotyping. Tag retention times for striped bass should be investigated to determine the

feasibility of these options for communal pond rearing, although elastomer tags have proven cost efficient and useful for identifying bluegill sunfish over periods of approximately 6 months (Dewey and Zigler 1996). The limitation of rearing space available to the National Breeding Program remains a critical determinant of the rearing approach. Only 16 0.1 ha fingerling production ponds are available at the NCSU PAFL. Until such a time that adequate numbers of Phase I rearing ponds become available to the breeding program, or until intensive larviculture techniques can be utilized with greater success to permit separate rearing of individual families, the second option, communal rearing of progeny groups, may continue to be necessary simply to produce adequate numbers of families for Phase II and Phase III performance evaluations. If larval families must be reared communally during Phase I, the use of microsatellite markers to determine which parents produced surviving offspring and in what proportions each parent contributed will be a necessary and effective means of determining parentage and broodstock contribution as well as for tracking pedigrees for effective management of the breeding program. Use of a multiplexed suite of highly variable microsatellite markers to identify individuals from groups of pooled families, such as was utilized in these experiments, would permit rapid genotyping at the lowest cost for the breeding program. Co-stocking of many even-aged families would be possible in the larval stage and genotypic identification could be made before the Phase II trials for a more balanced evaluation of families. Because thousands of fish are recovered from Phase I fingerling ponds, fingerlings to be used for Phase II progeny testing would have to be randomly sampled in adequate numbers to identify sufficient individuals from each family for subsequent performance evaluations. This method would require that fish be finclipped for DNA samples at the end of Phase I rearing and then marked with an inexpensive, short-

term physical tag to allow individual identification once genotypes were available. Alternatively, a form of mass selection could be employed, where genotypic identification of only the best performing individuals could be made at the end of Phase III rearing trials. Genotyping after selection would allow individual identification of this more limited number of individuals and would permit pedigree tracking for prevention of inbreeding in subsequent generations. The methodology used will be dependent on the goals of the breeding program as to whether progeny testing should be used to identify top performing families for breeding or whether only the top performing individuals, regardless of family, should be identified and used for breeding purposes.

Although the feasibility of communal rearing of striped bass in a selective breeding program has been demonstrated, the cost savings associated with pooling of multiple family groups during Phase I should be compared with the costs of genotyping that are necessary to determine parentage of communally reared fish after the Phase I period. Present genotyping costs (reagents and automated sequencer machine time only) for 6-7 loci are approximately $3.00/fish for our laboratory. By limiting the genotyping to only the phenotypically superior fish and using low cost student labor, genotyping costs might be competitive with the costs of water, feed, labor and supplies needed to maintain multiple ponds or tanks for separate larval rearing of individual families. A minimum estimate of the cost to produce a single 0.1 ha pond of Phase I fingerlings at PAFL is at least $700 per pond (A.S. McGinty, *personal communication*). With 16 ponds available at PAFL, costs for separate rearing of only 16 single parent crosses would easily exceed $11,000, roughly the cost of genotyping 3,700 fish. With the resultant gains in selection intensity and conservation of genetic variation taken into consideration, use of microsatellite-based communal rearing to produce many additional

families appears much more attractive. Application of microsatellite genotyping to identify communally reared individuals and to determine the degree of relatedness among potential spawners for 'walk-back' selection in a program of selective breeding has been proposed as a significant means of reducing costs and increasing selection intensities in an aquaculture setting since many more individuals and families could be maintained with communal rearing (Doyle and Herbinger 1995). In this selection scheme, many families are reared communally and then the largest individuals are genotyped in sequence ("walking back" from the largest fish in the size distribution) to determine their relatedness to one another. Genotyping proceeds until the required number of unrelated or non-sibling breeders has been identified. This method is estimated to be cost effective and to allow high selection intensities while simultaneously preventing inbreeding. Their simulations estimate that the number of genotyped individuals would be between a few hundred to one thousand to achieve selection intensities of 2-4 standard deviations and an effective number of breeders >80 fish. Use of this methodology would be substantially lower in cost than the separate rearing of the same number of unrelated families in Phase I.

In summary, although variation in growth and survival of progeny was detected among some striped bass families during Phase I rearing trials, there was limited evidence from these experiments that sire or strain influence early growth or survival. Further examination of these hypotheses with additional striped bass families in a more highly replicated experiment may provide useful information about Phase I survival and growth that is relevant to a selection program for these fish. The high degree of success in parentage determination for individuals co-stocked as larvae for rearing in a common environment

illustrates the feasibility of a microsatellite-based approach for performance evaluations and pedigree tracking for the National Breeding Program.

Table 8. Experimental crosses among striped bass broodstock to generate half-sibling families. Each dam was mated with six sires, two each from three stocks. Stocks used included a wild, Roanoke stock (R:F_0-97), a domestic Santee:Chesapeake stock (SC:F_1-94), a domestic Chesapeake stock (C:F_2-91). The (U) for dam 512C indicates strain of origin was unknown.

	DAM			
SIRE	512C (U)	2E55 (R:F_0-97)	152D (SC:F_1-94)	5F4B (R:F_0-97)
3B62 (R:F_0-97)			X	X
5442 (R:F_0-97)	X		X	X
4664 (R:F_0-97)		X		
7213 (R:F_0-97)		X		
2130 (R:F_0-97)	X			
3F11 (SC:F_1-94)	X		X	X
5C5D (SC:F_1-94)	X		X	X
292B (SC:F_1-94)		X		
7E27 (SC:F_1-94)		X		
5A46 (C:F_2-91)	X			
5709 (C:F_2-91)	X			
631D (C:F_2-91)		X	X	X
2A20 (C:F_2-91)		X	X	X

Table 9. Polymorphic microsatellite loci utilized for genotyping striped bass for parentage assignment with locus names, primer sequences, PCR annealing temperatures (T_A), allele size ranges in base pairs (bp), number of alleles observed and sources of loci.

Locus	Primer sequence (5'-3')	T_A	Size range (bp)	No. alleles	Source	GenBank accession no.
SB 6	F: ACAGCAAAGATAAACATCTG R: TTCATGATGTTTCACCAGG	49	183-230	10	Garcia de Leon et al. 1998	---
SB 91	F: AGACACCAGATAAGGAGA R: TAGATTCACACAAGGTGC	49	123-151	6	Roy et al. 2000	AF200743
SB 108	F: ACTCTCGTATCGAACCAT R: CTGGTCAAGCCTTTACTG	49	178-211	10	Wirgin (*pers. comm.*)	---
AT150-2#4	F: TATGACGCCATGTGTTGGCAC R: ATGTATGAGTTGATAGCATGAGG	58	148-156	4	Brown et al. 2003	AY248732
AG25-1#1	F: GCTTCCGCAAGTTTAGTTGC R: AACGCAGAATCCTGCCTGC	58	157-286	13	Brown et al. 2003	AY248735
MSM 1067	F: GGAATCAAATCCCTGCTGTTATAATCT R: CTATCTGGACTTTATCCCTACGAGTGA	58	190-208	4	Couch et al. 2006	BV678238

Table 10. Estimated pond stocking rates, fingerling recovery rates and survival by dam and pond; SE is the standard error for each estimate.

Dam	Pond	Pond area (ha)	Date of stocking	Estimated no. larvae stocked	SE	Date of harvest	Estimated no. fingerlings recovered	SE	No. days Phase I production	Estimated Phase I survival rate	SE
152D	A5	0.1	04/23/01	21,736	951	06/04/01	3,062	89	42	0.1410	0.0207
	A7	0.1	04/23/01	21,736	951	06/04/01	3,047	62	42	0.1402	0.0204
5F4B	A10	0.1	04/23/01	19,413	2,472	06/06/01	4,424	116	44	0.2279	0.0642
512C	A14	0.1	05/03/01	30,000	2,304	06/07/01	848	0	35	0.0283	0.0057
2E55	A15	0.1	05/03/01	30,000	973	06/06/01	1,121	18	34	0.0374	0.0057
	Keo	1.2	05/01/01	561,062	18,196	06/07/01	100,000	--	37	0.1782	--

Table 11. Numbers (*N*) of larvae and fingerlings assigned parentage of those sampled by dam for genotyping and estimated contribution (proportion) by sire for experimental crosses among striped bass broodstock. For dams 152D and 5F4B only, fingerlings were sampled within two size grades (categories <12 or >12) and numbers are reported by grade.

DAM	SIRE	Larvae		Fingerlings (<12 / >12)	
		Genotyped	Proportion (SE)	Genotyped	Proportion (SE)
512C (U)	5442 (R:F_0-97)	16	0.080 (0.019)	11	0.105 (0.030)
(research)	2130 (R:F_0-97)	47	0.236 (0.030)	38	0.362 (0.047)
	3F11 (SC:F_1-94)	21	0.106 (0.022)	8	0.076 (0.026)
	5C5D (SC:F_1-94)	10	0.352 (0.034)	36	0.343 (0.046)
	5A46 (C:F_2-91)	0	0	0	0
	5709 (C:F_2-91)	45	0.226 (0.030)	12	0.114 (0.031)
	N	199 of 200		105 of 105	
2E55 (R:F_0-97)	4664 (R:F_0-97)	19	0.098 (0.021)	16	0.127 (0.030)
(research)	7213 (R:F_0-97)	19	0.098 (0.021)	21	0.167 (0.033)
	292B (SC:F_1-94)	45	0.232 (0.030)	4	0.032 (0.016)
	7E27 (SC:F_1-94)	28	0.144 (0.025)	6	0.048 (0.019)
	631D (C:F_2-91)	39	0.201 (0.029)	70	0.556 (0.044)
	2A20 (C:F_2-91)	44	0.227 (0.030)	9	0.071 (0.023)
	N	194 of 199		126 of 128	
2E55 (R:F_0-97)	4664 (R:F_0-97)	21	0.109 (0.022)	50	0.202 (0.026)
(Keo)	7213 (R:F_0-97)	22	0.114 (0.022)	26	0.105 (0.020)
	292B (SC:F_1-94)	46	0.238 (0.031)	41	0.166 (0.024)
	7E27 (SC:F_1-94)	20	0.104 (0.022)	20	0.081 (0.017)
	631D (C:F_2-91)	49	0.254 (0.031)	67	0.271 (0.028)
	2A20 (C:F_2-91)	35	0.181 (0.028)	43	0.174 (0.024)
	N	193 of 196		247 of 250	
152D (SC:F_1-94)	3B62 (R:F_0-97)	82	0.258 (0.024)	34/27	0.321 (0.045) / 0.333 (0.052)
(pond A5)	5442 (R:F_0-97)	71	0.223 (0.023)	24/12	0.226 (0.041) / 0.148 (0.039)
	3F11 (SC:F_1-94)	61	0.192 (0.022)	26/17	0.245 (0.042) / 0.210 (0.045)
	5C5D (SC:F_1-94)	42	0.132 (0.019)	6/4	0.057 (0.022) / 0.049 (0.024)
	631D (C:F_2-91)	28	0.088 (0.016)	6/6	0.057 (0.022) / 0.074 (0.029)
	2A20 (C:F_2-91)	34	0.107 (0.017)	10/15	0.094 (0.028) / 0.185 (0.043)
	N	318 of 321		106/81 of 106/81	
152D (SC:F_1-94)	3B62 (R:F_0-97)	same as above		18/15	0.209 (0.044) / 0.117 (0.028)
(pond A7)	5442 (R:F_0-97)			16/26	0.186 (0.042) / 0.203 (0.036)
	3F11 (SC:F_1-94)			26/46	0.302 (0.050) / 0.359 (0.042)
	5C5D (SC:F_1-94)			8/10	0.093 (0.031) / 0.078 (0.024)
	631D (C:F_2-91)			10/8	0.116 (0.035) / 0.062 (0.021)
	2A20 (C:F_2-91)			8/23	0.093 (0.031) / 0.180 (0.034)
	N			86/128 of 86/128	
5F4B (R:F_0-97)	3B62 (R:F_0-97)	15	0.075 (0.019)	14/16	0.122 (0.032) / 0.158 (0.040)
(research)	5442 (R:F_0-97)	30	0.150 (0.025)	17/13	0.148 (0.036) / 0.129 (0.036)
	3F11 (SC:F_1-94)	29	0.145 (0.025)	31/21	0.270 (0.048) / 0.208 (0.045)
	5C5D (SC:F_1-94)	31	0.155 (0.026)	9/11	0.078 (0.026) / 0.109 (0.033)
	631D (C:F_2-91)	34	0.170 (0.027)	12/7	0.104 (0.030) / 0.693 (0.026)
	2A20 (C:F_2-91)	61	0.305 (0.033)	32/33	0.278 (0.049) / 0.327 (0.057)
	N	200 of 200		115/101 of 115/104	

Table 12. Genotypes at six microsatellite loci for striped bass broodstock used to produce experimental families. Alleles indicated in boldface type are unique within that group of six half-sibling families.

Sire	Dam	*SB91*	*SB108*	*SB6*	*AT150-2#4*	*AG25-1#1*	*MSM1067*	Stock
	5F4B	146 149	190 197	197 230	148 154	169 181	200 208	(R:F_0-97)
3B62		149 151	197 211	224 230	154 156	185 200	190 202	(R:F_0-97)
5442		151 151	195 197	197 228	148 154	181 185	200 208	(R:F_0-97)
3F11		146 146	182 197	202 214	150 154	192 192	190 208	(SC:F_1-94)
5C5D		146 149	187 197	197 214	154 156	196 200	200 200	(SC:F_1-94)
2A20		149 149	182 197	197 224	154 154	192 286	202 208	(C:F_2-91)
631D		133 146	182 203	204 224	154 154	157 181	200 202	(C:F_2-91)
	152D	146 146	190 203	197 214	150 154	192 192	200 208	(SC:F_1-94)
3B62		149 151	197 211	224 230	154 156	185 200	190 202	(R:F_0-97)
5442		151 151	195 197	197 228	148 154	181 185	200 208	(R:F_0-97)
3F11		146 146	182 197	202 214	150 154	192 192	190 208	(SC:F_1-94)
5C5D		146 149	187 197	197 214	154 156	196 200	200 200	(SC:F_1-94)
2A20		149 149	182 197	197 224	154 154	192 286	202 208	(C:F_2-91)
631D		133 146	182 203	204 224	154 154	157 181	200 202	(C:F_2-91)
	2E55	140 151	197 211	183 208	148 154	185 196	200 200	(R:F_0-97)
4664		140 149	197 197	183 218	154 154	185 189	200 200	(R:F_0-97)
7213		146 149	197 199	197 208	154 154	157 185	200 208	(R:F_0-97)
292B		123 149	190 190	197 214	154 156	196 200	208 208	(SC:F_1-94)
7E27		123 149	197 197	214 228	154 156	196 200	208 208	(SC:F_1-94)
2A20		149 149	182 197	197 224	154 154	192 286	202 208	(C:F_2-91)
631D		133 146	182 203	204 224	154 154	157 181	200 202	(C:F_2-91)
	512C	133 146	182 211	183 230	154 154	185 282	200 208	Unknown
2130		133 140	178 211	197 224	154 154	204 212	190 200	(R:F_0-97)
5442		151 151	195 197	197 228	148 154	181 185	200 208	(R:F_0-97)
3F11		146 146	182 197	202 214	150 154	192 192	190 208	(SC:F_1-94)
5C5D		146 149	187 197	197 214	154 156	196 200	200 200	(SC:F_1-94)
5A46		149 149	203 211	197 197	148 154	177 196	200 200	(C:F_2-91)
5709		149 151	190 197	197 197	154 154	196 212	200 202	(C:F_2-91)

Table 13. Log-likelihood ratio goodness of fit test results for Mendelian inheritance of six microsatellite loci in two striped bass families. Dam was 2E55 crossed with sires listed in table. Possible genotypes of progeny produced by these crosses, observed and expected (1:1 ratio) numbers of each genotype, and *G*-statistics with probabilities are shown in the table. Degrees of freedom for each test were $v=k$-1. There was no evidence of departure from Mendelian expectation for any locus in either family.

Family	Locus	Possible genotypes	Observed no. each genotype	Expected no. each genotype	G	Probability
292B	*SB91*	140 149	7	11.25	2.75	0.25<*P*<0.5
		123 140	14	11.25		
		123 151	13	11.25		
		149 151	11	11.25		
	SB108	190 197	25	22.5	0.56	0.5<*P*<0.75
		190 211	20	22.5		
	SB6	183 197	14	11.25	0.92	0.75<*P*<0.9
		183 214	10	11.25		
		197 208	11	11.25		
		208 214	10	11.25		
	AT150-2#4	148 154	10	11.25	-1.61	0.99<*P*
		148 156	12	11.25		
		154 154	10	11.25		
		154 156	12	11.25		
	AG25-1#1	185 196	10	11.25	-2.38	0.99<*P*
		185 200	13	11.25		
		196 196	13	11.25		
		196 200	6	11.25		
	MSM1067	200 208	45	45	0	0.99<*P*
2A20	*SB91*	140 149	28	24.5	1.00	0.25<*P*<0.5
		149 151	21	24.5		
	SB108	182 197	12	12.25	1.03	0.75<*P*<0.9
		182 211	12	12.25		
		197 197	10	12.25		
		197 211	15	12.25		
	SB6	183 197	10	12.25	3.75	0.25<*P*<0.5
		183 224	18	12.25		
		197 208	9	12.25		
		208 224	12	12.25		
	AT150-2#4	148 154	25	24.5	0.02	0.75<*P*<0.9
		148 156	24	24.5		
	AG25-1#1	185 192	8	12.25	4.72	0.1<*P*<0.25
		185 286	16	12.25		
		192 196	16	12.25		
		196 286	9	12.25		
	MSM1067	200 202	25	24.5	0.02	0.75<*P*<0.9
		200 208	24	24.5		

Table 14. Estimated survival by sire family (±SE). No standard error could be calculated for the commercial pond (Keo Fish Farms) due to the method of fingerling harvest.

DAM	SIRE	Estimated survival (SE)
512C (U)	5442 (R:F_0-97)	0.037 (0.016)
	2130 (R:F_0-97)	0.043 (0.012)
	3F11 (SC:F_1-94)	0.020 (0.009)
	5C5D (SC:F_1-94)	0.028 (0.007)
	5A46 (C:F_2-91)	0.000 (--)
	5709 (C:F_2-91)	0.014 (0.005)
2E55 (R:F_0-97)	4664 (R:F_0-97)	0.048 (0.017)
(research pond)	7213 (R:F_0-97)	0.064 (0.021)
	292B (SC:F_1-94)	0.005 (0.003)
	7E27 (SC:F_1-94)	0.012 (0.006)
	631D (C:F_2-91)	0.103 (0.023)
	2A20 (C:F_2-91)	0.012 (0.004)
2E55 (R:F_0-97)	4664 (R:F_0-97)	0.332
(commercial pond)	7213 (R:F_0-97)	0.165
	292B (SC:F_1-94)	0.124
	7E27 (SC:F_1-94)	0.139
	631D (C:F_2-91)	0.190
	2A20 (C:F_2-91)	0.171
152D (SC:F_1-94)	3B62 (R:F_0-97)	0.044 (0.008)
(pond A5)	5442 (R:F_0-97)	0.036 (0.008)
	3F11 (SC:F_1-94)	0.046 (0.010)
	5C5D (SC:F_1-94)	0.015 (0.007)
	631D (C:F_2-91)	0.023 (0.010)
	2A20 (C:F_2-91)	0.032 (0.011)
152D (SC:F_1-94)	3B62 (R:F_0-97)	0.136 (0.028)
(pond A7)	5442 (R:F_0-97)	0.070 (0.021)
	3F11 (SC:F_1-94)	0.115 (0.030)
	5C5D (SC:F_1-94)	0.039 (0.020)
	631D (C:F_2-91)	0.088 (0.039)
	2A20 (C:F_2-91)	0.182 (0.054)
5F4B (R:F_0-97)	3B62 (R:F_0-97)	0.033 (0.008)
	5442 (R:F_0-97)	0.034 (0.009)
	3F11 (SC:F_1-94)	0.064 (0.014)
	5C5D (SC:F_1-94)	0.028 (0.011)
	631D (C:F_2-91)	0.054 (0.019)
	2A20 (C:F_2-91)	0.035 (0.013)

Table 15. Least squares means (±SE) by sire for phenotypic trait values for experimental striped bass families.

DAM	SIRE	Body weight (g)	Total length (cm)	Condition factor
512C (U)	5442 (R:F_0-97)	0.32 (0.07)	3.12 (0.20)	0.92 (0.02)
	2130 (R:F_0-97)	0.28 (0.06)	3.06 (0.18)	0.92 (0.02)
	3F11 (SC:F_1-94)	0.46 (0.03)	3.57 (0.09)	0.91 (0.01)
	5C5D (SC:F_1-94)	0.32 (0.08)	3.19 (0.21)	0.90 (0.02)
	5A46 (C:F_2-91)	0.47 (0.03)	3.63 (0.09)	0.90 (0.01)
	5709 (C:F_2-91)	--	--	--
2E55 (R:F_0-97)	4664 (R:F_0-97)	0.46 (0.12)	3.65 (0.21)	0.92 (0.05)
(research pond)	7213 (R:F_0-97)	0.65 (0.10)	4.10 (0.17)	0.97 (0.04)
	292B (SC:F_1-94)	0.41 (0.14)	3.50 (0.26)	0.95 (0.06)
	7E27 (SC:F_1-94)	0.68 (0.06)	4.03 (0.11)	0.98 (0.02)
	631D (C:F_2-91)	0.59 (0.07)	3.88 (0.13)	0.97 (0.03)
	2A20 (C:F_2-91)	0.81 (0.03)	4.29 (0.06)	0.99 (0.01)
2E55 (R:F_0-97)	4664 (R:F_0-97)	0.36 (0.02)	3.20 (0.05)	1.09 (0.02)
(commercial pond)	7213 (R:F_0-97)	0.35 (0.01)	3.21 (0.03)	1.05 (0.01)
	292B (SC:F_1-94)	0.36 (0.01)	3.22 (0.03)	1.07 (0.01)
	7E27 (SC:F_1-94)	0.39 (0.02)	3.33 (0.04)	1.05 (0.02)
	631D (C:F_2-91)	0.41 (0.01)	3.39 (0.03)	1.06 (0.01)
	2A20 (C:F_2-91)	0.40 (0.01)	3.35 (0.03)	1.05 (0.01)
152D (SC:F_1-94)	3B62 (R:F_0-97)	1.28 (0.10)	4.90 (0.14)	1.03 (0.03)
	5442 (R:F_0-97)	1.25 (0.06)	4.78 (0.09)	1.06 (0.02)
	3F11 (SC:F_1-94)	1.39 (0.05)	4.98 (0.07)	1.06 (0.01)
	5C5D (SC:F_1-94)	1.24 (0.10)	4.83 (0.15)	1.03 (0.03)
	631D (C:F_2-91)	1.32 (0.06)	4.88 (0.09)	1.06 (0.02)
	2A20 (C:F_2-91)	1.44 (0.07)	5.05 (0.10)	1.05 (0.02)
5F4B (R:F_0-97)	3B62 (R:F_0-97)	1.36 (0.17)	5.01 (0.20)	1.04 (0.01)
	5442 (R:F_0-97)	1.35 (0.13)	4.92 (0.15)	1.05 (0.01)
	3F11 (SC:F_1-94)	1.31 (0.10)	4.92 (0.12)	1.04 (0.01)
	5C5D (SC:F_1-94)	1.57 (0.14)	5.20 (0.17)	1.04 (0.01)
	631D (C:F_2-91)	1.44 (0.12)	5.01 (0.14)	1.06 (0.01)
	2A20 (C:F_2-91)	1.50 (0.08)	5.10 (0.10)	1.02 (0.01)

Table 16. Least squares means (±SE) by strain for phenotypic trait values for experimental striped bass families. The (U) after dam 512C indicates that strain of origin is unknown.

DAM	SIRE	Body weight (g)	Total length (cm)	Condition factor
512C (U)	R:F_0-97	0.30 (0.03)	3.09 (0.11)	0.92 (0.01)
	SC:F_1-94	0.35 (0.02)	3.31 (0.07)	0.91 (0.01)
	C:F_2-91	0.38 (0.03)	3.41 (0.11)	0.90 (0.01)
2E55 (R:F_0-97)	R:F_0-97	0.61 (0.07)	3.92 (0.13)	0.96 (0.03)
(research pond)	SC:F_1-94	0.64 (0.06)	3.95 (0.11)	0.98 (0.02)
	C:F_2-91	0.77 (0.03)	4.22 (0.06)	0.98 (0.01)
2E55 (R:F_0-97)	R:F_0-97	0.35 (0.01)	3.21 (0.03)	1.06 (0.01)
(commercial pond)	SC:F_1-94	0.38 (0.01)	3.26 (0.03)	1.06 (0.01)
	C:F_2-91	0.41 (0.01)	3.36 (0.02)	1.05 (0.01)
152D (SC:F_1-94)	R:F_0-97	1.26 (0.02)	4.82 (0.03)	1.05 (0.01)
	SC:F_1-94	1.36 (0.02)	4.95 (0.02)	1.05 (0.01)
	C:F_2-91	1.38 (0.02)	4.96 (0.02)	1.06 (0.01)
5F4B (R:F_0-97)	R:F_0-97	1.02 (0.09)	4.49 (0.12)	1.04 (0.01)
	SC:F_1-94	1.05 (0.08)	4.53 (0.10)	1.03 (0.01)
	C:F_2-91	1.16 (0.07)	4.66 (0.08)	1.02 (0.01)
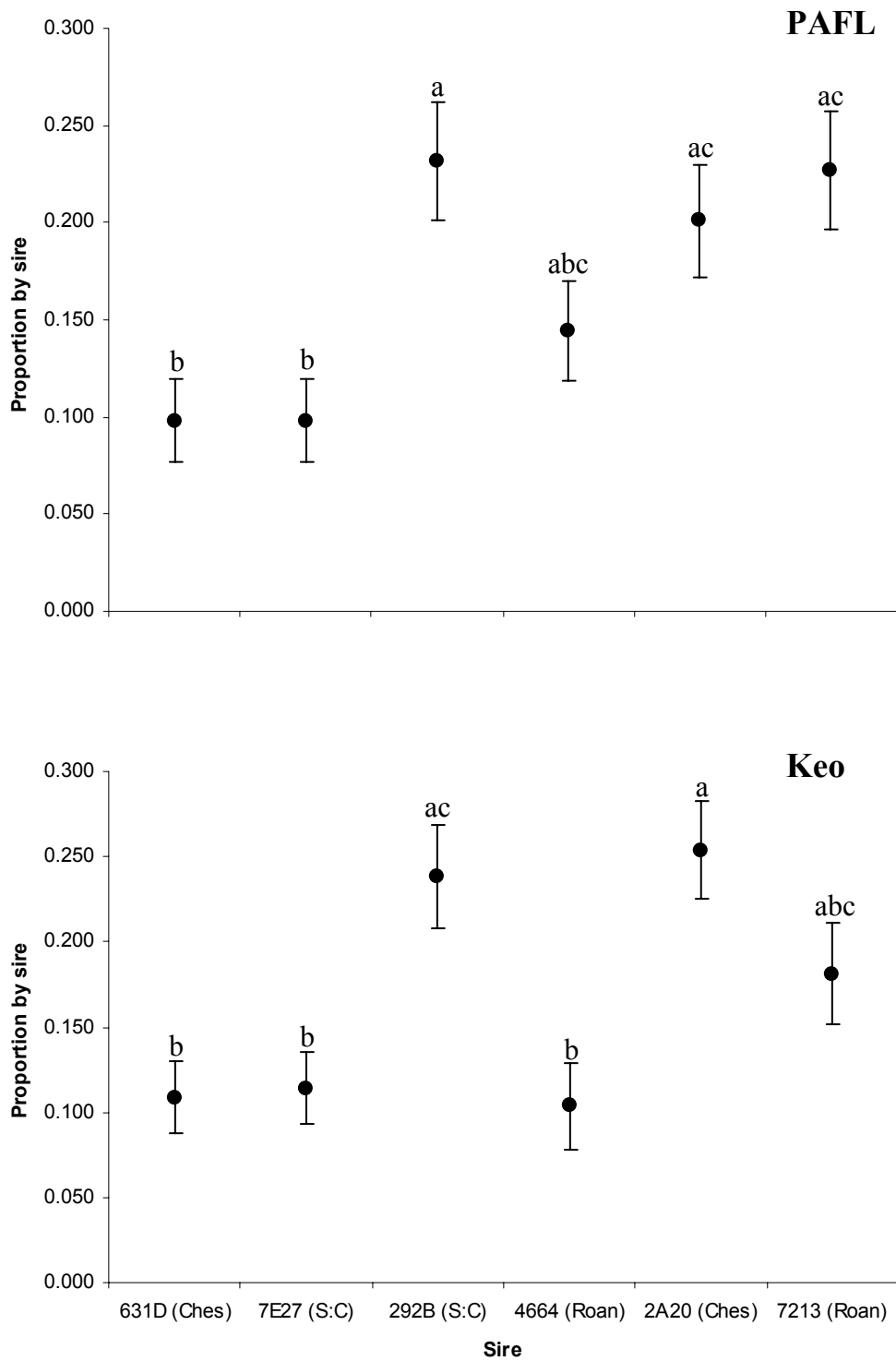
Figure 3. Proportional contribution (±) by sire to 2E55 larval groups reared at PAFL (top) and Keo Fish Farms (bottom). Proportions with shared letters over the error bars are not significantly different from one another after sequential Bonferroni adjustment.
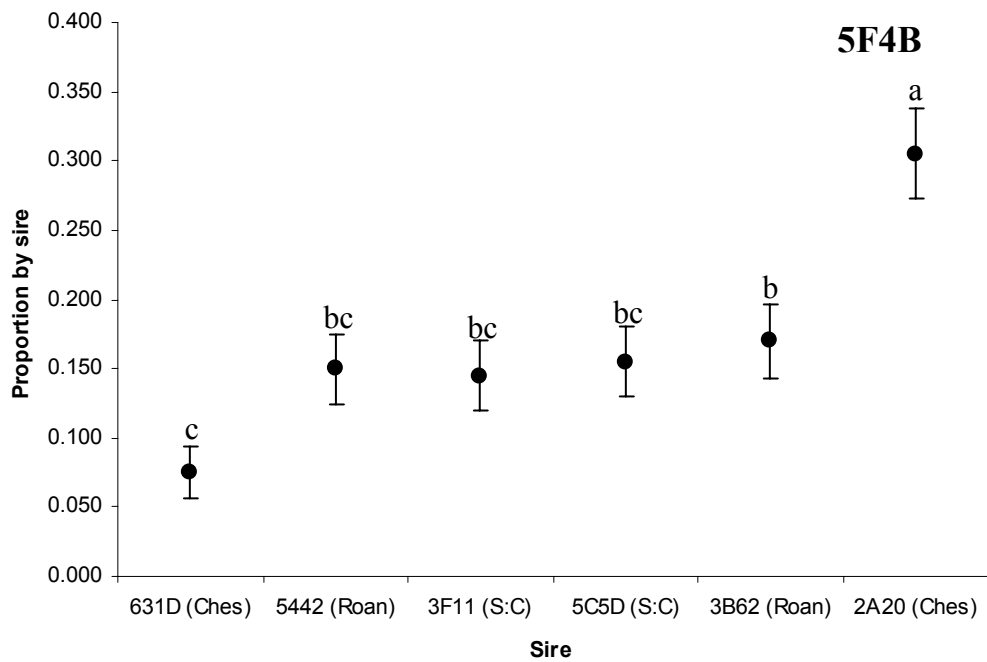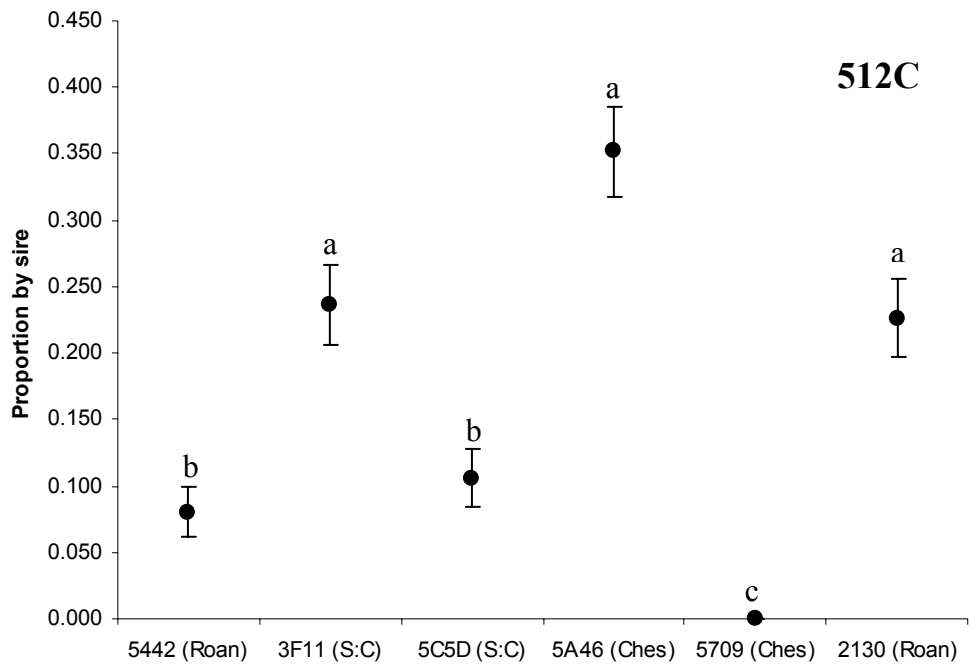
Figure 4. Proportional contribution (±) by sire to 512C (top) and 5F4B (bottom) larval groups reared at PAFL. Proportions with shared letters over the error bars are not significantly different from one another after sequential Bonferroni adjustment.
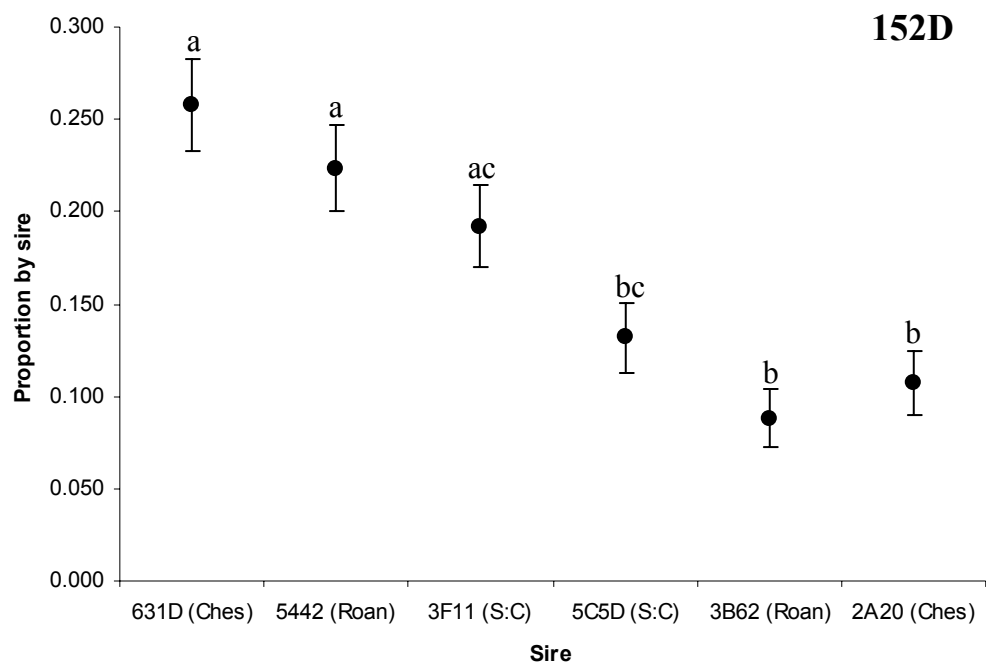
Figure 5. Proportional contribution (±) by sire to 152D larval group reared at PAFL. Proportions with shared letters over the error bars are not significantly different from one another after sequential Bonferroni adjustment.
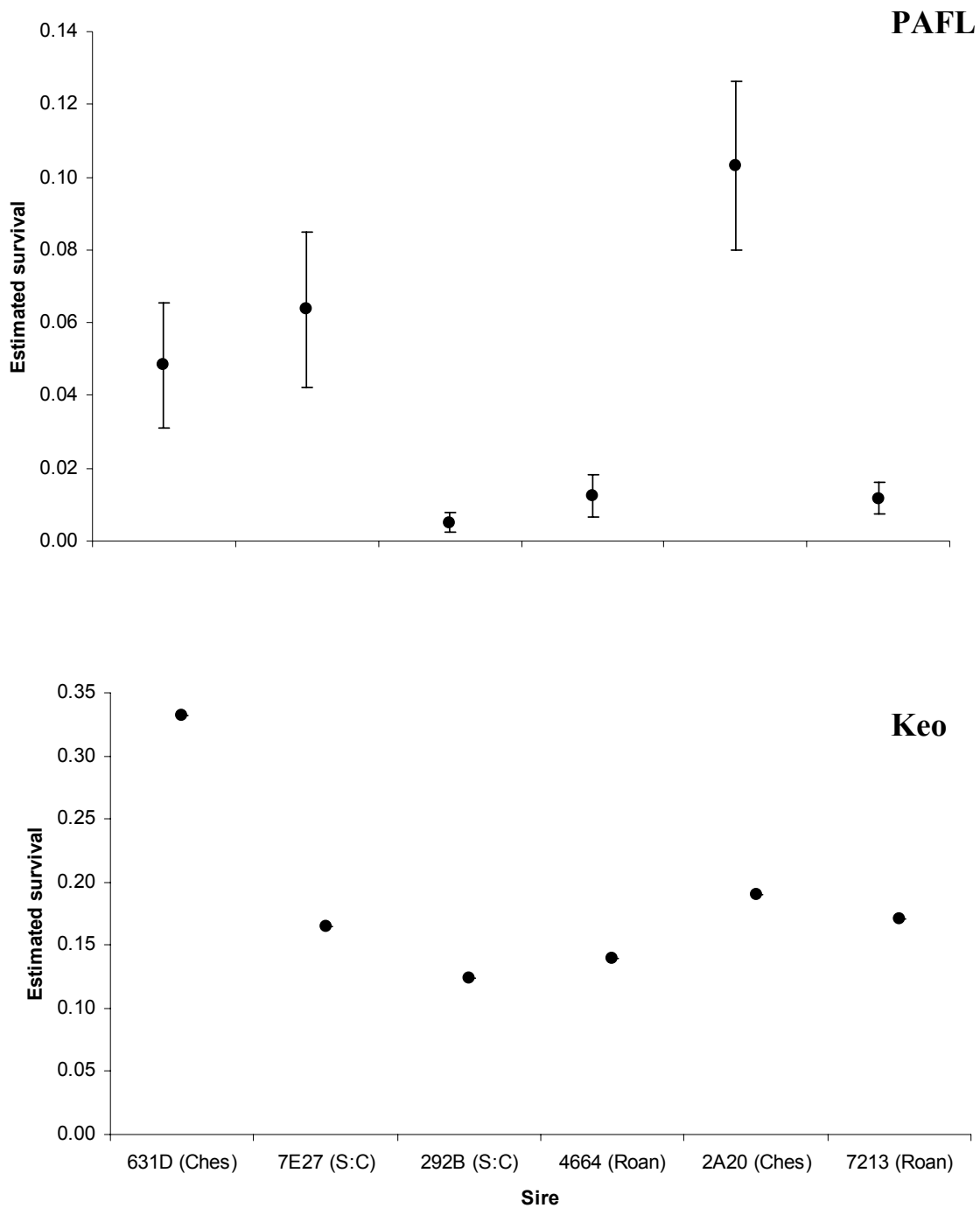
Figure 6. Estimated survival (±SE) by sire for PAFL 2E55 (top) and Keo 2E55 (bottom) Phase I fingerlings Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (S:C). Standard errors could not be calculated for Keo Phase I fingerlings. There were no pairwise significant differences detected by *Z*-tests for the PAFL pond.
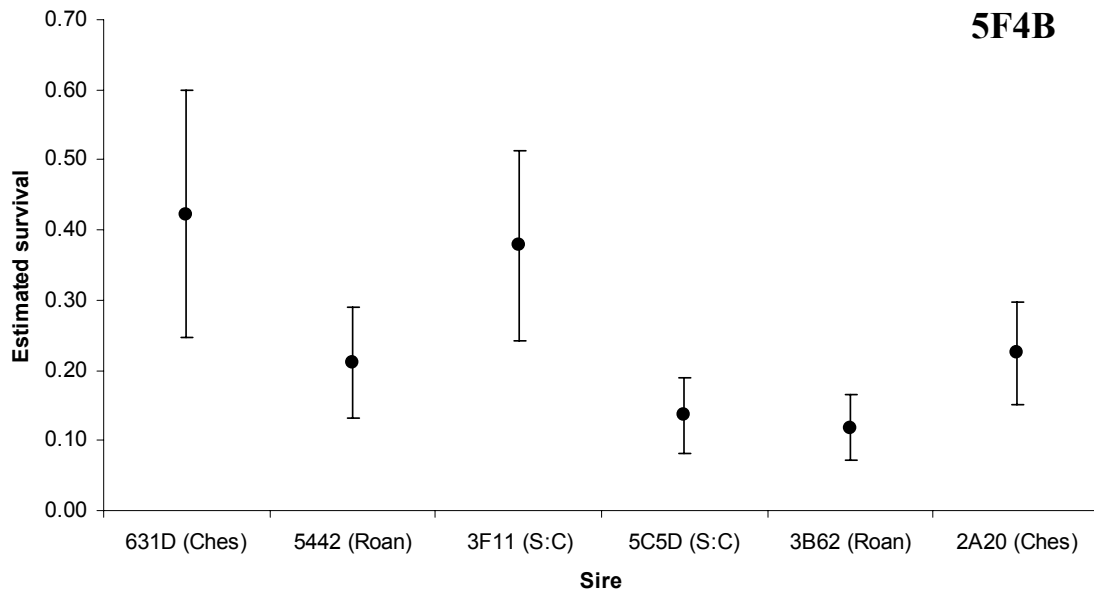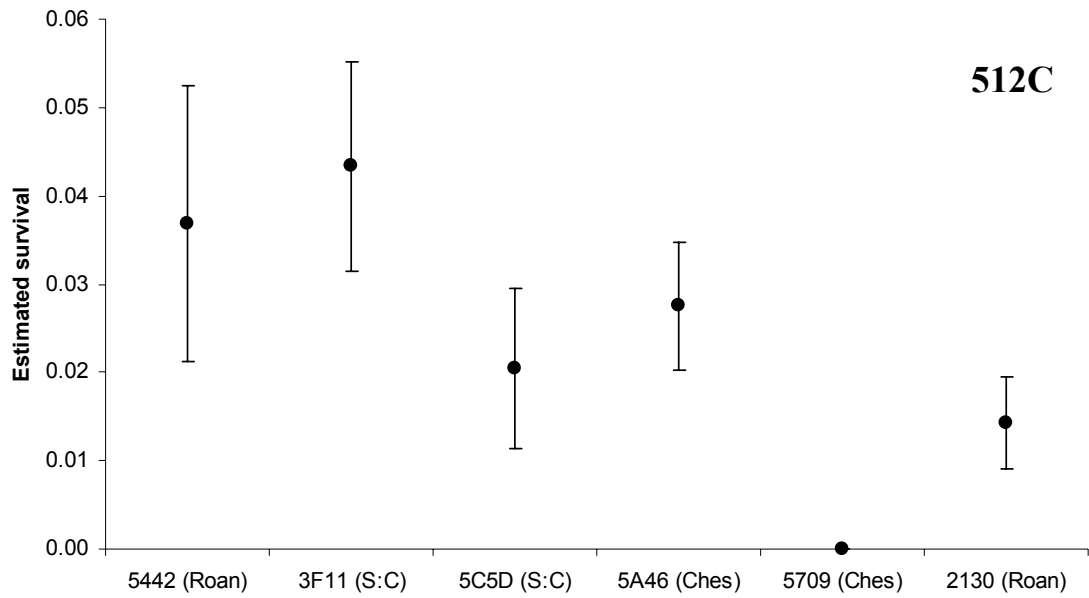
Figure 7. Estimated survival (±SE) by sire for dams 512C (top) and 5F4B (bottom) Phase I fingerlings. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (S:C). There were no significant differences pairwise differences detected for the 512C progeny. Differences between sire pairs for the 5F4B progeny were not significant after sequential Bonferroni correction.
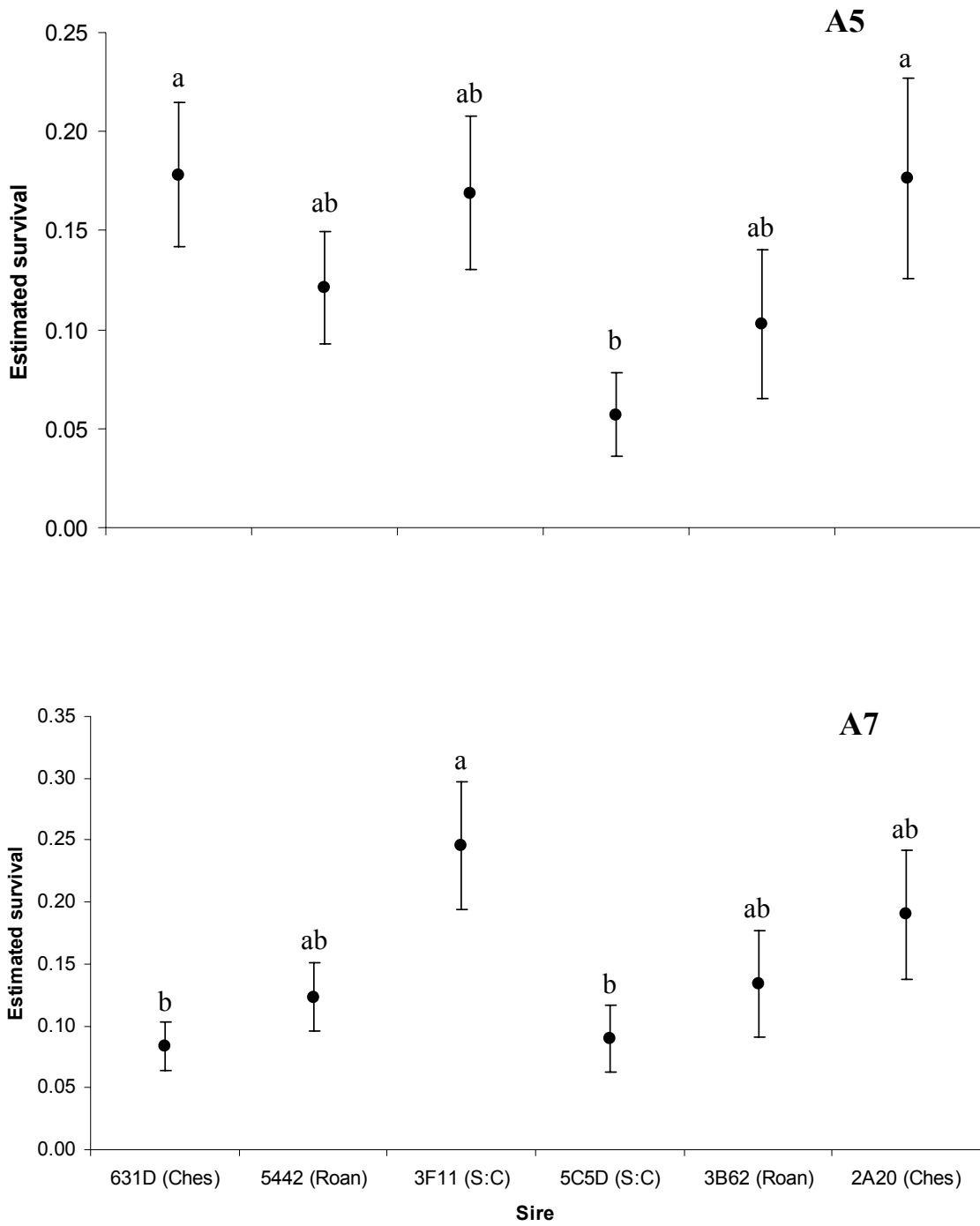
Figure 8. Estimated survival (±SE) by sire for dam 152D Phase I fingerlings in two ponds (A5 and A7). Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (S:C). Estimates with shared letters over the error bars are not significantly different after sequential Bonferroni correction.
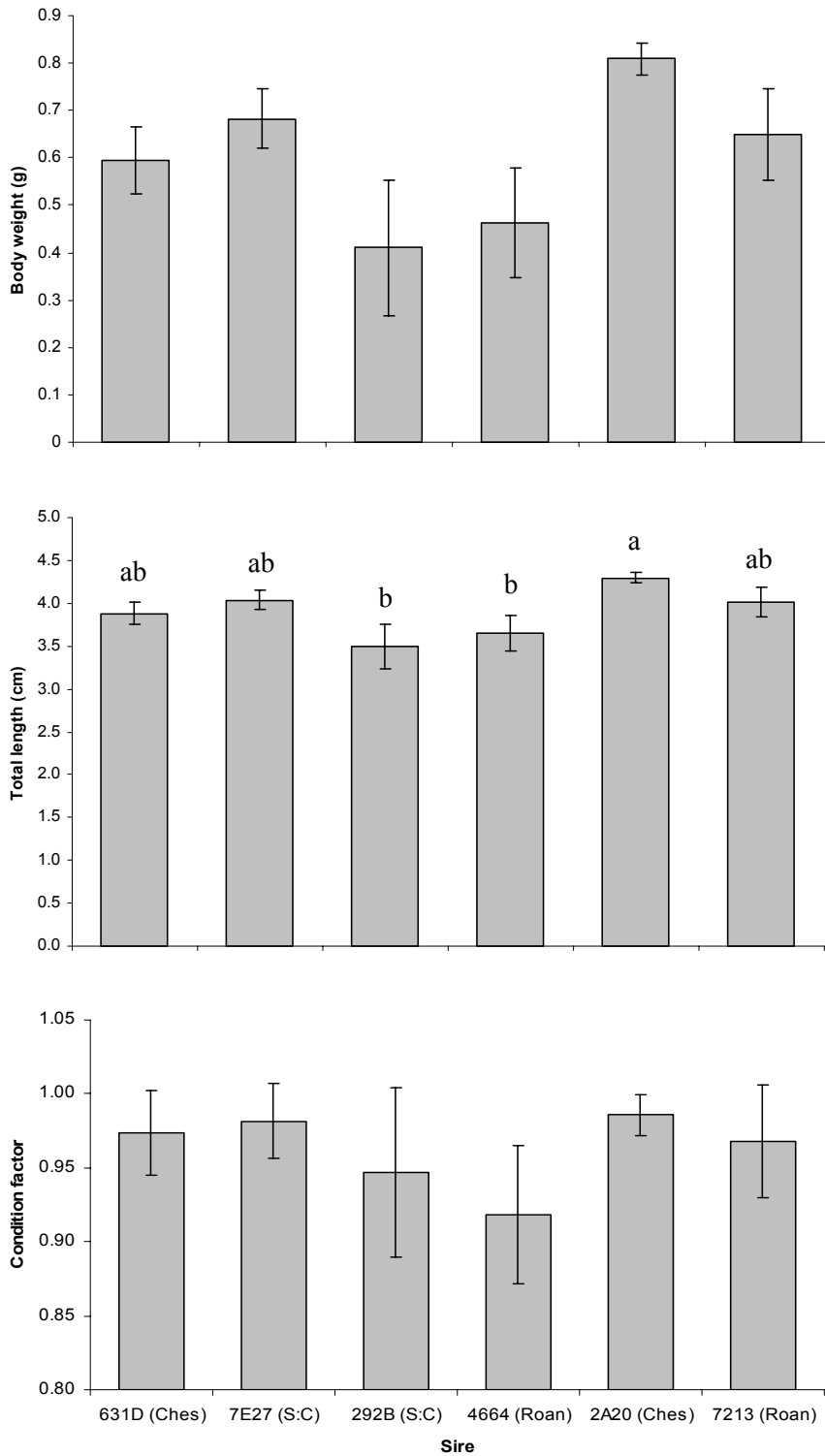
Figure 9. Least squares means for dam 2E55 PAFL progeny (±SE) for Phase I body weight, total length, and condition factor. Means with shared letters over the error bars are not significantly different. Roanoke sires are indicated by (Roan), Chesapeake sires by (Ches) and Santee:Chesapeake sires by (S:C).

Body weight (g)

0.5
0.5
0.4
0.4
0.3
0.3
0.2

a   ab   ab   ab   a   b

Total length (cm)

3.5
3.4
3.4
3.3
3.3
3.2
3.2
3.1
3.1
3.0

a   abc   bc   bc   a   b

Condition Factor

1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98

631D (Ches)   7E27 (S:C)   292B (S:C)   4664 (Roan)   2A20 (Ches)   7213 (Roan)
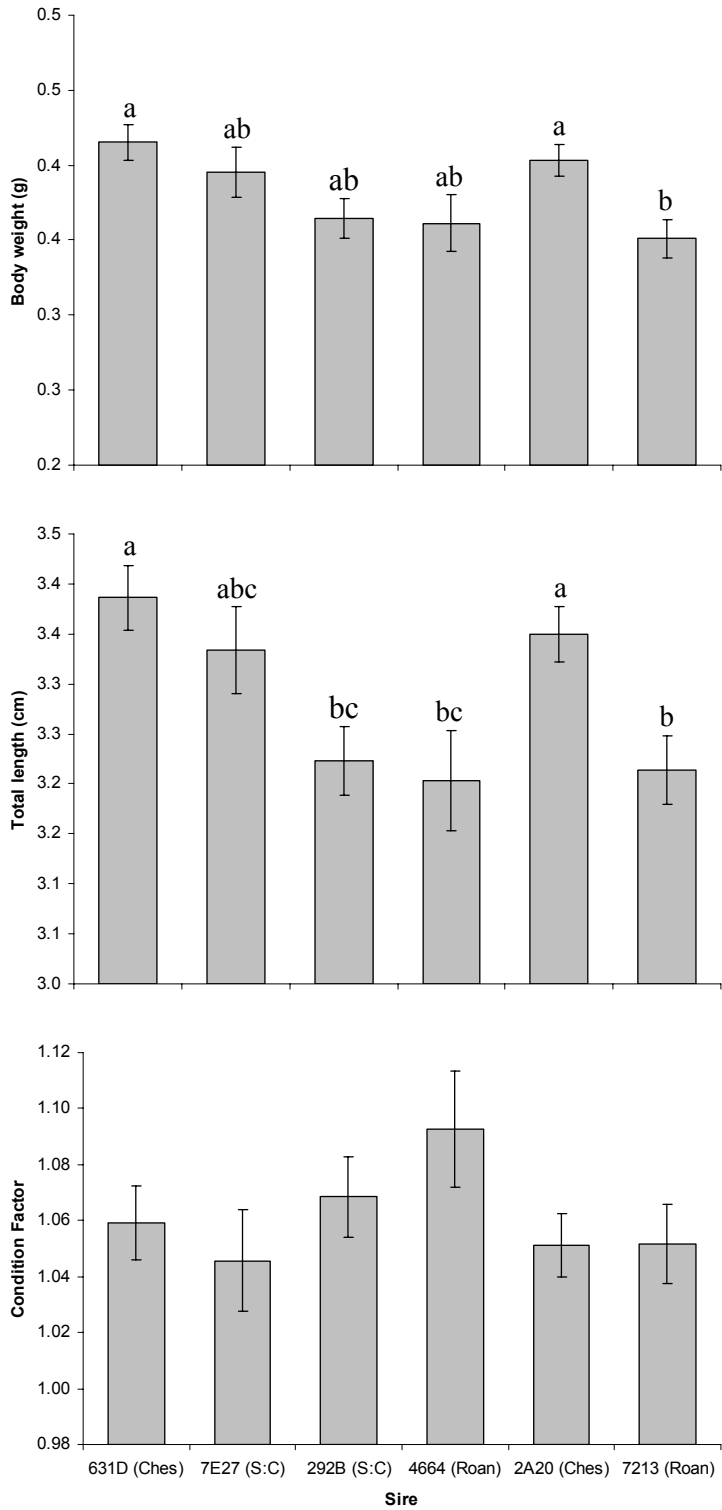
Sire

Figure 10. Least squares means for dam 2E55 Keo Fish Farms progeny (±SE) for Phase I body weight, total length, and condition factor. Means with shared letters over the error bars are not significantly different. Roanoke sires are indicated by (Roan), Chesapeake sires by (Ches) and Santee:Chesapeake sires by (S:C).
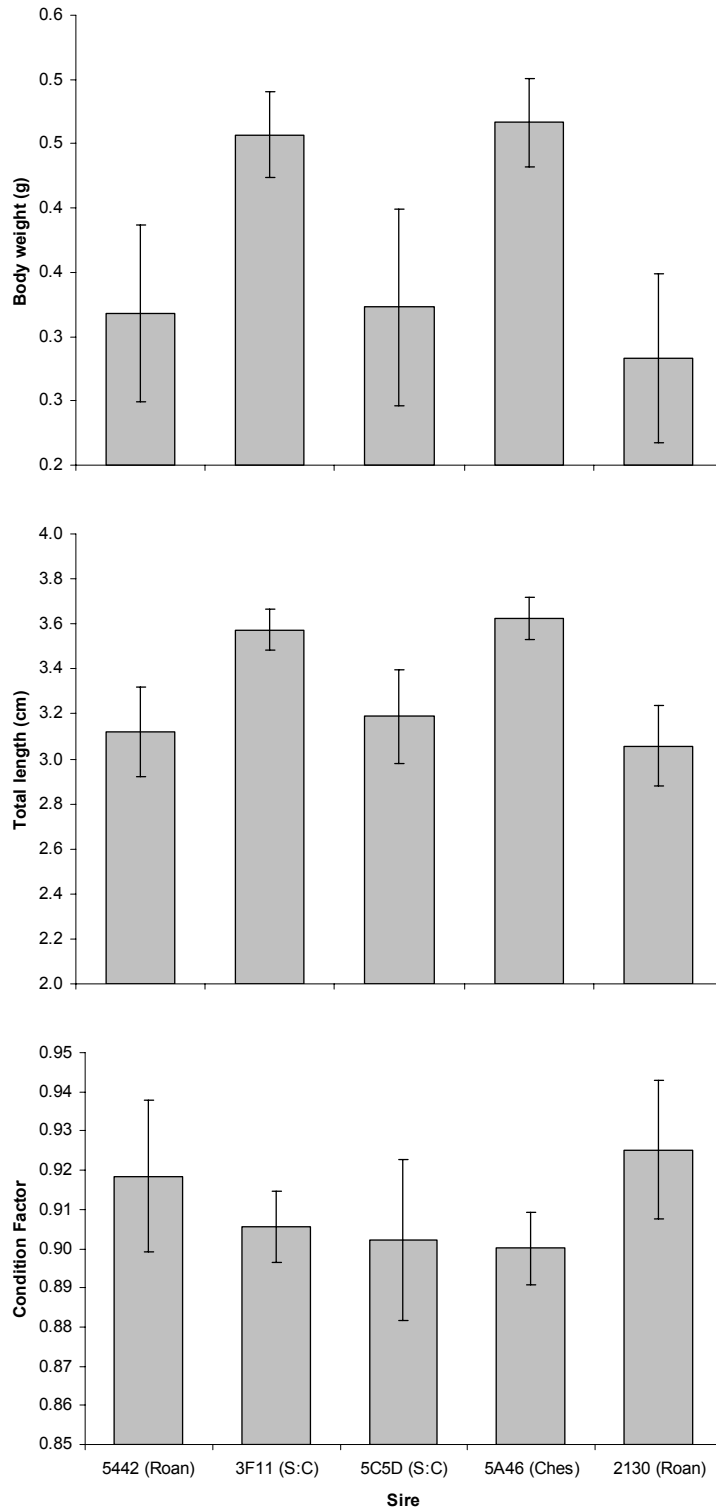
Figure 11. Least squares means for dam 512C progeny (±SE) for Phase I body weight, total length, and condition factor. Roanoke sires are indicated by (Roan), Chesapeake sires by (Ches) and Santee:Chesapeake sires by (S:C). There were no pairwise differences.
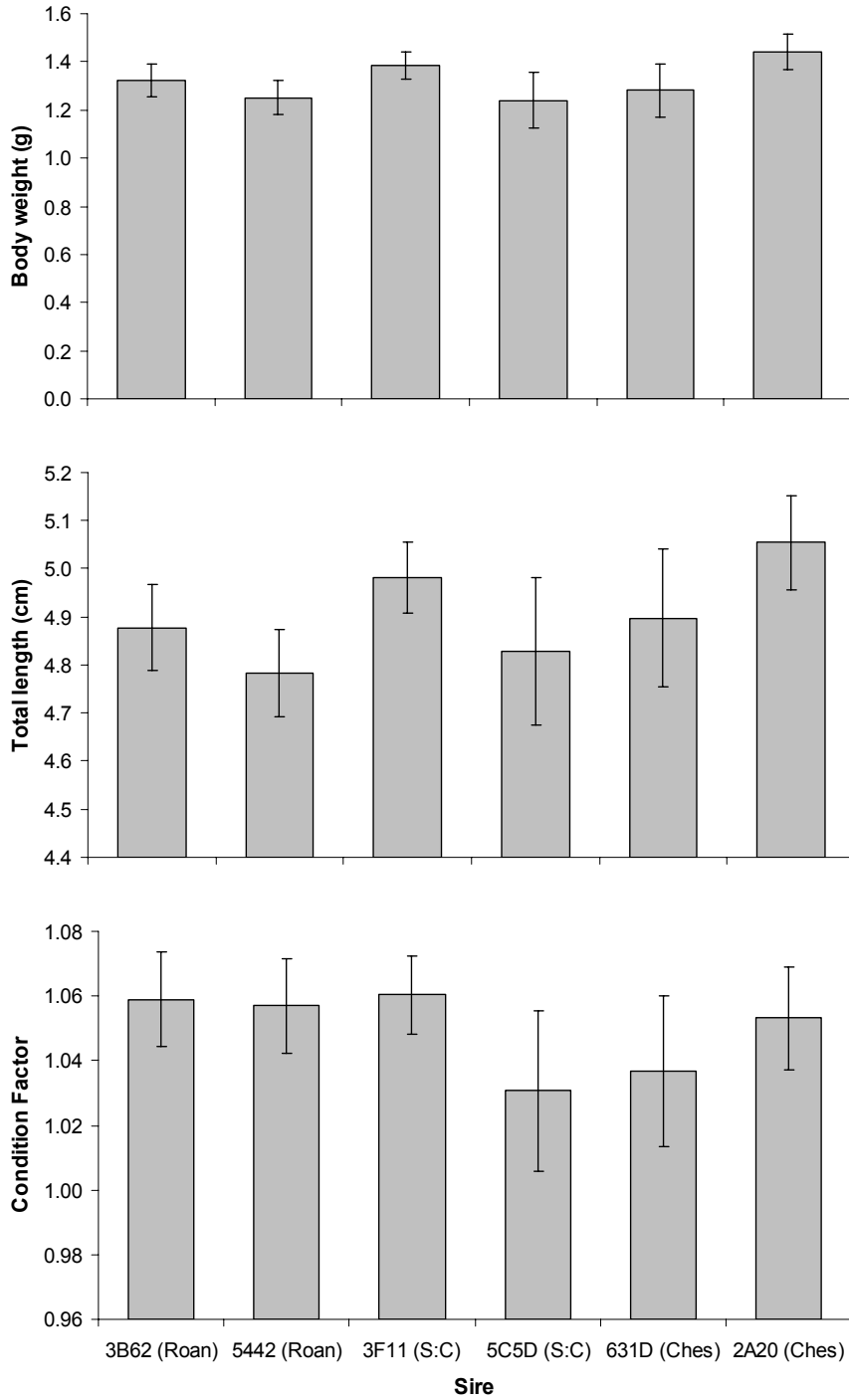
Figure 12. Least squares means for dam 152D progeny (±SE) for Phase I body weight, total length, and condition factor. Roanoke sires are indicated by (Roan), Chesapeake sires by (Ches) and Santee:Chesapeake sires by (S:C). There were no pairwise differences.
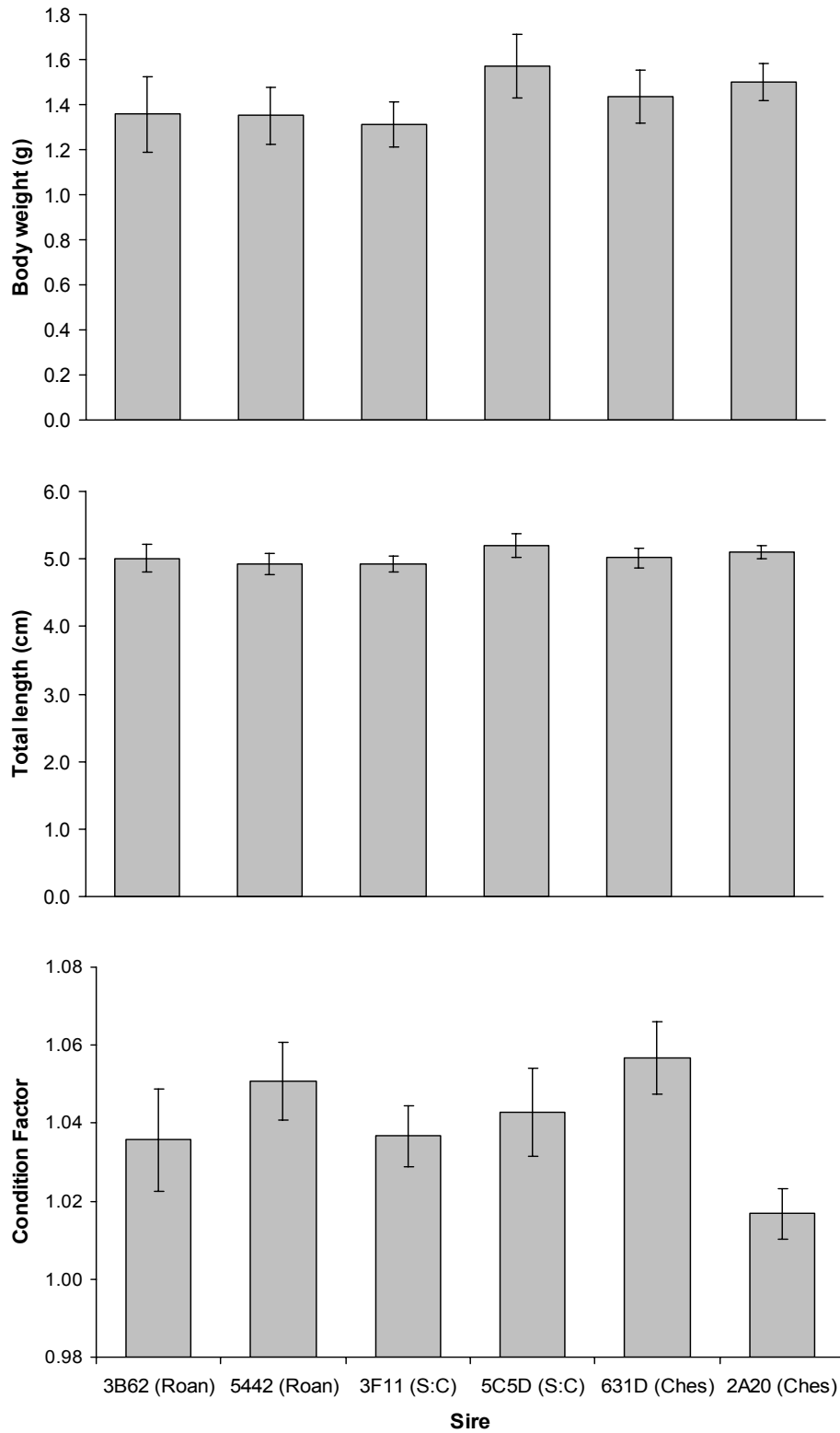
Figure 13. Least squares means for dam 5F4B progeny (±SE) for Phase I body weight, total length, and condition factor. Roanoke sires are indicated by (Roan), Chesapeake sires by (Ches) and Santee:Chesapeake sires by (S:C). There were no pairwise differences.

