Examining early growth genetic potential of two distinct localities of Florida Bass and an F1 hybrid bass (*Micropterus floridanus x Micropterus salmoides*)

by

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

Auburn, Alabama
December 12, 2020

Keywords: Largemouth Bass; Single Nucleotide Polymorphism; Common Garden Experiment

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Abstract

The pursuit of trophy black bass (*Micropterus spp.*) is a challenge many freshwater anglers embrace. In addition, the growth of a trophy-sized specimen is a challenge fishery and hatchery managers have striven to solve for years. Florida Bass (*Micropterus floridanus*) have been long heralded for their higher maximal growth potential in comparison with northern Largemouth Bass (*Micropterus salmoides*) when stocked into optimal environments. For decades, stocking programs employing Florida Bass have occurred throughout the Southeastern United States with conflicting results. More recently, there has been increased interest regarding the growth potential of F1 hybrid bass (*Micropterus floridanus x Micropterus salmoides*). This has been of interest due to its potential for replacing Florida Bass in stocking, particularly in parts of the Southeastern United States. In addition, no studies have compared growth performance among phenotypically and/or geographically-distinct Florida Bass populations. In this thesis, Chapter 1 encompasses an extensive literature review on the topic. Chapter 2 discusses the current study, a pond experiment aimed at exploring early growth genetic potential between two Florida Bass populations and an F1 hybrid bass (*M. floridanus x M. salmoides*). For the current study, one Florida Bass population was sourced from a central Florida phosphate mine lake, which will be referred to as South Pasture in the remainder of this thesis; another was an established commercial strain under long-term culture and selection at the American Sportfish Hatchery (ASFH) in Pike Road, AL. The F1 hybrid bass (*M. floridanus x M. salmoides*) population resulted from pure Florida Bass and northern Largemouth Bass on-site at the American Sportfish Hatchery. All utilized broodfish were confirmed in their classification as pure Florida Bass or northern Largemouth Bass via single nucleotide polymorphism (SNP) genotyping. Using a common garden experimental model, the two populations of Florida Bass and the F1 hybrid (*M. floridanus x M. salmoides*) were mixed into common pond environments with optimal forage densities as juveniles, where their growth was monitored over eight months. Passive integrated transponder (PIT) tags were utilized to properly identify and compare each population. We observed significantly greater ($p \leq 0.05$) final mean lengths (mm), weights (g), and relative weights (Wr) in F1 hybrid bass (*M. floridanus x M. salmoides*), in comparison to both populations of Florida Bass. F1 hybrid bass (*M. floridanus x M. salmoides*) also exhibited a significantly greater ($p \leq 0.05$) overall growth rate than either Florida Bass population. The ASFH population had a greater percentage change in weight gain (199.30%), than the South Pasture population (121.57%). However, the South Pasture population had a greater overall increase in weight (70 g). The ASFH population also had a greater percentage/overall increase in length than the South Pasture population. The study highlights the variability in early growth genetic potential within Florida Bass, as well as, ultimately, advances the development of next-generation F1 hybrid bass utilizing superior Florida Bass individuals/strains.

Acknowledgments

I would like to thank my advisor, Dr. Eric Peatman, for the opportunity to pursue my Master's degree at Auburn University. I would also like to thank Dr. Benjamin Beck for his unwavering time and support on this project, as well as allowing me the use of USDA-ARS Aquatic Animal Health Research facilities. I am thankful for Dr. Shawn McNulty providing his expertise in hatchery and pond management, as well as providing the fish used in this study. I wish to acknowledge Dr. Allen Davis for his time and assistance on this project, and Dr. Ian Butts for his guidance in statistical analyses.

I would also like to thank my lab members: Katherine Silliman, Honggang Zhao, Matthew Lewis, Sarah Johnson, and Megan Justice for their assistance in the lab and help during my time here at Auburn. I am also extremely grateful to have worked alongside Troy Bader, Lee Collins, Esau Arana, Michael Smallwood, and Osman Montero. I express my deepest appreciation for my fellow graduate students: Benjamin Prior, John Lewis, Cody Stites, João Reis, Michael Coogan, and Billur Kazaz for their suggestions and moral support. I am extremely grateful for my girlfriend Jaime Schussler, and her dog Maxx, for their constant love and support. Lastly, I would like to thank my parents, Bryan and Kelli Fewell, for their encouragement and advice throughout this journey and future endeavors.

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List of Abbreviations

ASFH	American Sportfish Hatchery
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
FB	Florida Bass
HCl	Hydrochloric Acid
LSM	Least Squares Means
MS-222	Tricaine Methanesulfonate
NaOH	Sodium Hydroxide
NB	Northern Largemouth Bass
PIT	Passive Integrated Transponder Tag
PCR	Polymerase Chain Reaction
SE	Standard Error
SNP	Single Nucleotide Polymorphism
Wr	Relative Weight
Ws	Standard Weight
USDA	United States Department of Agriculture

Chapter 1: Literature Review

Study Species

In 1802, the Largemouth Bass (*Micropterus salmoides*) and the Smallmouth Bass (*Micropterus dolomieu*) were the first black bass described by French naturalist Lacépède (Lacépède, 1802; Long et al., 2015; Taylor et al., 2019). Distinctions within the species of Largemouth Bass were first described by LeSueur (1822) when he identified the Florida Bass. It was not until over 100 years later that Bailey and Hubbs (1949) formally described them at the subspecies level by meristic and morphological techniques. At this time, Bailey and Hubbs (1949) considered there to be two subspecies of Largemouth Bass *(M. salmoides*): (1) Florida subspecies (*M.s. floridanus*) and (2) northern subspecies (*M.s. salmoides*). Recently, the Florida subspecies was elevated to a full species designation by Kassler et al. (2002) and Near et al. (2003) as a result of further molecular and morphological analyses. However, it should be noted that the American Fisheries Society currently recognizes Florida Bass as a provisional species, although it is expected to be recognized as a full species. Many individuals still refer to the bass as a subspecies, further complicating the name designation. However, in this thesis, Florida bass will be used to refer to *M.s. floridanus or M. floridanus* and northern Largemouth Bass to refer to *M.s. salmoides or M. salmoides*. If an unclear delineation between the species is noted, such as multi-generation hybridization, Largemouth Bass was used. When discussing this thesis research, "the current research study" is used.

Bailey and Hubbs (1949) hypothesized that an intergrade zone occurred as a result of the disappearance of an isolating barrier between the two subspecies, allowing both subspecies to mix in an accessible area. This intergrade zone was stated to encompass most of the state of Georgia and the eastern edge of Alabama (Bailey & Hubbs, 1949). The Florida Bass was believed to be endemic to peninsular Florida from the mouth of the St. Johns River to the Suwannee River system. Meanwhile the northern Largemouth Bass existed north and west of the Choctawhatchee Bay and Apalachicola River drainages in Florida, Alabama, and Georgia, in addition to the north and east of the Savannah River system in South Carolina (Bailey & Hubbs, 1949). This geographic designation of the intergrade zone lasted until Philipp et al. (1981, 1983) found that northern Largemouth Bass and Florida Bass had hybridized in a much larger area, and expanded the intergrade zone to include: northern Florida, Mississippi, Alabama, Georgia, South Carolina, North Carolina, Virginia, and other areas such as Texas and California where Florida Bass had been stocked. Philipp et al. (1983) used allozyme markers from 1,800 largemouth bass nationwide to draw these conclusions making this the most all-encompassing genetics study for largemouth bass. However, some essential areas such as coastal North and South Carolina were not included in the study. More recently, using microsatellite markers, Barthel et al. (2010) detected northern Largemouth Bass alleles in 12 populations of bass south of the Philipp et al. (1983) delineation of the intergrade zone, including intergrades in the St. Johns River and other central Florida river systems. As a result, Barthel et al. (2010) recently updated the intergrade zone description by stating: a) the St. John's River should now be considered an intergrade system; b) the Ocklawaha River and other central Florida systems should not be assumed to be pure Florida Bass systems; c) the geographic distance between pure Florida Bass populations in south Florida and intergrades alone may not be a reliable indicator of pure fish since intergrade populations were detected in south Florida.

