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STRIPED BASS STRAIN EFFECTS ON AQUACULTURE PERFORMANCE TRAITS, STRESS RESPONSIVENESS AND HYBRID OFFSPRING GROWTH

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ABSTRACT

STRIPED BASS STRAIN EFFECTS ON AQUACULTURE PERFORMANCE TRAITS, STRESS RESPONSIVENESS AND HYBRID OFFSPRING GROWTH

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University of New Hampshire, September 2019

Striped bass (*Morone saxatilis*) and their hybrids have been well studied and are widely cultured as a food and gamefish. Recent increases in striped bass aquaculture have generated interest in domestication programs where growth performance and stress tolerance are among the traits of interest. Unique geographic stocks, or strains have previously been identified and these populations will presumably exhibit distinct culture traits across different environments. Before application of genetic principles, domestication programs should begin by evaluating available strains for superior performance in the environments intended to be utilized during culture. In this effort, a series of experiments were conducted to better understand growth and stress in *Morone* culture with applications for genetic improvement. First, 7 strains of striped bass spanning the native range of the species and one domesticated strain were compared for growth at different salinities (0, 5, 30 ppt) until two years of age (~ 2.6 kg). Significant differences were found among and within strains for growth rate, feed conversion ratio and body shape but salinity had no effect on growth. Second, to observe paternal strain effects on hybrid growth, striped bass males representing four wild strains from the previous trials and the domestic strain were conditioned until maturity and manually spawned with white bass. Resulting hybrid offspring were grown communally in three size grade treatments (large, small, ungraded) and two culture environments (indoor recirculating tanks, outdoor tanks) until attaining market size (~ 680 g). Genotyping after final measurements permitted parental assignment of hybrids to

sires. Differences in final weight and body shape were found among hybrids with highly similar performance trends to the previously conducted pure striped bass trials, suggesting a paternal strain effect. In addition to growth comparisons, two studies were conducted to compare stress responsiveness of striped bass during exposure to common aquaculture conditions. The first experiment investigated techniques to mitigate stress during transportation and demonstrated synergistic effects of anesthetic drugs that improve fish welfare during handling. In the final study, a suite of physiological parameters were measured in 4 strains of striped bass after 14 weeks exposure to a repeated, acute net chase stressor, and compared to an unstressed control group. The study demonstrated reduced performance of most strains and differences were detected among some measured responses. No single strain (wild or domesticated) demonstrated maintenance of growth or immunocompetence, but correlations among stress biomarkers were identified and variations within strains described for potential future research. This work contributes to a growing body of literature on striped bass aquaculture that could be applied in the design of region-specific broodstock programs, general culture practices and stress physiology studies.

CHAPTER 1

INTRODUCTION

Life history

Striped Bass (*Morone saxatilis)* is an anadromous teleost with a native range spanning the entire Atlantic coast from the Canadian Provinces to the St. John's River in Florida and into the Gulf of Mexico (Merriman 1941; Setzler-Hamilton et al. 1980). For well over 100 years, striped bass have been stocked extensively for restoration efforts and sport fishing, including the pacific coast in 1879 where breeding populations now exist. Differentiation among such stocks has been demonstrated genetically (Wirgin et al. 1989, 1990, 1991; Waldman et al. 1996; Bielawski and Pumo 1997; Diaz et al. 1997) and from tagging studies (Setzler-Hamilton et al. 1980). Although numerous, smaller landlocked and non-migratory populations with unique life histories have been documented, the Atlantic coastal population is the most extensive, with the majority of stocks north of Cape Hatteras (North Carolina) undertaking annual feeding migrations to coastal waters as far north as the Canadian Maritime Provinces (Boreman and Lewis 1987). Substantial spawning populations historically occurred throughout the entire coast but are now limited to four primary systems including the Hudson River (New York), Delaware River (Delaware), Roanoke River (North Carolina) and tributaries of the Chesapeake Bay (Merriman 1941; Boreman and Lewis 1987; Dorazio et al. 1994). Many minor spawning populations also contribute to the migratory stock at unknown proportions but tributaries of the Chesapeake Bay system have historically maintained the most productive spawning stock on the Atlantic coast (Merriman 1941) and, depending on the year class, provide up to 90% of the Atlantic coast's annual harvests (Van Winckle et al. 1988).

Individuals spawn annually (iteroparous) in fresh to brackish water, between the months of April and June, with precise timing dependent on highly variable environmental conditions in nursery grounds (primarily temperature). Males usually mature between 2-4 years of age while females mature later between 4-8 years (Merriman 1941; Berlinsky et al. 1995) with lifespans lasting upwards of 30 years. Following spawning, embryos hatch within 1-4 days and the larvae readily adapt to elevated salinity allowing them to feed and grow efficiently within natal systems until the end of their second year (Lal et al. 1977, Secor et al. 2000, Setzler-Hamilton et al. 1980). At approximately 2 years of age, juveniles will join adults after the spawning season and engage in an annual migration into coastal waters to feed and overwinter (Merriman 1941).

Striped bass is considered to be an "inshore" fish spending most of the year feeding within 6-8 km from the nearest point of land. Precise habitat is determined by food availability and time of day but typical locations include sandy beaches, shallow bays or estuaries, above or around submerged rocky stretches and at the mouths of river systems leading to the open ocean (Bigelow and Schroeder 2002). Time of year has a significant effect on feeding activity, and the majority of heavy feeding occurs from the summer into the fall as fish prepare to spawn, with less activity from winter to spring. Studies of feeding behavior have shown stomach contents to be highly variable including items like lamprey, alewife, shad, herring, menhaden, smelt, silverside, mullet, sculpins, croaker, weakfish, flounders, lobsters, various crabs, shrimps, worms, squid, clams and small mussels (Bigelow et al. 2002). When feeding, the largest fish are found singly or in small groups while smaller fish (<3 kg) will often congregate in much larger schools giving them the nickname, "schoolies," by fishermen.

Fishery

Striped bass is arguably the most valuable inshore species of the Atlantic coast. It is highly prized by commercial and recreational fisherman alike as a food fish, due to its lean, white flesh with a delicate, sweet flavor that commonly fetches up to $20/lb at retail markets. Since colonial times, the striped bass fishery has been significant in Atlantic coastal communities as a food source and economic asset. Even the first public school of the country was partially funded by taxes on commercial sales of striped bass in 1670 (Pearson 1938). Initial concerns were expressed in the mid-1700s as the fishery began showing signs of depletion which worsened by the 19th century due to continued fishing pressure and spawning habitat destruction in the forms of dams and pollution (Pearson 1938; Richards and Rago 1999). Sporadic years of productivity managed to maintain the fishery, and from 1954 to 1970 the Chesapeake Bay produced multiple dominant year classes which led to a historical high in commercial harvest in 1973, with a landing of 6,700 metric tons (Boreman and Austin 1985). Post 1973, the tributaries of the Chesapeake failed to produce significant year classes and only 10 years later, the commercial landings crashed to less than 1,000 metric tons (Boreman and Austin 1985).

The rapid fishery collapse caused great concern among fishermen and scientists which prompted development of the Interstate Fisheries Management Plan for the Striped Bass, created by the Atlantic States Marine Fisheries Commission (ASMFC) in 1981 (ASMFC 1981). Due to the migratory nature of the species, effective fishery management was difficult because of necessary cooperation among Atlantic states. The ASMFC amended the plan three times by 1985 to restrict fishing practices and protect the vital Chesapeake stock (Richards and Rago 1999). Specific amendments included seasonal closures on spawning grounds, gear restrictions and increased minimum size limits intended to improve the adult chances for reproduction.

Moratoriums were declared by Delaware and Maryland in 1985 while other jurisdictions adopted size limits that exceeded the size of most fish, essentially closing the fishery (Richards and Rago 1999). The ASMFC's recommendations were strongly supported by the US Congress which passed the "Emergency Striped Bass Study" and the "Atlantic Striped Bass Conservation Act" in 1979 and 1984 respectively. The acts by Congress provided the necessary funding to improve population monitoring and research efforts into the causes of decline while threatening a federal moratorium on the fishery until regulations were in compliance with the council's recommendations. Monitoring research such as juvenile surveys were well funded, spawning stock samplings were conducted on major spawning grounds, fisheries statistics were greatly improved, various tagging studies were conducted coast wide, laboratory experiments tested the tolerances to relevant environmental stressors (Richards and Rago 1999) and maturation rates were updated (Berlinsky et al. 1995). Striped bass population response was immediate with many river systems showing signs of recovery by 1989. In addition to strict fishery and habitat management, hatcheries developed species specific protocols and stocked 7.5 million fingerlings into the Chesapeake system from 1985-1993, allowing for widespread successful restorations (Richards and Rago 1999).

Morone culture and hybridization

The earliest published report of successful striped bass egg production under artificial conditions was made in 1874 by Spencer Baird, the first commissioner of the newly created (February 1871) U.S. Commission of Fish and Fisheries. Ten years later, the first dedicated striped bass hatchery was built on the Roanoke River in Weldon, North Carolina (Worth 1884). The culture of striped bass was initially developed as a means of producing fish to enhance native coastal stocks, but was later expanded to include inland reservoirs (Bonn et al. 1976,

Stickney 1996). By the 1950s, new populations of striped bass were being established in man-made reservoirs throughout the southeastern United States, including the Santee-Cooper Reservoir of South Carolina. Techniques for hormone-induced spawning were developed in 1962, which led to the stocking of millions of striped bass fry annually (Stevens 1967).

In 1965, hybrid crosses between striped bass and other species of the genus *Morone* were being produced at South Carolina's Moncks Corner hatchery and considerable advancements in production protocols were made for hybrid striped bass fingerlings (Bishop 1968). The original objective of hybridization was to produce fish that had the size, fighting ability and food quality of the striped bass with the habitat adaptability and less stringent spawning requirements of the white bass (*Morone chrysops*; Bayless 1972, Harrell 1997, Rudacille and Kohler 2000). The first cross (original, palmetto) used a striped bass female and white bass male, but the reciprocal cross (sunshine bass) has become the industry standard for several reasons. First, with the decline of natural striped bass stocks, female broodstock became prohibitively difficult to obtain. Second, white bass mature at earlier ages and are smaller and easier to handle, and they have a lower mortality rate than striped bass following strip spawning. Additionally, white bass is often easier to stage and spawn following ovulation induction (Kohler et al. 1994, Smith et al. 1996, Harrell 1997). As hybrid production requires maintenance or acquisition of two species of broodstock, considerable research on both *Morone* species has been conducted, and entire books (Harrell et al. 1997) and culture method guidelines have been published (Bonn et al. 1976; Harrell et al. 1990). In addition to many studies focusing on striped bass nutrition (Woods and Soares 1996; Small and Soares 1998; Small et al. 2000), health (Plumb 1997), pond culture methodologies (Geiger et al. 1985; Geiger and Turner 1990; Harrell 1997), and stress mitigation (Harrell 1992; Harrell and Moline 1992), considerable research has been conducted on striped

bass reproduction (Berlinsky and Specker 1991; Jenkins-Keeran and Woods 2002; Reading et al. 2012). Several early studies demonstrated that hybrid striped bass exhibited heterosis for important production traits, including improved growth during the first two years, survival and disease resistance (Bishop 1968, Logan 1968, Kerby 1986), but comparisons to date have lacked proper replication, family organization and consistent environmental variables. In a study comparing domesticated striped bass to palmetto bass and backcross hybrid striped bass, Jenkins et al. (1998), observed that striped bass had significantly higher specific growth rates than palmetto bass, with the backcross group intermediate between them. In another study, that compared the growth of striped bass to hybrids, it was determined that the hybrid striped bass had lower metabolism than wild striped bass that resulted in higher growth rates (Tuncer et al. 1990). These studies, however, did not consider parentage or relatedness of the treatment fish.

During the decline of the wild commercial fishery, market opportunities for hybrids as a foodfish expanded greatly but have remained in relative stasis since about the year 2000 (Garber and Sullivan 2006). Today, the hybrid striped bass industry is fourth in value among finfish species, behind channel catfish, salmon, and rainbow trout. Without economic multipliers, the U.S. striped bass aquaculture industry's production was valued at an estimated $30 million including juvenile production of fry and fingerlings (Dasgupta and Thompson 2013). Hybrid striped bass production in 2012 totaled approximately 9 million pounds. Nearly 8 million pounds were produced domestically and an additional million pounds were produced in Italy, Israel, Germany and Mexico. Currently, 90% of U.S. production occurs in freshwater ponds while remaining producers use tanks with less than 1% using netpens or cages. Hybrids are usually grown for about 14-16 months (680 g), and the majority (~80%) are sold live or whole on ice into ethnic markets for a premium price (D'Abramo et al. 2002; Carlberg et al. 2005). As niche

markets were quickly saturated, further expansion of the hybrid industry became hindered by high production costs preventing the product's acceptance into traditional domestic retail outlets (Garber and Sullivan 2006).

In coastal states, consumers are accustomed to, and often prefer, pure-strain wild striped bass harvested from marine environments. Purebred striped bass, rather than hybrids, are also highly preferred in some lucrative ethnic markets, seafood restaurants and sushi bars, and unlike hybrids, can be grown in "open" systems (e.g., coastal areas) without the risk of genetic contamination of wild stocks. Marine striped bass culture has been initiated to meet this demand where fish are generally grown to larger sizes (2.2 kg) for whole, gutted, or filleted market forms (http://pacificoaquaculture.com). Pure and hybrid striped bass could maintain separate domestic industries with variable culture methods (mariculture, pond culture, recirculating systems) and market strategies (fillet, whole fish) that avoid direct competition. Sustainable, long term advancement in aquaculture production of both fish, however, requires commitments to genetic improvement and selective breeding practices. To initiate the process of domestication, a detailed understanding of genetic variability across the geographic range of the species, and information on the performance traits of stocks or strains of striped bass in different culture environments must be determined (Garber and Sullivan 2006; Ponzoni et al. 2013).

Previous studies have demonstrated differences in growth may occur among and within striped bass strains and that could be exploited through selective breeding practices. Jacobs et al. 1999 conducted growth studies comparing wild-caught fish obtained from New York, Maryland, South Carolina and Florida waters in two facilities. Growth rates were found to be significantly greater in Maryland and Florida fish compared to those from South Carolina and New York. Furthermore, variation was found within and among families and 3 stocks of fish captured from

different regions of the Chesapeake Bay (Woods et al. 1999). The growth variation among these populations was carefully evaluated for up to four generations removed from the founder population and demonstrated the great potential for selective breeding in this species.

Stress in aquaculture

Routine hatchery procedures, including vaccination, transporting, and grading, are stressors that activate the physiological stress response defined as a state of threatened homeostasis that is re-established by a complex suite of adaptive responses (Chrousos, 1998). These responses coordinate the animal's energy reserves away from normal bodily functions such as growth, immunocompetence, and reproduction and directs them towards coping with the stressor (Pickering 1993). If the adaptive response is successful, a return to pre-stressed conditions (homeostasis) is achieved, but if the stressor is too severe or long-lasting, the coping mechanisms become maladaptive and detrimental to the fish's performance (Barton and Iwama 1991; Barton 2002).

The stress response is under neuroendocrine control and categorized into three distinct phases collectively known as the General Adaptation Syndrome (GAS; Selye 1950, 1973). The primary response, or alarm reaction, is initiated by perception of the stressor and includes the immediate release of catecholamines (neural control) followed by activation of the hypothalamus-pituitary-interrenal gland (HPI) axis and release of corticosteroids (endocrine control) into circulation (Barton 2002). Homologous to the adrenal medulla in mammals, chromaffin cells in the anterior kidney contain stores of catecholamines (epinephrine) that are rapidly released upon direct sympathetic activation. Primary actions of epinephrine include increased respiration, stimulated oxygen uptake and mobilization of energy stores (glucose), collectively termed the "fight or flight" response (Bonga 1997). Although the actions of the

sympathetic nervous system provide the animal with the immediate means to cope with a stressor, the delayed endocrine stimulation of the HPI axis is equally important for longer term adaptation. The primary stress response is also categorized by the release of corticotrophin releasing hormone (CRH) from hypothalamic nuclei that is transported to the anterior pituitary (Barton 2002). The anterior pituitary releases Adrenocorticotrpic hormone (ACTH) into the blood stream, which stimulates interrenal cells of the anterior kidney. Homologous to the mammalian adrenal cortex, interrenal cells in fishes synthesize and release corticosteroids, one of which is cortisol (Barton and Iwama 1991; Mommsen et al. 1999). Cortisol has become the most widely studied hormones in the primary response, whose principle role is to ensure prolonged energy availability, primarily by promoting gluconeogenesis (Pickering 1990). The secondary, or resistance phase often results from influences in the primary phase and results in adjustments by the organism to regain homeostasis through metabolic, hematological and immunological changes (Mommsen et al. 1999). The tertiary stage is comprised of whole body responses in growth, disease resistance, behavior and ultimately survival (Barton 2002).

In a culture environment, exposure to acute or chronically-stressful conditions, may lead to varying elevations in cortisol (Barton and Iwama 1991). Regardless of the stressor's nature, inability of a fish to cope or adapt may lead to decreased somatic growth, immunosuppression, impaired reproductive capacity or death (Wendelaar Bonga 1997; Barton 2002). Although cortisol has become one the most widely studied hormone associated with the stress response due to predictable timing of release, relative ease of measurement, and demonstrated heritability in breeding programs, its correlations with aquaculture performance traits have remained difficult to interpret. In rainbow trout, ambiguous results were obtained, with some studies showing that fish with consistently low cortisol response grew at faster rates than those with a high responses,

while other studies suggested the opposite (Pottinger and Carrick 1999, Fevolden et al. 2002). Several studies have been conducted that compared the relative stress responsiveness among *Morone* species, and some of the disparate findings likely reflect genotypic differences. Davis and McEntire (2009) compared the cortisol stress response in striped, white and sunshine bass in response to net seining. Although they reported higher cortisol levels in striped bass, they interpreted their findings as speculative because of differences in broodstock origin (domestic vs. wild). Further, they concluded the only way to assign heritability of stress responsiveness is to make all possible crosses with individual high and low responding striped bass and white bass. Wang et al. (2004) observed differential cortisol responses among domesticated striped bass individuals from different families. In a later study, it was suggested that stress responsiveness may influence reproductive performance, but no information is currently available on its influence on growth (Castranova et al. 2005), or differences among strains. The varying results in stress research can be attributed to numerous factors including species specificity, nature of the stressor (acute, chronic, repeated), previous history of the fish under observation, health status and the great number of unknown interactions within the complex physiological response. Pickering (1981) accurately stated that "there are few concepts that have evoked as much discussion and disagreement as that of stress when applied to biological systems."

Inconsistent correlations between cortisol responsiveness and performance have been observed leading to a recent focus on a more complex selection trait termed "robustness" (Sadoul et al. 2015). This complex trait, first described in pigs, combines high production potential with resilience to stressors in a wide variety of environmental conditions (Knap 2005). It may be beneficial for aquaculture research to pursue a similar goal. Instead of drawing conclusions based on measures of the primary response, a suite of stress related biomarkers should be

examined in parallel with performance traits of interest in attempts to measure the response to the response.

Measurements during the secondary phase of the GAS such as alterations in glucose, hematological counts and expression levels of selected genes may provide insights into the precise mechanisms of reduced aquaculture performance during stress. A main role of the stress response is to provide the energy required to escape or cope with the stressor. In this effort, stored forms of energy, primarily glycogen, are liberated from the liver where both catecholamines and cortisol effectuate glycogenolysis making glucose readily available for muscle and other tissue types (Schreck and Tort 2016). Glucose levels are predictably elevated in the blood stream after exposure to the stressor, are simple to measure, and offer an indirect marker of the magnitude of the neuroendocrine response (Barton 2000; Pottinger and Carrick 1999). Although necessary to provide immediate energy, prolonged or exceptionally elevated glucose levels indicate that energy is not available for whole body functions, described in the tertiary phase, like growth and maintenance of the immune system.

Stress is known to alter hematological parameters through direct endocrine activity or indirectly through impaired energy state. Hematological parameters such as hematocrit and white blood cell counts are commonly measured to assess health in modern human and veterinary medicine, with a growing interest in aquaculture for their predictive nature of future performance (Davis et al. 2008). Adaptive changes in hematocrit and specific properties of erythrocytes occur during stress to increase oxygen availability to major organs which are easily measured in many species (Hrubec et al. 2001). Similarly, cortisol is shown to suppress lymphocyte production and activity, leading to a greater susceptibility to infection or disease (Schreck and Tort 2016). These hematological measures of the secondary phase in the GAS

provide practical, non-lethal means to monitor fish health and gauge future performance, but further research is needed to establish reference intervals for specific species of interest before applicable to the aquaculture industry.

The liver is a major target organ during times of stress and responsible for many of the associated metabolic adjustments such as alterations in appetite and growth (Aluru and Vijayan 2009). Cortisol modulation of nutrients, growth factors and hormones from hepatocytes leads to indirect and often detrimental effects on growth (Mommsen 2001; Davis 2006) but the precise mechanisms of such control remain unclear. During normal conditions, growth hormone (GH) from the pituitary, under highly complex regulation, binds receptors on hepatocytes (Jiao et al. 2006) to stimulate production of insulin- like growth factor 1 (*igf1*), the primary peptide hormone responsible for myogenesis in the vertebrate GH-IGF axis (Reinecke 2006; Picha et al. 2008). Igf1 has received considerable attention as a proposed biomarker of fish growth in numerous species and scenarios, but its regulation during stressful conditions is often difficult to determine unless combined with additional measures (Picha et al. 2008). Leptin is an anorexigenic peptide that has been well studied in mammals, known to modulate feed intake and maintain energy homeostasis with links to the stress axis, but only recently gained interest in aquaculture research (Bernier et al. 2012; Roubos et al. 2012; Baltzegar et al. 2014). During periods of catabolism and diminished energy reserves, leptin production decreases and triggers neural pathways to increase food consumption until energy reserves are restored (Ashima 2008). During positive energy status (anabolism), increased leptin production promotes energy expenditure, upregulates hypothalamic anorexigens and downregulates orexigens to decrease appetite (Ashima and flier 2000). Leptin activity during stress in fish is less clear, but similar effects observed during mammalian feeding have also been observed in rainbow trout (Murashita

et al. 2008; Madison et al. 2015) and goldfish (Volkoff et al. 2003) which include reduced feed intake. Although more complex to measure in comparison to glucose or blood cells, further research on the activity of growth factors like Igf1 and leptin under different stressors may provide details into the physiology of reduced performance and offer insights of how to mitigate the effects of stress during routine aquaculture practices.