CHAPTER 4

COMMUNAL REARING OF STRIPED BASS FOR EVALUATION OF GROWTH PERFORMANCE

DURING PHASE II PRODUCTION

ABSTRACT

A microsatellite marker-assisted communal rearing approach for Phase II performance evaluations of striped bass was utilized to examine paternal effects on growth of fingerlings reared in research ponds and in intensive culture in a commercial hybrid striped bass production tank. The objectives of the study were to confirm the feasibility of microsatellite genotyping for progeny identification in communal rearing of striped bass to approximately one year of age and to investigate sire-based genetic variation among striped bass families for Phase II growth traits. Use of microsatellite markers for parentage identification of individuals in mixed family groups was highly successful for families reared in both research ponds and on a large commercial tank farm. Performance trials revealed significant sire effects on variation in phenotypic traits related to growth and body shape, providing evidence that genetic variation underlying these traits is present in the captive NCSU striped bass broodstock and may be exploited in a program of selective breeding for the hybrid striped bass industry.

INTRODUCTION

Commercial production of hybrid striped bass (hybrid striped bass; white bass *Morone chrysops* x striped bass *M. saxatilis*) in ponds began in North Carolina in 1986 and has become the fourth most valuable form of finfish aquaculture in the United States (Pritchard 2005). However, unlike many other aquaculture industries for which domesticated strains and selective breeding programs have been utilized, the hybrid striped bass industry remains dependent on capture and spawning of wild broodfish for annual production of fingerlings. This dependence on fingerlings produced from wild-caught spawners precludes

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selective breeding and weds the hybrid striped bass industry to a wild cultivar with associated inefficiencies that lead to high production costs. Recent efforts by university and government researchers, along with many commercial hybrid striped bass farmers, have resulted in the establishment of a new National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry. The National Breeding Program is committed to domestication of both striped bass and white bass for commercial production of the hybrid and to selective improvement of the male parent, the striped bass, for improved production efficiency. To date, the National Breeding Program has targeted its efforts toward acquiring and domesticating numerous geographic strains of striped bass; developing molecular markers for progeny identification, pedigree tracking and linkage mapping; evaluating the effects of using domesticated parents for production of hybrid striped bass; and examining genetic and phenotypic variation among strains and families of striped bass (see Chapters 2 and 3; reviewed by Garber and Sullivan 2006).

The first phase of commercial hybrid striped bass production (Phase I) involves the stocking of 3-5 day old larvae into fertilized outdoor ponds where they feed on natural zooplankton. Phase I fingerlings are harvested after ~30-45 days (see Chapter 3). The later phases of hybrid striped bass farming are largely carried out either in outdoor ponds or tank systems. Tank culture of hybrid striped bass predominated from 1987 but pond culture comprised about 50% of production by 1996 and has since come to dominate the hybrid striped bass industry (Figure 1). Phase II pond culture begins with Phase I fingerlings of approximately 1-2 grams or more in body weight that have been trained to consume prepared feeds. Fingerlings are stocked into outdoor ponds at a rate of 10-15,000 fish per acre (25,000-37,500/hectare, ha) (Jenkins et al. 1988b; Hodson 1995) with densities below 12,500/ha

causing wider size variation (Smith et al. 1990) and lower average weights (Jenkins et al. 1988b) at harvest. The Phase II growing season generally begins in late summer to fall, depending on location, and culminates in the winter or early spring with harvest of 90-225g fish, termed Phase II fingerlings. The fish are seined from the ponds and slow-growing fish or "runts" (<60 g body weight), which generally make up 5-10% of the population (Hodson 1995), are removed using floating bar graders (Smith et al. 1990). Unlike the case with Phase I fingerling production, survival of the fish during Phase II is usually quite high, with ~85% survival expected during this rearing period (Kerby et al. 1987; Jenkins et al. 1988b; Hodson 1995) and fingerlings reaching 90-225 g in weight (Hodson 1995). The Phase II fingerlings are restocked into ponds for the final production period, Phase III, which ends in early fall when the fish reach market size at ~18 months of age (usually 680-900 g body weight). Hybrid striped bass produced in tanks are typically stocked as Phase I or Phase II fingerlings and the tank-reared fish are exposed to higher average water temperatures and substantially higher rearing densities than are pond-grown fish, can achieve faster growth rates, and generally are not graded but are instead divided into new tanks to reduce fish densities as the fish become larger in size. At optimal water temperatures, stocking densities, and feeding rates, tank reared fish can reach market size in just under one year (Rappaport 2003).

Rapid growth occurs during Phase II production with fingerlings typically growing ~100-200 fold in body weight. Fish that reach the rather broad weight range expected of Phase II fingerlings should be able to achieve market size in their second year (Hodson 1995), although culling of smaller fish from some production groups is often required. It is important that the genetic underpinnings of growth during Phase II be explored. Phenotypic variation in growth during Phase II that is related to genetic differences may be valuable for a

selective breeding program since, by selectively utilizing broodstock known to produce rapidly growing progeny, it may be possible to increase the percentage of high performance fish. Heritable variation in growth of advanced juveniles has been observed in a number of aquacultured fishes, including carp (Vandeputte et al. 2004), catfish (Dunham 1987); Atlantic cod (Gjerde et al. 2004), salmonids (Iwamoto et al. 1982; Herbinger et al. 1995; Gjedrem 2000) and African catfish (Volckaert and Hellemans 1999). Such genetic variation in production of striped bass or hybrid striped bass would indicate that growth-related traits are amenable to selective breeding. Gains in growth rate from selective breeding have been 10% per generation in rainbow trout (Kincaid 1977), 10% per generation in coho salmon (Hershberger et al. 1990), 10-30% per generation in Atlantic salmon (Gjedrem 1979) and 12-18% in catfish (Dunham 1987), illustrating the tremendous potential for rapid improvement that can be made through selection programs. Selective breeding of highly variable and unselected lines of striped bass is expected to yield similar heritable gains in growth rate.

Evidence of family or strain effects on striped bass growth performance is somewhat limited. Families produced from wild striped bass of several geographic strains were evaluated under intensive tank culture conditions by Jacobs et al. (1999), revealing significant differences between strains in growth to 150 days of age. Woods et al. (1999) compared growth rates of various families of striped bass in flow-through tank systems and found significant differences in body weight and length (measured at age 1) among several domesticated families of Chesapeake Bay origin. However, these authors saw no effect of family on growth of captive-bred F_1 generation fish produced from parents from Santee River in South Carolina. Additional studies are needed for striped bass during the Phase II and Phase III rearing periods. Detection of paternally-based or other familial differences in

growth of striped bass during these later life stages would provide additional evidence of genetic variation that could readily be exploited for selective breeding.

The practical uses of communal rearing protocols for performance testing of numerous families of striped bass were demonstrated in <u>Chapter 3</u>. Communal rearing techniques have been utilized for various aquaculture species in order to reduce the number of rearing units necessary for testing multiple families and to increase the number of families or groups that can be compared with available rearing units (Moav and Wohlfarth 1974; Dunham et al. 1982; McGinty 1983, 1987; Wohlfarth and Moav 1991). By utilizing communal rearing techniques, replicated progeny evaluations for three families evaluated in triplicate would require only three ponds rather than the nine ponds necessary if the same families were reared separately. Obviously, implementation of communal rearing can confer great advantages to a small aquaculture operation or a selective breeding program with limited facilities. Additionally, communal rearing can serve to decrease the effects environmental variation (e.g. pond effects) on phenotypic trait assessment since all families are reared under identical environmental conditions. For fish that are stocked into ponds at sizes too small for physical tagging, application of molecular markers as innate genetic tags can permit individual identification of each communally reared offspring. This technique has been applied in communal rearing evaluations of aquacultured fishes (Herbinger et al. 1995; Garcia de Leon et al. 1998; O'Reilly et al. 1998; Saillant et al. 2006) and was highly successful for determining parentage of communally reared striped bass larvae and fry (<u>Chapter 3</u>).

In the present study, a microsatellite marker-assisted communal rearing approach for Phase II performance evaluations of striped bass was utilized to examine paternal effects on

progeny growth in research ponds and in intensive culture in a commercial hybrid striped bass production tank. The objectives of the study were to confirm the feasibility of microsatellite genotyping for progeny identification in communal rearing of striped bass to approximately one year of age and to investigate sire-based genetic variation in Phase II growth traits among striped bass families. The hypotheses tested included:

1) There are significant differences in growth performance (length and weight, proxies for growth rate; shape, or condition factor; and incidence of external deformities) among paternal striped bass families during Phase II;

2) There are significant differences among paternal striped bass families in survival during Phase II;

3) There are significant changes in allele and genotype frequencies in mixed family groups of striped bass between the start and end of Phase II rearing due to differential survival of certain genotypes.

METHODS

Broodstock Genotyping—Prior to production of experimental families, PAFL striped bass broodstock ($N=120$) were genotyped at three polymorphic microsatellite primer loci (see Methods, Chapter 2) in order to evaluate the genetic variability of three broodstock strains, including captive striped bass of wild Roanoke River-Albemarle Sound origin (R:F_0-1997; captured wild as adults in 1997), F_2-generation Chesapeake Bay (C:F_2-1991) striped bass, and F_1-generation Santee x F_2-generation Chesapeake fish (SC:F_1-94).

Phase I Experimental Crosses—Experimental crosses among striped bass broodstock were conducted in April 2001 to produce even-aged half-sibling striped bass families for evaluation of survival and performance. Crosses were made using captive wild and domesticated broodstock held at the NCSU Pamlico Aquaculture Field Laboratory in Aurora, NC as detailed in Chapter 3, Methods. Fish were reared for 34-44 days through the Phase I production period then harvested by seining and graded by size using conventional bar graders. Fingerlings from the Keo Fish Farms commercial production pond (dam 2E55) and fingerlings from two dams reared at PAFL (152D and 5F4B) survived in adequate numbers for Phase II performance evaluations.

Phase II rearing: research ponds—

Dam 2E55—Approximately 20,000 Phase I fingerlings from dam 2E55 reared at Keo Fish Farms were trucked to PAFL on July 31 2001, graded and held in 2.97 m diameter flow-through tanks for approximately three weeks where they were trained to feed on artificial diets. During this period, fingerlings were graded every 2-3 days and fed at a rate of 11-14% of their body weight per day in order to equalize the size of fingerlings prior to stocking for Phase II production. It was assumed that there was no effect of compensatory gain, or accelerated growth after a period of food restriction, since all fish were the same age and feeding was minimally restricted. In catfish, there was no compensatory gain of feed restricted catfish compared to unrestricted catfish using a similar method of "multiple rearing" to force the fingerlings of varying ages to a common size prior to commencement of communal rearing activities (Dunham et al. 1982); instead, subsequent growth occurred at a rate based on the size of the fish rather than the age of the fish. Striped bass fingerlings (average weight 3.78 g) were hand-counted and stocked into triplicate 0.1 ha research ponds

(ponds A4, A11, and A14) at PAFL on August 23, 2001 at a stocking rate of 28,050 fish/ha, or 2,805 fish per pond. A single sample of fingerlings (n=250) was collected from the initial 2E55 progeny population immediately before fish were randomly divided into subgroups for stocking into replicate ponds. For this sample, phenotypic traits, including body weight (g), total length (cm), condition factor (Fulton-type condition factor [(weight/length^3)*100]), and incidence of external deformities (e.g., scoliosis, jaw or opercular malformations, and eye deformities) were measured. Tissue samples were collected and preserved in 70% ethanol for subsequent genetic analysis.

Dams 152D and 5F4B—Phase I fingerlings from two dams reared in research ponds at PAFL (5F4B and 152D) were fed and graded as described above and then were pooled in roughly equal numbers (n=1,410 fish for dam 152D; n=1,413 fish for dam 5F4B) for communal rearing in a 12-family group. The pooled fish (dam 152D, average fingerling weight 5.60 g; dam 5F4B, average fingerling weight 5.62 g) were stocked into duplicate 0.1ha ponds at PAFL on July 13, 2001. A total of 2,823 pooled fingerlings were stocked into each pond at a stocking rate of 28,230 fish/ha. Samples of the 12-family group were collected immediately before pond stocking for phenotypic and genetic analysis as described above (n=236 fish for pond A5; n=242 fish for pond A7).

Fingerlings stocked into the research ponds for Phase II rearing were fed initially at a rate of ~11% body weight per day. Feeding rate was gradually adjusted to 3% body weight/day. Ponds were sampled monthly in order to adjust feeding rates as the fish grew and water temperatures changed. Water quality parameters were managed controlled using standard hybrid striped bass pond management practices (Bonn et al. 1976; Geiger and Turner 1990; Hodson 1995) and ponds were hand-weeded to control submerged aquatic

vegetation or treated with Karmex ® DL (diuron, E.I. DuPont Canada Company, Missasaugua, Ontario) at 3,000 g/ha to control emergent aquatic and terrestrial vegetation.

All fingerlings from one pond (A5) stocked with the 12-family group, were lost in January 2002 due to a low dissolved oxygen event. Fish from the remaining research ponds were harvested at approximately one year of age by repeated seining, hand counted to determine percent survival, and then randomly sampled for genetic and phenotypic analysis as previously described. A total of 600 fish were sampled in May 2002 for each pond of dam 2E55 fingerlings for body weight, total length, condition factor, and incidence of external deformities to determine relative performance of sire families. Fin tissue was collected from each fish and individually preserved in 70% ethanol for subsequent genetic analysis. A total of 1,208 fish were sampled from the single remaining 12-family pond on March 27, 2002; however, due to the loss of the replicate 12-family replicate pond and limited resources for genotyping, this group was excluded from further analyses and data will be reported only for the 2E55 fingerlings. The surviving fingerlings in the 12-family group were utilized in Phase III rearing trials as described in Chapter 5.

Phase II rearing: commercial tank—Approximately 38,500 fingerlings produced from dam 2E55 were shipped from Keo Fish Farms to Kent SeaTech's (Mecca, CA) intensive rearing facility on 12 June 2001 for commercial tank culture. The fish at Kent SeaTech were held in two nursery tanks and trained on feed at 10% body weight/day for one month. Fingerlings were sampled to determine initial body weight and total length on July 12, 2001 and then an estimated 17,758 fingerlings were stocked into a 64,352 liter (17,000 gallon), 7.3m diameter commercial production tank at an average weight of 10.5 g/fish for intensive culture in a commercial hybrid striped bass production setting. Fish were fed

initially at a rate of 15% body weight per day for the first month and then adjusted to 1-3% body weight per day depending on the water temperature. The tank was sampled monthly to adjust feeding rates and water quality was managed using Kent SeaTech's proprietary intensive culture practices.

Performance data were collected from the Kent SeaTech fish on November 13, 2001 at 120 days after introduction into the production tank (or 7 months of age). The mean size of fish on this date (~150g) was expected to correspond to the size of Phase II pond reared fingerlings. Kent SeaTech staff provided Phase II sampling data on phenotypic traits, including total length, body weight, and condition factor, as well as corresponding tissue samples from 250 fish.

DNA Extraction and Microsatellite Genotyping—DNA was isolated from approximately 4 mg ethanol-preserved fin clip or muscle tissue samples using the PUREGENE® DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN). Extractions were performed according to the manufacturer's directions with omission of the RNase A step. DNA was rehydrated in 0.1X TE buffer and stored at -20 °C. The six microsatellite markers used for parentage assignment are the same as those used for Phase I parentage assignment (Chapter 3) and are listed in Table 9. These markers were selected in order to maximize the success of parentage assignment for communally reared striped bass. The markers had been optimized for multiplex PCR so that only two reactions were necessary to amplify all six loci. PCR amplification of microsatellite loci was carried out in 12μl reactions in 96-well PCR plates as described in Chapter 3, Methods. Electrophoresis data were collected and allele sizes were determined using ABI PRISM Genemapper version 3.0 software.

Statistical analyses—

Parentage assignment—Parentage assignment of offspring was based on genotypes for a minimum of five microsatellite loci. Individual samples that failed to amplify were re-extracted and re-genotyped; those for which full genotypes could not be determined for at least five loci were removed from subsequent analyses. Parentage of progeny was assigned using probability tests in PROBMAX2 version 1.2 software (Danzmann 1997) as described in Chapter 3, Methods. Progeny for which unambiguous parentage assignment could not be made (e.g., progeny assigned to more than one sire) were excluded from further analyses.

Population differentiation—Allele frequencies of larvae and fingerlings by pond were determined using GENEPOP version 3.4 software (http://wbiomed.curtin.edu.au/genepop/) (Raymond and Rousset 1995). Genic differentiation between initial and final Phase II fingerling populations was tested by calculating unbiased estimates of *P*-values of the probability test (Raymond and Rousset, 1995*a*) between samples by locus; Fisher's exact test was used to test differentiation across all loci and groups. Similarly, genotypic differentiation was tested by calculating unbiased estimates of the *P*-values of a log-likelihood based exact test (Goudet et al. 1996) by locus and globally by Fisher's test across loci and groups. Exact *P*-values were estimated using a Markov chain method (Guo and Thompson 1992) and all Markov chains consisted of 2000 dememorization steps, 1000 batches, and 2000 iterations to produce standard errors <0.001. Significance levels for multiple independent tests of allelic or genotypic differentiation were adjusted using sequential Bonferroni correction (Rice 1989).

Proportional contribution and survival—Proportional contribution of each strain and sire to the initial and final Phase II pond populations was estimated from genotyped

proportions of the samples. Heterogeneity Chi-square analysis (Zar 1984) was used to test the null hypothesis that the three pond samples from the end of Phase II came from the same population in order to determine if these samples could be pooled for analyses. Chi-square goodness of fit tests were used to test the null hypothesis that a sample contained equal proportions of progeny from all sires or sire strains. The proportion used to calculate expected frequencies for sire was 0.1667 (1/6) and the proportion used for strain was 0.3333 (1/3). Where the null hypothesis was rejected, Z-tests were used to test for pairwise differences in the proportional representation of sires, with sequential Bonferroni correction for multiple tests (Rice 1989). Overall survival for each pond was calculated from the initial and final counts for each pond. An estimation of survival could not be made for the commercial tank because the total number of fish remaining at the end of the Phase II rearing period was not determined. After progeny were assigned to parental pairs, survival by sire and strain were estimated for each pond of half-sibling families. Estimates of survival were made as described in Chapter 3, Methods with the exception that initial and final numbers of fish were not estimated but were instead hand-counted. Therefore, standard errors of the survival estimates were calculated using a Taylor series expansion (Seber 1982) as described in Chapter 3, Methods with the exception that zero was used for the variance terms for the initial and final fingerling numbers. In all cases where survival was estimated to be >1.0 due to low estimated proportions in the initial group and much higher estimated proportions in the final group, survival estimates were set to 1.0 and associated variances are calculated with these values. Differences in estimated survival among sire families were examined by Z-tests (Williams et al. 2002) as described in Chapter 3 with the covariance term removed as it was near zero for all comparisons. Because multiple pairwise tests were necessary to

compare sire families, significance levels were adjusted using sequential Bonferroni correction (Rice 1989). Differences in survival between pairs of strains also were compared by Z-tests using an unadjusted significance level of $\alpha=0.05$.

Paternal variation in body weight and total length—Phenotypic trait measures were evaluated for normal distribution by PROC UNIVARIATE in SAS version 9.1 software (Cary, NC). The Kolmorogov-Smirnov goodness-of-fit test for normal distribution, as well as histograms and normal probability plots, were used to evaluate normality of phenotypic distributions of length, weight and condition factor for all ponds at the beginning and end of Phase II and for the commercial tank at the end of Phase II. Least squares means were calculated for phenotypic traits. Overall differences in family means of pond-reared fish for body weight, total length, and condition factor were tested with analysis of variance (ANOVA) using PROC GLM in SAS with sire as a fixed effect at the start of Phase II (six sires; one sampled group) and sire and pond as fixed effects at the end of Phase II (six sires; three ponds). For the group evaluated at the start of Phase II, the error mean square for the entire experiment was used as the denominator for the F-tests. This method of analysis is considered a form of pseudoreplication (Hurlbert 1984) since fish are treated as the experimental unit, rather than rearing unit, and the measures on each fish constitute sub-sampling within the group; however, all progeny were reared under identical conditions and families that are compared within this group should share the same environmental variation in phenotype. For the three replicate ponds sampled at the end of Phase II, observations made on the fish within the three ponds also constitutes sub-sampling. Therefore, the effect of sire was tested using the error mean square for sire*pond as the denominator for the F-test (Steel and Torrie 1980). Interaction plots for sires and ponds were examined for each trait to

evaluate interactions among ponds and sires. The group reared in a single tank at Kent

SeaTech was evaluated with ANOVA using the fixed effect of sire at the end of Phase II with

the error mean square for the entire experiment as the denominator for the F-test. Groups

from PAFL and Kent also were evaluated by ANOVA as described above for differences in

phenotypic means by sire strain (Chesapeake, Roanoke, and Santee:Chesapeake). In cases

where significant overall differences were indicated by ANOVA ($P<0.05$), pairwise

differences among family means were investigated with the Tukey-Kramer multiple

comparison procedure in SAS. Pairwise comparisons were considered significant at $P<0.05$.

All phenotypic trait means are presented as least squares means plus or minus the standard

error (SE) of the mean.

RESULTS

Parentage assignment—In the initial PAFL sample of fingerlings before they were

stocked into Phase II research ponds, 244 fish were genotyped and 242 were attributed to

specific parental pairs. The two fish that could not be assigned to specific parental pairs were

assigned to the two Roanoke sires (5442 and 2130). In the research ponds at the end of Phase

II, a total of 608 fish were genotyped, and 602 could be attributed to a parental pair. Two fish

in pond A4 were discovered to be progeny from families that were not stocked into to the

pond but that likely belonged to two other dams from earlier Phase I rearing trials. The

remaining four unassigned fish were attributed to two sires (5442 and 2130) from the

Roanoke strain. Of 238 fish genotyped for the Kent SeaTech tank, 7 fish were removed from

further analyses. Two of these were assigned to two sires (Roanoke sires 5442 and 2130).

The remaining 5 unassigned fish were discovered to be hybrid striped bass, presumably from

an adjacent tank, due to the presence of white bass alleles for the *AT150-2#4* and *SB108* loci. In all, of the 1,083 striped bass genotyped from dam 2E55, 1,075 fingerlings (99.3%) were assignable to a specific sire.

Population differentiation—Differences in allelic and genotypic frequencies were examined between the initial and final Phase II fingerling populations at PAFL to investigate differences among populations from the time of pond stocking until Phase II harvest approximately 9 months later, as well as to examine the loss of alleles or genotypes from any group. No differences in allele or genotype frequencies were present after Bonferroni correction for multiple tests (*P*<0.0042) between the initial pond sample and the final Phase II samples. Only four genotypes were lost from the 57 genotypes detected in the initial Phase II population, one from the A4 pond population at locus *SB108*, one from the A11 population at *SB6*, and one each at locus *AG25-1#1* in the A11 and A14 ponds. However, no alleles were lost in any population during the course of Phase II pond rearing or as compared to the original 2E55 larval sample used to produce the Phase I fingerlings.

Proportional contribution—The proportional contribution of each sire was evaluated for the initial Phase II pond stocking group and within each pond and tank at the end of Phase II. Data on genotypic proportions within each sample are presented in Table 12 and described below.

Initial Phase II pond population—Chi-square testing revealed that proportional contribution varied by sire within the initial Phase II population, $X^2_{(4, N=242)}$ =44.50, *P*<0.0001. Significant differences in contribution between strain pairs also were noted, $X^2_{(1, N=242)}$ =24.16, *P*<0.0001. Proportional contributions by sire ranged from 0.07 (±0.02) for Roanoke sire 4664 to 0.29 (±0.03) for Chesapeake sire 2A20. Significant pairwise

differences were detected by *Z*-tests after sequential Bonferroni correction with the Chesapeake sire 2A20 having a greater contribution than did Roanoke sires 4664 (*P*<0.0033) and 7213 (*P*<0.0036). Santee:Chesapeake sires 7E27 and 292B also had higher contribution than did Roanoke sire 4664 (*P*<0.0038; *P*<0.0042, respectively) (Appendix Table IV). Pairwise comparison of contribution by strains revealed that the both the Chesapeake and Santee:Chesapeake progeny composed a significantly larger proportion of the initial pond sample than did the Roanoke progeny (*P*<0.0001).

Final Phase II populations, research ponds—Heterogeneity Chi-square analysis indicated that the three pond samples from the end of Phase II were not homogeneous, $X^2_{(10)}$=23.74, 0.05<*P*<0.01 (by sire) and $X^2_{(4)}$=9.55, 0.025<*P*<0.05 (by strain); therefore, the three pond samples were not pooled for Chi-square analysis of sire or strain frequencies. Examination of each pond by Chi-square goodness of fit tests revealed that proportional contribution by sire and by strain varied within each of the three ponds at the end of Phase II (*P*<0.001). Pairwise *Z*-tests indicated that Chesapeake sire 2A20 generally had large contributions in all ponds and Roanoke sires 4664 and 7213 generally had low contribution in ponds A4 and A11 (Appendix Table IV). By strain, Chesapeake sires comprised a larger proportion of the final pond samples than either of the other two strains. Roanoke progeny were lower in number than both other strains except in pond A11 where the Santee:Chesapeake and Roanoke contributions were not different from one another.

Final Phase II population, commercial tank—Chi-square analysis indicated that proportional contribution varied by sire within the Kent SeaTech commercial tank at the end of Phase II rearing (0.001<*P*<0.005). Chesapeake sire 631D had a lower contribution than did any other sires, and progeny contributions by Santee:Chesapeake sires 7E27 and 292B

were lower than those of several other sires (Appendix Table IV). By strain, the Chesapeake sires contributed a larger proportion of progeny than did the Roanoke ($P<0.001$) or Santee:Chesapeake ($P<0.003$) sires.

Estimated survival, research ponds—Cumulative survival in the three ponds ranged from 66.6% to 76.0% (Table 14). Estimated survival by sire family for each group of half-sibling families is illustrated for the Phase II ponds in Figure 14 and estimated survival ($\pm$SE) is tabulated for each sire family in Appendix Table V. Survival varied among sires within a pond, ranging from a low of 0.37 ($\pm$0.12) for Roanoke sire 7213 in pond A14 to a high of 1.00 ($\pm$0.21) for Chesapeake sire 631D (pond A4) and 1.00 ($\pm$0.23) for Roanoke sire 7213 in pond A11. After Bonferroni correction ($P<0.0033$), only one significant difference among sire pairs was detected by pairwise *Z*-tests (Appendix Table VI). This difference in survival occurred in pond A11 between Chesapeake sire 2A20, which had an estimated survival of 0.90 ($\pm$0.12), and Santee:Chesapeake sire 7E27, which had an estimated survival of 0.46 ($\pm$0.10). However, numerous pairwise differences were present at the significance level of $\alpha=0.05$ in all ponds. Roanoke sires 4664 and 7213 had generally low survival, except for sire 7213 in pond A11, and generally high survival (>70%) was observed in each pond for the Chesapeake sires 2A20 and 631D. In general, the best surviving progeny in each pond were sired by males from the Chesapeake strain (Figure 15).

Paternal variation in phenotypic traits—An extremely low incidence of deformities was observed in Phase II progeny with no fish from the initial sample for the research ponds having any deformities and only 5 of the 1,800 sampled fish from the end of Phase II having any deformity. Examination of differences in incidence of deformities among sire families was deemed unnecessary. Body weight, total length and condition factor were normally

distributed within the initial and final Phase II samples. Least squares means for all traits by sire family and by strain (± SE) are presented in Appendix Table VII.

Initial Phase II pond population—Within the initial Phase II sample, overall differences in mean total length, $F_{(5,236)}=2.77$, $P=0.0188$ and mean condition factor, $F_{(5,236)}=2.33$, $P=0.0430$ were detected among sire families. Body weights of the sire families ranged from 3.68g (±0.09) to 3.95g (±0.07). Total lengths ranged from 69.29cm (± 0.59) to 71.21cm (±0.36). For total length, no pairwise differences between sire families were evident with the Tukey multiple comparisons method (Figure 16). Means for condition factor among sires ranged from 1.07 (±0.01) to 1.10 (±0.01). For condition factor, pairwise differences were detected between two sires with progeny of Santee:Chesapeake sire 292B having greater condition factor than those of Chesapeake sire 2A20. Overall differences by sire strain were detected for body weight, $F_{(2,239)}=4.33$, $P=0.0142$, and total length, $F_{(2,239)}=4.39$, $P=0.0134$. Progeny of Santee:Chesapeake strain sires were heavier in body weight than those of Roanoke sires and both the Santee:Chesapeake and Chesapeake progeny were greater in total length than the Roanoke progeny (Figure 17).

Final Phase II population, research ponds—Overall differences in mean body weight, $F_{(5,10)}=3.96$, $P=0.0305$ and mean total length, $F_{(5,10)}=4.51$, $P=0.0206$ were detected among sire families. Body weights of the sire families ranged from 59.00g (±1.22) to 73.08g (±1.29). Total lengths ranged from 17.55cm (±0.09) to 18.70cm (±0.10). Pairwise differences between sire families were evident with the Tukey multiple comparisons method (Figure 18). Progeny of Santee:Chesapeake sire 7E27 had greater body weight and total length than Chesapeake sires 2A20 and 631D. No overall difference was observed for mean condition factor among sires, $F_{(5,10)}=3.05$, $P=0.0627$. Sire means for condition factor ranged from 1.06

(± 0.01) to 1.10 (± 0.01). No differences were detected by sire strain for body weight, $F_{(2,4)}=3.12$, $P=0.1524$, total length, $F_{(2,4)}=4.15$, $P=0.1059$, or condition factor, $F_{(2,4)}=1.25$, $P=0.3776$ (Figure 19)

Final Phase II population, commercial tank—Overall differences in mean body weight, $F_{(5,225)}=16.67$, $P<0.0001$, mean total length, $F_{(5,225)}=16.11$, $P<0.0001$, and mean condition factor, $F_{(5,225)}=10.89$, $P<0.0001$, were detected among sire families. Mean body weights of the sire families ranged from 122.26g (± 4.90) to 175.46g (± 5.18). Mean total lengths ranged from 20.47cm (± 0.21) to 22.59cm (± 0.23). For both body weight and total length, pairwise differences between sire families were evident with the Tukey multiple comparisons method (Figure 20). Progeny of sires Chesapeake sires 631D and 2A20, as well as those of Santee:Chesapeake sires 7E27 and 292B, were heavier in body weight and greater in total length than progeny of the Roanoke sires 4664 and 7213. Mean measures of condition factor among sire families ranged from 1.37 (± 0.02) to 1.50 (± 0.01). Pairwise differences in condition factor were present with progeny from Chesapeake sire 631D having greater condition factor than those of all other sires. Overall differences also were detected by sire strain for mean weight, $F_{(2,228)}=40.04$, $P<0.0001$, total length, $F_{(2,228)}=40.40$, $P<0.0001$, and condition factor, $F_{(2,228)}=13.99$, $P<0.0001$. Chesapeake progeny were heavier in body weight than those of the Santee:Chesapeake and Roanoke sire strains. Progeny of the Santee:Chesapeake sires also were heavier than Roanoke progeny. Santee:Chesapeake and Chesapeake progeny were longer in total length and also had greater condition factor than Roanoke progeny (Figure 21).

DISCUSSION

Parentage assignment and conservation of genetic diversity—Utilization of microsatellite markers for individual identification of communally reared striped bass for Phase II progeny performance evaluations proved successful in both research pond and commercial tank settings. In all, 1,083 striped bass fingerlings stocked together for communal rearing at six days after hatching were genotyped at 6 microsatellite loci. More than 99.3% (1,075) of these fingerlings were attributable to specific parental pairs. As was the case in Phase I rearing trials (see Chapter 3), this study demonstrates the utility of microsatellite markers for progeny identification in Phase II communal rearing of striped bass.

Progeny from all six sires utilized for production of the 2E55 families were present in the final Phase II pond and tank groups. There were no significant differences in allele or genotype frequencies between the initial Phase II pond sample and the final Phase II pond populations at PAFL. This result is likely due to high survival rates in each pond and to the absence of family-specific survival or selection acting at these loci. Only four genotypes were lost during Phase II pond rearing compared to the number of genotypes (*n*=57) observed in the sample collected immediately before the Phase II experiments. These losses may be attributable to low frequency genotypes which were lost during rearing due to Phase II mortality or to failure to detect the genotype in the samples due to limited sample sizes (~200 fish per pond). More importantly, no alleles were lost from the time the initial larval sample was collected at 4 days post hatch to the end of Phase II rearing at either 7 months of age (commercial tank) or one year of age (research ponds). This information reflects the efficacy of Kent SeaTech and PAFL fingerling rearing practices for conservation of genetic diversity during production of domesticated striped bass families.

Proportional contribution and survival—As expected during Phase II rearing, overall survival in the pond populations was generally high, ranging from 66.6% to 76.0%. Significant differences in proportional contribution by sire were present within the initial and final Phase II populations (Appendix Table IV), but survival by sire differed only within pond A11 after sequential Bonferroni correction for multiple Z-tests. In pond A11, survival of progeny from Chesapeake sire 2A20 exceeded that of Santee:Chesapeake sire 7E27. Evaluation of pairwise differences in survival using a level of significance uncorrected for multiple tests (α=0.05) revealed a number of additional pairwise differences (Appendix Table VI) with Chesapeake sire 631D having greater survival than all other sires in pond A4 and also having greater survival than the two Roanoke sires in pond A14. By contrast, survival in pond A11 was highest for Chesapeake sire 2A20 (also the most numerous group at the start of Phase II) and for Roanoke sire 7213.

Survival by sire was greater than 35% for all sires in each pond with the exception of the two Roanoke sires in ponds A4 and A14 and Roanoke sire 4664 in pond A11. Survival by strain was generally highest in the Chesapeake strain and lowest in the Roanoke strain, again with the exception of pond A11, where both the Roanoke and Chesapeake groups had higher survival than did the Santee:Chesapeake group. Differences in survival among sires may be due to inherent genetic differences among sires or strains in the ability to survive during the Phase II rearing period or to differences in competitive ability of the sire groups during communal rearing. However, differences in the general patterns of survival among replicate ponds, with some sires having very high survival in two ponds but not in the third, may indicate underlying differences in environmental conditions of the ponds to which some sires or strains were better suited. These different patterns of survival also may be due to chance,

particularly since a single representative sample was taken from the pooled fingerlings immediately before Phase II stocking, rather than individual samples being taken after the fingerlings were divided into subgroups for stocking into the three ponds, and due to chance those initial proportions by sire may not accurately represent the actual proportions stocked into each pond.

      Growth and shape-related phenotypic traits—Growth, as measured by mean body weight and total length, and body shape (mean condition factor) varied in the initial pond sample and also in the final pond populations. In the initial sample, body weight did not differ among sire families, but overall total length differed significantly by sire. However, no pairwise differences were observed by sire for total length. For condition factor, the single pairwise difference detected among sires was between Santee:Chesapeake sire 292B and Roanoke sire 2A20, with the Santee:Chesapeake progeny having greater mean condition factor than the 2A20 progeny. For the final Phase II pond groups, reared in ponds for approximately nine months, significant variation in trait means was present for both body weight and total length, but there was no difference in condition factor among sire families. The mean values for both weight and length were greatest for Santee:Chesapeake sire 7E27 and smallest for Chesapeake sires 631D and 2A20. Mean values for body weight and total length differed among sire families in the single commercial tank with the four Chesapeake and Santee:Chesapeake sires having greater weight and length than progeny from the two Roanoke sires. Progeny of Chesapeake sire 631D had significantly greater condition factor than any other sire family in the commercial tank. No differences in trait means by strain were found in the research ponds although differences were detected in the single commercial tank.

Moderate to high heritabilities for body weight have been reported for advanced juveniles in other fish species, indicating that the additive genetic variation exists for growth during this period and that growth related traits are amenable to selective breeding. Herbinger et al. (1995) communally reared rainbow trout and observed significant family effects on progeny growth. Volckaert and Hellemans (1999) reported that both body mass and body length, proxies for growth, were related to family and sire at 9 months of age in the African catfish. Heritability for body weight at 6 months of age in two populations of Atlantic cod was 0.29 ± 0.27 to 0.56 ± 0.26 (Gjerde et al. 2004). These populations evidenced a significant effect of sire during the first 6 months of life. Heritabilities in this range also have been reported for carp, with heritability for body weight at 8 weeks of age of 0.33 ± 0.08 and length of 0.33 ± 0.07 (Vandeputte et al. 2004). Although growth performance to 75 days appears to have a strong maternal component, significant effects of sire on growth also have been observed for black bream (Doupe and Lymberry 2005) during the period from 75-130 days of age. There have been no reports of heritability of growth-related traits for striped bass; however, genetic differences in juvenile growth was reported for striped bass from different latitudes (geographic strains) (Conover et al. 1997) and strain-specific growth performance has been reported for young of the year striped bass (Secor et al. 2001). Additionally, variation among families was seen within groups of yearling fish produced from Chesapeake Bay parents (Woods et al. 1999), and divergence in growth rates among striped bass from several Atlantic coast strains was reported by Jacobs et al. (1999). Although few families were examined in the present study, significant variation among striped bass families was present in striped bass reared in outdoor research ponds and in the commercial tank. There was a substantial effect of sire on growth performance during the

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Phase II rearing period, suggesting that genetic variation in growth within the captive striped bass broodstock at PAFL may be exploitable for selective breeding. Examination of additional striped bass families in replicated rearing units in commercial pond and tank settings will be necessary to fully assess the genetic variation of the captive strains and any possible genotype-environment interactions. Use of microsatellite markers and communal rearing should reduce the economic impact of such testing on the rearing capacity of participating commercial farms.

Recommendations for Phase II rearing trials for the National Breeding Program—In the present study, replicate ponds produced somewhat different results for Phase II fingerling growth traits. Fish from pond A14 were larger overall (body weight, total length), possibly due to a slightly longer Phase II rearing period or to different environmental conditions than in the other ponds. Interaction plots of sire trait means for the three ponds revealed that trait means were roughly parallel for most sires across ponds with the exception of Roanoke sire 4664, and perhaps Roanoke sire 7213, and the ANOVA revealed a significant interaction effect. The variation in performance of Roanoke progeny among the ponds may be due to environmental differences unique to the ponds to which these progeny were more sensitive than were progeny of the other sires. This effect is difficult to discern without testing additional family groups in replicated trials. However, it seems more likely that the differences were due to sampling error. Progeny of sire 4664 were quite low in number in the samples from each pond (<10 fish per pond) and comprised only 4.2% of the total number of fingerlings sampled (N=602). Similarly, progeny of sire 7213 numbered less than 20 fish in two of the ponds. The three Phase II ponds were all similar in area (0.1ha) and were managed as uniformly as possible. Additionally, all six families were reared together in each pond,

exposing sire families within a pond to the same environmental conditions and competitor strains. Despite attempts to minimize experimental error, pond-to-pond variation was present and it is clear that adequate replication of families in multiple ponds will be necessary to account for variation among rearing units when evaluating performance means during communal rearing trials. Qualitative differences among ponds were observed during the course of the rearing trials; *e.g.*, some ponds maintained high turbidity while others were fairly clear, and some ponds had greater growth of aquatic plants than others. Such unavoidable variation among rearing environments clearly affects absolute progeny performance and may influence relative progeny performance.

Large differences in absolute growth also were present between the research ponds and the commercial tank. Fish in the commercial tank were reared for a shorter period of time before Phase II sampling due to known differences in growth rate between the pond and tank rearing environments (S. Mitchell, *personal communication*). Fingerlings sampled at the end of 4 months in the commercial tank were expected to be similar in size to fingerlings sampled from the ponds at 9 months of age (~150g), but the tank-reared fish were approximately twice the average weight of the pond-reared fish. Kent SeaTech utilizes geothermal well water to maintain a constant 26-27 °C temperature in their production tanks (Rappaport 2003), thereby sustaining greater hybrid striped bass growth rates during the cooler fall and winter months than can pond producers for whom fingerling growth rates are limited by ambient temperatures. Fish densities also are greater in the Kent SeaTech tanks due to their ability to closely monitor and control water quality parameters. Clearly, environmental variables can greatly influence the performance of striped bass. The effects of feeding rate (Hung et al. 1993) and water quality parameters (Woods et al. 1985) on growth

have been demonstrated for both striped bass and hybrid striped bass and these variables should be taken into account when evaluating phenotypic means in various types of rearing units.

Unlike Phase II performance in the research ponds where trait values were lowest for the two Chesapeake sires, progeny growth performance in the Kent commercial tank was lowest for the two Roanoke sires. Variation in performance in different rearing systems may indicate the presence of genotype by environment interactions, where families (or genotypes) perform differently in one environment than they do in another environment, causing changes in rank order (order of merit) of the tested groups between environments (Falconer and Mackay 1996). Such variation must be considered and accounted for in subsequent progeny testing schemes and may impact selection protocols of the National Breeding Program since Phase II performance evaluations conducted in research ponds may not provide accurate predictions of fish performance in other rearing environments (e.g., commercial tanks, large ponds, raceways, or cages).

Genotype-environment interactions in strain growth performance have been reported by Iwamoto et al. (1986) for rainbow trout reared under several feeding and density regimes and the authors suggest that selection of strains adapted to specific growing environments may lead to increases in productivity. Similarly, Wohlfarth and Moav (1991) examined differences in performance of common carp reared in ponds and cages. These authors saw differences in rank order of the groups in the different environments and recommended performance testing in cages if rearing of selected carp is to be pursued in that environment. Such practices will be necessary for striped bass if the fish are to be selected in a culture facility that is different than the commercial production facility. Although the consistent

performance of genotypes must be examined in each culture setting, there is some evidence that rearing of striped bass under similar, though not identical, conditions can produce consistent ranking of groups. For striped bass reared in two tank culture facilities with measurably different water quality, density and husbandry practices, Jacobs et al. (1999) observed a high correlation between rank orders of mean family growth rates for fish reared from a ~50g starting weight for 150 days. It is possible that the effects of genotype-environment interactions could occur prior to the 50g starting weight used in their study (equivalent to Phase II pond production) but this hypothesis has not been tested. If genotype-environment interactions prove to be present for striped bass reared in more disparate settings, or for those reared in different environments during early life stages, evaluation of progeny performance traits must be carried out in the environment in which selected animals will be reared, under similar densities, water quality parameters, and ration levels. This likely will necessitate greater investment on the part of commercial farmers for use of rearing units, for assistance with sampling and phenotypic measures, and for return of future broodstock or cryopreserved gametes to PAFL or other breeding centers for subsequent reproduction. Use of communal rearing protocols will help to make such investments as inexpensive as possible for farmers.

For selective breeding purposes, the predictive value of Phase II on later culture phases would confer substantial benefits as breeders would save the time, expenses, and effort involved in rearing and performance testing fish during Phase III, enabling breeding programs to achieve more rapid gains. Vandeputte et al. (2002) observed the same strain rankings at 5 weeks of age as were seen in 2 summers-old carp. Evaluation of Phase III fingerlings in Chapter 5 will reveal if predictions of performance can be made for 18-month

old striped bass from Phase II performance data. Positive findings could greatly reduce costs for performance evaluations. If size differences (performance) in Phase II can ultimately predict performance differences later in life (size at the end of Phase III, for example), selection for top-performing individuals at early life stages may indirectly and fortuitously select for maximum growth in later stages, effectively increasing commercial production efficiency as well as accelerating the progress of selective breeding efforts.

Summary—In summary, use of microsatellite markers during Phase II communal rearing trials proved highly successful for progeny identification of striped bass reared in research ponds and in a commercial tank. The performance trials revealed significant sire effects on variation in phenotypic traits related to growth and body shape, providing evidence that genetic variation is present in the captive PAFL striped bass broodstock during this rearing period. Such variation should make possible the selective improvement of striped bass for aquaculture production.

Table 17. Estimated pond stocking rates, fingerling recovery rates and survival for three research ponds (A4, 11, 14) and a commercial production tank (Tank 40); fish were sampled but not harvested from Tank 40 thus survival could not be estimated.

Pond or tank	Date of stocking	No. fingerlings stocked	Date of harvest	No. fingerlings recovered	No. days in Phase II production	Phase I survival rate
A4	08/23/01	2805	05/13/02	2131	260	0.760
A11	08/23/01	2805	05/15/02	2013	262	0.718
A14	08/23/01	2805	05/23/02	1868	270	0.666
Tank 40	07/12/01	17,758	11/13/02	--	121	--

Table 18. Numbers (*N*) and estimated contributions by sire (proportions plus or minus standard errors) of 2E55 fingerlings assigned parentage in the initial and final Phase II pond sample and the final commercial tank sample. A4, 11 and 14 are designations for the research pond samples at the end of Phase II, Tank 40 is the commercial tank sample.

POND	SIRE	Larvae	
		Genotyped	Proportion (SE)
Initial pond sample	4664 (R:F_0-97)	17	0.070 (0.016)
	7213 (R:F_0-97)	28	0.116 (0.021)
	292B (SC:F_1-94)	47	0.194 (0.025)
	7E27 (SC:F_1-94)	47	0.194 (0.025)
	631D (C:F_2-91)	32	0.132 (0.022)
	2A20 (C:F_2-91)	71	0.293 (0.029)
	N	242 of 244	
A4	4664 (R:F_0-97)	9	0.045 (0.015)
	7213 (R:F_0-97)	18	0.090 (0.020)
	292B (SC:F_1-94)	36	0.179 (0.027)
	7E27 (SC:F_1-94)	39	0.194 (0.028)
	631D (C:F_2-91)	45	0.224 (0.029)
	2A20 (C:F_2-91)	54	0.269 (0.031)
	N	201 of 202	
A11	4664 (R:F_0-97)	8	0.040 (0.014)
	7213 (R:F_0-97)	36	0.180 (0.027)
	292B (SC:F_1-94)	27	0.135 (0.024)
	7E27 (SC:F_1-94)	25	0.125 (0.023)
	631D (C:F_2-91)	30	0.150 (0.025)
	2A20 (C:F_2-91)	74	0.370 (0.034)
	N	200 of 204	
A14	4664 (R:F_0-97)	8	0.040 (0.014)
	7213 (R:F_0-97)	13	0.065 (0.017)
	292B (SC:F_1-94)	34	0.169 (0.026)
	7E27 (SC:F_1-94)	39	0.194 (0.028)
	631D (C:F_2-91)	39	0.194 (0.028)
	2A20 (C:F_2-91)	68	0.338 (0.033)
	N	201 of 201	
Tank 40	4664 (R:F_0-97)	25	0.108 (0.020)
	7213 (R:F_0-97)	47	0.203 (0.026)
	292B (SC:F_1-94)	31	0.134 (0.022)
	7E27 (SC:F_1-94)	29	0.126 (0.022)
	631D (C:F_2-91)	42	0.182 (0.025)
	2A20 (C:F_2-91)	57	0.247 (0.028)
	N	231 of 233	
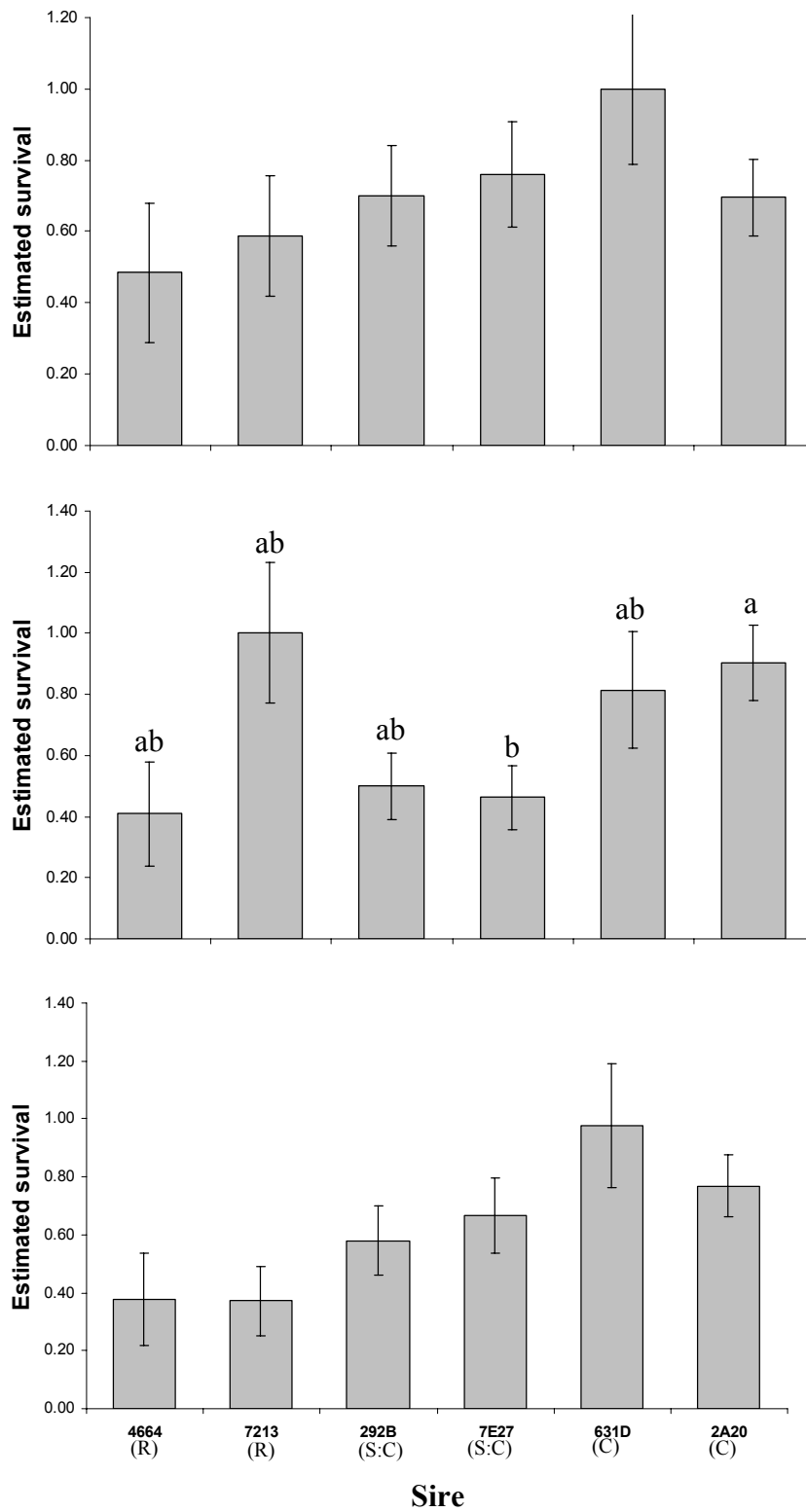
Figure 14. Estimated survival (±SE) by sire family for 2E55 Phase II final pond samples (top to bottom: A4, A11, A14). Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different after sequential Bonferroni correction.
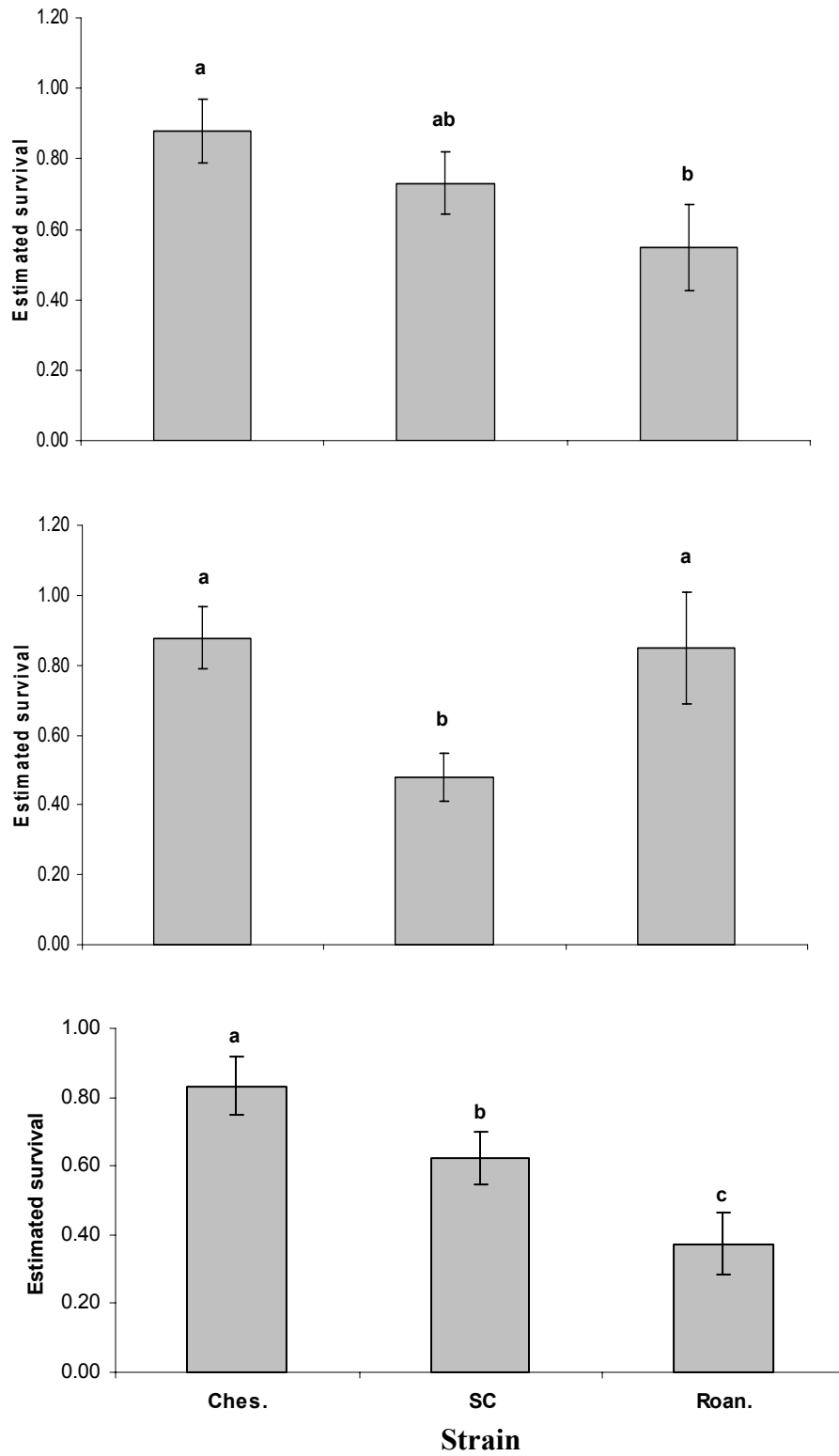
Figure 15. Estimated survival (±SE) by strain for 2E55 Phase II final pond samples (top to bottom: A4, A11, A14). Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different (*P*>0.05).
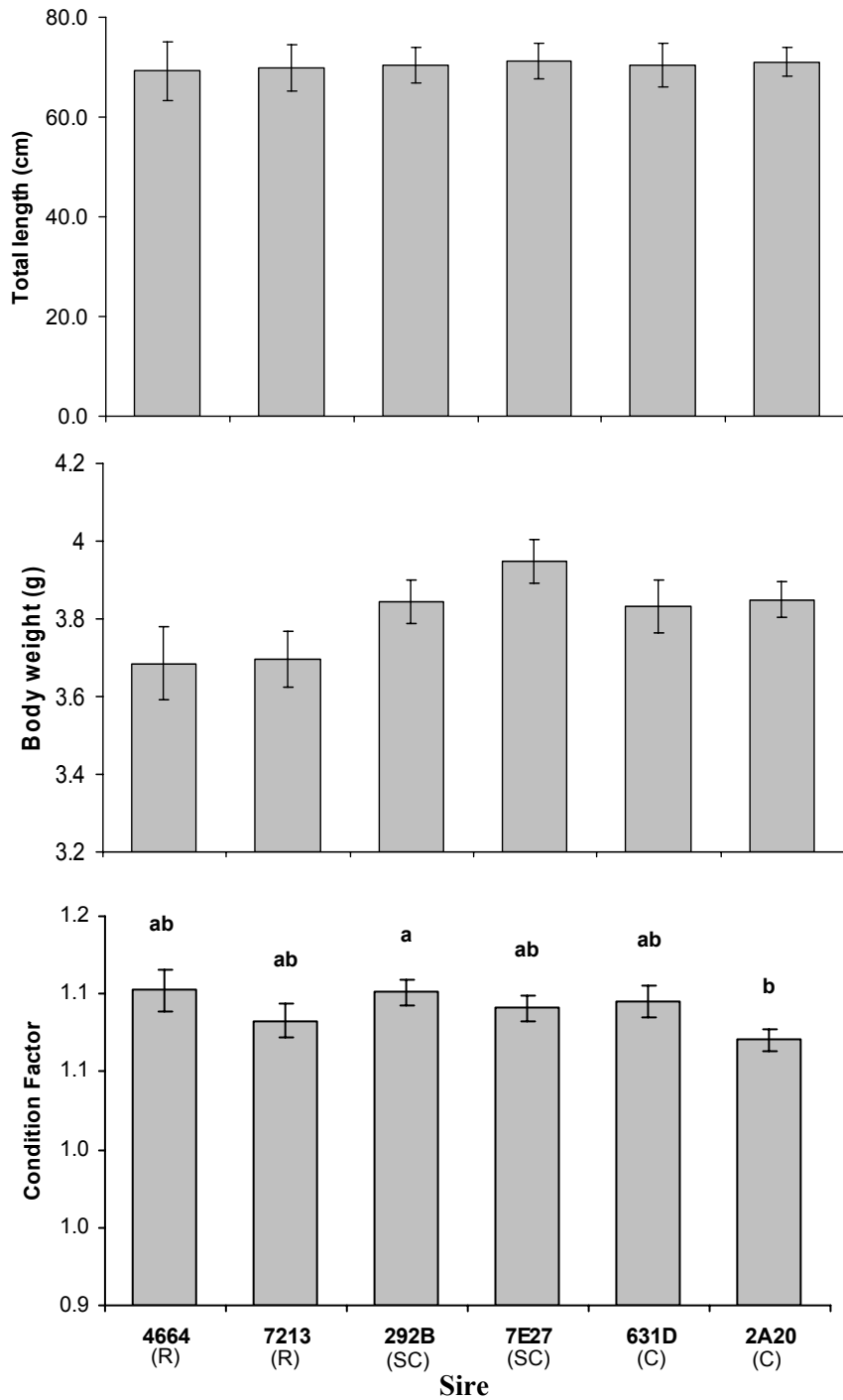
Figure 16. Least squares means (±SE) by sire family for 2E55 Phase II initial pond sample fingerling traits, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). There were no pairwise differences for total length and no overall difference for body weight. Means with shared letters over the error bars are not significantly different.
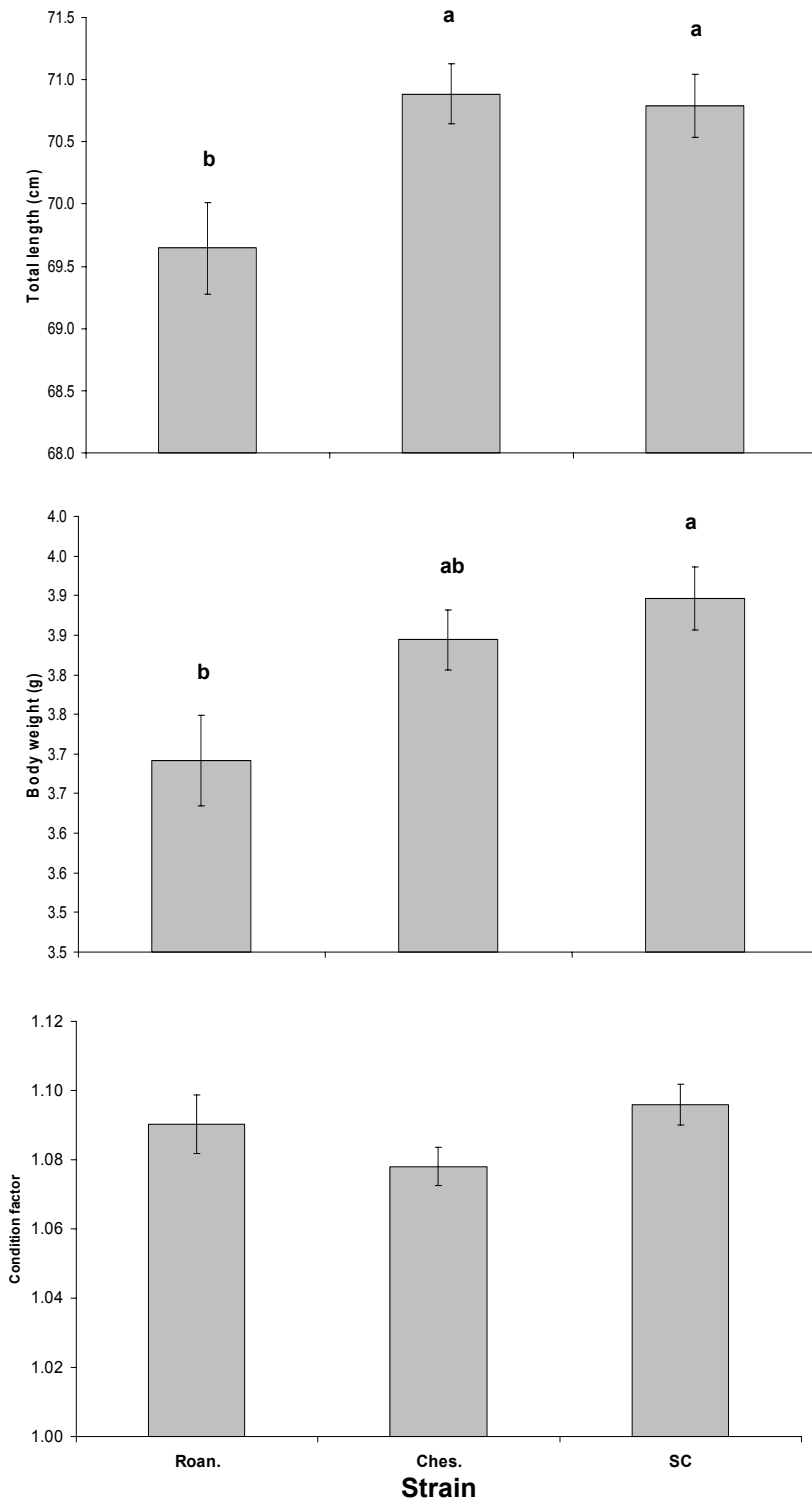
Figure 17. Least squares means (±SE) by strain for 2E55 Phase II initial pond sample fingerling traits, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan.), Chesapeake by (Ches.) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different. No effect of strain was present for condition factor.
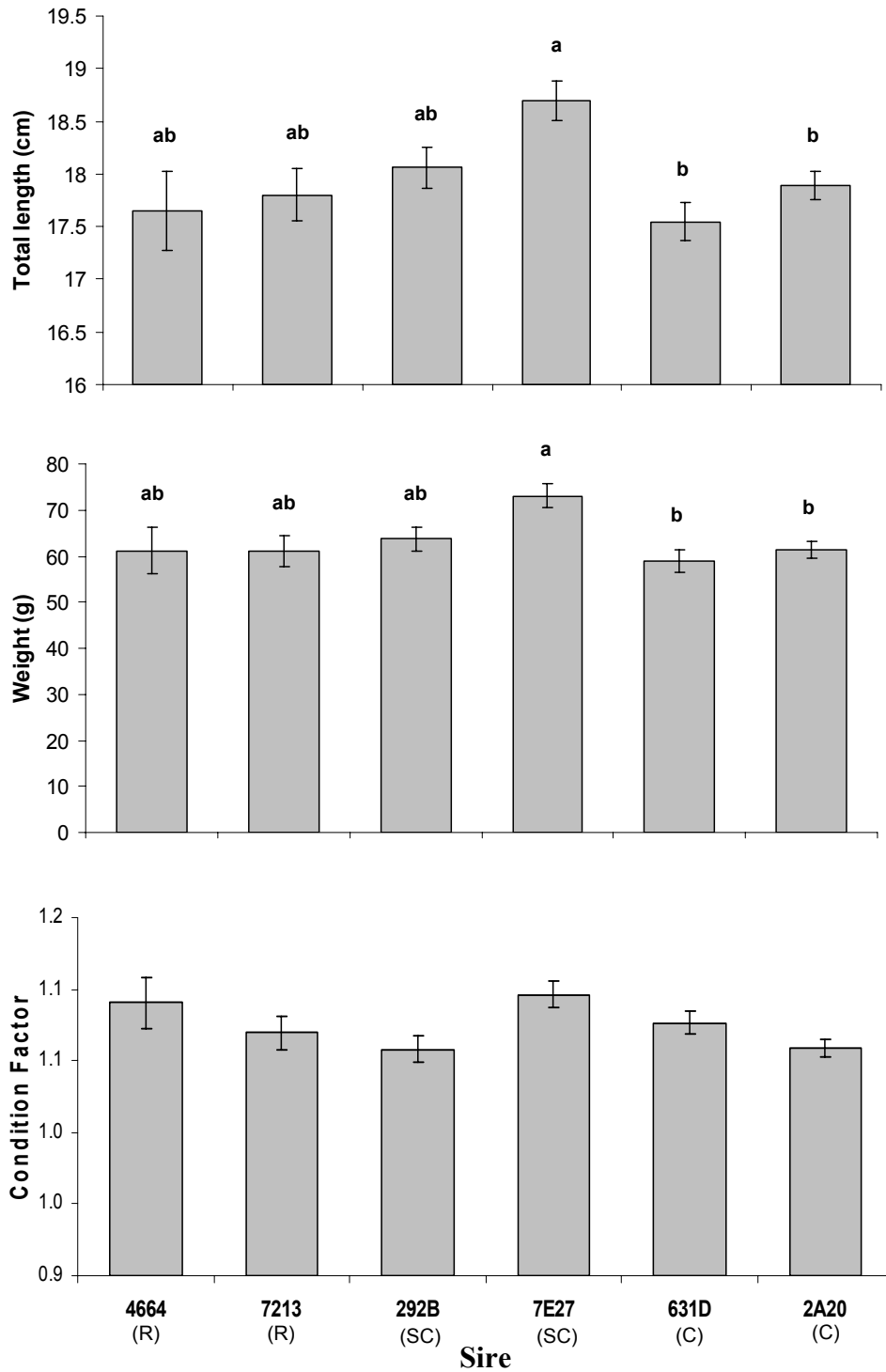
Figure 18. Least squares means (±SE) by sire family for 2E55 Phase II pond fingerling traits, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different. There was no effect of sire for condition factor.