Economic Importance as a Sportfish

Arguably the most sought-after group of freshwater sportfish in the world, black bass play a substantial economic role in the sportfish market. The establishment of angler groups such as the Bass Anglers Sportsman Society in 1968 and Fishing League Worldwide in 1979 led to increased conservation and stocking efforts throughout the country. Since this initial surge in popularity, angling clubs have continued to grow, tripling in number since 1980 (Caporelli, 2019). Clubs and tournament trails have spread to the college and high school level. Professional tournaments are regularly televised and live-streamed for at-home viewers. Tournaments are most prevalent in the southeast US, and hosting states reap their economic benefits as a result (Kerr & Kamke, 2003). Snellings (2015) found the 2013 annual tournament angling expenditures on Lake Guntersville in Alabama were $4.6 million dollars, with an estimated total economic benefit of $6.7 million dollars. In comparison, Sam Rayburn Reservoir in Texas had an estimated total economic value of tournament fishing at $31.1 million (Driscoll & Myers, 2014). As black bass tournament angling is predicted to increase, it is important the economic benefit it provides is protected by appropriate management and stocking practices (Kerr & Kamke, 2003; Schramm & Hunt, 2007; Driscoll & Myers, 2014).

To support the demand of recreational and tournament anglers, millions of bass are stocked into public waters, primarily by state agencies each year. For example, the Texas Parks and Wildlife Department (TPWD) produces and stocks between 6-8 million fry/fingerling bass per year (Caporelli, 2019). While most of these fish are produced at state hatcheries, private hatcheries do play an important role in the production of bass fingerlings and often supply state agencies with fish. In 2013, more than $14 million dollars was generated by bass produced in private hatcheries in the US (USDA, 2013; Caporelli, 2019). This represented 61% of all sportfish sales in the US, showing an increase of 32% since 2005 (USDA, 2013; Caporelli, 2019). As the sport of bass fishing continues to grow, further research is needed to ensure stocking and management programs are progressing at an equal rate. The current research study aimed to improve the knowledge of early growth genetics of bass, in order to aid in advancing next generation stocking practices through production of a more fit individual.

Early Growth Studies

Few studies have compared growth performance among phenotypically and/or geographically distinct Florida Bass populations. However, field observations that Florida Bass reach sizes greater than northern Largemouth Bass led to past studies focusing on growth differences among species and their reciprocal F1 hybrid crosses (Note: the female parent is represented first): *M. floridanus x M. salmoides and M. salmoides x M. floridanus*. Clugston (1964) compared the growth of Florida Bass fingerlings to northern Largemouth Bass fingerlings in pond experiments in Fort Lauderdale, FL. Northern Largemouth Bass fingerlings were imported from Iowa, but the origin of Florida Bass fingerlings was not provided. Given the updated knowledge of the intergrade zone in Florida as described by Barthel et al. (2010), this may have been a flaw in the study as the Florida Bass may have not been pure. Overall, the fastest growth recorded for a northern Largemouth Bass fingerling was 736.8 g in a year (Clugston, 1964). This surpassed Florida Bass whose fastest recorded growth reached 564 g after approximately fourteen months. However, the fingerlings were not stocked at the same time or same size for direct comparison. Florida Bass were stocked at 52 mm in length in April of 1960, whereas northern Largemouth Bass were stocked at 58 mm in length in July of 1960. However, both species were reported to be approximately the same size in May of 1961.

Zolczynski and Davies (1976) performed their own pond experiments in Alabama and found over six months that northern Largemouth Bass had increased early growth in comparison to Florida Bass and the F1 hybrid bass (*M. floridanus x M. salmoides*). For their study, Florida Bass broodfish were sourced from Lakes Ivanhoe and Fairview in central Florida, and northern

Largemouth Bass broodfish sourced from Lake Martin in Alabama. Gowan (2015) found the mean northern Largemouth Bass allele frequency in Lake Martin to be 0.53, indicating that bass in Lake Martin were essentially a 50/50 northern Largemouth Bass and Florida Bass split. This indicated that the anticipated pure northern Largemouth Bass brood fish were most likely intergrades (multi-generation hybrids of Florida and northern Largemouth Bass). The ponds were initially stocked with Bluegill (*Lepomis macrochirus*) and Fathead Minnows (*Pimephales promelas*), the same forage base used in the current research study (Zolczynski & Davies, 1976). The fingerling bass were all between 19-25 mm when initially stocked. Fish grow-out occurred over six months. The overall mean weight change for the northern Largemouth Bass, Florida Bass, and F1 hybrid bass was 225.0 g, 174.7 g, and 158.2 g, respectively. Based on the slower initial growth of Florida Bass, they concluded that Florida Bass possibly live longer in order to reach the larger maximal sizes observed by anglers. They also concluded that northern Largemouth Bass were more easily caught due to their increased aggressiveness and likely would not have the chance to live as long as Florida Bass. Although Zolczynski and Davies (1976) study does not compare growth specifically within the Florida strain, it serves as a good reference to the current research study due to its similarities in initial forage base and geographic location.

During the 1970's, Inman et al. (1977) evaluated the growth of Florida Bass, northern Largemouth Bass and the F1 hybrid (*M. floridanus x M. salmoides*) in a 3.64-hectare lake located in north central Texas. Northern Largemouth Bass were obtained from two nearby Texas lakes, Florida Bass were sourced from an experimental Texas lake, and F1 hybrids were collected from the Jasper State Fish Hatchery in Texas. As with other growth studies during this time period, no genetic analyses were performed to verify the purity of the fish. The Florida Bass and F1 hybrids were both stocked at an age of one year, and the northern Largemouth Bass were stocked at a comparable size, but their age could not be confirmed. Average stocking lengths and weights are shown in Table 1.

Table 1: Average stocking lengths (mm) and weights (g) of bass in Inman et al. (1977).

Species	Average Length (mm)	Average Weight (g)
Northern Largemouth Bass	193	173
Florida Bass	182	129
F1 hybrid[1]	198	168

[1](*M. floridanus x M. salmoides*)

Fish were marked by injecting a dye before release, and yearly electrofishing surveys were conducted to monitor the growth of the fish over the course of three years. Initially, it appeared the northern Largemouth Bass had the greatest size difference, but nearly two years after their initial stocking F1 hybrids experienced growth rates almost three times higher than northern Largemouth Bass. Florida Bass exhibited growth rates almost twice that of northern Largemouth Bass. Final average lengths and weights are shown in Table 2.

Table 2: Average final lengths (mm) and weights (g) of bass in Inman et al. (1977).