Selective breeding and domestication

There has been a rapid increase in global aquaculture production during recent decades due to the demand for quality protein and the decline in wild fisheries. Although aquaculture has been successful at filling this void, culture of many species is still dependent on the unsustainable collection of wild broodstock and results in unpredictable offspring performance. Selective breeding and domestication are the most effective and sustainable means to improving productivity and achieve long term gains (Ponzoni et al. 2013). Due to biological factors including high fecundity and relatively short generation intervals in fish, rapid improvements have been documented, such as an average 10–20% increase in growth rate per-generation, which is 5 to 6 times greater than that obtained for any terrestrial farm animals (Gjedrem and Baranski, 2009). Despite these results, less than 10% of aquaculture production is derived from genetically improved stocks (Gjedrem et al. 2012). Growth rate is often the most desirable phenotype and selection for growth has proven effective for several major aquacultured finfish species including brook trout *Salvelinus fontinalis* (Embody and Hyford 1925), Atlantic salmon *Salmo salar* (Friars et al. 1995), channel catfish *Ictalurus punctatus* (Rezk et al. 2003), Nile tilapia *Oreochromis niloticus* (Eknath 1993) red sea bream *Pragus major* (Murata et al. 1996) and yellow perch *Perca flavescens* (Rosauer et al. 2011). Although growth is the most common, other major phenotypes include survival, feed conversion ratio (FCR), fillet yield/quality, disease

resistance, stress tolerance, morphological traits and various reproductive traits. The initial step towards broodstock development of any species entails extensive comparisons of performance traits among strains from different geographic regions in the specific environments intended to be utilized during culture (Ponzoni et al. 2013). Although strain specific differences in growth have been documented for striped bass (Jacobs et al. 1999; Woods 2001), little attention has been given to effects of different environments or additional traits such as stress responsiveness.

Objectives

In this dissertation, four studies were conducted with the goal of identifying aquaculture traits within and between geographic strains of striped bass for application in selective breeding and general culture. The information from this research in conjunction with substantial, recent advances in genetic/genomic resources (Reading et al., 2012; Li et al., 2014; Abdelrahman et al., 2017; Fuller et al., 2018; Zhao, 2019) will aid future, focused broodstock development.

Hypotheses

1. Natural variations in life history and habitat among geographic strains of striped bass will influence performance in culture. Long term growth trials will identify aquaculture traits among striped bass strains and their hybrid offspring in different culture environments.
2. Differences in stress responsiveness exist within and among striped bass strains. Measurements of physiological indicators across all stages of the stress response during exposure to different stressors will reveal new insights into the topic with specific applications to aquaculture.

Chapter overview

To test these hypotheses, a series of four experiments were conducted. In Chapter 2, long term growth trials were used to compare performance of seven wild strains spanning the entire natural range of the species and one domesticated line spawned at the North Carolina State University (NCSU) Pamlico Aquaculture Field Laboratory as a part of the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry.* A pair of consecutive, two year trials, tracking growth rates and other performance indicators in recirculating aquaculture systems at different salinities (0, 5, 30 ppt) were conducted. This chapter was peer reviewed and published in the journal *Aquaculture* (Kenter et al. 2018). In Chapter 3, male striped bass from different strains were used to produce hybrids and investigate the paternal effect on growth performance of juveniles in recirculating systems and outdoor production environments. Chapter 3 was a follow up study to Chapter 2 and is currently in the final stages of editing before submission to the journal *Aquaculture*. Chapter 4 investigated novel anesthetic approaches to mitigate stress during transportation and common aquaculture procedures using a wild and domestic strain of striped bass. This chapter was peer-reviewed and published in the *North American Journal of Aquaculture* (Kenter et al. 2019). Chapter 5 describes a 14-week study comparing stress responsiveness among three selected wild and one domesticated strain exposed to a repeated, acute net chase stressor. A manuscript from this chapter was submitted to the journal of *Fish Physiology and Biochemistry* on July 14, 2019 for peer review (Kenter et al. in review).

CHAPTER 2

STRAIN EVALUATION OF STRIPED BASS (*Morone saxatilis)* CULTURED AT DIFFERENT SALINITIES

Introduction

Striped bass (*Morone saxatilis*) is an anadromous fish indigenous to the Atlantic coast of North America and the eastern Gulf of Mexico (Setzler-Hamilton et al. 1980) that has been the focus of aquaculture efforts since the 1800s (Worth 1884). The Atlantic striped bass population extends from the Miramichi River (New Brunswick, Canada) south to the St. John's River in northern Florida and spawning once occurred in costal freshwater rivers throughout this range (Boreman and Lewis 1987). Today, the major spawning grounds on the U.S. Atlantic coast are limited to tributaries of the Chesapeake Bay (Virginia and Maryland) and the Hudson River, although smaller spawning populations exist in several other rivers, including the Roanoke River (North Carolina), the Delaware River and the Kennebec River (Maine). In the Canadian provinces, spawning populations are now limited to the Miramichi River (New Brunswick) and Shubenacadie River (Nova Scotia) (Cook et al. 2010). Striped bass have also been introduced to many freshwater lakes and reservoirs throughout the United States, the Pacific Ocean as well as waters in several other countries (Froese et al. 2007).

Hybridization of striped bass with other *Morone* species, particularly the stenohaline freshwater white bass (*Morone chrysops*), has been conducted since the 1960s, largely because hybrids demonstrated superior growth, disease resistance, and tolerance to higher water temperatures when grown in freshwater ponds (Harrell et al. 1990; Harrell 1997). The hybrid striped bass industry is currently fourth in value among United States finfish species, behind channel catfish (*Ictalurus punctatus*), salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus*

mykiss) and they are also cultured in Mexico, Portugal, France, Germany, Italy, Israel, South Vietnam, China, Taiwan, and Russia (FAO 2014). As hybrid production requires maintenance or acquisition of two different species of broodstock, considerable research on both *Morone* species has been conducted, and several books (Harrell et al.1997b) and culture method guidelines have been published (Bonn et al. 1976; Harrell et al. 1990). In addition to many studies focusing on striped bass nutrition (Dougall et al. 1996; Small and Soares 1998; Small et al. 2000), feeding (Huang et al. 1993), health (Harms et al. 1996), pond culture methodologies (Geiger et al. 1985a; Geiger and Turner 1990), larval rearing techniques (Geiger et al. 1985b; Robichaud and Peterson 1998) and stress mitigation (Harrell 1992; Harrell and Moline 1992), considerable research has been conducted on striped bass reproduction and controlled spawning (Hodson and Sullivan 1993; Weber et al. 2000; Jenkins-Keeran and Woods 2002; Clark et al. 2005; Reading et al. 2012).

Hybrid striped bass are cultured in 20 states, particularly in the South and Midwest of the United States, in constructed, inland freshwater ponds. The fish are usually grown for about 14-16 months, and are marketed whole at approximately 680 g (D'Abramo et al. 2002). In coastal states, consumers are accustomed to, and often prefer, pure-strain wild striped bass harvested from marine environments. Purebred striped bass, rather than hybrids, are also highly preferred in some lucrative ethnic markets, seafood restaurants and sushi bars, and unlike hybrids, can be grown in "open" systems (e.g., coastal areas) without the risk of genetic contamination of wild stocks. Marine striped bass culture has been initiated to meet this demand, and for these purposes, fish are generally grown to larger sizes (2.2 kg) for whole, gutted, or filleted market forms (http://pacificoaquaculture.com).

Despite the extensive research that has been conducted on striped bass culture, farmers

generally continue to rely on wild-caught broodstock for production needs. This dependence on wild broodstock is common in aquaculture where only about 8.2% of global production is derived from domesticated species (Gjedrem et al. 2012). The use of wild or unselected broodstock results in considerable variability in desirable traits, and to improve juvenile performance some growers have relied on hybridization, chromosome set manipulations and production of monosex populations. These practices must be repeated for each generation, however, but long term gains are possible by selective breeding, which has been common practice for terrestrial agriculture species for centuries (Abdelrahman et al. 2017). Selection for enhanced growth has proven effective for several aquacultured finfish species including brook trout (*Salvelinus fontinalis*) (Embody and Hyford 1925), Atlantic salmon (Gunnes and Gjedrem 1978), channel catfish (Rezk et al. 2003), Nile tilapia (*Oreochromis niloticus*) (Eknath 1993), red sea bream (*Pragus major*) (Murata et al. 1996), and yellow perch (*Perca flavescens*) (Rosauer et al. 2011). An initial step in this process entails comparing growth performance of strains originating from different geographic regions in controlled conditions (Ponzoni et al. 2013). While growth comparisons of striped bass strains have been reported previously (Jacobs et al. 1999, Woods 2001), the current study was conducted to compare strain performance in fresh, brackish, and salt water to market size (two years) and also included a strain of striped bass that has been domesticated in culture for about six generations.

Methods

Juvenile Strain Acquisition

Fingerling striped bass (0.5-4.0 g), bred in captivity during two consecutive natural (spring) spawning seasons at state, federal, private, and university hatcheries in Texas (TX), Florida (FL), South Carolina (SC), Virginia (VA), Delaware (DE), North Carolina (NC) and Nova Scotia (NS) were transported to the Ritzman Aquaculture Laboratory ((University of New

Hampshire (UNH), Durham, NH, USA)) for two growth trials (Table 1). Striped bass broodstock were wild-caught from the spawning grounds except the NS broodstock that were wild-captured as juveniles and maintained in captivity. The striped bass strains used in these trials were selected because of their geographic distribution, hatchery availability, and growth performance in initial trials.

The domesticated striped bass line (DOM) was originally created from hundreds of outcrosses of six distinct striped bass strains (Canada, Pacific Ocean, Roanoke River, Chesapeake Bay, Santee-Cooper Reservoir, and Florida-Gulf of Mexico) in the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* at the North Carolina State University (NCSU) Pamlico Aquaculture Field Laboratory (Aurora, NC). The DOM striped bass were mass selected for performance and body conformation in fresh and brackish water aquaculture systems for ~8-9 generations and primarily serve as broodstock for producing the hybrid striped bass cross. Juvenile DOM striped bass were produced at NCSU and transported to UNH. Fin clips (caudal fin) from all broodfish were collected and stored in 95% ethanol for family identification by microsatellite genotyping upon conclusion of the growth trials.

Table 1
Strain, river source, spawn date(s), parental crosses and age at acquisition (days post spawn; dps) of striped bass cultured in two growth trials.

	Strain	River or Source	Spawn date	Crosses	Shipping Age (dps)
Trial 1	TX	Lake Livingston Tailrace	4/8/2013	20	38
	FL	Ochlockonee	4/7- 4/8/2013	7	38-39
	SC	Santee Cooper	3/31, 4/8, 4/11, 4/25/2013	20	48-73
	VA	Mattaponi and Roanoke	4/16 (Mattaponi), 5/6/2013 (Roanoke fish)	9	45-65
	DE	Delaware	5/9/2013	2	112
Trial 2	FL	Ochlockonee	4/10/2014	10	40
	SC	Santee Cooper	4/3- 4/18/2014	20	68-83
	NC	Neuse and Tar	4/23 (Neuse), 4/26/2014 (Tar)	7	88-91
	VA	Mattaponi	4/15/2014	7	51
	NS	Shubenacadie	5/21-6/19/2014	15	55-84
	DOM	NC State University	5/28/2014	9	86

Culture Conditions and sampling protocols

One year growth comparison-Trial 1

During Trial 1, a comparison of five strains was conducted in simulated marine conditions (30 ppt salt) until fish reached approximately one year of age. Juvenile fish were maintained by strain in 15 x 1500 L tanks that were constructed into five identical and independent, three-tank recirculating systems. All systems were equipped with screen filters, bead filters, biological filters, protein skimmers, ultra violet (UV) sterilizers and temperature control. Upon arrival, the five strains (DE, VA, SC, FL, TX) were randomly assigned to three tanks each. The, temperature was maintained at 20-21°C throughout the trial. Seawater was trucked from Little Bay, Durham NH (~25-28 ppt), filtered, chlorinated, aerated, dechlorinated with sodium thiosulfate, and brought to 30ppt with the addition of Hi-Grade sodium chloride (Cargill, Minneapolis, MN). Water quality was monitored daily for temperature, dissolved oxygen, pH, and salinity. Total ammonia and nitrite were monitored weekly.

The fish were hand fed marine finfish diets (Skretting, Europa; St. Andrew, NS, Canada; 50% minimum (min.) crude protein and 18% min. crude fat) twice daily (six days/week). Daily feeding rates were normalized on a percent body weight basis beginning at 3% and decreasing to 2% and 1.25% at about 250 and 300 days post spawn (dps), respectively. The initial stocking density was high (213 fish/tank) and was periodically reduced (final density 40 fish/tank) to avoid growth plateaus or deteriorating water quality due to overcrowding. The culling events were staggered, based on average age of the strain, and occurred at around 180, 280 and 320 dps. Individuals were randomly selected during culling events in an effort to maintain equivalent representation of all families.

At 30-day intervals, a subset of 20-50% of fish per tank were netted, anesthetized with 150 mg/L, buffered, tricaine methanesulfonate (Tricaine-S, Western Chemical, Ferndale, WA), weighed to the nearest 0.1 g, and total length measured to the nearest 1.0 mm. From these measurements, tank biomass was calculated and daily feed rations were recalculated. Upon conclusion of the experiment, all fish were sampled as above and specific growth rate (SGR), feed conversion ratio (FCR), and condition factor (K) were calculated as described below. Stripe patterns were also described using a qualitative rating of 0, 1 or 2. A rating of 0 indicated, unbroken, parallel lines; 2 indicated a highly irregular, checkered pattern; and 1 was intermediate between the two.

Fin clips were collected from the upper lobe of caudal fins off all juvenile striped bass during final measurements and 50% of these were later extracted and genotyped to determine family origin (n=300, 60 fish per strain).

Common garden growth comparison-Trial 1

During the 280 dps culling event in the experiment described above, a random subsample from VA, SC, FL, and TX strain fish were implanted with PIT tags and stocked together ("common garden design") into a single recirculating system with three x 5000 L tanks. The DE fish were omitted from this part of the study because they were significantly younger and smaller than the fish representing the other four, similarly sized strains. The water conditions were maintained as described above. Each tank was initially stocked with 20 fish/strain, but were reduced to 10 fish/strain at 540 dps to maintain water quality. As above, individuals from each strain were randomly selected during this culling event to maintain equivalent representation of families. The fish were fed to apparent satiation twice daily, six days/week and received Skretting, Europa (270-500 dps) and Bio Brood (Bio-Oregon, Westbrook, ME; 12 mm, 48% min. crude protein and 24% min. crude fat) until the conclusion of the study (about 750 dps). This study was terminated when the fish were approximately two years of age, at which time all fish were weighed, total lengths recorded, and fin clips collected.

Upon conclusion of the study, a random subsample of each strain (n=8 fish) were euthanized and "skin on", trimmed fillet yields were compared. The same individual filleted all fish by hand to avoid inter-investigator variation. Coefficient of variations between left and right fillets were calculated for each fish and were below 5% for all individuals included in analyses.

<u>One year growth comparison-Trial 2</u>

During Trial 2, a comparative growth study was conducted at UNH and The University of Maryland Crane Aquaculture Facility (UMD), using fish originating from NS, VA, NC, SC, FL and DOM broodstock (Table 1). Based on density observations during year 1 trials, at UNH, 40 fish from NS, VA, FL and DOM were stocked into 21 x 1500 L tanks, three tanks per strain that were incorporated into seven identical and independent recirculating aquaculture systems.

Three systems were maintained at 0 ppt and four at 30 ppt to allow strain comparisons to be conducted between these two water salinities. Due to a limited number of fish, the NS strain was only evaluated in marine conditions. Fish from VA, NC, SC, FL, and DOM were shipped to UMD, stocked into 15 x 2000 L tanks (40 fish per tank; 3 tanks per strain) and maintained at 20-21ºC, and 5 ppt. The tanks at UMD were incorporated into a single recirculating system with mechanical and biological filtration, foam fractionation and UV sterilization. At UNH, the fish were fed as described in growth comparison Trial 1 above, and at UMD, the fish were fed the same formulated diet, once daily to apparent satiation, five days per week. Feed consumption was recorded daily at both facilities and used to calculate FCR, as described below. Growth data were collected at 30-day intervals from 50% and 100% of the fish in each tank at UMD and UNH, respectively, as described above. The studies were terminated when the fish were one year (365 days) of age, at which time, all fish were weighed and lengths recorded for growth comparisons. Fin clips were collected from all fish and 50% of these were randomly selected for extraction, genotyping and family analysis (UNH, n=420, 120 fish each from VA, FL, TX and 60 fish from NS; UMD, n=300, 60fish from each strain). Upon conclusion of this experiment, the fish reared at UMD were PIT tagged and transported back to UNH for a common garden growth comparison.

Common garden growth comparison-Trial 2

Striped bass that were reared at UMD (24 fish each from the VA, NC, SC, FL, and DOM strains) and UNH (24 fish from NS strain) during Trial 2 were grown in a common garden experiment, as described in 2.2.2 above. Using these fish afforded a comparison of all strains, but minimized the number of strains grown at different facilities through one year of age. Feeding, sampling, and water conditions were all consistent with the previous common garden trial, but

initial densities were decreased to avoid culling mid-trial. Due to variations among strains and the common garden design, it was not possible to sample all strains at the same age without excessive handling and changes in density. Therefore, all fish were grown for an additional 365 days, until they were approximately two years of age.

Upon conclusion of the trial, a random subsample of each strain (n=8 fish) was again compared for fillet yield as well as carcass yield, head weight, visceral weight, and visceral fat. Carcass yield was determined by eviscerating the fish and subtracting the weight of viscera (liver, gastrointestinal tract, spleen, and gonads) from total body weight. Once the viscera were removed and weighed in a beaker, the beaker was placed in a low heat oven overnight at 30°C until the visceral fat melted. Melted visceral fat was sieved and weighed separately from the viscera. As in Trial 1, the same individual filleted all fish and the coefficient of variation between left and right fillets was below 5% for each fish included in the analyses.

Growth and morphometric calculations

Specific growth rates (SGR) were calculated for the strain comparison and common garden studies using the formula: [ln(Wf)-ln(Wi)]/T]*100, where T is time in days and ln(Wf) and ln(Wi) are final and initial fish weights, respectively. Feed conversion ratios (FCR) were calculated using the formula: (feed intake/n)/(Wf-Wi), where n is the number of fish in a given tank and Wf and Wi are final and initial fish weights, respectively. For the common garden trials, condition factor (K) was calculated to quantify body shape using the formula: $(W/(L\text{^}3)*10^4)$; where W is weight and L is total length. Absolute growth rates for fish in Common Garden-Trial 2 were calculated using the formula: (Wf-Wi)T, where T is time in days and Wf and Wi are final and initial fish weights, respectively. The final weights based on absolute growth rates were normalized among strains to 750 dps for statistical comparisons.

Microsatellite Genotyping Analysis

Genomic DNA was extracted from fin clip samples using the Qiagen DNeasy 96 Blood and Tissue Kit (Qiagen Inc. Hilden, Germany) following the manufacturer's protocol. Genotyping was performed with 11 microsatellite markers: MSM 1144, MSM 1095, MSM 1096, MSM 1067, MSM 1094, MSM 1168, MSM 1208 and MSM 1243 of Couch et al. (2006) and MSM 1526, MSM 1592 and MSM 1357 of Rexroad et al. (2006). DNA was amplified by polymerase chain reactions (PCR) using fluorescent-dye-labeled primers (FAM, HEX and NED) in 12.5µl reaction volumes. Reactions contained 1.5-2.0 µl DNA template, 5X Go Taq Flexi colorless buffer (Promega), 2 mg/ml bovine serum albumin (BSA; multiplex one only), 0.06 mM deoxynucleotide triphosphates (dNTPs), 2 mM $MgCl_2$, 0.5 U Taq Polymerase (Promega), and variable amounts of primer, as shown in Table 2. PCRs were performed in an Eppendorf thermal cycler (Eppendorf Inc. Westbury, NY) with cycling conditions as follows for multiplex one: denaturation at 95°C for 15min, 35 cycles of 94°C for 30s, 45s at 60°C and 30s at 72°C, and final elongation at 72°C for 10mins. Multiplex two: denaturation at 95°C for 15min, 30 cycles of 94°C for 30s, 45s at 58.5°C and 30s at 72°C, and final elongation at 72°C for 10mins.

Table 2
Multiplex reactions used for striped bass family assignment including locus name, primer concentration and dye label.

	Locus	Primer Conc.	Dye
Multiplex 1	MSM 1144	0.4µM	FAM
	MSM 1526	0.96µM	HEX
	MSM 1095	0.24µM	FAM
	MSM 1096	0.48µM	HEX
	MSM 1067	0.48µM	NED
	MSM 1243	0.16µM	HEX
Multiplex 2	MSM 1094	0.2µM	HEX
	MSM 1168	0.3µM	FAM
	MSM 1208	0.7µM	FAM
	MSM 1592	0.6µM	NED
	MSM 1357	0.3µM	FAM

The resulting PCR product was diluted at a ratio of 2:8 in Hi-Di formamide. Plates were shipped to Yale University's, DNA Analysis Facility (New Haven, CT) for fragment analysis using an automated DNA sequencer (3730xl 96-capillary genetic analyzer; Applied Biosytems, Foster City, CA). Peaks were scored manually using Peak scanner version 2.0 software (Applied Biosystems) and raw scores sorted into allelic bins.

Family assignment

Parental assignments (both maternity and paternity) were conducted for all genotyped juveniles (n=1,020 fish). Genotypes of the progeny were compared to those of the broodstock candidate parents using CERVUS version 3.0.7 software (Marshall et al. 1998). CERVUS calculates the log-likelihood of each candidate parent being the true parent and uses simulations to determine the level of confidence in the parentage assignment. A genotyping error rate of 1% was assumed and the proportion of parents sampled was 100%. For offspring with parental assignment confidence < 80%, we also conducted sibship assignment using the maximum likelihood method implemented in COLONY version 2.0.6.3 (Jones and Wang 2010). COLONY can

determine the number of parents for a set of progeny for which not all parents have been sampled and it can use these estimated parental genotypes to reconstruct sibship groups

Statistical analyses

Statistical comparisons of growth parameters (SGR, FCR) were conducted using the monthly measurements on replicate tanks (7-9 sampling events) until strains attained one year of age. Common garden comparisons were conducted when fish were initially stocked and again once they attained two years of age. During Trial 1 (experiment 2.2.2), a mid point sampling was also conducted during the culling event (500 dps). Analyses of these measurements and final weight, condition factor and fillet characteristics were performed using one-way ANOVA. Tukey's pairwise comparison of means was applied if ANOVA output indicated a significant ($P<0.05$) overall model difference among strains.

Additional statistical analyses were conducted for both trials to determine the influence of family and strain on performance, as measured by final weight at one year of age. Families within strains were reared together such that representation was entirely unknown until measurements and fin clips were collected at final time points. Therefore, variance estimates due to family could only be performed on final weights when the parentage of an individual was assigned by genotype.