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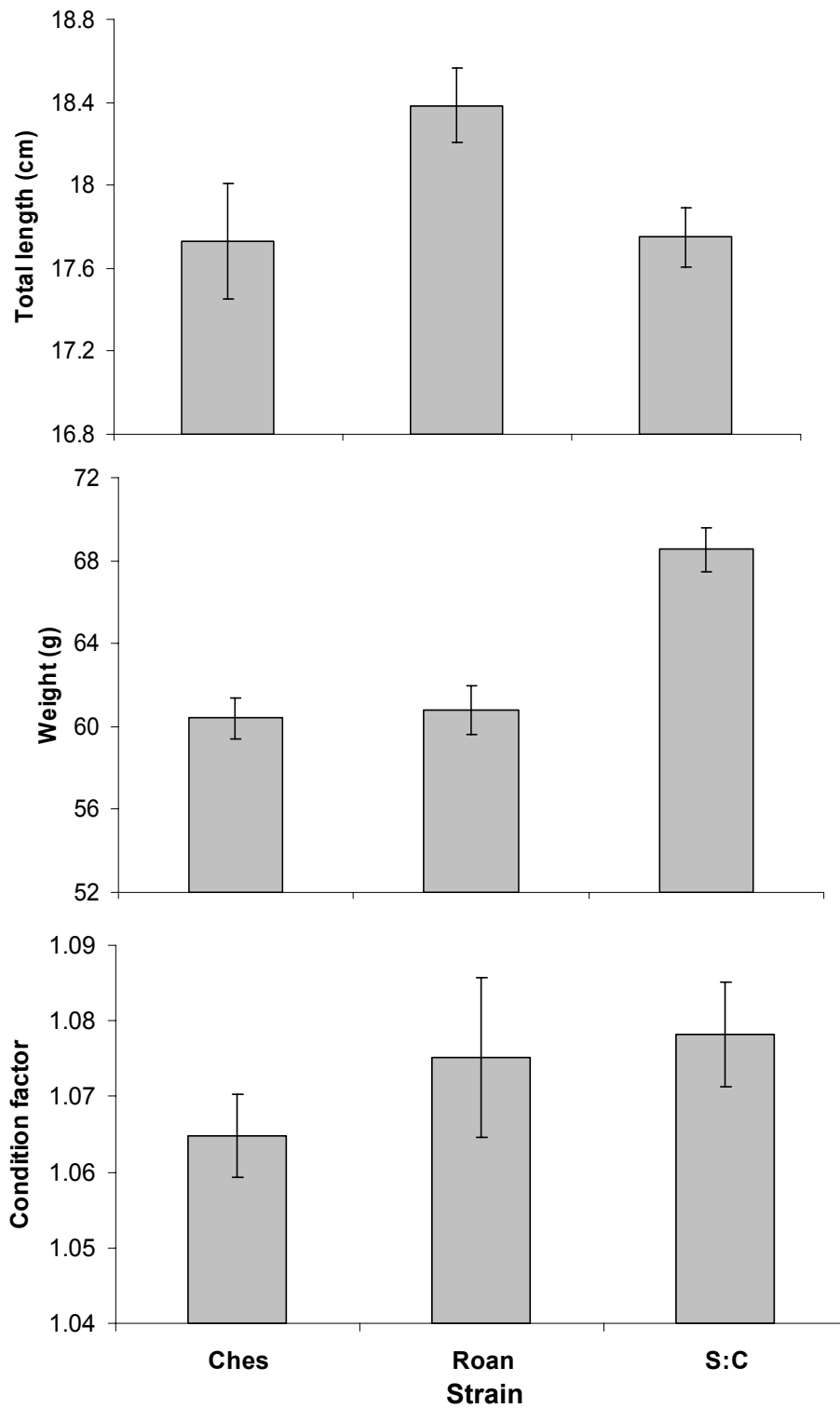
Figure 19. Least squares means (±SE) by strain for 2E55 Phase II final pond sample fingerling traits, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (S:C). The effect of strain was not significant for any trait.
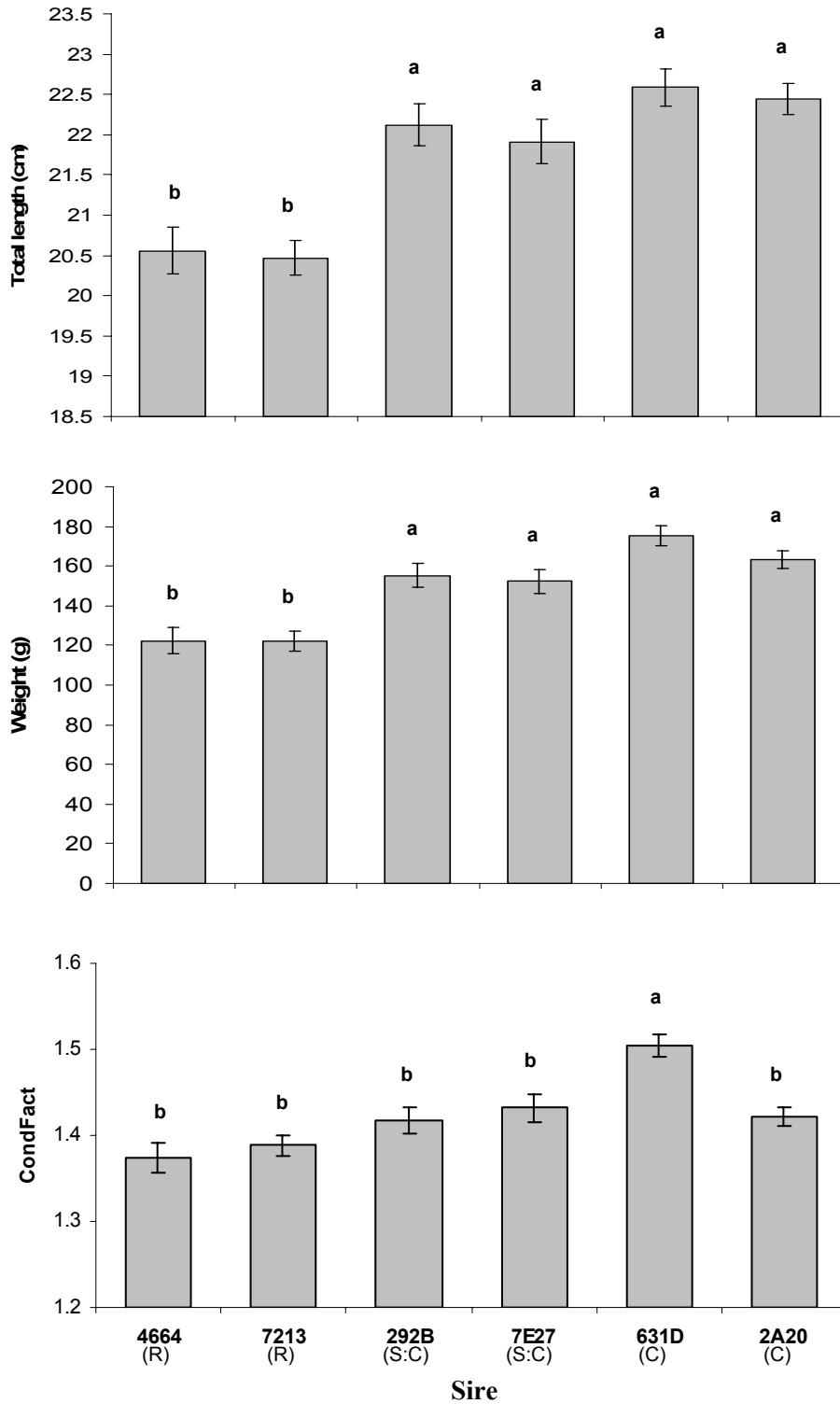
Figure 20. Least squares means (±SE) by sire family for 2E55 Phase II commercial tank fingerling traits, including body weight, total length, and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (S:C). Means with shared letters over the error bars are not significantly different.
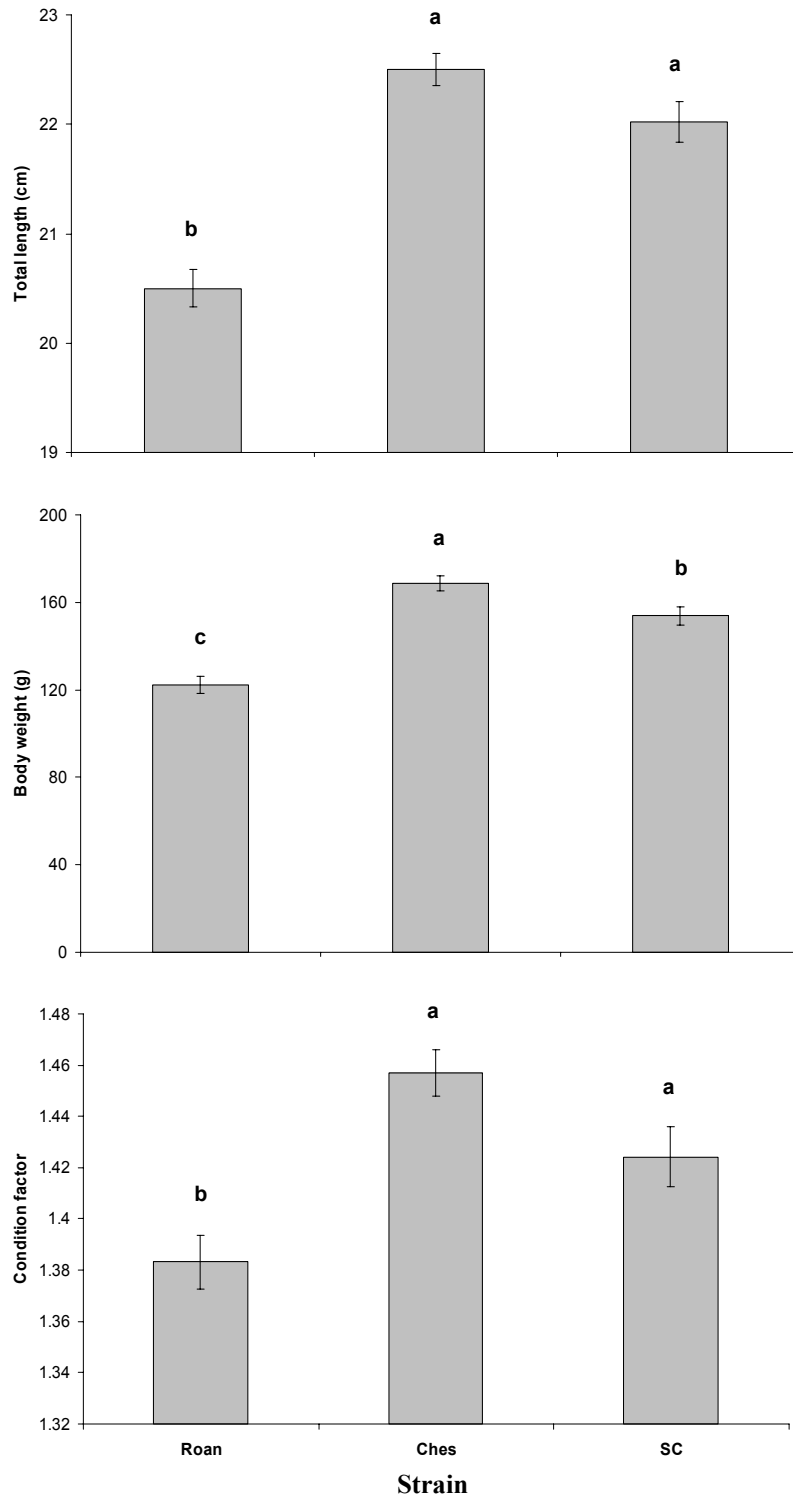
Figure 21. Least squares means (±SE) by strain for 2E55 Phase II final commercial tank sample fingerling traits, including body weight, total length, and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.

CHAPTER 5

PHASE III PERFORMANCE EVALUATIONS OF COMMUNALLY REARED STRIPED BASS AT

RESEARCH AND COMMERCIAL SCALE

ABSTRACT

Sustainable aquaculture production of hybrid striped bass requires domestication and selective breeding to eliminate dependence on wild broodstock and to maximize production efficiency. Currently, resource limitations prohibit the individual rearing of multiple larval families for performance testing, necessitating a breeding program based on communal rearing of progeny groups using high-resolution molecular markers as innate genetic tags for progeny identification. To evaluate the genetic basis of several commercially important performance characteristics for Phase III, or market-size, striped bass, performance traits were examined for eighteen half-sibling families reared in research ponds. Six of the families also were reared in a commercial tank production system. Six microsatellite markers were utilized as innate genetic tags for parentage identification and 99.7% of offspring could be unambiguously attributed to specific parental pairs. Mean growth performance and carcass characteristics differed significantly by genetic background in each environment and variation in measures of antimicrobial peptide activity, a potential measure of innate disease resistance, differed by strain within the research ponds. In general, progeny of domesticated Santee:Chesapeake sires out-performed those of other strains both at research-scale and in the commercial tank. In research ponds, performance of fish as yearlings (Phase II) allowed prediction of performance at Phase III (18-20 months of age). Application of large-scale communal rearing trials based on microsatellite markers for progeny identification should be a viable approach in a selective breeding program for striped bass. Results from performance evaluations provide fundamental information needed to accelerate selective breeding and to increase production efficiency for the hybrid striped bass industry.

The hybrid striped bass (striped bass, *Morone saxatilis* x white bass, *Morone chrysops*, and reciprocal cross) has been cultivated as a gamefish in the United States since 1965 (Bishop 1968). Severe declines in wild striped bass populations in the 1970s and resultant harvest restrictions provided the stimulus for commercial aquaculture production of the hybrid striped bass. Hybrid striped bass initially were produced in earthen ponds but have since been successfully reared as foodfish in intensive tank and raceway systems, as well as in both freshwater and marine cages. At present, production of hybrid striped bass is the fourth most valuable form of finfish aquaculture in the United States (Pritchard 2005). However, the hybrid striped bass industry remains largely dependent on wild striped bass and white bass broodfish for annual fingerling production, resulting in production inefficiencies that limit expansion of the industry. Growers and researchers recognize that continued reliance on wild broodfish cannot support industry growth or increased production efficiency (Woods 2001). Instead, expansion of hybrid striped bass farming will require full domestication and genetic improvement of these fishes. Accordingly, industry producers and university and federal researchers initiated a National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry (hereafter referred to as the National Breeding Program) in 2002. Captive striped bass broodstocks are currently under domestication at NCSU and the University of Maryland and some domesticated fish are being held on several commercial farms.

It is well recognized by the hybrid striped bass industry that domestication and genetic improvement of the parental species of the hybrid is necessary for realizing sustainable production and promoting industry expansion. Economists at Kent SeaTech

Corporation predict that production costs for hybrids could be reduced by approximately 12% if selective breeding can yield just 20% faster growth rates (J. Carlberg, personal communication). Gains in growth rates of this magnitude have been achieved through only 1-2 generations of selective breeding of other aquaculture species, including salmon (Gjedrem 1979, Hershberger et al. 1990), trout (Kincaid et al. 1977), and catfish (Dunham 1987). Most finfish for which selection trials have been made appear to respond with gains of 10-20% per generation (Refstie et al. 1997), as do shellfish (Calvo et al. 2003). Knibb (2000) estimates that even modest genetic gains of 10% by breeding could double profits for marine aquaculture companies, and these genetic gains will compound for each generation of selection. For Atlantic salmon, a selective breeding program produces a cost benefit ratio of 1:15 (Gjedrem 1997). Because the hybrid striped bass industry mainly utilizes wild broodfish for spawning, there is much untapped genetic potential for development of a superior cultivar and the potential economic returns from domestication and selective breeding could be equally dramatic.

Direct perpetuation of favorable traits in hybrid striped bass lines is not possible, despite the fertility of the hybrid, since the F_2 offspring exhibit lower hatchability, decreased larval viability and decreased growth (Smith and Jenkins 1984) compared to the F_1 hybrids. Because the hybrid striped bass industry relies on the striped bass as the male parent for the production of hybrids, selective improvement will be largely focused on striped bass. These efforts should result in production of improved hybrid striped bass and will permit dissemination of cryopreserved gametes from improved male lineages. Although methods for cryopreservation of eggs or embryos have not been achieved for striped bass, techniques for both short term storage and long term cryopreservation of striped bass semen have been

developed (Kerby et al. 1985; Jenkins-Keeran et al. 2001; Jenkins-Keeran and Woods, 2002a,b; He and Woods 2003; Thirumala et al. 2006). The USDA National Animal Germplasm Program has committed to archiving striped bass samples for conservation of valuable germplasm resources. Distribution of striped bass semen from NCSU broodstock already has been demonstrated for fresh, extended striped bass semen (C.V. Sullivan and A.S. McGinty, *unpublished data*) as well as for cryopreserved semen (C.V. Sullivan and L.C. Woods III, *unpublished data*). In addition to benefits related to the ease of preserving and distributing male gametes from improved striped bass lines, striped bass males become reproductively mature at an earlier age than do females (2-3 years rather than 4-7 years), and this shorter generation time should prove beneficial in a breeding program.

The commercial production of hybrid striped bass begins with acquisition of broodstock for production of hybrid larvae. Gravid female white bass are induced to ovulate with injections of human chorionic gonadotropin (hCG; Kohler et al. 1997) and eggs are manually stripped and fertilized in vitro with milt from striped bass males. The majority of fry produced by the hybrid striped bass industry are stocked into outdoor earthen ponds which have been fertilized and prepared in order to stimulate production of zooplankton that serve as forage for the young fish (Geiger 1985). Fingerlings are harvested at approximately one gram or more in body weight, trained to accept artificial feeds, and then sold to producers for growout in outdoor ponds or commercial tank or raceway systems for Phase II production, during which time they grow to 90-225 g body weight. Fish are harvested at the end of Phase II at six to twelve months of age and are reduced in density for final growout. Small fish are culled and then the Phase II fingerlings are stocked into ponds at a rate of 7,500 to 10,000 fish per hectare and reared until they reach market size (568-618 grams) at

approximately 18 months of age (Hodson 1995). Approximately 66% of hybrid striped bass foodfish production is carried out in outdoor ponds, 32% occurs in tank systems and the remainder of production is in cages (J. Carlberg, *personal communication*).

Evaluation of specific phenotypic measures at the end of Phase III is critical since this is the time when fish are sold to market. Growth performance and feed conversion efficiency (unit gain in fish body mass /unit feed consumed) were identified as traits of great importance to producers in surveys of hybrid striped bass growers conducted in the late 1990's (Harrell and Webster 1997) and during the 2002 inaugural meeting of the National Breeding Program. Heritability estimates for growth rate on the order of 0.2-0.5 have been reported for salmonids (Iwamoto et al. 1982; Gjedrem 2000), catfish (Dunham 1987) and European sea bass (Saillant et al. 2006) and lines of catfish selected for increased body weight have shown increased feed conversion efficiencies (Dunham and Smitherman 1983). A strong relationship between growth performance and feed conversion efficiency also has been reported for salmon (Thodeson et al. 2001) and rainbow trout (Henryon et al. 2002). These results suggest that selection for improved growth performance may result in better feed conversion efficiencies for striped bass. Gains in growth rate from selective breeding have been 10% per generation in rainbow trout (Kincaid 1977; Kincaid 1983) and coho salmon (Hershberger et al. 1990), 10-30% per generation in Atlantic salmon (Gjedrem 1979) and 12-18% in catfish (Dunham 1987), illustrating the tremendous potential for rapid improvement that can be made through selection programs. Selective breeding of highly variable and unselected lines of striped bass is expected to yield similar heritable gains.

Other traits determined by market preferences also are important for Phase III performance assessments. Currently, ~83% of farmed hybrid striped bass are sent to market

alive and ~17% are sold whole on ice (J. Carlberg, *personal communication*). Although

hybrid striped bass are not usually sold wholesale as fillets, high meat yield and better

allocation of energy to muscle production as opposed to gonadal growth or fat deposition

remain important traits for marketing these fish in gourmet restaurant and live fish markets.

Sexual dimorphism in growth also may be important as some evidence suggests that female

body weight exceeds male body weight at harvest in both striped bass and hybrid striped bass

(Harrell 1997; Woods et al. 1999; S.J. Mitchell, *personal communication*). Additional market

traits of interest include external morphological characters such as body shape and the

incidence of physical deformities. Heritability for body shape (body depth) was 0.15-0.49 in

carp (Ankorion 1966) and this trait has proven amenable to selection in carp. Because most

hybrid striped bass are sold whole and unprocessed, body shape may be an important trait for

selective improvement.

Due to the crowded and stressful conditions of commercial fish culture, stress

tolerance and disease resistance also are critical traits for improvement. One of the greatest

challenges to the hybrid striped bass industry, especially in intensive tank production, is the

control of disease (Plumb 1997; J. Carlberg, *personal communication*). Genetic variation in

disease resistance has been demonstrated for both salmon (Gjedrem et al. 1995) and for

rainbow trout (Dorson et al. 1995; Henryon et al. 2002). Similar variation in striped bass

could prove very valuable for a striped bass breeding program. One possible measure of

disease resistance and associated stress tolerance is measurement of antimicrobial peptide

activity (Robinette et al. 1998). Antimicrobial substances present on the mucosal surfaces of

fish are an important component of the innate immune system of fishes and function as a

non-specific first line of defense against microbial invasion (Robinette et al. 1998; Ellis

2001). Many such antimicrobial substances have been identified in fishes, including histone-like proteins that function as broad-spectrum antibiotics and that augment the action of other defense molecules (Patrzykat et al. 2001). Channel catfish skin contains at least three such proteins (Robinette et al. 1998) and histone-like antimicrobial peptides have been found in the skin, gills and spleen of rainbow trout (Noga et al. 2001; Fernandes et al. 2002), coho salmon (Patrzykat et al. 2001), halibut (Birkemo et al. 2003), cod (Bergsson et al. 2005) and hybrid striped bass (Noga et al. 2001). In both trout and hybrid striped bass, these peptides are lethal to the ectoparasitic protozoan *Amyloodinium ocellatum*, an important parasite in warm water aquaculture (Noga et al. 2001). The histone-like proteins of catfish also are toxic to bacteria and water molds (Robinette et al. 2001) and those of trout (Fernandes et al. 2002) and cod have antibacterial properties (Bergsson et al 2005). Patrzykat et al. (2001) reported that histone-like antimicrobial peptides in coho salmon mucus and blood confer protection against two common fish pathogens, *Vibrio anguillarum* and *Aeromonas salmonicida*. Genetic variation in antimicrobial activity has been demonstrated for lysozyme, another broad-spectrum antimicrobial peptide, in rainbow trout and Atlantic salmon (Grinde et al. 1988; Balfry et al. 1997; Fevolden et al. 2002). Because improvement of disease resistance has been identified as a high priority for the hybrid striped bass industry (Garber and Sullivan 2006; National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry, 2002 meeting minutes), evaluation of variation in disease resistance or stress tolerance will be an important goal of the National Breeding Program. Use of an easily measured, non-destructive method for evaluating disease resistance and stress tolerance, such as measurement of antimicrobial peptide activity, may provide a good alternative to use of challenge tests or other means which result in mortality or infection of potential broodstock.

Genetic variation in phenotypic traits is necessary for the selective improvement of economically important aspects of fish performance. Unusually low genetic variation has been reported to characterize the striped bass (Grove et al. 1976; Sidell et al. 1980; Waldman et al. 1988), and both striped bass and white bass exhibit extraordinarily limited genetic diversity as compared to other marine and anadromous fishes (DeWoody and Avise 2000). However, significant genetic differentiation has been detected among geographic strains of striped bass (Wirgin et al. 1991; Wirgin and Maceda 1991; Diaz et al. 1997; Wirgin et al. 1997a,b; Roy et al. 2000) by the use of molecular markers, indicating that adequate genetic variation may be present for selective improvement. Additionally, research has revealed performance differences among geographic stocks and families of captive (Woods et al. 1999, Jacobs et al. 1999; Woods 2001), and wild (Conover et al. 1997; Brown et al. 1998; Secor et al. 2000) striped bass and wild white bass (Kohler et al. 2001). Variation in growth rate, feed conversion efficiency, tolerance of culture conditions, and disease resistance in the parent species suggests that these fish will respond strongly to selection (Garber and Sullivan 2006). The largest and most diverse repository of captive striped bass is located at the NCSU Pamlico Aquaculture Field Laboratory (PAFL) in Aurora, North Carolina. Identification of genetic and phenotypic variation within captive striped bass broodstock is a key goal of the National Breeding Program.

Performance evaluations of captive striped bass will allow investigation of the genetic underpinnings of variation in commercially important phenotypic traits, a critical first step in developing a long-term selective breeding program to genetically improve and further domesticate *Morone* species. Because maintenance of multiple families in separate tanks or ponds would require far greater resources than are currently available to the National

Breeding Program, the use of communal rearing techniques offers promise for selective breeding of striped bass. Using these techniques, multiple families can be stocked into the same pond or tank environment and reared under identical environmental conditions. Communal rearing techniques can thereby reduce the number of rearing units necessary for production of many families and increase the number of families or groups that can be compared (Moav and Wohlfarth 1974; McGinty 1987). Additionally, by rearing all families in the same environment, the environmental component of phenotypic variation among families can be removed, unmasking genetic contributions to growth and other commercially important performance traits with greatly reduced tank or pond effects. Communal stocking of common carp in ponds (Moav and Wohlfarth 1974) and cages (Wohlfarth and Moav 1991) has proven that mixed, as compared to separate, rearing is a valuable and efficient method for performance testing of numerous family groups of fish and this principle also has been demonstrated in catfish (Dunham et al. 1982) and tilapia (McGinty 1983, 1987).

For striped bass, which must be stocked into outdoor ponds as larvae when they are far too small for physical tagging, the use of highly polymorphic genetic markers will be necessary for parentage identification during communal performance evaluations and for pedigree tracking in a program of selective breeding. The proven utility of microsatellite markers makes them appealing as DNA markers for striped bass. Microsatellites are often extremely polymorphic and have proven effective for analysis of kinship and parentage (Herbinger et al. 1995; Garcia de Leon et al. 1998; O'Reilly et al. 1998; DeWoody et al. 1998) in both wild and captive fishes. Microsatellite markers developed for striped bass have been shown to be polymorphic in white bass and hybrid striped bass as well (Couch et al. 2006). The usefulness of microsatellites for progeny identification in striped bass was

demonstrated in Chapters 3 and 4, and these markers should be useful not only for individual identification during striped bass performance evaluations but also for subsequent pedigree tracking in a selective breeding program for *Morone* species. Use of microsatellite marker-assisted communal rearing techniques for striped bass is expected to permit meaningful comparisons of performance among fish from numerous families during all phases of production. Evaluation of communal rearing techniques and data from family performance comparisons conducted during Phase III rearing should provide valuable information for design of a selective breeding program for striped bass.

Bosworth and colleagues reported that body weight at 40 days is a good predictor of weight at later life stages (Bosworth et al 1997) and Saillant et al. (2006) have observed a strong correlation of body weight at 341 days post fertilization to body weight at 818 days post fertilization in the European sea bass. In carp, weight gain is positively correlated with initial weight, so common carp with higher initial weights grow faster than those stocked at lower weights in both earthen ponds and in cages (Wohlfarth and Moav 1993; Moav and Wohlfarth 1985). Assessing performance of market-sized fish at Phase III will enable testing of the predictive value of earlier performance, (e.g., Phase II, Chapter 4) on trait values at final harvest. The ability to predict the performance of fish at the end of Phase III (18-20 months of age) from performance at the end of Phase II (7-12 months of age) would permit breeders to select broodstock whose offspring perform well in Phase II for production of fish with superior phenotypes for market. This development could greatly reduce the expense of a selective breeding program for striped bass by eliminating the final six months of growout in performance trials, resulting in savings for labor and feed, freeing pond or tank rearing units

for other evaluations, and accelerating the pace of selective improvement for the breeding program.

In the present study, a microsatellite marker-assisted communal rearing approach was utilized to examine paternal effects on striped bass progeny growth during the final stage, Phase III, of the hybrid striped bass commercial production cycle. Fish were reared extensively in research ponds and intensively in a commercial hybrid striped bass production tank. The primary goals of this study were to confirm the utility of microsatellite genotyping for progeny identification in communally reared striped bass at harvest and to investigate sire-based genetic variation in commercially important phenotypic traits within a captive striped bass broodstock population. The hypotheses tested included:

1) There are significant differences in performance during production in Phase III among paternal striped bass families;

2) Performance at the end of Phase II production (approximately one year of age) can predict performance at the end of Phase III production;

3) Performance in research ponds can predict performance in commercial tank culture.

METHODS

Broodstock Genotyping—Prior to production of experimental families, PAFL striped bass broodstock ($N=120$) were genotyped at three polymorphic microsatellite primer loci (see Methods, Chapter 2) in order to evaluate the genetic variability of the broodstock strains. Broodstock were highly variable at all the three loci, in most cases facilitating the use of

males with unique alleles for at least one microsatellite locus so that parentage of communally reared progeny could be unambiguously determined.

Production of Fingerlings for Phase III Performance Evaluations—Experimental crosses among striped bass broodstock were conducted in April 2001 to produce even-aged half-sibling striped bass families for evaluation of survival and performance. Crosses were made using captive wild and domesticated broodstock held at the NCSU Pamlico Aquaculture Field Laboratory (PAFL) in Aurora, NC as described in Chapter 3, Methods. Broodstock used to produce the experimental families included striped bass of wild Roanoke River-Albemarle Sound origin (R:F_0-1997; captured wild as adults in 1997), F_2-generation Chesapeake Bay (C:F_2-1991) striped bass, and F_1-generation Santee x F_2-generation Chesapeake fish (Table 1). Dams were crossed with six sires, two each from the three broodstock strains, and fry were reared by dam through Phase I in 0.1ha earthen research ponds at PAFL or in a 1.2ha commercial pond at Keo Fish Farms (Keo, Arkansas). Phase I fingerlings were harvested, graded by size, and trained on prepared feed. Fingerlings from the Keo Fish Farms commercial production pond (from dam 2E55) and those produced from dams 152D and 5F4B reared at PAFL survived in adequate numbers for Phase II performance evaluations.

For Phase II production, fingerlings from Keo Fish Farms were delivered to PAFL and stocked into triplicate 0.1 ha research ponds. Fish were harvested from ponds at approximately one year of age and sampled for genetic and phenotypic analysis as described in Chapter 4. Fingerlings produced from dam 2E55 also were reared in a commercial tank at Kent SeaTech Corporation's (Mecca, CA) intensive rearing facility. Fingerlings were sampled from the tank at approximately seven months of age in order that fish body weights

would roughly correspond to those expected after Phase II rearing in ponds. Fingerlings from two other dams (5F4B and 152D) reared in research ponds during Phase I were pooled and reared in duplicate ponds as a 12-family group during Phase II production. Fingerlings from one of these ponds were retained for subsequent Phase III trials.

Phase III Rearing Trials—

Research ponds—Phase II fingerlings produced from the 2E55 dam and recovered from the PAFL research ponds were re-stocked shortly after Phase II sampling at a rate of 10,000 fish/ha (1,000 fish per pond) into triplicate research ponds (Table 17). Average weights of the fingerlings were 59.3g (pond A4), 60.3g (pond A11) and 73.1g (pond A14). Fingerlings from the 12-family group were stocked into duplicate research ponds (ponds A1 and A2) at a rate of 8,700/ha (870 fish per pond) and at an average body weight of 131.4g. Fish were fed at a rate of ~1-3% body weight per day. Ponds were sampled monthly in order to adjust feeding rates according to average fish body weights and water temperatures. Water quality parameters were managed using standard hybrid striped bass pond management practices (Bonn et al. 1976; Geiger and Turner 1990; Hodson 1995) and aquatic vegetation was controlled by application of Karmex ® DL (diuron, E.I. DuPont Canada Company, Missasaugua, Ontario) at 3,000g/ha or by hand removal of weeds.

The Phase III fish were fasted for 24 hours then harvested from the ponds by seining during mid-November 2002 to early January 2003 when they were 19-20 months of age. Ponds were drained to recover all remaining fish and all fish were individually counted to determine percent survival for each pond. Fish were transferred from the pond to an outdoor flow-through tank (7.3 m diameter x 0.92 m deep) for short-term containment prior to sampling.

Commercial tank—Fish produced from dam 2E55 family that were stocked into the commercial production tank for Phase II rearing were maintained in the same tank for Phase III performance evaluations. Feeding rates were approximately 1-3% body weight/day and the tank was sampled monthly to adjust feeding rates according to the body weight of the fish. Water quality was managed using Kent SeaTech's proprietary intensive culture practices. Fish were fasted for 24 hours then harvested in October 2002 at approximately 18 months of age by drawing down the water level in the tank and then collecting the fish with trammel nets. Fish were transferred to smaller nursery tanks for short-term holding prior to sampling.

Phenotypic trait evaluations—

Growth, shape and energy allocation—Phase III fish were evaluated for several growth-related performance traits, including total length (cm) and body weight (g). Total lengths were measured to the nearest 0.1cm and weight was measured to the nearest 0.1g. Condition factor (Fulton-type condition factor [(weight/length^3) x 100]), a measure of body shape, was calculated from the measures of length and weight. A total of 600 progeny of dam 2E55 were sampled from each of the thre ponds at PAFL and 500 of the fish produced from dam 2E55 dam were sampled from the commercial production tank at Kent SeaTech Corporation. A total of 401 fish from the 12-family group at PAFL were measured for these same traits. Fillet weights also were collected for 373 fish in the commercial tank by a professional fish filleter hired from a Los Angeles seafood processing company. Both left and right fillet weights were collected and percentage fillet weight was calculated for each fish [(total fillet weight/body weight) x 100] to the nearest 0.1g. For family 2E55, four hundred fish per pond at PAFL and 500 fish from the commercial tank at Kent SeaTech were

dissected for measures of visceral weight (included stomach, intestines, liver, spleen, gall bladder and mesenteric fat) and gonad weight in order to calculate viscerosomatic and gonadosomatic indices [VSI, (visceral weight/body weight) x 100; GSI, (gonad weight/body weight) x 100]. Viscera and gonad weights were measured to the nearest 0.01g. Because VSI and GSI percentage data were less than 20%, values were square root transformed prior to analysis to normally distribute the data (Steel and Torrie 1980). All fish were finclipped and fin tissue samples were individually preserved in 70% ethanol for later genetic analysis. External deformities, including abnormalities of the eyes, head (jaw and operculum deformities) and spine, were recorded for all sampled fish.

The gender of each fish that was dissected was determined by visual examination of the gonads. In cases where fish were immature and gender could not be determined, bilateral samples of gonad tissue were collected and preserved in clearing fixative (ethanol:formalin:acetic acid; 6:3:1 v/v) and in 4F:1G fixative (4% paraformaldehyde, 1% glutaraldehyde in phosphate buffer; MacDowell and Trump 1976) for subsequent microscopic or histological identification. Samples in clearing fixative were examined at 100X magnification using a dissecting stereomicroscope fitted with a calibrated ocular micrometer. A droplet of glycerol was added to the sample to fully clear the tissue in order to better reveal cellular features. Gender was identified by the presence or absence of primary growth oocytes, which were identified based on their large size (diameter). Prior to gender differentiation, tissue from testes and ovary appear indistinguishable at 100X magnification; however, in differentiated fish, no cells larger than 15 μm diameter are visible in male samples while ovarian tissue can contain cells (oocytes) from 50 μm to more than 800 μm in diameter (Jackson and Sullivan 1995). Samples preserved in 4F:1G fixative were embedded

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in paraffin, sectioned using a microtome at 5µm, and stained with toluidene blue. Histology was performed at the NCSU College of Veterinary Medicine in the Laboratory for Advanced Electron and Light Optical Methods. Histological slides were examined under a light microscope for gender identification.

Antimicrobial peptide activity—Gill tissue samples were collected from progeny of the 2E55 dam from two PAFL ponds (60 fish per pond) and from the commercial tank (120 fish) for analysis of antimicrobial peptide activity using a radial immunodiffusion assay (Hultmark et al. 1982; Robinette and Noga 2001) of gill extracts. To minimize the effects of stress on the outcome of the antimicrobial peptide assay, all samples were collected within 1 hour of crowding in the seine or of removal from the tank (E.M. Noga, *personal communication*). Fish were netted from the pond seine or tank and immediately anesthetized in an oxygenated ice slurry. Approximately 50µl of clean gill filament tissue was collected from each fish. The gill sample was blotted to remove excess water or blood and placed into 150µl of sterile 1% acetic acid solution in a 1.7ml microcentrifuge tube. Samples were immediately boiled in a water bath for 5 minutes then frozen on dry ice or liquid nitrogen for transport to the laboratory. Boiled tissue samples were homogenized on ice with a microtube tissue pestle for 3-4 minutes or until tube contents were homogeneous. Following centrifugation at 13,000 x g for 10 minutes at 4 °C, the supernatant extract was removed and placed into a sterile microcentrifuge tube for storage at -80 °C. Pelleted gill tissue samples were retained at -80 °C in the event re-extraction was necessary.

In preparation for radial immunodiffusion assays, *Escherichia coli* (D31 stock) were grown overnight in tripticase soy broth with 0.5% NaCl to an optical density at 570 nm of 0.098-0.12 as measured on a UV-Vis 120 spectrophotometer (Shimadzu Scientific

Instruments, Columbia, MD). One milliliter of streptomycin sulfate (10 mg/ml; final concentration 0.1 mg/ml; Sigma-Aldrich Co., St. Louis, MO) and *E. coli* (10% of final volume) were added to 55 °C Luria-Bertani agar then plated onto sterile gridded (10x10) petri plates. Plates were allowed to dry for approximately 10 minutes at room temperature and were then stored at 4 °C until use later the same day. Each petri plate was warmed to room temperature for 5 minutes and then a vacuum-driven gel immunodiffusion punch was used to create 36 2.5mm sample wells in the hardened agar. Gill extracts from each striped bass were loaded into the wells in duplicate (3µl per well). Histone 2B-like protein (H2B) from calf thymus (Boehringer-Mannheim, Indianapolis, IN) was serially diluted (1X to 32X) in 0.01% acetic acid and loaded into duplicate wells on each plate as a positive control. Two wells per plate were loaded with 0.01% acetic acid as a negative control. Plates were incubated for 18 hours at 37 °C and the antimicrobial activity of each sample was evaluated by measuring the diameter of cleared zones to 0.1mm with electronic digital calipers (three measures per sample replicate). Replicates within 0.2 mm average diameter of one another were averaged to create a single measure of the diameter of the cleared zone for each fish. Sample replicates not within 0.2 mm of one another were re-assayed. A standard curve was created for each plate from measurements of H2B samples and the data were log-transformed before analysis to create linear standard curves. The undiluted H2B standard was arbitrarily assigned an activity of 100 units and measures of clearing zone diameters for each fish sample were converted to units of antimicrobial peptide activity by reference to the standard curve. Antimicrobial peptide activity data were square root transformed to produce normal distributions (Zar 1984).

DNA Extraction—DNA was isolated from approximately 4 mg ethanol-preserved fin tissue using the PUREGENE® DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN). Samples were blotted dry and extractions were performed according to the manufacturer's directions with omission of the RNase A step. DNA was rehydrated in 0.1X TE buffer and stored at -20 °C. Genomic DNA was extracted from broodstock blood samples (see Methods, Chapter 2) using a phenol: chloroform extraction procedure modified from Saghai-Maroof et al. (1984). Broodstock DNA was solubilized in 100µl 1X TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) buffer and diluted 1:20 in sterile water before use.

Microsatellite Genotyping—Six microsatellite markers were used for parentage assignment in striped bass and are listed in Table 9. These markers were selected in order to maximize the success of parentage assignment for communally reared striped bass. The markers were optimized for multiplex PCR so that only two reactions were necessary to amplify all six loci. Primers were labeled with fluorescent tags so that all loci could be pooled for fluorescent genotyping. PCR amplification of microsatellite loci was carried out in 12µl reactions in 96-well PCR plates (see Chapter 3, Methods). Sterile deionized water was used as a negative control for each 96-well plate of PCR to ensure that reagents were not contaminated and a positive control, consisting of a broodstock DNA sample of known quality, was used on each plate to verify offspring DNA quality and concentration. An internal fluorescent size standard, GeneScan™ -500 LIZ (0.5µl; Applied Biosystems), was added to each 0.6µl sample of clean PCR product for accurate and consistent scoring of alleles. Samples were chemically denatured by addition of 9.0µl Hi-Di Formamide (Applied Biosystems) and heat denatured at 95 °C for 5 minutes. Electrophoresis data were collected and allele sizes were determined using ABI PRISM Genemapper version 3.0 software.

Statistical analyses—

Assignment of parentage—Parentage assignment of offspring was based on genotypes for a minimum of five microsatellite loci. Individual samples that failed to amplify were re-extracted and re-genotyped; those for which genotypes could not be determined were removed from subsequent analysis. Parentage of progeny was assigned using probability tests in PROBMAX2 version 1.2 software (Danzmann 1997). The genotypes of any unassigned progeny (probability <1.0 of belonging to a single parental pair) were evaluated for allele calling or data entry errors then re-genotyped if necessary. Progeny for which unambiguous parentage assignment could not be made (e.g., progeny assigned to more than one sire) were excluded from analyses of sire effects.

Proportional contribution and survival by sire and strain—Proportional contribution of each sire and strain to the final Phase III populations were estimated from genotyped proportions of the samples. Heterogeneity Chi-square analysis (Zar 1984) was used to test the null hypothesis that the three 2E55 pond samples from the end of Phase III came from the same population. Chi-square goodness of fit tests (Zar 1984) were used to test the null hypothesis that a sample contained equal proportions of progeny from all sires or strains. The proportion used to calculate expected frequencies for sire from the total sampled was 0.1667 (1/6) and the proportion used for strain was 0.3333 (1/3). Where the null hypothesis was rejected, Z-tests were used to test for pairwise differences between the proportional contributions of individual pairs, as described in Chapter 3, with sequential Bonferroni correction for multiple tests (Rice 1989).

Overall survival for each pond was calculated from the initial and final counts for each pond. An estimation of survival could not be made for the commercial tank because fish

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remaining in the tank at the end of the Phase II and Phase III rearing periods were not completely enumerated. After progeny were assigned to parental pairs, survival by sire was estimated for each pond of half-sibling families. Estimates of survival by sire family and strain were made as described in <u>Chapter 3, Methods</u> with the exception that initial and final numbers of fish were not estimated but were instead hand-counted. Standard errors of the survival estimates were calculated using a Taylor series expansion (Seber 1982) as described in <u>Chapter 3</u> with zero used for the variance terms for the initial and final fish numbers since fish were hand-counted. In all cases where survival was estimated to be >1.0 due to low estimated proportions in the initial group and much higher estimated proportions in the final group, survival estimates were set to 1.0 and associated variances are calculated with these values. Differences in estimated survival among sire families were examined by Z-tests (Williams et al. 2001) with the covariance term removed as it was near zero for all comparisons. Because multiple pairwise tests were necessary to compare sire families, significance levels were adjusted using sequential Bonferroni correction (Rice 1989). Differences in survival between pairs of strains also were compared by Z-tests using an unadjusted significance level of $\alpha = 0.05$.

Population differentiation—Allele frequencies of fish by pond and tank were determined using GENEPOP version 3.4 software (http://wbiomed.curtin.edu.au/genepop/) (Raymond and Rousset 1995a). Genic differentiation between Phase II and Phase III populations of family 2E55 was tested by calculating unbiased estimates of P-values of the probability test (Raymond and Rousset, 1995b) between samples by locus; Fisher's exact test was used to test differentiation across all loci and groups. Similarly, genotypic differentiation was tested by calculating unbiased estimates of the P-values of a log-likelihood based exact

test (Goudet et al. 1996) by locus and globally by Fisher's test across loci and groups. Exact *P*-values were estimated using a Markov chain method (Guo and Thompson 1992) and all Markov chains consisted of 2000 dememorization steps, 1000 batches, and 2000 iterations to produce standard errors <0.001. Significance levels for multiple independent tests of allelic or genotypic differentiation were adjusted using sequential Bonferroni correction (Rice 1989).

Paternal variation in phenotypic traits—All phenotypic trait measures, including length, weight, condition factor, VSI, GSI (by gender), fillet percentage, and antimicrobial peptide activity were examined for normal distribution using PROC UNIVARIATE in SAS version 9.1 software (Cary, NC). The Kolmorogov-Smirnov goodness-of-fit test for normal distribution, as well as histograms and normal probability plots, were used to evaluate normality of phenotypic distributions for all ponds and for the commercial tank at the end of Phase III. Residuals and expected values were calculated using the general linear model procedure (PROC GLM) and plotted in PROC GPLOT to visualize diagnostic residual plots for evaluation of the constancy of error variances (Kutner et al. 2005). Sires with fewer than three progeny sampled for antimicrobial peptide activity were removed from analyses. A WEIGHT statement was utilized to control variance among the sire families for this trait due to wide variation in numbers of progeny. Least squares means were calculated for all phenotypic traits.

Family 2E55 Phase III research ponds—Overall differences in sire family means of total length, body weight, condition factor, VSI and antimicrobial peptide activity for pond-reared progeny of dam 2E55 were tested in a randomized block design where ponds were the blocks and all treatments (sire families) were assigned to each pond. In this way, all families

were evaluated under identical environmental conditions within each pond and the experimental unit of pond was replicated to enable detection of variation among ponds. Interaction plots were examined for each trait to investigate possible interactions among sires and ponds. Analysis of variance (ANOVA) was carried out using PROC GLM. Sire, pond and gender were evaluated as fixed effects in a split-plot analysis with sire and pond in the main plot and gender in the sub-plot. The effect of sire was tested using the mean squared for sire x pond (experimental error) as the denominator for the F-test, and the effects of gender and sire x gender were tested using the mean square for sire x pond x gender in the denominator of the F-test (Kuehl 1994). Effects of strain (Chesapeake, Roanoke or Santee:Chesapeake) also were examined by split-plot ANOVA. In cases where significant overall differences were indicated by ANOVA ($P<0.05$), pairwise differences among family means were investigated with the Tukey-Kramer multiple comparison procedure in SAS 9.1. Pairwise comparisons were considered significant at $P<0.05$. All phenotypic trait means are presented as untransformed least squares means plus or minus the standard error (SE) of the mean.

The effect of sire on GSI was examined separately by gender using single factor ANOVA with sire and pond as fixed effects. For females, the effect of sire on GSI was tested using the mean square for sire x pond (experimental error) as the denominator for the F-test (Steel et al. 1997) since the ponds were subsampled. For males, two-factor ANOVA using the fixed effects of sire and pond was carried out using the mean values of GSI for each sire family within each pond (a total of 18 means) in order to normalize the data since the means should be normally distributed according to the Central Limit Theorem (Milton 1980). The mean square of sire x pond was used as the denominator for the F-test in the ANOVA.

Interaction plots were examined for possible interactions between sire and pond on mean GSI. Tukey's test for additivity was used to test for interactions between the two factors (Kutner et al. 2005) prior to ANOVA.

Differences in the proportion of each gender within sire families were evaluated by Chi-square goodness of fit tests with the PROC FREQ procedure in SAS 9.1 to examine the null hypothesis that there were equal proportions of males and females for all sires. Heterogeneity Chi-square analysis (Zar 1984) was first used to test the null hypothesis that the three pond samples from the end of Phase III came from the same population. Where the null hypothesis of equal proportions of males and females for all sires was rejected, Chi-square tests with the Yates correction for continuity (Zar 1984) were used to evaluate the equality of gender proportions within each sire family.

The interaction of Phase and sire was examined for the three research ponds in order to assess the predictive value of Phase II sire family performance on Phase III performance. Trait means for body weight and total length, two traits easily measured at both Phases, were log-transformed and evaluated with a repeated measures design (Kutner 2005). Log-transformation of data was necessary to stabilize the variances between the two Phases (Snedecor and Cochran 1980) since measures for the fish differed greatly between Phases due to substantial growth of the fish during Phase III. In the repeated measures ANOVA, the effects of sire and pond were tested using the mean square for sire x pond in the denominator of the F-test; the effects of Phase and sire x Phase were tested using the mean square for sire x pond x Phase for the F-test. The absence of a significant interaction effect between sire and Phase may indicate that performance at Phase II (approximately one year of age) can be used to predict performance in Phase III (approximately 18 months of age).

Family 2E55 Phase III commercial tank—Trait data for the progeny of dam 2E55 reared in a single tank at Kent SeaTech were evaluated by ANOVA using the fixed effects of sire and gender. Because there was only one tank of fish and all fish were reared under identical conditions within the same tank, the error mean square for the experiment was used as the denominator of the F-tests. Effects of strain were examined similarly. Differences in the proportion of each gender within sire families were evaluated by Chi-square goodness of fit tests (Zar 1984) as described above.

12-family group Phase III research pond—The 12-family group of the mixed progeny of two dams and six sires was analyzed with a two-factor ANOVA using sire and dam as fixed effects. Interactions between sire and dam also were investigated. Because only one pond of fish remained at the end of Phase III and all fish were reared under identical conditions within the same pond, the error mean square for the experiment was used as the denominator for the F-tests.

RESULTS

Parentage assignment—From the sample of Phase III fish produced from dam 2E55 and reared in the PAFL research ponds, 1000 fish were genotyped and 996 were attributed to specific parental pairs. The four fish which could not be assigned to a secific parental pair had genotypes corresponding to both of the Roanoke sires (5442 and 2130). In the sample of fish from the Kent SeaTech tank, 409 fish were genotyped. Of these, 389 could be assigned to a specific pair of striped bass parents. Three fish were attributed to both the 5442 and 2130 (Roanoke) sires and 17 fish were discovered to be hybrid striped bass due to the presence of white bass alleles at four of the six loci. In the sample of fish from the 12-family group

reared in the PAFL pond, 401 fish were genotyped. Of these, 188 were attributed to dam 152D and 213 fish were attributed to dam 5F4B. All 401 fish from the 12-family group could be unequivocally assigned to specific parental pairs. In all, of the total 1,793 Phase III striped bass genotyped, 1,782 fingerlings (99.4%) were assigned to specific sire-dam pairs.

Population differentiation—Differences in allelic and genotypic frequencies were examined for progeny of dam 2E55 between the final Phase II fingerling populations and the harvest of the final Phase III populations 7 months later for the research ponds or 11 months later for the commercial tank. No differences in allele or genotype frequencies were present after sequential Bonferroni correction for multiple tests between the Phase II sample and the final Phase III sample for either the commercial tank or the research pond A4, although significant differences were present for pond A14 and pond A11. Of the 34 genotypes detected in the initial larval group shipped to Keo Fish Farms for Phase I production of the fingerlings used in these trials, only one genotype was lost in the final Phase III populations. This loss occurred in pond A14 at locus *SB6*. All of the genotypes that were not detected in the Phase II population (Chapter 4, Results) were found to be present in the Phase III groups, likely the result of genotyping larger samples of fish in Phase III. Importantly, no alleles were lost in any population during the course of Phase III pond rearing or as compared to the original sample of three-day old larvae which contained 57 alleles.

Proportional contribution and survival—The proportional contribution of each sire to the Phase III populations was evaluated for the research ponds and the commercial tank. Data on genotypic proportions within each sample are presented in Table 20. Survival ranged from 86-94% in ponds for the progeny of dam 2E55, and was 0% and was 46% for the two ponds of fish from the 12-family group. All fish from the 12-family group in pond A1 were lost due

to a bloom of the toxic dinoflagellate, *Gyrodinium galatheanum*, in June 2002 and an estimated 300 or more fish in the second 12-family pond (A2) died in July 2002 due to low dissolved oxygen event following copper sulfate treatment for control of aquatic weeds.

Proportional contribution, family 2E55 research ponds—Heterogeneity Chi-square analysis indicated that the three pond samples from the end of Phase II were not homogeneous, $X^2_{(10)}$=22.83, 0.01<P<0.025; therefore, the three pond samples treated separately for Chi-square analysis of sire or strain frequencies. Examination of each pond by Chi-square goodness of fit tests revealed that proportional contribution by sire and by strain varied within each of the three ponds at the end of Phase II (P<0.001). Pairwise *Z*-tests indicated that Chesapeake sire 2A20 generally had large contributions in all ponds and Roanoke sires 4664 and 7213 generally had low contribution in all ponds (Appendix Table VII). By strain, Chesapeake sires generally comprised a larger proportion of the final pond samples than either of the other two strains. Roanoke progeny were lower in number than both other strains in all ponds.

Proportional contribution, 2E55 commercial tank—Chi-square analysis indicated that proportional contribution varied by sire within the Kent SeaTech commercial tank at the end of Phase III rearing $X^2_{(5, N=389)}$=30.92, P<0.001. Chesapeake sires 631D and 2A20 had generally higher contributions to the Phase III sample and the contribution of Roanoke sire 4664 was lower than the contributions of most other sires (Appendix Table VII). By strain, the Chesapeake sires contributed a larger proportion of progeny than did the Roanoke (P<0.0001) or Santee:Chesapeake (P<0.0001) sires.

Proportional contribution, 12-family group, research pond—Proportional contribution to the pond differed by sire ($X^2_{(5, N=401)}$ = 100.11, P<0.001) but not by dam ($X^2_{(1, N=389)}$ = 1.32,

0.25<P<0.50). Within pond A2, the contribution of the two Chesapeake sires and

Santee:Chesapeake sire 3F11 were generally high while those of the Roanoke sires and

Santee:Chesapeake sire 5C5D were low (Appendix Table VII). Within each dam,

proportional contribution also differed by sire (dam 152D: $X^2_{(5, N=188)}$ = 71.21, P<0.001; dam

5F4B: $X^2_{(5, N=213)}$ = 78.92, P<0.001). With Santee:Chesapeake dam 152D, Chesapeake sire

631D had the greatest contribution, closely followed by Chesapeake sire 2A20.

Santee:Chesapeake sire 3F11 also had generally high contribution. With Roanoke dam 5F4B,

Chesapeake sire 2A20 and Santee:Chesapeake sire 3F11 had the highest contributions.

Within each dam, proportional contribution differed by strain (dam152D: $X^2_{(2, N=188)}$ = 57.71,

P<0.001; dam 5F4B: $X^2_{(2, N=213)}$ = 25.35, P<0.001). In each case, the Chesapeake and

Santee:Chesapeake strains had higher contributions to the total group than did the Roanoke

strain.

      <u>Estimated survival, family 2E55 research ponds</u>—Cumulative survival of fish in the

three research ponds ranged from 85.8% to 93.7% (Table 19). Estimated survival by sire

family for each group of pond-reared half-sibling families is illustrated for the Phase III in

Figure 23 and estimated survival (±SE) is reported for each sire family in Table 21. Progeny

survival varied among sires, ranging from a low of 0.27 (±0.07) for Chesapeake sire 631D in

pond A11 to a high of 1.00 for several sires in each pond. Several significant differences

among sire pairs in ponds A4 and A11 were detected by pairwise Z-tests (Appendix Table

VIII). In pond A4, survival of progeny from sire Roanoke sire 7213, estimated to be 0.42

(±0.10), was lower than that of Chesapeake sire 2A20, estimated at 0.87 (±0.11). In pond

A11, survival of progeny from Chesapeake sire 631D, (0.27 ±0.07) was lower than for

progeny of Santee:Chesapeake sires 292B (0.82 ± 0.16) as well as for offspring of and

Chesapeake sire 2A20 (1.00 ± 0.14). Survival of progeny of Santee:Chesapeake sires 7E27

and 292B also was lower than that of Chesapeake sire 2A20. In general, the best surviving

progeny in each pond were sired by males from the Chesapeake or Santee:Chesapeake strains

(Figure 24).

Paternal variation in phenotypic traits—Phenotypic traits were normally distributed

for all the Phase III groups with the exception of GSI for pond reared males from the 2E55

family. Least squares means for all traits by sire family are presented in Tables 22 and 23.

Phenotypic traits that were transformed for data analysis (e.g., VSI, GSI, antimicrobial

peptide activity) are presented in tables and figures as least squares means of the

untransformed data.

Family 2E55 Phase III research ponds— At the end of Phase III rearing in the

research ponds, overall differences in all phenotypic traits were detected among families

produced from dam 2E55. All trait means differed among the three replicate ponds and

means differed by gender for several traits. Interaction effects among sire and pond were not

present for any trait.

A significant effect of sire family was present for total length $F_{(5,10)}$=49.21, P<0.0001.

Lengths ranged from 30.48 (±0.22) to 32.68cm (±0.09) and pairwise differences among sire

families were detected (Figure 24). Progeny of Santee:Chesapeake sire 7E27 were longer

than progeny of all the other sires. Progeny of Santee:Chesapeake sire 292B were greater in

total length than progeny of Chesapeake sires 631D and 2A20 and than progeny of Roanoke

sire 4664. A significant effect of strain also was present for total length, $F_{(2,4)}$=69.01,

P=0.0008, with Santee:Chesapeake progeny being longer than progeny of the other two

strains (Figure 25). Mean total length differed among ponds, $F_{(2,10)}$=227.30, P<0.0001, with

fish from pond A14 having higher mean total length than those in both other ponds (Figure

26). There was no difference in total length between males and females (Figure 27).