Species	Average Length (mm)	Average Weight (g)
Northern Largemouth Bass	317	907
Florida Bass	344	1076
F1 hybrid[1]	350	1309

[1](*M. floridanus x M. salmoides*)

Clugston (1964) and Zolczynski and Davies (1976) observed similar increased early-growth in northern Largemouth Bass as Inman et al. (1977). However, they used fingerlings and their grow out times were a year or less. No significant growth advantages were displayed by Florida Bass or F1 hybrids in their studies (Clugston, 1964; Zolczynski & Davies, 1976). Inman et al. (1977) showed that growth advantages of the Florida Bass and F1 hybrids were not apparent until after age three. Zolczynski and Davies (1976) and Inman et al. (1977) performed catchability experiments between the species and found differing results. Zolczynski and Davies (1976) found evidence that Florida Bass were harder to catch in their ponds than northern Largemouth Bass, whereas Inman et al. (1977) found no significant differences in catchability between the two species. It is worth noting these three studies took place in southern states: Florida, Alabama, and Texas, where thermal conditions roughly equivalent to native geographic locations of Florida Bass exist.

Isely et al. (1987) became the first to use a modern genetic analysis to identify bass used in an early growth study. Young of the year bass were collected by seining or electrofishing from four farm ponds in central Illinois that were stocked between 1982 and 1984 with Florida Bass, northern Largemouth Bass, and/or both reciprocal F1 hybrids. Electrophoretic techniques, following the methods in Philipp et al. (1979, 1983), were used to identify stocks with three diagnostic loci used to differentiate Florida Bass from Lake Dora, Florida, and northern Largemouth Bass from Bone Lake, Wisconsin. Backcrosses of the fish were possible as lakes were sampled annually over a three-year time span. Overall, they found that northern Largemouth Bass were significantly larger in total length and weight at the end of the study than Florida Bass. They also found that these differences were independent of age differences, providing further proof of genetic differences underlying a dissimilarity in growth rates between the species (Isely et al., 1987). F1 hybrids were found to be intermediate in growth between the other two species as well. Due to the location of the study, the northern ponds may not have adequately simulated the Florida Bass's native environment, impacting the growth of the Florida Bass and F1 hybrid. It is worth noting that the production of intergrades and backcrosses increased over time in the ponds, which has more recently sparked discussion of potential negative impacts (Philipp et al., 2002; Goldberg et al., 2005).

Williamson and Carmichael (1990) looked at the suitability of Florida Bass, northern Largemouth Bass, and both reciprocal F1 hybrids as an aquaculture species. The Florida Bass used in the study were sourced from an impoundment at Texas A&M University Aquaculture Research Center near College Station, Texas. However, they originated from a stock in Florida. The northern Largemouth Bass were one generation removed from an impoundment in northeastern Texas, and the F1 hybrid bass were from broodstock at the Inks Dam National Fish Hatchery near Burnet, Texas (Williamson et al., 1986b). Fish were confirmed genetically pure by electrophoretic analysis using tissue as described by Carmichael et al. (1986). Fish were stocked into seventeen 0.04 ha production ponds, fourteen of the ponds were stocked separately by strain and fish weighed 1.2-1.3 g on average at stocking. The remaining three ponds were stocked with all strains, similar to this research study, and had average weights of 1.9-2.0 g. All fish were feed trained prior to stocking, so no forage base was present in the ponds. Differences in feed training success were found with northern Largemouth Bass exhibiting the highest training ability and Florida Bass the lowest. Ponds were drained before bass stocking. The separate strain ponds were harvested after 111 days, and the northern Largemouth Bass were found to grow to sizes significantly larger than the Florida Bass and both reciprocal F1 hybrid crosses, as shown in Table 3.

Table 3: Mean harvest weights (g/fish) of bass stocked in separate strain ponds (Williamson & Carmichael, 1990).

Species	Average Weight (g)
Northern Largemouth Bass	117.3
F1 hybrid[1]	90.9
Florida Bass	78.3
F1 hybrid[2]	74.5

[1](*M. salmoides x M. floridanus*)
[2](*M. floridanus x M. salmoides*)

Similar results were seen in ponds stocked with all bass communally that were harvested after 127 days. The northern Largemouth Bass again grew much more quickly than the other strains, as shown in Table 4.

Table 4: Mean harvest weights (g/fish) of bass stocked in communal ponds (Williamson & Carmichael, 1990).

Species	Average Weight (g)
Northern Largemouth Bass	147.9
F1 hybrid[1]	108.5
Florida Bass	90.0
F1 hybrid[2]	84.9

[1](*M. salmoides x M. floridanus*)
[2](*M. floridanus x M. salmoides*)

This provided further evidence of the increased growth advantage of northern Largemouth Bass at a very early age, as observed in earlier studies (Clugston, 1964; Zolczynski & Davies 1976; Inman et al., 1977; Isely et al., 1987). Still, no comparisons within the Florida Bass strain itself were tested or identified.

In a more generalized pond experiment, Kleinsasser et al. (1990) used 0.04-0.48-ha ponds in Texas to evaluate 2nd year growth characteristics of Florida Bass, northern Largemouth Bass, and both F1 hybrid crosses. The origins of the fish are the same as in Williamson and Carmichael

(1990) as previously described, and with further detail in Williamson et al. (1986b). Ten ponds were used in total for the experiment. Ponds 1-4 were stocked at a density of 400 bass per hectare (16 fish per pond). Ponds 5-8 were stocked at a density of 800 bass per hectare (32 fish per pond). Pond 9 was stocked at a density of 340 bass per hectare, and pond 10 was stocked at a density of 275 bass per hectare. Fathead Minnows and Goldfish (*Carassius auratus*) were stocked into ponds 1-4 and replenished when it appeared their numbers became low; however, it is noted that forage became depleted due to its lack of availability during the summer. Ponds 5-8 also received an equal stocking of prey, and ponds 9 and 10 already contained Mosquitofish (*Gambusia affinis*) and Goldfish, so no prey was added. Bass were stocked communally for ponds 1-8 in December of 1984, and three subsequent seines were conducted until they were harvested in December the following year. Ponds 9 and 10 were stocked in January of the same year and harvested the following January. Overall, the F1 hybrid bass (*M. floridanus x M. salmoides*) had the highest average weight by the end of the experiment at 471.0 g, and the Florida Bass had the lowest average weight at 287.4 g (Kleinsasser et al., 1990). The authors did caution that the Florida Bass may have performed poorly due to multiple factors, including not being able to adjust to a small hatchery pond type of environment. The authors also performed a catchability experiment, and like some previous studies found that the northern Largemouth Bass was more susceptible to angling than the Florida Bass (Zolczynski & Davies 1976; Kleinsasser et al., 1990).

Overall, Clugston (1964), Zolczynski and Davies (1976), and Inman et al. (1977) observed that northern Largemouth Bass exhibited increased early growth in comparison to Florida Bass. Zolczynski and Davies (1976) also found that the F1 hybrid bass (*M. floridanus x M. salmoides*) experienced the slowest growth, whereas over a longer growout Inman et al.

(1977) found evidence that Florida Bass or F1 hybrids experienced increased growth advantages in comparison to northern Largemouth Bass. These early studies did not use molecular means to confirm the purity of the bass used in their experiments. As previously discussed, this draws into question some of their findings and conclusions. In the 1980's and early 90's molecular analysis was performed to ensure genetically pure fish were used in experiments. Isely et al. (1987) was the first to use electrophoretic techniques to verify bass used in an early growth study. Isley et al. (1987) found evidence the northern Largemouth Bass exhibited increased early growth over Florida Bass and F1 hybrids similar to Williamson and Carmichael (1990) and previous studies (Clugston, 1964; Zolczynski & Davies, 1976). The geographic differences between these studies should be considered, as one occurred in Illinois and the other in Texas (Isley et al., 1987; Williamson & Carmichael, 1990). In contrast, Kleinsasser et al. (1990) observed that F1 hybrid bass (*M. floridanus x M. salmoides*) outperformed northern Largemouth Bass and Florida Bass during a short growout period in Texas ponds. The genetic purity of the fish used in this study was also confirmed by electrophoretic analyses. All the studies only analyzed the growth of Florida Bass from one specific locality and compared them to northern Largemouth Bass or F1 hybrid crosses. They failed to compare Florida Bass parallel to other geographical localities. Conversely, the current research study uses a parallel comparison.