A linear mixed-model analysis (REML) was used to test for differences among final weights and calculate components of variance. Any families represented by a single juvenile were excluded from the analysis. For Trial 1 data, strain was treated as a fixed effect while family nested within strain and tank nested within strain were random effects. For Trial 2, experimental differences between facilities (UNH and UMD), such as system design, husbandry practices and strain inclusion prohibited direct statistical comparisons. Final weights at UMD

were analyzed separately from UNH using the same model applied in Trial 1. At UNH, salinity, strain and their interaction were included as fixed effects while family and tank nested within strain were designated as random effects. Analyses were performed in JMP (13).

Results

Growth evaluations

One year growth comparison-Trial 1

Growth rates were similar among strains up to one year of age during the first trial, but a significant difference in SGR was found between DE and SC (ANOVA, $P<0.05$) in marine conditions (Table 3). Mortalities occasionally occurred after a sampling event, but they were unrelated to strain or salinity treatments and survival was above 98%. No differences were found in FCR among strains (ANOVA, $P=0.064$), but condition factors differed. FL had the deepest bodied individuals ($K=1.35 \pm 0.01$) while fish from DE had the most streamlined body shape ($K=1.16 \pm 0.01$) (Table 3). Stripe patterns differed among strains and DE and VA had the most parallel, unbroken lines compared to other strains (Fig. 4).

Common garden growth comparison-Trial 1

Growth rates differed during the second year and despite the similar age of fish, final weights of fish from VA, FL, and TX were greater than those from SC (Fig. 1; Table 4). Differences in condition factor K (ANOVA, $P<0.05$) also were evident at the conclusion of the trial (Table 5), but did not influence fillet yield.

Although significant in the mixed-model (REML, $P=0.0231$), the random effect of family nested within strain only accounted for 25.6% of the total variance in final weights among strains. Tank nested within strain did not have a significant effect on final weight (REML, $P=0.386$) and accounted for only 3.2% of total variance.

One year growth comparison-Trial 2

Striped bass survival was greater than 95% in the second trial and mortalities were not strain-related. The DOM striped bass had better growth and feed conversion compared to most wild strains reared at both UNH and UMD (Tables 6 and 7). The DOM strain FCR (0.98 ± 0.01) in marine conditions was significantly lower than all wild strains at UNH in both salinities (ANOVA, P=0.0004). A similar FCR (0.98 ± 0.04) was observed at UMD in brackish conditions, which was lower than all wild strains and significantly better than that found in SC and FL fish. Growth rates among strains were similar at UNH, but the DOM fish exhibited the greatest SGR (ANOVA, $P<0.05$) in marine systems compared to all wild strains, and compared to VA in freshwater conditions. SGRs were overall lower at UNH compared to UMD where VA fish had the highest SGR followed by the DOM fish. Fish cultured at UMD had greater final weights compared to the same strains at UNH (Fig. 3). Condition factors differed among strains (ANOVA, $P<0.05$) and FL consistently had deeper bodied individuals and VA the most streamlined.

Common garden growth experiment- Trial 2

Figure 2 shows the divergence in average weights among strains when grown for 2 years to larger market sizes. The DOM fish, which had the highest SGR during the first year of salt water culture, did not maintain their growth advantage after the second year (Table 8). The NC and FL strains had higher SGRs than the DOM fish and the FL fish finished the trial with the highest final weights. The NS fish had the lowest final weight, followed by SC. Similar to results from the 1 year growth trial, FL had the highest condition factor and NC and VA were still the most streamlined (Table 8). Condition factor was not correlated with fillet yield, but FL had smaller head weights than VA fish and greater proportional visceral weight than VA and SC fish

(Table 9). Lower head weights also contributed to lower head on, carcass yield of FL fish compared to DOM, VA, and SC (ANOVA, $P<0.05$). Similar to the first trial, random effect of family was significant (REML, $P=0.0157$) but did not account for the majority of variance at the UMD facility (22.4%). The effect of tank nested within strain was not significant (REML, $P=0.0941$). At UNH, salinity alone and the interaction of strain by salinity were not significant (REML, $P<0.05$) contributors to final weight. Family nested within strain was again significant (REML, $P=0.0143$), but only accounted for 22.9% of variance which is in agreement with results at UMD. Tank effect was again insignificant (REML, $P=0.1636$), accounting for 5.1% of the variance.

Table 3

Feed conversion, specific growth rates and condition factors for striped bass strains grown in marine conditions in Trial 1 (One year growth comparison). Means (± standard error) followed by different letters indicate significant differences between strains ($P < 0.05$).

	Delaware	**Virginia**	**S. Carolina**	**Florida**	**Texas**
FCR	1.13 ± 0.06	1.13 ± 0.03	1.20 ± 0.06	1.10 ± 0.04	1.14 ± 0.05
SGR	1.33 ± 0.14^{A}	1.01 ± 0.08^{AB}	0.93 ± 0.07^{B}	1.11 ± 0.07^{AB}	1.11 ± 0.10^{AB}
Condition Factor (K)	1.16 ± 0.01^{D}	1.25 ± 0.01^{B}	1.2 ± 0.01^{C}	1.35 ± 0.01^{A}	1.28 ± 0.01^{B}

Table 4

Age, survival, final weights, specific growth rates and condition factors for striped bass grown in conditions marine conditions in Trial 1 (Common garden growth experiment). Means (± standard error) followed by different letters indicate significant differences between strains ($P < 0.05$).

	Virginia	S. Carolina	Florida	Texas
Age (dps)	740	756	760	759
Final Weight (kg)	2.79 ± 0.08^{A}	2.20 ± 0.08^{B}	2.56 ± 0.08^{A}	2.74 ± 0.09^{A}
SGR (~500dps)	0.72 ± 0.02^{B}	0.65 ± 0.02^{C}	0.69 ± 0.01^{BC}	0.8 ± 0.01^{A}
SGR (~750dps)	0.55 ± 0.01^{A}	0.49 ± 0.01^{B}	0.52 ± 0.01^{AB}	0.54 ± 0.01^{A}
Condition Factor (K)	1.41 ± 0.01^{BC}	1.38 ± 0.02^{C}	1.70 ± 0.04^{A}	1.51 ± 0.03^{B}
Survival	30/30	26/30	29/30	30/30

Figure 1

Mean weight (g) by age (dps) for striped bass grown in marine conditions in Trial 1.

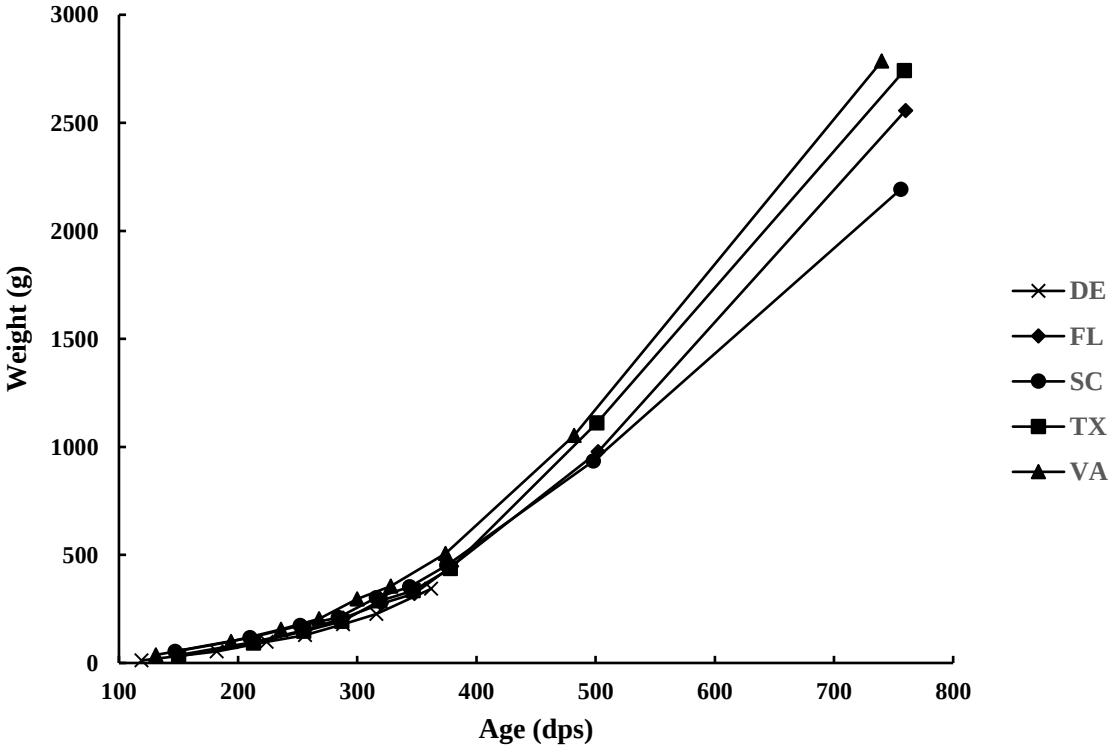


Table 5

Age, final weights, condition factors and fillet yields for striped bass grown in marine conditions in Trial 1 (Common garden growth experiment). Means followed by different letters indicate significant differences between strains ($P < 0.05$; N=8 fish/strain).

	Virginia	S. Carolina	Florida	Texas
Age (dps)	740	756	760	759
Final weight (kg)	2.90 ± 0.10^{A}	2.06 ± 0.13^{B}	2.72 ± 0.18^{A}	2.81 ± 0.17^{A}
Condition factor	1.41 ± 0.02^{C}	1.36 ± 0.02^{C}	1.76 ± 0.05^{A}	1.56 ± 0.05^{B}
Fillet yield %	40.2 ± 0.4	40.8 ± 0.6	40.7 ± 0.4	40.8 ± 0.6

Table 6

Feed conversion ratios, specific growth rates and condition factors for striped bass grown in marine conditions and freshwater conditions at UNH in Trial 2 (One year growth comparison). Means (± standard error) followed by different letters indicate significant differences between strains ($P < 0.05$).

UNH, Marine					**UNH, Freshwater**		
	Domestic	**Canada**	**Virginia**	**Florida**	**Domestic**	**Virginia**	**Florida**
FCR	0.98 ± 0.01^{A}	1.14 ± 0.02^{BC}	1.14 ± 0.03^{BC}	1.18 ± 0.03^{C}	1.05 ± 0.02^{AB}	1.16 ± 0.02^{BC}	1.14 ± 0.02^{BC}
SGR	1.42 ± 0.01^{A}	1.26 ± 0.02^{BC}	1.16 ± 0.03^{C}	1.25 ± 0.05^{BC}	1.34 ± 0.03^{AB}	1.15 ± 0.02^{C}	1.23 ± 0.03^{BC}
Condition Factor	1.34 ± 0.01^{BC}	1.31 ± 0.01^{C}	1.14 ± 0.01^{D}	1.36 ± 0.01^{B}	1.54 ± 0.01^{A}	1.32 ± 0.01^{BC}	1.56 ± 0.01^{A}

Table 7

Feed conversion ratios, specific growth rates and condition factors for striped bass grown in brackish conditions at UMD in Trial 2 (One year growth comparison). Means (± standard error) followed by different letters indicate significant differences between strains ($P < 0.05$).

UMD, Brackish					
	Domestic	**Virginia**	**N. Carolina**	**S. Carolina**	**Florida**
FCR	0.98 ± 0.04^{A}	1.13 ± 0.05^{AB}	1.15 ± 0.06^{AB}	1.17 ± 0.07^{B}	1.21 ± 0.10^{B}
SGR	1.64 ± 0.03^{B}	1.75 ± 0.02^{A}	1.42 ± 0.002^{D}	1.53 ± 0.02^{C}	1.61 ± 0.005^{BC}
Condition Factor	1.35 ± 0.01^{B}	1.17 ± 0.01^{D}	1.14 ± 0.005^{E}	1.24 ± 0.01^{C}	1.38 ± 0.01^{A}

Table 8

Age, survival, final weights, age adjusted final weights, specific growth rates and condition factors for striped bass grown in marine conditions in Trial 2 (Common garden growth experiment). Means (± standard error) followed by different letters indicate significant differences ($P < 0.05$). Survival was 100% survival for all strains.

	Domestic	Canada	Virginia	N. Carolina	S. Carolina	Florida
Age (dps)	714	706	757	748	762	762
Final Weight (kg)	2.48 ± 0.08^{B}	1.69 ± 0.08^{C}	2.50 ± 0.08^{B}	2.43 ± 0.06^{B}	2.26 ± 0.09^{B}	2.85 ± 0.10^{A}
Adjusted Weight (kg)	2.68 ± 0.09^{A}	1.85 ± 0.09^{C}	2.46 ± 0.08^{AB}	2.44 ± 0.06^{AB}	2.19 ± 0.08^{BC}	2.76 ± 0.10^{A}
SGR (~24month)	0.40 ± 0.01^{BC}	0.35 ± 0.01^{C}	0.43 ± 0.01^{AB}	0.46 ± 0.01^{A}	0.45 ± 0.01^{A}	0.45 ± 0.01^{A}
Condition Factor (K)	1.51 ± 0.02^{B}	1.43 ± 0.03^{BC}	1.34 ± 0.02^{C}	1.35 ± 0.02^{C}	1.50 ± 0.03^{B}	1.71 ± 0.03^{A}

Figure 2

Mean weight (g) by age (dps) for striped bass grown in marine conditions in Trial 2.

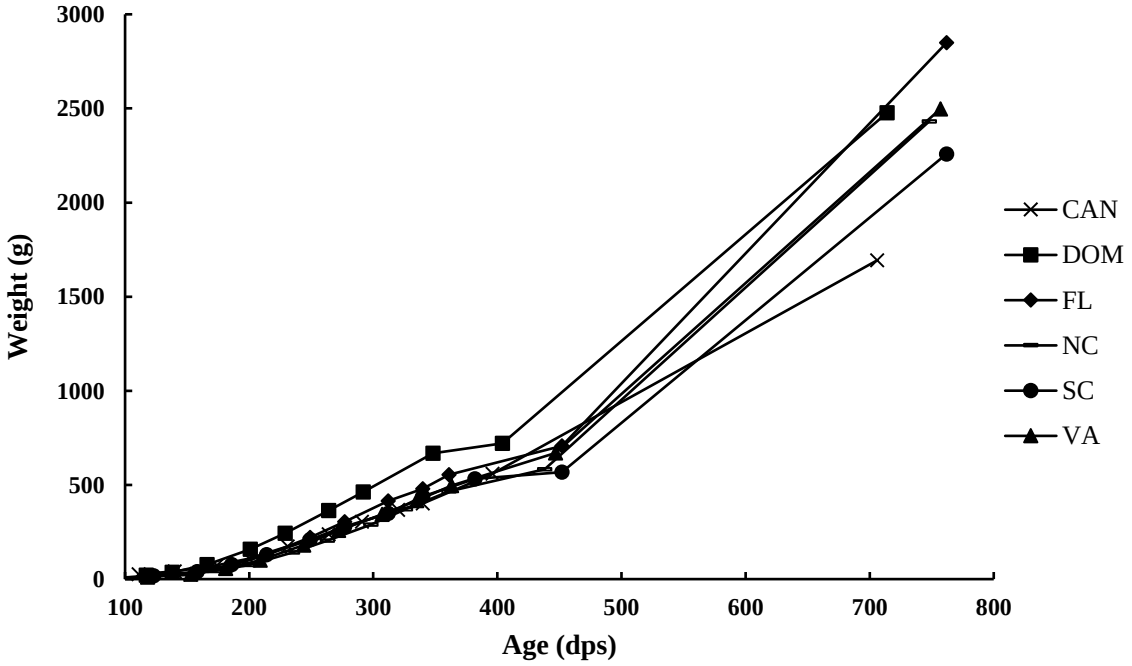


Table 9

Age, final weights, condition factors, skin on fillet yields, carcass yields, head weight percentages, total visceral percentages and percent visceral fat for striped bass grown in marine conditions in Trial 2 (Common garden growth experiment). Means (± standard error) followed by different letters indicate significant differences ($P < 0.05$).

	Domestic	**Canada**	**Virginia**	**N. Carolina**	**S. Carolina**	**Florida**
Age (dps)	714	706	757	748	762	762
Final weight (kg)	2.47 ± 0.10 ^{AB}	1.73 ± 0.15 ^{C}	2.52 ± 0.19 ^{AB}	2.60 ± 0.10 ^{AB}	2.06 ± 0.15 ^{BC}	2.75 ± 0.16 ^{A}
Condition factor (K)	1.50 ± 0.02 ^{B}	1.42 ± 0.04 ^{BC}	1.33 ± 0.03 ^{C}	1.38 ± 0.03 ^{BC}	1.41 ± 0.04 ^{BC}	1.78 ± 0.04 ^{A}
Fillet yield %	42.6 ± 0.68	41.0 ± 0.60	42.0 ± 0.47	42.9 ± 0.53	43.7 ± 0.65	42.1 ± 1.19
Head on carcass yield %	90.3 ± 0.49 ^{A}	88.9 ± 0.61 ^{AB}	90.5 ± 0.37 ^{A}	89.9 ± 0.26 ^{AB}	90.3 ± 0.30 ^{A}	88.5 ± 0.43 ^{B}
Head weight %	27.8 ± 0.44 ^{AB}	27.6 ± 0.58 ^{AB}	28.1 ± 0.54 ^{A}	26.8 ± 0.39 ^{AB}	27.2 ± 0.40 ^{AB}	25.8 ± 0.57 ^{B}
Viscera %	9.44 ± 0.29 ^{AB}	10.4 ± 0.34 ^{AB}	9.13 ± 0.39 ^{B}	9.44 ± 0.25 ^{AB}	9.21 ± 0.20 ^{B}	10.7 ± 0.43 ^{A}
Visceral fat %	63.9 ± 2.6	61.5 ± 3.5	64.2 ± 4.0	67.8 ± 4.4	64.5 ± 3.2	70.0 ± 2.5

Figure 3

Mean weights (g) of common strains grown at UNH and UMD in Trial 2 (One year growth comparison). UNH salinity treatments are grouped and independent t-test differences are designated by an asterisk ($P<0.05$).

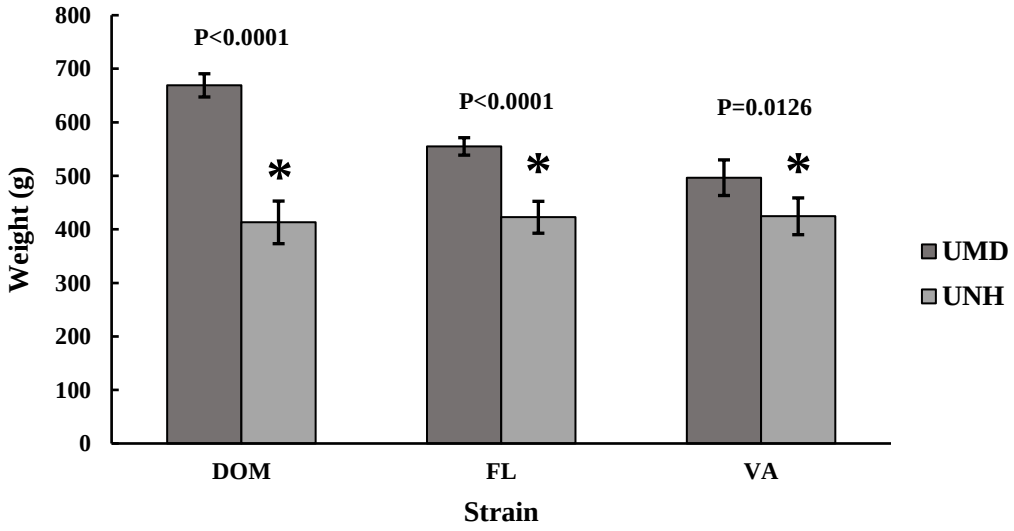


Figure 4
Stripe patterns described among strains in Trial 1 (One year growth comparison) 0 = unbroken, parallel pattern, 1= slight breaks and gaps, 2= highly irregular and checkered. Images of a 2 and 0 are shown for comparison.

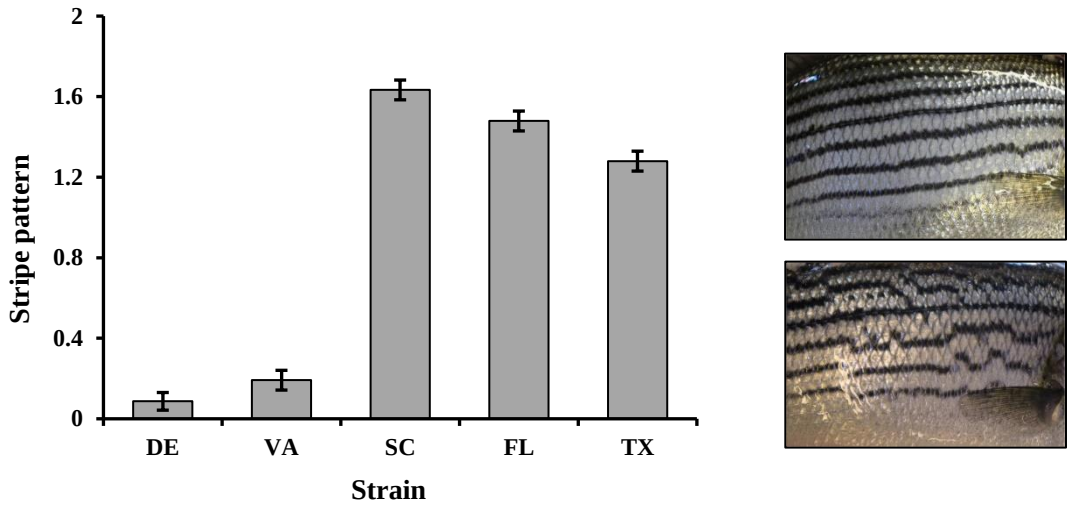


Figure 5
Condition factor and general morphological differences observed in Trials 1-2. Images of VA (top) and FL (bottom) shown for comparison at one year of age.