Mean body weight, $F_{(5,10)}$=131.98, P<0.0001 differed by sire family. Body weights

ranged from 348.8g (±6.01) to 435.8g (±2.42). Pairwise differences among sires were

detected with the Tukey multiple comparisons method (Figure 24). Progeny of

Santee:Chesapeake sires 292B and 7E27 had the heaviest body weights among the sire

families. Significant differences also were present by strain, $F_{(2,4)}$=108.13, P=0.0003, with

Santee:Chesapeake progeny having greater body weights than progeny of the other strains

(Figure 25). Mean body weight differed among ponds, $F_{(2,10)}$=778.71, P<0.0001, with fish

from pond A14 having higher mean body weight than those from both other ponds and fish

from pond A11 having higher mean body weight than those from pond A4 (Figure 26).

Gender effects were significant for mean body weight, $F_{(1,10)}$=7.13, P>F=0.0235. Males had

a higher mean body weights (386.46g ± 3.11) than females (373.56g ± 3.69) (P=0.0235)

(Figure 27).

Overall differences among sire families also were present for mean condition factor,

$F_{(5,10)}$=26.76, P<0.0001. Measures by sire family ranged from 1.18 (±0.004) to 1.24 (±0.004).

Progeny of Santee:Chesapeake sire 7E27 had higher condition factor than those of

Chesapeake sire 2A20 or Roanoke sire 7213 (Figure 24). Progeny of sires 292B

(Santee:Chesapeake), 631D (Chesapeake) and 7213 (Roanoke) all had higher condition

factor than progeny of Chesapeake sire 2A20. A significant effect of strain on condition

factor was present as well, $F_{(2,4)}$=132.37, P=0.0002, with progeny of the Santee:Chesapeake

strain having greater condition factor than progeny of the other two strains and those of the

Roanoke strain having higher condition factor than progeny of the Chesapeake strain (Figure

25). Mean condition factor differed among ponds, $F_{(2,10)}=230.50$, $P<0.0001$. Fish from pond A14 had higher mean condition factor than those from both other ponds and fish from pond A11 had higher mean condition factor than those from pond A4 (Figure 26). Gender effects were significant for mean condition factor, $F_{(1,10)}=8.76$, $P=0.0143$. Males had higher mean condition factors (1.22 ± 0.005) than females (1.20 ± 0.006) ($P=0.0143$) (Figure 27).

Mean viscerosomatic index (VSI) differed among sire families, $F_{(5,10)}=7.76$, $P=0.0032$, and ranged from 9.63 (± 0.09) to 10.36 (± 0.14). Pairwise differences among sire families were detected, with progeny of the Roanoke sire 7213 having lower VSI than those of Santee:Chesapeake sire 292B, Chesapeake sire 2A20 and Roanoke sire 4664 (Figure 28). There was no difference in mean VSI by strain (Figure 29). Mean VSI differed among ponds, $F_{(2,10)}=128.77$, $P<0.0001$, with fish from pond A14 having higher mean VSI than those from both other ponds and fish from pond A11 having higher mean body weight than those from pond A4 (Figure 30). Gender effects were significant for mean VSI, $F_{(1,10)}=8.55$, $P=0.0152$. Females had higher mean VSI (10.16 ± 0.06) than males (9.92 ± 0.05) ($P=0.0152$) (Figure 31).

Overall differences by sire family in antimicrobial peptide activity were not present in the family 2E55 Phase III progeny sampled from the two research ponds $F_{(4,4)}=4.73$, $P=0.0808$ (Figure 28). There was a significant effect of strain on antimicrobial peptide activity, $F_{(2,2)}=56.98$, $P=0.0172$. The progeny of the Santee:Chesapeake and Chesapeake strains both had higher mean antimicrobial peptide activity than did those of the Roanoke strain (Figure 29). Mean activity differed by pond, $F_{(1,4)}=35.01$, $P=0.0041$ (Figure 30). There was no interaction of sire and pond. There was no difference in antimicrobial peptide activity by gender (Figure 31).

Effects of sire, pond and strain on GSI were evaluated by gender. There was a significant effect of sire family on female GSI, $F_{(5,10)}$=25.10, P<0.0001. Progeny of Chesapeake sire 2A20 had greater GSI than those of all other sires. Progeny of Santee:Chesapeake sire 292B and Chesapeake sire 631D both had greater GSI than progeny of Santee:Chesapeake sire 7E27 or Roanoke sire 7213 (Figure 32). Differences in mean GSI were present by strain, $F_{(2,4)}$=26.25, P=0.0050, and pairwise differences were detected between the Chesapeake strain (greater GSI) and both other strains (Figure 33). Mean GSI also differed by pond, $F_{(2,10)}$=19.22, P=0.0004. Females reared in pond A4 had greater mean GSI than those reared in pond A11 or A14, and mean GSI for fish in pond A11 was greater than that of fish from pond A14 (Figure 34).

No interaction of sire and pond in their effects on male GSI was detected using Tukey's test for additivity (Kutner et al. 2005), F^*=0.5966 <$F_{(0.95, 1,9)}$=5.12, P>0.25. Therefore, effects of sire and pond could be evaluated with a two-factor ANOVA for fixed effects with the mean square of the interaction as the denominator of the F-test for each factor's main effects (Kutner et al. 2005). Significant effects of both sire, $F_{(5,10)}$=8.83, P=0.0020, and pond, $F_{(2,10)}$=43.79, P<0.0001, were present. Male progeny of Santee:Chesapeake sire 7E27 had a higher GSI than those of Roanoke sire 7213 and Chesapeake sire 631D (Figure 32). There was no significant effect of strain on GSI, $F_{(2,4)}$=2.16, P=0.2310 (Figure 33). Males from pond A4 males had average GSI of 1.02 ($\pm$ 0.06) which was greater than that of males from pond A11 (0.45 $\pm$ 0.06) or pond A14 (0.35 $\pm$ 0.06) (Figure 34).

Use of the heterogeneity Chi-square goodness of fit tests for examination of gender proportions by sire family revealed that there was no difference among ponds in the

proportion of each gender. Pond data were pooled for subsequent analyses. Chi-square analysis indicated that the proportion of each gender was not the same across all sire families, $X^2_{(5, N=996)}$=226.69, P<0.001. Within sire families, Chi-square tests revealed that there were significant differences in proportions of males and females for all sires except Roanoke sire 4664 (Table 24). The two Chesapeake sire families each had larger numbers of females than males while the Santee:Chesapeake families and the remaining Roanoke family had larger numbers of males than females.

A low incidence of deformities was observed in Phase III progeny of the 2E55 dam. Only 18 of the 996 fish sampled from the research ponds had any external deformity. Further examination of differences in incidence of deformities among family 2E55 Phase III sire families was therefore deemed to be unnecessary.

A total of 50 fish larger than 530g body weight (top 12% of group by weight) from pond A14 were retained at PAFL as future broodstock. These fish were PIT-tagged and genotyped to determine their sire. All remaining sampled and unsampled fish were delivered to Carolina Fisheries and marketed whole on ice or live as foodfish.

Family 2E55 Phase III Commercial Tank—Overall differences in mean total length, $F_{(5,375)}$=18.07, P<0.0001, mean body weight, $F_{(5,375)}$=12.72, P<0.0001, mean condition factor, $F_{(5,375)}$=3.59, P=0.0035, and mean VSI, $F_{(5,369)}$=13.70, P<0.0001, were detected among sire families. The effect of sire was not significant for mean percent fillet.

Mean total lengths ranged from 38.20cm (± 0.38) to 41.69cm (± 0.26). Pairwise differences among sire families were present (Figure 35). Progeny of the two Santee:Chesapeake sires (7E27 and 292B) and Chesapeake sire 2A20 had greater total length than those of the two Roanoke sires (4664 and 7213) or than those of Chesapeake sire 631D.

Progeny of Chesapeake sire 631D and Roanoke sire 7213 both had greater total length than those of Roanoke sire 4664. Overall differences also were detected by strain for mean total length, $F_{(2,381)}$=32.97, P<0.0001, with progeny from the Santee:Chesapeake strain having greater total length than those of the other two strains and those of the Chesapeake sires also having greater length than offspring from the Roanoke strain (Figure 36). Significant effects of gender were not present for mean total length (Figure 37).

Mean body weights of fish in the sire families ranged from 762.54g (± 29.50) to 1004.68g (± 23.12). Pairwise differences between sire families were evident with the Tukey multiple comparisons method (Figure 35). Progeny of Santee:Chesapeake sire 7E27 were heavier than those of Chesapeake sire 631D and than those of the two Roanoke sires. Progeny of Santee:Chesapeake sire 292B also were heavier than those of the Roanoke sires. The progeny of Chesapeake sire 631D had greater mean body weight than those of Roanoke sire 4664, and Chesapeake progeny of sire 2A20 had greater body weight than those of Roanoke sire 7213. Overall differences also were detected by strain for mean body weight, $F_{(2,381)}$=27.37, P<0.0001, with progeny from the Santee:Chesapeake sires having greater body weight than those of the other two strains. Progeny of the Chesapeake sires were heavier than those from Roanoke sires (Figure 36). Significant effects of gender were not present for mean body weight (Figure 37).

Mean measures of condition factor among sire families ranged from 1.30 (±0.02) to 1.38 (±0.01). Pairwise differences in condition factor were present (Figure 35) with progeny from Chesapeake sire 631D and Santee:Chesapeake sire 7E27 having greater condition factor than those of Chesapeake sire 2A20. No overall differences were detected by strain (Figure 36) or by gender (Figure 37) for condition factor.

Mean values for percent fillet weight did not differ by sire (Figure 38) or by strain (Figure 39) but there was a significant effect of gender, $F_{(1,304)}$=4.38, P=0.0372. Male fish had greater mean percent fillet (43.74% ± 0.14) than did females (43.34% ± 0.12) (P=0.0372) (Figure 40).

Mean VSI of the sire families ranged from 8.84 (± 0.12) to 10.36 (±0.18). Progeny of Roanoke sire 4664 had greater VSI than did progeny of Santee:Chesapeake sire 7E27 or Roanoke sire 7213, and progeny of Roanoke sire 7213 had a lower VSI than those of all other sires (Figure 38). Overall differences were detected by strain for VSI, $F_{(2,375)}$=7.59 P=0.0006, with progeny from the Chesapeake and Santee:Chesapeake strain having higher mean VSI than those of the Roanoke strain (Figure 39). There was no significant effect of gender on VSI in the commercial tank (Figure 40).

Only 25 fish of the 120 fish sampled from the commercial tank had detectable levels of gill antimicrobial peptide activity. Of these, three or fewer progeny were sampled for two of the sire families (7E27 and 2A20) and data for these families were removed from the analysis. No overall differences in antimicrobial peptide activity were detected by sire, strain or gender for the remaining families from the commercial tank (Figure 38-40).

Effects of sire and strain on mean GSI were evaluated by gender. For females in the commercial tank, there was a significant effect of sire on mean GSI, $F_{(5,219)}$=15.36, P<0.0001. Pairwise comparison revealed that progeny of Chesapeake sires 631D and 2A20 and Santee:Chesapeake sire 292B all had greater mean GSI than did progeny of the two Roanoke sires (4664 and 7213) (Figure 41). Progeny of the Chesapeake sires also had greater mean GSI than those of Santee:Chesapeake sire 7E27 (Figure 10I). Significant effects of strain on mean female GSI also were present, $F_{(2,222)}$=35.37, P<0.0001. Progeny of the

Chesapeake strain had greater GSI than the other two strains and Santee:Chesapeake strain fish had greater mean GSI than did those of the Roanoke strain (Figure 42).

For males in the commercial tank, there were significant effects of sire on mean GSI, $F_{(5,156)}$=8.54, P<0.0001, with male progeny of the two Santee:Chesapeake sires and Chesapeake sire 2A20 all having greater mean GSI than progeny of Roanoke sire 7213 (Figure 41). The effect of strain on GSI also was significant for males, $F_{(2,159)}$=14.21, P=<0.0001. Progeny of the Santee:Chesapeake strain had greater GSI than those of the Roanoke strain (Figure 42).

Chi-square analysis indicated that the proportion of each gender was not the same across all sire families within the commercial tank, $X^2_{(5, N=387)}$=108.77, P<0.001. Within sire families, Chi-square tests revealed that there were significant differences in proportions of males and females for both Chesapeake sires and for Santee:Chesapeake sire 7E27 (Table 24). Progeny of the two Chesapeake sire families had larger numbers of females than males while more males than females were identified for the Santee:Chesapeake family. A single hermaphrodite fish was identified in the commercial tank sample; this fish was sired by Chesapeake sire 2A20.

A low incidence of deformities was observed in Phase III progeny of dam 2E55 that were reared in the commercial tank. Because only 17 of the 373 fish sampled had external deformities, evaluation of differences in the incidence of deformities among the sire families was considered to be unnecessary.

Approximately 350 fish from the Kent SeaTech commercial tank were retained as future broodfish. These animals were hand-selected by the Kent SeaTech broodstock manager, Steven J. Mitchell, based on body size, the absence of any physical deformities and

the absence of subcutaneous hemorrhages at the base of the fins or on the abdomen of the fish, indicative of past infection with *Streptococcus iniae*. With the exception of approximately 100 fish retained by Kent Sea Tech at their Mecca, CA farm, the broodstock were trucked to Keo Fish Farms (Keo, AR) the week after they were selected. The fish had an average body weight of 889g and an average total length of 41.4cm. Of these, approximately 102 fish were then hauled from Keo Fish Farms to PAFL where they were PIT-tagged and genotyped; all twenty-eight of the fish remaining at Kent SeaTech were genotyped in 2003.

Predictive value of Phase II for Phase III—The interaction of rearing Phase and sire was examined for progeny of dam 2E55 from the research ponds in order to assess the predictive value of Phase II sire family performance on Phase III performance. The data on total length, body weight and condition factor collected at the end of Phase II and Phase III were log transformed and sire performance was evaluated across both rearing Phases. There was no significant interaction effect between sire and Phase for total length, $F_{(5,10)}=0.30$, $P=0.9048$, body weight, $F_{(5,10)}=0.76$, $P=0.5976$, or condition factor, $F_{(5,10)}=0.47$, $P=0.7916$. Plots of total length and body weight by sire family across the two production Phases reveal near parallelism with similar rank orders of sire means between Phases, particularly for the two top-performing Santee:Chesapeake sires (Figure 43).

12-family Phase III research pond—Significant overall differences between sire families in mean total length, $F_{(5,369)}=4.29$, $P=0.0008$, mean body weight, $F_{(5,369)}=3.38$, $P=0.0053$, and mean condition factor, $F_{(5,369)}=12.65$, $P<0.0001$, were detected for the 12-family group. However, significant differences also were detected by dam for mean body weight, $F_{(1,369)}=13.87$, $P=0.0002$, and for mean total length, $F_{(1,369)}=10.77$, $P=0.0011$. There

was no difference by dam for condition factor. Since only 401 fish from the 12-family group survived, gender data were not collected from the 12-family fish in order to retain fish larger than 575g (~10% of the population, top 22.5% by body weight) as potential broodstock (*n*=41 fish).

Mean total length by sire ranged from 32.13cm (± 0.36) to 33.80cm (± 0.26) in the 12-family group. Pairwise differences were detected by the Tukey multiple comparisons test (Figure 44). Progeny of Santee:Chesapeake sire 3F11 were heavier than those of Roanoke sire 5442 or Santee:Chesapeake sire 5C5D. Differences also were present by strain, $F_{(2,375)}$=3.52, *P*=0.0307, but no pairwise differences among strains were present (Figure 45). Differences by sire also were present within each dam. For the Santee:Chesapeake dam 152D, progeny of Santee:Chesapeake sire 3F11, Roanoke sire 3B62 and Chesapeake sire 2A20 were longer than those of Santee:Chesapeake sire 5C5D (Figure 46). There were no differences in total length by strain for progeny of dam 152D (Figure 47). For Roanoke dam 5F4B, progeny of Santee:Chesapeake sire 3F11 were longer than those of Roanoke sire 5442 (Figure 48). By strain, the progeny of dam 5F4B that were sired by Santee:Chesapeake sires were longer than those of the Roanoke strain (Figure 49). Between the two dams, progeny of Santee:Chesapeake dam 152D were longer (33.32 cm ± 0.22) than those of the Roanoke dam (32.39 cm ± 0.18) (*P*=0.0011) (Figure 50).

Over both dams in pond A2, mean body weight by sire ranged from 417.24g (± 15.36) for the Santee:Chesapeake sire 5C5D to 481.30g (± 11.09) for the Santee:Chesapeake sire 3F11. Within the total group, pairwise differences among sires were detected by the Tukey multiple comparisons test (Figure 44). Progeny of sire 3F11 were heavier in body weight than were progeny from Santee:Chesapeake sire 5C5D or Roanoke sire 5442.

Differences also were present by strain, $F_{(2,375)}$=3.47, P=0.0321, but no pairwise differences among strains were present (Figure 45). Differences by sire were present for body weight within each dam's progeny group. For Santee:Chesapeake dam 152D, progeny of Santee:Chesapeake sire 3F11 and Chesapeake sires 631D and 2A20 were heavier than those of Santee:Chesapeake sire 5C5D (Figure 46). There were no pairwise differences by strain for dam 152D (Figure 47). For Roanoke dam 5F4B, progeny of Santee:Chesapeake sire 3F11 were heavier than those of Roanoke sires 5442 and 3B62 (Figure 48). By strain, the progeny of dam 5F4B that were sired by Santee:Chesapeake sires were heavier than those of the other two strains (Figure 49). Between the two dams, progeny of the Santee:Chesapeake dam were heavier (464.71g ± 9.27) than those of the Roanoke dam (420.40g ± 7.46) (P=0.0002) (Figure 50).

Condition factor by sire ranged from 1.19 (± 0.006) to 1.26 (± 0.008) within the 12-family group with pairwise differences among several sire pairs (Figure 44). Progeny of Chesapeake sire 631D had greater condition factor than those of Chesapeake sire 2A20, Santee:Chesapeake sire 3F11 or Roanoke sire 3B62. Progeny of both Santee:Chesapeake sires (5C5D and 3F11) and Roanoke sire 5442 also had greater condition factor than those of Chesapeake sire 2A20. There was no difference in condition factor by strain (Figure 45). Differences by sire also were detected within each dam. For Santee:Chesapeake dam 152D, progeny of Chesapeake sire 631D had greater condition factor than those of Chesapeake sire 2A20 or Santee:Chesapeake sire 3F11 (Figure 46). Progeny of Roanoke sires 5442 and 3B62 also had greater condition factor than those of Chesapeake sire 2A20. There was no difference in condition factor by strain for dam 152D (Figure 47). For the Roanoke dam, progeny of Chesapeake sire 631D had greater condition factor than progeny of Chesapeake

sire 2A20 and Roanoke sire 3B62 (Figure 48). There was no difference in condition factor by strain for dam 152D (Figure 49). There also was no difference in condition factor between progeny of the two dams (Figure 50).

Deformities were present in 85 of the 401 fish from the 12-family group. Chi-square analysis indicated that sire did not have a significant effect on incidence of deformities ($X^2_{(5, N=382)}$=10.16, P=0.0708). However, there was a significant effect of dam on incidence of deformities ($X^2_{(1, N=383)}$=10.48, P=0.0012). In the 175 sampled progeny of Santee:Chesapeake dam 152D, 38.9% had external deformities while in the 208 sampled progeny of Roanoke dam 5F4B, 23.6% fish had deformities. High incidences of deformities, in excess of 25%, were observed for both of these dams after production Phase I and it not unexpected that large numbers of fish with these deformities were retained from Phase I.

Examination of interaction plots revealed possible interactions between dam and sire for the 12-family group. Significant interaction effects of dam and sire were detected by two-factor ANOVA for total length, $F_{(5,369)}$=2.91, P=0.0137, and body weight, $F_{(5,369)}$=2.83, P=0.0159. Logarithmic and square root transformation of the data (Kutner et al. 2005) did not remove the interaction effects. Pairwise comparison did not reveal significant differences in performance of any sire family between the two dams. In general though, Santee:Chesapeake sire 5C5D produced better performing progeny when crossed with the Roanoke dam 5F4B (e.g., body weight 434.98g) than with the Santee:Chesapeake dam (399.51g), while the Santee:Chesapeake sire 3F11 produced progeny that performed quite similarly (483.98g and 478.62g, respectively) when crossed with either dam (Figures 51-52). The remaining four sires all produced generally better-performing progeny when crossed with the Santee:Chesapeake dam (see Table 22). There also were significant interaction

effects for strain (of sire) and dam on body weight, $F_{(2,375)}$=6.31, P=0.0020, total length,

$F_{(2,375)}$=5.37, P=0.0050, and condition factor, $F_{(2,375)}$=3.67, P=0.0264 (Figures 53-54).

Pairwise differences in performance were present between dams for total length for the

Roanoke strain, for body weight for both the Roanoke and the Chesapeake strain, and for

condition factor for the Chesapeake strain. In each case, the progeny of the

Santee:Chesapeake dam out-performed those of the Roanoke dam. Therefore, for the six

crosses evaluated in this project, sire by dam and sire by strain interaction effects indicate

that variation among cross and strain combinations are present and should be evaluated in

more detail.

DISCUSSION

Parentage assignment and communal rearing—The use of six microsatellite markers

for parentage identification in striped bass proved highly successful for Phase III communal

rearing trials in both research ponds and in a commercial production tank. Genotyping and

parentage assignment success were near 100% for striped bass reared in groups of 6 families

and in groups of 12 families. Of the 1,000 genotyped progeny of the 2E55 dam that were

reared in triplicate research ponds at PAFL, 996 (99.6%) could be unequivocally assigned to

specific parental pairs. For the Kent SeaTech Corporation commercial tank, 392 progeny of

the 2E55 dam were genotyped and 389 (99.2%) were attributed to specific parental pairs. The

genotyping protocol also identified 17 hybrid striped bass which were inadvertently stocked

into the commercial production tank. The microsatellite profiles of these hybrids contained

numerous alleles not present among the possible parents and most of these alleles were

characteristic of white bass. In the 12-family group reared at PAFL, parentage was unequivocally determined for 100% of the 401 genotyped progeny of two dams and six sires. Use of only six microsatellite markers to determine parentage of 6- and 12-family groups of communally reared striped bass, and the high success of parentage assignment, along with the detection of unrelated hybrid progeny, demonstrate the feasibility of this approach for communal rearing for striped bass performance evaluations.

Population differentiation—Differences between Phase II and Phase III allelic and genotypic distributions were examined for the family 2E55 groups reared at PAFL and at Kent SeaTech. For one PAFL pond and for the commercial tank, there was no difference in genetic constitution between the end of Phase II and the end of Phase III, likely due to high survival in Phase III and to random, rather than family-specific, losses of individuals during this rearing period. The remaining two ponds had different genetic compositions between Phase II and Phase III; however, these differences were due to deviations at a single (and different) locus for each pond, *SB91* for pond A11 and *SB108* for pond A14 and they provide little evidence of locus-specific changes in genotype or allele frequencies due to selection acting on any individual genotypes. All of the four genotypes that were not detected in the Phase II pond samples were found to be present in the Phase III sample. These genotypes may have gone undetected in the Phase II groups due to lower sample sizes (~200 fish for Phase II and ~300-400 fish for Phase III). Importantly, only one genotype that was present in the initial larval population at three days post hatch was not found in the Phase III sample (pond A14) and all 57 alleles that were present in both the initial larval group were present in the final Phase III sample of market size fish. The retention of all 57 alleles throughout the 18-20 month rearing period provides evidence that genetic variation can be maintained

throughout the production cycle and reflects the effectiveness of hatchery and pond rearing practices at PAFL for maintaining genetic diversity during communal rearing of striped bass progeny.

Proportional representation and survival—Microsatellite genotyping also allowed determination of the proportional representation of each family within the mixed progeny groups, revealing unequal numbers of progeny by sire in many cases. In family 2E55 fish reared in the PAFL research ponds, progeny of Chesapeake sire 2A20 outnumbered those in the other sire groups and Roanoke progeny were generally low in number. Similar results were seen in the commercial tank sample. For the 12-family group, progeny of the Santee:Chesapeake sire 3F11 were present in large numbers for dam families and Roanoke progeny were generally fewer in number. These inequalities were likely retained from the earlier production Phases. The family 2E55 Phase I group reared at Keo Fish Farms and then transferred to PAFL and Kent SeaTech for subsequent rearing trials had high survival within the Chesapeake sire families (although survival also was high in the Roanoke sire families) (Chapter 3, Results), and the family 2E55 Phase II pond groups had significantly higher survival of Chesapeake sire progeny than seen for the other sire groups (Chapter 4, Results). Similarly, for the 12-family group, the 3F11 sire had high Phase I progeny survival (Chapter 3, Results) when crossed with either dam. Since survival during Phase III was generally high for all of the family 2E55 fish reared in research ponds (85.8-93.7%), the differences in numerical proportions for Phase III probably reflect variation in initial representation and survival trends from earlier Phases rather than any noteworthy trends in Phase III.

Survival of family 2E55 fish differed by sire within all three research ponds and survival differed by strain in two ponds. Progeny of Roanoke sire 7213 had the lowest

survival in pond A4 and those of Chesapeake sire 631D had the lowest survival in the other two ponds, but nearly all sire families had >80% survival in each pond. Differential survival by sire family does not appear to be an important factor in Phase III rearing of striped bass.

Phenotypic variation—A primary goal of this study was to determine if significant variation in performance traits is present within the PAFL captive broodstock which might be exploited for selective improvement of striped bass. Significant differences were observed by sire family for nearly all phenotypic traits examined during Phase III rearing in both the 2E55 family and the 12-family PAFL pond groups, as well as in the commercial pond, providing evidence that paternal variation in offspring performance during Phase III exists among captive PAFL striped bass.

Significant pond to pond variation for most traits examined here signifies the importance of replicated communal evaluations to account for variation among rearing units. Although there were differences in the mean starting weight of fish reared in each pond, qualitative differences among ponds also were observed during the course of the rearing trials; *e.g.*, some ponds maintained high turbidity while others were fairly clear, and some ponds had greater growth of aquatic plants than others. There was a significant difference in mean dissolved oxygen concentrations among the ponds ($F_{(2,353)}$=3.53, P<0.030) (Appendix Table XII), and other, non-measured environmental factors, may have varied as well. Such unavoidable variation among rearing environments which were treated as similarly as possible reinforces the necessity of replicating communal rearing units, especially for calculations of absolute trait means and for comparisons of groups of fish reared in different ponds.

Growth-related traits—For the progeny of the 2E55 dam reared in ponds, the fish with the best growth (body weight and total length) performance tended to be offspring of the two Santee:Chesapeake sires. In the commercial tank, progeny of the Santee:Chesapeake sires, as well as those of Chesapeake sire 2A20, had better performance than those of the other sires. For the 12-family group, offspring of one of the Santee:Chesapeake sires had better growth performance than those of two other sires (one Santee:Chesapeake and one Roanoke sire). Within dams, progeny of Roanoke sire 3B62, Santee:Chesapeake sire 3F11, and of the Chesapeake sires all had better growth than progeny of the remaining sires when crossed with the Santee:Chesapeake dam. When crossed with the Roanoke dam, progeny of Santee:Chesapeake sire 3F11 again had high growth performance as compared to offspring of the Roanoke sires. By strain, the family 2E55 Santee:Chesapeake group outperformed the other two strains in both the research ponds and the commercial tank, and for the 12-family group the same strain out-performed the Roanoke group within the Roanoke dam family and performed equally well when crossed with either dam. The number of families examined here is small, but these results provide evidence that the domesticated Santee:Chesapeake lineage may produce better quality progeny than those sired by the wild Roanoke strain or of the Chesapeake strain.

Although Woods (2001) reported greater growth performance (length and weight) among market-sized striped bass from a domesticated Chesapeake strain and wild Chesapeake Bay fish have been shown to have better growth than that of several other Atlantic coast strains in intensive culture recirculating systems (Jacobs et al. 1999), Chesapeake progeny have been observed to have poor growth performance at PAFL. These discrepancies may be due to the evaluation of wild rather than domesticated fish (in the case

of Jacobs et al. 1999). They also may be related to the genetic background of the domesticated Chesapeake fish used in this study which were produced from a very limited group of parents (see Chapter 2). These fish also were domesticated under indoor tank conditions rather than in the outdoor pond and tank environment at PAFL. The effect of passive selection during domestication may have produced improvements in some PAFL groups and a more detailed investigation of variation within and among strains would be useful for a breeding program for striped bass.

The Santee:Chesapeake strain has performed well in PAFL rearing conditions (C. V. Sullivan and A.S McGinty, *personal communication*) and domestication selection for this strain at PAFL may have conferred some advantage to the progeny of these broodstock for captive rearing conditions. As noted, greater growth performance (length and weight) was reported by Woods (2001) for striped bass from a domesticated Chesapeake strain compared to progeny of wild parents, and wild versus domesticated lines of other cultured species, including salmon and catfish, can have markedly different performance in growth (Gjedrem 2000; Dunham and Smitherman 1983; Hershberger et al. 1990; Fleming et al. 2002). Domestication, without any directed selection, appears to confer an average increase of 3% per generation for growth of catfish, and salmon which have been domesticated for seven generations have three times faster growth as well as higher concentrations of growth hormone than their wild founder stock (Fleming et al. 2002). Hodson et al. (2000) demonstrated equal or greater survival and performance of hybrid striped bass produced from captive-bred versus wild broodfish at both research and commercial scale. Performance of progeny from domesticated striped bass broodstock strains has not been rigorously evaluated in comparison to either wild or domesticated strains for any *Morone* species and

comprehensive strain evaluations should provide additional benefit for selective improvement of striped bass. Examination of additional striped bass families from these strains in replicated rearing units in commercial pond and tank settings will be necessary to fully assess the genetic variation of growth performance within and among the captive broodstock groups.

Estimates of heritability for growth performance have not been generated for striped bass, and should be possible using a communal rearing approach to maximize the numbers of crosses that can be examined. For tilapia, heritability of mean body weight is 0.61 and 0.24 for males and females, respectively (Velasco et al. 1995). For Atlantic salmon, heritabilities for weight at harvest ranged from 0.41 ± 0.18 to 0.60 ± 0.18 (Fjalestad et al. 1996), and for rainbow trout, heritability estimates for body weight and body length were 0.35 and 0.53, respectively (Henryon et al. 2002). These traits also were highly correlated with feed conversion efficiency (Thodeson et al. 2001; Henryon et al. 2002). Similarly, in other studies of rainbow trout, feed conversion efficiency was demonstrated to be high for faster-growing fish (Overturf et al. 2003). Heritabilities for body weight in Chinook salmon strains ranged from 0.24 to 0.36 with small differences among strains (Winkelman and Peterson 1994) and a similar heritability (0.25) was estimated for body weight in carp (Gjedrem 1983). The magnitude of these heritabilities indicates that selective breeding can result in substantial gains for growth-related traits in these species. Although heritability of growth-related traits could not be estimated for striped bass in this study, the evidence of substantial variation among striped bass sires suggests that genetic variation is present among captive PAFL striped bass. If additive genetic variance comparable to that for the species described above is

present for striped bass, this variation should permit substantial gains to be made through selective breeding.

Condition factor—For condition factor, a measure of body shape, progeny of the 2E55 dam reared in ponds that were sired by Santee:Chesapeake sire 7E27 had higher condition factor (or a deeper body shape) than those of Chesapeake sire 2A20 or Roanoke sire 7213. In the commercial tank, offspring of Santee:Chesapeake sire 7E27, as well as Chesapeake sire 631D, had higher condition factor than progeny of the other Chesapeake sire. By strain, Santee:Chesapeake progeny had the highest condition factor in ponds but there was no difference in the commercial tank. For the 12-family group, the progeny of Chesapeake sire 631D had higher condition factor than those of Santee:Chesapeake sire 3F11 or than those of the other Chesapeake sire, and this pattern was similar within the progeny of each dam. Overall, there was no difference in condition factor among strains for the 12-family group. Genetic variation in body shape may be an important trait for a program of selective breeding since the United States hybrid striped bass industry markets more than 80% of its fish whole to live markets (Carlberg and VanOlst 2004) where appearance and body shape can be quite important for sales. Condition factor also may be a measure of general health since fish with low condition factor may be long and slender in appearance due to poor nutrition. Sire-based variation in condition factor was present in the families of striped bass evaluated in this study.

Viscerosomatic index—Viscerosomatic index (VSI), or the percentage of the total weight that is comprised of viscera, may be a trait of interest for a selective breeding program for *Morone* species (Garber and Sullivan 2006) since allocation of energy to viscera (intraperitoneal fat, liver) rather than to production of lean muscle may reduce the dress-out

percentage of the fish. For progeny of dam 2E55, VSI was lowest in both the ponds and the commercial tank for progeny of Roanoke sire 7213. By strain, VSI was lowest in the commercial pond for the Roanoke group but no difference among strains was seen in the research ponds. VSI was not examined for the 12-family group. Values for VSI were similar to those reported for white bass by Rudacille and Kohler (2000) and by Kohler et al. (2001) for sunshine bass. Variation in weight of intraperitoneal fat and in the amount of lipid and glycogen stored in the liver can contribute to variation in VSI among families. This variation may be the result of genetic differences in patterns of energy storage among families or may be due to poor nutrition. The latter does not appear to be the case for the Roanoke sire 7213 as the progeny of this sire did not have the lowest body weight, total length or condition factor among the families and in some cases these fish had higher performance than those of other sire groups. Genetic contributions to VSI have not been investigated for striped bass. Among reciprocal hybrid striped bass produced by three geographic strains of white bass, no variation was detected in mean VSI by strain (Kohler et al. 2001).

Fillet percent—No difference in mean percentage fillet weight was observed by sire in the Kent SeaTech commercial tank sample. Kohler et al. (2001) reported differences in percent fillet by strain of white bass used to produce hybrid striped bass although no difference was seen for percent fillet between sunshine and palmetto bass (Rudacille and Kohler 2000). Low heritabilities for dress-out weight also have been reported in other fish species (Tave 1993).

Antimicrobial peptide activity— As evaluated here, antimicrobial peptide activity is a measure of histone-like proteins that function as an initial defense against microbial or parasitic invasion in fishes (Robinette et al. 1998). Chronic stresses, such as low water

quality or crowding, have been shown to depress levels of antimicrobial peptide activity (Robinette and Noga 2001). Although 120 fish were sampled for gill antimicrobial peptide activity from the commercial tank, only 25 samples produced measurable activity in the radial diffusion assay. This result may be due to the depression of antimicrobial peptide activity of the fish due to stress associated with rearing in the intensive commercial setting, where fish are maintained at high densities and at somewhat sub-optimal water quality. Many of the striped bass sampled at Kent SeaTech had signs of past *Streptococcus iniae* infection (S. Mitchell, *personal communication*), indicative of physical stress due to disease and perhaps the result of chronic levels of stress that caused immune suppression. Stress has been implicated in the compromise of disease resistance in fishes (Anderson et al. 1990) and immune suppression due to stress has been shown to cause increased susceptibility to infectious diseases in chinook salmon (Maule et al. 1989), rainbow trout (Fevolden et al. 1992), and carp (Yin et al. 1995). In the commercial tank sample, therefore, chronic stress may have reduced gill antimicrobial activity to nearly undetectable levels. By contrast, striped bass reared in the research ponds at lower density (and likely lower levels of culture stress) had higher levels of antimicrobial peptide activity, which was measurable in 116 of the 120 fish sampled. Because antimicrobial peptide activity is fairly easily measured, this method may serve as a simple measure of general disease resistance or stress tolerance for a program of selective breeding for striped bass.

For progeny of dam 2E55, gill antimicrobial peptide activity did not differ by sire in either the research pond or the commercial tank environments. Activity in the research ponds was greatest within the Santee:Chesapeake 292B sire family (5.99 units $\pm$ 0.34) compared to a range of 3.48 $\pm$ 0.39 to 5.2 $\pm$ 0.43 for the other sire families. Investigation of antimicrobial

peptide activity with a larger sample of fish from replicate ponds should provide additional information on the sire-based variation present for this trait. However, antimicrobial peptide activity did differ by strain within striped bass in the PAFL ponds. Progeny of the two domesticated groups, the Santee:Chesapeake and the Chesapeake strains, had greater gill antimicrobial peptide activity than progeny of the wild Roanoke strain. This effect may be due to the fact that these two strains have been domesticated over several generations and may be better adapted to captivity with correspondingly lower levels of stress. Although the number of families examined is small and the number of fish sampled by family is fairly low, this is the first known report of genetic variation in antimicrobial histone-like peptides in fish. More detailed examination of this trait with greater numbers of families and greater sample sizes may be a valuable investment as disease resistance is one of the most important traits identified for striped bass improvement.

Improving general resistance to disease by selecting for increased antimicrobial peptide activity may be more beneficial in an aquaculture setting than selection for resistance against a single disease since cultured fish are exposed to such a wide range of pathogens. Investigations of other broad-spectrum antimicrobial peptides for such improvements have proven promising. Activity of lysozyme, a type of non-specific antimicrobial peptide present in the skin, gills, and alimentary tract (Robinette et al. 1998), has been reported to have a significant sire by dam effect in rainbow trout (Grinde et al. 1988) and has a moderate heritability of 0.19 ± 0.11 in Atlantic salmon (Fevolden et al. 1994). This estimate is considerably larger than that for other measures of disease resistance or stress response, including cortisol levels (Fevolden et al. 1994) or antibody concentrations after disease challenge (Fjalestad et al. 1996). These authors suggested that selective breeding for

improved lysozyme production would be feasible for trout. Similarly, Lund and colleagues (1995) saw variation in lysozyme activity among families of salmon, and directional selection for low or high lysozyme activity has been shown to improve (or reduce) resistance to *Aeromonas salmonicida* infection in salmon and to alter immunoglobulin M production (Røed et al. 2002). Selection of brown trout for high levels of the mucus antimicrobial substance, precipitin, resulted in production of progeny that were more resistant to *Furunculosis* infection (Cipriano and Hartwell 1986) and the trait was highly correlated to survival. Strain differences in antibody levels have been observed for rainbow trout exposed to infectious hematopoietic necrosis virus (Overturf et al. 2003). Family differences in physiological stress response in striped bass were reported by Wang et al. (2004). Although only three families were evaluated, 5 of 6 fish determined to be "low responders" (least affected by stress) by measures of post-stress plasma cortisol levels were from a single family. These fish also were significantly longer and had higher condition factor than the other families. The authors suggest that that there may be adequate inter-family variation in stress response in striped bass for selective improvement. Clearly, a more detailed evaluation of antimicrobial peptide activity in striped bass is warranted in order to estimate the source of the variation of the trait and to determine the value of this trait for selective improvement of disease resistance. Investigation of the action of other antimicrobial peptides in striped bass, for example, lysozyme or the recently identified moronecidin (Lauth et al. 2002, 2005) or hepcidin (Silphaduong and Noga 2001), also may prove to be promising avenues of research, as may variation based on major histocompatibility complex (MHC) class genotypes as demonstrated in Chinook salmon (Pitcher and Neff 2006). Variation in MHC class II sequences has been described for striped bass (Walker and McConnell 1994). Because even

very small differences in environmental conditions can influence antimicrobial peptide activity (Grinde et al. 1988), use of the communal rearing approach, wherein all fish are exposed to identical environmental conditions, should prove quite useful for such investigations.

Gonadosomatic index—GSI, or gonadosomatic index, was examined by gender for fish produced from the 2E55 dam in the research ponds and in the commercial tank. In both environments, GSI was highest for female progeny of the Chesapeake sires and GSI was generally low for the Roanoke families. For pond reared males, GSI was highest for progeny of the Santee:Chesapeake sire 7E27 and lowest for progeny of Chesapeake sire 631D and Roanoke sire 7213; there was no difference in mean GSI by strain. In the commercial tank, male GSI was highest for progeny of Chesapeake sire 2A20 and for the two Santee:Chesapeake sires while offspring of Roanoke sire 7213 had the lowest GSI. There also was a significant difference among strains in the commercial tank, with the Santee:Chesapeake strain having higher GSI than the Roanoke strain. Kohler et al. (2001) reported that mean GSI differed among geographic strains of white bass used to produce hybrid striped bass. Higher GSI is the result of greater gonad weight, and those families with higher GSI likely are responding to environmental cues from photoperiod and temperature that cause gonadal growth, which is initiated just after the autumnal equinox in response to the seasonal decrease in day length and water temperature (Woods and Sullivan 1993; Sullivan et al. 1997). The greater GSI measured for families of the two domesticated striped bass strains compared to that of the wild Roanoke strain is unsurprising based on maturity schedules reported for wild and captive striped bass. Wild striped bass males generally mature within three years, and although females are mature by seven years of age, maturity

for a small percentage of females can occur as early as three years (Sullivan et al. 1997). Captive striped bass generally mature earlier than wild fish (Smith and Jenkins 1988). Early maturity has been observed for captive-reared Chesapeake striped bass at Crane Aquaculture Facility in Maryland and for various captive lineages at PAFL (Hodson and Sullivan 1993; Sullivan et al. 1997). At these facilities, males may spermiate at or before two years of age and some females may mature as early as three years of age. In the progeny from dam 2E55 that were sampled at PAFL, only three males were observed to be spermiating, but all three fish were sired by either a Santee:Chesapeake or Chesapeake sire. At Kent SeaTech, no male fish were spermiating. Although fish at PAFL were sampled during December and January, after gonadal growth likely had commenced, fish at Kent SeaTech were sampled in October, prior to exposure to the environmental cues for gonadal growth. Additionally, fish at Kent SeaTech are reared under ambient photoperiod but under water temperatures that are semi-controlled and these fish likely do not receive proper environmental cues to stimulate gametogenesis under these conditions. Two females with mature gonads were identified within the commercial tank sample; both were later determined to be hybrid striped bass.

Although early maturity may hasten the pace of selective improvement, since gains may be achieved in fewer generations, early maturity in fishes also is associated with faster growth. For example, growth rate determines the incidence of maturation and the degree of investment in gonadal growth in Atlantic salmon (Adams and Thorpe 1989). Similarly, in Arctic charr, female GSI is predicted by growth rate during the months prior to gonadal maturation (Adams and Huntingford 1997). Age at sexual maturity also is correlated with body size for rainbow trout (Martyniuk et al 2003; Kause et al. 2003), with achievement of some minimum body size necessary before sexual maturity can occur. In a study of

maturation in tilapia, the fastest growing fish also matured earliest, and offering a higher percentage of protein in the diet resulted in more rapid growth and earlier maturation (Al Hafedh et al. 1999). Selective breeding trials in tilapia have indicated that early maturation is correlated with growth rate in females (Longalong et al. 1999), although faster growing trout may have a higher threshold body size for gonadal maturation than slower growing fish (Gall 1986). Moderate heritabilities have been reported for age and size at sexual maturity (0.15 ± 0.02 and 0.20 ± 0.05, respectively; Eknath et al. 1995) in tilapia and similar values have been estimated for age at sexual maturity in rainbow trout (0.12-0.34, Kause et al. 2003; 0.20, Gjerde and Gjedrem 1984). Heritability for age at maturity in salmon was 0.48 ± 0.20 (Gjerde 1984). A similar correlation for maturation and growth rate in striped bass would imply that selection for early maturity may yield benefits for more rapid growth as well as yielding more rapid returns of selection by reducing generation intervals. Although the sire families with the highest body weights and lengths also had the highest GSI, the correlation between growth and maturity has not been demonstrated for striped bass. If these traits are proven to be highly correlated, selection for early maturity may produce faster growing striped bass. This approach may be advantageous since hybrid striped bass are generally harvested during the second autumn of their lives, near or shortly after gonadal growth has begun but well before final maturation and ovulation may be induced by longer day lengths and warming water temperatures in the spring. Should the fish be harvested during the spring of their third year, however, there is a risk that female fish may release their eggs immediately prior to harvest, resulting in a loss in body weight and an emaciated appearance just before they are delivered to market. This occurs in hybrid striped bass (L. Brothers, Carolina Fisheries, *personal communication*), many of which mature in their second year.

However, unlike hybrid striped bass, which mature earlier than striped bass and also may volitionally ovulate in ponds, striped bass generally will not release their eggs in captivity without exogenous hormonal stimulation. Production of more rapidly maturing striped bass broodstock may therefore be of benefit to the hybrid striped bass farming industry. A change in marketing from whole fish to the sale of fillets would necessitate reconsideration of selection for earlier reproduction and concomitant faster growth since reduced fillet size and quality has been found for sexually maturing fish such as Atlantic salmon (Aksnes et al. 1986).

Gender differences in performance traits—Sexual dimorphism has been reported for a number of fish species. Males are larger than females in tilapia (Toguyeni et al. 1997), salmonids (Crandell and Gall 1993; Bonnet et al. 1999; Fleming and Gross 1994), and catfish (Goudie et al. 1994). Females are larger than males in European eel (Roncarati et al. 1997) and Eurasian perch (Fontaine et al. 1997). Gender dimorphism in growth also has been reported for the European sea bass (*Dicentrarchus labrax*; Pavlidis et al. 2001; Saillant et al. 2001b, 2002, 2006), a species closely related to the striped bass, where females can grow 21-67% faster than males in body weight. Growth dimorphism is greatest early in life (up to ~10 months of age) then appears to stabilize (at ~20-32 months of age) (Saillant et al. 2001b). Dimorphism appears to be the result of growth differences due to phenotypic sex, as male fish which are sex-reversed also grow faster than phenotypically male fish (Saillant et al. 2001b). Dimorphism also is more pronounced in sea bass reared at higher temperatures (20-25 °C) common in sea bass aquaculture, indicating a gender by environment interaction (Saillant et al. 2002). In hybrid striped bass and striped bass, the female also is reported to be the larger sex (Harrell 1997). In commercial culture at Kent SeaTech Corporation, female

reciprocal cross hybrid striped bass can be up to 36% heavier in body weight than males (S.J. Mitchell, *personal communication*). Davis and Ludwig (2004) reported that male reciprocal hybrids grow faster than females in the first year of life but that females grow faster in the second year.

In the present study, there were few differences in any trait by gender with the exception of body weight and condition factor in pond-reared fish from the 2E55 family, where males were heavier than females and had greater condition factor and females had greater VSI, and in the commercial tank, where males had greater percent fillet than females. It should be noted that for the pond-reared striped bass sampled at the end of Phase III, males averaged only 387g body weight while the females averaged 376g in weight. These weights are considerably lower than those of market-sized fish (568-681g) and sexually dimorphic growth may not be manifested until fish reach larger sizes or greater ages. Interestingly, in the commercial tank, where average weight of fish exceeded 800g, there also was no difference in growth-related traits by gender. It is possible that the earlier sexual development of the hybrid striped bass results in a more marked gender dimorphism during commercial production while the later maturing striped bass do not manifest this difference until later in life. Higher female than male VSI in the pond samples may be a sign of the potential for greater growth by females due to greater digestive capacity, which has been observed in both tilapia (Toguyeni et al 1997) and European sea bass (Saillant et al. 2001b). The difference in percent fillet by gender, as seen in the Kent SeaTech sample, has not been reported for striped bass or white bass but the percentage difference observed here was very small, with mean percent fillet of 47.8% in males and 47.1% in females. The effect of genetic background on gender dimorphism in striped bass is unknown. There was no sire by gender

interaction for the pond-reared 2E55 family although the interaction was significant for the commercial tank.

Sex ratios—For both pond- and tank-reared progeny of dam 2E55, sex ratios were skewed in several of the sire families, especially for progeny of the two Chesapeake sires and of Santee:Chesapeake sire 7E27. The Santee:Chesapeake and Roanoke families generally had greater proportions of males than females. Conversely, the Chesapeake families had higher proportions of females than males. Similar disparities in sex ratios among sire families have been reported for the closely related European sea bass (Saillant et al. 2002, 2006). The result for Chesapeake fish is similar to that observed in wild European sea bass (Arias 1980, cited in Pavlidis et al. 2001), a species closely related to the striped bass, where females often outnumber males, especially at greater ages. However, many farmed sea bass populations are skewed in the opposite way, similar that seen in the Santee:Chesapeake strain, with higher percentages of males. This effect may be caused by conditions of captivity that alter the process of sex differentiation (Saillant et al. 2002). Although the process of gender differentiation has not been thoroughly investigated for striped bass, both genetic and environmental factors influence phenotypic sex in sea bass. Greater proportions of females are produced at lower temperatures, similar to those experienced by wild sea bass, and male-biased sex ratios are produced at higher temperatures (Blázquez et al. 1998; Pavlidis et al. 2001; Koumoundouros et al. 2002; Saillant et al. 2002), such as those experienced in commercial culture. The proportion of each sex also appears to be strongly influenced by parental effects, with significant additive sire and dam effects on progeny sex ratio (Saillant et al. 2002). Significant dam-temperature and sire-temperature interactions also are present for sea bass, indicating that the progeny of some sires and dams have greater sensitivity to

temperature influences on gender than do others. These results suggest that directional selection for sex ratio might be possible for sea bass, similar to the sex-ratio modifications suggested by Wohlfarth and Wedekind (1991) for tilapia. Saillant and colleagues (2002) suggested that non-sensitive lines might be produced for developing monosex populations of sea bass for aquaculture, for experimental investigations, or for eliminating temperature-dependent effects on sex determination in captive populations. Kohler et al. (2001) reported no difference in sex ratios among hybrid striped bass produced by several geographic strains of white bass, but the effects of genetic background on gender ratios is unknown for striped bass. The variation in sex ratios among the six striped bass sires in this study may indicate that modifications in gender ratios might be possible for a striped bass breeding program.

Alternatively, the skew in sex ratio for striped bass may be due to the standard husbandry practice of grading fingerlings several times at the end of Phase I to reduce cannibalism and remove stunted, smaller fish. If, as Davis and Ludwig (2004) observed in hybrid striped bass, male striped bass grow faster than females in the first year, the process of grading may selectively remove many smaller female fish, reducing the incidence of females at harvest. However, these authors did not observe any change any sex ratio in commercial ponds due to grading. In reciprocal hybrid striped bass sampled from PAFL from 40 days of age until 176 days of age, gender identification was possible as early as 140 days of age (B. Davis and C.V. Sullivan, *unpublished data*). Contrary to the results of Davis and Ludwig (2004), female hybrid striped bass were longer than males at younger ages. The genetic background of these fish was not determined and any association of genetic background with sex ratios in *Morone* species remains to be verified.

Predictive value of Phase II for Phase III—The interaction of rearing Phase and sire

was examined for progeny of dam 2E55 reared in research ponds in order to assess the

predictive value of Phase II sire family performance for Phase III performance. The

phenotypic traits of total length and body weight were examined since those traits can be

easily and non-destructively measured on both Phase II and Phase III striped bass. Data from

fish reared during Phase II (Chapter 4) in the same ponds were used for this analysis. For the

three PAFL research ponds, there was no significant interaction effect between sire and

Phase for total length or body weight. This information provides evidence for the

predictability of final harvest trait means from Phase II data collected 6-7 months prior to

harvest. In particular, examination of these traits by sire family across the two production

Phases revealed that rank orders of progeny performance for the top two sire families, each

from the Santee:Chesapeake strain, was the same for both Phase II, when fish were

approximately one year old, and Phase III, when fish were 19-20 months old. This

information may be very valuable for selective breeding since the Phase III performance of

families that are tested in PAFL research ponds may be predicted well before the fish reach

the end of Phase III rearing. Should the predictive nature hold for rearing of striped bass in

commercial ponds, the environment where more than 50% of foodfish are produced, or in

commercial tanks, breeders could greatly reduce the time and expense of performance testing

striped bass for market traits. Vandeputte et al. (2002) observed the same strain rankings for

body weight at five weeks of age as were seen in two summers-old carp and Saillant et al.

(2006) have observed a strong correlation of growth performance from 341 days post

fertilization to 818 days post fertilization in sea bass. Furthermore, Bosworth et al. (1997)

reported that weight at 40 days is a good predictor of weight at later life stages in hybrid

striped bass. Taken with the predictability of performance observed in other fishes, the results seen in the striped bass research ponds indicate that the predictive value of Phase II for Phase III is promising and worthy of more thorough investigation in both commercial ponds and tanks.

Use of research ponds for performance evaluations—Interestingly, when family Phase III trait means for total length and body weight are plotted for the research ponds and commercial tank (Figure 55), the two families with the highest performance in ponds (Santee:Chesapeake families) also performed best in the dramatically different growing conditions of the commercial production tank. The family with the lowest growth performance (Roanoke sire 4664) also was the worst performer in both the ponds and the tank. Although these effects were examined only for one tank and rank orders change somewhat for the other three sires, these results may prove valuable for a striped bass selective breeding program since they suggest that Phase III performance in the intensive commercial tank setting may be predicted by performance in the small research ponds. If this proves to be the case upon evaluation of additional families, this information may eliminate the necessity for testing progeny performance of some traits in the tank environment. Instead, traits could be evaluated much more economically in small research ponds. However, significant genotype environment interactions have been shown for growth-related traits among fish strains (Iwamoto et al. 1986; Wohlfarth and Moav 1991) and within species (Saillant et al. 2006). Until such interactions are evaluated in striped bass, all traits of importance should be evaluated in both types of rearing environments.

Sire-dam interactions—Examination of interaction plots between the dams and sires for the 12-family group revealed significant interaction effects for total length and body

weight; however, there were no differences in performance of any sire family between the two dams. In general, one Santee:Chesapeake sire produced better performing progeny when crossed with the Roanoke dam (mean body weight 434.98g) than with the Santee:Chesapeake dam (mean body weight 399.51g) while the other Santee:Chesapeake sire produced progeny that performed quite similarly (mean body weights 483.98g or 478.62g) when crossed with either dam. Although not significantly different between dams, progeny of the remaining four sires all had higher trait values when crossed with the Santee:Chesapeake dam. Strain-dam interaction also was seen in the 12-family group, with significant differences between progeny of the two dams when the sires originated from either the Roanoke or Chesapeake strain. In all of these cases, progeny of the Santee:Chesapeake dam had higher performance than those of the Roanoke dam.

The variation in performance between some sire-dam and strain-dam combinations observed here indicates that further investigation of cross combinations is needed for captive strains of striped bass. Such investigations should provide valuable information about the breadth of variation present within the captive broodstock and about the value of crosses within particular strains of striped bass. Heterosis has been reported for crosses among carp (Moav and Wohlfarth 1974) and catfish (Dunham 1986) strains, and the effects of sire-dam, strain-dam and strain-strain interactions should be evaluated in greater detail in a striped bass selective breeding program.

Potential broodstock selected from performance tested groups—For the 102 striped bass selected as potential broodstock at Kent SeaTech Corporation and returned to PAFL, genotyping indicated that more than 36% were from the Santee:Chesapeake sires, 58% were from the Chesapeake sires and 6% were from the Roanoke sires. Only six fish from the

Roanoke strain were selected as broodfish. The group of ~100 broodfish retained at Kent SeaTech was not sampled or genotyped until 2003. During that time, the group suffered losses due to disease and culling of fish. In this group, 57% of the remaining fish were Santee:Chesapeake and 32% were from the Chesapeake sires. No fish from the Roanoke sire 4664 remained and only 11% were from the other Roanoke sire. In the sample of broodfish reared at PAFL, 81% were from the Santee:Chesapeake strain and 17% were from the Chesapeake lineage. Only one fish from a Roanoke sire was chosen as a broodfish and no fish were chosen from Roanoke sire 4664. All of these broodfish were selected by visual inspection on the basis of body size and general health, with no foreknowledge of their genetic background. Differences in proportions among strains of striped bass chosen as broodstock reflect the results of phenotypic analyses described for Phase III, with progeny of the Santee:Chesapeake sires having better growth performance in the research ponds and progeny of the Santee:Chesapeake and one Chesapeake sire having the best performance in the commercial tank. In general, fish sired by Roanoke males performed poorly overall.

Hybrid striped bass progeny have been produced from both PAFL and Kent SeaTech 2E55 family broodstock for comparison against industry standard hybrids produced from wild parents. The results of these investigations should be available in Fall 2007. Evaluation of numerous families during a selective breeding program will be necessary in order to avoid reductions in genetic diversity due to selection of broodfish from only a few top sires. Considerations for such a breeding program are discussed in Chapter 6. Performance of select sires for production of hybrid striped bass and evaluations of improved striped bass compared with hybrids will be important for the National Breeding Program.

In summary, use of microsatellite markers during Phase III communal rearing trials proved a practical approach for progeny identification of striped bass performance tested in communal groups within several research ponds and in a commercial tank. Parentage of individuals reared in both 6- and 12-family communal groups were identified with a high degree of success. Application of microsatellite markers and a communal rearing approach should reduce the impact of such testing on the rearing capacity of participating commercial farms and evidence of adequate additive genetic variation should enable selective improvement of striped bass. Analysis of performance traits revealed significant sire effects on variation in phenotypic traits related to growth performance, body shape, and various carcass traits, providing evidence that genetic variation is present within the captive PAFL striped bass broodstock population for future selection efforts. In particular, progeny of domesticated sires from a Santee:Chesapeake broodstock group produced at PAFL demonstrated high performance both in research ponds and in a commercial production tank. Such variation may be exploited in a program of selective breeding for striped bass to benefit the United States hybrid striped bass industry. The similarity of sire rankings for growth-related traits between the PAFL research ponds and the commercial tank suggests promise for economical evaluation of such traits at research scale, rather than at commercial scale. Additionally, the predictive value of Phase II phenotypic trait means for Phase III performance in research ponds indicates that selective breeding of striped bass might be accelerated by the ability to predict the value of phenotypic traits six to eight months earlier than Phase III rearing trials can be accomplished. Further investigation of these promising avenues of research should be included in microsatellite marker-assisted communal rearing trials of striped bass in the National Breeding Program.

ACKNOWLEDGMENTS

Table 19. Estimated pond stocking rates, fingerling recovery rates and survival for five research ponds and a commercial production tank (Tank40); fish were sampled but not fully harvested from Tank 40, thus survival could not be estimated.

Pond or tank	Dam	Date of stocking	No. fingerlings stocked	Date of harvest	No. fingerlings recovered	No. days Phase III production	Phase III survival rate
A1	152D + 5F4B	04/10/02	870	--	--	--	0.000
A2	152D + 5F4B	04/10/02	870	11/16/02	401	216	0.461
A4	2E55	05/24/02	1000	12/13/02	936	229	0.936
A11	2E55	05/23/02	1000	01/06/02	858	223	0.858
A14	2E55	05/30/02	1000	12/09/02	937	189	0.937
Tank 40	2E55	11/13/02	~17,758 (initial no. Phase I fish)	10/29-31/02	--	346-348	--

Table 20. Numbers (*N*) and estimated contributions by sire (proportions plus or minus standard errors) of fingerlings assigned parentage in the Phase III research pond samples for dam 2E55 (ponds A4, A11, A14), dams 152D and 5F4B (pond A2) and the final 2E55 commercial tank sample (tank 40).

POND	SIRE	Larvae	
		Genotyped	Proportion (SE)
A4	4664 (R:F_0-97)	14	0.047 (0.012)
	7213 (R:F_0-97)	24	0.080 (0.016)
	292B (SC:F_1-94)	64	0.214 (0.024)
	7E27 (SC:F_1-94)	54	0.181 (0.022)
	631D (C:F_2-91)	40	0.134 (0.020)
	2A20 (C:F_2-91)	103	0.344 (0.027)
	N	299 of 299	
A11	4664 (R:F_0-97)	11	0.037 (0.011)
	7213 (R:F_0-97)	32	0.108 (0.018)
	292B (SC:F_1-94)	51	0.172 (0.022)
	7E27 (SC:F_1-94)	60	0.202 (0.023)
	631D (C:F_2-91)	21	0.071 (0.015)
	2A20 (C:F_2-91)	122	0.411 (0.028)
	N	297 of 301	
A14	4664 (R:F_0-97)	16	0.040 (0.010)
	7213 (R:F_0-97)	34	0.085 (0.014)
	292B (SC:F_1-94)	94	0.235 (0.021)
	7E27 (SC:F_1-94)	93	0.232 (0.021)
	631D (C:F_2-91)	41	0.102 (0.015)
	2A20 (C:F_2-91)	122	0.305 (0.023)
	N	400 of 400	
Tank 40	4664 (R:F_0-97)	32	0.082 (0.014)
	7213 (R:F_0-97)	72	0.185 (0.020)
	292B (SC:F_1-94)	64	0.164 (0.019)
	7E27 (SC:F_1-94)	53	0.136 (0.017)
	631D (C:F_2-91)	84	0.216 (0.021)
	2A20 (C:F_2-91)	84	0.216 (0.021)
	N	389 of 392	
A2	5442 (R:F_0-97)	43	0.107 (0.015)
	3B62 (R:F_0-97)	29	0.072 (0.013)
	3F11 (SC:F_1-94)	77	0.192 (0.020)
	5C5D (SC:F_1-94)	40	0.100 (0.015)
	631D (C:F_2-91)	86	0.214 (0.020)
	2A20 (C:F_2-91)	126	0.314 (0.023)
	N	401 of 401	

Table 21. Estimated survival (SE) by sire and strain for progeny of dam 2E55 reared in three Phase III research ponds, A4, A11 and A14.

POPULATION	SIRE or STRAIN	ESTIMATED SURVIVAL
Pond A4	4664 (R:F_0-97)	1.000 (0.434)
	7213 (R:F_0-97)	0.417 (0.103)
	292B (SC:F_1-94)	1.000 (0.210)
	7E27 (SC:F_1-94)	1.000 (0.224)
	631D (C:F_2-91)	0.835 (0.187)
	2A20 (C:F_2-91)	0.871 (0.106)
	R:F_0-97	0.541 (0.109)
	SC:F_1-94	1.000 (0.139)
	C:F_2-91	0.861 (0.078)
Pond A11	4664 (R:F_0-97)	0.710 (0.312)
	7213 (R:F_0-97)	1.000 (0.280)
	292B (SC:F_1-94)	0.823 (0.162)
	7E27 (SC:F_1-94)	0.893 (0.165)
	631D (C:F_2-91)	0.271 (0.067)
	2A20 (C:F_2-91)	1.000 (0.136)
	R:F_0-97	0.925 (0.211)
	SC:F_1-94	0.859 (0.102)
	C:F_2-91	0.839 (0.078)
Pond A14	4664 (R:F_0-97)	0.942 (0.400)
	7213 (R:F_0-97)	1.000 (0.314)
	292B (SC:F_1-94)	1.000 (0.180)
	7E27 (SC:F_1-94)	1.000 (0.170)
	631D (C:F_2-91)	0.495 (0.102)
	2A20 (C:F_2-91)	0.845 (0.105)
	R:F_0-97	1.000 (0.245)
	SC:F_1-94	1.000 (0.108)
	C:F_2-91	0.717 (0.064)

Table 22. Least squares means (SE) for phenotypic trait values for Phase III experimental striped bass families by dam and rearing unit. Remaining missing values indicate that data were not collected for a group.