Stocking Florida Bass Outside its Native Range

As the potential growth advantages of Florida Bass became more apparent, interest in stocking Florida Bass outside their native range increased. This coincided with a surge in reservoir development across the United States. More recently, interest in stocking F1 hybrid bass (*M. floridanus x M. salmoides*) into areas lacking Florida Bass genetics has also grown. However, there are conflicting views regarding stocking Florida Bass and F1 hybrid bass (*M.*

floridanus x M. salmoides) in non-native waters. Heterosis or hybrid vigor has been inconclusive in multiple studies using F1 hybrid bass (both reciprocal crosses) (Williamson and Carmichael, 1986a; Cooke et al., 2001; Philipp et al., 2002; Cooke and Philipp, 2006; Allen et al., 2009). However, in other crosses such as hybrid catfish (*Ictalurus punctatus x Ictalurus furcatus*), increased fitness has been observed (Dunham et al., 1990; Dunham and Brummett, 1999; Li et al., 2004). As a result, this has led to an increase in genetic research examining the differences between Florida Bass and northern Largemouth Bass in addition to both reciprocal F1 hybrid bass crosses.

Philipp et al. (1983) performed a biogeochemical genetic evaluation on Florida Bass and northern Largemouth Bass using gel electrophoresis to analyze phenotypes at 28 enzyme loci. Significant differences were found at two loci that could be used to tell the two species' contributions to any specific population (Philipp et al., 1983). These findings led to the expansion of the geographic area of the intergrade zone first described by Bailey and Hubbs (1949). Overall, Philipp's studies suggested that genetics may influence the thermal tolerances of bass affecting their growth in different environments (Philipp et al., 1981, 1983, 1985). Philipp et al. (1983) also stated that intergrade fish would likely not be suitable for stocking in northern or southern areas because introducing Florida Bass alleles into a population of northern Largemouth Bass could reduce the overall fitness of the fish.

At this time, many of Philipp's conclusions were based on large-scale geographic data, and not parallel comparisons of the fish. Fields et al. (1987) performed an actual direct comparison between species in aquarium experiments. Bass were subjected to a temperature increase of 0.2°C/min at the following acclimation temperatures: 8, 16, 24, and 32°C, and 1°C/day for the 32°C. The bass's critical and thermal maximums were recorded. Fields et al.

(1987) found that Florida Bass had a greater tolerance than northern Largemouth Bass at high temperatures (24 and 32°C), but the difference was not as significant at lower temperatures. Fields et al. (1987) also stated they believed there were likely significant differences between stocks of the same species. For example, a pure northern Largemouth Bass from New York and a pure northern Largemouth Bass from Texas would likely be adapted to their native thermal environments. However, Fields et al. (1987) agreed with Philipp's consensus that stocking bass outside of their native environments was detrimental to populations.

Further research by Koppelman et al. (1988) produced confounding information that there were no significant thermal tolerance differences between the Florida Bass, northern Largemouth Bass, and both reciprocal F1 hybrid bass crosses. The study used bass acclimated to 8, 16, 20, and 32°C, similarly to Fields et al. (1987). However, Koppelman et al. (1988) came to two conclusions: (1) seasonal differences may have produced confounding results of the bass's final thermal preferences, as their data collected during the Spring and Fall was found to be significantly different or (2) there were no significant differences in the thermal preferences between species. The latter provided support for the survival ability of Florida Bass following stocking in nonnative waters.

Maceina et al. (1988b) analyzed Aquilla Lake, a Texas reservoir newly introduced with Florida Bass, for gene flow and life history impacts. Florida Bass were stocked from 1982 to 1985 and originated from the TPWD fish hatchery in Huntsville, Texas. A population of native northern Largemouth Bass inhabited the watershed before the dam was constructed. Bluegills were the only forage species stocked into the ponds. Maceina et al. (1988b) found the genetic inflow from Florida Bass occurred very quickly, and the survival rates for Florida Bass and F1 hybrid bass were higher than the native northern Largemouth Bass. Analysis of the relative weights between the fish showed that Florida Bass were in the worst health condition with the lowest relative weights, F1 hybrid bass had intermediate relative weights, and northern Largemouth Bass had the heaviest relative weights (Maceina & Murphy, 1988a). This observation provided further evidence that there could be genetic differences controlling the performance of the bass.

Philipp and Whitt (1991) performed experiments in central Illinois that analyzed the overwinter survival of northern Largemouth Bass, Florida Bass, and both reciprocal F1 hybrids over three years in 0.08-hectare ponds. Northern Largemouth bass broodstock were collected from Bone Lake, Wisconsin and Florida Bass were collected from Lake Dora, Florida. Reciprocal F1 hybrids were produced from these broodstock. Using electrophoretic techniques, the broodfish and 100 fingerlings from each stock were confirmed for their genetic purity. For evaluation of over-winter survival, several ponds were stocked during the Fall with Fathead Minnows and Crayfish as their main prey. The remaining ponds were stocked in the spring with Bluegills, Fathead Minnows, and Lake Chubsuckers (*Ermyzon sucetta*) as prey. All ponds were drained and harvested the following Fall. Philipp and Whitt (1991) found that Florida Bass had decreased survival in comparison to northern Largemouth Bass overwinter, and F1 hybrids had intermediate survival. Furthermore, northern Largemouth bass also maintained significantly larger total lengths and weights than the other stocks. Philipp and Whitt (1991) proceeded to criticize Maceina et al. (1988b) findings due to their small sample size and collection occurring during bass spawning season. They concluded this may have influenced Florida Bass and F1 hybrids to appear healthier, as northern Largemouth Bass spawned earlier in the experiment (Maceina et al., 1988b).

Maceina later refuted some of Philipp's conclusions by arguing that the ponds used in Philipp and Whitt (1991) were too small and temperature fluctuations would be far greater than in larger reservoirs. In addition, he argued that the most suitable strain of bass for lotic systems should be stocked, since many more had been built in recent years (Maceina & Murphy, 1992). Philipp defended his pond experiments in Illinois as well as other criticisms mentioned by Maceina and Murphy. He felt Maceina and Murphy believed in uprooting the native genetics of a fishery, in hopes that the fishery may improve overall (Maceina & Murphy, 1992).

As stocking programs had been in place for several decades, further research on the impacts of these practices were performed on reservoir systems, even outside of the continental United States. For example, island reservoirs in Puerto Rico were each stocked with Florida Bass, northern Largemouth Bass, and intergrade Largemouth Bass (Neal & Noble, 2002). At age 2, Florida Bass accounted for 76% of the studies catch and after year 3 they were 100% of the catch (Neal & Noble, 2002). This indicated that the Florida Bass exhibited the greatest longevity overall. In comparison, the relative weights of the intergrades were higher than northern Largemouth Bass and Florida Bass for both years 1 and 2 (Neal & Noble, 2002). However, most of the intergrade Largemouth Bass contained more Florida Bass alleles than northern Largemouth Bass, indicating that most were not true F1 hybrid bass. As a result, there was a strong indication that stocking Florida Bass may provide better results than F1 hybrid bass in tropical climates.