Discussion

Despite slight differences, growth rates of cultured striped bass were comparable among most strains with final weights of approximately 460 g and 2.5 kg at the age of one and two years, respectively. These growth rates and final weights exceeded those reported in previous striped bass strain comparisons conducted in recirculating systems (Jacobs et al. 1999; Woods et al. 2001). Purebred striped bass raised in recirculating systems in the present study attained the typical hybrid market size (680 g or 1.5 lbs.) in less time (15-16 months) than required for some hybrids (Kohler et al. 2000). For commercial production, farmers typically grow hybrid striped bass in earthen ponds, because they grow more rapidly at an early age and demonstrate greater stress tolerance in commercial operations (Kerby and Harrell 1990; Noga et al. 1994; Harrell 1997). Direct comparisons between purebred and hybrid striped bass using half sibling families or selected broodstock have not been conducted (Garber and Sullivan 2006), however, and the improved growth attained by heterosis at younger ages may be lost after the first year due to the genetic influences of the smaller white bass parent (Kohler 1997). While stable environmental conditions likely contributed to the growth rates achieved in this study, controlled hybrid and purebred striped bass comparisons are warranted. The growth rates demonstrated in this study were also greater than those reported for European seabass (*Dicentrarchus labrax*), at (500-700 g; 22 months, 1160 g; 45 months) and Gilt-head bream (*Sparus aurata*), (400-500 g; 12 months) in recirculating systems (Saillant et al. 2001; Gorshkov et al. 2004; Gines et al. 2004), although differing experimental conditions limit a direct comparison between these studies.

The striped bass compared in these studies represent migratory and resident (non-migratory) strains that cover the majority of the Atlantic range. Although many strains were similar in growth performance, slight differences were observed and likely attributable to

dissimilar life histories and adaptive variation. Conover (1997) compared growth rates of striped bass larvae, under controlled conditions, from six locations spanning their natural range. He found fish from Nova Scotia and New York (northernmost strains) had the greatest growth compared to North Carolina, South Carolina and Gulf populations. He proposed that striped bass exhibit countergradient variation in growth rates in which fish from northern latitudes grow more rapidly than southern conspecifics to compensate for a shorter first growing season. (Conover 1990; 1997). While the New York (Hudson River) strain was not available for comparison in the present study, fish derived from the Shubenacadie River (Nova Scotia, Canada) were compared in the second growth trial. These fish represent one of northernmost, non-migratory populations of striped bass (Cook et al. 2006), which has been shown to be genetically distinct from other striped bass populations (Wirgin et al. 1993). In the present study, this strain was found to be slow growing when cultured to market sizes, results similar to those obtained by Woods (2001). The juveniles grown in the present study were already 84 dps when acquired and cultured intensively during their larval stages. Evidence of countergradient variation, therefore, may have been obscured by culture conditions and late date of acquisition.

The striped bass from the Santee Cooper (SC) river system have been land-locked and genetically isolated since the construction of the Pinopolis dam in 1941 (Scruggs 1956). A previous study by Secor et al. (2000) compared the growth of young (45-90 dps) striped bass from this population with those from the Chesapeake Bay across a range of temperatures and salinities. Irrespective of environmental conditions, fish from the Chesapeake grew significantly greater than those from the Santee Cooper and these finding were later confirmed for older fish (Jacobs et al. 1999; Woods 2001). In the present study, the Santee Cooper population did not

perform as well as the other strains (VA, FL, TX) in the first two year trial, and similar to the Canadian strain, did not attain final weights of at least 2.4 kg in the second trial.

The striped bass historically found in the Gulf of Mexico were non-migratory and isolated from Atlantic coastal populations. Overfishing and destruction of spawning habitat in the 1950s and 1960s led to the loss of all natural reproduction except in a few Florida river systems (Wooley & Crateau 1983). In effort to restore the Gulf coast populations, 1.8 million Santee-Cooper fry and 125,000 fingerlings were released into the Apalachicola, Chattahoochee and Flint Rivers between 1965-76 (Nicholson 1986). Despite these introductions the FL fish maintained genetic distinctiveness, but the western gulf Texas, Louisiana and Mississippi River strains were found to be genetically similar to the Atlantic coast populations (Diaz et al. 1997; Dunham et al. 1988; Wirgin et al. 1991,1997; Wirgin and Maceda 1991). Both the FL and TX gulf strains evaluated in the growth comparison trials described here performed well despite genetic mixing with slower growing Santee-Cooper fish. The unique morphology (e.g. high condition factor) of the FL fish may be a useful selection trait for specific ethnic markets.

The DOM fish have been continuously bred at North Carolina State University's, Pamlico Aquaculture Field Lab since the early 1990s (~8-9 generations) with the objective of providing a reliable source of broodstock, to the hybrid striped bass aquaculture industry. Originally created by outcrossing several strains, this domestic strain has been spawned, pond-raised, and undergone both directional (e.g., large body size, deformity-free, short generation time) and passive (e.g., feed entrainment, stress tolerance) selection. The DOM fish grew faster and had lower FCRs than most wild strains up to one year of age across salinities, but failed to maintain their growth advantage in the second year marine, common garden trial. This may have been due to inadvertent selection for freshwater conditions, but no growth advantage was

detected in the first year salinity comparison trial. Alternatively, the second year growth performance may reflect persistence of genetic founder effects from slower growing strains (NS, NC, and SC) that have managed to escape elimination through the mass selection scheme. Family-based selection has been utilized with other important finfish species such as tilapia and carp (Bentsen et al. 1998; Gjerde et al. 2002). As this selection method offers many advantages over mass or individual selection (Lind et al. 2012), it should be considered for future striped bass broodstock development. For the growth parameters analyzed, significant differences were found among strains but the influence of families within strains accounted for 22.4 – 25.6% of the variation in our mixed model analysis. Strains with more than ten parental crosses often had limited representation among siblings and unequal sample sizes made it difficult to describe within strain differences past the mixed model variance output. Effect of family was considerably lower than that observed in previous studies (59-63% Jacobs et al. 1999; Woods 2001), but still suggests that family selection may be beneficial for improving broodstock programs. Future studies should determine the heritability of specific growth parameters in this species to maximize selection efficiency.

Growth trials for striped bass raised in salt water conditions were previously reported (Turner and Farley 1971; Secor et al. 2000) and commercial striped bass culture in marine net pens has recently been initiated to supply the sushi and fresh fillet markets (http://pacificoaquaculture.com). In the present study, no differences in striped bass growth were found between culture in salt or freshwater. These results are consistent with previous findings with several striped bass strains. Duston et al. (2004) examined the growth of striped bass of different sizes from the Shubenecadie River in Nova Scotia in fresh or saltwater (30ppt). Among most size classes, no differences in growth were observed in 115 day trials. Similarly, Secor et

al. (2000) found no salinity preferences (0.5, 7.0 and 15 ppt) by striped bass juveniles (45 dph) from the Santee Cooper river system and Chesapeake Bay in short (15 day) growth trials. Although similar growth performances were found among strains raised at the different facilities, the mean final weights of all strains were significantly greater for fish raised at UMD in 5 ppt compared to those raised at UNH (0 and 30 ppt). This may be attributable to system and husbandry differences rather than salinity, as the fish at UMD were raised in larger tanks and fed to apparent satiation (5 days/week) compared to feeding on a percentage of body weight basis at UNH. Although salinity did not affect growth in the present study, several studies have shown that the addition of salts can mitigate stress responses and improve survival of striped bass and their hybrids in adverse conditions (Grizzle et al. 1992; Mazik et all. 1991; Harrell 1992; Wallin and Van Den Avyle 1995) and may improve flavor (Drake et al. 2006).

Several morphological differences among striped bass strains were found in these studies that may influence marketability. Broken stripe patterns were more conspicuous on fish from SC, FL and TX. These patterns are often associated with hybrid striped bass and their occurrence in wild striped bass may suggest an intended backcrossing had occurred. For decades, hybrid striped bass were routinely stocked as a gamefish in waters where interactions with anadromous striped bass may occur (Harrell 1993) and evidence has shown them closely overlapping in diet and behavior within the Chesapeake (Patrick and Moser 2001). Their reproductive viability (Harrell 1984), genetic capacity to backcross (Bayless 1968), and participation in spawning runs synchronized with natural striped bass (Bishop 1968; Moser and Ross 1993; Patrick and Moser 2001) may have potential effects on certain populations. Results indicated that fish from DE and VA had consistently parallel lines while SC, FL and TX were often irregular. A genetic analysis was not performed during these studies to check wild individuals for white bass alleles as

described by Woods et al. (1995), but such an approach could be considered to screen individuals for purity prior to any future selective breeding efforts.

Fish from FL had the greatest condition factor K regardless of age or year class, but no differences in skin on, trimmed fillet yield were found among strains, which averaged about 42%. Similar findings were reported in *Morone* hybrids (McEntire 2015), the closely related European sea bass (Vandeputte et al. 2014), and the yellow perch; Rosauer et al. 2011), where differences in body shape did not influence the amount of harvestable muscle tissue. In many breeding programs, selection for smaller head size is common, as it may lead to greater overall fillet yield (Jiang 2008). Future studies should determine the heritability of this trait in striped bass, since the difference in head sizes observed between the FL and VA strains may serve as a starting point for future selection. Skin on fillet yields (>40%) in the present were greater than those observed in both *Morone* hybrids (Bosworth 1998), three strains of tilapia (Rutten et al. 2004), and channel catfish and their hybrids (Li et al. 2004). Considerable amounts of visceral fat were also observed in this study and may reflect the high lipid content (18% and 24%) of the diets compared to common *Morone* commercial diets containing approximately 10% (Wang et al. 2006). No differences were found among strains, however, and in other species (e.g., rainbow trout, hybrid catfish) the amount of visceral fat did not influence muscle accretion (Jobling et al. 1998; Li et al. 2004).

Relying on wild broodstock spawned at multiple hatcheries created challenges for the design of these experiments and required assumptions for common environmental conditions. The natural spawning times of the strains used in this study varied by latitude and resulted in age differences among the fry juveniles used in growth trials. To compensate for these differences, strains were isolated for their first year of growth, thinning events and feeding ration changes

were staggered and growth period durations were normalized. The number of families represented also varied by strain and was dependent on hatchery practices and wild broodstock availability. For this reason, strain comparisons were repeated across years in the cases of VA, SC, and FL, which permitted greater family representation and confirmed growth performance rankings. The common temperature chosen for these studies (20-21°C) may also have resulted in suboptimal growth during the early life stages, as several studies have shown that temperature preferences decrease changes as striped bass age. For instance, small striped bass juveniles (< 5-40 g) of several strains grew optimally at 24-28 °C (Kellogg and Gift 1983, Secor 2000, Duston et al. 2004) while larger juveniles and sub-adults preferred temperatures closer to 20°C (Cheek at al. 1985; Duston et al. 2004).

Conclusions

Growth rates of striped bass cultured in recirculating systems were high and similar across salinity treatments. Growth differences among strains became more pronounced during the second year of culture and although morphological differences were evident, they did not influence fillet yield. These results suggest that strain-specific family-based selection may be beneficial for striped bass broodstock development but heritability estimates for relevant production traits must be determined to facilitate these efforts.

CHAPTER 3

PATERNAL STRAIN EFFECTS ON GROWTH AND BODY SHAPE IN HYBRID STRIPED BASS (*Morone chrysops* ♀ *x Morone saxatilis* ♂)

Introduction

Interspecific hybridization has been applied to many aquaculture species in an effort to produce offspring with performance traits superior to those of either parental species (Bartley et al. 2000). Improved growth is one trait of considerable importance in aquaculture. Examples of food fish that have demonstrated growth benefits from hybrid development include silver carp x bighead carp (*Hypophthalmichthys molitrix x Aristichthys nobilis*) (Hulata 1995), channel catfish x blue catfish (*Ictalurus punctatus x Ictalurus furcatus*) (Smitherman et al. 1983), and gilthead seabream x red seabream (*Sparus aurata* x *Pagrus major*) (Knibb et al. 1998). The advantages gained through hybridization are offset in some cases, however, by the additional expenses involved in maintaining two species of broodstock, reduced reproductive performance, and additional labor for manual spawning and *in vitro* fertilization.

Beginning in the 1960s hybrid crosses between striped bass (*Morone saxatilis*) and other species of the genus *Morone* were being produced, and because preliminary findings indicated that hybrids exhibited greater tolerance to extremes in temperature and dissolved oxygen than either of its parents, it was thought that hybrids were better suited for many aquaculture systems (Logan 1968). The most common cross made was between striped bass males and white bass (*Morone chrysops*) females ("sunshine bass") because many producers found that spawning white bass females required less expertise than for striped bass (Smith 1988). Today, the hybrid striped bass (HSB) industry is fourth in value among finfish species in the US, behind only channel catfish, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

Relatively high production costs have prevented HSB from entering many mainstream markets and this is in part due to variation in juvenile growth performance resulting from spawning undomesticated broodstock (Woods 2001; Garber and Sullivan 2006). Hybrid striped bass are poised for genetic improvement with well-established husbandry protocols (Harrell et al. 1990; Hodson 1995) and substantial, recent advances in genetic/genomic resources (Reading et al. 2012; Li et al. 2014; Abdelrahman et al. 2017; Fuller et al. 2018; Zhao 2019). Strain-specific differences in growth performance have been documented in striped bass (Jacobs et al. 1999; Woods 2001; Kenter et al. 2018), heritability estimates for production traits have been calculated at different life stages in hybrid offspring (Wang et al. 2006; Lochmann and Goodwin 2015) and differences in weight and carcass yield were demonstrated in HSB produced using three geographic strains of white bass dams, but no studies have examined differences originating from sires (Kohler et al. 2001). The purpose of the present study was to compare growth between graded and ungraded HSB in two production environments among half-sib families sired by four wild striped bass strains and a 5th generation domesticated broodstock.

Methods

Broodfish acquisition

Fingerling striped bass, bred in captivity during the natural (spring) spawning seasons at state and federal hatcheries in Virginia (VA), South Carolina (SC), Florida (FL) and Texas (TX) were transported to the University of New Hampshire (UNH) Ritzman Aquaculture Laboratory (Durham, NH, USA) and grown communally for two years in recirculating systems as previously described (Kenter et al. 2018). The PIT tagged fish were transported to the North Carolina State University's Pamlico Aquaculture Field Laboratory (PAFL; Aurora, NC, USA) and grown for an additional year in flow-through culture tanks (38,433 L) with well water. Male striped bass were

identified by PIT tag and moved to an indoor recirculating system (3,555 L) in the late winter (February), and the water was maintained at 10-12 °C and 10-12 ppt salinity with photoperiod. In the spring, the three year old fish were in spawning condition and were used in the trials in addition to two-year old domestic (DOM) males already present at the facility. The 5th generation DOM males were part of the cohort used for annual production as a part of the *National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry* (Reading et al. 2018). Captive-bred 9th generation, age 3, female white bass from the Artesian Aquafarms LLC (South Mills, NC, USA) were used as dams to produce the reciprocal-crossed hybrid (sunshine bass) by *in vitro* fertilization. All female white bass were held in the conditioning tanks at PAFL as described above for striped bass males, except that they were held in 5 ppt salinity or less.

Spawning Preparations and Procedures

Beginning in April, fish were maintained under ambient photoperiod and gradually conditioned for spawning by increasing temperature 1 °C and decreasing salinity 1 ppt per day until 18-20 °C and 0.5-0.0 ppt were attained (Hodson and Sullivan 1993; Sullivan et al. 2003). Approximately 40 hrs prior to spawning, striped bass sires (3.73 kg ± 0.18, Mean ± SEM) were anaesthetized (150 mg/L, buffered MS-222, Tricaine-S, Syndel USA, Ferndale, WA), injected in the dorsal lymphatic sinus with 165 IU hCG/kg body weight (Chorulon®, Merck Animal Health, Madison, NJ, USA) to facilitate spermiation and sorted into four indoor staging tanks (3,555 L) (Harrell 1997). The staging tanks each held a single sire per strain (n= 4/strain) with the exception of FL where only two eligible fish were available. A total of 12 adult, white bass dams (0.82 kg ± 0.03) were maintained in a single flow-through raceway (630 L) and injected with 300 IU/kg of hCG approximately 24 hrs prior to strip spawning to induce ovulation.

On the morning of spawning, sires were captured by netting from their staging tanks, anesthetized and milt collected by applying gentle pressure on the ventral surface. The milt was stored inactive at 4 °C in separate, sterile 15 ml conical tubes and motility was verified by activating a subsample with water and viewing under a compound microscope (200x magnification). Dams were checked for ovulation every 2 hrs throughout the day by applying slight pressure to the ventral surface. Ovulating females were removed from the tank by netting, uniquely fin clipped for future parentage assignment and dry strip spawned into a Teflon-coated pan (McGinty and Hodson 2008). Eggs were divided into five equal aliquots that each received 1 ml of the previously collected milt from each sire. Hatchery water was added to activate the sperm while pans were gently swirled to initiate fertilization. Fertilized eggs from each resulting cross were poured into separate, aerated McDonald hatching jars and treated with tannic acid (150 mg/L) to reduce adhesiveness (Rottman et al. 1988; McGinty and Hodson 2008). McDonald jars were maintained separately until fertilization for each cross was confirmed using a compound microscope, based on proper embryo development. The viable embryos produced from a single female (5 half-sibling crosses) were then pooled into one McDonald jar for the remaining incubation period.

Larval culture and recirculating aquaculture growout

After a 48-hr incubation, larvae hatched into individual aquaria (90 L each) where total fry production could be enumerated for each female. Estimates were made by counting the number of viable, swim-up fry in each of three triplicate subsamples of a known volume (approximately 50 ml each) from each aquarium, and then multiplying the average of the three subsamples by the total volume for the aquaria. HSB fry were stocked into three replicate 0.25-acre earthen ponds (n= 25,000/pond) using fry produced from each spawn to ensure adequate

and equivalent pooling for family representation during fingerling production. Ponds were filled and initially fertilized with 34 kg cottonseed meal approximately 7 days prior to fry stocking and then fertilized with 1.4 kg inorganic phosphate fertilizer, 3.2 kg pelleted alfalfa meal, and 9.5 kg cottonseed meal every 7 days thereafter.

To observe the effect of size grading on long-term growth performance, HSB were seined from each pond, during early phase I (0.75-1.0 g/fish), pooled with equal pond representation and either graded into large and small cohorts, or left ungraded. Grading was conducted using a floating bar grader (5.6 and 6.4 mm, Aquatic Solutions, Des Moines, IA, USA) and average weight at harvest was 1.48 ± 0.76 g. Fish from each group were shipped to UNH and treated in an aerated bath containing 200 ppm formalin for 1 hr upon arrival. All fish were anesthetized as previously described, weighed to the nearest 0.1 g, and total length measured to the nearest 1.0 mm prior to stocking indoor recirculating systems. Fish from the small and large grades were randomly assigned to 6 x 1500 L tanks (40 fish/tank; 3 tanks/grade) that were constructed into two identical and independent, three-tank recirculating systems. Ungraded HSB were stocked at a similar density into 5 x 5000 L tanks (130 fish/tank) housed in two identical and independent, three tank systems. All systems were equipped with screen filters, bead filters, biological filters, protein skimmers, ultra violet (UV) sterilizers and temperature control. Salinity was maintained at 10 ppt with the addition of Hi-Grade sodium chloride (Cargill, Minneapolis, MN) for the initial two weeks to mitigate handling stress before a gradual reduction to freshwater (0 ppt) by the end of the first month. Temperature was kept at 22-23 °C throughout the trial. Water quality was monitored daily for temperature, dissolved oxygen, pH, and salinity. Total ammonia and nitrite were monitored weekly.

All tanks at UNH were hand fed commercial finfish diets (Skretting, Europa; St. Andrew, NS, Canada; 50% minimum (min.) crude protein and 18% min. crude fat) twice daily to apparent satiation. Growth trials continued until each respective size class reached the standard industry marketable weight (1.5 lb; 680 g; D'Abramo and Frinsko, 2008) determined by periodically measuring subsamples of fish (10%) from each culture tank during the last two months of growout. Final measurements were conducted on all individuals and fin clip samples were collected from the upper lobe of caudal fins and stored in 95% ethanol to determine parentage.

A mortality event due to bacterial infection was observed in the ungraded hybrids post-stocking at UNH. To eradicate the pathogen, symptomatic individuals were culled from the systems and finclips were collected (as described above) from symptomatic and dead fish (n= 30 each) to identify infected strains. Six fish were submitted for pathogen identification by bacterial culture and 16S rRNA sequencing (Fish Vet Group, Portland, ME, USA).

Outdoor growout

The remaining large grade fish (n= 2,630) were grown at PAFL in earthen ponds until approximately seven months of age. A random subset (n= 375) was then stocked into flow-through tanks (3 x 2,400 L) for an additional four months at which time density was reduced by a final transfer to larger tanks (3 x 5,800 L). The fish were hand fed a diet containing 42% min. crude protein and 16% min crude fat, once daily (Zeigler Gold 42/16 feed (Zeigler Bros, Inc. Gardners, PA, USA). Final measurements were taken when fish attained market size and fin clip samples collected as described above.

Microsatellite Genotyping Analysis

Genomic DNA was extracted from fin clip samples using the Qiagen DNeasy 96 Blood and Tissue Kit (Qiagen Inc. Hilden, Germany) following the manufacturer's protocol.

Genotyping was performed with 9 microsatellite markers in two multiplexing reactions. DNA was amplified by polymerase chain reactions (PCR) using fluorescent-dye-labeled primers (FAM, HEX and NED) in 12.5 and 15 µl reaction volumes for multiplex 1 and 2 respectively. Multiplex 1 contained: 1.5 µl DNA template, 5X Go Taq Flexi colorless buffer (Promega), 0.2 mg/ml bovine serum albumin (BSA), 0.06 mM deoxynucleotide triphosphates (dNTPs), 2 mM $MgCl_2$, 0.5 U Taq Polymerase (Promega), and variable amounts of primer, as shown in Table 1. Multiplex 2 contained: 2 µl DNA template, 5X Go Taq Flexi colorless buffer, 0.2 mg/ml BSA, 2.5 mM dNTPs, 2.5 mM $MgCl_2$, 1 U Taq Polymerase, and variable primer concentrations. PCRs were performed in an Eppendorf thermal cycler (Eppendorf Inc. Westbury, NY) with cycling conditions as follows for multiplex one: 95 °C for 15min, 35 cycles of 94 °C for 30 s, 45 s at 60 °C and 30 s at 72 °C, and final elongation at 72 °C for 10mins. Multiplex two: 95 °C for 5 min, 30 cycles of 95 °C for 45 s, 45 s at 50.9 °C and 45 s at 72 °C, and final elongation at 72 °C for 10 mins.

Table 1
Multiplex reactions used for HSB family assignment including locus name, primer concentration, dye label and reference.

	Locus	Primer Conc.	Dye	Reference
Multiplex 1	MSM 1067	0.48µM	NED	Couch et al. 2006
	MSM 1094	0.2µM	HEX	Couch et al. 2006
	MSM 1095	0.24µM	FAM	Couch et al. 2006
	MSM 1144	0.4µM	FAM	Couch et al. 2006
Multiplex 2	MSM 1168	0.2µM	FAM	Couch et al. 2006
	SB 83	0.2µM	NED	Han et al. 2000
	SB 91	0.2µM	HEX	Roy et al. 2001
	HSB 1B	0.2µM	FAM	Ross et al. 2004
	HSB 6C	0.3µM	FAM	Ross et al. 2004

The resulting PCR product was diluted at a ratio of 0.5:9.5 in Hi-Di formamide. Plates were shipped to the Yale University DNA Analysis Facility (New Haven, CT) for fragment analysis using an automated DNA sequencer (3730xl 96-capillary genetic analyzer; Applied Biosytems, Foster City, CA). Peaks were scored manually using Peak scanner version 2.0 software (Applied Biosystems) and raw scores sorted into allelic bins.