DAM	N	SIRE	Body weight (g)	Total length (cm)	Condition factor	VSI
2E55 (R:F_0-97) (research pond A4)	14	4664 (R:F_0-97)	300.66 (20.18)	29.45 (0.50)	1.16 (0.02)	9.70 (0.33)
	24	7213 (R:F_0-97)	329.78 (9.85)	30.50 (0.24)	1.16 (0.01)	9.28 (0.16)
	64	292B (SC:F_1-94)	345.97 (7.79)	30.80 (0.19)	1.18 (0.01)	9.58 (0.13)
	54	7E27 (SC:F_1-94)	382.85 (6.99)	31.90 (0.17)	1.17 (0.01)	9.33 (0.11)
	40	631D (C:F_2-91)	310.18 (11.60)	29.70 (0.29)	1.18 (0.01)	9.28 (0.19)
	103	2A20 (C:F_2-91)	305.38 (6.31)	29.99 (0.16)	1.12 (0.01)	9.55 (0.10)
2E55 (R:F_0-97) (research pond A11)	11	4664 (R:F_0-97)	326.22 (15.27)	30.01 (0.38)	1.20 (0.02)	10.35 (0.25)
	32	7213 (R:F_0-97)	355.31 (11.18)	30.93 (0.28)	1.20 (0.01)	9.51 (0.18)
	51	292B (SC:F_1-94)	361.32 (6.67)	30.98 (0.16)	1.21 (0.01)	9.97 (0.11)
	60	7E27 (SC:F_1-94)	405.28 (7.21)	32.04 (0.18)	1.22 (0.01)	9.76 (0.12)
	21	631D (C:F_2-91)	335.33 (13.61)	30.22 (0.34)	1.20 (0.02)	9.87 (0.22)
	122	2A20 (C:F_2-91)	326.53 (6.85)	30.22 (0.17)	1.18 (0.01)	9.94 (0.11)
2E55 (R:F_0-97) (research pond A14)	16	4664 (R:F_0-97)	419.38 (13.33)	31.98 (0.33)	1.28 (0.02)	11.03 (0.22)
	34	7213 (R:F_0-97)	448.70 (10.44)	32.75 (0.26)	1.28 (0.01)	10.10 (0.17)
	94	292B (SC:F_1-94)	482.44 (5.48)	33.50 (0.14)	1.28 (0.01)	10.84 (0.09)
	93	7E27 (SC:F_1-94)	519.34 (5.64)	34.10 (0.14)	1.31 (0.01)	10.71 (0.09)
	41	631D (C:F_2-91)	447.52 (8.50)	32.57 (0.21)	1.29 (0.01)	10.88 (0.14)
	122	2A20 (C:F_2-91)	438.04 (6.31)	32.80 (0.16)	1.24 (0.01)	10.97 (0.10)
2E55 (R:F_0-97) (commercial tank)	32	4664 (R:F_0-97)	762.54 (29.50)	38.20 (0.38)	1.34 (0.02)	10.36 (0.18)
	72	7213 (R:F_0-97)	855.22 (19.45)	39.90 (0.25)	1.33 (0.01)	8.84 (0.12)
	64	292B (SC:F_1-94)	982.34 (20.13)	41.70 (0.26)	1.34 (0.01)	9.95 (0.12)
	53	7E27 (SC:F_1-94)	1004.68 (23.13)	41.80 (0.30)	1.37 (0.01)	9.60 (0.14)
	84	631D (C:F_2-91)	903.93 (24.50)	40.16 (0.32)	1.38 (0.01)	9.66 (0.15)
	84	2A20 (C:F_2-91)	944.80 (29.23)	41.60 (0.38)	1.30 (0.02)	9.98 (0.18)

Table 22, continued.

DAM	N	SIRE	Body weight (g)	Total length (cm)	Condition factor	VSI
152D (SC:F_1-94) (research pond A2)	6	3B62 (R:F_0-97)	489.90 (38.41)	33.95 (0.91)	1.24 (0.02)	--
	25	5442 (R:F_0-97)	468.30 (19.21)	33.23 (0.45)	1.25 (0.01)	--
	30	3F11 (SC:F_1-94)	483.98 (17.18)	33.78 (0.41)	1.22 (0.01)	--
	16	5C5D (SC:F_1-94)	399.51 (24.29)	31.67 (0.57)	1.23 (0.02)	--
	64	631D (C:F_2-91)	479.84 (12.25)	33.43 (0.29)	1.27 (0.01)	--
	47	2A20 (C:F_2-91)	466.75 (14.69)	33.83 (0.35)	1.19 (0.01)	--
5F4B (R:F_0-97) (research pond A2)	23	3B62 (R:F_0-97)	389.29 (20.53)	31.97 (0.48)	1.18 (0.01)	--
	18	5442 (R:F_0-97)	376.42 (22.18)	31.15 (0.52)	1.23 (0.02)	--
	47	3F11 (SC:F_1-94)	478.62 (14.03)	33.82 (0.33)	1.23 (0.01)	--
	24	5C5D (SC:F_1-94)	434.98 (18.82)	32.59 (0.44)	1.24 (0.01)	--
	22	631D (C:F_2-91)	427.00 (20.53)	32.30 (0.48)	1.25 (0.01)	--
	79	2A20 (C:F_2-91)	416.10 (10.79)	32.53 (0.26)	1.19 (0.01)	--

Table 23. Least squares means (SE) and sample sizes by sire for gonadosomatic index (GSI) and antimicrobial peptide (AMP) activity for Phase III experimental striped bass families. The missing value for antimicrobial peptide activity in pond A14 (--) is due to inadequate sampling of fish ($n=2$) from the 4664 sire family in both ponds. Remaining missing values indicate that trait data were not collected for a group.

DAM	SIRE	N (male/female)	GSI (male/female)	N	AMP units of activity
2E55 (R:F_0-97)	4664 (R:F_0-97)	21 / 10	1.10 (0.14) / 0.33 (0.04)	2	--
(research pond A4)	7213 (R:F_0-97)	9 / 2	0.61 (0.09) / 0.28 (0.02)	6	2.43 (1.26)
	292B (SC:F_1-94)	35 / 16	1.26 (0.07) / 0.40 (0.02)	9	4.21 (1.15)
	7E27 (SC:F_1-94)	39 / 21	1.53 (0.07) / 0.35 (0.01)	14	3.49 (1.06)
	631D (C:F_2-91)	8 / 13	0.69 (0.15) / 0.40 (0.02)	5	3.35 (1.35)
	2A20 (C:F_2-91)	20 / 102	0.92 (0.10) / 0.44 (0.01)	24	3.76 (1.32)
2E55 (R:F_0-97)	4664 (R:F_0-97)	16 / 8	0.41 (0.13) / 0.31 (0.03)	--	--
(research pond A11)	7213 (R:F_0-97)	10 / 4	0.30 (0.11) / 0.23 (0.02)	--	--
	292B (SC:F_1-94)	40 / 24	0.44 (0.07) / 0.34 (0.01)	--	--
	7E27 (SC:F_1-94)	33 / 21	0.68 (0.07) / 0.29 (0.01)	--	--
	631D (C:F_2-91)	4 / 36	0.27 (0.21) / 0.36 (0.01)	--	--
	2A20 (C:F_2-91)	17 / 86	0.58 (0.10) / 0.41 (0.01)	--	--
2E55 (R:F_0-97)	4664 (R:F_0-97)	26 / 8	0.23 (0.13) / 0.25 (0.02)	2	--
(research pond A14)	7213 (R:F_0-97)	10 / 6	0.22 (0.08) / 0.23 (0.02)	4	4.54 (1.33)
	292B (SC:F_1-94)	58 / 36	0.42 (0.06) / 0.30 (0.01)	11	7.78 (1.10)
	7E27 (SC:F_1-94)	61 / 32	0.51 (0.05) / 0.25 (0.01)	13	4.90 (1.46)
	631D (C:F_2-91)	14 / 27	0.22 (0.11) / 0.28 (0.01)	6	6.20 (1.05)
	2A20 (C:F_2-91)	20 / 102	0.51 (0.10) / 0.32 (0.01)	20	6.65 (1.51)
2E55 (R:F_0-97)	4664 (R:F_0-97)	47 / 25	0.20 (0.01) / 0.41 (0.03)	4	2.64 (0.57)
(commercial tank)	7213 (R:F_0-97)	20 / 11	0.17 (0.01) / 0.40 (0.02)	6	1.08 (0.81)
	292B (SC:F_1-94)	39 / 25	0.22 (0.01) / 0.52 (0.02)	5	0.92 (0.78)
	7E27 (SC:F_1-94)	36 / 17	0.21 (0.01) / 0.46 (0.02)	1	--
	631D (C:F_2-91)	12 / 72	0.18 (0.01) / 0.55 (0.01)	7	0.89 (0.75)
	2A20 (C:F_2-91)	8 / 75	0.22 (0.01) / 0.53 (0.01)	2	--

Table 24. Numbers of male and female progeny for each sire family by rearing unit with calculated Chi-square values and P-values. Gender ratios are different at $P \leq 0.05$.

DAM	SIRE	NO. FEMALES	NO. MALES	X^2	P-VALUE
2E55 (R:F_0-97)	4664 (R:F_0-97)	12	29	6.24	0.25<P<0.50
(research ponds)	7213 (R:F_0-97)	26	64	15.21	<0.0001
	292B (SC:F_1-94)	76	133	15.00	<0.0001
	7E27 (SC:F_1-94)	74	133	16.25	<0.0001
	631D (C:F_2-91)	76	26	23.54	<0.0001
	2A20 (C:F_2-91)	290	57	155.11	<0.0001
2E55 (R:F_0-97)	4664 (R:F_0-97)	11	21	2.53	0.75<P>0.90
(commercial tank)	7213 (R:F_0-97)	26	46	5.01	0.25<P>0.50
	292B (SC:F_1-94)	25	39	2.64	0.75<P>0.90
	7E27 (SC:F_1-94)	17	36	6.11	0.01<P>0.025
	631D (C:F_2-91)	72	12	41.44	<0.0001
	2A20 (C:F_2-91)	75	8	52.48	<0.0001
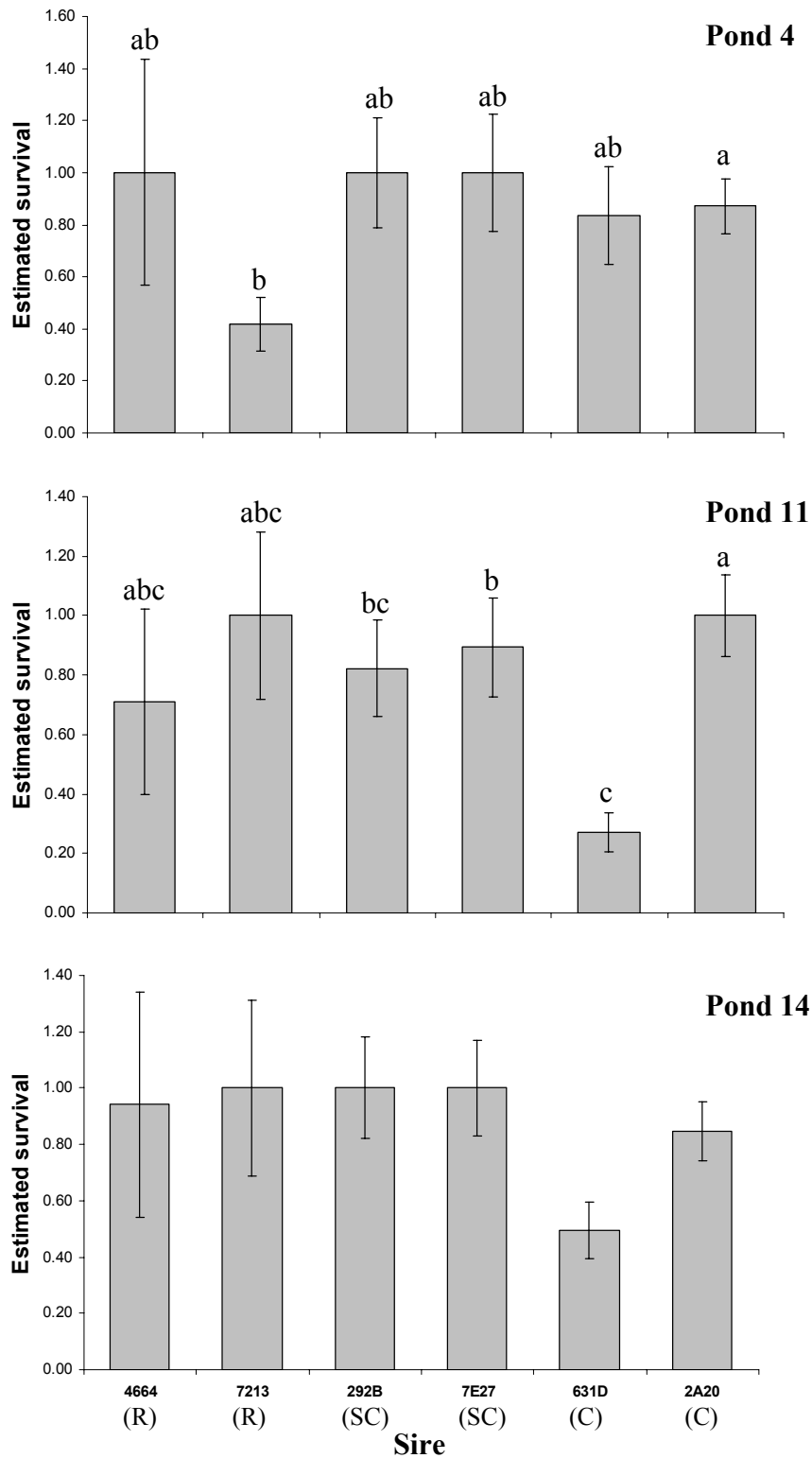
Figure 22. Estimated survival (±SE) by sire family for 2E55 Phase III pond samples. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Estimates with shared letters over the error bars are not significantly different.
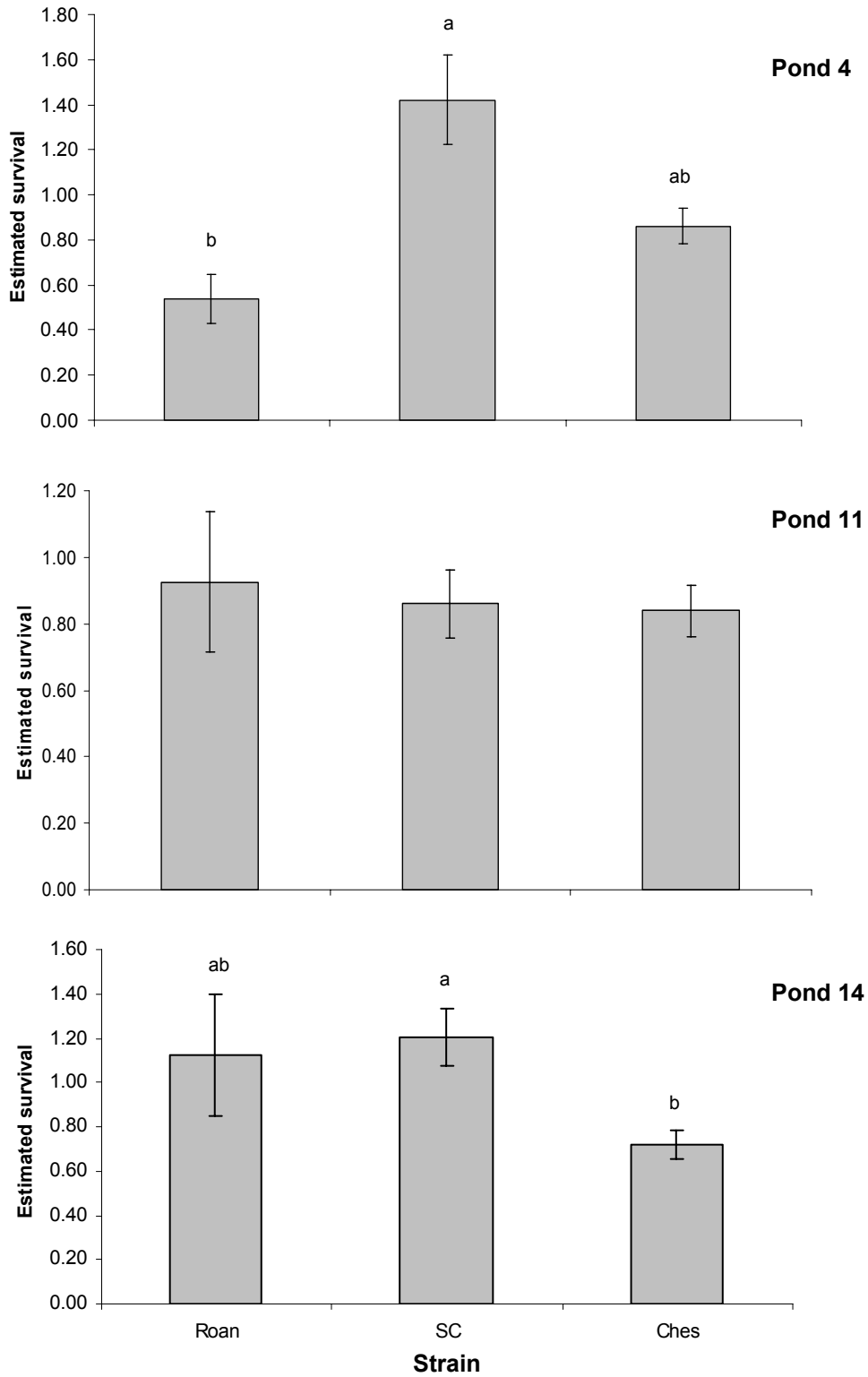
Figure 23. Estimated survival (±SE) by strain for 2E55 Phase III pond samples. Strains are Chesapeake (Ches.), Santee:Chesapeake (S:C), and Roanoke (R). Means with shared letters over the error bars are not significantly different.
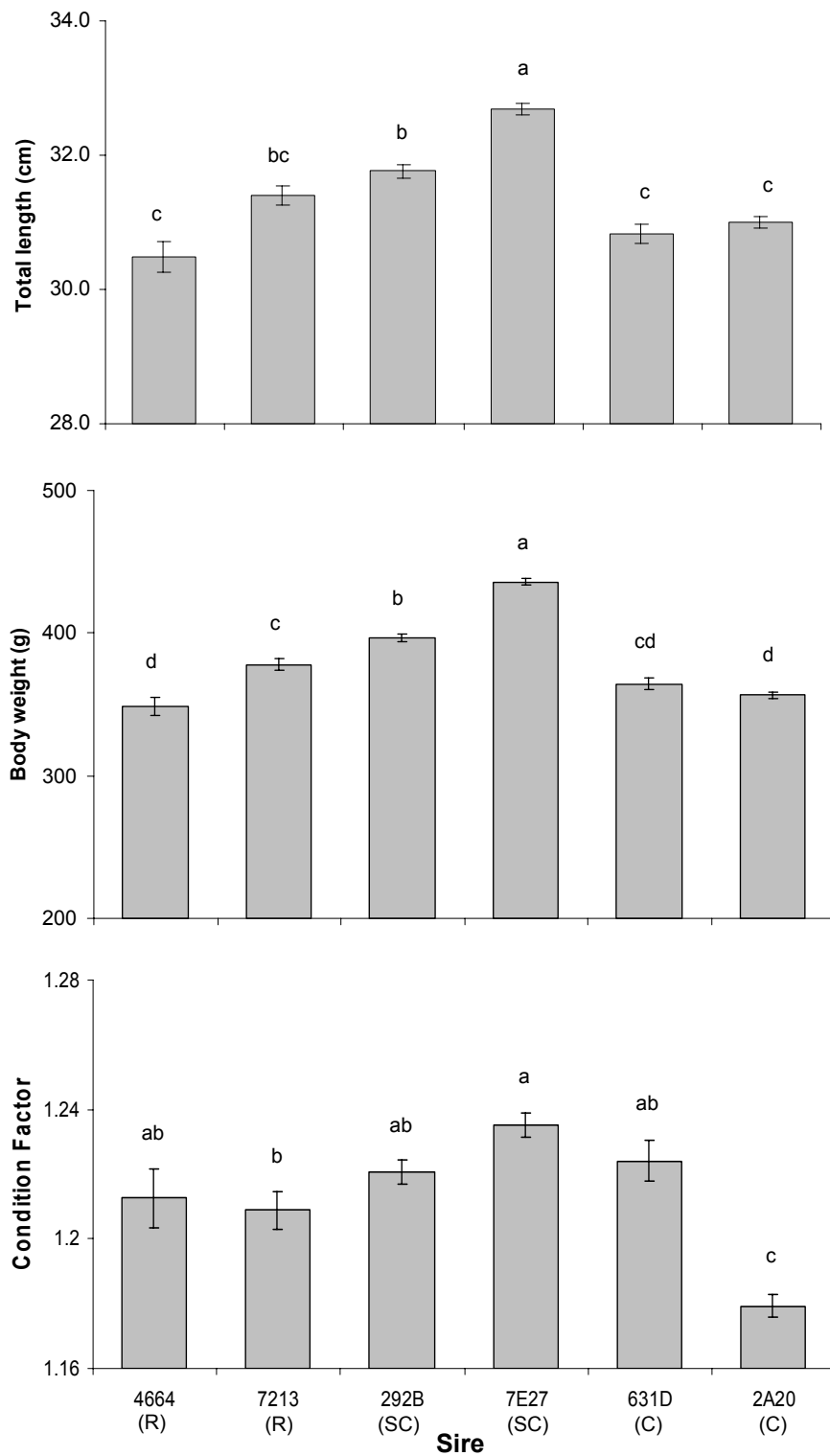
Figure 24. Least squares means (±SE) by sire family for 2E55 Phase III pond harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
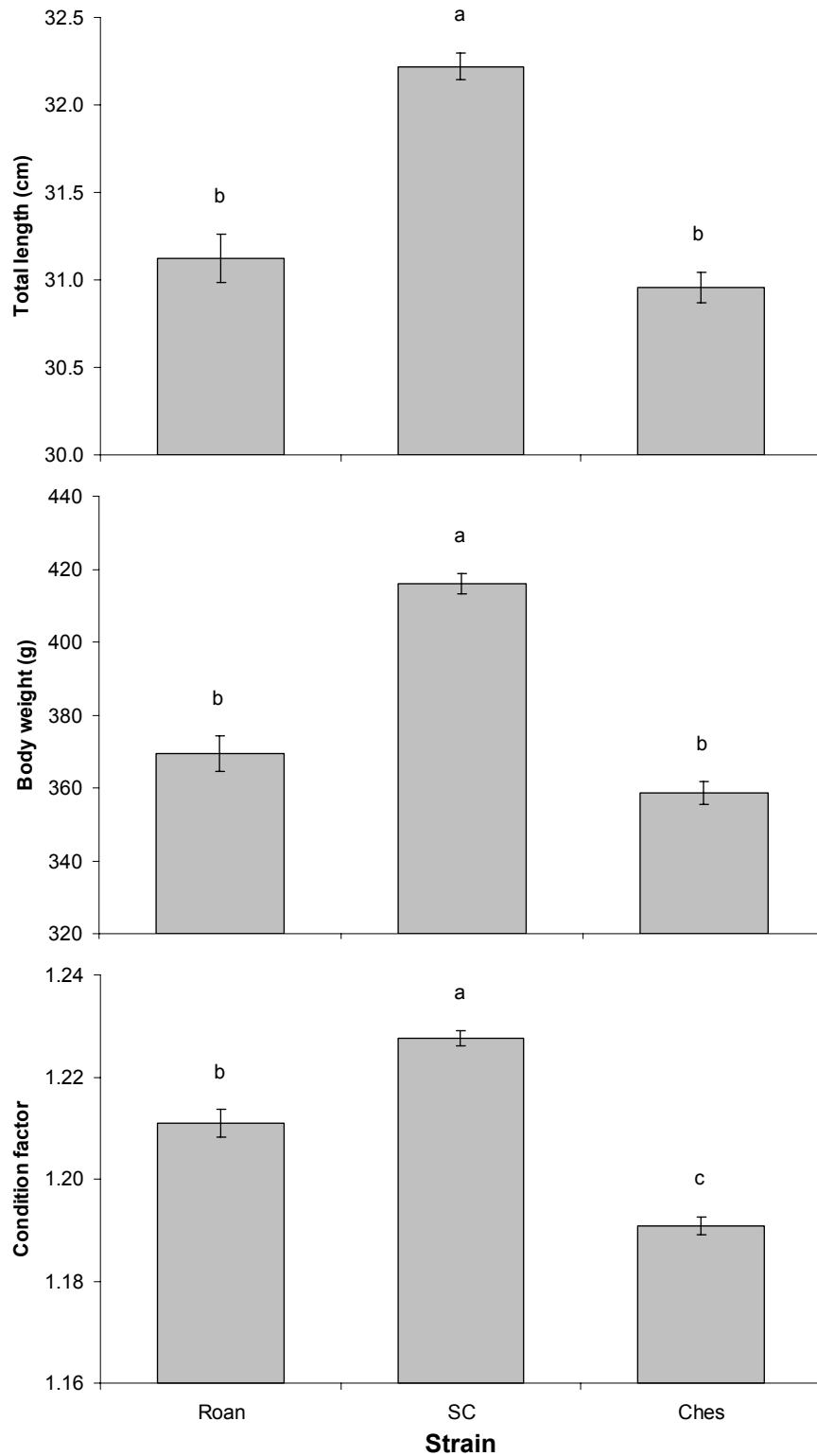
Figure 25. Least squares means (±SE) by strain for 2E55 Phase III final pond harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
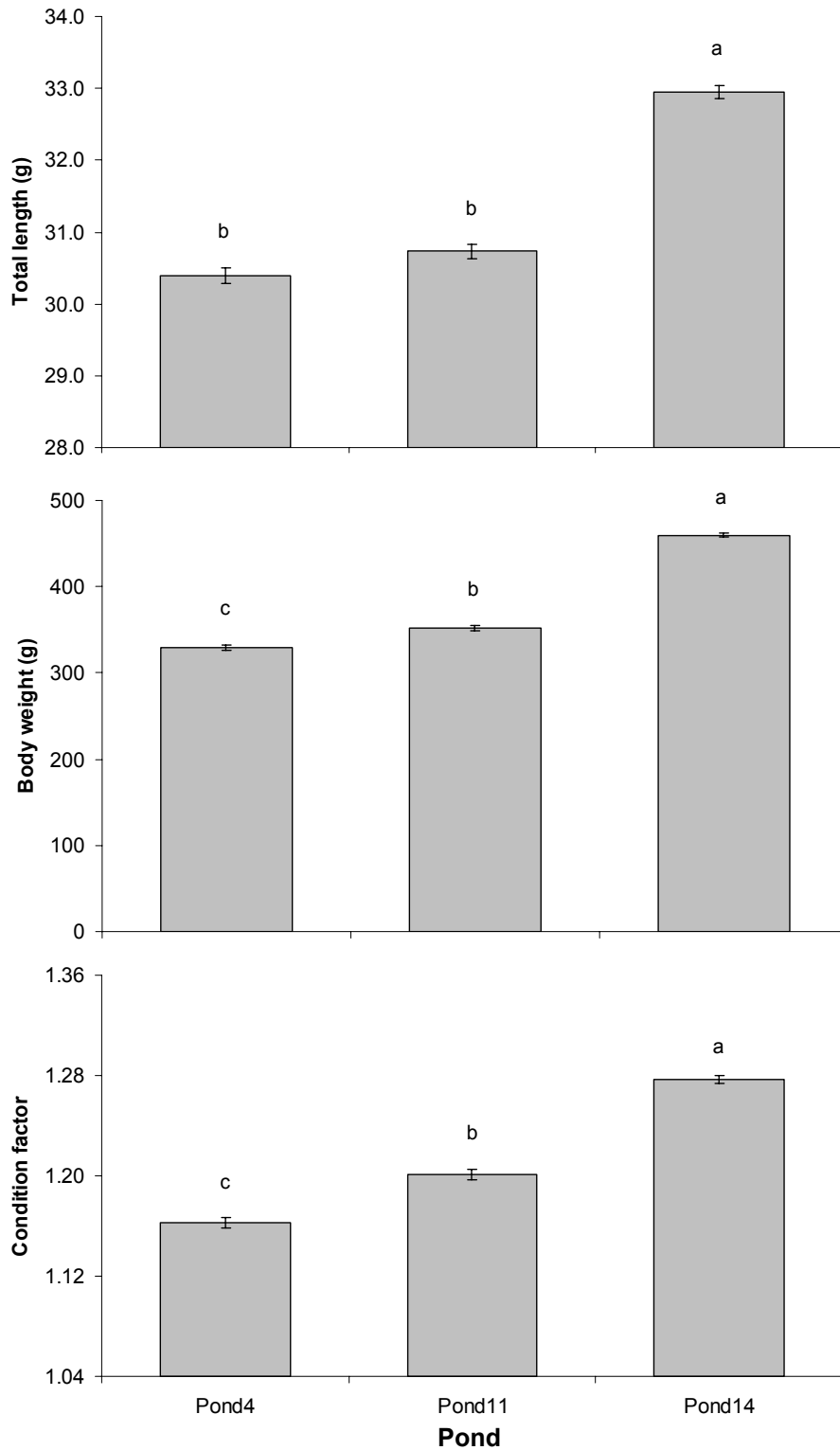
Figure 26. Least squares means (±SE) by pond for 2E55 Phase III final pond fingerling traits, including total length, body weight and condition factor. Means with shared letters over the error bars are not significantly different.
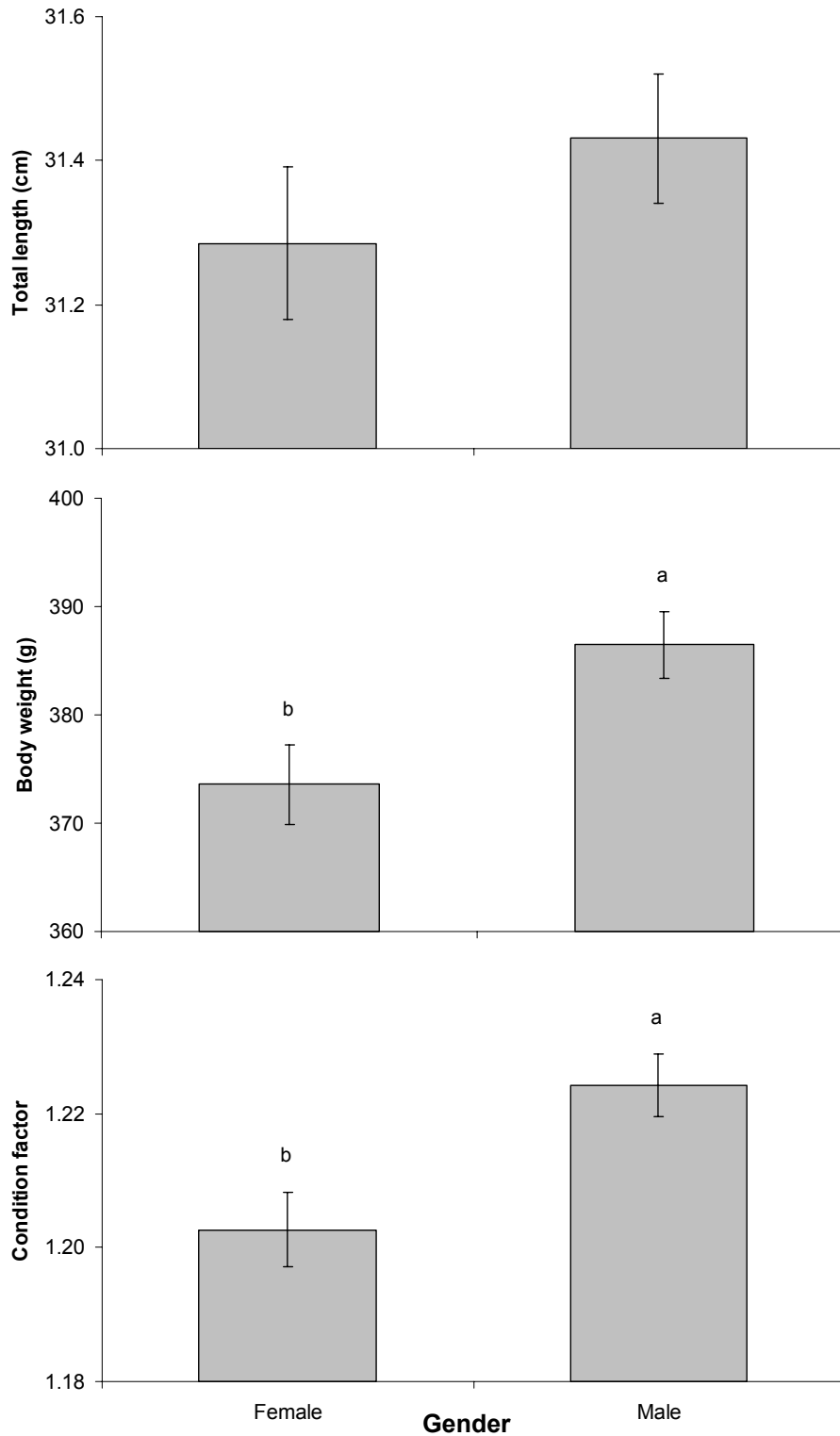
Figure 27. Least squares means (±SE) by gender for 2E55 Phase III final pond fingerling traits, including total length, body weight and condition factor. Means with shared letters over the error bars are not significantly different.
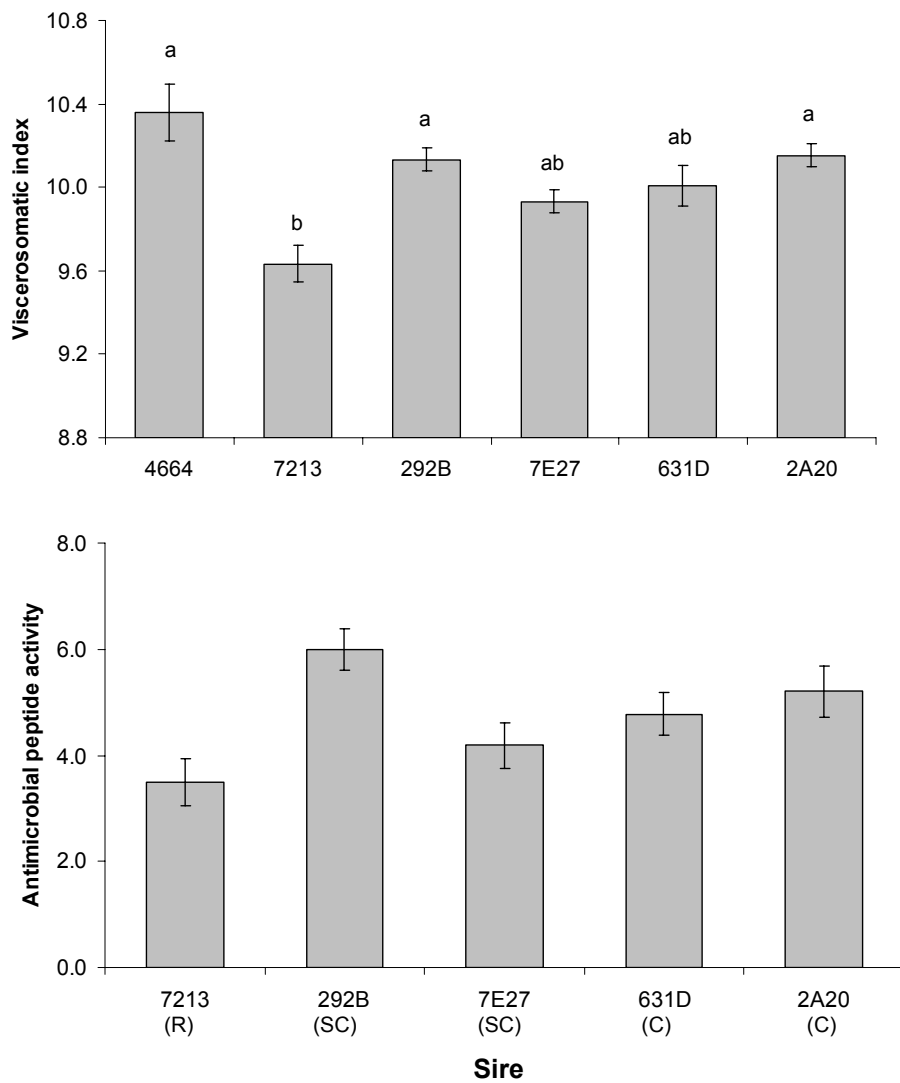
Figure 28. Least squares means (±SE) by sire family for 2E55 Phase III pond fingerling traits, including viscerosomatic index and antimicrobial peptide activity. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
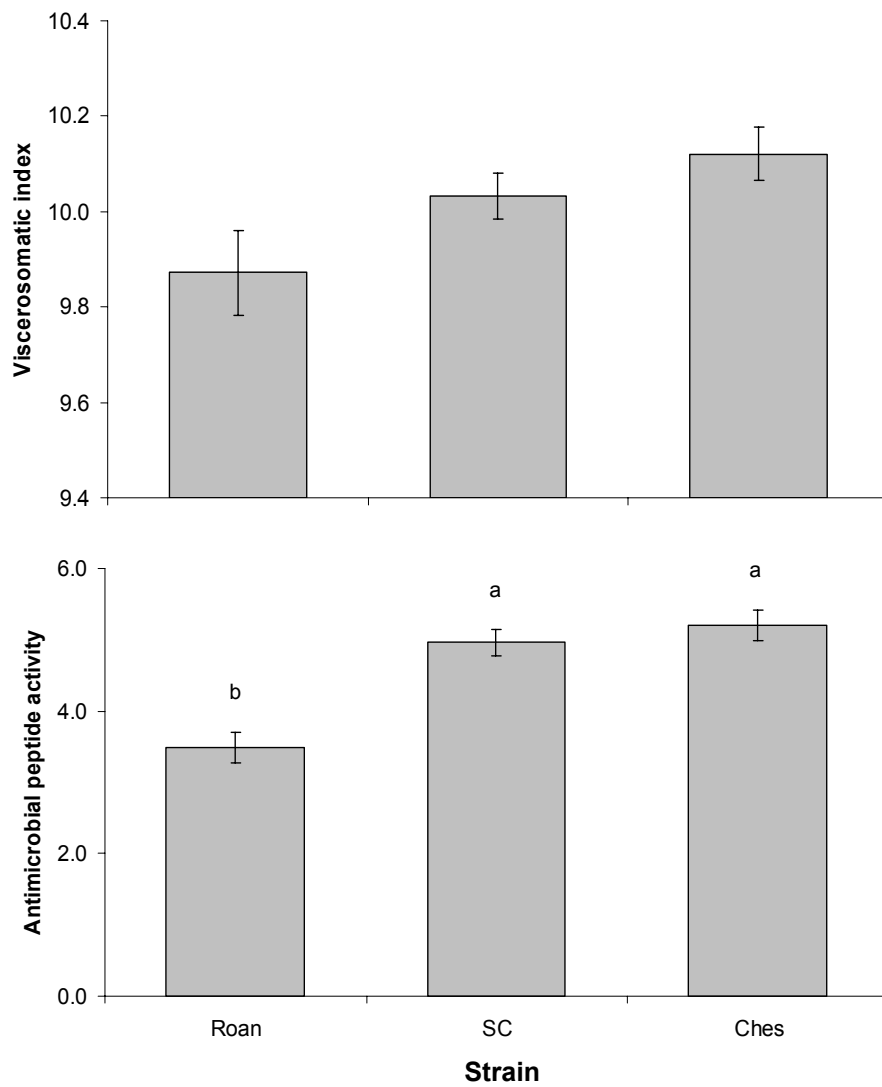
Figure 29. Least squares means (±SE) by strain for 2E55 Phase III final pond viscerosomatic index and antimicrobial peptide activity. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
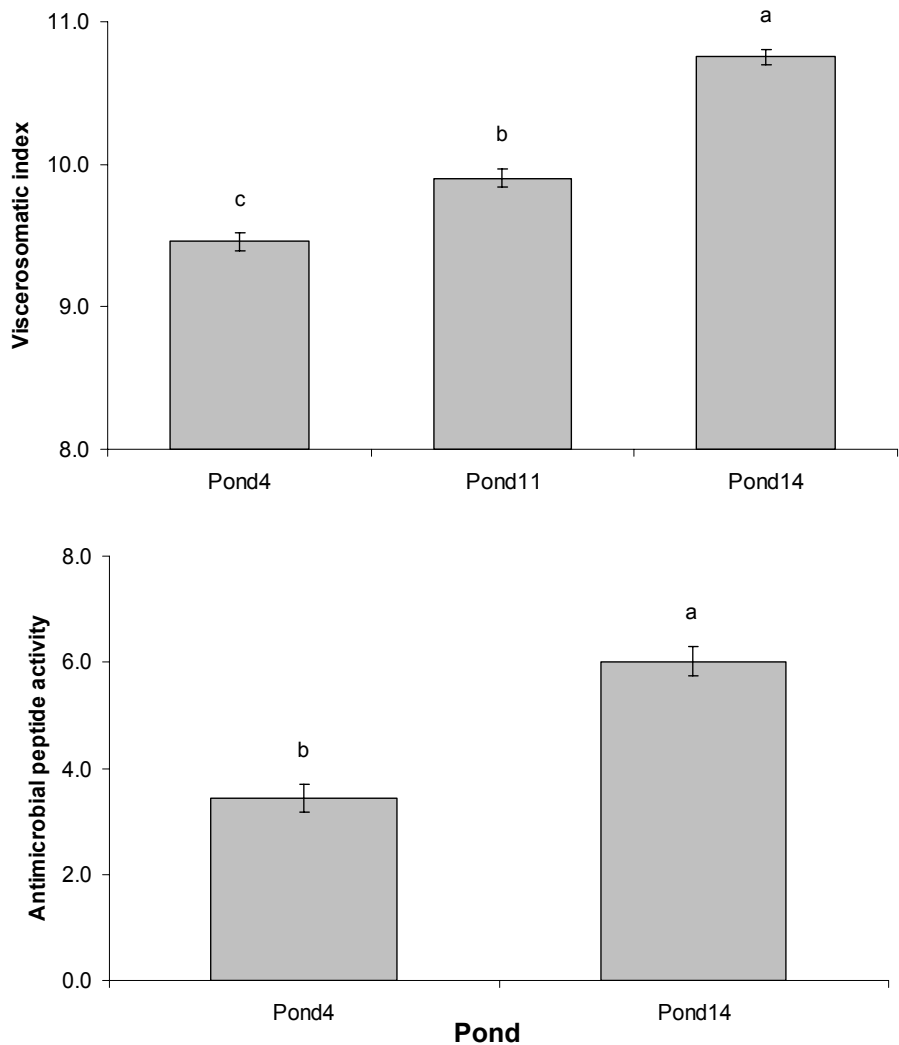
Figure 30. Least squares means (±SE) by pond for 2E55 Phase III final pond viscerosomatic index and antimicrobial peptide activity. Means with shared letters over the error bars are not significantly different.
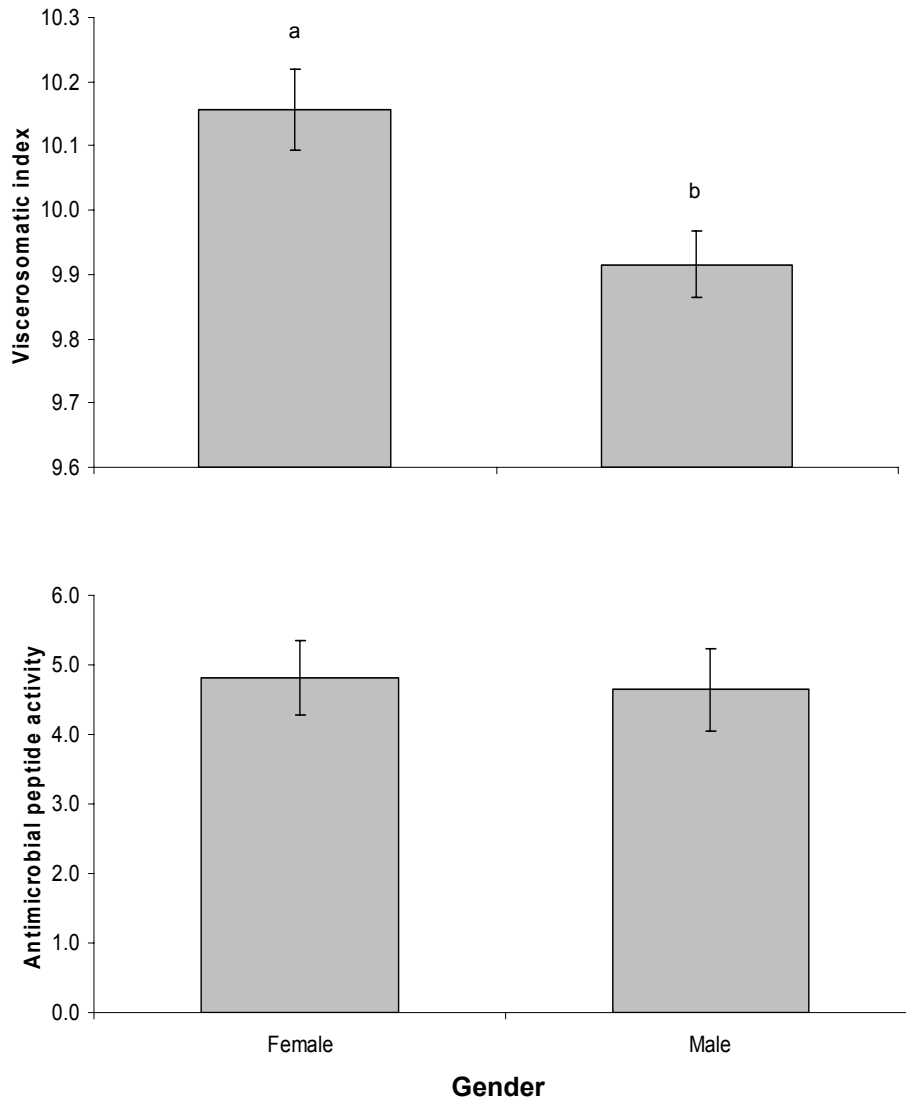
Figure 31. Least squares means (±SE) by gender for 2E55 Phase III final pond viscerosomatic index and antimicrobial peptide activity. Means with shared letters over the error bars are not significantly different.
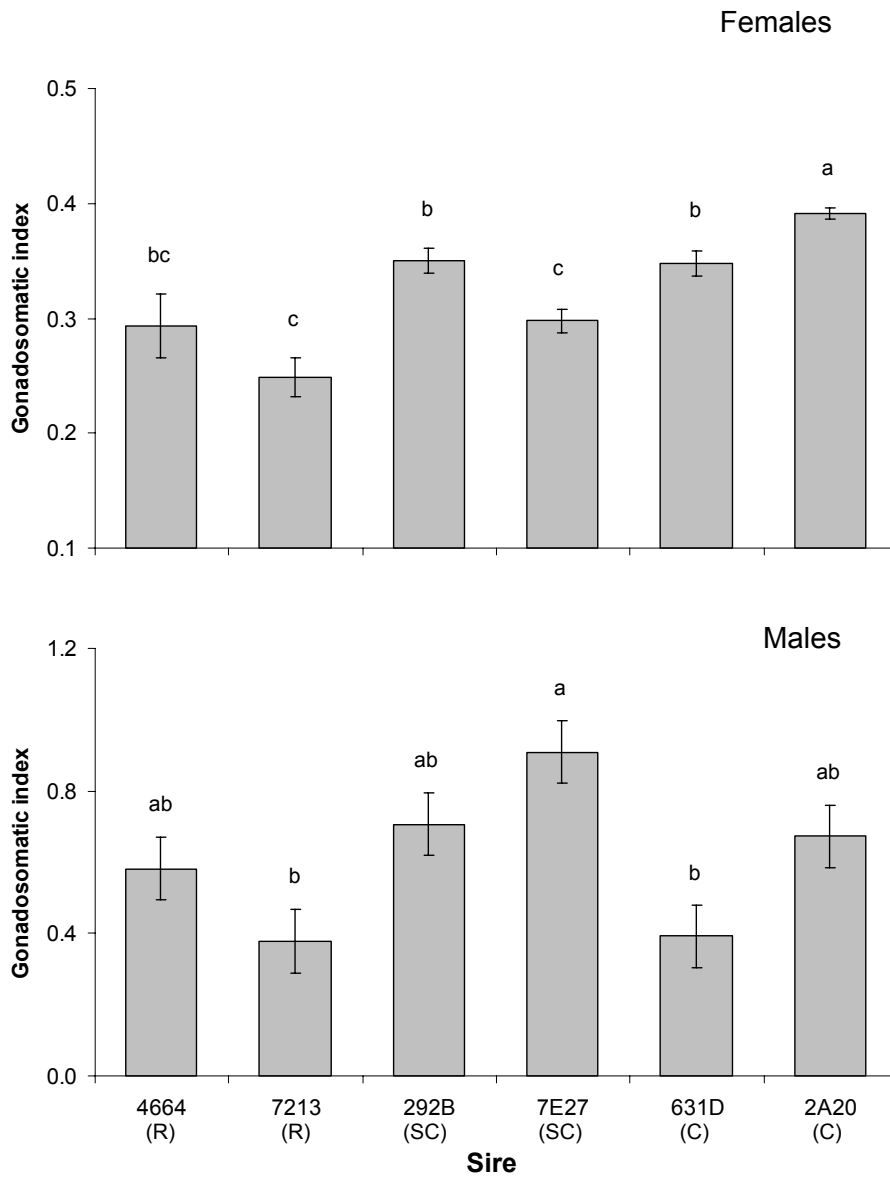
Figure 32. Least squares means (±SE) by gender for 2E55 Phase III pond harvest gonadosomatic index. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
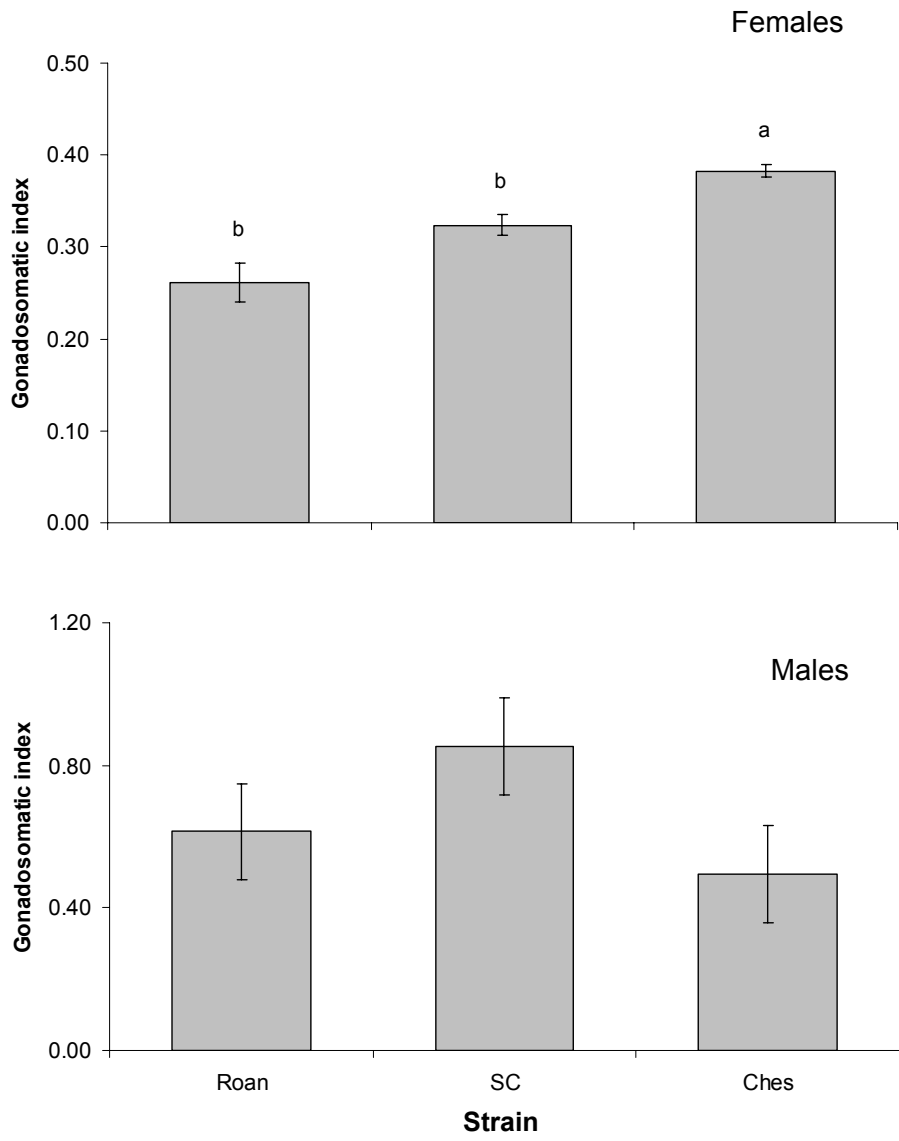
Figure 33. Least squares means (±SE) by strain for 2E55 Phase III final pond gonadosomatic index for females and males. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
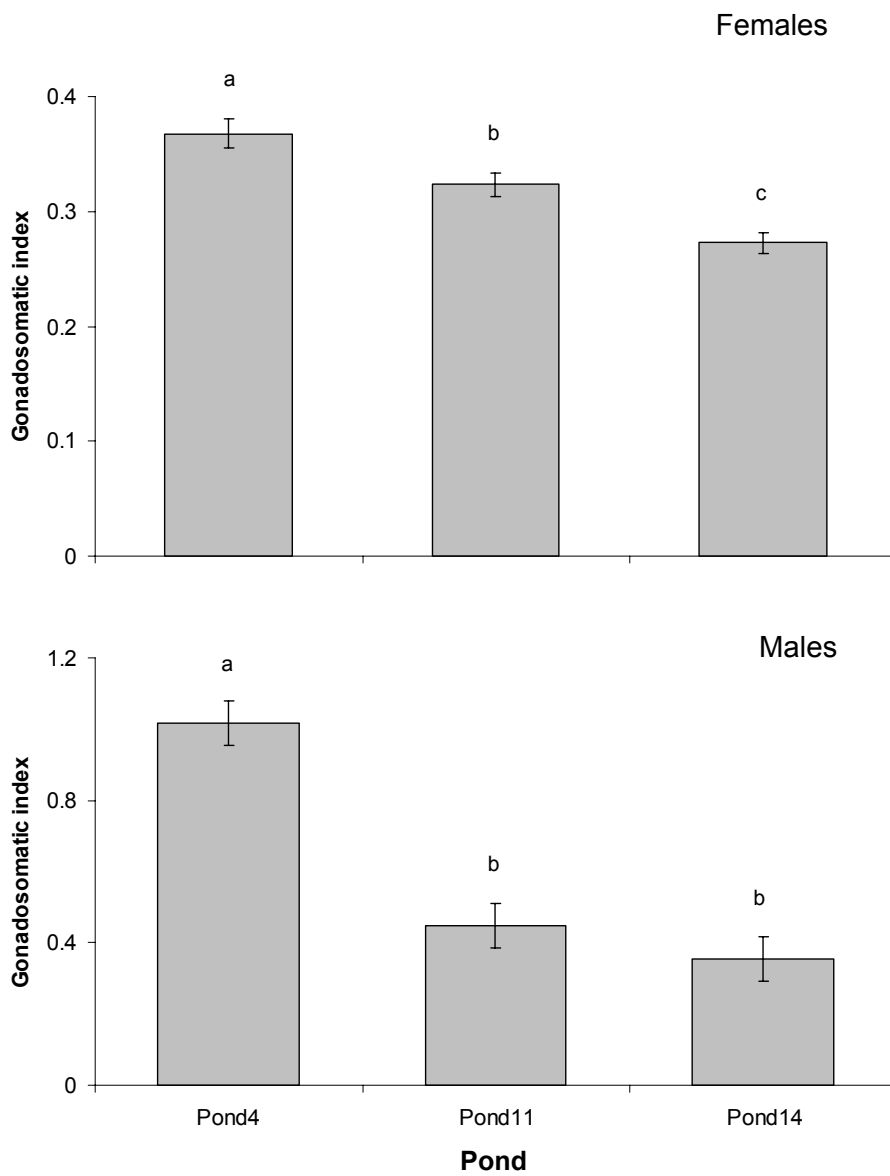
Figure 34. Least squares means (±SE) by gender for gonadosomatic indices of 2E55 Phase III final pond. Means with shared letters over the error bars are not significantly different.
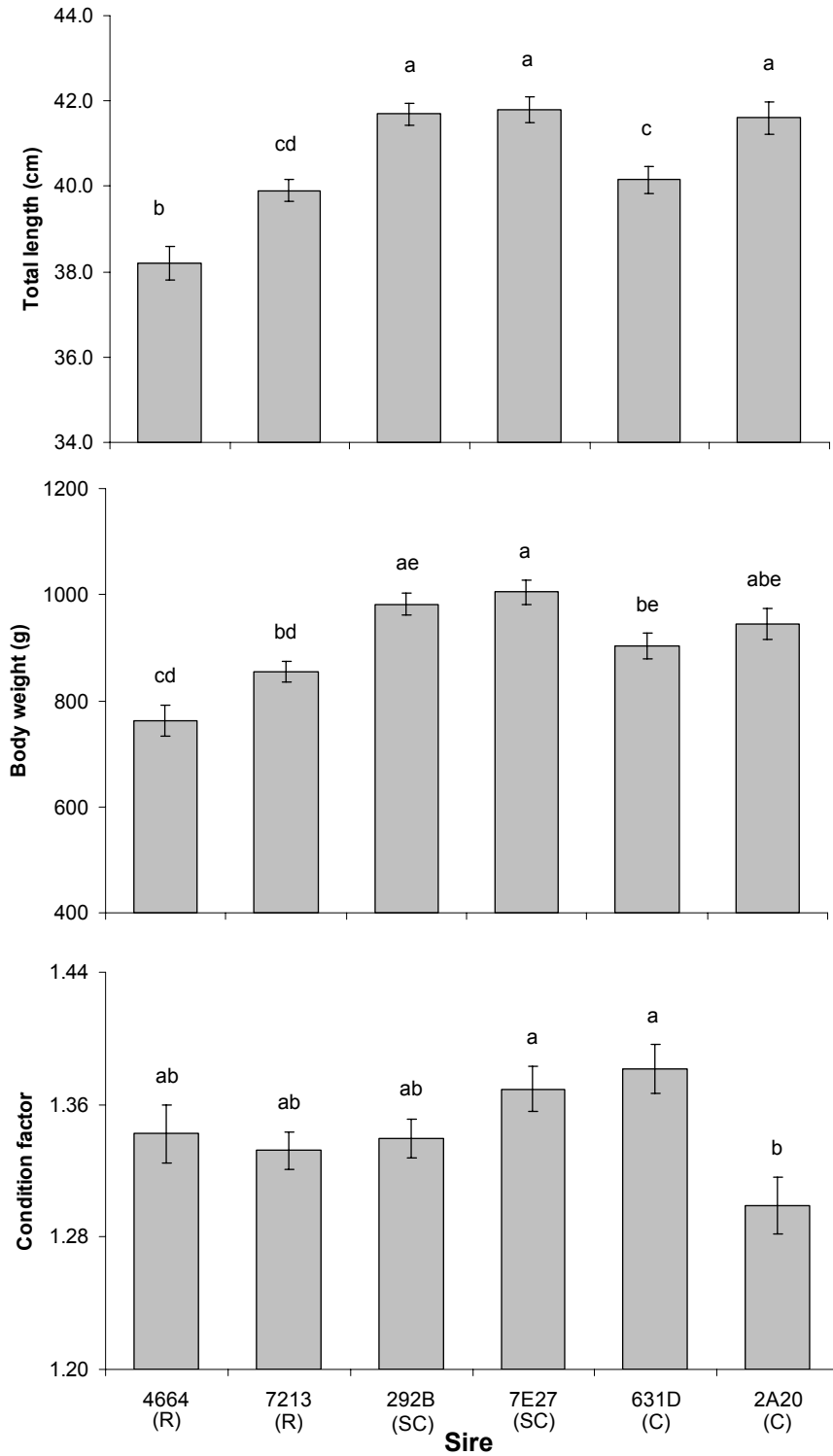
Figure 35. Least squares means (±SE) by sire family for 2E55 Phase III commercial tank harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
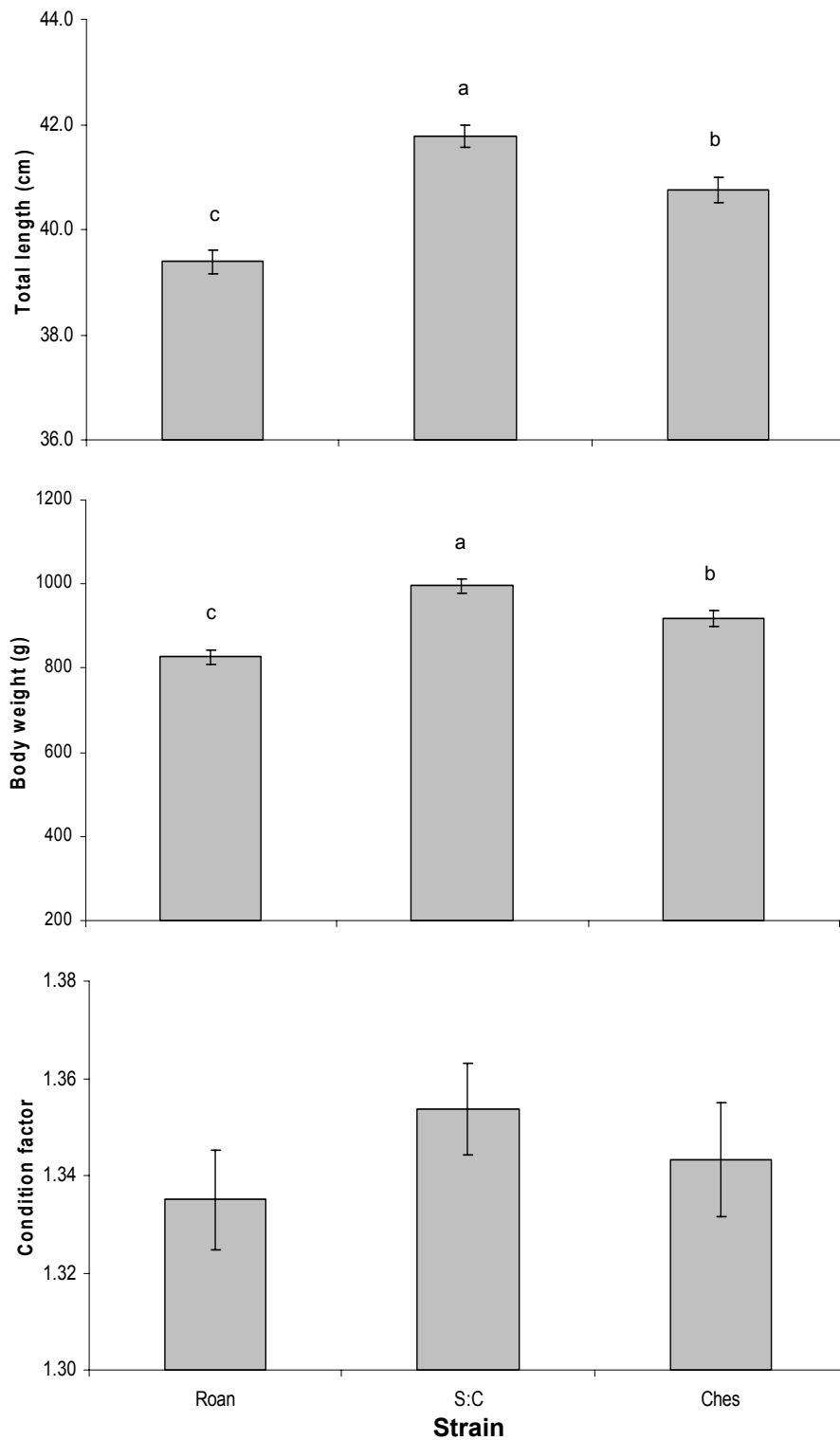
Figure 36. Least squares means (±SE) by strain for 2E55 Phase III commercial tank harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
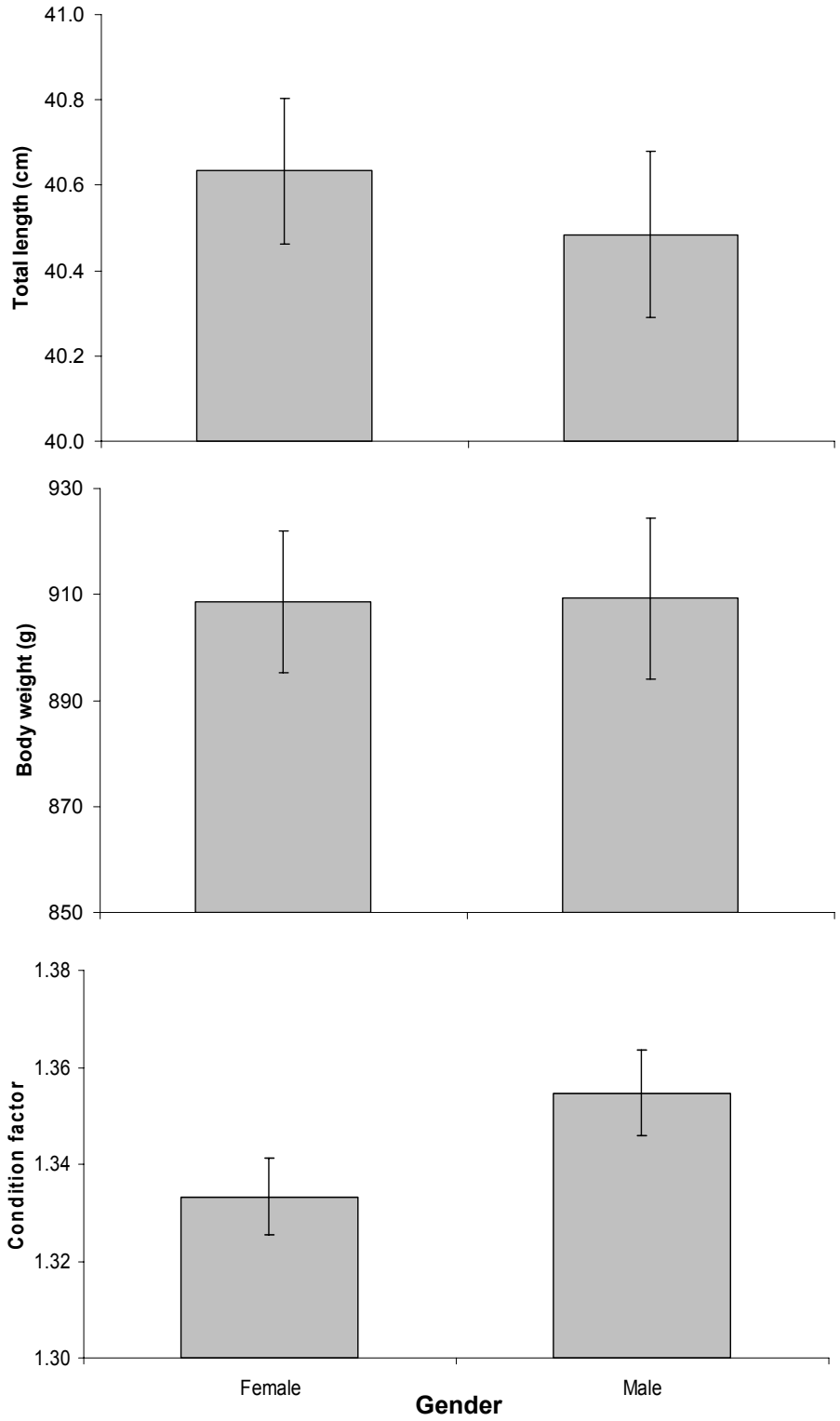
Figure 37. Least squares means (±SE) by gender for 2E55 Phase III commercial tank harvest traits, including total length, body weight and condition factor. Gender was not significantly different for any trait.
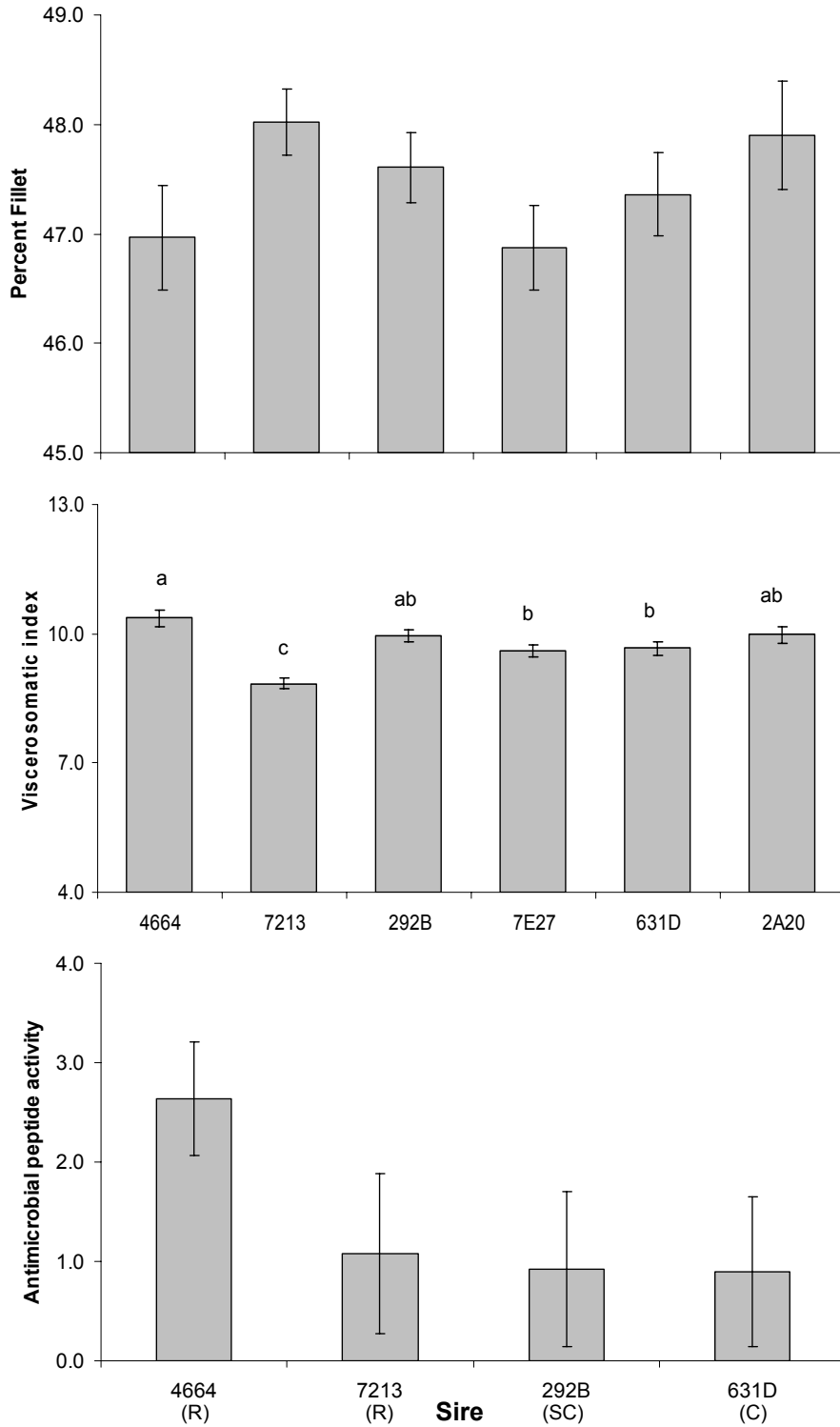
Figure 38. Least squares means (±SE) by sire family for 2E55 Phase III commercial tank harvest traits, including percent fillet, viscerosomatic index and antimicrobial peptide activity. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
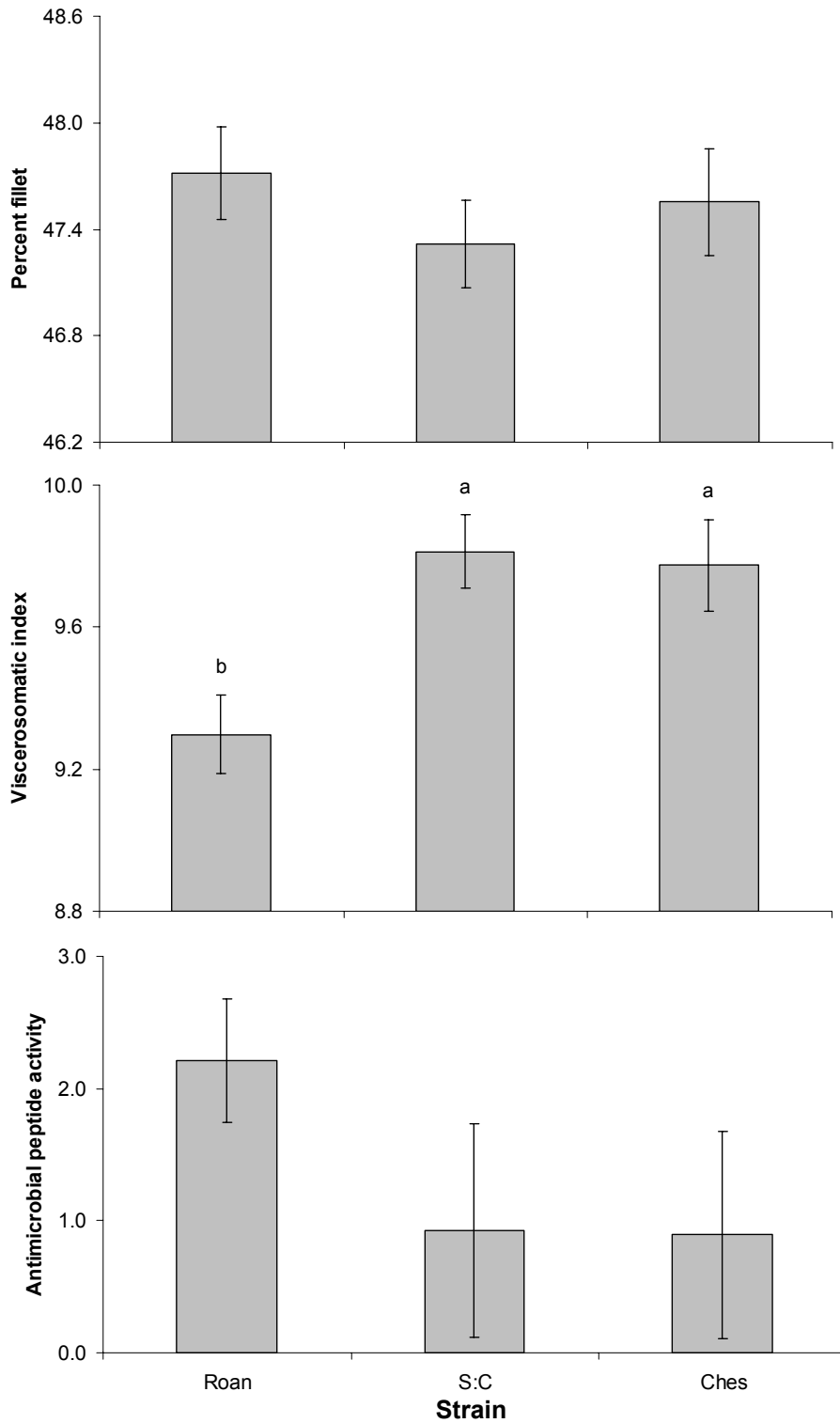
Figure 39. Least squares means (±SE) by strain for 2E55 Phase III commercial tank harvest traits, including percent fillet, viscerosomatic index and antimicrobial peptide activity. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
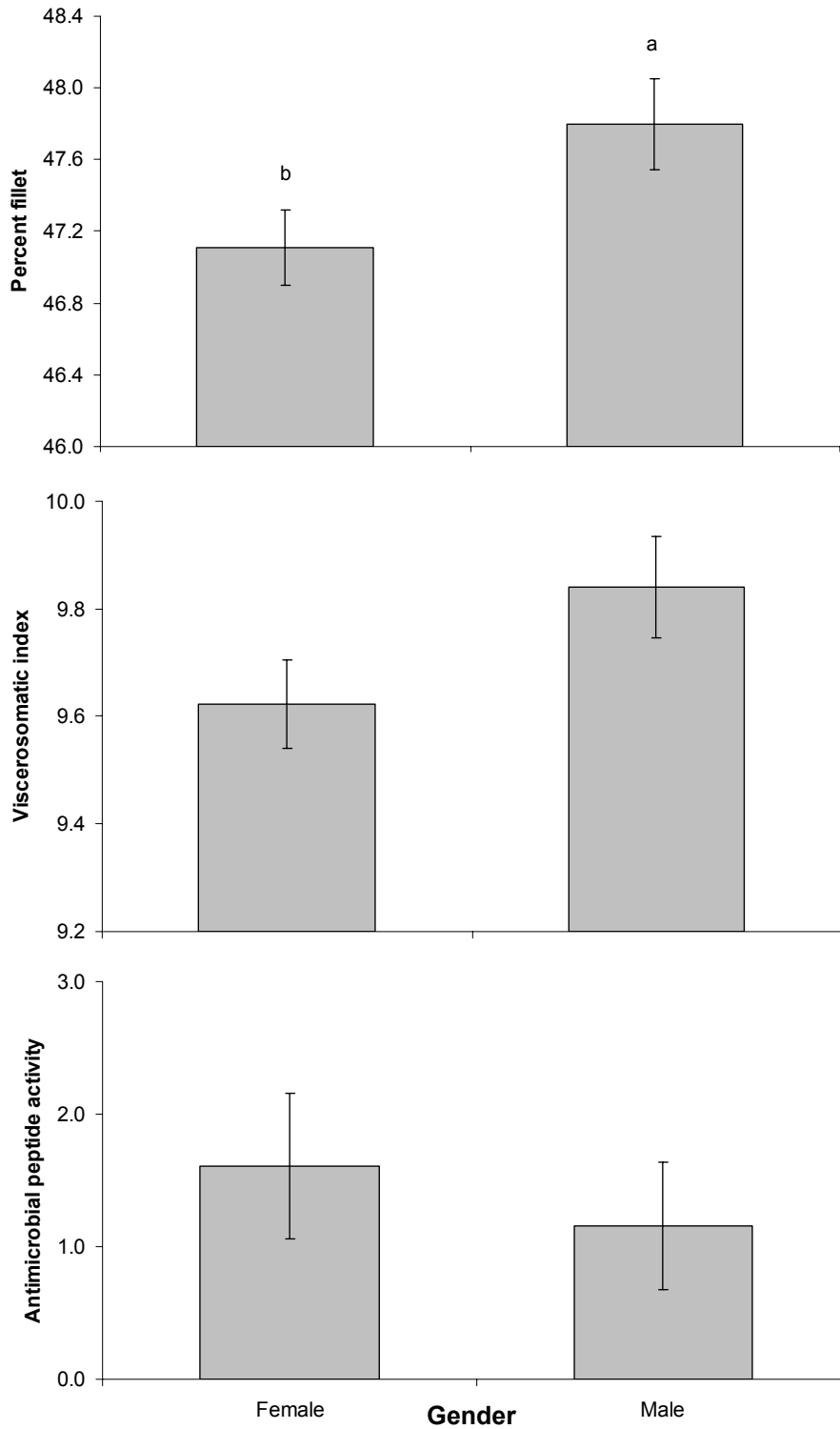
Figure 40. Least squares means (±SE) by gender for 2E55 Phase III final commercial tank harvest traits, including percent fillet, viscerosomatic index and antimicrobial peptide activity. There was no effect of gender on viscerosomatic activity or antimicrobial peptide activity.
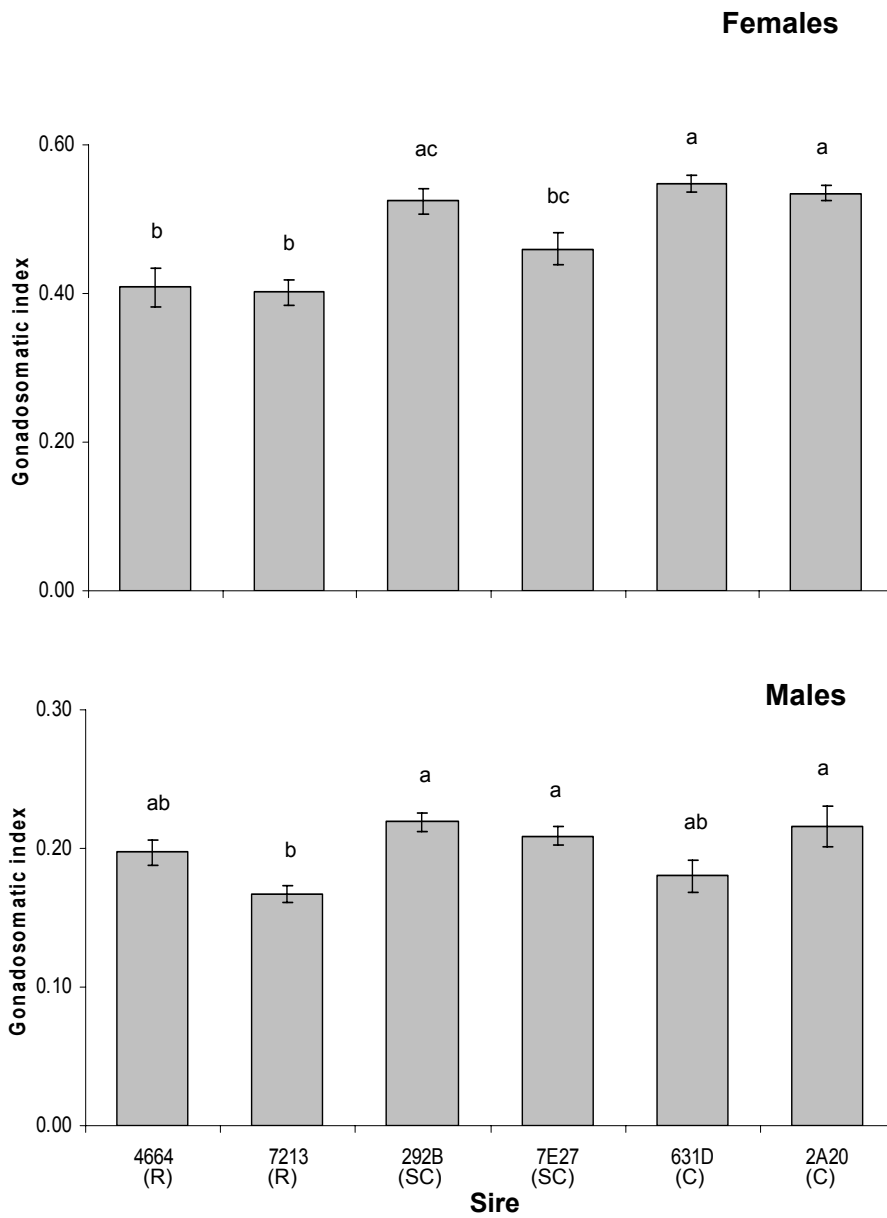
Figure 41. Least squares means (±SE) by gender for 2E55 Phase III commercial tank harvest gonadosomatic index. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.