In another study on Tennessee's Chickamauga Reservoir, fingerlings of Florida Bass, northern Largemouth Bass, and F1 hybrid bass (*M. salmoides x M. floridanus*) were all stocked and their survival was analyzed, however not at a level to differentiate between the three bass (Hoffman & Bettoli, 2005). All bass fingerlings were marked with oxytetracycline prior to stocking for positive identification. A blind test resulted in 97% of the fish being properly identified (Hoffman & Bettoli, 2005). It appeared stocked fish had an initial length advantage over native fish in the first summer; however, their capture rates decreased after the first year (Hoffman & Bettoli, 2005). It is of interest that despite having an initial growth advantage over wild fish, the stocked fish did not exhibit increased survival. It was not stated where the broodfish for the fingerlings originated. If the Florida Bass individuals used were outsourced from other areas such as Florida this may have impacted the survivability of the fish. Genetic differences resulting from different geographic areas may not only affect the growth of fish, but also their survival ability in non-native environments. Philipp et al. (2002) observed no growth advantages for Florida Bass outside of their native range. Florida Bass stocked into Illinois ponds lost over half of their relative fitness when compared to native northern Largemouth Bass populations (Philipp et al., 2002).

Recently, genetics have been used to (1) identify the impacts of Florida Bass stocking programs and (2) advance conservation genetics of black bass. Li et al. (2015) further advanced genetic identification by developing single nucleotide polymorphism (SNP) markers and developed an initial 25 SNP panel to better differentiate Florida Bass, northern Largemouth Bass, and introgressed fish. This panel was successfully tested on hatchery and wild bass populations and demonstrated Florida Bass stockings over the years in Alabama had significantly changed population genetics in some of these drainages (Li et al., 2015). A similar 35 SNP panel was used to test the broodfish in the current research study to ensure purity. Zhao et al. (2018) expanded the initial SNP panel (Li et al. (2015)) to assign parentage to Florida Bass offspring using a total of 58 SNPs. This is a valuable tool for Florida Bass genetic enhancement for hatchery production. A SNP panel such as this can identify which parental fish produced the most-fit offspring. Although most SNP developments benefited Florida Bass, northern Largemouth Bass, and their intergrades, Thongda et al. (2019) developed a panel that could delineate between 15 different species of black bass and show levels of hybridization. This was essential in aiding the conservation of black bass species threatened by introductions of non-native black bass. In general, Thongda et al. (2019) demonstrated how SNPs could be utilized in conservation and management efforts of threatened species.

Several studies used the SNP procedures developed by Li et al. (2015) and Zhao et al. (2018) to analyze the impacts of Florida Bass stockings. Hargrove et al. (2019) tracked the introgression of Florida Bass alleles into 13 South African lakes from past introductions. Hargrove et al. (2019) found significant variation between lakes regarding hybridization, with some populations containing a high majority of Florida Bass or northern Largemouth Bass alleles. This study served as the first analysis of northern Largemouth Bass and Florida Bass introgression outside of the United States (Hargrove et al., 2019).

Hargrove et al. (2020) evaluated if long-term Florida Bass stocking in Lake Chickamauga, TN had resulted in significant contributions to tournament angled fish. Fin clips were collected from both angler and electrofishing captured fish for genetic testing. No significant differences were found between both angler and electrofishing catches. The most abundantly caught fish was a northern Largemouth Bass x F1 hybrid with this group totaling 41% of electrofishing samples and 43% of angling samples. This study provided evidence that F1 (and later-stage) hybrids may contribute a significant portion of tournament catches on a lake with a long-term Florida Bass stocking program (Hargrove et al., 2020). As the cost effectiveness and efficiency of SNP technologies for monitoring bass genetics continue to improve, state and private agencies will have much more ease in performing detailed analysis of black bass populations scaling from large reservoirs to small impoundments (Johnson, 2019).

Study Objective

The overall objective of the current research study was to investigate the potential for differences in early growth genetic potential within the Florida Bass (*M. floridanus*) strain, as well as in comparison to an F1 hybrid bass (*M. floridanus x M. salmoides*). This research should lead to advancements in the development of next-generation F1 hybrid bass utilizing superior Florida Bass individuals/strains. More specifically, this study used a common garden experimental model on ponds to monitor growth of two localities of Florida Bass and an F1 hybrid bass with passive integrated transponder (PIT) tags.

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Chapter 2: Examining early growth genetic potential of two distinct localities of Florida Bass and an F1 hybrid bass (*Micropterus floridanus x Micropterus salmoides*)

Abstract

Florida Bass (*M. floridanus*) have long been recognized for their higher maximal growth potential in comparison with northern Largemouth Bass (*M. salmoides*) when stocked into optimal environments. Current studies have failed to compare growth performance among phenotypically and/or geographically-distinct Florida Bass populations. In the current study, we evaluated two Florida Bass populations and one F1 hybrid bass population (*M. floridanus x M. salmoides*). One Florida Bass population was sourced from a central Florida phosphate mine lake, South Pasture. Another was an established commercial strain under long-term culture and selection at the American Sportfish Hatchery (ASFH) in Pike Road, AL. The F1 hybrid bass (*M. floridanus x M. salmoides*) population resulted from crossing pure Florida Bass and northern Largemouth Bass on-site at American Sportfish Hatchery. All utilized broodfish were confirmed in their classification as pure Florida Bass or northern Largemouth Bass via single nucleotide polymorphism (SNP) genotyping. Using a common garden experimental model, the two populations of Florida Bass and the F1 hybrid were tagged with passive integrated transponder (PIT) tags as juveniles and mixed into common pond environments with optimal forage densities. Their growth was monitored over an 8-month period by conducting four seine samples. We observed significantly greater ($p \leq 0.05$) final mean lengths (mm), weights (g), and relative weights (Wr) in F1 hybrid bass (*M. floridanus x M. salmoides*), in comparison to both populations of Florida Bass. F1 hybrid bass (*M. floridanus x M. salmoides*) also exhibited a significantly greater ($p \leq 0.05$) overall growth rate than either Florida Bass population. Differences between Florida Bass populations were observed as well. The ASFH population had a greater percentage change in weight gain (199.30%) than the South Pasture population

(121.57%). However, the South Pasture population had a greater overall increase in weight (70 g). The ASFH population also had a greater percentage/overall increase in length than the South Pasture population. The study provides evidence of variability in early growth genetic potential within Florida Bass and opens the door to advancing the development of next-generation F1 hybrid bass by maximizing superior Florida Bass individuals/strains.

Introduction

Black bass (*Micropterus spp.*) are one of the most desired freshwater sportfish among anglers across the world. Anglers have created a fast-expanding industry resulting in an annual economic benefit in excess of millions of dollars for states and local communities with thriving bass populations (Kerr & Kamke, 2003; Schramm & Hunt, 2007; Driscoll & Myers, 2014). Although sometimes unrecognized by the angling public, in the scientific community, Largemouth Bass are accepted as two distinct species: northern Largemouth Bass (*M. salmoides*) and Florida Bass (*M. floridanus*) (Kassler et al., 2002; Near et al., 2003; Barthel et al., 2010). Florida Bass have been long heralded for their higher maximal growth potential when stocked into optimal environments in comparison to northern Largemouth Bass. This observation has led to numerous research studies examining growth differences between the two species as well as their reciprocal F1 hybrids. Much of the conventional wisdom regarding bass genetics has been based on these studies. However, these findings may be flawed because the genetic origin of the fish in the study could not be confirmed (Clugston, 1964; Zolczynski & Davies 1976; Inman et al., 1977).