Family assignment

Parental assignments (both maternity and paternity) were conducted for all genotyped juveniles (n = 1,024). Genotypes of the progeny were compared to those of the broodstock candidate parents using CERVUS version 3.0.7 software (Marshall et al. 1998). CERVUS calculates the log-likelihood of each candidate parent being the true parent and uses simulations to determine the level of confidence in the parentage assignment. A genotyping error rate of 1%

was assumed and the proportion of parents sampled was 100%. For offspring with parental assignment confidence < 80%, an additional sibship analysis was conducted using the maximum likelihood method implemented in COLONY version 2.0.6.3 (Jones and Wang 2010). COLONY can determine the number of parents for a set of progeny for which not all parents have been sampled and it can use these estimated parental genotypes to reconstruct sibship groups. Results from the two methods were cross referenced and in the event of inconsistency across methods the genotypes were manually compared for mismatches as a final quality control measure to ensure confidence in assignments.

Growth performance calculations and statistical analyses

To evaluate the influence of sire strain on growth performance (paternal effect), two growth metrics– final weight, and body shape – were compared among fish assigned to the five different sires. Final weight and total body length were measured on replicate tanks when fish attained average market size (~680). Condition factor (K) was calculated to quantify body shape using the formula: $(W/(L\text{^}3) * 10^4)$; where W is weight and L is total length. One-way ANOVA was used to test for differences in these two metrics among fish sired by the five different strains. Tukey's pairwise comparison of means was applied if the ANOVA omnibus test indicated a significant ($P < 0.05$) difference among sire strain.

An additional linear mixed-model analysis (REML) was used to test for differences among final weights in the ungraded group and calculate components of variance. Families and strains were reared communally such that representation was unknown until measurements and fin clips were collected at market size. Therefore, variance estimates due to family could only be performed on final weights when the parentage of individual hybrids were assigned by genotype. Strain was treated as a fixed effect while sire nested within strain and dam were included as

random effects to describe influence of paternal and maternal effects beyond that of the sire strain. Analyses were performed in JMP (13).

Results

Viable fry from 11 of 12 female white bass ranged from 5,797 to 82,143 (25,779 ± 6814, Mean ± SEM). Fertilization prior to embryo pooling did not indicate any differences in sperm quality among the males (N=18; data not shown). Of the 53 hybrid crosses stocked into recirculating systems, survival was > 95% in all size grades with no differences among families. Large, small and ungraded treatments took approximately 12, 14 and 17 months, respectively, to attain market size in recirculating systems (Table 2). Final weights differed among strains in the ungraded group, such that FL hybrids (850.1 ± 269.9 g) were larger than all other strains ($p=0.0001$; Table 2). There were no differences in final weights of hybrids sired by the different strains for the small and large graded groups. Condition factor (K) differed among strains and FL were the deepest bodied (~1.8) in the small and ungraded treatments. VA males consistently sired the most streamlined (≤1.6) hybrids across all size grades.

Bacterial cultures from skin lesions and kidney swabs confirmed Motile Aeromonas Septicemia (*Aeromonas sp.)* as the opportunistic disease post-experimental stocking. Equal proportions of dead and symptomatic fish were found among strains during the early mortality event, except only 1 FL (symptomatic) fish was found (Table 4).

The large graded treatment maintained in outdoor tanks required 15 months to attain market size and the DOM strain fish were significantly larger (697.3 ± 110.3 g) than the other strains (Table 3). The DOM fish had highly variable growth performance and this strain produced both the smallest (343 g) and largest (951 g) individuals (Table 3). FL sires produced deeper bodied individuals (1.60 ± 0.08) compared to VA, SC and TX ($p<0.0001$), but similar to

DOM (1.55 ± 0.08; Table 3). Random effects of dam (p=0.3783) and sire nested within strain (p=0.1002) did not have a significant influence on final weight and accounted for only 1.7% and 7.7% of the total variance, respectively.

Table 2
Mean (± SD) final weight (g), minimum individual weight (g), maximum individual weight (g), and condition factor (K) of three HSB size grades produced by five different sire strains and grown in recirculating aquaculture systems. Different letters denote significant differences among strains and within size grades (ANOVA and Tukey's, $p<0.05$).

Strain	N	Weight ± SD (g)	Min (g)	Max (g)	K ± SD
Ungraded (~17 months to market size)					
DOM	80	689.6 ± 273.3^{B}	207.0	1322.3	1.58 ± 0.22^{C}
FL	90	850.1 ± 269.9^{A}	288.7	1403.6	1.81 ± 0.14^{A}
SC	155	729.4 ± 253.8^{B}	221.7	1363.2	1.65 ± 0.19^{B}
TX	160	765.9 ± 246.9^{AB}	200.6	1474.0	1.64 ± 0.17^{BC}
VA	122	729.1 ± 216.1^{B}	237.3	1336.2	1.60 ± 0.17^{BC}
Small Grade (~14 months to market size)					
DOM	20	675.4 ± 227.5^{A}	98.7	1055.4	1.66 ± 0.23^{AB}
FL	12	759.4 ± 151.8^{A}	382.1	933.9	1.80 ± 0.16^{A}
SC	26	636.6 ± 175.0^{A}	270.0	975.4	1.65 ± 0.13^{B}
TX	25	659.4 ± 92.7^{A}	543.2	840.0	1.64 ± 0.11^{B}
VA	35	613.3 ± 122.1^{A}	396.8	864.9	1.59 ± 0.11^{B}
Large Grade (~12 months to market size)					
DOM	31	670.4 ± 148.3^{A}	355.1	904.0	1.62 ± 0.14^{A}
FL	8	579.2 ± 80.9^{A}	495.0	691.7	1.64 ± 0.09^{A}
SC	12	616.5 ± 117.1^{A}	475.9	869.2	1.62 ± 0.09^{A}
TX	38	642.6 ± 105.6^{A}	443.0	845.9	1.59 ± 0.08^{A}
VA	30	591.1 ± 113.5^{A}	317.5	769.2	1.47 ± 0.08^{B}

Table 3

Mean (± SD) final weight (g), minimum individual weight (g), maximum individual weight (g), and condition factor (K) of large grade HSB grown in outdoor tanks. Different letters denote significant differences among strains and treatments (ANOVA and Tukey's, $p<0.05$)

Strain	N	Weight ± SD (g)	Min (g)	Max (g)	K ± SD
Large Grade (~15 months to market size)					
DOM	72	697.3 ± 110.3^{A}	343	951	1.55 ± 0.08^{AB}
FL	14	585.4 ± 76.3^{B}	463	704	1.60 ± 0.08^{A}
SC	18	618.1 ± 138.1^{B}	449	921	1.51 ± 0.08^{BC}
TX	32	611.8 ± 95.2^{B}	388	790	1.48 ± 0.10^{C}
VA	44	582.5 ± 75.5^{B}	423	768	1.45 ± 0.09^{C}

Table 4

Mortalities and symptomatic individuals identified by genotype during the *Aeromonas* outbreak in ungraded hybrids. No statistical comparisons performed.

Strain	Mortalities	Symptomatic
DOM	6	3
FL	0	1
SC	11	5
TX	5	9
VA	8	12

Discussion

The results of this study demonstrated that HSB growth is influenced by sire strain, culture environment and grading strategies. Large grade hybrids attained market size sooner than small and ungraded fish, and the shorter production cycle of large grade fish favored the domestic strain (DOM) hybrids in outdoor tanks but not significantly so in recirculating systems ($p = 0.0772$). In treatments that required longer to achieve market size (small and ungraded), wild sired HSB generally grew larger and displayed less variation among individual final weights.

Growth performance of the parental strains used to produce sunshine bass in this study was previously documented (Kenter et al. 2018). In that study, the DOM striped bass grew faster and had lower feed conversion ratios than most wild strains up to one year of age, but did not maintain their growth advantage in the second year. The findings of the current study were consistent with those of Kenter et al. (2018), as the DOM fish were generally larger in the treatment requiring the shortest durations to market size (large grade), but longer grow-out showed wild-strain hybrids to achieve greater final weights beyond 14 months of culture. The growth rates of both the hybrids and the parental striped bass strains that were used as sires were similar (~ 500 g at one year of age) when cultured in the same recirculating systems (Kenter et al. 2018). As striped bass were grown at a slightly lower temperature than the hybrids (20-21 vs. 22-23 °C) but still achieved similar growth rates, their comparative value as a commercial aquaculture species should be re-evaluated in common garden growth trials.

As with growth, the overall trends in condition factor (K) among hybrids were similar to those found in pure striped bass strains, with FL producing the deepest bodied individuals, VA the most streamlined and SC, TX and DOM, often intermediate between them. The trends in

final weight and K observed among hybrid offspring were highly similar to the previously described sire's strain of origin (Kenter et al. 2018), suggesting a genetic effect of strain. The mixed model analysis supports this contention with little contribution of individual dams or sires within strains to the variance among final weights. Additionally, the breeding design used in the present study successfully controlled for dam effects by crossing multiple males with the same female producing half sibling families. These results contribute to previous work with hybrid that suggested stronger dam effects compared to sire on final weight, K and dressout parameters (Kohler et al. 2001; Wang et al. 2007), though the sires used in those studies were of unknown origins with no history of performance traits. Although individual growth history of dams was unknown in the current study, they were selected based on a common genetic background and size.

Commercial hybrid production generally requires 16-18 months in outdoor production systems and about 12 months in recirculating systems (Rudacille and Kohler 2000) which is similar to the times required in the current study. Large, small, and ungraded fish attained market size in about 12, 14, and 17 months of age, respectively, in freshwater, 22-23 °C recirculating systems. This temperature was selected to ensure optimal water quality, feed conversion and health but is below what has been shown to be optimal for growth (25-27 °C; Hodson 1990; Woiwode and Adelman 1991). This suggests that further reductions in production time are possible in optimally performing, recirculating systems. Evidence for genotype x environment interactions were observed in the large grade hybrids between production systems. DOM-sired hybrids were larger than wild strain-sired hybrids grown in the outdoor systems, suggesting that directional selection may be favorable in particular culture systems or environments (Gjedrem et al. 2005). Small grade fish grown in recirculating systems attained

market size about 2 months later than the large grade, which would likely correspond to 3-4 months in outdoor systems. Despite growth to a marketable size, economic evaluations must be conducted to determine if the increased costs associated with extended grow-out (e.g. feed, increased mortality) would be cost-effective on a commercial basis with conventional pond culture. The ungraded fish required the longest to attain average market size (~ 17 months) possibly due to feed competition or a combination of social factors. These findings in the difference in growth performance among size grades and ungraded fish support the continued practice of size grading during commercial farm operations (Potthoff and Christman 2006; D'Abramo and Frinsko 2008).

A primary goal in HSB improvement has been to reduce inherent size variation among offspring through domestication of parental species (Woods, 2001; Garber and Sullivan, 2006). Currently, the highest prices are received for uniform fish (~680 g;1.5 lb) live or whole on ice, while oversized fish are processed for fillets and command lower prices (Carlberg et al. 2005; Garber and Sullivan 2006**)**. DOM-sired hybrids displayed the highest ranges in final weight and body shape compared to those sired by first generation captive wild strains, which may be attributable to the high levels of genotypic variation currently present in the DOM striped bass line. The DOM fish used here are 5^{th} generation captive bred and 2 generations removed from collapse of 400 different genetic crosses representing 6 geographic strains of wild striped bass collected from the Atlantic Coast (Reading et al. 2018). This study should be replicated with future generations of DOM striped bass to further assess progress in selective breeding to produce superior hybrid striped bass and striped bass cultivars

Finally, the relative absence of FL hybrids displaying symptoms of furunculosis may suggest inherited disease resistance, and larger, controlled, studies are warranted to investigate

this as a selection trait in broodstock development programs. Additional future research should determine feed conversion ratios among strains and if the observed differences in K correlate with dressout traits that were not investigated in this study.

CHAPTER 4

TRANSPORT STRESS MITIGATION AND THE EFFECTS OF PRE-ANESTHESIA ON STRIPED BASS (*Morone saxatilis)*

Introduction

Fish handling during routine aquaculture practices such as grading, tagging and transport may impose varying levels of stress that can lead to undesirable physiological consequences (Barton and Iwana 1991). Stress responses in fishes, as well as other vertebrates, have been well studied and include activation of the hypothalamic-pituitary-interrenal axis that results in release of glucocorticoids (Mommsen et al. 1999). The inability of a fish to cope following a severe stressor may lead to decreased growth rate, immunosuppression, impaired reproductive capacity or death (Wendelaar Bonga 1997; Barton 2002). To mitigate stress, anesthesia has become a common practice in aquaculture, but responses to specific anesthetic agents vary widely among species, life stages and environmental conditions (Zahl et al. 2012).

During human and veterinary medical procedures, combinations of anesthetics agents, that differ in their modes of action (sedation, immobilization, analgesia), may be used to provide more effective results than single agents alone (Zahl et al. 2012). Despite a suite of anesthetics available for aquaculture species, researchers often rely on single anesthetic compounds due to limited information available about species-specific, synergistic efficacy and regulatory restrictions. Tricaine methanesulfonate (MS-222) and metomidate hydrochloride (Aquacalm^{TM}), are two anesthetics commonly used in aquaculture for different scenarios requiring anesthesia. MS-222, the only anesthetic currently legally available in the US for foodfish, is used in a variety of situations and is effective in inducing paralysis and blocking nociception. Adverse effects of this anesthetic, however, include reducing osmoregulatory function and exacerbated cortisol

release (Carter et al. 2011). Metomidate is a fast acting, nonbarbituate, hypnotic that is effective in inducing sedation and suppressing cortisol, but has limited analgesic properties (Olsen et al. 1995; Small 2003; Davis and Griffin 2004). The efficacy of these anesthetics used alone has been investigated for many commercially important fishes, but further study is required to fully understand their potential under different environmental conditions, with different stressors, and in combination with other agents.

Striped Bass have been well studied and widely cultured as a food and gamefish, but their relatively high sensitivity to stressors in captivity compared to hybrid Striped Bass (Striped Bass *M. saxatilis* x White Bass *M. chrysops*) has limited their use for commercial production (Noga et al. 1994; Reubush and Heath 1997). Efforts have been made to mitigate the detrimental effects of stress in Striped Bass with the use of salts, anesthesia, electroanesthetia and alterations in water hardness (Gilderhus et al. 1991; Mazik et al. 1991; Harrell 1992; Lemm 1993; Jenning and Looney 1998; Woods et al. 2008). While much of this research has focused on optimizing doses of specific anesthetics and improving immediate post-stress survival, less attention has been focused on correlating acute stressors with subsequent fish behavior/performance or determining the synergistic effects of combined anesthetics.

The purpose of the present study was to investigate the use of MS-222 and metomidate during common aquaculture stressors on market-size Striped Bass. In the first experiment, stress responsiveness of wild and domesticated Striped Bass strains was compared following transportation with, and without, the addition of low doses of anesthetics and/or salt. The second experiment examined the potential benefits of "pre-anesthetizing" fish prior to netting and handling.

Methods

Fish Acquisition

Striped Bass were bred in captivity during their respective spawning seasons at the Welaka National Fish Hatchery (Welaka, Florida) and North Carolina State University (NCSU) Pamlico Aquaculture Field Laboratory (Aurora, NC). The Florida fish were produced from wild-caught, Gulf of Mexico broodstock and the domesticated line was initially produced from hundreds of outcrosses of six distinct Striped Bass strains (Canada, Pacific Ocean, Roanoke River, Chesapeake Bay, Santee-Cooper Reservoir, and Florida-Gulf of Mexico) and mass selected for performance traits for ~8-9 generations. Fingerlings (<2 g) were transported to the Ritzman Aquaculture Laboratory (University of New Hampshire (UNH), Durham, NH, USA) and cultured by strain, in 1500 L grow-out tanks (20-21°C, 0 ppt salinity and 12:12 photoperiod), that were incorporated into recirculating systems, for one year. At that time, strains were removed from their respective tanks, implanted with PIT tags and transferred into 5000 L tanks which were part of a larger recirculating system (20-21°C and 10 ppt). Fish were cultured for an additional two months until outdoor trials were conducted (July).

Transportation Experiment

Twelve individuals from each strain (Mean ± SEM = 1,061.1 ± 75.6 g) were quickly netted, identified by PIT tag and stocked into a pair of adjacent 1500 L staging tanks (6 fish/strain/tank, 12 fish total) with matching water quality parameters. After a 48 hr recovery period without feeding, fish from both tanks were netted and moved to separate, insulated, 311 L transport tanks (Bonar Plastics, Chicago, IL, USA) on the bed of a full-size pickup truck. The two transport tanks were each initially filled halfway with 150 L of 0 ppt water at 20-21°C and either remained as such or were modified to one of the following additional treatments prior to

loading the fish: brackish (10 ppt) water with no anesthetic, fresh (0 ppt) or brackish (10 ppt) water with metomidate hydrochloride (1 mg/L, Aquacalm^{TM}, Western Chemical, Ferndale, WA, USA), or buffered tricaine methanesulfonate (25 mg/L, MS-222, Tricaine-S, Western Chemical, Ferndale, WA). Salinity levels were raised with the addition of fine-ground sodium chloride (Hi-Grade, Cargill, Minneapolis, MN, USA).

Anesthetic doses were determined in preliminary trials with fish not included in the study. Concentrations of MS-222 and metomidate were selected to induce stage I anesthesia (Zahl et al. 2012) where respiratory rates decreased and swimming activity ceased, but fish maintained equilibrium in the water column.

The fish were moved from staging tanks to the transport tanks in less than two minutes, covered, and transported at the same time of day (10:00; to standardize diurnal cortisol patterns), under similar weather conditions. Each transportation trial was conducted by the same individual, along the same route, that consisted of 15 mins of driving, followed by a 15 min stationary pause, which was repeated, for one hour total transportation. Total transport time was determined based on a preliminary time course trial (data not shown) which demonstrated cortisol levels peaking in magnitude at one hour post stressor in both strains of Striped Bass. During stationary pauses, fish behavior was observed and dissolved oxygen levels were monitored and maintained (7-10 mg/L) through slightly ajar covers without disturbing the fish. Temperature and pH were recorded at the beginning and end of each trial but remained stable throughout the study. Upon completion of the trials and arrival at the Aquaculture Research Center (ARC, UNH), the transport tanks were opened, and three random fish/strain from each treatment were stunned by a blow to the head, bled from caudal vessels using heparinized syringes and euthanized with an overdose of buffered MS-222 (150 ppm). Blood was transferred

to heparinized, 1.5 ml Eppendorf tubes containing 70 µl Aprotonin (Sigma Aldrich, St. Louis, MO, USA), transported to the laboratory on ice, and centrifuged 8,000 × g for 10 min at 4°C. The plasma was stored at -70°C for future cortisol analysis.

The remaining three fish/strain were stocked into a 750 L tank which was part of a nine tank recirculating system maintained at 20-21°C and 0 ppt salinity. After 24 hrs, the fish were hand fed daily (Skretting, Europa; St. Andrew, NS, Canada; 50% minimum (min.) crude protein and 18% min. crude fat) to apparent satiation for seven days to monitor survival, feed consumption, relative health, and behavior. Three replicates were conducted for each transportation treatment (six fish/strain/tank, 36 fish total/treatment).

Pre-Anesthesia experiment

Cortisol levels were measured in FL strain fish anesthetized with a single anesthetic compound, or two compounds in succession. To observe the effects of single anesthetic compounds, two pairs of fish were moved from the 5000 L community tank into 1500 L staging tanks maintained at 20-21°C and 10 ppt salinity, as above. After a 48 hr recovery period, the fish were netted and transferred to 150 L baths containing either metomidate (5 ppm) or buffered tricaine (MS-222, 150 ppm) and supplemental aeration. Induction times (Stage III-1: loss of equilibrium, slowed ventilation rate, reaction to strong tactile stimuli; Zahl et al. 2012) were recorded. To determine the depth of anesthesia and tactile responsiveness, the anesthetized fish were placed flat on a measuring board for 30 s, a sharp flick was applied to the ventral caudal peduncle, and the presence or absence of a reflex response was recorded. The fish were then carefully transferred to a static, 750 L recovery bath containing fresh water and supplemental oxygen and recovery time to pre-anesthetized conditions (Stage 0: normal swimming behavior; Zahl et al. 2012) was recorded. After one hour in the recovery bath, the fish were quickly netted

and euthanized for blood samples as previously described to measure magnitude in cortisol response. The process was repeated three times for each anesthetic agent (two fish/replicate, six fish total/anesthetic).

A similar protocol was followed to test the effectiveness of combinations of anesthetic agents using pre-anesthetic sedation. Groups of three fish were moved from the 5000 L community tank into 1500 L staging tanks, as above, and allowed to recover for 48 hrs before experimental treatments were applied. Water flow to the tanks was stopped and a low, pre-anesthetic dose of Metomidate (1 ppm) or MS-222 (25 ppm) was added directly to the culture tank without disturbing the fish. After 15 min of exposure, when normal swimming activity ceased and ventilation rates were slowed, the three individuals were transferred directly to the 150 L bath containing the final concentrations of either metomidate (5 ppm) or buffered MS-222 (150 ppm). Stage III-1 induction times were again monitored, reflex reactions checked and recovery to stage 0 in the 750 L bath recorded. Fish remained in the recovery bath for one hour prior to euthanasia and bleeding for cortisol analysis.

Cortisol Analysis

Plasma cortisol levels were measured using a direct enzyme linked immunosorbent assay (ELISA), following protocols adapted from the Smithsonian's National Zoological Park endocrine workbook (Brown et al. 2003). The assays were validated for parallelism using pooled plasma from more than ten individuals before any comparisons among experimental samples were made. Intra-and inter- assay coefficients of variation were 4.01% ± 1.35% and 7.67% ± 0.60%, respectively (Mean ± SEM). The lowest and highest detectable limits of the assay were 3.9 and 1000 pg, respectively.