Females

Males
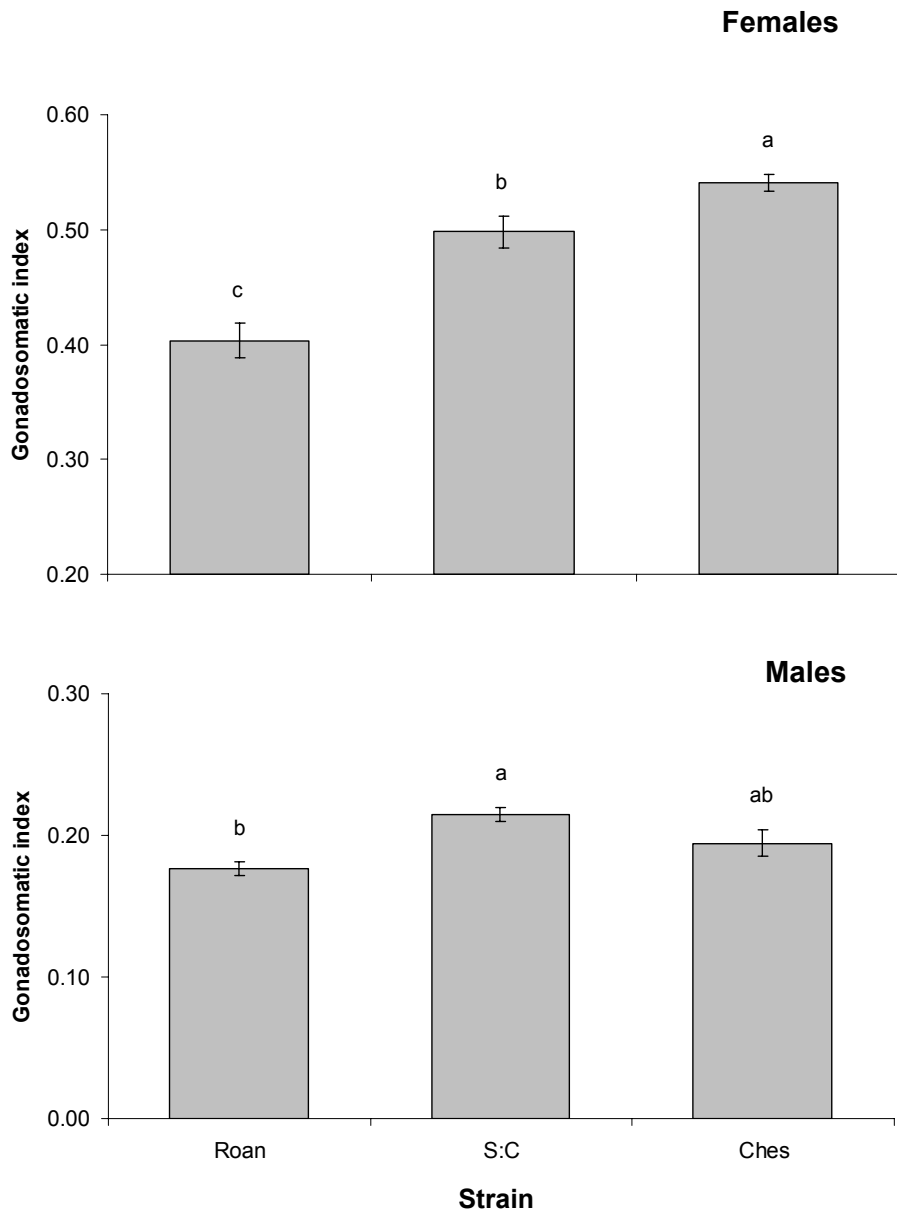
Figure 42. Least squares means (±SE) by gender for 2E55 Phase III commercial tank gonadosomatic indices. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
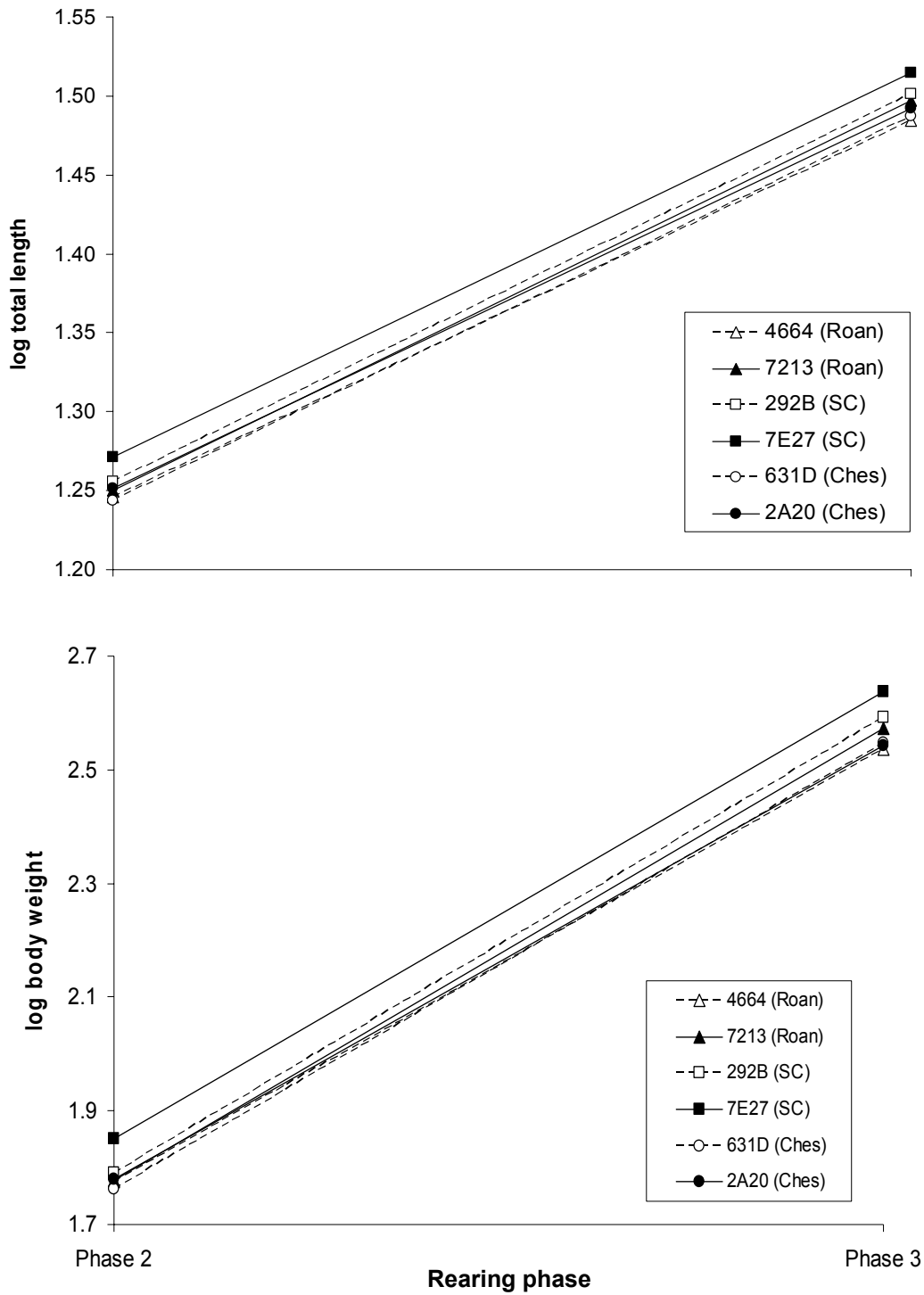
Figure 43. Interaction plots for least squares means of 2E55 Phase III traits by sire family across two rearing Phases in research ponds. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).
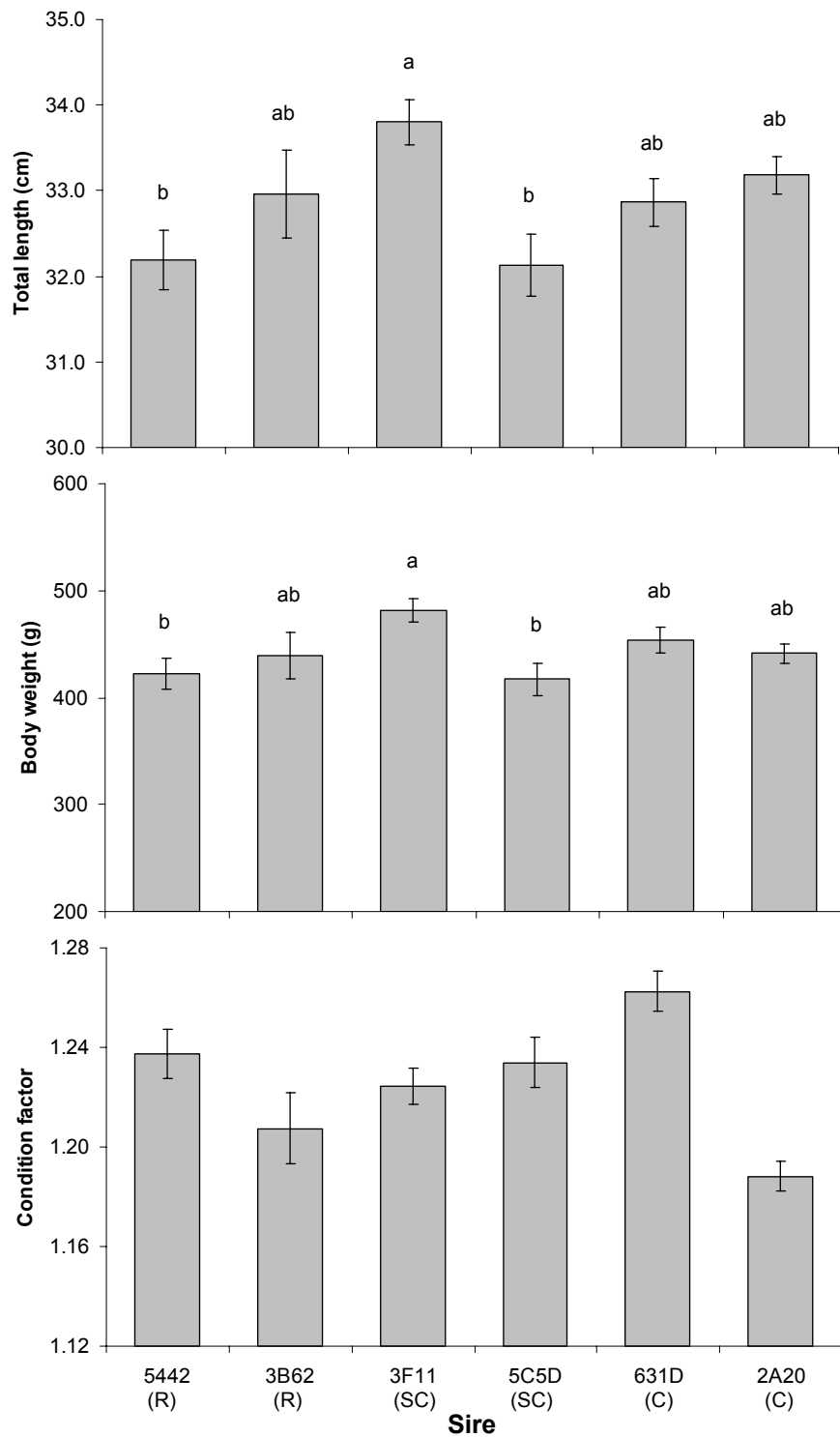
Figure 44. Least squares means (±SE) by sire family for Phase III harvest traits in the overall 12-family group, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
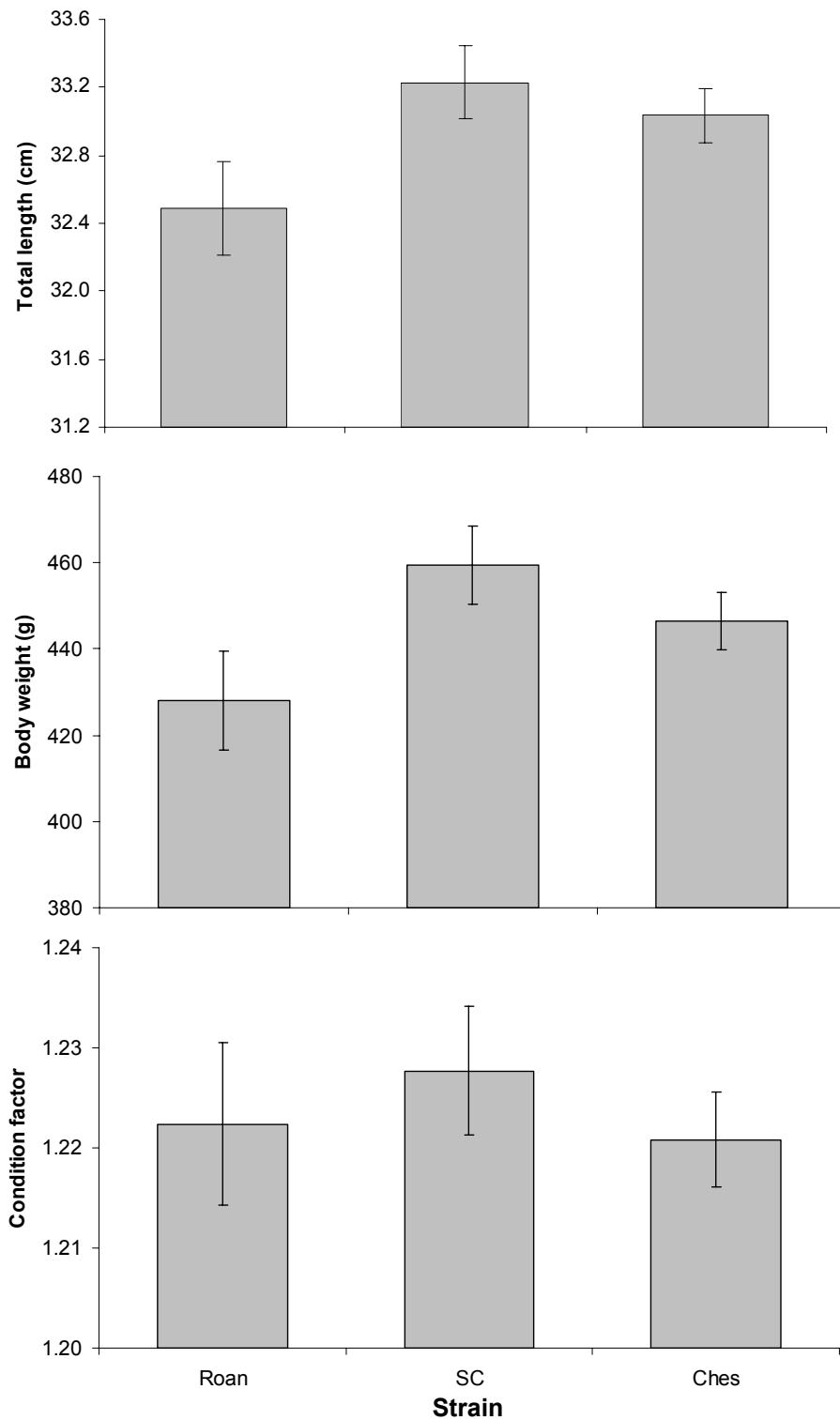
Figure 45. Least squares means (±SE) by sire strain for Phase III final pond harvest traits in the overall 12-family group, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).
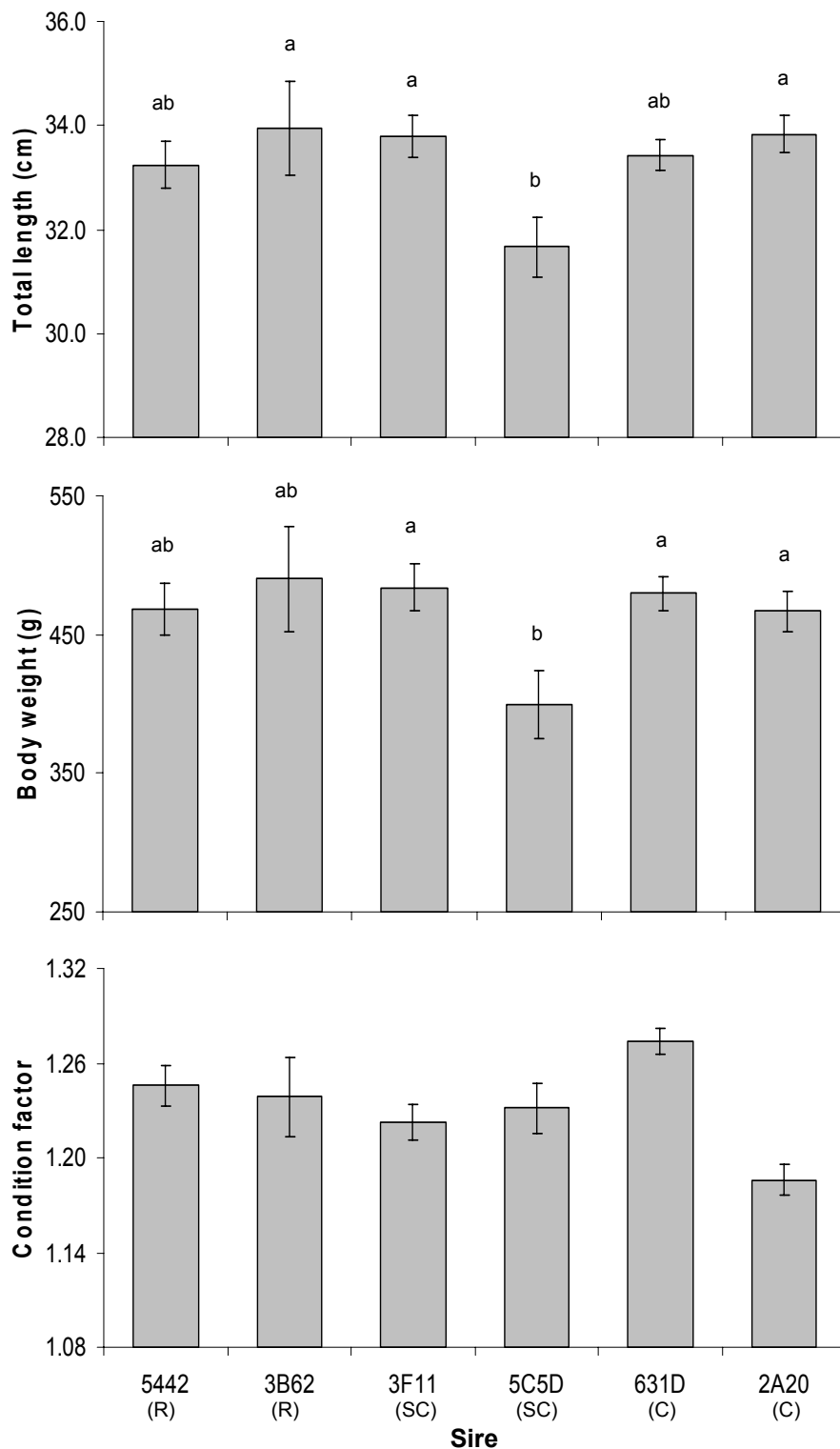
Figure 46. Least squares means (±SE) by sire family for Santee:Chesapeake dam 152D (12-family group) Phase III harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
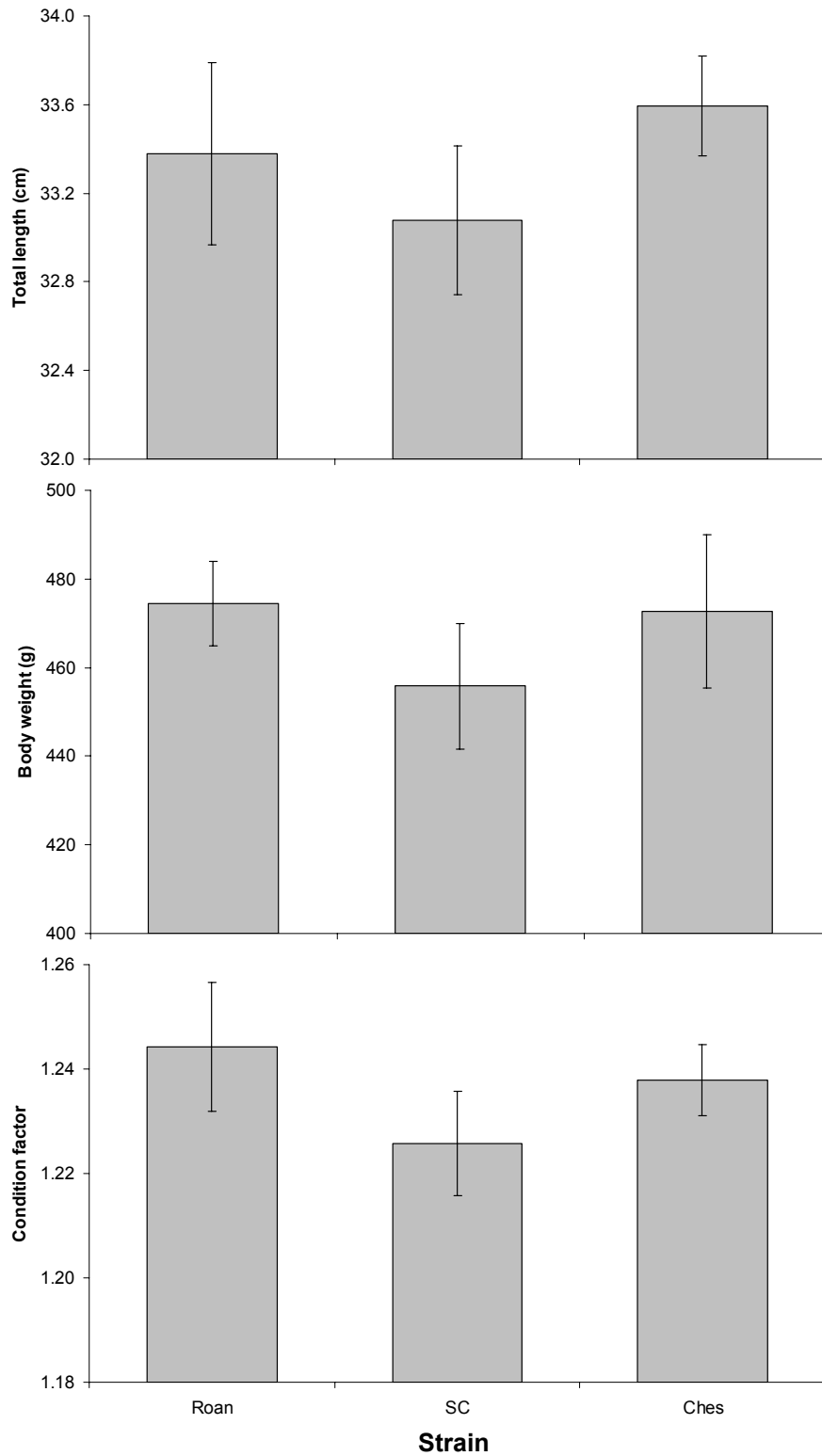
Figure 47. Least squares means (±SE) by sire strain for Santee:Chesapeake dam 152D (12-family group) Phase III final harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).

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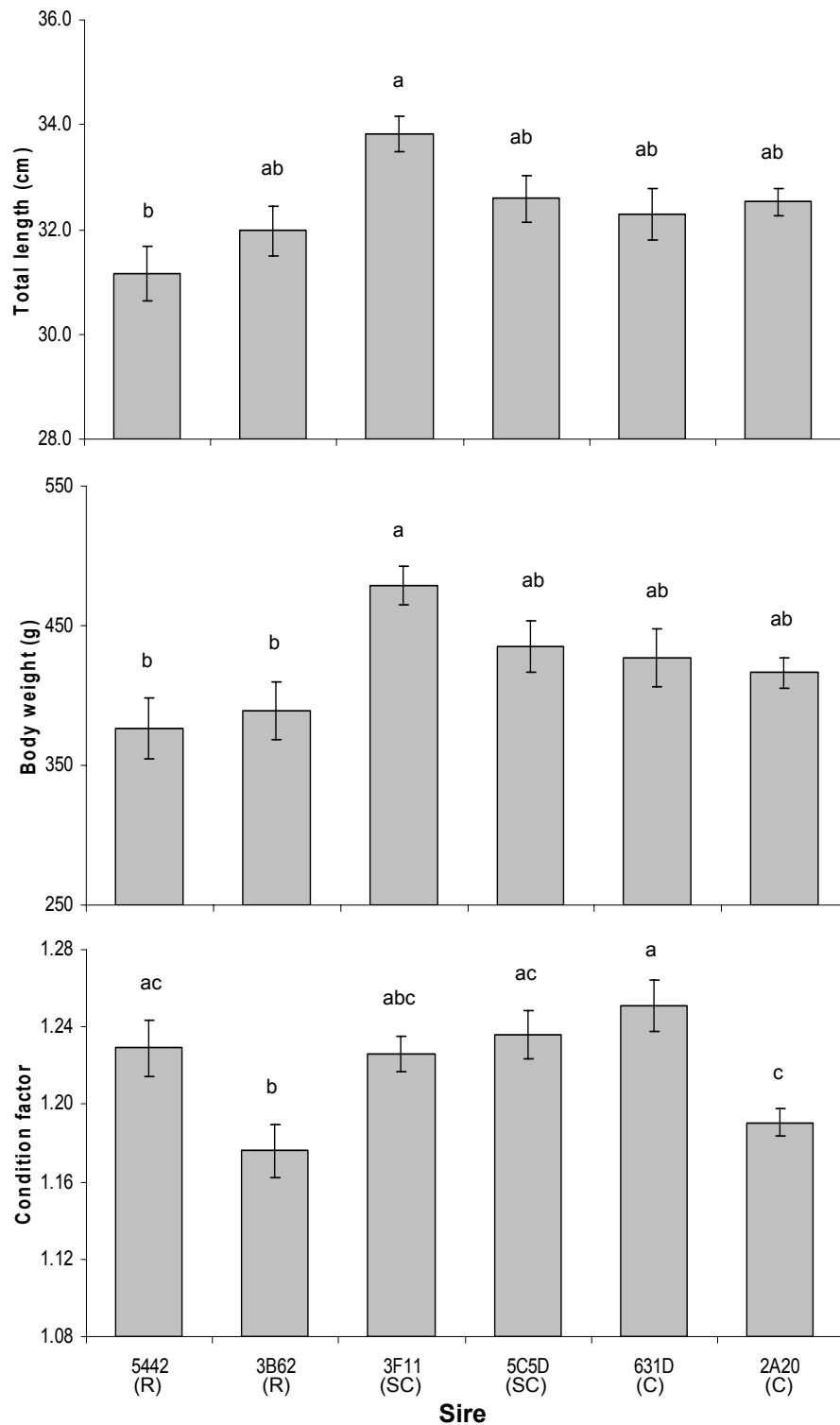
Figure 48. Least squares means (±SE) by sire family for Roanoke dam 5F4B (12-family group) Phase III harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (R), Chesapeake by (C) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
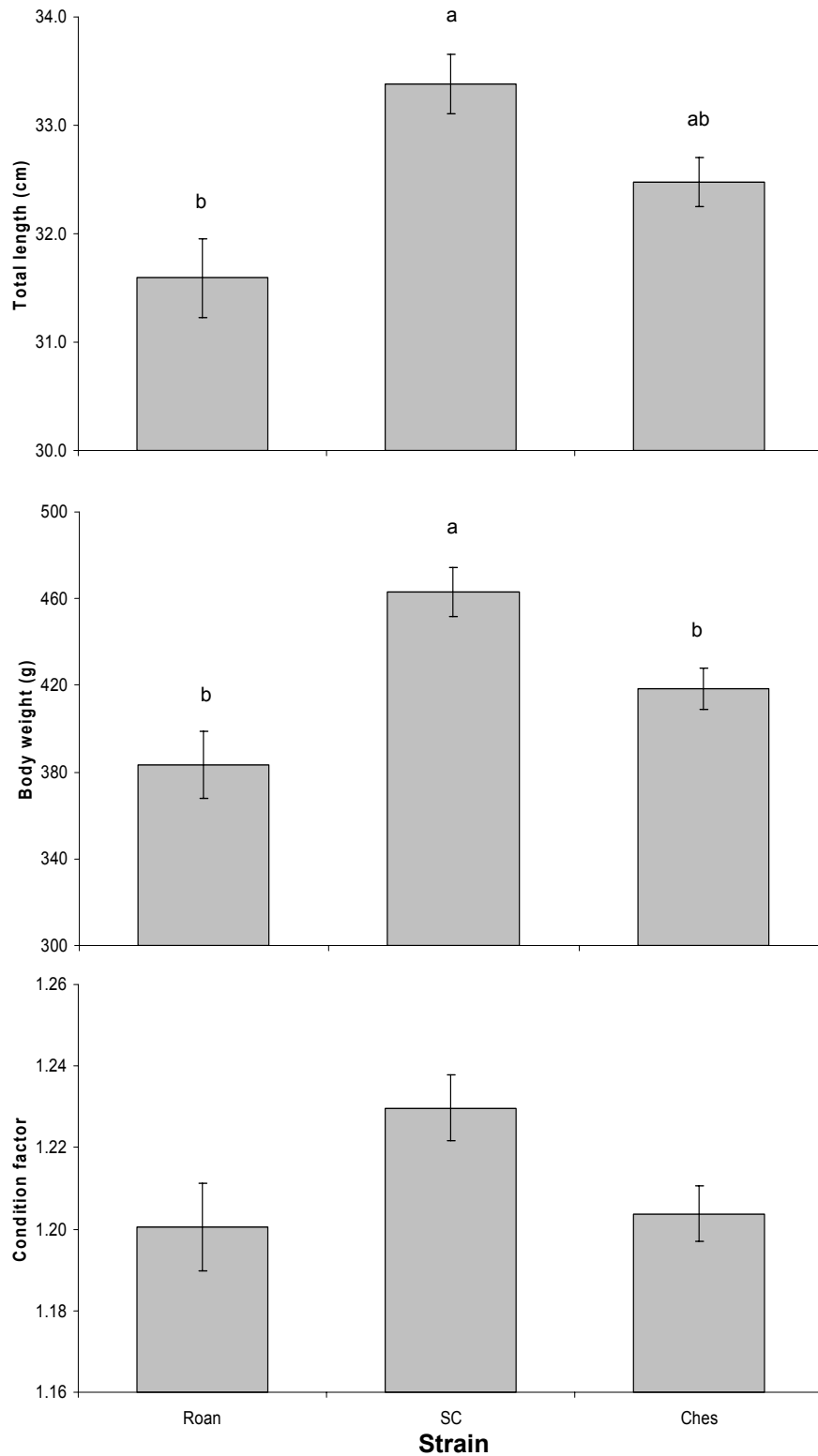
Figure 49. Least squares means (±SE) by sire strain for Roanoke dam 5F4B (12-family group) Phase III final harvest traits, including total length, body weight and condition factor. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC). Means with shared letters over the error bars are not significantly different.
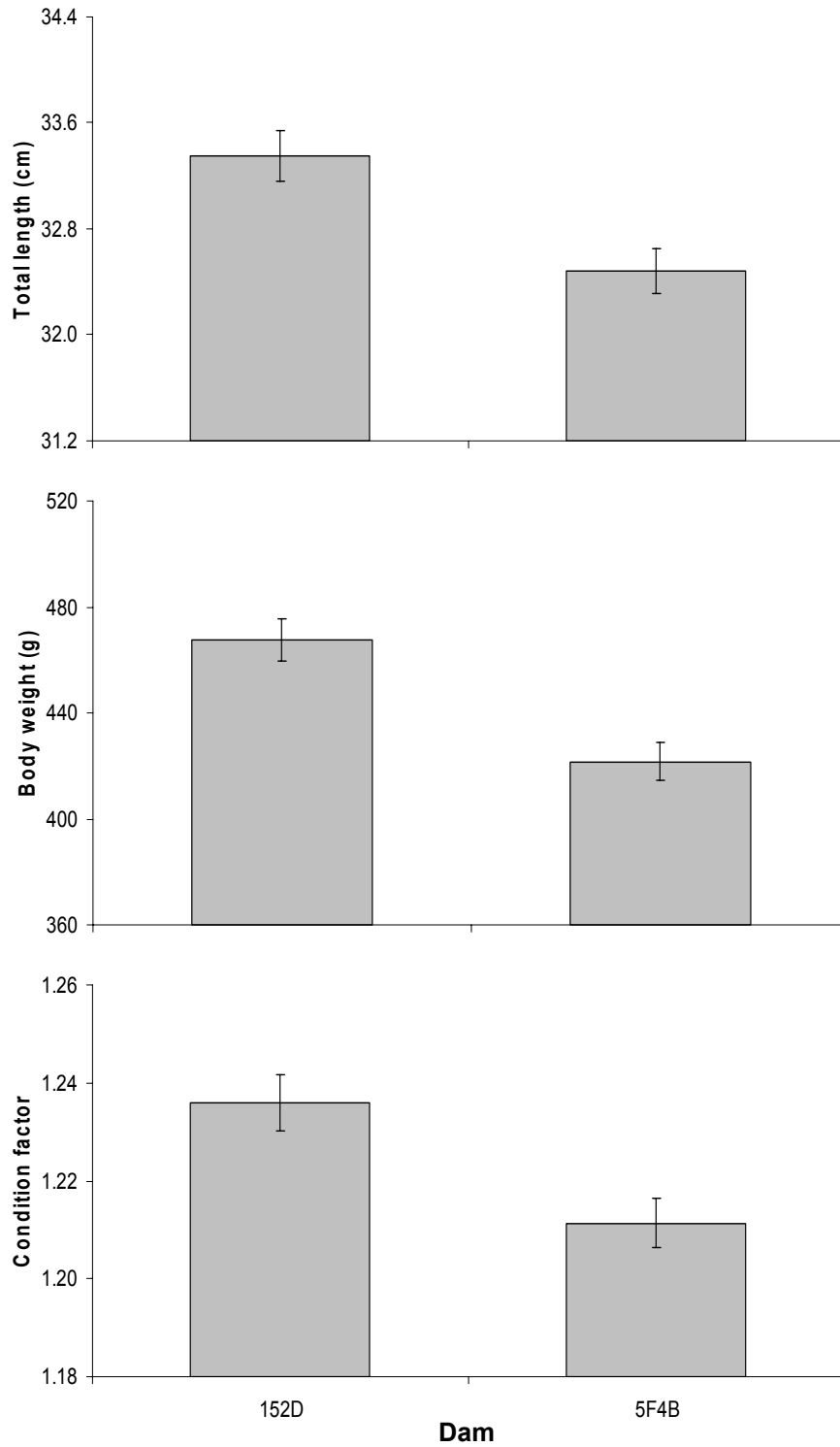
Figure 50. Least squares means (±SE) for Santee:Chesapeake dam 152D and Roanoke dam 5F4B (12-family group) Phase III final harvest traits, including total length, body weight and condition factor. Means with shared letters over the error bars are not significantly different.
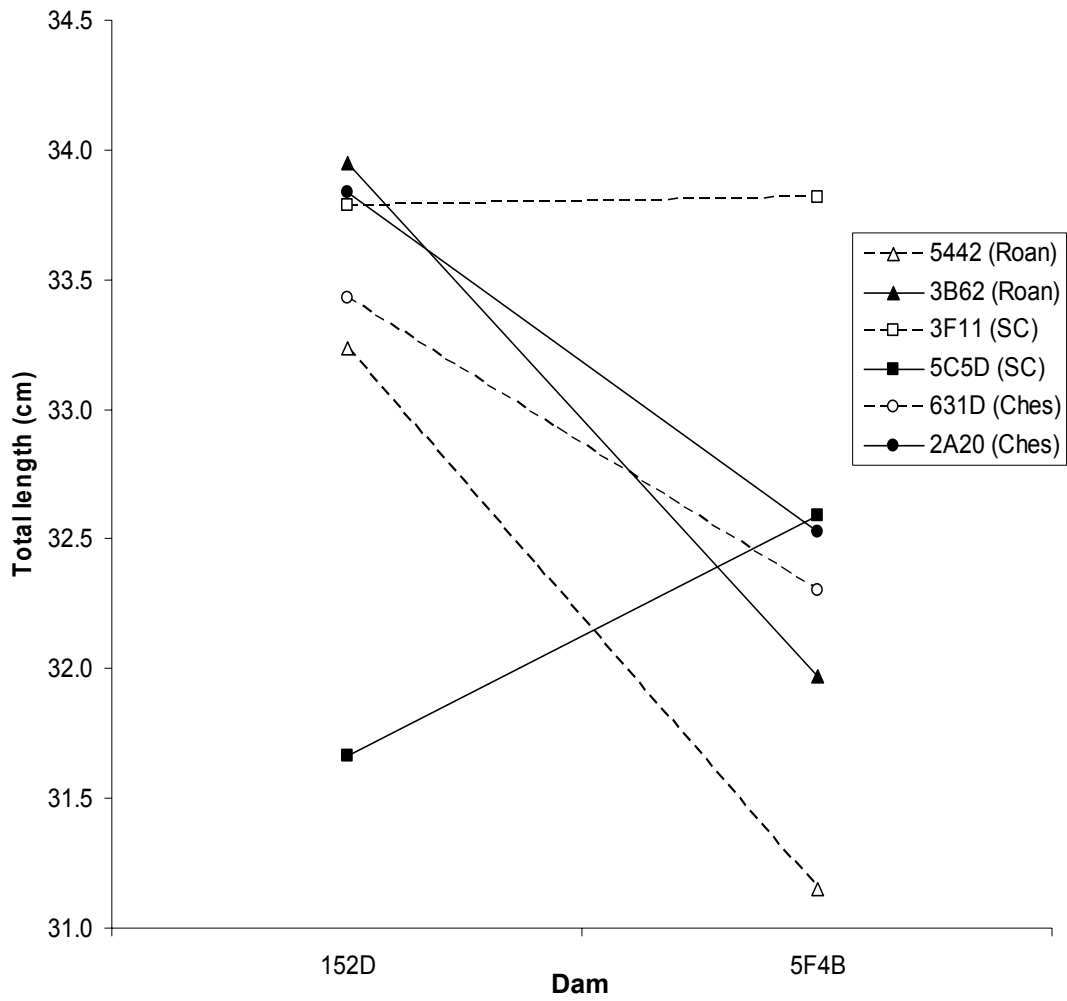
Figure 51. Interaction plot of least squares means of total length for six sires crossed with two dams, Santee:Chesapeake dam 152D and Roanoke dam 5F4B. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).
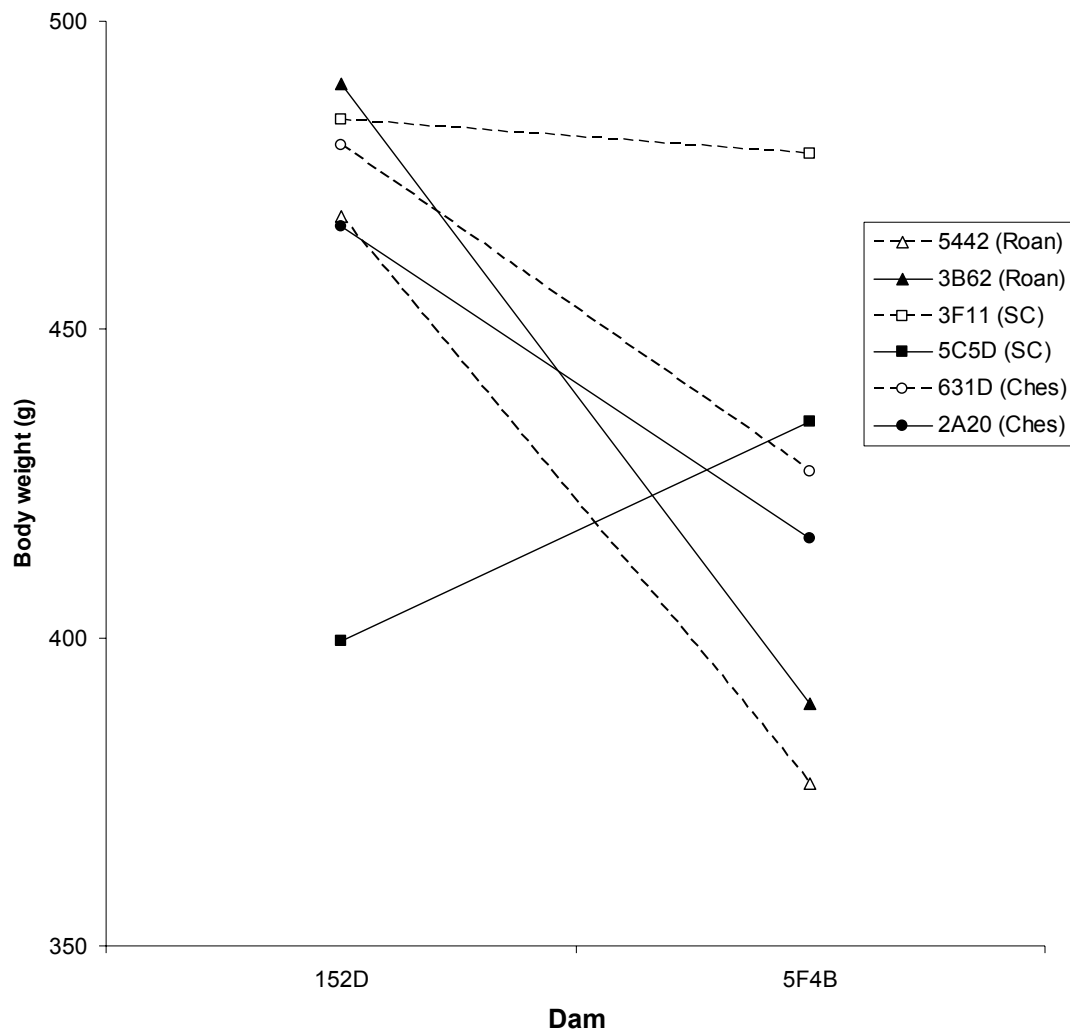
Figure 52. Interaction plot of least squares means of body weight for six sires crossed with two dams, Santee:Chesapeake dam 152D and Roanoke dam 5F4B. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).
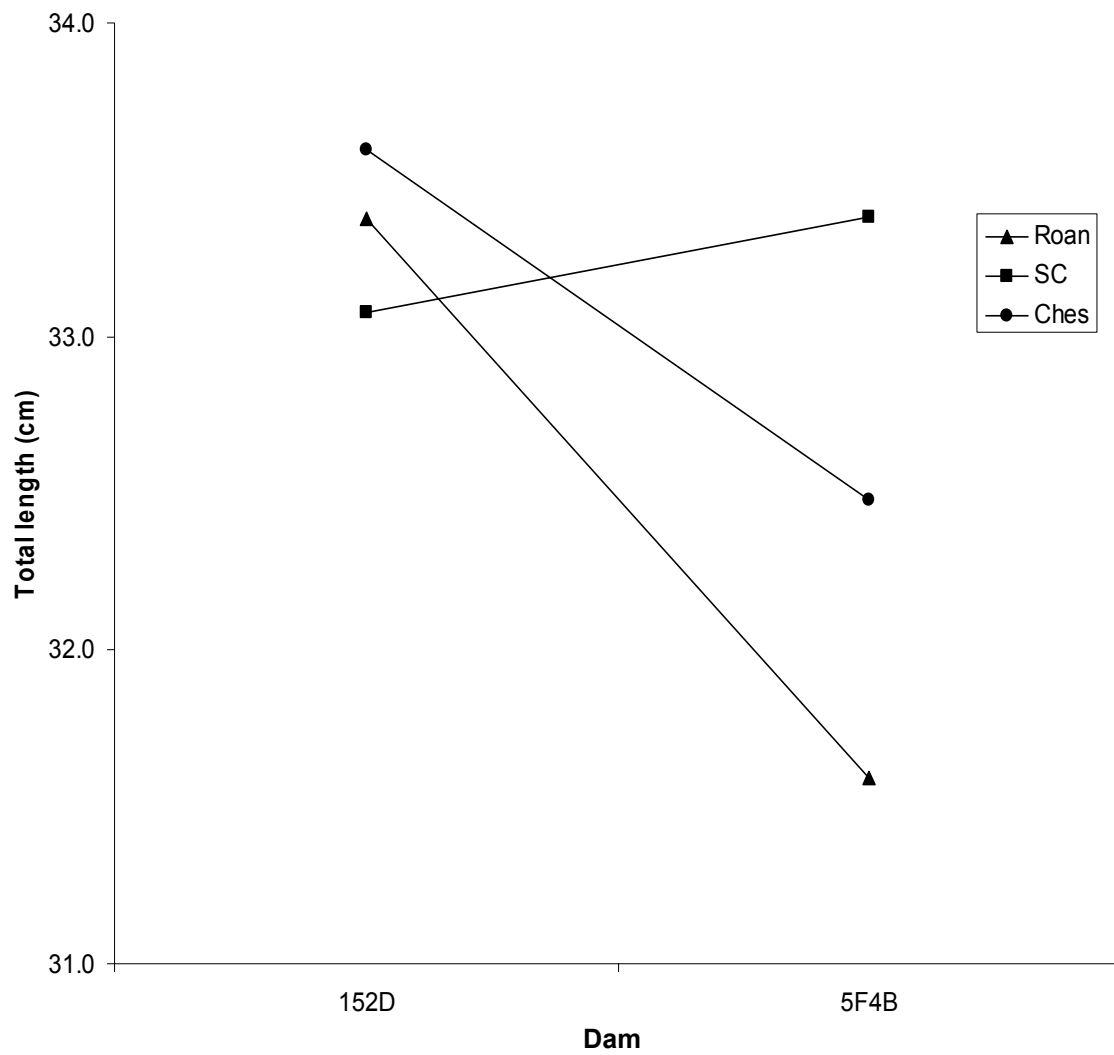
Figure 53. Interaction plot of least squares means of body weight for three strains of sires crossed with two dams, Santee:Chesapeake dam 152D and Roanoke dam 5F4B. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).
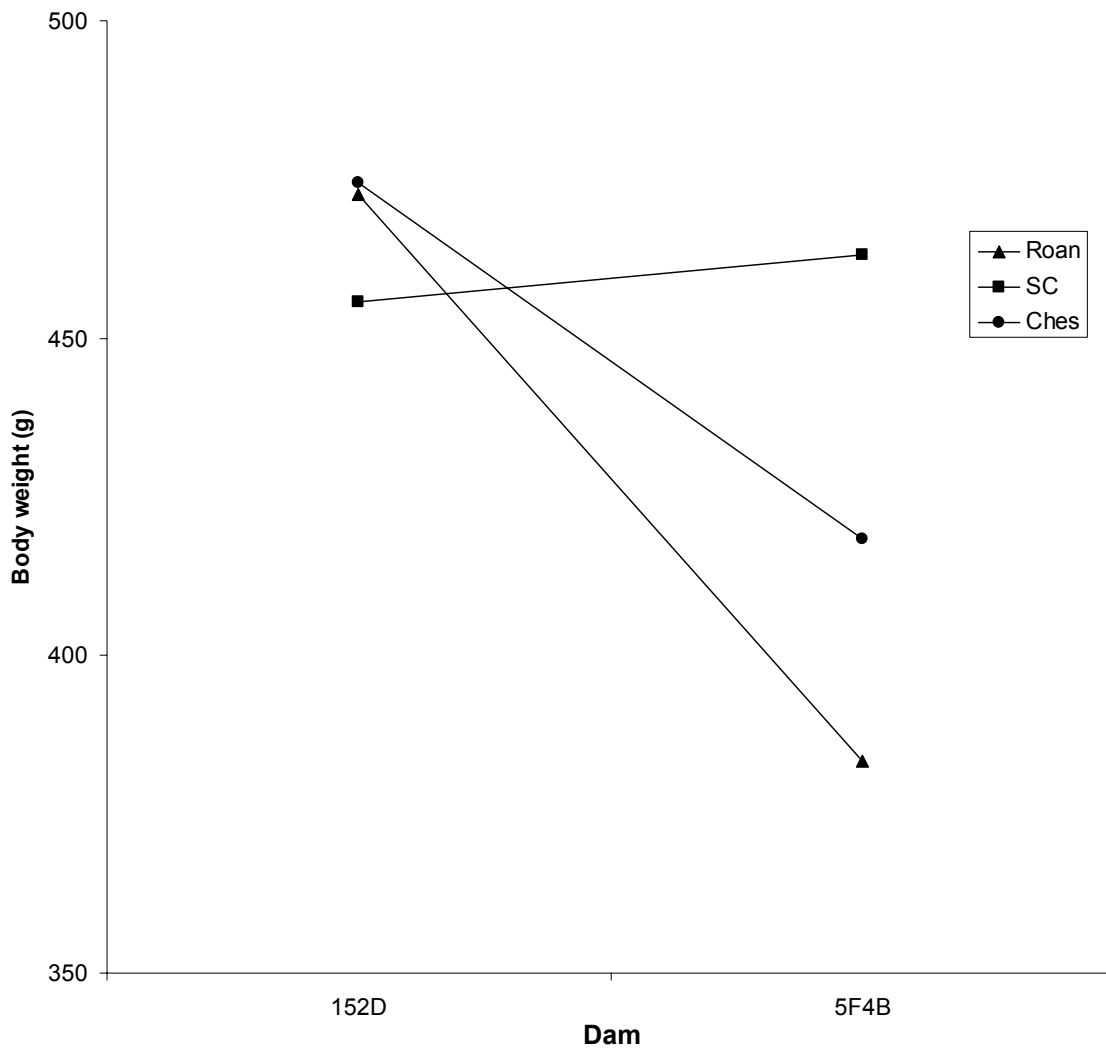
Figure 54. Interaction plot of least squares means of body weight for three strains of sires crossed with two dams, Santee:Chesapeake dam 152D and Roanoke dam 5F4B. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).
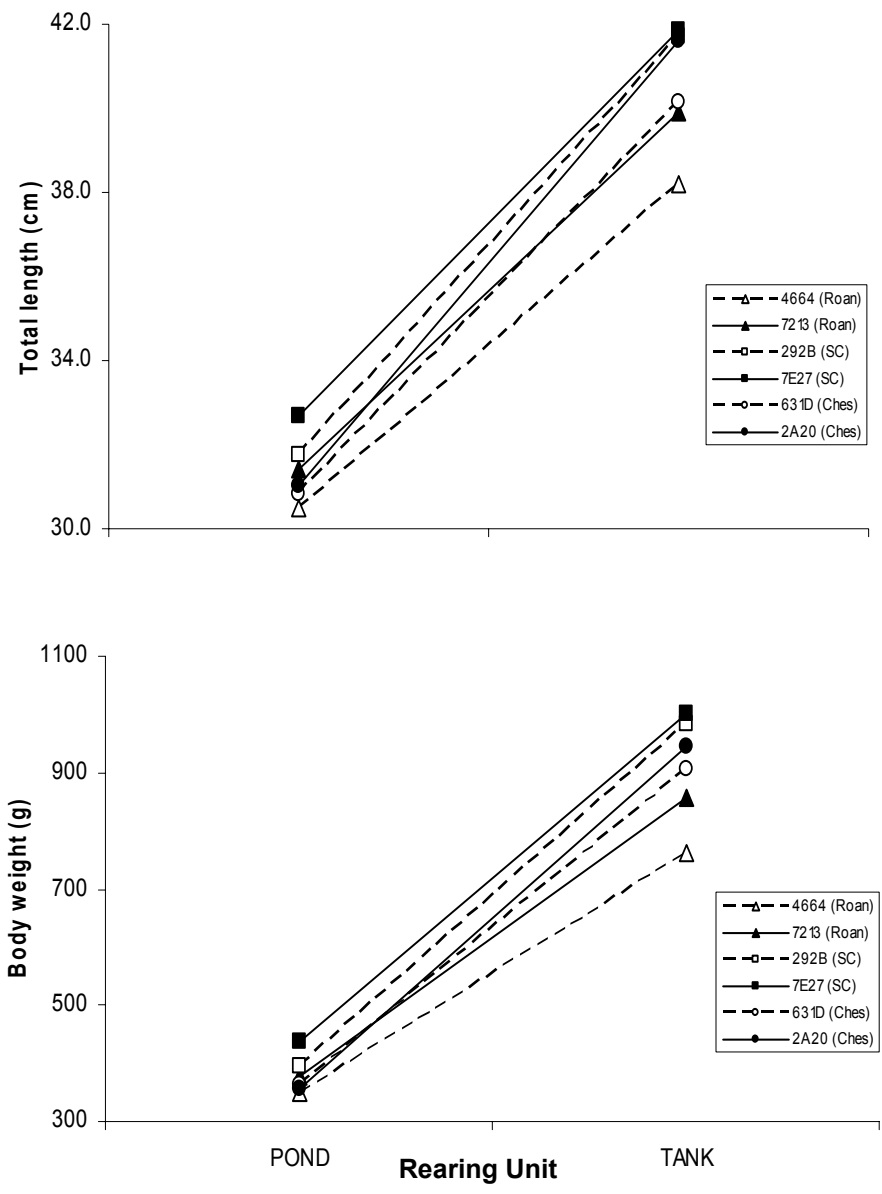
Figure 55. Least squares means for 2E55 sire family traits, including body weight and total length, in PAFL research scale ponds and a commercial tank at Kent SeaTech Corporation. Roanoke sires are indicated by (Roan), Chesapeake by (Ches) and Santee:Chesapeake by (SC).