As genetic analyses advanced and became available for fisheries applications, growth studies implemented electrophoretic techniques, as first described by Philipp et al. (1983), to genetically verify fish. Isley et al. (1987) and Williamson and Carmichael (1990) found evidence that northern Largemouth Bass exhibited increased early growth over Florida Bass and F1 hybrids. It should be noted, geographical differences were present, as Isley's study took place in Illinois, and Williamson's in Texas. In contrast, Kleinsasser et al. (1990) found F1 hybrid bass (*M. floridanus x M. salmoides*) outperformed northern Largemouth Bass and Florida Bass in Texas ponds.

Further genetic research examining the differences between Florida Bass and northern Largemouth Bass continued, as the interest in public and private of stocking Florida Bass into areas outside of their native range grew. Many studies aimed to identify the relative fitness of Florida Bass stocked into non-native environments. These studies sparked a still ongoing debate among biologists, resource managers, and the angling public (Maceina et al., 1988b; Philipp and Whitt, 1991; Maceina & Murphy, 1992). Recently, the development of single nucleotide polymorphism (SNP) markers and panels have increased the accuracy of genetic identification while lessening the cost and effort required in identifying Florida Bass, northern Largemouth Bass, and their intergrades (Li et al., 2015; Zhao et al., 2018). In general, this advancement has been a valuable tool for Florida Bass genetic selection during hatchery production, as well as for state agencies establishing genetic baselines and investigating the impacts of stocking Florida Bass and F1 bass into their public waterways.

Past studies have treated Florida Bass (and, for the most part northern Largemouth Bass) as a single invariant population, when their widespread geographic ranges (with associated water quality, temperature, forage differences etc.) would predict that variations in performance would exist among populations within the species. Therefore, in the current study, two Florida Bass populations and one F1 hybrid (*M. floridanus x M. salmoides*) population were compared. The results of this study provide an updated genetic assessment of early growth between Largemouth Bass species and within the Florida Bass species itself. In addition, these findings should aid in the development of next-generation F1 hybrid bass (*M. floridanus x M. salmoides*) utilizing superior Florida Bass populations and individuals.

Materials and Methods

SNP Genotyping/Purity Confirmation

In late February 2019, broodfish were obtained from two specific localities. One population of Florida Bass originated from central Florida phosphate mine lake, South Pasture (27º 35´ 44.18″ N, 82º 03´ 09.49″ W). The second population of Florida Bass and northern Largemouth Bass were an established commercial strain under long-term culture and selection at the American Sportfish Hatchery, located in Pike Road, AL. All utilized broodfish were confirmed in their classification as pure Florida Bass or northern Largemouth Bass via SNP genotyping. F1 hybrid bass (*M. floridanus x M. salmoides*) resulted from crossing a mixed Florida Bass line (ASFH, S. Pasture, and Duette) with an ASFH line of northern Largemouth Bass. Several batches of F1 hybrid fingerlings were also confirmed of their purity via SNP genotyping.

For this process, fin clips were collected from each broodfish and stored in 95% ethanol. A digestion method utilizing sodium hydroxide (NaOH) and hydrochloric acid (HCL) was used to extract deoxyribonucleic acid (DNA) from the samples. For this method, 100 µl of NaOH was added to a 2 mL centrifuge tube containing the fin clip. The sample was vortexed and placed in an incubator at 95 ºC for 1 hr. After samples were broken down, they were placed in a refrigerator for 5 min to cool and 100 µl of HCL was added to each sample. The tubes were vortexed again, and then centrifuged at 8,000 rpm for 1 min. The samples were then stored at 4 ºC.

Using a NanoDrop ND-2000 UV-VIS Spectrophotometer, the DNA concentrations (ng/ul) and purity ratios (260/230, 260/280) were estimated. Next, the samples were SNP genotyped on the 35-plex FLNB SNP panels developed by Zhao et al. (2018) using the Agena MassARRAY iPLEX platform following the manufacturer's protocol (Agena Bioscience® Inc., San Diego, CA). To perform the SNP genotyping process, two µl of each DNA sample was transferred using a multi-channel pipette into a 96-well plate and then subjected to three polymerase chain reactions (PCR). The first reaction used designed primers and the iPLEX Gold Reagent Kit to amplify target regions of our markers according to manufacturer protocol (Gabriel et al., 2009). Parameters for the first PCR are as follows: pre-denaturation at 94 ºC for 2 min, 45 cycles of denaturation at 94 ºC for 30 s, annealing at 56 ºC for 30 s, extension at 72 ºC for 1 min, and a final extension at 72 ºC for 5 min. The second PCR used shrimp alkaline phosphatase to remove unincorporated dNTPs from PCR amplification products. Parameters for the second PCR are as follows: enzyme activation at 37 ºC for 40 min, and enzyme degradation at 85 ºC for 5 min. The third PCR extended the primer by one mass-modified nucleotide depending on allele and assay design (Gabriel et al., 2009). Parameters for the third PCR are as follows: pre-denature at 94 ºC for 30 s, 45 cycles of denaturation at 94 ºC for 5 s, annealing at 52 ºC for 5 s, extension at 80 ºC for 5 s, and final extension at 72 ºC for 5 min.

Once the PCR reactions were completed, 41µl of HPLC grade water was added to all 96 wells. SpectroCLEAN resin was then added to the wells to remove unwanted salts that could possibly cause distortion later in the analysis process. The plate was then rotated at 360º for 20 min and spun down in a centrifuge at 2000 rpm for 5 min. The Agena MassARRAY Nanodispenser transferred samples from 96-well plate to a silica chip using the capillary action of slotted pins and contact dispersing for nanovolumes (Gabriel et al., 2009). The chip was then placed into the MassARRAY compact mass spectrometer. Each sample was excited with a laser under vacuum by the matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) method (Gabriel et al., 2009). SNP genotypes were called using the SEQUENOM SYSTEM TYPER 4.0 Analysis software. Genotypes were categorized based on the following allele significances: conservative, moderate, aggressive, and user call.

The genotypes were then analyzed in RStudio (version 1.0.136) and compared to a reference genotype of a pure northern Largemouth Bass (NB) and a pure Florida Bass (FB). (Table 5; Zhao et al., 2018). For every genotype, the NB allele frequency, the FB allele frequency, heterozygous (HE) allele frequency, and homozygous allele frequency were computed using the following formulas:

$$FB\ Allele\ Frequency\ =\ ((FB\ SNPs)\ +\ (1/2 * HE\ SNPs)\ /\ Total\ Scored\ SNPs)$$

$$NB\ Allele\ Frequency\ =\ ((NB\ SNPs)\ +\ (1/2 * HE\ SNPs)\ /\ Total\ Scored\ SNPs)$$

$$Heterozygous\ Allele\ Frequency\ =\ ((HE\ SNPs)\ /\ Total\ Scored\ SNPs)$$

$$Homozygous\ Allele\ Frequency\ =\ ((FB\ SNPs\ +\ NB\ SNPs)\ /\ Total\ Scored\ SNPs)$$

Frequencies were used to calculate the percentages of FB, NB, heterozygosity, and homozygosity for each individual by multiplying the frequencies by 100% (Davis, 2018; Johnson, 2019).