The assay performance was monitored with duplicate pool samples included on each 96 well plate. Experimental plasma samples (50 µl) were double-extracted using 1.0 mL of diethyl ether, dried at 37°C in a water bath, stored at –20°C, and reconstituted in 400 µl of assay buffer (0.1 M phosphate, 0.15 M NaCl, 1 mL/L bovine serum albumin, pH 7.0). NUNC 96-well Maxisorp plates (Thermo Fisher Scientific, Waltham, Massachusetts) were coated with 1:8500-diluted rabbit anticortisol antibody (University of California, Davis, California) and incubated overnight at 4°C. Plates were washed with 0.15M NaCl and 0.5 mL/L Tween 20 in a microplate washer (Thermo Labsystems, OPSYS MW; Thermo Fisher Scientific) and blotted dry. Samples, cortisol standards (0.078–20 ng/mL; Sigma Aldrich), and cortisol–horseradish peroxidase conjugate (University of California Davis) were added and incubated for 1hr at room temperature. Plates were again washed, incubated with substrate buffer [0.05 M citric acid, 6.0 mM H2O2, 0.4 mM 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), pH 4.0] for 15 min with gentle shaking, and absorbance was read at 405 and 620 nm with a Synergy 2 Plate Reader (BioTek, Winooski, Vermont). Background absorbance (620 nm) was subtracted from readings at 405 nm prior to analyses, and cortisol standard curves were generated using a five-parameter logistic model in GEN 5 software (BioTek).

Statistical Analysis

Comparisons among all treatments were performed using one way ANOVA and Tukey's pairwise comparison of means was applied if significant differences were detected ($P<0.05$). Differences between strains in the simulated transport experiment were determined by a Student's t-test.

Results

Transportation experiment

After one hour of transport, plasma cortisol levels were similar between strains (Figure 1; $P=0.4547$) but significant differences were found among treatments (Figure 2; $P<0.0001$). Cortisol levels following transport with MS-222 in fresh and brackish water (94.96 ± 12.9 and 68.59 ± 9.3 ng/ml) did not differ from each other or from levels following transport in freshwater alone (79.13 ± 12.3 ng/ml). The use of metomidate was consistently effective in reducing the cortisol responsiveness as compared to MS-222 and freshwater alone. Cortisol levels following transport in brackish water (45.34 ± 6.3 ng/ml) were similar to most other treatments but were lower compared to MS-222 in freshwater (Figure 2).

Survival rates were all 100% and no observations of ill-health were detected within seven days of the transportation treatments. Feed consumption was highly variable within treatments and no significant differences were detected at one, three or seven days post-transport (Table 1).

Pre-anesthesia experiment

The addition of a pre-anesthetic dose directly to the culture tank followed by the same agent in a bath produced no reduction in circulating cortisol compared to directly transferring the fish without pre-anesthesia (Figure 3). The treatments using metomidate alone (4.6 ± 1.1 ng/ml) were found to significantly reduce plasma cortisol compared to those using MS-222 alone (37.6 ± 7.1 ng/ml) and metomidate pre-anesthesia followed by MS-222 (45.7 ± 8.3 ng/ml). MS-222 pre-anesthesia followed by metomidate resulted decreased cortisol levels relative to pre-treatment with MS-222 or Metomidate followed by MS-222 (Figure 3; $P<0.05$).

Stage III-1 induction and stage 0 recovery times generally increased with exposure to metomidate. The longest anesthesia induction and recovery times observed were for MS-222

pre-anesthesia followed by metomidate (302 ± 8 and 606 ± 13 s respectively) and metomidate pre-anesthesia followed by a metomidate (288 ± 32 and 663 ± 6 s; P<0.0001). Anesthesia with MS-222 alone produced the shortest induction and recovery times (97 ± 16 and 154 ± 19 s) and tactile responsiveness was absent. The response to stimuli was completely absent when fish were exposed to MS-222 at either point in the anesthesia process (Table 2).

Figure 1
Mean (± SEM) plasma cortisol concentrations between FL and Domestic strains with transportation treatments grouped. T-test (P= 0.4547).

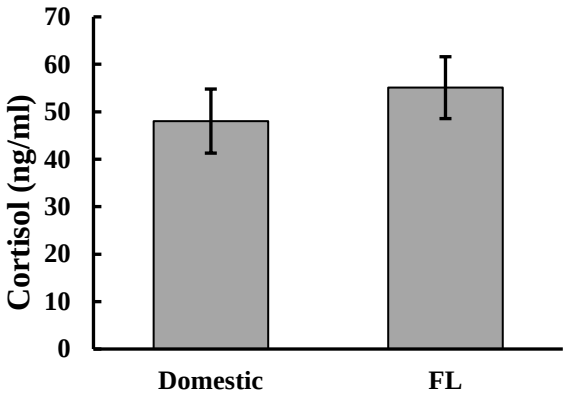


Fig. 2
Mean (± SEM) plasma cortisol concentrations one hour post-transport. Different letters denote significant differences among treatments (P < 0.05).

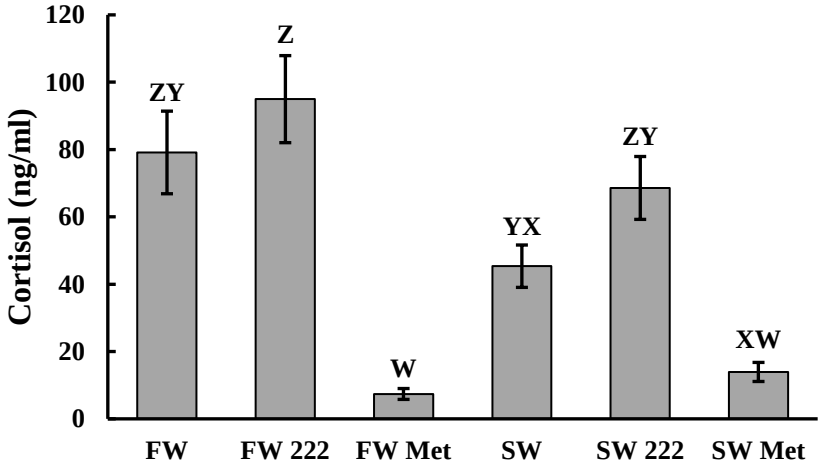


Table 1
Feed consumption (grams) at 1, 3 and 7 days post-transport (Mean ± SEM). No significant differences were found among treatments (P > 0.05).

Treatment	1 day	3 day	7 day
FW	0.5 ± 0.5	12.8 ± 8	123.8 ± 25.6
FW 222	0	22.5 ± 11	117.75 ± 19.8
FW Met	4 ± 2	46.8 ± 13.8	131.75 ± 44.8
SW	0	6.3 ± 6.3	89 ± 23
SW 222	1.8 ± 1.8	22.3 ± 15.9	84.5 ± 41
SW Met	0.5 ± 0.5	36.5 ± 25	153.75 ± 29.8

Fig. 3
Mean (± SEM) plasma cortisol concentrations one hour post-anesthetic treatment. Different letters denote significant differences among treatments (P < 0.05).

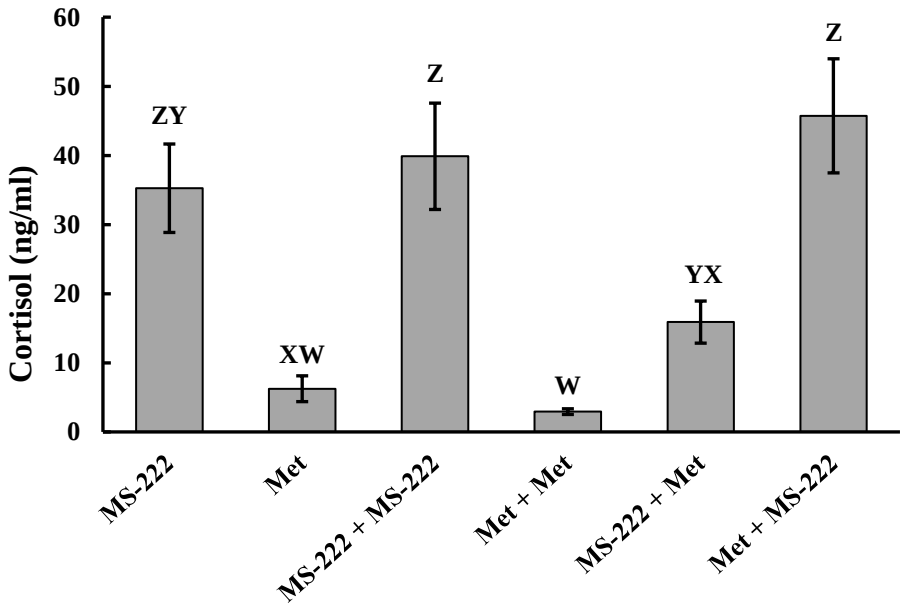


Table 2
Anesthetic treatment, induction and recovery times (seconds), and response to stimuli (caudal peduncle flick). Means (± SEM) followed by different letters denote significant differences among treatments ($P < 0.05$).

Treatment	Induction Time (s)	Recovery Time (s)	Response
MS-222	97 ± 16 z	154 ± 19 z	-
Met	186 ± 17 y	336 ± 10 y	+
Met + MS-222	132 ± 15 zy	192 ± 12 z	-
MS-222 + Met	302 ± 8 x	606 ± 13 x	-
MS-222 + MS-222	108 ± 7 zy	415 ± 43 y	-
Met + Met	288 ± 32 x	663 ± 6 x	+

Discussion

In contrast to fishes, the practice of anesthesia is well understood in modern human and veterinary medicine where administration protocols are specifically tailored to the individual based on size and level of anesthesia/analgesia required (Zahl et al. 2012). The species diversity and range of environmental conditions encountered in different aquaculture operations, however, requires an understanding of species and life stage-specific anesthesia dose requirements, to attain practical induction and recovery times (Mattson and Riple 1989; Keene et al. 1998; Mylonas et al 2005). Only somewhat recently have protocols been developed that combine drugs of different properties to achieve more complete anesthesia than can be achieved with a single compound. Drug combinations and environmental manipulations may potentiate each other, thereby reducing dosage requirements, shortening induction times and synergistically preventing stress or pain responses (Zahl et al. 2009; Zahl et al. 2011; Sneddon 2012).

In the current study, the efficacy of MS-222 and metomidate, administered alone or in combination, were evaluated for their ability to mitigate cortisol stress responsiveness and pain during transport and handling. MS-222 and metomidate differ in their mechanism of actions and

elicit different physiological responses. MS-222 acts by blocking voltage-gated sodium channels and thereby prevents the propagation of action potential in excitable cell types (Frazier and Narahashi 1975; Carmichael 1985; Burka et al. 1987). The rapid inhibition of nerve function results in the predictable stages of anesthesia including loss of equilibrium, paralysis and nociception obstruction (Rang et al. 2003). In contrast, Aquacalm^{TM} stimulates the activity of inhibitory gamma-aminobutyric acid type A ($GABA_A$) receptors, modulates inhibitory gamma-aminobutyric acid type A receptors (Grasshoff et al. 2006), and permits immobilization at higher dosages. The drug has questionable analgesic properties but has the potential to mitigate stress during transport due to its ability to suppress cortisol synthesis at low concentrations (Olsen et al. 1995; Davis and Griffin 2004; King et al. 2005; Berlinsky et al. 2016).

Previous studies with Striped Bass and hybrid Striped Bass examined the effectiveness of a suite of anesthetics and salt treatments (5 and 10 ppt) on mitigating cortisol stress responses associated with capture, transportation and handling (Davis et al. 1982, Harrell and Moline 1992, Harrell 1992, Davis and Griffin 2004). These studies demonstrated that, with the exception of Metomidate, all salt and anesthetic treatments result in elevated cortisol following administration of the stressors. Furthermore, some of the anesthesia treatments (e.g. MS-222) exacerbated cortisol release (Harrell 1992). Our results are consistent with these previous studies and transporting fish in the presence of MS-222 or adding the anesthetic to the culture water prior to netting, both resulted in elevated cortisol. The addition of Metomidate prior to, or immediately after, netting, however, did not elevate cortisol. Also, consistent with previous studies, MS-222 was effective in blocking nociception and a combination of both anesthetics blocked both nociception and cortisol synthesis. Since no attempts were made to minimize effective doses, the induction and recovery times for the combined drugs were longer than those of the individually

administered compounds. Further research should focus on optimizing the combined treatments to minimize effective dosages.

Despite the acute stressors applied during these studies, fish resumed feeding 48-72 hrs post-stressor and no adverse health effects were observed within seven days after the experimental treatments. The immunosuppressive effects of cortisol are well documented (Barton et al. 1987; Pickering and Pottinger 1989), but cortisol is also adaptive and may not reduce immunocompetency following a short-term acute stressor (Davis and Griffin 2004). It has been suggested that a transient, relatively small cortisol release, due to stress, may actually be beneficial to survival, and that cortisol suppression by metomidate may impair surgical or post-stress recovery (Ledingham and Watt 1983; Davis and Griffin 2004). The beneficial effects of corticosteroid release may explain why no differences in cortisol response were observed between Striped Bass domesticated for many generations and those spawned from wild-caught fish. The benefits of cortisol suppression by metomidate may lie in the expected duration and magnitude of the stressor, and its use, where permissible, may be beneficial in appropriate circumstances.

CHAPTER 5

STRESS, GROWTH AND IMMUNE INTERACTIONS IN FOUR STRAINS OF STRIPED BASS (*Morone saxatilis)*

Introduction

Finfish aquaculture requires routine practices such as handling, grading, transport and general system maintenance, which may induce stress and lead to suboptimal production. The physiological stress response is defined as a state of threatened homeostasis which must be re-established by a complex suite of adaptive responses (Chrousos 1998). Stress responses are under neuroendocrine control and divert an animal's energy reserves away from normal bodily functions such as growth, immune system maintenance, and reproduction and directs them towards coping with the stressor (Pickering 1993). If the adaptive response is successful, a return to pre-stressed conditions (homeostasis) is achieved, but if the stressor is too severe or long-lasting, the coping mechanisms become maladaptive and detrimental to the fish's performance (Barton and Iwama 1991; Barton 2002).

The stress response is categorized into three distinct phases collectively known as the General Adaptation Syndrome (GAS; Selye 1950, 1973). The primary response, or alarm reaction, is the neural activation of the hypothalamus-pituitary-interrenal gland (HPI) axis and release of catecholamines (adrenaline and noradrenaline) followed by production of the glucocorticoid, cortisol (Wendelaar Bonga 1997; Mommsen et al. 1999). Cortisol is the major corticosteroid in teleosts and has been the focus of aquaculture research for decades, due to its predictive responsiveness following acute stress and relative ease of measurement (Barton and Iwama 1991). Following the primary response, the secondary, or resistance stage, includes adjustments by the organism to regain homeostasis through metabolic, hematological and

immunological changes (Mommsen et al. 1999). The tertiary stage is comprised of whole body responses in growth, disease resistance, behavior and ultimately survival (Barton 2002). A better understanding of the mechanisms involved in the stress response is a priority for aquaculturists and necessary for selective breeding programs.

Growth performance differences have been reported among populations, or strains within a species, for commonly cultured fish including Atlantic salmon (*Salmo salar*)(Gunnes and Gjedrem 1978), Nile tilapia (*Oreochromis niloticus*)(Eknath et al. 1993), common carp (*Cyprinus carpio*) (Bakos and Gorda 1995) and more recently striped bass (*Morone saxatilis*)(Jacobs et al. 1999; Woods 2001; Kenter et al. 2018). Once identified, phenotypes including growth can be selected to create domesticated lines with improved production. Although many studies have focused on growth as a selection trait, very few have compared stress responsiveness in broodstock development. The magnitude of the primary cortisol response has been shown to be highly heritable and used to create high or low responder lines of rainbow trout (*Oncorhynchus mykiss)* (Pottinger and Carrick 1999; Fevolden et al. 2002). Inconsistent correlations between high and low cortisol responsiveness and growth have been observed and recent attention has focused on a more complex selection trait termed "robustness" (Sadoul et al. 2015). This complex trait, first described in pigs, combines high production potential with resilience to stressors in a wide variety of environmental conditions (Knap 2005).

The aim of the present study was to characterize the stress response among three wild striped bass strains and a 5th generation domestic strain, mass selected for general aquaculture performance (growth rate and body conformation). The wild strains were chosen based on differences in life history, geographic location and unpublished behavioral differences previously observed during growth trials (Kenter et al. 2018). Parameters of interest were monitored across

the primary (cortisol), secondary (glucose, hematological counts, hepatic gene expression) and tertiary (growth, survival) stages, to compare strain responses to a repeated, acute stressor.

Materials and Methods

Fish and Experimental Design

Fingerling striped bass (0.5-4.0 g), bred in captivity during the natural (spring) spawning seasons at federal, state and university hatcheries in Florida (FL), Virginia (VA) Nova Scotia (CAN) and North Carolina (DOM) were transported to the Ritzman Aquaculture Laboratory ((University of New Hampshire (UNH), Durham, NH, USA)). Wild striped bass broodstock (FL, VA) were caught from the spawning grounds except the CAN broodstock that were wild-captured as juveniles and raised in captivity. The DOM striped bass were produced at North Carolina State University's (NCSU) Pamlico Aquaculture Field Laboratory (Aurora, NC) as a part of the National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry. Juveniles were initially maintained by strain and grown to a suitable market size (902.4 ± 21.2 g; Mean ± SEM) in separate 1500 L tanks that were constructed into independent, three-tank recirculating systems (Kenter et al. 2018). All systems were equipped with screen filters, bead filters, biological filters, protein skimmers, ultra violet (UV) sterilizers and temperature control.

Stress experiments were conducted in the UNH Aquaculture Research Center in an indoor, recirculating system comprised of six 1500 L tanks and filtration components described above. Each tank housed six fish/strain (24 fish/tank) that were weighed and PIT- tagged for individual identification. Fish were allowed two weeks of acclimation to the system's photoperiod (12 hr light: 12 hr dark), temperature (20-21°C) and salinity (10 ppt) prior to the

onset of experimental treatments. All research followed UNH Institutional Animal Care and Use Committee guidelines.

Three tanks were left undisturbed (control group) throughout the entirety of a 14 week trial and the other three (stressed group) were exposed to a repeated, acute stressor: 1 min of vigorous chasing with a hand held dip net. Chasing events took place on Mondays and Thursdays at a consistent time of day (12:45) to account for diurnal rhythms in stress or growth signals. Fish in all tanks were fed to satiation twice daily with a 9 mm Skretting, Europa marine finfish diet (50% min. crude protein and 18% min. crude fat) every day except Saturdays. Feed amounts were recorded daily to track consumption rates between treatment groups.

Sampling procedures

At the conclusion of the 14 week trial, one tank from each treatment was randomly selected for baseline cortisol measurements (t = 0 hr). To inhibit cortisol release in the fish during netting, 5 mg L^{-1} Metomidate hydrochloride (Aquacalm^{TM}, Syndel USA, Ferndale, Washington) was first added to each tank (Kenter et al. 2019). Anesthetized fish were then euthanized by cranial percussion, and blood was collected (2 ml) from caudal vessels using heparin-containing syringes. An aliquot (1 ml) was transferred to heparinized, 1.5 ml tubes on ice containing 70 µl Aprotonin (Sigma Aldrich, St. Louis, MO, USA), transported to the laboratory, and centrifuged 8,000 × g for 10 min at 4 °C. Resulting plasma was stored at -20 °C for later cortisol and glucose analyses. Remaining blood (1 ml) was transferred into a MiniCollect® tube (Greiner Bio-One) pre-coated with EDTA and refrigerated for hematological comparisons. Additionally, liver samples were dissected from the same fish, submerged in sterile, 1.5 ml tubes containing RNA*Later*®, (ThermoFisher Scientific) held on dry ice and then stored at -80 °C for quantitative real-time PCR (QPCR).

Fish in the remaining two tanks in each treatment were subjected to a one-minute net chase and bled at either one (t = 1 hr) or three (t = 3 hr) hours post-stressor. Metomidate was added to the tanks prior to chasing, as described above, to inhibit cortisol release.

All fish were weighed and total length measured to calculate specific growth rates (SGR) and feed conversion ratio (FCRs). FCR was calculated by treatment and not by individual strain, since feed consumption was calculated by tank, with multiple strains grown communally. FCR was calculated using the formula: (feed intake/n)/(Wf-Wi) where n is the number of fish in a given tank. SGR was calculated using the formula ((ln(Wf)-ln(Wi))/T)*100; where T is time in days and ln (Wf) and ln(Wi) are final and initial fish weights, respectively. Condition factor (K) was used to quantify body shape differences between strains. Condition factor was calculated with the formula $((W/(L^3)*100{,}000))$; where W is weight and L is total length.

Repeated cortisol measurement study

To further characterize responsiveness among and within strains, a second study was conducted using repeated cortisol measurements and 15 individuals from each strain (DOM, FL, VA, CAN) that were not included in the above study. Strains were housed separately in four 1500 L tanks that were part of a larger recirculating system with water quality parameters described above. Fish were PIT tagged so that cortisol measurements could be made among and within strains. The fish were exposed to three, one minute net chase events at one month intervals at a consistent time of day (12:00). One mL of blood was collected from caudal vessels using heparin-containing syringes from all individuals, one hour after each stress event. Blood samples were centrifuged and resulting plasma was stored as described below for cortisol analysis.

Cortisol analysis

Plasma cortisol levels were measured using a direct enzyme linked immunosorbent assay (ELISA), following protocols adapted from the Smithsonian's National Zoological Park endocrine workbook (Brown et al. 2003) and described by Berlinsky et al. 2016. Initially, the assays were validated for parallelism using pooled plasma from more than ten individuals before comparisons among experimental samples were made. The assay quality was monitored by using duplicate pool samples included on each plate. Experimental plasma samples (50 µl) were double-extracted using 1.0 mL of diethyl ether, dried at 37 °C in a water bath, stored at –20 °C, and reconstituted in 400 µl of assay buffer (0.1 M phosphate, 0.15 M NaCl, 1 mL L^{-1} bovine serum albumin, pH 7.0). NUNC 96-well Maxisorp plates (Thermo Fisher Scientific, Waltham, Massachusetts) were coated with 1:8500-diluted rabbit anticortisol antibody (University of California, Davis, California) and incubated overnight at 4 °C. Plates were washed with 0.15 M NaCl and 0.5 mL L^{-1} Tween 20 in a microplate washer (Thermo Labsystems, OPSYS MW; Thermo Fisher Scientific) and blotted dry. Samples, cortisol standards (0.078–20 ng mL^{-1}; Sigma Aldrich), and cortisol–horseradish peroxidase conjugate (University of California Davis) were added and incubated for 1 hr at room temperature. Plates were washed again, incubated with substrate buffer [0.05 M citric acid, 6.0 mM H2O2, 0.4 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), pH 4.0] for 15 min with gentle shaking, and absorbance was read at 405 and 620 nm with a Synergy 2 Plate Reader (BioTek, Winooski, Vermont). Background absorbance (620 nm) was subtracted from readings at 405nm prior to analyses, and cortisol standard curves were generated using a five- parameter logistic model in GEN 5 software (BioTek). Intra- and interassay coefficients of variation for cortisol assays were 7.4% ± 1.4% and 7.5 ± 0.6%, respectively.