CHAPTER 6

SUMMARY OF DISSERTATION FINDINGS AND RECOMMENDATIONS FOR FUTURE RESEARCH FOR

SELECTIVE IMPROVEMENT OF STRIPED BASS

The preceding chapters of this dissertation outline the need for selective breeding for *Morone* species and provide substantial information to support the goals of the National Program of Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry. Currently, resource limitations prohibit the individual rearing of multiple larval families for striped bass performance testing, necessitating a breeding program that is based on communal rearing of progeny groups with high-resolution molecular markers as genetic "tags" for progeny identification. As described in the previous chapters and summarized below, such a program appears to be highly feasible, and numerous genetic markers are now available to support these goals. Additionally, the demonstration of genetic variation within and among captive broodstock strains and the evidence of paternal variation in economically important performance traits provide valuable first principles information for development and execution of an efficient selective breeding program for striped bass.

Chapter 1—Chapter 1 provided an introduction to the rationale for the dissertation research. This chapter introduced information regarding the biology and distribution of wild striped bass, an overview of the production cycle of the hybrid striped bass, and the need for domestication and selective genetic improvement of the parent species of the hybrid to enable more efficient and sustainable aquaculture production of these fish.

Chapter 2—In Chapter 2, genetic variation was examined within and among three captive striped bass broodstock strains available to the National Breeding Program, one wild (Roanoke F_0) strain, and two domestic (Chesapeake F_2 and Santee x Chesapeake F_1) strains.

Three microsatellite DNA loci available at the time of the study were utilized to estimate the genetic variability of the strains for a program of domestication and selective improvement. All microsatellite loci were highly polymorphic in the striped bass broodstock population and average heterozygosity was generally high.

Significant differences in allelic and genotypic distributions were present for all strain pairs. Overall, the broodstock population appears to have moderately high genetic diversity. The differences in strain allele frequency distributions and the presence of unique alleles observed in each strain may serve to facilitate identification of strain origins, determination of relationships among strains and families within the captive broodstock population, and tracking of introgression among strains. Genetic variation evident within the captive striped bass strains should be exploitable in a selective breeding program for improvement of commercially valuable phenotypes such as growth, feed conversion efficiency, disease resistance and carcass traits. Using an adequate number of parents, crosses among the distinct broodstock strains may increase the genetic diversity available for selection, providing a genetically heterogeneous founder population from which to initiate directed selection. Examination of genetic variability at additional loci would bolster the utility of this information for future breeding efforts. Hundreds of additional microsatellite loci have been developed since the execution of this research and evaluation of broodstock genetic variation at another four to six multiplexed loci would require minimal additional investment of time or resources.

Chapter 3—Utilizing genotyped striped bass broodstock, investigations in Chapter 3 addressed the feasibility of using microsatellite markers to support communal rearing

protocols for performance evaluations of striped bass. Genetic variation in growth-related

phenotypic traits and in survival was examined in communally reared families of striped

bass. Challenges associated with captive spawning of the striped bass and with survival of

larvae and fingerlings limited the number of families which could be examined, as well as the

level of replication of the experiments. However, twenty-four experimental crosses were

successfully generated from domesticated and wild strains of striped bass and stocked by

dam in 6-family groups into outdoor pond mesocosms. Larvae stocked at 4-6 days post hatch,

far too small for physical tagging, were reared together until harvest at 34-40 days after

hatching. Parentage of pooled progeny was determined by microsatellite genotyping and

more than 99% of progeny could be attributed to specific sire-dam pairs using only six

microsatellite markers. The high degree of success in parentage determination in a common

environment illustrates the feasibility of a microsatellite-based communal rearing approach

for performance evaluations and pedigree tracking in the National Breeding Program.

Although variation in growth of progeny was observed in striped bass populations during

Phase I production, there was limited evidence from these experiments that the effects of sire

or strain influence early growth or survival. Examination of additional families in a more

highly replicated design should provide further information on the influence of genetic versus

random environmental effects in these early life stages.

Chapter 4—Chapter 4 continued family performance evaluations for six families of fish

produced from dam 2E55 which were reared both in research ponds at PAFL and in a

commercial production tank at Kent SeaTech Corporation. A microsatellite marker-assisted

communal rearing approach for performance evaluations in striped bass was utilized to

examine paternal effects on progeny growth of fingerlings during the second stage of commercial culture, Phase II. Again, the objectives of the study were to confirm the feasibility of microsatellite genotyping for progeny identification in striped bass that are communally reared to approximately one year of age and to investigate genetic variation among striped bass families that underlies Phase II traits. Use of microsatellite markers for parentage identification of individuals in mixed family groups proved highly successful for families reared in both ponds and in the commercial tank. Additionally, replicated performance trials revealed significant sire effects on variation in phenotypic traits related to growth and body shape, providing evidence that genetic variation is present in the captive NCSU striped bass broodstock. This variation in economically important production traits may be exploited in a program of selective breeding for striped bass.

Chapter 5—In Chapter 5 of the dissertation, performance traits were examined for eighteen half-sibling families reared in research ponds in order to evaluate the genetic basis of commercially important performance characteristics measured at the end of production Phase III in market-size striped bass. Six of the families also were reared in a commercial tank production system. Using six microsatellite markers as innate genetic tags for parentage assessment, more than 99.7% of offspring could be unambiguously attributed to specific parental pairs, including progeny reared in 6- and 12-family communal groups. Mean growth performance and carcass characteristics differed significantly by genetic background in both the pond and tank environments. Contrary to previous observations of sexual dimorphism in growth traits for hybrid striped bass and European sea bass, where females are larger than males, sexual dimorphism was not detected in the commercial tank and males were larger

than females in the research ponds. Effects of fish size and environmental conditions should be investigated more fully in order to determine if dimorphic performance is important in cultured striped bass. Large variation in gender ratios observed in this study may indicate that genetic variation in gender ratios could be exploited for improvement of striped bass or hybrid striped bass growth performance. Histone-like antimicrobial peptide activity, a potential measure of innate disease resistance or stress response, differed by strain within the research ponds, indicating a promising line of future research for the breeding program. Use of a communal rearing approach, wherein all fish are exposed to identical environmental conditions, should prove quite useful for such investigations.

The interaction of rearing Phase and sire was examined for a group of six families reared in three research ponds in order to assess the predictive value of sire family performance during Phase II on their performance during Phase III. Total length and body weight were examined since these phenotypic traits of are easily and non-destructively measured. Examination of data on progeny of dam 2E55 from three research ponds revealed no significant interaction effect between sire and Phase. This information provides evidence for the predictability of final harvest trait means in research ponds from Phase II data collected 6-7 months prior to harvest. Examination of these traits by sire family across the two culture Phases revealed that rank orders for performance of the top two sire families was the same for both Phase II, when fish were approximately one year old, and Phase III, when fish were 19-20 months old. This observation suggests that the Phase III performance of families tested in research scale ponds may be predicted well before the fish reach the end of the Phase III rearing period, resulting in substantial savings in time, costs of labor and feed, and use of rearing units.

Taken together with the predictability of performance observed in other fishes across growing stages, the results seen in striped bass research ponds indicate that examination of the predictive value of Phase II performance for Phase III striped bass merits more thorough investigation. Also worthy of note is the fact that Phase III trait means plotted for the research ponds and the commercial tank reveal that the two families with the highest performance in ponds (Santee:Chesapeake families) also performed best in the dramatically different growing conditions of the commercial production tank. The family with the worst growth performance also was the lowest performer in both the ponds and the tank. These results may prove valuable for selective breeding of striped bass since they suggest that Phase III performance of families in the intensive commercial tank setting may be predicted by their performance in small research ponds. If this proves to be the case upon evaluation of additional families with greater replication, it may be unnecessary to test progeny performance of certain traits in this commercial environment.

Variation in performance between some sire-dam and strain-dam combinations observed in the 12-family group indicates that further investigation of cross combinations is needed for captive strains of striped bass. Such investigations should provide valuable information regarding genetic variation present within the captive broodstock groups and regarding the value of specific crosses within and among particular strains of striped bass. The effects of sire-dam, strain-dam and strain-strain interactions should be evaluated in greater detail as the effects of crossing among striped bass strains may be suitable for achieving short-term breeding gains. Differences between strains in growth and tolerance of temperature and salinity have been shown for striped bass but the effect of crosses among strains is unknown. In trout, crosses among strains or stocks was suggested as a means for

increasing genetic variability in the broodstock population (Iwamoto et al. 1986; Silverstein et al. 2004) and crossbreeding of particular strains can result in heterosis for phenotypic traits in catfish (Dunham 1986), common carp (Wohlfarth 1993; Hulata 1995), rainbow trout (Linder et al. 1983) and Pacific oyster (Hedgecock et al. 1996). Crossbreeding of different geographic stocks striped bass may be an avenue for short term improvement in a breeding program as various stocks have proven to differ in growth performance (Conover et al. 1997; Brown et al. 1998; Woods et al. 1999, Jacobs et al. 1999; Secor et al. 2000) and crossing of captive stocks may increase the genetic and phenotypic variation available for subsequent improvement.

Application of large-scale communal rearing trials based on microsatellite markers for progeny identification should be a viable approach in a selective breeding program for striped bass. Results from performance evaluations described in <u>Chapter 5</u> provide fundamental information needed to accelerate selective breeding and to increase production efficiency for the hybrid striped bass industry and highlight several areas of promising future research. Access to hundreds of new microsatellite markers recently developed for striped bass (Rexroad et al. 2006; Couch et al. 2006) and implementation of high-throughput fluorescent genotyping methods developed during the course of this dissertation research should enable careful tracking and management of captive striped bass genetic resources in a selection program based on communal rearing.

Approaches for selective breeding of striped bass—The appropriateness of various selective breeding approaches for the hybrid striped bass industry was recently reviewed by Garber and Sullivan (2006). Their recommendations indicate that mass selection should yield

benefits to the industry through improvement of both parental species of the hybrid. The details of this approach within the National Breeding Program have not yet been finalized and evaluation of some common methods of selection may be beneficial. Individual, or mass, selection involves choosing individuals with the greatest values for phenotypic traits for use as the next generation of broodstock. Mass selection is generally preferred for selective breeding because it is simple and often produces rapid selective improvements (Falconer and Mackay 1996). Family selection, in which entire families are selected or rejected based on their phenotypic trait means, is useful for traits with low heritability and for traits for which there is little variation due to common environment. For this selection method, large families are needed to evaluate the trait and numerous families must be reared to minimize losses of genetic diversity (since only a limited number of the families will be selected) and to ensure reasonable selection intensity. Rearing of many large families to accommodate this method can be costly although a communal rearing approach may offset some of these costs. Within-family selection, a method of selection in which individuals that most greatly exceed their family's trait mean are selected, can eliminate concerns about phenotypic variation due to environmental differences among families since all individuals within a family are reared in a common environment and a given number of individuals is selected from within each family. Importantly, this method allows for each family to contribute equally to the subsequent generation, which contributes to the maintenance of adequate effective population size and genetic diversity and, since all families are utilized, fewer families must be maintained than for family selection. A modification of the within-family method was suggested by Doyle and Herbinger (1995) for selective breeding of fish species and may be worthy of consideration for striped bass. In their "walk-back" selection method, superior individuals are

selected from a given population of communally reared families by choosing the largest individual as a breeder. That animal is genotyped, and then the next largest animal is chosen and genotyped. If the second fish is closely related to the first, it is rejected, but if it is more distantly related (or from another family), it is retained as a broodfish. Selection continues by "walking back" from the upper tail of the phenotypic distribution in this way until the desired number of breeders has been identified. This method takes advantage of the large family sizes characteristic of fish species and minimizes inbreeding among close relatives while at the same time allowing for high selection intensities. The method of selection to be utilized for striped bass improvement has not yet been finalized, but exploration of mass selection and a walk-back type of within-family selection appear to be most promising given the current limited resources of the National Breeding Program.

Utilizing body weight data generated from the replicated ponds of Phase III fish (progeny of dam 2E55), I evaluated an individual, or mass, selection scheme compared to a modified form of within-family selection to investigate the utility of these approaches for striped bass. Of particular interest were the proportional representation of sires under each selection approach and the trait means of the resultant "selected" broodstock. For each pond at PAFL, the largest 60 fish by body weight were selected to represent a population of mass selected broodfish. To represent a type of within-family selection for these same ponds that would theoretically minimize relatedness and loss of genetic variation among this generation of breeders, the top ten fish from each family were selected. This approach is similar to Doyle and Herbinger's (1995) "walk-back" selection with the exception that multiple fish from each family were selected in order to produce the requisite number of breeders since so

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few families were available for consideration. Each selection scheme resulted in retention of 180 fish from the original pond-reared 2E55 progeny.

Trait means for each pond before selection and for each pond after selection, as well as proportional representation by each sire, are shown in Table 25. Use of the mass selection scheme resulted in an average increase in mean body weight of 89.3g per pond for the selected individuals as compared to the mean body weight of the ponds before selection. Use of the modified within-family selection method, where the ten largest fish from each sire family were selected from each pond, resulted in an average increase in body weight of 53.9g per pond. The mass selection method produced highly variable proportional representation by sire, with some sires having no offspring or very few offspring in the selected group. Due to their larger size, the Santee:Chesapeake 7E27 sire family was highly represented in the final mass selected group while the Roanoke 4664 sire group and the Chesapeake 631D group were quite under-represented. In fact, only the two Santee:Chesapeake sires exceeded 10% representation in the final collection of 180 mass-selected broodstock, and they represent 26% (sire 292B) and 55% (sire 7E27) of the final selected group. Conversely, the modified within-family selection method produced equivalent representation from each sire family with each sire's progeny accounting for 16.7% of the final collection of broodstock.

The higher selected mean for body weight is characteristic of mass selection protocols as compared to other selection methods, including family selection, since the former method relies only on the individual's performance (Falconer and Mackay 1996). In the present case, the restriction to selecting the top ten individuals from each family resulted in a reduction in the mean of the selected fish since many individuals with high trait values were discarded in order to preserve genetic variation. In both cases, the proportion of the overall population

selected is the same, 18%, but the selection of the very best performing individuals from each pond at the end of Phase III by mass selection yields the greatest mean body weight for the selected groups and would presumably yield the most rapid response to selection but the coincident loss of genetic diversity should be considered. However, this simulation is an artificial one and in an actual striped bass selective breeding program, individuals from many more families would be evaluated. Selection from additional families should result in better maintenance of genetic diversity in a mass selected group. Application of a mass selection scheme should yield rapid gains in performance traits and examination of numerous communally reared families should provide the National Breeding Program with the means to achieve selective improvement of striped bass while minimizing losses of genetic diversity. Development of a control line of unselected striped bass will be necessary to monitor changes in phenotypic means and genetic variation from generation to generation in the breeding program. In support of selective breeding goals, use of microsatellite markers for individual identification would allow breeders to track broodstock pedigrees and to avoid crosses between closely related individuals and communal rearing will reduce the numbers of rearing units necessary for performance evaluations.

Other planned investigations—Additional investigations using data from this dissertation research are planned. One such area is that of paternal variation in striped bass body conformation. The hybrid striped bass industry is largely based on sale of live fish to seafood markets or on sale of whole fish on ice for the restaurant trade. In each case, market preferences are based largely on the external appearance of the fish, with body shape important in both markets. For live sales, a rounder fish shape is preferred (higher condition

factor) since these fish often are prepared for presentation at table and conformity to the size and shape of the serving platter is preferred. For sales of whole fish on ice to restaurants, a longer and leaner body shape is preferred (lower condition factor) for preparation and presentation of fillets. Evaluation of genetic variation in body shape as measured by condition factor was carried out in this dissertation research and sire-based genetic variation was present. A more detailed examination of differences in body conformation is possible by using landscape-based morphometrics based on quantitative analysis of digital images of striped bass. A landscape-based thin-plate spline technique demonstrated for *Morone* larvae by Fulford and Rutherford (2000) has already been utilized for digital images collected for Phase III hybrid striped bass, revealing evidence of genetic and gender differences in body conformation (A.F. Garber and C.V. Sullivan, *unpublished data*). In the present study, similar data were collected from maternal family 2E55 striped bass at the end of Phase III and full analysis of this data may provide further insight into body shape variation of *Morone* species. Briefly, striped bass were stunned in an oxygenated slurry of ice and water and small pins were used to mark eight landmarks on each fish's body. Landmarked points included the pre-operculum, the insertion point of the first dorsal spine, the insertion point of the first spine of second dorsal fin, the center of the caudal peduncle, the insertion point of the first spine of the anal fin, the anterior insertion point of the pelvic fin, the anterior-dorsal insertion point of the pectoral fin, the posterior edge of the upper jaw, and the center of the eye. Fish were then photographed individually with a 5cm ruler for consistent scaling. Images were collected using a stand-mounted Nikon Coolpix 4500 digital camera with 4 megapixel digital zoom set for wide angle photographs using a remote release. Three to four images were collected for each fish. A total of 400 fish per pond were photographed at PAFL and 426

were photographed at Kent Sea tech. Genotyping of these fish is complete. Full analysis of these data is expected to illuminate gender and paternal variation in body shape for the striped bass parent and should provide useful information for selective breeding of striped bass for body shape traits.

In an additional line of research, striped bass families produced for this dissertation project will be utilized for linkage mapping. In a project entitled "Building a Superior Striped Bass: A Genome Map for Accelerated Selective Breeding" and funded by the National Oceanic and Atmospheric Administration's Marine Aquaculture Initiative, families of maternal family 2E55 fish produced during this study will be utilized as reference families for production of the first genetic linkage map for striped bass. Access to hundreds of microsatellite markers developed for striped bass in our laboratory (Couch et al. 2006; Rexroad et al. 2006) will be assessed for allelic polymorphism in parents of the reference families. Markers which prove polymorphic will be utilized to genotype their progeny. Our focus on striped bass for construction of the genetic map is based on the fact that the dissemination of improved germplasm to the industry will be most efficiently accomplished by providing cryopreserved striped bass semen, since striped bass is utilized as the male parent of the commercially produced hybrid striped bass. Molecular markers available for striped bass also can be utilized effectively in the other parent, the white bass, as well as in hybrid striped bass, and as *Morone* appears to be a young genus of closely related species, a genetic linkage map developed for striped bass may prove useful for other *Morone* species.

Genetic linkage is the non-random association between loci and linkage between molecular markers or between markers and genes controlling a trait are of great value for selective improvement of species. At the genetic level, linkage occurs when two loci are on

the same chromosome. The closer the loci are to one another, the lower the likelihood of recombination between them during meiosis. This results in linkage disequilibrium in which alleles for the loci segregate in the gametes more often than is predicted by chance. Linkage associations can be detected by statistical analysis using marker genotypes from mapping reference families. Linkage disequilibrium between marker loci allows researchers to evaluate the inheritance of alleles in reference families and to determine the order and relative spacing of genetic markers along the chromosomes. For many agricultural species, linkage maps have proven to be effective tools for the rapid identification of genomic regions associated with important commercial traits and characterization of the genes underlying those traits. This information may then be used to implement marker-assisted selection (MAS) for improved production efficiency. Linkage maps also can be useful in studies of genome structure and evolution, positional cloning of genes, evaluation of adaptive traits in wild populations, and for comparative mapping among species. Thus, a striped bass linkage map will provide a valuable genetic resource not only for aquaculture, but also for fisheries science and marine conservation genetics.

Although classical selection can be used to produce better-performing offspring, economically important phenotypic traits are usually polygenic; they are controlled by numerous genes, with each gene contributing to a fraction of the overall phenotype. Such traits exhibit a range of expression and are measurable, or quantitative. Indirect selection of individuals as broodstock solely on the basis of observed phenotype, as is now widely practiced in aquaculture, is an inefficient method for genetic improvement, requiring months to years for some trait evaluations. Inheritance of those phenotypes may be unpredictable due to the individual heritability of specific traits and the number of genes involved. Use of

genetic markers linked to quantitative trait loci (QTL), or loci linked to traits that contribute to quantitative characters, may allow direct selection for traits of interest because the phenotype of the individual is known relative to its marker genotype. If the marker and QTL are closely associated, the marker genotype of the fish can be used in the absence of phenotypic information to more accurately select breeders that will pass on the preferred phenotype to their offspring. Ultimately, use of MAS based on QTL for striped bass may provide an effective means of selecting for preferred traits without complicated test-crosses and lengthy evaluations of progeny to ascertain parental performance. Availability of MAS for striped bass will require determination of chromosomal (or linkage) relationships among genetic markers using mapping reference families. Appropriate markers will then be analyzed for associations with economically important traits that are difficult or impossible to non-lethally measure on individual fish in a selection program, such as gender, fillet yield and disease resistance. If markers exhibit a high degree of correlation with specific traits, individuals with certain marker genotypes can be chosen for selective breeding to rapidly magnify traits of interest in the striped bass broodstock population. Marker-assisted selective breeding can implemented within the context of the ongoing selection program, permitting us to add mapped QTL to phenotype in a selection index for the breeding values of individual fish (Poompuang and Hallerman 1997).

Access to hundreds of microsatellite markers and to several well-characterized full-sibling intercross reference families permits development of a linkage map for striped bass. Use of archived samples of DNA and phenotypic data from these families will dramatically reduce the time, labor and costs typically necessary for a mapping project of this magnitude. Determination of recombination frequencies is most easily accomplished in crosses using

individuals with disparate performance traits, e.g., between highly inbred lines. As such lineages are not currently available for *Morone* species, it should be most efficient to exploit the dramatic differences in performance traits among captive striped bass strains. Use of crosses among these phenotypically and genetically disparate sire groups will facilitate evaluation of marker linkage relationships and QTL in the reference family populations as progeny of these inter-strain crosses should satisfy the requirements for performance and genetic differences between lines that are required for both linkage mapping and discovery of QTL. Intercrosses of this nature have been utilized successfully in linkage mapping for channel catfish (Waldbieser et al. 2001), Mediterranean seabass (Chistiakov et al. 2004, 2005) and salmon (Moen et al. 2004a). This map should provide a significant genetic resource that will prove critically important for successful marker-assisted selective breeding and functional genomics studies of farmed striped bass and hybrid striped bass.

Conclusion—In summary, this dissertation research has demonstrated the feasibility of communal rearing methodologies for striped bass performance evaluations based on microsatellite marker genotyping for progeny identification. Evidence of genetic variation within and among broodstock strains and variation in economically important performance traits as revealed in these studies provides useful information for development of a rational and efficient program for selective breeding of striped bass and for further investigations. Use of pond rearing techniques for production of striped bass broodstock demonstrated by Hodson and Sullivan (1993) and confirmed in this study should provide a viable means of producing captive and domesticated broodstock for the hybrid striped bass industry and selection of these animals should yield gains in production. Although additional research is

necessary, several authors have suggested that, as a cultivar, the striped bass may be superior to the current hybrid striped bass product and improved striped bass may be particularly desirable in some market sectors (Smith et al. 1985; Jenkins et al. 1988; Garber and Sullivan 2006). Use of selectively improved striped bass would eliminate the need for maintaining two parental species for hybrid production and would reduce environmental concerns about genetic contamination resulting from hybrid striped bass escaping into the wild. The studies detailed in this dissertation support the goals of the National Program of Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry and provide critical information to facilitate decision-making regarding future selective breeding of striped bass.

Table 25. Number, proportion and trait means (body weight) by sire for three ponds using a mass selection versus a modified family selection protocol for choosing striped bass broodfish from the 2E55 family for the next generation. Mean body weight for the pond before selection (Avg. before selection) and mean for the selected individuals (Avg. after selection) are shown for each pond.

Pond	Avg. before selection	Avg. after selection	SIRE					
			4664 (R:F_0-97)	7213 (R:F_0-97)	292B (SC:F_1-94)	7E27 (SC:F_1-94)	631D (C:F_2-91)	2A20 (C:F_2-91)
Top 60 fish per pond								
A4								
N			1	5	12	35	0	7
Avg. body weight (g)	334.7	410.9	383.8	400.9	416.6	416.3	--	385.4
Proportion by sire			0.017	0.083	0.020	0.583	--	0.117
A11								
N			0	3	16	31	2	8
Avg. body weight (g)	351.2	436.1	--	415.9	429.3	448.0	412.5	420.9
Proportion			--	0.050	0.270	0.520	0.030	0.130
A14								
N			1	2	19	33	3	2
Avg. body weight (g)	468.9	575.6	539.7	557.0	568.5	584.7	555.2	557.4
Proportion by sire			0.017	0.033	0.317	0.550	0.050	0.033
Avg. overall		474.2						
Top 10 fish per family								
A4								
N			10	10	10	10	10	10
Avg. body weight (g)	334.7	386.7	344.2	385.2	422.9	455.1	341.2	381.7
Proportion by sire			0.167	0.167	0.167	0.167	0.167	0.167
A11								
N			10	10	10	10	10	10
Avg. body weight (g)	351.2	389.9	338.5	393.6	444.3	493.1	371.9	415.6
Proportion by sire			0.167	0.167	0.167	0.167	0.167	0.167
A14								
N			10	10	10	10	10	10
Avg. body weight (g)	468.9	539.8	451.4	508.1	587.7	633.7	525.7	532.3
Proportion by sire			0.167	0.167	0.167	0.167	0.167	0.167
Avg. overall		438.8						

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APPENDICES

Appendix Table I. *P*-values for pairwise Z-tests of estimated proportions by sire and strain to Phase I larval populations. Proportional contributions with * after the *P*-value are significant after sequential Bonferroni correction. The presence of a (–) indicates *P*-values >0.05. Sires in boldface type have the higher estimated proportional representation within a pair.

POPULATION	SIRE PAIR	Z	P-VALUE
2E55	4664 (R:F_0-97) – 7213 (R:F_0-97)	2.1013	0.0179
(PAFL pond)	4664 (R:F_0-97) – 292B (SC:F_1-94)	2.2222	0.0132
	4664 (R:F_0-97) – 7E27 (SC:F_1-94)	1.4039	--
	4664 (R:F_0-97) – 631D (C:F_2-91)	1.4039	--
	4664 (R:F_0-97) – 2A20 (C:F_2-91)	1.4816	--
	7213 (R:F_0-97) – 292B (SC:F_1-94)	0.1208	--
	7213 (R:F_0-97) – 7E27 (SC:F_1-94)	3.4952	0.0002*
	7213 (R:F_0-97) – 631D (C:F_2-91)	3.4952	0.0002*
	7213 (R:F_0-97) – 2A20 (C:F_2-91)	0.6193	--
	292B (SC:F_1-94) – 7E27 (SC:F_1-94)	3.6160	0.0001*
	292B (SC:F_1-94) – 631D (C:F_2-91)	3.6160	0.0001*
	292B (SC:F_1-94) – 2A20 (C:F_2-91)	0.7401	--
	7E27 (SC:F_1-94) – 631D (C:F_2-91)	0.0000	--
	7E27 (SC:F_1-94) – **2A20 (C:F_2-91)**	2.8778	0.0020*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	2.8778	0.0020*
2E55	4664 (R:F_0-97) – 7213 (R:F_0-97)	2.1978	0.0179
(commercial pond)	4664 (R:F_0-97) – **292B (SC:F_1-94)**	3.5726	0.0002*
	4664 (R:F_0-97) – 7E27 (SC:F_1-94)	0.3269	--
	4664 (R:F_0-97) – 631D (C:F_2-91)	0.1652	--
	4664 (R:F_0-97) – **2A20 (C:F_2-91)**	3.9287	<0.0001*
	7213 (R:F_0-97) – 292B (SC:F_1-94)	1.3784	--
	7213 (R:F_0-97) – 7E27 (SC:F_1-94)	1.8736	0.0307
	7213 (R:F_0-97) – 631D (C:F_2-91)	2.0342	0.0212
	7213 (R:F_0-97) – 2A20 (C:F_2-91)	1.7337	0.0418
	292B (SC:F_1-94) – 7E27 (SC:F_1-94)	3.2501	0.0006*
	292B (SC:F_1-94) – 631D (C:F_2-91)	3.4099	0.0003*
	292B (SC:F_1-94) – 2A20 (C:F_2-91)	0.3545	--
	7E27 (SC:F_1-94) – 631D (C:F_2-91)	0.1618	--
	7E27 (SC:F_1-94) – **2A20 (C:F_2-91)**	3.6064	0.0002*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	3.7661	0.0001*
512C	5442 (R:F_0-97) – **2130 (R:F_0-97)**	4.1203	<0.0001*
(research pond)	5442 (R:F_0-97) – **3F11 (SC:F_1-94)**	4.3574	<0.0001*
	5442 (R:F_0-97) – 5C5D (SC:F_1-94)	0.8639	--
	5442 (R:F_0-97) – **5A46 (C:F_2-91)**	6.9661	<0.0001*
	5442 (R:F_0-97) – 5709 (C:F_2-91)	4.1712	<0.0001*
	2130 (R:F_0-97) – 3F11 (SC:F_1-94)	0.2378	--
	2130 (R:F_0-97) – 5C5D (SC:F_1-94)	3.2779	0.0005*
	2130 (R:F_0-97) – 5A46 (C:F_2-91)	2.7916	0.0026
	2130 (R:F_0-97) – 5709 (C:F_2-91)	7.6256	<0.0001*
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	3.5160	0.0002*
	3F11 (SC:F_1-94) – 5A46 (C:F_2-91)	2.5512	0.0054
	3F11 (SC:F_1-94) – 5709 (C:F_2-91)	7.8443	<0.0001*
	5C5D (SC:F_1-94) – **5A46 (C:F_2-91)**	6.1173	<0.0001*
	5C5D (SC:F_1-94) – 5709 (C:F_2-91)	4.8454	<0.0001*
	5A46 (C:F_2-91) – 5709 (C:F_2-91)	10.3916	<0.0001*

Appendix Table I, continued.

POPULATION	SIRE PAIR	Z	P-VALUE
5F4B	5442 (R:F_0-97) – 3B62 (R:F_0-97)	0.5457	--
(research pond)	5442 (R:F_0-97) – 3F11 (SC:F_1-94)	0.1410	--
	5442 (R:F_0-97) – 5C5D (SC:F_1-94)	0.1391	--
	5442 (R:F_0-97) – 631D (C:F_2-91)	2.3905	0.0084
	5442 (R:F_0-97) – **2A20 (C:F_2-91)**	3.7622	0.0001*
	3B62 (R:F_0-97) – 3F11 (SC:F_1-94)	0.6867	--
	3B62 (R:F_0-97) – 5C5D (SC:F_1-94)	0.4067	--
	3B62 (R:F_0-97) – 631D (C:F_2-91)	2.9284	0.0017*
	3B62 (R:F_0-97) – **2A20 (C:F_2-91)**	3.2130	0.0007*
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	0.2800	--
	3F11 (SC:F_1-94) – 631D (C:F_2-91)	2.2513	0.0122
	3F11 (SC:F_1-94) – **2A20 (C:F_2-91)**	3.9039	<0.0001*
	5C5D (SC:F_1-94) – 631D (C:F_2-91)	2.5275	0.0057
	5C5D (SC:F_1-94) – **2A20 (C:F_2-91)**	3.6224	0.0001*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	6.1322	<0.0001*
152D	**5442 (R:F_0-97)** – 3B62 (R:F_0-97)	4.7872	<0.0001*
(research ponds)	5442 (R:F_0-97) – 3F11 (SC:F_1-94)	0.9785	--
	5442 (R:F_0-97) – 5C5D (SC:F_1-94)	3.0300	0.0012*
	5442 (R:F_0-97) – 631D (C:F_2-91)	1.0213	--
	5442 (R:F_0-97) – 2A20 (C:F_2-91)	4.0012	<0.0001*
	3B62 (R:F_0-97) – **3F11 (SC:F_1-94)**	3.8148	0.0001*
	3B62 (R:F_0-97) – 5C5D (SC:F_1-94)	1.7782	0.0375
	3B62 (R:F_0-97) – **631D (C:F_2-91)**	5.8098	<0.0001*
	3B62 (R:F_0-97) – 2A20 (C:F_2-91)	0.8025	--
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	2.0518	0.0202
	3F11 (SC:F_1-94) – 631D (C:F_2-91)	2.0009	0.0228
	3F11 (SC:F_1-94) – 2A20 (C:F_2-91)	3.0251	0.0013*
	5C5D (SC:F_1-94) – **631D (C:F_2-91)**	4.0549	<0.0001*
	5C5D (SC:F_1-94) – 2A20 (C:F_2-91)	0.9787	--
	631D (C:F_2-91) – 2A20 (C:F_2-91)	5.0257	<0.0001*

Appendix Table II. *P*-values for pairwise Z-tests of estimated survival by sire and strain during Phase I rearing. Pairwise comparisons with * after the *P*-value are significant after sequential Bonferroni correction. The presence of a (–) indicates *P*-values >0.05. Sires in boldface type have the higher estimated survival within a pair.

POPULATION	SIRE PAIR	*Z*	*P*-VALUE
2E55 (research pond)	4664 (R:F_0-97) - 7213 (R:F_0-97)	0.0437	--
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.0631	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94	1.1198	--
	4664 (R:F_0-97) - 631D (C:F_2-91)	1.0059	--
	4664 (R:F_0-97) - 2A20 (C:F_2-91)	1.3217	--
	7213 (R:F_0-97) - 292B (SC:F_1-94)	0.6984	--
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	1.1368	--
	7213 (R:F_0-97) - 631D (C:F_2-91)	1.0285	--
	7213 (R:F_0-97) - 2A20 (C:F_2-91)	1.3323	--
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	1.3004	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.2427	--
	292B (SC:F_1-94) - 2A20 (C:F_2-91)	1.4377	--
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	0.2675	--
	7E27 (SC:F_1-94) - 2A20 (C:F_2-91)	0.4866	--
	631D (C:F_2-91) - 2A20 (C:F_2-91)	0.7172	--
512C	5442 (R:F_0-97) - 2130 (R:F_0-97)	0.8794	--
	5442 (R:F_0-97) – 3F11 (SC:F_1-94)	0.1885	--
	5442 (R:F_0-97) – 5C5D (SC:F_1-94	0.5935	--
	5442 (R:F_0-97) – 5A46 (C:F_2-91)	0.3241	--
	2130 (R:F_0-97) – 3F11 (SC:F_1-94)	1.1126	--
	2130 (R:F_0-97) – 5C5D (SC:F_1-94)	0.3772	--
	2130 (R:F_0-97) – 5A46 (C:F_2-91)	0.7747	--
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	0.8153	--
	3F11 (SC:F_1-94) – 5A46 (C:F_2-91)	0.5428	--
	5C5D (SC:F_1-94) – 5A46 (C:F_2-91)	0.3461	--
5F4B	5442 (R:F_0-97) – 3B62 (R:F_0-97)	1.0127	--
	5442 (R:F_0-97) – 3F11 (SC:F_1-94)	1.0670	--
	5442 (R:F_0-97) – 5C5D (SC:F_1-94	0.7816	--
	5442 (R:F_0-97) - 631D (C:F_2-91)	1.0923	--
	5442 (R:F_0-97) - 2A20 (C:F_2-91)	0.1283	--
	3B62 (R:F_0-97) – 3F11 (SC:F_1-94)	1.8191	0.0351
	3B62 (R:F_0-97) – 5C5D (SC:F_1-94)	0.2557	--
	3B62 (R:F_0-97) - 631D (C:F_2-91)	1.6686	0.0475
	3B62 (R:F_0-97) - 2A20 (C:F_2-91)	1.2318	--
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	1.6626	0.0485
	3F11 (SC:F_1-94) - 631D (C:F_2-91)	0.1965	--
	3F11 (SC:F_1-94) - 2A20 (C:F_2-91)	0.9975	--
	5C5D (SC:F_1-94) - 631D (C:F_2-91)	1.5517	--
	5C5D (SC:F_1-94) - 2A20 (C:F_2-91)	0.9759	--
	631D (C:F_2-91) - 2A20 (C:F_2-91)	1.0334	--
152D (pond A5)	5442 (R:F_0-97) – 3B62 (R:F_0-97)	0.4006	--
	5442 (R:F_0-97) – 3F11 (SC:F_1-94)	0.9859	--
	5442 (R:F_0-97) – 5C5D (SC:F_1-94	1.8187	0.0344
	5442 (R:F_0-97) - 631D (C:F_2-91)	1.2291	--
	5442 (R:F_0-97) - 2A20 (C:F_2-91)	0.9423	--
	3B62 (R:F_0-97) – 3F11 (SC:F_1-94)	1.2306	--

Appendix Table II, continued.

POPULATION	SIRE PAIR	Z	P-VALUE
	3B62 ($R:F_0$-97) – 5C5D ($SC:F_1$-94)	1.0645	--
	3B62 ($R:F_0$-97) - 631D ($C:F_2$-91)	1.4501	--
	3B62 ($R:F_0$-97) - 2A20 ($C:F_2$-91)	1.1691	--
	3F11 ($SC:F_1$-94) – 5C5D ($SC:F_1$-94)	2.5343	0.0057
	3F11 ($SC:F_1$-94) - 631D ($C:F_2$-91)	0.1758	--
	3F11 ($SC:F_1$-94) - 2A20 ($C:F_2$-91)	0.1142	--
	5C5D ($SC:F_1$-94) - **631D ($C:F_2$-91)**	2.8828	0.0020*
	5C5D ($SC:F_1$-94) - **2A20 ($C:F_2$-91)**	2.1736	0.0150
	631D ($C:F_2$-91) - 2A20 ($C:F_2$-91)	0.0331	--
152D (pond A7)	5442 ($R:F_0$-97) – 3B62 ($R:F_0$-97)	0.2706	--
	5442 ($R:F_0$-97) – 3F11 ($SC:F_1$-94)	2.1006	0.0179
	5442 ($R:F_0$-97) – 5C5D ($SC:F_1$-94	0.8722	--
	5442 ($R:F_0$-97) - 631D ($C:F_2$-91)	1.1524	--
	5442 ($R:F_0$-97) - 2A20 ($C:F_2$-91)	1.1308	--
	3B62 ($R:F_0$-97) – 3F11 ($SC:F_1$-94)	1.6678	0.0475
	3B62 ($R:F_0$-97) – 5C5D ($SC:F_1$-94)	0.8733	--
	3B62 ($R:F_0$-97) - 631D ($C:F_2$-91)	1.0520	--
	3B62 ($R:F_0$-97) - 2A20 ($C:F_2$-91)	0.8279	--
	3F11 ($SC:F_1$-94) – 5C5D ($SC:F_1$-94)	2.6974	0.0035*
	3F11 ($SC:F_1$-94) - 631D ($C:F_2$-91)	2.9466	0.0016*
	3F11 ($SC:F_1$-94) - 2A20 ($C:F_2$-91)	0.7659	--
	5C5D ($SC:F_1$-94) - 631D ($C:F_2$-91)	0.1619	--
	5C5D ($SC:F_1$-94) - 2A20 ($C:F_2$-91)	1.7158	0.0427
	631D ($C:F_2$-91) - 2A20 ($C:F_2$-91)	1.9071	0.0281

Appendix III

Manuscript for microsatellite marker development published by the journal *Molecular Ecology Notes* (*http://www.springerlink.com/(bqlnop551ldvytz2h44cvf45)/app/home/issue.asp)*. Unlike the published manuscript, Table 1 includes all 149 markers developed in this study.

ISOLATION AND CHARACTERIZATION OF 149 NOVEL MICROSATELLITE DNA MARKERS FOR
STRIPED BASS, *MORONE SAXATILIS*, AND CROSS-SPECIES AMPLIFICATION IN WHITE BASS, *M.
CHRYSOPS*, AND THEIR HYBRID

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ABSTRACT

To support detailed genetic analysis of striped bass (*Morone saxatilis*) and white bass
(*M. chrysops*), we isolated 153 microsatellite loci from repeat-enriched striped bass
DNA libraries. Of these, 147 markers amplified in striped bass (average 4.7 alleles per
locus) and 133 in white bass (average 2.2 alleles per locus). One-hundred twenty-two
markers amplified in their hybrid. Development of new microsatellite markers will
facilitate evaluations of genetic structure in wild populations and will support pedigree
analysis and linkage mapping for selective breeding.

The anadromous striped bass (*Morone saxatilis*) is native to coastal regions of eastern North America from Nova Scotia to Florida and within the Gulf of Mexico west to Louisiana. This species supported valuable commercial and recreational fisheries but experienced significant population declines in the mid-1970's due to habitat degradation and overfishing. Population bottlenecks and supplementation of depleted stocks with non-native fish likely have altered the historic population genetic structure of striped bass. Additionally, widespread introductions of a fertile hybrid striped bass (HSB; white bass *M. chrysops* X *M. saxatilis*) for stock enhancement and recreational fishing may have permitted introgression with wild *Morone* species. Although genetic differentiation has been detected among several geographic strains of *M. saxatilis*, unusually low genetic variation appears to characterize this species (Waldman *et al.* 1988) and has limited the number of informative molecular markers available for detailed population genetic analysis.

Numerous high-resolution molecular markers also are needed for pedigree tracking in aquacultured populations of *Morone* species. Declines in wild striped bass harvests created market demand for production of HSB as foodfish. Until recently, this industry remained largely dependent on wild fish for broodstock. Efforts toward sustainable aquaculture of HSB by domestication and selective breeding of the parent species are now underway and require highly polymorphic markers for progeny identification.

To date, 46 published microsatellite markers have been characterized for *Morone* species. Only 12 markers have ≥6 alleles in striped bass and 4 have ≥3 alleles for white bass (see review in Garber and Sullivan, *in press*). Additional informative markers are necessary not only to support detailed genetic analyses for conservation and management of wild populations, but also for selective breeding and linkage mapping in aquaculture.

Microsatellite markers were developed from a repeat-enriched striped bass DNA library using a protocol by Ostrander *et al.* (1992) modified by Westerman *et al.* (2005). Genomic DNA (100 μg) was extracted from whole blood from a striped bass and digested with *Sau*3A1 and *Bam*H1 (Invitrogen). Three enriched libraries containing CA:GT repeats were screened. One library (SB-PE1) was 25% enriched and two (SB-PE2 and SB-PE7) were >60% enriched.

Sequencing of clones was carried out using an ABI Prism® 3700 DNA Analyzer (Applied Biosystems) and sequence analysis was performed with Vector NTI Suite 7.0 (Invitrogen). Sequences were aligned and primers were designed for unique, high-quality sequences with Oligo® 6.0 software (Molecular Biology Insights).

PCR amplification of microsatellite loci was carried out in 10 μl reactions containing 1.0 μl DNA (~10 ng/μl), 2 mM $MgCl_2$, 48.2 μM each dNTP (Promega), 1 μl 10X buffer (QIAGEN), 0.48 μM forward primer (Integrated DNA Technologies), 0.50 μM reverse primer with 5' fluorescent label (Applied Biosystems), and 0.48 U HotStar *Taq* DNA polymerase (QIAGEN). Thermal cycling parameters consisted of 95 °C for 15 min, 35 cycles each of 94 °C for 30 s, annealing temperature for 30 s, and 72 °C for 30 s, followed by 1 cycle of final elongation at 72 °C for 10 min. Amplification was

performed either in multiplexed sets of two to four markers or singly. Amplified products were run on an ABI Prism® 3700 DNA analyzer with GENESCAN^{TM} 500 LIZ^{TM} size standard, and alleles were identified using GeneMapper® software version 3.0 (Applied Biosystems).

A total of 138 primer pairs from the SB-PE2 and SB-PE7 libraries were evaluated for polymorphism using a geographically diverse screening panel of DNA sampled from wild and captive-bred striped bass (n=15) and white bass (n=6). Two hybrids were included for evaluation of amplification. Fifteen additional markers from the SB-PE1 library were screened before the full panel was available. In all, 153 microsatellite loci were evaluated (GenBank Accession numbers BV678169-BV678309; BV678652-BV678663).

Only four markers failed to reliably amplify in either species (GenBank Accession numbers BV678253, -83, -89, and -97). Detailed information for 149 amplifying markers can be found in the primer database (http://tomato.bio.trinity.edu/home.html). For striped bass, 147 markers amplified successfully with a range of 1-17 alleles per locus and an average of 4.7 alleles per locus. In white bass, 133 markers amplified successfully with 1-10 alleles per locus (average 2.2 alleles per locus). Seventy-one markers were polymorphic in both species; 50 markers had ≥6 alleles in striped bass and 38 had ≥3 alleles in white bass. Of the 122 markers amplifying in both parent species and the hybrid (2 alleles detected, 1 attributable to each parent species), 45 produced non-overlapping, species-specific allele size ranges (≥10 bp difference between parental species) and may be useful for detecting introgression.

Observed and expected heterozygosities, Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were assessed using GENEPOP (http://wbiomed.curtin.edu.au/genepop/). Significance was evaluated after correction for multiple tests (Rice 1989). Overall average heterozygosity was 0.56 for striped bass and 0.45 for white bass. Significant deviations from HWE were observed for seven loci in striped bass. These deviations may indicate the presence of null alleles or result from pooling of samples from various geographic locations by species for analysis. No significant linkage disequilibrium was detected. Data for a subset of the markers are presented in Table 1. These 29 markers amplified in both parents and in the hybrid and had expected heterozygosities ≥ 0.75 in striped bass; as such, these markers should prove especially useful for genetic evaluations in both wild and captive populations.

Contribution of numerous new microsatellite markers for *Morone* species provides necessary molecular tools for detailed genetic analysis of stock structure in wild populations and for selective breeding and linkage mapping in aquaculture.

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Table 1. Summary data for 149 microsatellite loci screened in striped bass (SB) and white bass (WB) with observed allele size ranges (NA represents no amplification), number of individuals assayed (*N*), number of alleles observed (*k*), observed (H_O) and expected (H_E) heterozygosities (boldface numbers represent significant deviations from HWE), and PCR annealing temperatures (T_A) in °C. Non-amplifying loci in the hybrid are indicated with an asterisk. Loci examined in multiplex PCR (MP) that share a common number were amplified successfully in a multiplex set; those with a dash were evaluated singly.