Table 5: List of the 35 SNP markers with fixed allelic differences used to determine purity and hybridization of Florida Bass and northern Largemouth Bass. The reference genotypes displayed are those of a pure Florida Bass (*M. floridanus*), pure northern Largemouth Bass (*M. salmoides*), and a pure F1 hybrid.

Markers	Florida Bass	Northern Largemouth Bass	F1 Hybrid Bass
X2FLContig12388	T	A	TA
X2FLContig124	T	C	TC
X2FLContig132	G	A	GA
X2FLContig18667	G	A	GA
X2FLContig19961	T	C	TC
X2FLContig2242	T	C	TC
X2FLContig2279	T	G	TG
X2FLContig2283	T	G	TG
X2FLContig2861	A	G	AG
X2FLContig288	A	T	AT
X2FLContig31979	T	A	TA
X2FLContig3379	G	A	GA
X2FLContig4936	C	T	CT
X2FLContig5713	A	G	AG
X2FLContig692	C	T	CT
X2FLContig9758	G	T	GT
X2FLContig987	G	A	GA
X2FLContig8717	T	C	TC
FLContig11272	T	C	TC
FLContig1595	T	C	TC
FLContig16665	A	C	AC
FLContig17151	A	T	AT
FLContig1811	C	A	CA
FLContig1826	T	A	TA
FLContig298	A	G	AG
FLContig21621	G	A	GA
FLContig21676	G	A	GA
FLContig21917	C	T	CT
FLContig2635	A	G	AG
FLContig3296	T	G	TG
FLContig3616	A	G	AG
FLContig4773	C	T	CT
FLContig4919	T	G	TG
FLContig6127	C	G	CG
NBContig12358	G	A	GA
Fixed Alleles	**35**	**35**	**35**

Pond Experiment

All procedures involving the handling and treatment of fish used during this study were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC) prior to initiation. The procedures are as follows:

In mid-April 2019, broodfish were moved to individual spawning tanks with 22°C well water to promote spawning. Eight pairs of fish were stocked into each tank. Spawntex spawning mats produced by Pentair® (45.72 x 60.96 cm) were placed in these tanks for the bass to lay their eggs (~10,000 per mat). Mats with eggs were collected and hung in cages (to prevent escapement of fry) for 3 days while eggs hatched. Once fry were observed to swim in the cages, they were transported to ponds for further growout throughout May. A 50.8 mm size was reached in early June. All spawning practices took place at the American Sportfish Hatchery in Pike Road, AL.

On September 6th, 2019 500 Florida Bass of each locality and the F1 hybrid bass were stocked into three separate ponds at the United States Department of Agriculture (USDA) facility in Auburn, AL. These ponds were stocked with 3.63 kg of forage consisting of Bluegills and Fathead Minnows on March 18th, 2019. An additional 45 kg of Fathead Minnows and ten Grass Carp (*Ctenopharyngodon idella*) per pond were stocked on June 24th, 2019.

Figure 1: Aerial photograph of USDA facility.

On December 12th, 2019 the growout ponds were seined and the three populations of fish were held in their own individual tanks. Due to the lack of numbers found in the two Florida Bass population growout ponds, some fish had to be supplemented from a pole barn facility at Auburn's Upper Fisheries Station, where extra bass from each population were held in tanks. Each of these fish were then randomly selected, anesthetized using tricaine methanesulfonate (MS-222), and intramuscularly passive integrated transponder (PIT) tagged. The tag number, length, weight, and population of each fish was then recorded using a Trovan® FishReader W system with ZeusCapture software. Fish were then placed into a holding tank to recover before being transported to their subsequent study pond. This process was completed by stocking a specific population on a per pond basis before beginning on the next population to prevent any mix-ups. The use of a common garden experimental model reduced the impacts of any individual pond variability on results. Thirty fish from each population were stocked into 4 separate study ponds.

Figure 2: Aerial photograph of the four study ponds used at the USDA facility.

Aeration was provided by ¾ hp/120V Kasco Marine® High Oxygen Transfer Aerators during nighttime hours (7pm-6:30am) throughout the study. Ponds were fertilized late-March 2020 at a rate of 0.68 kg per pond with Perfect Pond Plus® fertilizer and 4.73 L per pond of CAL FLO® liquid limestone to promote algal blooms. To ensure adequate forage, supplemental forage stockings also took place. On January 18th, 2020 15.87 kg of Golden Shiners (*Notemigonus* *crysoleucas*) and on May 1st, 2020 5.90 kg of Fathead Minnows (*P. promelas*) were stocked into each study pond. Over the course of the experiment daily dissolved oxygen (DO) was monitored with a YSI® Pro20, and temperatures were recorded with ONSET® HOBO Water Temp Pro v2 loggers.

Four sample seines occurred on the following dates: February 25th, April 6th, June 15th, and August 11th, 2020. On each day, an additional seine pull per pond could occur to maximize fish recapture if low numbers were found in the initial seine pull. Data collection followed the same procedure during as initial stocking. During the June 15th harvest, ten fish were randomly selected from each study pond and euthanized using MS-222 for sex determination.

Statistical Analyses

The standard weight (Ws) regression equation:

$$log10Ws = -5.528 + 3.273\, log10Total\ Length$$

was used to calculate relative weight (Wr) index (Henson, 1991). Length, weight, and relative weight (Wr) data was modeled with auto regression 1 using a repeated measures factorial analysis of variance (ANOVA) with a mixed procedure in SAS version 9.4. Non-normal data was observed, so a $\log_{10}$ transformation was applied prior to conducting Tukey's Honest Significant Differences (HSD) post-hoc tests and p-values. A general linear slopes model, including a Student's t-test, was used to identify significant differences between the slopes of regression lines (Zar, 1996). Single-factor ANOVAs with a mixed procedure and Tukey's HSD post-hoc tests were also used to identify differences between growth rates, sex, and biomass values, and any data that was non-normal was $\log_{10}$ transformed. A p-value of less than 0.05 was considered statistically significant for all tests.

Results

Pond Parameters

Hardness and alkalinity were monitored for each of the experimental ponds. Hardness and alkalinity values fell within 30-55 ppm, which is representative of small impoundments in the Piedmont region of Alabama. Recorded values are shown in Table 6.

Table 6: Hardness (ppm) and alkalinity (ppm) in study ponds over the course of the experiment.

Date	Hardness	Alkalinity
1/25/19	30	30
3/27/20*	40	40
5/14/20	35-45	40-45
8/5/20	45-50	45-55

**After the addition of 0.68 kg per pond of Perfect Pond Plus® fertilizer and 4.73 L per pond of CAL FLO® liquid limestone*

Pond temperatures were recorded at 30-minute intervals throughout the study. Temperature ranged from 12-32° C over the eight-month time span. Values missing from mid-June to mid-July were due to removal of ONSET® HOBO Water Temp Pro v2 loggers during this time. Pond temperatures are plotted in Figure 3.