Hematological Comparisons

Immunocompetence under repeated acute stress was estimated among strains using hematological parameters. Previously refrigerated blood samples were processed within 48 hrs of collection and assessed for Hematocrit (Hct), total red blood cell count (RBC), and total white blood cell count (WBC) which included indistinguishable thrombocytes.

Hct (%) was determined by standard protocols in microhematocrit tubes after centrifugation for five minutes at 12,000 x g and read on a microcapillary reader (International Equipment Company, Boston, MA, USA). Each sample was assessed in triplicate tubes for a mean value and expressed as a percentage.

RBC and WBC were determined manually using a Neubauer hemocytometer. Briefly, blood was diluted 1:400 using Natt-Herrick's solution (Natt and Herrick 1952) and allowed 5 mins to incorporate the stain before loading into the hemocytometer. Four replicate counts were made on each sample and calculations were reported in cells ml^{-1} using the formula (Average cell number x dilution factor)/0.0001 ml.

RNA extractions and cDNA synthesis

Liver samples were removed from RNA*Later*®, blotted dry, and cut into a 0.3 cm^3 section before being added to 500 µl cold Tri Reagent (Sigma-Aldrich, St. Louis, MO, USA). The tissue was homogenized using microtubes and pestles (Fisher Scientific, Pittsburgh, PA, USA) and brought to 1.0 ml with cold Tri Reagent. Extractions were performed using standard phenol/chloroform procedures (Molecular Research Center, Cincinnati, OH, USA) with the addition of a third precipitation step, modified from Picha et al. 2008, to remove glycogen contamination. Briefly, a polyvinylpyrrolidone (PVP) solution (2% PVP, 1.4 M NaCl) was

added to the RNA samples, incubated for 10 min at room temperature, centrifuged at 15,000 x g for 15 min, and the supernatant transferred to a new tube. A 5 M LiCl solution (200 µl) was added and incubated on ice for 20 min, prior to centrifugation at 15,000 x g for 15 min at 4 °C. The supernatant was vacuum-aspirated, and the RNA pellet dissolved in sterile water. Total RNA quantity and quality were assessed using a BioTek Epoch Plate Reader with Take 3 Module and 1.0% agarose gel electrophoresis. Total RNA (2.5 µg) was DNase-treated using the Promega RQ1 RNase-free DNase kit (Promega Corp., Madison, WI, USA), and cDNA synthesis was performed using 1.8 µg of DNase-treated RNA, 2.5 µM oligo dT primer (20 mer), and 200 units of Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA).

Real time quantitative PCR (QPCR)

Stress effects on insulin-like growth factor 1 (*igf1*) and leptin (*lep*) gene expression were assessed using QPCR and previously established primer sets in striped bass (Picha et al. 2008, Won et al. 2012). Beta actin (*actb*) was also used as a reference gene (Won et al. 2012). QPCR assays were performed using a StepOne Plus Real Time PCR System and the FAST SYBR™ Green Master Mix (Applied Biosystems). Reactions consisted of 10 µl total volume, with 1.33 µl diluted cDNA template and 0.5 µM primer concentration. Each assay was run under standard cycling conditions (95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60°C for 1 min), except for reference gene *actb*, which required a 57 °C annealing temperature. All assays were followed by dissociation curve analysis and exhibited only single peak amplification. All samples were assayed in duplicate (diluted 1/40, 1/50, or 1/100, depending on assay) and triplicate relative standard curves (diluted 1/5–1/1280) were made from pooled cDNA samples represented across treatments. Optimized linear standard curves consisted of five or six points, and all assays exhibited approximately 90–110% PCR efficiency. PCR products from each assay were also

electrophoresed in 2% agarose gels to confirm amplification of the intended target, and no contamination was evident in all standard qPCR negative controls (no template and no reverse transcriptase).

Table 1
Gene symbols, primer sequences, GenBank accession numbers, product sizes (bp), annealing temperatures and literature references

Gene symbol	Primer sequence (5'-3')	GenBank Acc. No.	bp	Annealing temp (°C)	Reference
actb	F: AGCCAACAGGGAGAAGATGA R: TGGGGCAATGATCTTGATCT	L36342	657	57	Won et al. 2012
igf1	F: TTGTGTGTGGAGAGAGAGGCTTT R: TGACCGCCGTGCATTG	AF402670	67	60	Picha et al. 2008
lep	F: CAGCGACCCAAGCTTTCAGT R: CACGGTGTGAATGAACTTTTTCC	JF919618.1	71	60	Won et al. 2012

Statistical analyses

Statistical comparisons of growth parameters (SGR, FCR, K) were conducted using the initial stocking measurements and final measurements collected on individuals at the conclusion of the trial. Hematological and plasma comparisons (PVC, RBC, WBC, cortisol, glucose) were conducted at the conclusion of the trial among strains and between treatments. At the conclusion of the repeated cortisol measurement study, following the third stress event, mean cortisol values were calculated for individual fish and strains. Individual means were ranked to identify fish with consistently high or low responses based on standard deviations (± 1) from their strain's overall average. Plasma cortisol and glucose values were square root and log transformed respectively to achieve normality. Analyses of these measurements and daily feed consumption were performed using one-way ANOVA among strains or Students t-test between treatments.

Tukey's pairwise comparison of means was applied if ANOVA output indicated a significant ($P<0.05$) overall difference among strains.

QPCR results were analyzed using the Pfaffl method for relative quantification (Pfaffl, 2001). Individual expression levels were: 1) calibrated to the DOM control group mean, 2) normalized to *actb*, to compensate for differences in cDNA synthesis efficiency among samples, and 3) expressed relative to the DOM control group (set to 1.0), to enhance data presentation. Relative expression values of assays were log transformed prior to analyses to satisfy assumptions of normality or equal variance.

All data were expressed as mean (± SEM) and analyzed using one-way ANOVAs in JMP Pro 13.0 (SAS Institute, Cary, NC, USA), followed by Tukey's post hoc tests. Phenotypic correlations among the physiological parameters described above, within the respective treatments and timepoints, were performed without regard to fish strain (n=24 fish/treatment/timepoint). Pearson correlations and associated p-values were conducted in R v. 3.5.3 (R Core Team 2019) and calculated using the *rcorr* function in the *Hmisc* package (Harrell Jr., 2019).

Results

Control and stressed fish exhibited similar feed consumption, except on net-chased days, when stressed individuals significantly reduced their feed intake ($p < 0.05$, Fig. 1). The fish in stressed tanks exhibited a significantly higher FCR than controls (1.82 ± 0.13 and 1.37 ± 0.02 respectively; $p = 0.03$, Fig. 2C), and all strains except CAN had lower specific growth rates (SGR) when stressed (Fig. 2A, Table 2). Among strains in the control treatment, SGRs were largely higher and more variable, with DOM, FL, and VA fish exhibiting greater growth than CAN ($p<0.0001$). Condition factor (K) generally decreased within strains and between

treatments by the conclusion of the trial, but only the DOM strain exhibited a significant effect of stress on K ($p<0.0001$, Fig. 2B, Table 2).

Many hematological parameters were different between treatments and among individual strains. While hematocrit (Hct %) did not differ (Fig. 3A, Table 2), RBC counts were higher in stressed fish compared to controls ($p = 0.03$, Fig. 3B). Differences among individual strains, however, were not observed ($p = 0.0183$, Table 2). RBC and Hct % were positively correlated with each other in the control group only ($p = 0.0023$, Table 3). In contrast, WBC counts were both greatly reduced in the stressed treatment ($p < 0.0001$, Fig. 3C) and different among strains ($p = 0.0004$, Table 2). Control FL and VA fish exhibited the overall greatest WBC counts (2.05 ± 0.38 and 1.80 ± 0.25 $x10^5$ μL^{-1}, respectively), that were higher than those in respective stressed individuals (0.99 ± 0.09 and 0.83 ± 0.11 $x\ 10^5$ μL^{-1}, Table 2). WBC counts in control DOM and CAN fish were not reduced when stressed (Table 2) and were not correlated with other physiological parameters in stressed individuals (Table 3).

Hepatic gene expression patterns exhibited few differences between control and stressed fish (Fig. 4). *Igf1* expression was lower in the stressed treatment ($p = 0.0445$, < 2 fold), but not different within most strains and was not correlated with individual SGRs ($p = 0.2123$). Only CAN fish exhibited a two- fold, *igf1* decrease when stressed. Leptin expression was not influenced by treatment or strain ($p = 0.1979$ and 0.2459 respectively) but was strongly, negatively correlated with SGR and K in stressed fish ($r = -0.58$, $r = -0.55$ respectively, Table 3).

Plasma glucose and cortisol responsiveness to the net chase stressor during the first study was consistent among strains, with the exception of CAN, which showed no elevation in either parameter (Fig. 5A-B; $p = 0.581$). The elevated cortisol was detected in control fish only, however, as DOM, FL, and VA strains exhibited no significant cortisol peak at any time point in

stressed tanks during final sampling (Fig. 5C, E, G). Blood glucose patterns did not differ within strains or between treatments (Fig. 5D, F), except in stressed CAN and DOM fish, which were not elevated, post-stressor (Fig. 5B, H). The baseline (time = 0 hr) glucose and cortisol levels within treatments were not correlated with each other but glucose showed a positive correlation with *igf1* expression in the control fish only ($p = 0.0198$, Table 3). At one hour post-stressor, there was a positive correlation between peak cortisol and glucose levels ($r = 0.60$, $p = 0.0025$, Table 4) and cortisol and SGR ($r = 0.46$, $p = 0.0281$) in the control group fish. These correlations were not present at three hours post- stressor (data not shown). There were no significant correlations among cortisol, glucose and SGR in fish from the stressed treatment at either time point.

Significant differences were found among strains in the repeated cortisol study with CAN displaying a lower cortisol response (9.56 ± 1.89 ng mL^{-1}) to an acute stressor than other strains ($p < 0.0001$, Fig 6). Individuals within strains generally had consistently elevated cortisol levels across stress events and at least three fish per strain could be **r**anked as high (+ 1 SD; 54.9 ± 2.53 ng mL^{-1}) or low responders (- 1 SD; 11.7 ± 1.66 ng mL^{-1}) . No habituation in cortisol response was identified, as 1 hr cortisol levels in DOM, FL, and VA strains were consistently elevated (~30-40 ng mL^{-1}) after each stress event compared to the baseline 1 hr levels in stressed strains from the first study (~4 ng mL^{-1}) (Figs. 5 and 6).

Figure 1
Mean (± SEM) feed consumption (g) to satiation by day with net-chased events occurring on Mondays and Thursdays. Asterisks denote significant differences between treatments and within days. T-test (P<0.05)

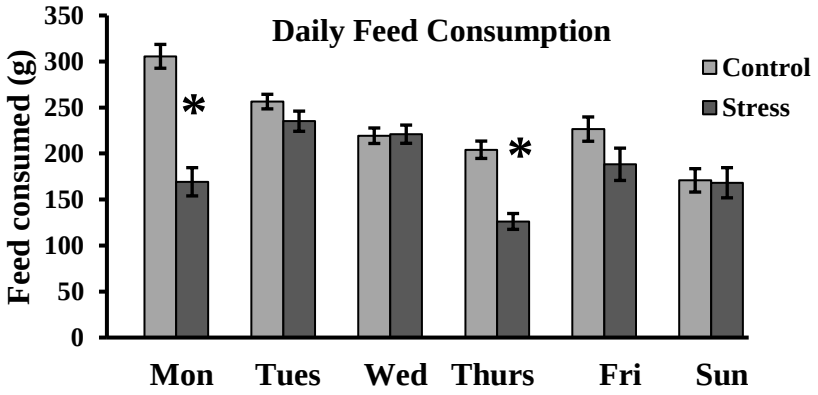


Figure 2
Mean (± SEM) growth parameters (a; SGR b; K and c; FCR) between control and stress treatments. Asterisk denotes significant difference. T-test (P= 0.03)

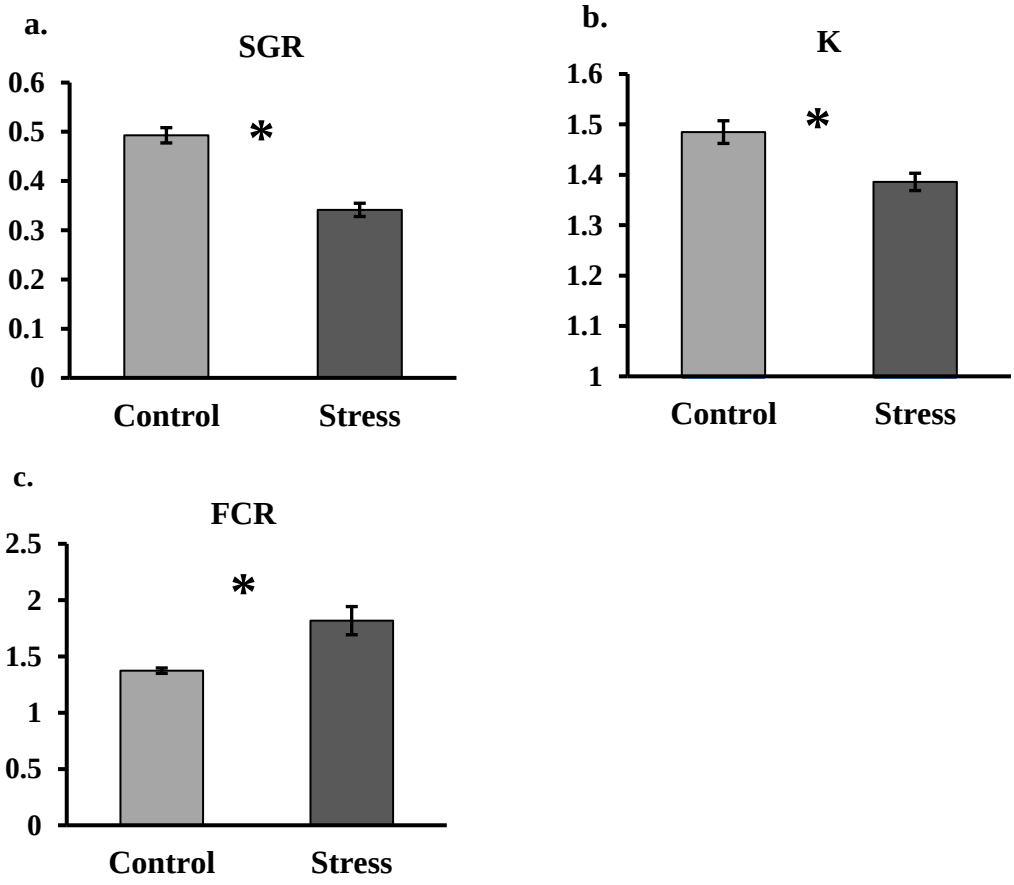


Figure 3
Mean (± SEM) hematological parameters (a; Hct b; RBC and c; WBC) between control and stress treatments. Asterisk denotes significant difference. T-test ($P<0.05$)

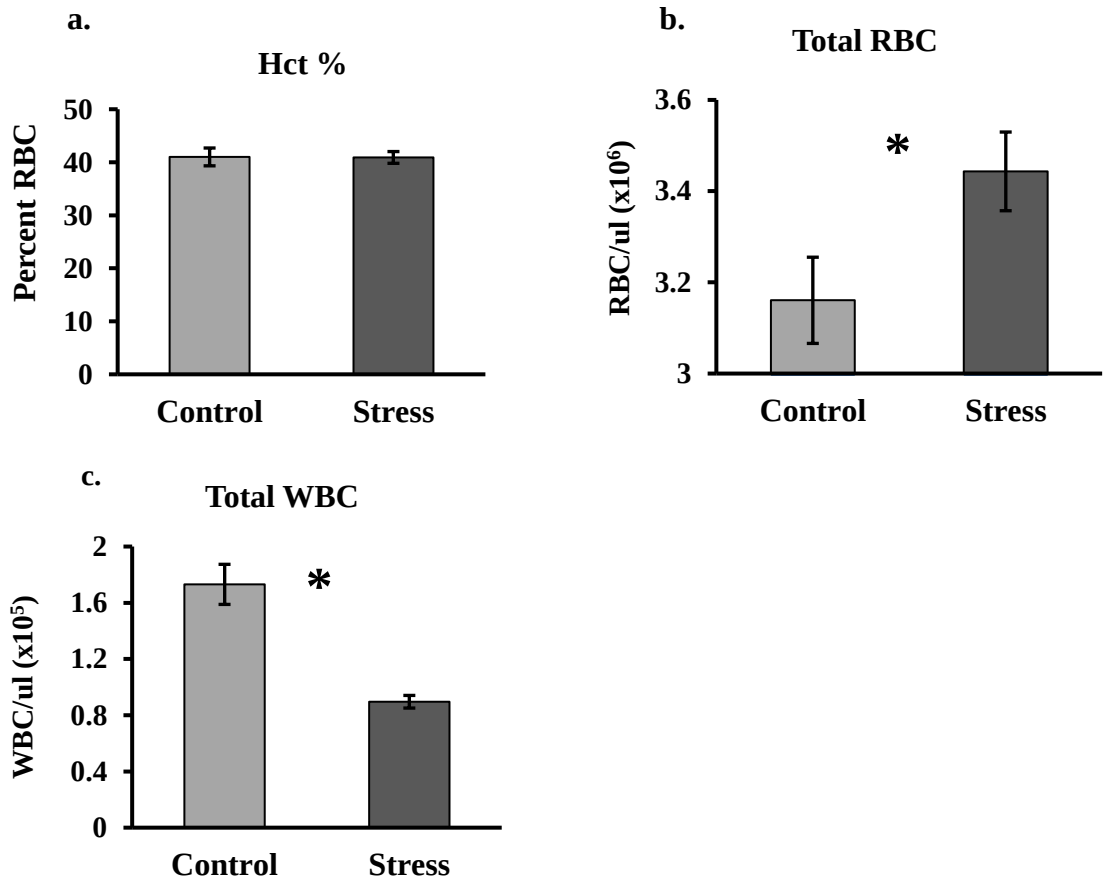


Table 2

Mean (± SEM) specific growth rate (SGR), condition factor (K), hematocrit (Hct %), total red blood cells (RBC) and total white blood cells (WBC). Different letters denote significant differences among strains and treatments (ANOVA and Tukey's, $p<0.05$)

	Control				**Stress**			
	DOM	**FL**	**VA**	**CAN**	**DOM**	**FL**	**VA**	**CAN**
SGR	0.49 ± 0.02^{AB}	0.53 ± 0.02^{A}	0.56 ± 0.02^{A}	0.39 ± 0.03^{BC}	0.34 ± 0.02^{C}	0.34 ± 0.02^{C}	0.36 ± 0.03^{C}	0.33 ± 0.03^{C}
K	1.55 ± 0.03^{A}	1.64 ± 0.04^{A}	1.36 ± 0.02^{CD}	1.35 ± 0.03^{CD}	1.41 ± 0.02^{BC}	1.51 ± 0.03^{AB}	1.26 ± 0.02^{D}	1.36 ± 0.03^{C}
Hct (%)	40.0 ± 3.3	39.7 ± 5.34	42.1 ± 2.66	42.1 ± 2.15	39.6 ± 1.95	43.8 ± 2.38	37.0 ± 1.05	43.2 ± 2.44
RBC (x $x10^6$/µL)	2.91 ± 0.15	2.96 ± 0.19	3.24 ± 0.21	3.53 ± 0.12	3.50 ± 0.13	3.60 ± 0.26	3.12 ± 0.09	3.55 ± 0.14
WBC ($x10^5$/µL)	1.60 ± 0.24^{ABC}	2.05 ± 0.38^{A}	1.80 ± 0.25^{AB}	1.48 ± 0.27^{ABCD}	0.91 ± 0.09^{CD}	0.99 ± 0.09^{BCD}	0.83 ± 0.11^{D}	0.85 ± 0.06^{CD}

Table 3

Correlation matrices of physiological parameters measured in the Control and Stress treatments at t = 0 hr (n = 24 fish/treatment). Significance level is indicated as P<0.001 (***), P<0.01(**), P<0.05(*)

Control

	Cortisol	Glucose	SGR	K	RBC	WBC	*igf1*	*lep*	PCV
Cortisol	1.00	0.16	0.10	0.22	-0.23	0.07	0.20	0.28	-0.24
Glucose		1.00	-0.02	0.15	-0.03	-0.02	**0.47***	0.20	0.04
SGR			1.00	**0.82*****	-0.26	0.31	0.10	-0.20	0.20
K				1.00	-0.33	0.10	0.36	-0.06	0.21
RBC					1.00	0.24	0.13	-0.22	**0.59****
WBC						1.00	-0.27	**-0.40***	0.34
Igf1							1.00	0.32	0.21
lep								1.00	-0.21
PCV									1.00

Stress

	Cortisol	Glucose	SGR	K	RBC	WBC	*igf1*	*lep*	PCV
Cortisol	1.00	0.27	0.20	0.14	0.19	-0.11	-0.13	0.16	0.24
Glucose		1.00	0.37	0.36	0.34	0.07	0.05	-0.04	0.35
SGR			1.00	**0.77*****	0.15	0.12	-0.22	**-0.58****	0.06
K				1.00	0.17	0.04	-0.15	**-0.55****	0.15
RBC					1.00	0.26	-0.05	-0.18	0.21
WBC						1.00	-0.21	0.15	0.06
igf1							1.00	-0.01	-0.04
lep								1.00	-0.02
PCV									1.00

Table 4

Correlation matrices of physiological parameters measured in the Control and Stress treatments at t = 1 hr (n = 24 fish/treatment) Significance level is indicated as P<0.001 (***), P<0.01(**), P<0.05(*)

Control

	Cortisol	Glucose	SGR
Cortisol	1	**0.60****	**0.46***
Glucose		1	0.37
SGR			1

Stress

	Cortisol	Glucose	SGR
Cortisol	1	0.16	-0.03
Glucose		1	0.33
SGR			1

Figure 4

Mean (± SEM) hepatic gene expression of insulin-like growth factor 1 (*igf1*) and leptin (*lep*) at t = 0 hr (n = 24 fish/treatment) within treatments (left) and across strains (right). Asterisk denotes significant difference. T-test (P<0.05)