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	*N/k* SB WB	H_O/H_E SB WB	T_A	MP
MSM1065	BV678236	F: ATGCCCTAGTCCAGATACACA	$(CA)_{16}$	240-248	6/2	1/0.56	58	--
		R: AATACAGCATGACGGTGTTTC		225-234	15/3	1/0.58		
MSM1066	BV678237	F: CCACTCAGCTCGTGCTTT	$(CA)_{25}$	275-287	6/3	0.33/0.55	58	--
		R: CTCCCAGTCATGCGATGT		265	33/1	--		
MSM1067	BV678238	F: GGAATCAAATCCCTGCTGTTATAATCT	$(CA)_{14}(GT)_{11}$	190-210	6/5	0.83/0.8	58	--
		R: CTATCTGGACTTTATCCCTACGAGTGA		154-159	33/2	0.71/0.49		
MSM1068	BV678239	F: TCTTTGTGCGACTTCAAGGTTTAGGAC	$(CA)_{21}$	144-163	6/3	1/0.69	58	--
		R: TTTGTTTCCCTGCATGTGTTTGTATGT		220-228	10/3	0.9/0.62		
MSM1069	BV678240	F: GCTGTTTATGTCCCAATAGAG	$(GT)_{16}$	265	3/1	--	58	--
		R: GAGATTATCCAAGGCCATAGT		294	4/1	--		
MSM1073	BV678309	F: CTGATTGGGTCTGTAATAGTCTTG	$(CT)_{21}$	116-120	5/2	0.4/0.36	58	--
		R: AAAGACAGGCACAGTGAGACATAC		151-161	33/4	0.58/0.57		
MSM1074	BV678241	F: TTTCTCACTCTCTCCCATGCCCTC	$(GT)_{14}$	160-174	3/2	0.67/0.53	58	--
		R: ATGTAAGGCCTGGAGTCCGTCAAC		NA	4/0	--		
MSM1075	BV678242	F: CTTCTTTTCTCCTCCTCCAGCACAATC	$(AC)_{29}$	212-226	3/3	0.67/0.73	58	--
		R: CTCTTGCTGTAAAATTTCTCGCCAAGT		192-197	10/2	0.4/0.36		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1076	BV678243	F: TTTCTCTCGGAACATCATTGCTATCTG	$(GT)_{21}$	185	3/1	--	58	--
		R: CTGAGGCTACAATACAGGCACTGGTAA		205-207	4/2	0.5/0.43		
MSM1077	BV678244	F: TTAAAGGAAAACTGTTTGCAACTCCGA	$(CA)_{22}$	216-220	5/3	1/0.73	58	--
		R: CACGCAGTATTTAAGCAATTTGCATGT		202-212	6/2	0.17/0.17		
MSM1078	BV678245	F: GCAGGACTCCCGTGAAATACAACC	$(GT)_{10}$	125	3/1	--	58	--
		R: AATTGAGAGGCCTTGGCTAGCATC		132-141	4/2	1/0.6		
MSM1079	BV678246	F: CGCCGAAAAGACACAGTTTAC	$(GT)_{11}$	250	3/1	--	58	--
		R: CTCCTGCTGGAAAAACTGATG		237	4/1	--		
MSM1080	BV678247	F: GTCGATTCGCTCACATATTGG	$(CA)_9$	198-206	3/2	1/0.6	58	--
		R: ATAGGCAGCCGCTGTTATAAA		201-210	4/3	1/0.68		
MSM1081	BV678248	F: TGTGTGTAAATGTCAAGGGTGTAA	$(GT)_9$	142	3/1	--	58	--
		R: TGCACCATTTTAATTTAGTGTGAG		164	4/1	--		
*MSM1082**	BV678249	F: GGACGATTGCGAGTTAAT	$(GT)_9$	102-105	3/2	0.67/0.53	58	--
		R: ATGTGACAAGTACGCGAG		116-124	4/3	0.67/0.6		
MSM1083	BV678169	F: ACAGAGCTTATCACCGTGCAG	$(CA)_{13}$	119-130	15/3	0.6/0.54	62	1
		R: AAGAACGTTGCCAACTCCC		115-119	6/2	1/0.55		
*MSM1084**	BV678170	F: CATTGATATTGCAGCAGCCCTAAC	$(TG)_{17}$	218-248	15/10	0.77/0.89	58	--
		R: CTGATCACCGTCCTTTGAGCTTTA		NA	6/0	--		
MSM1085	BV678171	F: TCTTTTATTTTTAGCCTCATTCAGACTGAT	$(CA)_{31}$	144-189	15/13	0.73/0.9	58	--
		R: CAGCAACAGATGATGGTCAAGTATG		109-111	6/2	0.33/0.3		
MSM1086	BV678172	F: CCCTTTTGTAGCCCTGCACTTGTA	$(CA)_{21}$	156-164	15/4	0.53/0.67	58	--
		R: CGGAGGGTTAGCCATGCATTT		139	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1087	BV678173	F: GAGTTCTGCTGAGGCTATTGA	(CA)$_{22}$	100-135	15/6	0.53/0.54	58	--
		R: GGCAACATCCTGATAGTATGTG		100-102	6/2	0/0.3		
MSM1088	BV678174	F: ACGTCAGAGCAGTGGGGTAA	(TG)$_{15}$	171-196	15/6	0.77/0.71	58	--
		R: CGACAGTCAAGTCAACATGCTT		208-231	6/5	0.67/0.83		
MSM1092	BV678175	F: CACTCTGGTTTACTGAATAAGCTCC	(CA)$_{28}$	183-216	15/7	0.64/0.79	58	--
		R: GTGCAGCCACAGTGTGTCTAC		152	6/1	--		
MSM1093	BV678176	F: TACACACAAGCCCAAACGACA	(CA)$_{14}$	182-195	15/5	0.6/0.69	58	--
		R: ACGTTAAGGCAGTCGCAGGT		170-174	6/2	1/0.55		
MSM1094	BV678177	F: TCCATCCCATCCTCTGTATC	(CA)$_{25}$	125-157	15/7	0.85/0.83	58	--
		R: GCCTCTCTGAGCTTATCCCTA		160-183	6/9	1/0.95		
MSM1095	BV678178	F: TGATAGCTGTGGTTACTGGTTG	(TG)$_{28}$	155-188	15/8	0.93/0.81	60	--
		R: AGGCTGATGCTGCAGTTATT		147-170	6/3	0.33/0.59		
MSM1096	BV678179	F: GACATGCACAGAGACAAATG	(CA)$_{25}$	182-200	15/7	0.73/0.8	60	--
		R: CAAGCTCCAGTCTATAACAGC		180	6/1	--		
MSM1097	BV678180	F: GTGTGTTTCAACCGGTGTC	(TG)$_{13}$	136-151	15/6	0.67/0.69	60	--
		R: AGAAGGAGCAGCATAGACCTAA		121-125	6/2	0.33/0.3		
MSM1098	BV678181	F: GCTGCAAGGATTAAACACTCAC	(CA)$_{18}$	151-160	15/4	0.79/0.69	60	--
		R: GCTCCCTCTGATGGTCTTGTA		138-143	6/2	1/0.55		
MSM1099	BV678182	F: CAATCCAAGTTTCACTGCGTGT	(CA)$_{20}$	122-130	15/4	0.53/0.62	60	--
		R: CCCCTTTTTCCGTTTCTATGC		118	6/1	--		
MSM1100	BV678183	F: CTGTTTGGCATGCAGGTAGGAA	(TG)$_{20}$	172-176	15/3	0.33/0.54	60	--
		R: AGCCCGCTCTGACCACTTAAGA		166	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1101	BV678184	F: TGGTAAGTGTCCGCTTCATAC	$(TG)_{15}$	141-177	15/5	0.57/0.53	60	--
		R: TGATACACTGAGCTGATGTCCT		162-168	6/2	0.33/0.55		
MSM1102	BV678185	F: AGAGAGACTGGATGATACGG	$(GT)_{16}$	142	15/1	--	60	--
		R: GACTAAGCAGGATTACTCAAGG		118-122	6/2	1/0.56		
MSM1104	BV678186	F: TTCTGCCTGAGGAGCTACA	$(CCT)_8$	144	15/1	--	60	--
		R: GAATAGGTCACACAGCGTTG		144	6/1	--		
MSM1106	BV678187	F: CTTGCTTTGCCGTGTG	$(GT)_{20}$	140-152	15/4	0.27/0.70	60	--
		R: CTGGTGTTGGCGTCTATG		180-182	6/2	0.17/0.17		
MSM1107	BV678188	F: GATAACCTATAGGCCACGTTG	$(GT)_{13}$	144-225	15/8	0.6/0.76	60	--
		R: TTCACAAGACTGCACGTACA		129	6/1	--		
MSM1108	BV678189	F: GACTGTATTTGGGCGTGACTTC	$(GT)_{15}$	130-136	15/3	0.07/0.13	60	--
		R: CCCACCAGTGTGAATAATTACACC		126	6/1	--		
MSM1109	BV678190	F: TTCCACTGTGTGTAAACCACC	$(GT)_{17}$	116-120	15/3	0.43/0.52	61	--
		R: CTGAAGGCCCTGACATGTAC		110	6/1	--		
MSM1110	BV678191	F: ACAAGCTGGATGACGTGG	$(GT)_{17}$	109-159	15/6	0.67/0.72	61	--
		R: TCACTGTGCGCTCAACC		101-138	6/5	1/0.83		
MSM1111	BV678192	F: GAGACCACGGCTGTGTCGAG	$(AC)_{19}$	161-171	15/5	0.73/0.64	61	--
		R: GTGCATGTGAGCCAGTGTTCAA		144-148	6/2	1/0.55		
MSM1114	BV678193	F: GGCACTCTTTAGAACCTGTTA	$(CA)_{17}$	176-187	15/4	0.6/0.63	61	--
		R: GCATGCCCCTTTACTTT		185-187	6/2	0.67/0.55		
MSM1115	BV678194	F: TAAGAGCTCCTGCTGTACACTCGC	$(CA)_{14}$	120-151	15/5	0.64/0.7	61	--
		R: GAATCCCCTCTTCCCCATTGAC		129-133	6/2	0.33/0.3		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1117	BV678195	F: CGGCTCCAAATATCGGCTCA	$(GT)_{21}$	160-186	15/4	**1/0.61**	61	--
		R: CAGGTAAGCCCATCAGTGACATCA		168-197	6/3	0.5/0.62		
MSM1118[*]	BV678196	F: GTTGCTTCTGTTGTTGCTG	$(CT)_{32}$	144-161	15/2	**1/0.52**	68	2
		R: GCAGAAACCTCGGACAG		130-140	6/3	1/.67		
MSM1119	BV678197	F: TCAGTTCTTCCCACGCAAGC	$(CA)_{18}$	150-156	15/3	0.53/0.57	61	--
		R: ACAGTAGCTCCATTTGCGGTCA		140-166	6/4	0.33/0.7		
MSM1120	BV678198	F: AGCCTGGCCTTTTACCCTACACACT	$(CA)_{19}$	NA	15/0	--	68	--
		R: AATGTGGGTGGGGTGCATGT		144-157	6/3	0.67/0.73		
MSM1121	BV678199	F: GTGGTGTTATTCGGTGTTTAG	$(GT)_{15}$	239-257	15/4	0.27/0.36	61	--
		R: CATTTCCTGTCTGATGCC		249-251	6/2	0.2/0.2		
MSM1122	BV678653	F: AGTGCCATGAAAAAGTGCTTTG	$(GT)_{16}$	176-198	15/7	0.47/0.65	64	3
		R: ATTAACCTCAGCACATCCTGTCAC		196-198	6/2	0.17/0.17		
MSM1123	BV678200	F: CTATTGAGGGATTGCAGAGCTACACTT	$(CA)_{27}$	231-251	15/8	0.4/0.67	61	--
		R: GCAAGGAAATGATGTGGACTGG		228-230	6/2	0/0.36		
MSM1124	BV678201	F: GACTAGTTTCTGGTTTGGCCGGAGT	$(CA)_{17}$	130-161	15/3	0.47/0.6	61	--
		R: CCTGGAACCTGTGCGTAATCTTACTGT		155-161	6/2	0.17/0.17		
MSM1125	BV678202	F: AGCCTCTCCACACACTACAGA	$(GT)_{18}$	129-139	15/4	0.8/0.66	61	--
		R: GCAACTCGAACCAATCAGA		159-161	6/2	0.17/0.17		
MSM1129	BV678203	F: CACAAACACAGCACGCTGAA	$(CA)_{20}$	178-190	15/3	0.29/0.26	61	--
		R: GCGGCTTGTTTGTCCATAATG		170-172	6/2	0.33/0.48		
MSM1131	BV678204	F: GAAAACAAACATGAACCTGAGACTGC	$(CT)_{12}(CA)_{25}$	163-177	15/6	0.36/0.62	61	4
		R: CGCTGTGATTTGACTGAAACTCG		171-181	6/3	0.5/0.62		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1132	BV678205	F: CTACAGACTTGATAAGATGGGTTGG	$(GT)_{20}$	125-146	15/5	0.21/0.52	61	--
		R: TCAACTAACCAGCGTCAATGTC		137-166	6/3	0.17/0.44		
MSM1133	BV678654	F: GCCCAGTGGTAATTTAGCAGT	$(TG)_{16}$	143-147	15/2	0.07/0.07	61	--
		R: CTATGAAATGAAACGCTGCC		139-160	6/4	0.67/0.64		
MSM1134[*]	BV678206	F: ACTTTTCTGTCAGGACACAGC	$(AC)_{36}$	186-222	15/10	0.5/0.92	61	--
		R: CGATGGCAGCTTACATAGG		147	6/1	--		
MSM1135[*]	BV678207	F: AAGGGTTTGAGGAAGACAAC	$(CA)_{44}$	163-189	15/9	0.75/0.89	61	--
		R: GCAGCCAGTCACTATCTTATG		NA	6/0	--		
MSM1136	BV678208	F: AGAAGAGGGCAGACATTAGTC	$(CA)_{19}$	165-184	15/4	0.45/0.4	61	5
		R: CTTTGTATGTAAGCGTGTGC		167	6/1	--		
MSM1137	BV678209	F: GCAGGCAGGTTTTATCTAGGTTAG	$(CA)_{35}$	153-240	15/16	0.79/0.92	55	6
		R: ACACTCTCTGCCCTTTGAGTTC		125	6/1	--		
MSM1138	BV678652	F: GGCCACCTTCAACTAACATACTTC	$(TG)_{17}$	184-192	15/5	0.5/0.75	61	5
		R: CGCTCCGTGTCTTGTCTAAAT		159-167	6/3	0.67/0.62		
MSM1139	BV678210	F: TCTTTCCCAGCAGTGAACAAACTAT	$(AC)_{34}$	171-201	15/8	0.77/0.82	61	--
		R: GCTGTGGCCAAATTATTGTAGTCAG		166-172	6/3	0.17/0.44		
MSM1140	BV678211	F: GCCAAGCCATTGCATTATCCCATT	$(AC)_{17}$	179-209	15/8	0.6/0.78	61	7
		R: TCACTCCTCATGCCACTTTCGACC		158-178	6/5	0.67/0.79		
MSM1142	BV678212	F: TGCCAGGAGATTAGATAGCTTGCAC	$(AC)_{25}$	158-162	15/3	0.43/0.66	61	--
		R: CGAAATTGGACTTGGCGAAATC		122	6/1	--		
MSM1143	BV678213	F: CTGGAGACAATCAATAGCTG	$(AC)_{20}$	87-119	15/6	0.29/0.68	55	8
		R: GAAAGTTTCCACCTCATACC		85	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1144	BV678214	F: CAGTGGGAGGGAGAGTAAATA	$(AC)_{25}$	115-150	15/10	0.92/0.88	61	5
		R: GCAGGATAGGAATCAGTCG		175-183	6/3	0.5/0.44		
MSM1145	BV678215	F: CTCCTCAAAATGTGTGACCC	$(CA)_{43}$	155-293	15/17	0.92/1.03	55	8
		R: TGCAGTGTTGATCAGGTTACAG		202-253	6/8	0.5/0.93		
MSM1146	BV678216	F: GCGCTCCATGTACAACCA	$(GT)_{18}$	183-197	15/3	0.13/0.49	61	5
		R: ATCCTCTAATGTCCCGAAACAC		179	6/1	--		
MSM1147	BV678217	F: TCTGCCAGTCTTCCTTGTAAGC	$(CA)_{20}$	158	15/1	--	61	9
		R: TTGCCCTGTGACGTTCAAC		164	6/1	--		
MSM1148	BV678218	F: TTGAGACAAAGCCGTGACGAGAC	$(CA)_{17}$	139-145	15/4	0.8/0.63	61	7
		R: ATTGATTTCTCCGACAGCGTGTG		152-158	6/2	0.17/0.17		
MSM1149	BV678219	F: GAAAAGCACTCAGAGGAACACACGC	$(AC)_{18}$	188-194	15/4	0.6/0.49	68	2
		R: GTCAACACTCACAGCAAGACACTGACA		178	6/1	--		
MSM1150[*]	BV678220	F: CTGCGACAACAGGAAGCTAAC	$(GT)_{19}$	151-153	15/2	0.14/0.25	61	4
		R: CAATCCCCCGAGTGAACTT		NA	6/0	--		
MSM1151[*]	BV678221	F: TGAAGGAGCTTCTGAACCTC	$(CA)_{26}$	209-247	15/11	0.79/0.88	61	10
		R: CAGTACTTACATGGCTGTAGGG		NA	6/0	--		
MSM1152	BV678222	F: TGAACTACAGCCTATACCAGA	$(CA)_{23}$	197-249	15/10	**0.25/0.87**	61	11
		R: AGAGTCAAGAACCTTGTGG		179	6/1	--		
MSM1153	BV678223	F: CCCACATAGTAGTGATCAGCACACCGT	$(CA)_{16}$	156	15/1	--	61	11
		R: GAGGTTTCTTGCCCAATGCATATTTG		142-147	6/2	0.17/0.17		
MSM1154	BV678655	F: AACTCCTGGTTGGTCCACAGCTTG	$(CA)_{40}$	194-196	15/2	0/0.15	62	12
		R: TTGTGCGTCTCCACCGGTTG		157	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1155	BV678224	F: GTGCTCGTACCTGAAAAGTACACATGC	$(CA)_{23}$	154-181	15/8	0.57/0.83	61	13
		R:CAGCCTAACAAATTAAACACCATTATGCAG		159-163	6/2	0.17/0.41		
*MSM1156**	BV678225	F: AAGGAGGTCGAGTGGTAATTCC	$(CA)_{57}$	140-225	15/13	0.71/0.93	61	14
		R: GTCTGGGATTTGTGCTCGTC		NA	6/0	--		
MSM1157	BV678226	F: TGTCTGAGCAGGATGCTTACC	$(CA)_{34}$	165-200	15/12	0.67/0.9	64	3
		R: GCCCATTAGCTTTTGTAGCAAC		140	6/1	--		
*MSM1158**	BV678227	F: GGTCAGGAAGAGTTCATCAA	$(GT)_{31}$	143-181	15/6	0.71/0.73	61	10
		R: GTCCTCCTCTCTTCACTTGTAA		NA	6/0	--		
*MSM1159**	BV678656	F: ACATGGACAGCTGTCAAAC	$(CA)_{27}$	178-214	15/10	0.93/0.85	61	15
		R: CAAGCTATAGGGAGTGTTCAG		NA	6/0	--		
MSM1161	BV678228	F: TTCGACCTCGCCAACTTC	$(CT)_{14}(CA)_{10}$	154-188	15/8	0.93/0.88	61	--
		R: TCGGGTTCTCTAAAGCTACCTG		171-175	6/3	0.2/0.6		
*MSM1162**	BV678229	F: GGGAGGCCATCGATTAT	$(TC)_{34}$	173-185	15/7	0.8/0.88	61	10
		R: GAGAGAAAGTGAGAGGAATGAG		144-156	6/3	0.17/0.71		
MSM1163	BV678230	F: GGCGTGCAAATAGGATGTAAGC	$(AC)_{14}$	193-205	15/3	0.79/0.55	61	13
		R: TAAGACCAGTGGCAGAGTCGTG		257-317	6/10	0.67/0.97		
MSM1164	BV678231	F: CTCCTGCTGATGATATGATACG	$(GT)_{16}$	171-190	15/3	0.21/0.44	61	4
		R: ACAGTGCCCACTAACCCA		196	6/1	--		
MSM1165	BV678232	F: TCGGTCAGAGTGAGCTCAGAGT	$(AC)_{50}$	213-231	15/6	0.57/0.8	61	9
		R: CAGGTTACAACGACCACGACA		160-162	6/2	0.33/0.3		
MSM1166	BV678233	F: CTGAGGTCTCAACACATTCAGT	$(CA)_{18}$	176-207	15/7	0.67/0.84	61	14
		R: TCAGTAACCAAACACTCCCTG		182	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1167	BV678234	F: ACAGCAGCTCTCATGCACACA	$(AC)_{21}$	152-158	15/3	0.47/0.39	64	3
		R: CAGCCCTTCCTGTCGCTTTT		147	6/1	--		
*MSM1168**	BV678235	F: GAGAACGGAGCCGACATCA	$(CA)_{27}$	132-155	15/6	0.73/0.82	62	1
		R: CATGAAAATGGGTCCTATGGGA		141-143	6/2	0/.67		
MSM1170	BV678292	F: TGCACCTGAACGCACCTTTAA	$(GT)_{15}$	224-232	15/4	0.69/0.62	61	16
		R: GCACAAGCGTCAGAAGTTGGA		243-245	6/2	0.4/0.53		
MSM1171	BV678293	F: TCCCTGTAGTATGCGCTC	$(CA)_{16}$	188	15/1	--	61	17
		R: CCAGAGTGGAGGTATGCT		196-198	6/2	0.17/0.41		
MSM1172	BV678294	F: GGGTAACATTTGCTTTTCGCCTAGTTT	$(CA)_{16}$	167-173	15/4	0.33/0.45	61	17
		R: AGACACTGACCTCTTCATCACAGATGC		151	6/1	--		
*MSM1173**	BV678295	F: TCACAAACTCACCGCTACACA	$(GT)_{13}$	119-204	15/3	0.36/0.55	61	18
		R: CGCGTTTGGAGGAAGTTATTC		190	6/1	--		
MSM1174	BV678296	F: CGCTCCGTCACTACAATCCTA	$(CA)_{18}$	179-189	15/5	0.55/0.67	61	19
		R: CGCATGTGTAAAACCCTCGTA		194	6/1	--		
*MSM1176**	BV678298	F: CCAGCCAGAACCTGTGAGTAA	$(CA)_{30}$	170-185	15/6	0.44/0.56	61	18
		R: TCGGGAGGGTAATAGTGTTGA		160	6/1	--		
MSM1177	BV678299	F: TAAATACGCACGACGGTCAGG	$(GT)_{11}$	179-183	15/3	0.14/0.26	62	12
		R: AGTGGAGGGAGAAACGCAAGA		210	6/1	--		
MSM1179	BV678300	F: CGCCGGTAAGCCTTTATATCGTTACTC	$(CAAA)_7$	213-219	15/3	0.79/0.62	61	12
		R: CTGCTGTTTTCTCCTGTGTTGCTGTAG		216	6/1	--		
MSM1180	BV678301	F: TACAAACAAACTGATGGACAGACGGAC	$(TAA)_{10}$	197	15/1	--	64	20
		R: AAGAACTGCATACCCCACGTAACTCTC		211-220	6/4	0.17/0.56		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1181	BV678302	F: GTTTAACTGGACCAAAGGGGACAT	$(TTGAA)_7$	214-220	15/3	0.64/0.57	61	--
		R: GCTGAGGGGTCTGTTATCTAGCAG		210-216	6/2	0.83/0.53		
MSM1182	BV678303	F: GCATTTAAGTTGGCACCGTAG	$(CA)_{17}(GA)_{13}$	216-224	15/3	0.62/0.52	61	--
		R: TCTAGGCCATTAGGTTGCAAT		275-279	6/3	0.2/0.6		
MSM1183	BV678304	F: GAGGCAGAGGGGAGGAAAGTTCAC	$(CA)_{15}(GA)_7$	206-234	15/7	0.43/0.62	64	20
		R: TGTCTGCTGATAACCAATCATCGG		221-223	6/2	0.33/0.3		
MSM1184	BV678305	F: TCATGGAGGAGAGTGAGCTAGAGA	$(GT)_{18}$	140-154	15/5	0.27/0.31	61	21
		R: CATACAGGACCGCAGGAGTAGATA		162-174	6/4	0.33/0.76		
MSM1185	BV678306	F: AACAAAATACACCCAACTATCTCACGC	$(CA)_{28}$	166-199	15/7	0.53/0.71	61	22
		R: AAACCACCACAGTGTCAGCTTCTAGTC		120-125	6/2	0.33/0.3		
MSM1186	BV678307	F: TATGGAGGTGGTTTAGGGTCT	$(CA)_{25}$	192-212	15/6	0.67/0.75	63	23
		R: TCAGGAGTTACAGAACGGAGA		192	6/1	--		
MSM1187[*]	BV678308	F: AATCAGGTCCCACCAAATCAATTC	$(CAA)_9$	226-232	15/3	0.43/0.36	61	--
		R: GGCTGCTGCTTCTTCACTGTACAC		229-231	6/4	0.5/0.45		
MSM1191	BV678262	F: CAACATCTTCCTGATATTCCC	$(GT)_{12}$	240-250	15/3	0.33/0.48	61	15
		R: CTTAACCGTGAGTTATTCCGA		229	6/1	--		
MSM1192	BV678263	F: GTGCTGAAGAGAGATGAGTTTGGT	$(CT)_{30}$	236-242	15/4	0.6/0.57	61	24
		R: CCATTAGAGAAGAAGAACGCAGAG		260-272	6/6	0.33/0.88		
MSM1193	BV678264	F: ACTCAGTTACTCAACGCCCTC	$(CA)_{20}$	122-145	15/7	0.71/0.79	61	25
		R: CCACTGGGCTTTGTCTAACTC		130	6/1	--		
MSM1194	BV678265	F: CACATCAGCCTTCATTACCAC	$(GT)_{30}$	223-258	15/7	**0.33/0.78**	61	14
		R: TGTGAGCAATAAACTGATGCC		225-231	6/3	0.17/0.44		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1195	BV678266	F: TTGGGAGTAAACATTCTCTGTGTAGGC	$(GT)_{21}$	220-222	15/2	0/0.13	61	26
		R: AATAGTTTTGGTAGTGCTAGCTTCGGG		214-216	6/2	0.5/0.53		
*MSM1196**	BV678267	F: CCTTTGTCTCTGTCTTTGTCC	$(CT)_{19}$	121-127	15/3	0.29/0.4	61	15
		R: ACTTGATCTTGGCTCTAGGTG		148	6/1	--		
*MSM1197**	BV678268	F: AGACTGAACTCAGAGAGGACCGAG	$(GT)_{15}$	247	15/1	--	61	24
		R: CCTTTAAACAGCGTTACAGCAATG		NA	6/0	--		
MSM1200	BV678269	F: AAGCTTTGAGTTCCCACACCC	$(CA)_{14}$	233-244	15/3	0.33/0.3	61	26
		R: TCTTGTGTTGCCTGGTAACCC		258-260	6/2	0.33/0.3		
*MSM1201**	BV678270	F: CAGACTTTCCTAAACCCCCAGATATCA	$(CA)_{24}$	248-276	15/4	0.33/0.4	61	27
		R: GATGCCAGATGAGACTTGTGAAGTGTT		NA	6/0	--		
MSM1202	BV678282	F: CATCGACAATACATGCACTTG	$(GT)_{17}$	118-143	15/4	0.33/0.31	61	21
		R: AACCAGCCCATTCAGTTACAT		137-139	6/2	0.33/0.48		
*MSM1206**	BV678284	F: TTCCCCTCTTGTCCCATAGGCT	$(GT)_{16}$	236-242	15/3	0.27/0.57	61	21
		R: CGGCTTCACAGAAACAACGTCA		NA	6/0	--		
*MSM1207**	BV678285	F: CCCCTCTCTCAGCAGAAAGTAAAT	$(GT)_{21}$	207-223	15/4	0.47/0.65	61	28
		R: TGCTTGTCCTGGTGTAAGTACAAA		NA	6/0	--		
MSM1208	BV678286	F: AACTCAAACTGCAGCGTTCTC	$(TA)_{31}$	171-195	15/7	0.86/0.81	61	16
		R: CTCCTGACCAAGGCAATATGT		175-207	6/7	0.75/0.93		
MSM1209	BV678287	F: TGCTGCTCAGTGATTATACTCA	$(GT)_{15}$	195-225	15/6	0.53/0.56	61	19
		R: GGAAAACATGAAGAAGTAGCCT		197	6/1	--		
MSM1211	BV678288	F: GAGAGAGCGAGAGCATTG	$(CA)_{20}$	173-179	15/3	0.4/0.58	61	17
		R: TGAGTAATGAACGGGCTT		166	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1213	BV678250	F: GATCTCAGATGGGAGCGAATC	$(CA)_{36}$	227-231	15/3	0.07/0.4	61	29
		R: GGCAATGATGTGGAGTCAAGA		222	6/1	--		
MSM1215	BV678251	F: ACAGCTGTTTGACCAGGTAAG	$(CA)_{36}$	184-201	15/4	0.5/0.46	63	23
		R: ACCCAGTTCCCTCTGATGTCG		151	6/1	--		
MSM1216	BV678252	F: AGGGGTGGGTTTCACTCTCTATTC	$(CA)_{31}$	214-216	15/2	0.27/0.24	61	7
		R: AGTGTGGTTGCTCACAACAACAAG		215-231	6/4	0.67/0.77		
MSM1218	BV678254	F: ATGATTTCAGAACCGGAGACC	$(CA)_{25}$	169-182	15/3	0.43/0.47	61	25
		R: CTGATGCTCGTCTGTAAAGGC		161	6/1	--		
MSM1219	BV678255	F: AGAGAAACTCTCAGGCTATCT	$(TA)_{22}$	210	15/1	--	61	29
		R: ACATACAAAGCTGGTAATGAC		210	6/1	--		
MSM1220[*]	BV678256	F: TAATCTAACAGCGCACATGA	$(CA)_{29}$	242-269	15/6	0.67/0.81	61	15
		R: TGTCACAAAATTCAAGGTCC		NA	6/0	--		
MSM1221[*]	BV678257	F: TAATCATCCCCAGGTGACTAG	$(GT)_{28}$	240-252	15/5	0.53/0.78	61	7
		R: AGTGTCTTGTGGGTAATGGAG		NA	6/0	--		
MSM1222	BV678258	F: GAGTGGGAATGAAGCGAT	$(CA)_{14}$	178-184	15/2	0.07/0.19	61	24
		R: GAAGTTTGTGTTCGGCTG		174	6/1	--		
MSM1223[*]	BV678259	F: TCTGTCTCTCTGCTTTTTCATCGC	$(CT)_{18}$	NA	15/0	--	61	25
		R: TTCGCATATTATGAGTTCGACCGT		94	6/1	--		
MSM1224[*]	BV678260	F: GTGTCCCCCATCTGTCATT	$(CA)_{26}$	243-249	15/3	0.33/0.58	61	29
		R: CCCTCGGTCTATTGCTCTC		232	6/1	--		
MSM1226	BV678261	F: CATATTTACTGTGGTGGTCTCC	$(CA)_{21}$	180-200	15/4	0.4/0.5	61	24
		R: AGTCTCTTTGAGTTAGCGGTTC		136-155	6/3	1/0.62		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1228	BV678271	F: CTGACTGGTGGGACTAGGACA	$(CA)_{17}$	243-245	15/2	0.4/0.5	61	22
		R: TGGCCTAACTGTTGGTTGATT		255-257	6/2	0.5/0.53		
MSM1229	BV678272	F: ACCTGGGTGAGTCAACTTTAG	$(GT)_6(AT)_{11}$	122-140	15/8	**0.46/0.86**	63	23
		R: AAAGTTCCCACAGCTACTCAT		120	6/1	--		
MSM1230	BV678273	F: CACCAGACTCCCTTTTAATCACAT	$(GT)_{28}$	108-170	15/12	0.71/0.83	55	6
		R: TCATGGAGAATTTTGTTGTCAACT		154-156	6/2	0/0.48		
MSM1231	BV678274	F: CAACACAGCGAAAGATAAGCA	$(GT)_{20}$	117-126	15/3	0.6/0.45	61	28
		R: AAAGAGGCTGGAACAGATTCA		105-109	6/3	0.83/0.59		
MSM1232	BV678275	F: GCCTCTTTCTCTCTTCCCTAACCAGC	$(CT)_{19}(CA)_{13}$	231-233	15/2	0.36/0.49	61	4
		R: TTCATATCGAACAGCCAGCCTATCAA		233	6/1	--		
MSM1233	BV678276	F: TGACAAACAGAGAGCGTG	$(GT)_{23}$	150	15/1	--	61	14
		R: ATGTTGCGCTATAATGCC		156-158	6/2	0.33/0.48		
MSM1234	BV678657	F: CTGCACCTCTAGGAGTCCACA	$(CT)_{32}$	177	15/1	--	61	11
		R: AGTGGTGATGGCAGGATAATG		171-177	6/2	0.83/0.53		
MSM1235	BV678658	F: GGGACAGAATGAGGCTTGTCT	$(GT)_{32}$	231-233	15/2	0.13/0.13	61	21
		R: TCTCAAAGAACGCCCCTAAAG		225	6/1	--		
MSM1236	BV678659	F: GTATAATTTAGCAAAGCGACTGAG	$(TAA)_{19}$	197-212	15/4	0.42/0.64	61	26
		R: GAGCTTCCATAAAATACACAATGA		203-212	6/3	0.8/0.62		
MSM1237	BV678660	F: GAATCCTGGAGTTCACACA	$(CA)_{17}$	116-143	15/5	0.64/0.6	61	9
		R: CGTGGGTCTCGTCAGTATA		106	6/1	--		
MSM1238	BV678277	F: ATGTACGGACATCCCCTGCTATGA	$(CA)_{20}$	228-234	15/2	0.2/0.52	61	27
		R: ATCAGATGGGCTGGGAGTTACTGG		218	6/1	--		

Locus	GenBank Accession no.	Primer sequences (5'-3')	Repeat motif	Allele size range (bp) SB WB	N/k SB WB	H_O/H_E SB WB	T_A	MP
MSM1239	BV678278	F: GTTGCCATTGTCACGCCAGTA	$(CA)_{28}$	224-250	15/8	**0.47/0.81**	61	17
		R: TTTCTTCACGCCCGCTGATTA		240-246	6/4	0.17/0.74		
MSM1240[*]	BV678661	F: TTGCACGCTTCTGTGGTAGTT	$(CA)_{35}$	188-209	15/7	0.6/0.83	61	27
		R: GTCCCAAATTGCCACGTTTAT		NA	6/0	--		
MSM1241[*]	BV678279	F: ACTGGCACGGATAGCGATGAG	$(CA)_{22}$	161-204	15/7	0.6/0.75	61	26
		R: CAGGCAAACGCTGACAGACTG		197-213	6/4	0/0.8		
MSM1242	BV678662	F: ATAGTGAGGATGGAATAGATAGATGG	$(CA)_{19}$	122-134	15/5	0.6/0.72	61	28
		R: ATGTGTCTATGAATTAATCCGTCTTT		115-117	6/2	0.17/0.17		
MSM1243	BV678663	F: GTTGCTGCTTTAGGTTGGACA	$(CA)_{18}$	222-244	15/6	0.79/0.81	61	16
		R: TTGTGTGAGCAATTAGAGCGA		224-230	6/2	0.67/0.48		
MSM1244[*]	BV678280	F: AGTTGTGTGATGTGGTCATTTT	$(GT)_8(AT)_5$	230-238	15/4	0.13/0.36	61	28
		R: GGTCAGTGTGTAAGGGTGTAAA		NA	6/0	--		
MSM1245	BV678281	F: TGCTGAAATGAGAGTGATGT	$(GA)_{13}$	121	15/1	--	61	27
		R: GTCGGTCTTGGTCATCTAAG		121	6/1	--		
MSM1246	BV678290	F: CGAGAGCTGATTATGTGTGGTCAT	$(CA)_{30}$	214-238	15/8	**0.33/0.83**	61	19
		R: CATTAGCAGCAGGACCTGATGTAA		181-189	6/4	0.67/0.71		
MSM1247	BV678291	F: GCTCTTCTTGTCTGCAGGGATGAT	$(CA)_{39}$	194-214	15/2	0.07/0.07	61	22
		R: TCCAAGTTCCACTCTCAGGACCTT		133	6/1	--		

Appendix Table IV. Values for the *Z*-test and associated *P*-values for pairwise comparisons of proportional contribution by sire and strain for Phase II. Sire comparisons that are significant after sequential Bonferroni correction are indicated by * after the *P*-value. Sires or strains in boldface type have the higher contribution of a pair. The presence of a – indicates *P*-values >0.05.

POPULATION	SIRE or STRAIN PAIR	*Z*	*P*-VALUE
Initial pond sample	4664 (R:F_0-97) - **7213 (R:F_0-97)**	1.73	0.0418
	4664 (R:F_0-97) - **292B (SC:F_1-94)**	4.09	<0.0001*
	4664 (R:F_0-97) - **7E27 (SC:F_1-94)**	4.09	<0.0001*
	4664 (R:F_0-97) - **631D (C:F_2-91)**	2.27	0.0116
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	6.65	<0.0001*
	7213 (R:F_0-97) - **292B (SC:F_1-94)**	2.40	0.0082
	7213 (R:F_0-97) - **7E27 (SC:F_1-94)**	2.40	0.0082
	7213 (R:F_0-97) - 631D (C:F_2-91)	0.55	--
	7213 (R:F_0-97) - **2A20 (C:F_2-91)**	5.00	<0.0001*
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.00	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.85	0.0322
	292B (SC:F_1-94) - **2A20 (C:F_2-91)**	2.56	0.0052
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	1.85	0.0322
	7E27 (SC:F_1-94) - **2A20 (C:F_2-91)**	2.56	0.0052
	631D (C:F_2-91) - **2A20 (C:F_2-91)**	4.42	<0.0001*
	(C:F_2-91) - (SC:F_1-94)	0.83	--
	(C:F_2-91) - (R:F_0-97)	5.93	<0.0001
	(SC:F_1-94) - (R:F_0-97)	5.05	<0.0001
Pond A4	4664 (R:F_0-97) - **7213 (R:F_0-97)**	1.80	0.0359
	4664 (R:F_0-97) - **292B (SC:F_1-94)**	4.37	<0.0001*
	4664 (R:F_0-97) - **7E27 (SC:F_1-94)**	4.74	<0.0001*
	4664 (R:F_0-97) - **631D (C:F_2-91)**	5.46	<0.0001*
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	6.50	<0.0001*
	7213 (R:F_0-97) - **292B (SC:F_1-94)**	2.66	0.0039*
	7213 (R:F_0-97) - **7E27 (SC:F_1-94)**	3.04	0.0012*
	7213 (R:F_0-97) - **631D (C:F_2-91)**	3.77	0.0001*
	7213 (R:F_0-97) - **2A20 (C:F_2-91)**	4.82	<0.0001*
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.38	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.12	--
	292B (SC:F_1-94) - **2A20 (C:F_2-91)**	2.17	0.0150
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	0.74	--
	7E27 (SC:F_1-94) - **2A20 (C:F_2-91)**	1.78	0.0375
	631D (C:F_2-91) - 2A20 (C:F_2-91)	1.04	--
	(C:F_2-91) - (SC:F_1-94)	2.43	0.0075
	(C:F_2-91) - (R:F_0-97)	8.39	<0.0001
	(SC:F_1-94) - (R:F_0-97)	5.72	<0.0001
Pond A11	4664 (R:F_0-97) - **7213 (R:F_0-97)**	1.90	0.0287
	4664 (R:F_0-97) - **292B (SC:F_1-94)**	3.00	0.0013*
	4664 (R:F_0-97) - **7E27 (SC:F_1-94)**	2.72	0.0033*
	4664 (R:F_0-97) - **631D (C:F_2-91)**	2.34	0.0096
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	10.04	<0.0001*
	7213 (R:F_0-97) - 292B (SC:F_1-94)	0.65	--
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	0.80	--
	7213 (R:F_0-97) - 631D (C:F_2-91)	0.40	--
	7213 (R:F_0-97) - **2A20 (C:F_2-91)**	2.74	0.0031*

POPULATION	SIRE or STRAIN PAIR	Z	P-VALUE
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.60	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	0.39	--
	292B (SC:F_1-94) - **2A20 (C:F_2-91)**	12.08	<0.0001*
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	0.65	--
	7E27 (SC:F_1-94) - **2A20 (C:F_2-91)**	13.05	<0.0001*
	631D (C:F_2-91) - **2A20 (C:F_2-91)**	5.54	<0.0001*
	(C:F_2-91) - (SC:F_1-94)	5.53	<0.0001
	(C:F_2-91) - (R:F_0-97)	6.54	<0.0001
	(SC:F_1-94) - (R:F_0-97)	0.94	--
Pond A14	4664 (R:F_0-97) - 7213 (R:F_0-97)	1.12	--
	4664 (R:F_0-97) - **292B (SC:F_1-94)**	4.34	<0.0001*
	4664 (R:F_0-97) - **7E27 (SC:F_1-94)**	4.96	<0.0001*
	4664 (R:F_0-97) - **631D (C:F_2-91)**	4.96	<0.0001*
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	8.27	<0.0001*
	7213 (R:F_0-97) - **292B (SC:F_1-94)**	3.30	0.0005*
	7213 (R:F_0-97) - **7E27 (SC:F_1-94)**	3.94	<0.0001*
	7213 (R:F_0-97) - **631D (C:F_2-91)**	3.94	<0.0001*
	7213 (R:F_0-97) - **2A20 (C:F_2-91)**	7.27	<0.0001*
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.65	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	0.65	--
	292B (SC:F_1-94) - **2A20 (C:F_2-91)**	3.97	<0.0001*
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	0.00	--
	7E27 (SC:F_1-94) - **2A20 (C:F_2-91)**	3.32	0.0005*
	631D (C:F_2-91) - **2A20 (C:F_2-91)**	3.32	0.0005*
	(C:F_2-91) - (SC:F_1-94)	3.46	0.0003
	(C:F_2-91) - (R:F_0-97)	10.36	<0.0001
	(SC:F_1-94) - (R:F_0-97)	6.44	<0.0001
Tank 40	4664 (R:F_0-97) - **7213 (R:F_0-97)**	2.85	0.0022*
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.86	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94)	0.58	--
	4664 (R:F_0-97) - **631D (C:F_2-91)**	2.26	0.0119
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	3.96	<0.0001*
	7213 (R:F_0-97) - 292B (SC:F_1-94)	2.00	0.0028*
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	2.27	0.0116
	7213 (R:F_0-97) - 631D (C:F_2-91)	0.59	--
	7213 (R:F_0-97) - 2A20 (C:F_2-91)	1.11	--
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.28	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.41	--
	292B (SC:F_1-94) - **2A20 (C:F_2-91)**	3.11	0.0009*
	7E27 (SC:F_1-94) - **631D (C:F_2-91)**	1.68	0.0465
	7E27 (SC:F_1-94) - **2A20 (C:F_2-91)**	3.39	0.0003*
	631D (C:F_2-91) - **2A20 (C:F_2-91)**	1.71	0.0436
	(C:F_2-91) - (SC:F_1-94)	3.88	0.0001
	(C:F_2-91) - (R:F_0-97)	2.62	0.0044
	(SC:F_1-94) - (R:F_0-97)	1.24	--

Appendix Table V. Estimated survival (± SE) by sire and strain for three Phase II research ponds.

POPULATION	SIRE or STRAIN	ESTIMATED SURVIVAL
Pond A4	4664 (R:F_0-97)	0.48 (0.19)
	7213 (R:F_0-97)	0.59 (0.17)
	292B (SC:F_1-94)	0.70 (0.14)
	7E27 (SC:F_1-94)	0.76 (0.15)
	631D (C:F_2-91)	1.00 (0.21)
	2A20 (C:F_2-91)	0.70 (0.11)
	R:F_0-97	0.55 (0.12)
	SC:F_1-94	0.73 (0.09)
	C:F_2-91	0.88 (0.09)
Pond A11	4664 (R:F_0-97)	0.41 (0.17)
	7213 (R:F_0-97)	1.00 (0.23)
	292B (SC:F_1-94)	0.50 (0.11)
	7E27 (SC:F_1-94)	0.46 (0.10)
	631D (C:F_2-91)	0.81 (0.19)
	2A20 (C:F_2-91)	0.90 (0.12)
	R:F_0-97	0.85 (0.16)
	SC:F_1-94	0.48 (0.07)
	C:F_2-91	0.88 (0.09)
Pond A14	4664 (R:F_0-97)	0.38 (0.16)
	7213 (R:F_0-97)	0.37 (0.12)
	292B (SC:F_1-94)	0.58 (0.12)
	7E27 (SC:F_1-94)	0.66 (0.13)
	631D (C:F_2-91)	0.98 (0.21)
	2A20 (C:F_2-91)	0.77 (0.11)
	R:F_0-97	0.37 (0.09)
	SC:F_1-94	0.62 (0.08)
	C:F_2-91	0.83 (0.08)

Appendix Table VI. *P*-values for pairwise Z-tests of estimated survival by sire and strain for Phase II. Sires or strains in boldface type have the higher contribution of a pair. Sire comparisons that are significant after sequential Bonferroni correction are indicated by * after the *P*-value. The presence of a (–) indicates *P*-values >0.05.

POPULATION	SIRE or STRAIN PAIR	Z	P-VALUE
Pond A4	4664 (R:F_0-97) - 7213 (R:F_0-97)	0.40	--
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.90	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94	1.13	--
	4664 (R:F_0-97) - **631D (C:F_2-91)**	1.80	0.0359
	4664 (R:F_0-97) - 2A20 (C:F_2-91)	0.95	--
	7213 (R:F_0-97) - 292B (SC:F_1-94)	0.51	--
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	0.76	--
	7213 (R:F_0-97) - 631D (C:F_2-91)	1.53	--
	7213 (R:F_0-97) - 2A20 (C:F_2-91)	0.54	--
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.29	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.18	--
	292B (SC:F_1-94) - 2A20 (C:F_2-91)	0.03	--
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	0.94	--
	7E27 (SC:F_1-94) - 2A20 (C:F_2-91)	0.35	--
	631D (C:F_2-91) - 2A20 (C:F_2-91)	1.29	--
	(C:F_2-91) - (SC:F_1-94)	1.17	--
	(C:F_2-91) - (R:F_0-97)	2.16	0.0154
	(SC:F_1-94) - (R:F_0-97)	1.19	--
Pond A11	4664 (R:F_0-97) - **7213 (R:F_0-97)**	2.05	0.0202
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.44	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94	0.26	--
	4664 (R:F_0-97) - 631D (C:F_2-91)	1.58	--
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	2.36	0.0091
	7213 (R:F_0-97) - 292B (SC:F_1-94)	1.94	0.0262
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	2.10	0.0179
	7213 (R:F_0-97) - 631D (C:F_2-91)	0.62	--
	7213 (R:F_0-97) - 2A20 (C:F_2-91)	0.36	--
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.24	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.42	--
	292B (SC:F_1-94) - **2A20 (C:F_2-91)**	2.46	0.0069
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	1.61	--
	7E27 (SC:F_1-94) - **2A20 (C:F_2-91)**	2.74	0.0031*
	631D (C:F_2-91) - 2A20 (C:F_2-91)	0.40	--
	(C:F_2-91) - (SC:F_1-94)	3.53	0.0002
	(C:F_2-91) - (R:F_0-97)	0.15	--
	(SC:F_1-94) - **(R:F_0-97)**	2.11	0.0174
Pond A14	4664 (R:F_0-97) - 7213 (R:F_0-97)	0.03	--
	4664 (R:F_0-97) - 292B (SC:F_1-94)	1.03	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94	1.41	--
	4664 (R:F_0-97) - **631D (C:F_2-91)**	2.26	0.0119
	4664 (R:F_0-97) - **2A20 (C:F_2-91)**	2.04	0.0207
	7213 (R:F_0-97) - 292B (SC:F_1-94)	1.23	--
	7213 (R:F_0-97) - **7E27 (SC:F_1-94)**	1.66	0.0485
	7213 (R:F_0-97) - **631D (C:F_2-91)**	2.47	0.0068
	7213 (R:F_0-97) - **2A20 (C:F_2-91)**	2.46	0.0069
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.49	--

Appendix Table VI, continued.

POPULATION	SIRE or STRAIN PAIR	Z	P-VALUE
	292B (SC:F_1-94) - 631D (C:F_2-91)	1.63	--
	292B (SC:F_1-94) - 2A20 (C:F_2-91)	1.17	--
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	1.25	--
	7E27 (SC:F_1-94) - 2A20 (C:F_2-91)	0.49	--
	631D (C:F_2-91) - 2A20 (C:F_2-91)	0.87	--
	(C:F_2-91) - (SC:F_1-94)	1.86	0.0314
	(C:F_2-91) - (R:F_0-97)	3.70	0.0001
	(SC:F_1-94) - (R:F_0-97)	2.07	0.0192

Appendix Table VII. Least squares means (±SE) for phenotypic trait values for experimental striped bass families reared in research ponds or a commercial tank.

DAM	SIRE	Body weight (g)	Total length (cm)	Condition factor
2E55 (R:F_0-97)	4664 (R:F_0-97)	61.08 (5.04)	17.65 (0.37)	1.09 (0.02)
(Pond A4)	7213 (R:F_0-97)	61.14 (3.36)	17.80 (0.25)	1.07 (0.02)
	292B (SC:F_1-94)	63.68 (2.58)	18.06 (0.19)	1.06 (0.01)
	7E27 (SC:F_1-94)	73.08 (2.54)	18.70 (0.19)	1.10 (0.01)
	631D (C:F_2-91)	59.00 (2.39)	17.55 (0.18)	1.08 (0.01)
	2A20 (C:F_2-91)	61.42 (1.81)	17.88 (0.13)	1.06 (0.01)
2E55 (R:F_0-97)	4664 (R:F_0-97)	122.29 (6.72)	20.56 (0.30)	1.37 (0.02)
(commercial tank)	7213 (R:F_0-97)	122.26 (4.90)	20.47 (0.21)	1.39 (0.01)
	292B (SC:F_1-94)	155.24 (6.03)	22.12 (0.26)	1.42 (0.02)
	7E27 (SC:F_1-94)	152.24 (6.23)	21.91 (0.27)	1.43 (0.02)
	631D (C:F_2-91)	175.46 (5.18)	22.59 (0.23)	1.50 (0.01)
	2A20 (C:F_2-91)	163.49 (4.45)	22.44 (0.20)	1.42 (0.01)

Appendix Table VIII. Values for the Z-test and associated P-values for pairwise comparisons of proportional contribution by sire and strain to Phase III populations. A dashed line (--) indicates P-values >0.05. Proportional contributions with * after the P-value are significant after Bonferroni correction. Strain comparisons are significant at $P<0.05$. Sires or strains in boldface type have the higher contribution of a pair.

POPULATION	SIRE OR STRAIN PAIR	Z	P-VALUE
Pond A4	4664 (R:F_0-97) – **7213 (R:F_0-97)**	1.68	0.0465
(Dam 2E55)	4664 (R:F_0-97) – **292B (SC:F_1-94)**	6.27	<0.0001*
	4664 (R:F_0-97) – **7E27 (SC:F_1-94)**	5.27	<0.0001*
	4664 (R:F_0-97) – **631D (C:F_2-91)**	3.75	0.0001*
	4664 (R:F_0-97) – **2A20 (C:F_2-91)**	9.90	<0.0001*
	7213 (R:F_0-97) – **292B (SC:F_1-94)**	4.70	<0.0001*
	7213 (R:F_0-97) – **7E27 (SC:F_1-94)**	3.68	0.0001*
	7213 (R:F_0-97) – **631D (C:F_2-91)**	2.12	0.0170
	7213 (R:F_0-97) – **2A20 (C:F_2-91)**	8.35	<0.0001*
	292B (SC:F_1-94) – 7E27 (SC:F_1-94)	1.03	--
	292B (SC:F_1-94) – 631D (C:F_2-91)	2.60	0.0047*
	292B (SC:F_1-94) – **2A20 (C:F_2-91)**	3.59	0.0002*
	7E27 (SC:F_1-94) – 631D (C:F_2-91)	1.58	--
	7E27 (SC:F_1-94) – **2A20 (C:F_2-91)**	4.63	<0.0001*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	6.23	<0.0001*
	(C:F_2-91) – (SC:F_1-94)	2.44	0.0073
	(C:F_2-91) – (R:F_0-97)	10.11	<0.0001
	(SC:F_1-94) – (R:F_0-97)	6.62	<0.0001
Pond A11	4664 (R:F_0-97) – **7213 (R:F_0-97)**	3.36	0.0004*
(Dam 2E55)	4664 (R:F_0-97) – **292B (SC:F_1-94)**	5.50	<0.0001*
	4664 (R:F_0-97) – **7E27 (SC:F_1-94)**	6.41	<0.0001*
	4664 (R:F_0-97) – **631D (C:F_2-91)**	1.82	0.0344
	4664 (R:F_0-97) – **2A20 (C:F_2-91)**	12.22	<0.0001*
	7213 (R:F_0-97) – **292B (SC:F_1-94)**	2.26	0.0119*
	7213 (R:F_0-97) – **7E27 (SC:F_1-94)**	3.20	0.0007*
	7213 (R:F_0-97) – 631D (C:F_2-91)	1.59	--
	7213 (R:F_0-97) – **2A20 (C:F_2-91)**	8.98	<0.0001*
	292B (SC:F_1-94) – 7E27 (SC:F_1-94)	0.95	--
	292B (SC:F_1-94) – 631D (C:F_2-91)	3.82	0.0001*
	292B (SC:F_1-94) – **2A20 (C:F_2-91)**	6.64	<0.0001*
	7E27 (SC:F_1-94) – 631D (C:F_2-91)	4.75	<0.0001*
	7E27 (SC:F_1-94) – **2A20 (C:F_2-91)**	5.66	<0.0001*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	10.56	<0.0001*
	(C:F_2-91) – (SC:F_1-94)	3.10	0.0010
	(C:F_2-91) – (R:F_0-97)	9.49	<0.0001
	(SC:F_1-94) – (R:F_0-97)	5.67	<0.0001
Pond A14	4664 (R:F_0-97) – **7213 (R:F_0-97)**	2.64	0.0041*
(Dam 2E55)	4664 (R:F_0-97) – **292B (SC:F_1-94)**	8.35	<0.0001*
	4664 (R:F_0-97) – **7E27 (SC:F_1-94)**	8.27	<0.0001*
	4664 (R:F_0-97) – **631D (C:F_2-91)**	3.46	0.0003*
	4664 (R:F_0-97) – **2A20 (C:F_2-91)**	10.59	<0.0001*
	7213 (R:F_0-97) – **292B (SC:F_1-94)**	5.91	<0.0001*
	7213 (R:F_0-97) – **7E27 (SC:F_1-94)**	5.83	<0.0001*
	7213 (R:F_0-97) – 631D (C:F_2-91)	0.85	--
	7213 (R:F_0-97) – **2A20 (C:F_2-91)**	8.17	<0.0001*
	292B (SC:F_1-94) – 7E27 (SC:F_1-94)	0.08	--

POPULATION	SIRE OR STRAIN PAIR	Z	P-VALUE
Pond A14	**292B (SC:F_1-94)** – 631D (C:F_2-91)	5.08	<0.0001*
(Dam 2E55)	292B (SC:F_1-94) – **2A20 (C:F_2-91)**	2.24	0.0125*
	7E27 (SC:F_1-94) – 631D (C:F_2-91)	5.00	<0.0001*
	7E27 (SC:F_1-94) – **2A20 (C:F_2-91)**	2.32	0.0102*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	7.35	<0.0001*
	(C:F_2-91) – **(SC:F_1-94)**	2.00	0.0228
	(C:F_2-91) – (R:F_0-97)	9.54	<0.0001
	(SC:F_1-94) – (R:F_0-97)	9.78	<0.0001
Tank 40	4664 (R:F_0-97) – **7213 (R:F_0-97)**	4.26	<0.0001*
(Dam 2E55)	4664 (R:F_0-97) – **292B (SC:F_1-94)**	3.52	0.0002*
	4664 (R:F_0-97) – **7E27 (SC:F_1-94)**	2.42	0.0078
	4664 (R:F_0-97) – **631D (C:F_2-91)**	5.33	<0.0001*
	4664 (R:F_0-97) – **2A20 (C:F_2-91)**	5.33	<0.0001*
	7213 (R:F_0-97) - 292B (SC:F_1-94)	0.76	--
	7213 (R:F_0-97) – 7E27 (SC:F_1-94)	1.86	0.0314
	7213 (R:F_0-97) - 631D (C:F_2-91)	1.08	--
	7213 (R:F_0-97) – 2A20 (C:F_2-91)	1.08	--
	292B (SC:F_1-94) – 7E27 (SC:F_1-94)	1.10	--
	292B (SC:F_1-94) – **631D (C:F_2-91)**	1.83	0.0336
	292B (SC:F_1-94) – **2A20 (C:F_2-91)**	1.83	0.0336
	7E27 (SC:F_1-94) – **631D (C:F_2-91)**	2.93	0.0017*
	7E27 (SC:F_1-94) – **2A20 (C:F_2-91)**	2.93	0.0017*
	631D (C:F_2-91) – 2A20 (C:F_2-91)	0.00	--
	(C:F_2-91) – (SC:F_1-94)	4.06	<0.0001
	(C:F_2-91) – (R:F_0-97)	4.88	<0.0001
	(SC:F_1-94) – (R:F_0-97)	0.98	--
Pond A2	**5442 (R:F_0-97)** – 3B62 (R:F_0-97)	1.73	0.0418
(Dams 152D + 5F4B)	5442 (R:F_0-97) – **3F11 (SC:F_1-94)**	3.39	0.0003*
	5442 (R:F_0-97) – 5C5D (SC:F_1-94)	0.35	--
	5442 (R:F_0-97) – **631D (C:F_2-91)**	4.18	<0.0001*
	5442 (R:F_0-97) – **2A20 (C:F_2-91)**	7.43	<0.0001*
	3B62 (R:F_0-97) -- **3F11 (SC:F_1-94)**	5.08	<0.0001*
	3B62 (R:F_0-97) – 5C5D (SC:F_1-94)	1.38	--
	3B62 (R:F_0-97) – **631D (C:F_2-91)**	5.86	<0.0001*
	3B62 (R:F_0-97) – **2A20 (C:F_2-91)**	9.11	<0.0001*
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	3.73	0.0001*
	3F11 (SC:F_1-94) – 631D (C:F_2-91)	0.79	--
	3F11 (SC:F_1-94) – **2A20 (C:F_2-91)**	4.02	<0.0001*
	5C5D (SC:F_1-94) – **631D (C:F_2-91)**	4.52	<0.0001*
	5C5D (SC:F_1-94) – **2A20 (C:F_2-91)**	7.77	<0.0001*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	3.22	0.0006*
	(C:F_2-91) – (SC:F_1-94)	7.97	<0.0001
	(C:F_2-91) – (R:F_0-97)	11.10	<0.0001
	(SC:F_1-94) – (R:F_0-97)	3.33	0.0004
Pond A2	**5442 (R:F_0-97)** – 3B62 (R:F_0-97)	3.62	0.0001*
(Dam 152D)	5442 (R:F_0-97) – 3F11 (SC:F_1-94)	0.73	--
	5442 (R:F_0-97) – 5C5D (SC:F_1-94)	1.49	--

POPULATION	SIRE OR STRAIN PAIR	Z	P-VALUE
	5442 (R:F_0-97) – **631D (C:F_2-91)**	4.88	<0.0001*
	5442 (R:F_0-97) – **2A20 (C:F_2-91)**	2.92	0.0018*
	3B62 (R:F_0-97) -- **3F11 (SC:F_1-94)**	4.31	<0.0001*
	3B62 (R:F_0-97) – **5C5D (SC:F_1-94)**	2.21	0.0136
	3B62 (R:F_0-97) – **631D (C:F_2-91)**	8.37	<0.0001*
	3B62 (R:F_0-97) – **2A20 (C:F_2-91)**	6.40	<0.0001*
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	2.22	0.0132
	3F11 (SC:F_1-94) – **631D (C:F_2-91)**	4.14	<0.0001*
	3F11 (SC:F_1-94) – **2A20 (C:F_2-91)**	2.19	0.0143
	5C5D (SC:F_1-94) – **631D (C:F_2-91)**	6.37	<0.0001*
	5C5D (SC:F_1-94) – **2A20 (C:F_2-91)**	4.39	<0.0001*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	1.93	0.0268
	(C:F_2-91) – (SC:F_1-94)	8.34	<0.0001
	(C:F_2-91) – (R:F_0-97)	9.46	<0.0001
	(SC:F_1-94) – (R:F_0-97)	1.67	0.0475
Pond A2	5442 (R:F_0-97) – 3B62 (R:F_0-97)	0.99	--
(Dam 5F4B)	5442 (R:F_0-97) – **3F11 (SC:F_1-94)**	3.98	<0.0001*
	5442 (R:F_0-97) – 5C5D (SC:F_1-94)	0.98	--
	5442 (R:F_0-97) – 631D (C:F_2-91)	0.66	--
	5442 (R:F_0-97) – **2A20 (C:F_2-91)**	7.50	<0.0001*
	3B62 (R:F_0-97) -- **3F11 (SC:F_1-94)**	3.17	0.0008
	3B62 (R:F_0-97) – 5C5D (SC:F_1-94)	0.16	--
	3B62 (R:F_0-97) – 631D (C:F_2-91)	0.16	--
	3B62 (R:F_0-97) – **2A20 (C:F_2-91)**	6.68	<0.0001*
	3F11 (SC:F_1-94) – 5C5D (SC:F_1-94)	3.02	0.0013*
	3F11 (SC:F_1-94) – 631D (C:F_2-91)	3.33	0.0004*
	3F11 (SC:F_1-94) – **2A20 (C:F_2-91)**	3.44	0.0003*
	5C5D (SC:F_1-94) – 631D (C:F_2-91)	0.31	--
	5C5D (SC:F_1-94) – **2A20 (C:F_2-91)**	6.53	<0.0001*
	631D (C:F_2-91) – **2A20 (C:F_2-91)**	6.84	<0.0001*
	(C:F_2-91) – (SC:F_1-94)	3.35	0.0004*
	(C:F_2-91) – (R:F_0-97)	6.46	<0.0001*
	(SC:F_1-94) – (R:F_0-97)	2.99	0.0014*

Appendix Table IX. *P*-values for pairwise *Z*-tests of estimated survival by sire and strain for Phase III. The presence of a (–) indicates *P*-values >0.05. Proportional contributions with * after the *P*-value are significant after Bonferroni correction. Strain comparisons are significant at *P*<0.05. Sires or strains in boldface type have the higher estimated survival within a pair.

POPULATION	SIRE or STRAIN PAIR	*Z*	*P*-VALUE
Pond A4	4664 (R:F_0-97) - 7213 (R:F_0-97)	1.31	--
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.00	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94)	0.00	--
	4664 (R:F_0-97) - 631D (C:F_2-91)	0.35	--
	4664 (R:F_0-97) - 2A20 (C:F_2-91)	0.29	--
	7213 (R:F_0-97) - **292B (SC:F_1-94)**	2.48	0.0066
	7213 (R:F_0-97) - **7E27 (SC:F_1-94)**	2.36	0.0091
	7213 (R:F_0-97) - **631D (C:F_2-91)**	1.96	0.0250
	7213 (R:F_0-97) - **2A20 (C:F_2-91)**	3.06	0.0011*
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.00	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	0.59	--
	292B (SC:F_1-94) - 2A20 (C:F_2-91)	0.54	--
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	0.57	--
	7E27 (SC:F_1-94) - 2A20 (C:F_2-91)	0.52	--
	631D (C:F_2-91) - 2A20 (C:F_2-91)	0.17	--
	(C:F_2-91) **- (SC:F_1-94)**	2.63	0.0043
	(C:F_2-91) - (R:F_0-97)	2.38	0.0087
	(SC:F_1-94) - (R:F_0-97)	3.90	<0.0001
Pond A11	4664 (R:F_0-97) - 7213 (R:F_0-97)	0.69	--
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.32	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94)	0.52	--
	4664 (R:F_0-97) - 631D (C:F_2-91)	1.37	--
	4664 (R:F_0-97) - 2A20 (C:F_2-91)	0.85	--
	7213 (R:F_0-97) - 292B (SC:F_1-94)	0.55	--
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	0.33	--
	7213 (R:F_0-97) - 631D (C:F_2-91)	2.53	0.0057
	7213 (R:F_0-97) - 2A20 (C:F_2-91)	0.00	--
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.30	--
	292B (SC:F_1-94) - 631D (C:F_2-91)	3.13	0.0008*
	292B (SC:F_1-94) - 2A20 (C:F_2-91)	0.84	--
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	3.50	0.0002*
	7E27 (SC:F_1-94) - 2A20 (C:F_2-91)	0.50	--
	631D (C:F_2-91) - **2A20 (C:F_2-91)**	4.82	<0.0001*
	(C:F_2-91) - (SC:F_1-94)	0.16	--
	(C:F_2-91) - (R:F_0-97)	0.38	--
	(SC:F_1-94) - (R:F_0-97)	0.30	--
Pond A14	4664 (R:F_0-97) - 7213 (R:F_0-97)	0.12	--
	4664 (R:F_0-97) - 292B (SC:F_1-94)	0.13	--
	4664 (R:F_0-97) - 7E27 (SC:F_1-94)	0.13	--
	4664 (R:F_0-97) - 631D (C:F_2-91)	1.08	--
	4664 (R:F_0-97) - 2A20 (C:F_2-91)	0.23	--
	7213 (R:F_0-97) - 292B (SC:F_1-94)	0.00	--
	7213 (R:F_0-97) - 7E27 (SC:F_1-94)	0.00	--
	7213 (R:F_0-97) - 631D (C:F_2-91)	1.53	--
	7213 (R:F_0-97) - 2A20 (C:F_2-91)	0.47	--
	292B (SC:F_1-94) - 7E27 (SC:F_1-94)	0.00	--

Appendix Table IX, continued

POPULATION	SIRE or STRAIN PAIR	Z	P-VALUE
	292B (SC:F_1-94) - 631D (C:F_2-91)	2.44	0.0073
	292B (SC:F_1-94) - 2A20 (C:F_2-91)	0.74	--
	7E27 (SC:F_1-94) - 631D (C:F_2-91)	2.55	0.0054
	7E27 (SC:F_1-94) - 2A20 (C:F_2-91)	0.78	--
	631D (C:F_2-91) - **2A20 (C:F_2-91)**	2.39	0.0084
	(C:F_2-91) - **(SC:F_1-94)**	2.25	0.0122
	(C:F_2-91) - (R:F_0-97)	1.43	--
	(SC:F_1-94) - (R:F_0-97)	0.28	--

Appendix Table X. Water quality parameters by date (in year 2002) for three PAFL research ponds during Phase III rearing, including ambient temperature (°C) and dissolved oxygen (mg/L).

Date	Temp	Pond4	Pond11	Pond14
2-Jan	4.2	8.4	11.7	12.2
14-Jan	7.3	18.6	10.4	12.1
16-Jan	8.4	17.8	9.0	12.6
18-Jan	9.6	17.6	7.7	12.7
24-Jan	10.3	11.8	7.6	10.9
28-Jan	11.5	14.0	10.3	--
29-Jan	12.1	13.0	11.1	10.6
30-Jan	14.3	12.0	11.7	11.1
1-Feb	17.0	9.3	8.7	9.9
2-Feb	15.6	8.0	8.2	8.5
3-Feb	13.0	9.2	11.5	9.4
4-Feb	10.2	8.7	11.4	9.2
5-Feb	4.6	11.2	11.8	10.6
7-Feb	7.3	12.5	13.2	12.4
8-Feb	7.1	11.5	11.8	11.0
9-Feb	8.6	12.4	12.4	11.6
10-Feb	10.9	13.3	13.5	13.3
11-Feb	11.8	10.0	10.6	10.3
12-Feb	10.2	9.3	10.6	10.3
13-Feb	10.9	10.4	10.5	11.3
14-Feb	9.6	10.0	10.4	10.4
15-Feb	10.1	10.6	10.4	10.6
16-Feb	12.2	10.3	10.0	10.8
17-Feb	10.9	10.0	9.2	9.3
18-Feb	7.0	10.5	9.8	10.0
19-Feb	8.2	11.6	9.8	10.2
20-Feb	9.6	11.0	9.0	9.5
22-Feb	13.3	8.8	6.9	8.6
25-Feb	9.3	10.8	8.9	9.5
26-Feb	11.3	11.9	8.3	10.0
27-Feb	12.5	9.9	7.9	8.9
18-Mar	15.5	7.1	7.5	5.5
19-Mar	15.3	9.0	9.0	8.5
31-Mar	14.0	8.9	9.0	8.8
18-Jun	26.8	5.0	4.4	4.3
24-Jun	29.0	--	--	--
25-Jun	28.2	5.9	5.7	5.6
26-Jun	28.5	5.3	5.4	5.1
27-Jun	27.7	6.3	5.5	4.8
1-Jul	28.5	5.6	5.8	4.4
3-Jul	28.5	4.8	6.0	4.3
4-Jul	28.8	4.8	7.3	4.2
10-Jul	26.6	5.0	7.5	4.4
12-Jul	24.7	7.0	7.4	6.0
19-Jul	29.3	5.8	4.5	4.0
22-Jul	29.3	6.6	5.0	5.8

Appendix Table X, continued.

Date	Temp	Pond4	Pond11	Pond14
23-Jul	28.5	6.1	4.1	4.1
24-Jul	28.0	6.4	4.7	4.2
25-Jul	27.2	4.7	3.6	3.4
26-Jul	27.2	6.0	5.0	3.9
29-Jul	29.5	6.0	5.5	3.5
30-Jul	30.9	4.9	3.5	3.2
31-Jul	31.5	3.8	3.4	3.3
1-Aug	30.0	3.6	2.6	3.2
2-Aug	30.2	2.8	5.1	4.0
3-Aug	29.5	5.0	4.2	5.1
4-Aug	23.7	5.8	5.6	4.7
5-Aug	28.5	6.8	5.7	4.4
6-Aug	28.7	6.1	4.2	4.8
7-Aug	25.2	7.6	5.4	6.1
8-Aug	24.0	7.1	4.9	6.8
9-Aug	23.5	6.6	6.4	6.7
12-Aug	26.6	6.4	6.2	5.9
14-Aug	28.0	5.8	5.5	6.2
15-Aug	27.2	5.2	5.0	5.2
16-Aug	27.4	5.0	4.1	4.7
19-Aug	28.5	6.1	5.0	5.0
20-Aug	28.6	5.5	5.4	4.7
21-Aug	28.4	5.1	4.4	5.3
22-Aug	28.1	5.7	4.9	5.2
23-Aug	28.6	5.7	4.7	5.1
26-Aug	28.9	5.0	4.1	5.3
27-Aug	27.7	4.2	4.7	4.8
28-Aug	27.1	4.5	4.4	5.0
29-Aug	26.1	5.0	4.5	4.4
30-Aug	26.5	5.4	5.3	6.0
3-Sep	24.6	7.2	6.6	6.6
4-Sep	26.6	6.4	5.2	5.4
5-Sep	27.6	6.7	5.3	5.8
6-Sep	27.1	7.3	5.2	5.7
9-Sep	25.3	6.8	5.9	5.3
10-Sep	25.1	6.3	5.8	5.3
11-Sep	23.9	6.4	5.7	3.9
12-Sep	24.1	6.5	6.3	2.8
13-Sep	24.0	6.5	6.1	6.3
16-Sep	26.1	5.4	5.1	4.7
17-Sep	25.7	5.1	5.0	4.9
18-Sep	26.1	5.4	5.0	4.7
19-Sep	25.4	5.4	4.9	4.6
20-Sep	26.2	6.0	5.3	4.7
23-Sep	27.4	5.1	4.7	4.7
24-Sep	25.4	4.9	4.1	5.1
25-Sep	24.3	6.0	5.7	4.8

Appendix Table X, continued.

Date	Temp	Pond4	Pond11	Pond14
26-Sep	24.2	5.5	5.1	4.4
30-Sep	24.3	6.2	6.9	5.0
1-Oct	24.5	5.8	6.5	5.1
2-Oct	25.2	5.4	5.5	4.5
3-Oct	26.2	5.1	4.5	4.1
4-Oct	27.0	5.1	4.7	3.7
7-Oct	25.2	5.4	5.5	5.8
8-Oct	24.3	5.4	4.6	5.0
9-Oct	21.9	5.9	5.2	4.9
10-Oct	22.4	6.0	5.4	4.8
11-Oct	23.1	5.1	4.2	5.5
14-Oct	22.2	6.8	6.8	5.4
15-Oct	18.9	7.6	7.9	6.9
16-Oct	19.0	8.0	8.5	5.9
17-Oct	18.4	7.5	7.3	5.4
18-Oct	18.0	7.5	7.8	5.9
25-Oct	17.2	7.8	7.0	6.8
28-Oct	18.9	7.6	6.5	6.4
29-Oct	18.4	7.8	7.3	7.0
30-Oct	17.4	8.2	7.6	6.5
31-Oct	14.0	9.7	9.8	7.3
4-Nov	13.1	10.0	10.7	8.9
5-Nov	13.1	10.4	11.2	9.2
6-Nov	14.5	9.2	9.3	8.0
7-Nov	12.9	10.1	9.8	8.8
8-Nov	12.5	9.9	10.4	9.0
11-Nov	17.9	8.3	9.7	6.6