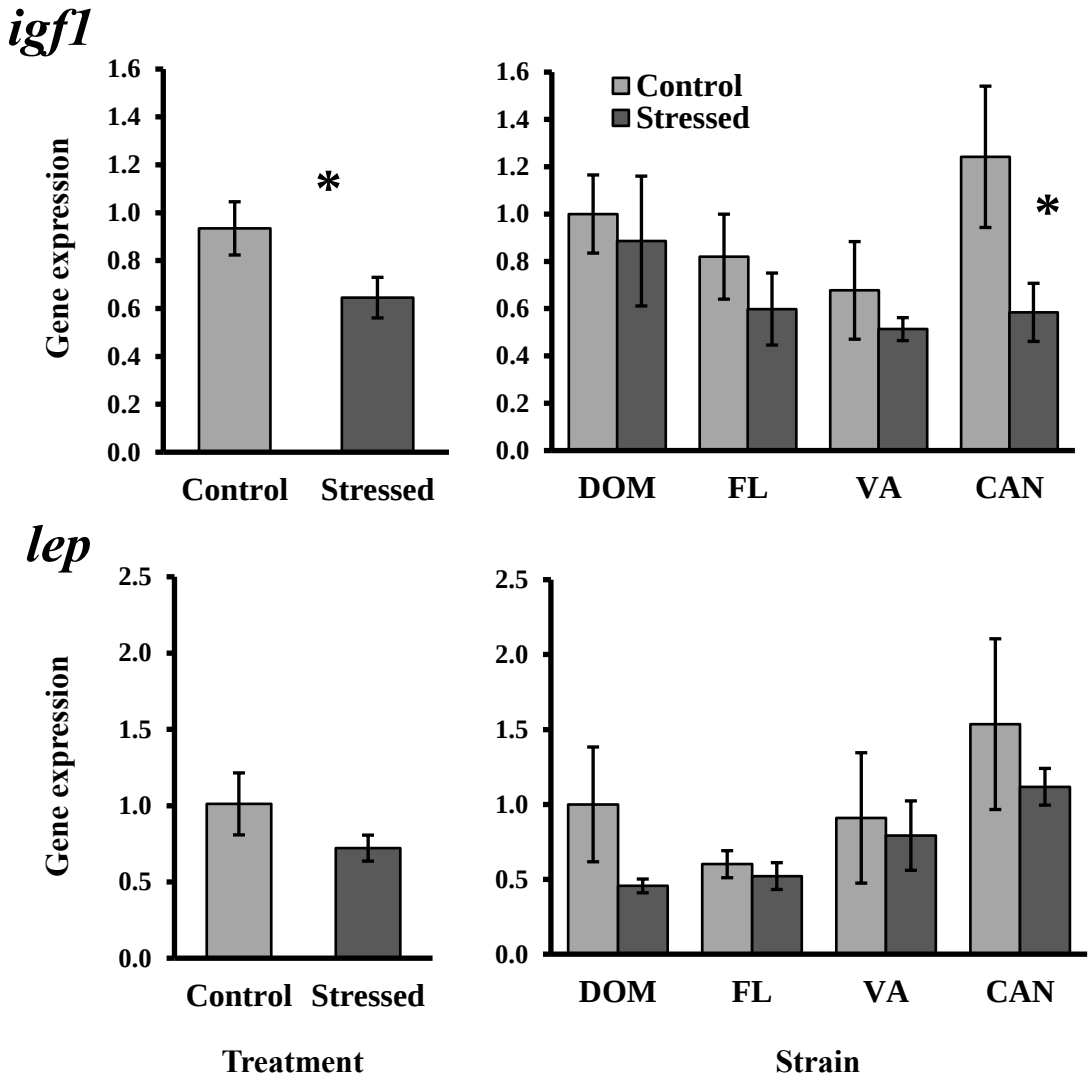


Figure 5

Mean (± SEM) plasma cortisol (ng mL^{-1}; a,c,e,g) and glucose (mM; b,d,f,h) values at 0, 1 and 3 hours post stressor at the conclusion of the 14 week trial. Different letters denote significant differences within strains and across treatments (ANOVA and Tukey's, $p<0.05$)

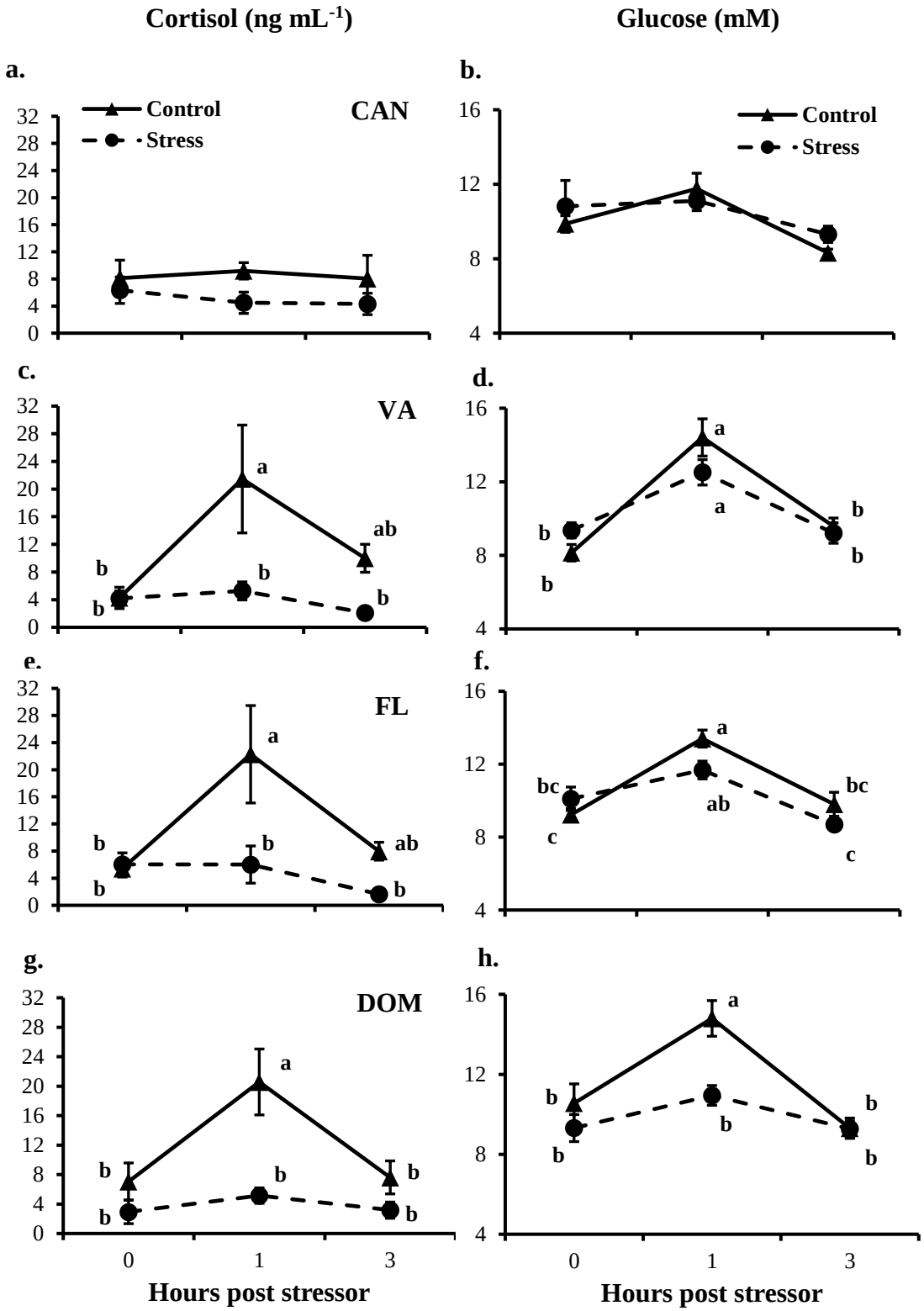
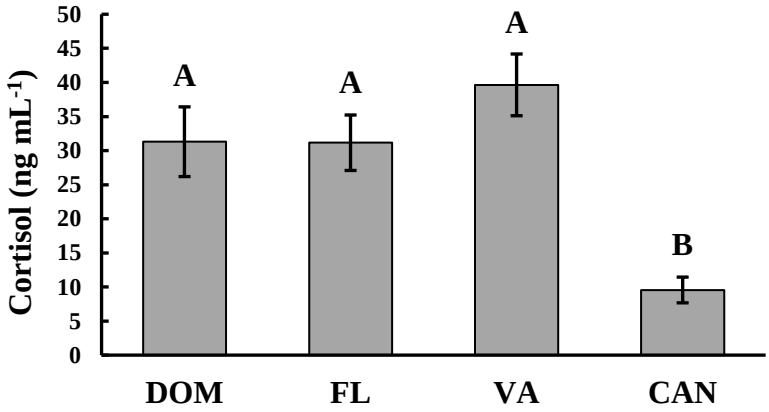


Figure 6

Mean (± SEM) plasma cortisol (ng mL^{-1}) across three measurements (monthly) in strains from the repeated cortisol measurement study. Different letters denote significant differences among strains (ANOVA and Tukey's, $p<0.05$)



Discussion

The results of this study demonstrated that the repeated acute stressor applied was sufficient to elicit a physiological stress response that reduced SGR, K, and *igf1* expression, elevated FCR and suppressed WBCs, but had variable effects among striped bass strains. Stress elicited differential responses among strains across all stages of the GAS that may prove useful in broodstock development, but a single biomarker was insufficient for evaluating stress sensitivity among groups or individuals. No single strain displayed a clear difference across all measured parameters when exposed to the repeated acute stressor. Instead, complex associations across parameters observed suggest that a suite of robustness traits should be examined during broodstock development, as has been undertaken with terrestrial livestock breeding programs (Gamborg and Sandoe 2005; Knap 2005; Ellen et al. 2014; Sadoul et al. 2015).

Feed intake was similar between stressed and undisturbed fish on all days except those when the (net chase) stressor was applied, at which times stressed fish consumed less than controls. Although strain-specific measurements could not be attained because of the communal rearing experimental design, qualitative feeding behavior, post-stressor, could be observed because of morphological differences (i.e. stripe patterns, K, head size) present among strains (Kenter et al. 2018). These observations indicated that the CAN strain maintained normal feeding behavior, post-stressor, while the others crowded towards the back of tanks and were hesitant to pursue pellets. The poor feeding response, post-stressor, in all strains except CAN, was likely responsible for the depressed SGRs observed, and is consistent with results obtained in other marine species, such as Atlantic salmon (McCormick et al. 1998) and European sea bass (*Dicentrarchus labrax*)(Leal et al. 2011).

Other than a significant difference in K, no observational or behavioral differences were noted between treatments, nor was health apparently compromised. To gauge immunocompetence, a suite of basic hematological parameters were measured (secondary stress response) with implications for aquaculture performance. RBC counts and Hct can be elevated during the secondary stage of the stress to increase systemic oxygen availability during a period of higher metabolic demand (Cnaani et al. 2004). Results from this study showed significantly elevated, baseline RBC values in the stressed treatment which were correlated with Hct, and consistent with observations in hybrid striped bass (Hrubec et al. 2001). The relationship between RBC and Hct can be difficult to interpret during stress because of hemodilution or hemoconcetration caused by impaired osmoregulation, splenic contraction, stress polycythemia (Haux et al. 1985; Morgan and Iwama 2011) and erythrocyte swelling caused by the actions of epinephrine (Nikinmaa 1982). Although there were no differences in RBC counts among strains due to high individual variation, it is interesting to note the similar changes in RBC counts between the stressed and unstressed fish in the DOM and FL strains. The nearly identical RBC counts and cortisol levels between treatments in the CAN strain likely contribute to the correlation between these parameters. The physiological variation observed may be due to local adaptations by strains to perform in their native environments, since lower oxygen conditions are likely more common in the Gulf of Mexico (FL) and earthen production ponds (DOM) compared to the cold, coastal waters of Canada. It is possible that the subtle strain-specific physiological differences could be exploited by selective breeding for specific aquaculture settings.

Total WBC counts have been shown to be depressed during times of stress leading to greater susceptibility to disease (Tort 2011). The results of this study support this finding with depressed WBC counts in the stressed treatment compared to controls. When strains were

observed independently, all showed consistent numerical decreases in WBC counts (approximately 50% of control fish) that were significantly different in only the FL and VA strains. CAN stressed fish also showed some evidence of a small, but insignificant decline in WBC counts ($p = 0.0697$) despite the consistency in cortisol levels between treatments. If measured at additional timepoints, changes in WBC counts may provide a practical, non-lethal stress indicator and predictor of robustness.

The liver is a major target organ during stress events and is responsible for many of the associated metabolic adjustments (Aluru and Vijayan 2009). Cortisol can have suppressive effects on somatic growth by binding to intracellular hepatocyte receptors and disrupting normal transcriptional control of metabolic enzymes, hormones and growth factors. (Mommsen 2001; Davis 2006). Leptin is an anorexigenic peptide that has been well studied in mammals and is known to modulate feed intake and maintain energy homeostasis with links to the stress axis, but only recently gained interest in aquaculture research (Bernier et al. 2012; Roubos et al. 2012; Baltzegar et al. 2014). In mammals, leptin production decreases during periods of catabolism and diminished energy reserves which triggers neural pathways to increase food consumption until energy reserves are restored (Ahima 2008). During positive energy status (anabolism), increased leptin production promotes energy expenditure, upregulates hypothalamic anorexigens and downregulates orexigens to decrease appetite (Ahima and Flier 2000). The actions of leptin during stress events have not been well characterized in fish, but appear similar to those in mammals following exogenous administration as shown in rainbow trout (Murashita et al. 2008; Madison et al. 2015) and goldfish (Volkoff 2006). Won et al. (2012) cloned and characterized striped bass leptin, showed exclusive expression in the liver, and observed its roles during fasting of interspecies (white bass x striped bass) hybrids. Fasting significantly reduced hepatic mRNA

expression, which increased to near control levels upon re-feeding, and thereby supported leptin's positive association with endogenous energy reserves. Our results show a strong correlation with growth (SGR, K) but no differences between treatments or within strains of striped bass after 14 weeks of repeated stress. Based on these results, leptin regulation may be more closely tied to energy state rather than the HPI axis during repeated, acute stress. These results differ from those in rainbow trout in which *lep* increased following exogenous cortisol administration (Madison et al. 2015) and the differences may be due to the nature of stressor applied, current energy state of fish under observation, and differences in time points. Future research on appetite regulation during stress should continue to focus on additional anorexigenic and orexigenic hormones.

During unstressed periods, growth hormone (GH), under highly complex regulation, is secreted from the anterior pituitary and stimulates hepatic production of insulin- like growth factor 1 (Igf1), a primary peptide hormone involved in vertebrate myogenesis (Duan 1997; Reinecke 2006; Picha et al. 2008). As Igf1 is constitutively released during production, its transcription and translation rates are essentially equivalent and bioactivity is controlled by a suite of binding proteins that control its plasma clearance rates (Beckman 2011). In the present study, overall hepatic *igf1* expression and growth rates were significantly lower in the stressed treatment compared to the control which agrees with previous, effects *in vitro* studies (Philip and Vijayan 2015; Faught and Vijayan 2016; Shepherd et al. 2018) and *in vivo* (Saera-Vila et al. 2009) among species. In the CAN strain, however, stressed fish showed lower *igf1* than controls, despite being the only strain without measurable differences in circulating cortisol or SGRs. Future work on hepatic derived growth markers should focus on circulating hormone

levels, receptor activity and binding proteins (IGFBPs) *in vivo* that may directly link the HPI and GH-IGF axes.

Elevated cortisol levels, which have most frequently been used as a stress biomarkers were positively correlated with SGR in the control group, but not correlated with performance traits in the stressed tanks after 14 weeks of repeated net chasing. Cortisol has been shown to activate gluconeogenic mechanisms causing a shift in energy substrates away from carbohydrates and towards lipids and amino acids (Wendelaar Bonga 1997). It has also been shown to increase metabolic rates, reduce nutrient absorption, mobilize energy stores and interfere with growth signals from the somatotropic axis (Barton et al. 1987; Mommsen et al. 1999; Kajimura et al. 2003; Bernier et al. 2004). While elevated cortisol is typically a reliable indicator for HPI activation, the CAN strain did not exhibit a measurable increase in plasma cortisol at 1 or 3 hr post stressor while the other strains were largely similar and predictable. This divergent response was unique to the CAN strain and may reflect either a resistance to specific aquaculture-related stressors or an underlying chronic stress in both treatments resulting in an overall muted acute cortisol response as has been shown in other species (Barcellos et al. 1999; Barton et al. 1987). Also, the parents of the CAN fish, unlike VA and FL, were wild-captured as juveniles and maintained in captivity until maturity before producing the juveniles used in this study. The difference in culture history prior to juvenile acquisition may have contributed an additional confounding factor that altered the strain's perception of stress compared to others. Overall, the CAN-specific stress response difference may be a combination of environmental and genetic factors and requires additional study from a larger population-wide sample.

With the exception of CAN, fish in this study appear to have become habituated to the bi-weekly stressor over the course of the trial, as evidenced by the stable cortisol levels prior to and following the stressor in the stressed treatment group. In the repeated cortisol sampling study, habituation to the reduced frequency (monthly) stressor was not evident and elevated cortisol measurements from all three events were comparable to the control group in the first experiment. CAN fish did not demonstrate cortisol responsiveness but at least three fish from the other strains could be categorized as high or low responders based on previously described criteria. Habituation to stressors has been shown previously in striped bass (Wang et al. 2004) and salmonids (Barton et al. 1987; Schreck et al. 1995). Further research on the correlations between cortisol and performance traits is warranted and may prove useful in striped bass selective breeding protocols (Pottinger and Carrick 1999; Fevolden et al. 2002). Elevated glucose levels 1 hour post-stressor may have been induced by catecholamines, rather than cortisol, (Van Raaij et al. 1995, 1996; Wang et al. 2004), but were not measured in this study. Irrespective of their origin, glucose elevation in the VA, FL, DOM strains may have contributed to the diminished SGRs observed, as elevated plasma glucose has been shown to inhibit food intake through hypothalamic glucosensing and modulation of appetite-regulating genes (Riley et al. 2009; Madison et al. 2015).

Conclusions

Overall, no single wild or domesticated strain displayed superior robustness during exposure to a bi-weekly net chase stressor. CAN fish had a highly unique stress response across most stages of GAS, but their slow growth in culture has been demonstrated previously (Woods 2001; Kenter et al. 2018, present study) and may limit their aquaculture potential. The DOM strain, mass selected for growth and general morphology did not exhibit better maintenance of

growth rate or K than first generation striped bass cultured from the Gulf of Mexico (FL) or Atlantic coast (VA).

Although variable among individuals, peak cortisol response was correlated with growth performance only in the control fish prior to stressor habituation. This further demonstrates that cortisol is only a single component in the complex GAS and its selection as a sole phenotype in broodstock development, is questionable. Future stress studies should continue to focus on correlations between measures of the GAS across all stages and aquaculture performance traits that may be useful in domestication efforts. The growing literature base on strain specific performance of striped bass under different conditions represents an opportunity to create or select robust lines of fish for specific culture environments.

CHAPTER 6

CONCLUSIONS

Collectively, the research in this dissertation demonstrated differences in performance traits among strains of striped bass and their hybrids in multiple environments, developed protocols for managing stress in the production of the species and advanced our current understanding of stress responsiveness in fishes.

Wild striped bass strains from different river systems displayed differences in growth and body conformation that may be attributed to genetic background or local adaptations to their natural environment, and these traits were heritable in hybrid offspring. The ability of the species to perform similarly in fresh, brackish and marine salinities demonstrated opportunities for culture in a variety of environments. Results agreed with previous strain comparisons (Jacobs et al. 1999; Woods 2001) at identifying consistently slow growing populations (CAN, NC, SC) that have limited potential in farming compared to most central Atlantic (DE, VA) or Gulf coast populations (FL, TX). The domestic line was compared alongside wild strains for the first time since its founding, and was found to have growth and feed conversion advantages during their first year of life that were not maintained during longer growth trajectories. Trends in growth and body shape among hybrid offspring were similar to those of strains used to spawn them suggesting a paternal strain effect on both phenotypes that outweighed individual parental effects. These studies demonstrated the responsiveness of DOM hybrid striped bass to selection pressure from the breeding program for rapid early growth in outdoor culture environments. Impressive growth performance and less variation in final weights was shown in wild strains when grown indoors demonstrating the importance of comparisons in multiple environments to establish effective breeding programs.

The aquaculture potential documented among wild strains establishes the foundation for region-specific broodstock programs dependent on the production systems of interest for culture. A hypothetical US mariculture industry could now utilize fast growing, native stocks to the Atlantic (DE, VA) or Gulf coast (FL,TX), that avoid the risk of genetic contamination during escapes, a common concern in open ocean farming. Future research should continue to investigate strain-specific performance across environments and begin to apply modern genetic techniques like marker assisted selection to better understand domestication for *Morone* culture.

The results from stress studies further demonstrated that the stress response is a complex, adaptive mechanism and a single measure cannot be used to estimate its effects on aquaculture performance. Cortisol levels naturally fluctuate during normal biological processes and are a common biomarker to quantify stress (Barton and Iwama 1991) with known maladaptive consequences. During prolonged or severe stress, it promotes hyperglycemia and suppresses performance but it is more often a single component of the primary response with minimal negative effects. The transportation trial showed substantial differences in cortisol among treatments and methods to reduce its elevation, but they did not result in observed health or feeding benefits. The 14-week net chase study found individual cortisol levels to be correlated with growth rate in the control fish, but the relationship was absent after repeated exposure in the stressed treatment. Hematological parameters such as blood cell counts were the most consistent indicators of distress among and within strains and required less expertise to measure. Hematological analyses should continue to be studied in fish so that it may be used as a routine, practical diagnostic tool for stress and health assessments (Hrubec et al. 2001). No single wild or domesticated strain displayed measurable performance advantages during the routine aquaculture stressors (transportation, netting) in these studies which suggests inadvertent co-selection for

such specific tolerances may not be present in the breeding program. Stress and aquaculture research will benefit from inclusion of more parameters across stages of the GAS as has been done with terrestrial agriculture for future selection of "robust" animals (Sadoul et al. 2015). Overall, the results in the present studies advance the current status of *Morone* culture and contribute to a foundation of information to be used in broodstock development programs.

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APPENDICES

APPENDIX A. ANIMAL CARE AND USE DOCUMENTATION

University *of* New Hampshire

Research Integrity Services, Service Building
51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

27-Jan-2017

Berlinsky, David L
Dept of Biological Sciences
Rudman Hall
Durham, NH 03824-2618

IACUC #: 160110
Project: Stress Responsiveness in Striped Bass
Next Review Date: 26-Jan-2018

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your request for a time extension for this protocol. Approval is granted until the "Next Review Date" indicated above. You will be asked to submit a report with regard to the involvement of animals in this study before that date. If your study is still active, you may apply for extension of IACUC approval through this office.

The appropriate use and care of animals in your study is an ongoing process for which you hold primary responsibility. Changes in your protocol must be submitted to the IACUC for review and approval prior to their implementation.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact me at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Dean Elder, D.V.M.
Vice Chair

cc: File