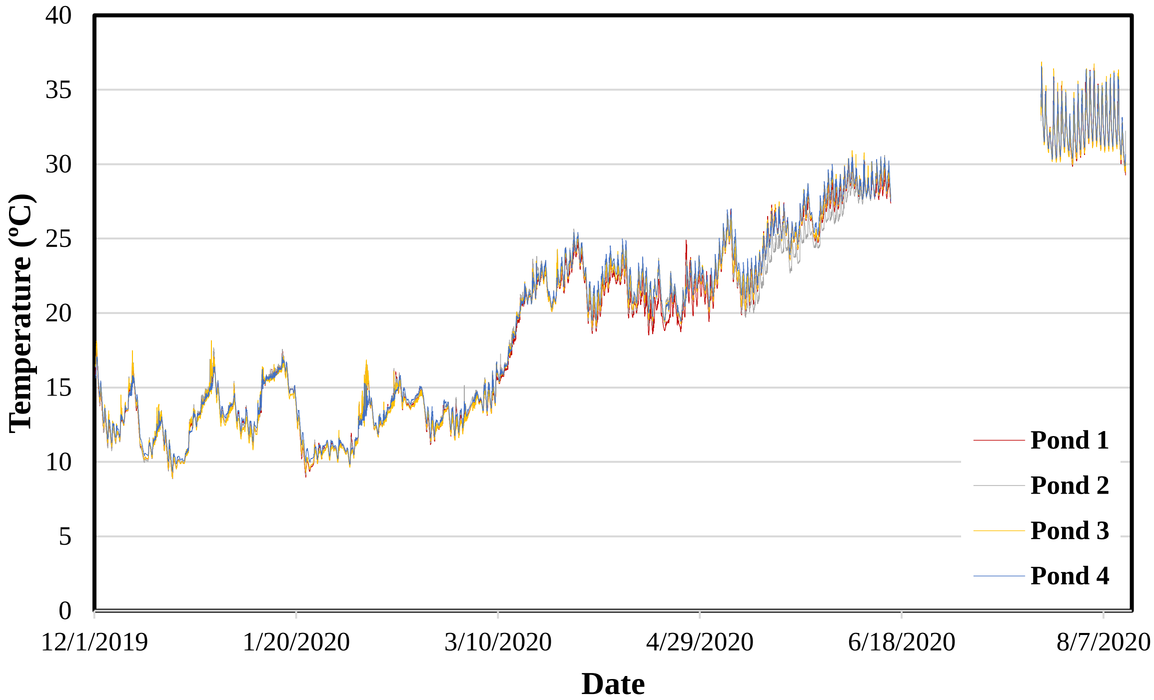


Figure 3: Temperature (°C) of study ponds from December 12, 2019 to August 11th, 2020.

Water visibility, as measured with a secchi disk, varied throughout the study as a function of the phytoplankton and zooplankton bloom. Average secchi depths are shown in Table 7. Pond 4 had the highest overall average secchi depth at 85 cm, Pond 3 had the lowest overall secchi depth at 53 cm.

Table 7: Average secchi depth (cm) per pond over the course of the study.

Sample Period	Pond 1	Pond 2	Pond 3	Pond 4
December-February	116	81	55	131
March-April*	48	58	53	72
May-June	54	30	50	66
Overall	67	56	53	85

**The addition of 0.68 kg per pond of Perfect Pond Plus® fertilizer and 4.73 L per pond of CAL FLO® liquid limestone occurred late-March.*

Recorded dissolved oxygen concentrations (ppm) were highest during the Winter months of 2019-2020. Throughout Spring 2020 they remained ~8 ppm as aided by aerators during night.

Dissolved oxygen concentrations were sampled primarily in the afternoon over a six-month period of the study are shown in Figure 4.

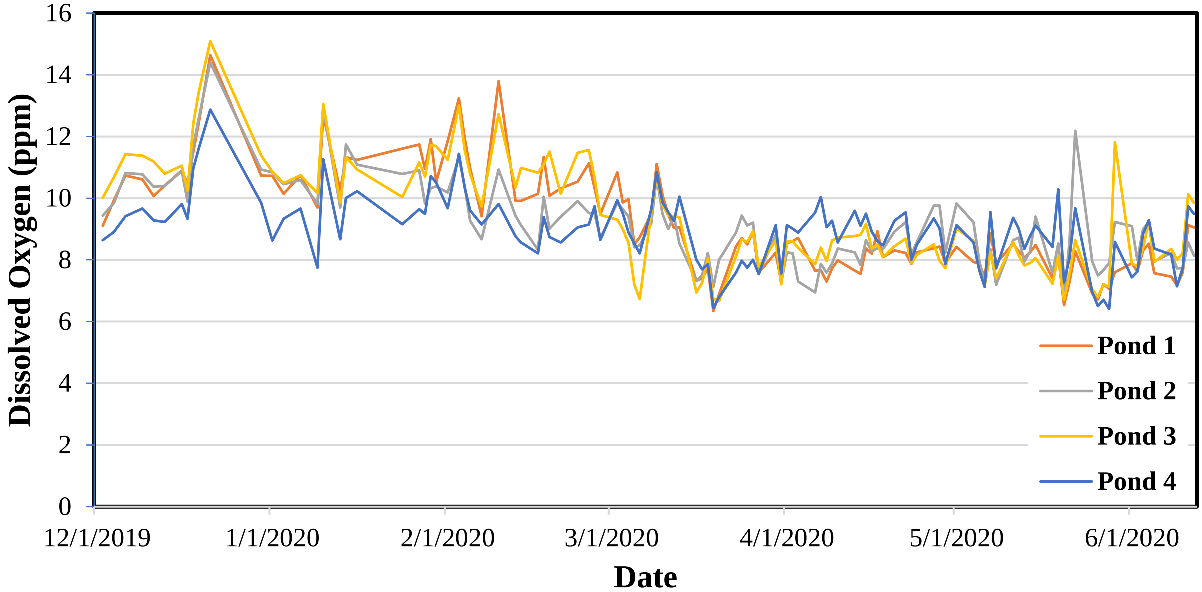


Figure 4: Dissolved oxygen concentrations (ppm) in study ponds from December 12, 2019 to June 15th, 2020.

Length Among Populations

Over the course of the study, mean total lengths (mm) were consistent among each population across all study ponds. However, ASFH populations were significantly smaller ($p \leq 0.05$), than South Pasture and F1 hybrids (*M. floridanus x M. salmoides*) at initial stocking. This overall pattern remained constant until the third seine at which point F1 hybrids (*M. floridanus x M. salmoides*) were observed to be significantly larger ($p \leq 0.05$) than both ASFH and South Pasture populations. Mean total lengths (mm) between ponds over time are shown in Table 8. Standard errors are shown in Appendix 1.

Table 8: Mean total length (mm) between populations and ponds. Statistical comparisons were conducted among genetic groups at each seine sampling timepoint (columns). Bolded values within each column with different letters are significantly different ($p < 0.05$).

Population	Pond	Mean total length (mm)				
		Initial Stocking	Seine 1	Seine 2	Seine 3	Seine 4
ASFH	1	135.33	151.52	170.00	197.73	200.33
	2	145.17	166.88	175.63	205.19	215.71
	3	127.83	142.62	180.45	212.22	205.00
	4	114.83	133.48	169.38	198.24	211.54
	Overall	**130.79 x**	**148.96 x**	**173.86 x**	**203.31 x**	**208.30 x**
F1	1	156.67	179.82	206.67	240.95	251.43
	2	156.50	182.80	216.67	249.29	261.92
	3	165.00	185.20	211.50	248.70	252.19
	4	164.50	190.17	221.36	259.47	270.45
	Overall	**160.67 y**	**184.58 y**	**215.24 y**	**249.35 y**	**258.06 y**
South Pasture	1	156.17	175.42	200.00	215.00	208.57
	2	160.33	175.89	201.36	212.11	211.92
	3	173.67	182.75	212.50	226.00	230.00
	4	154.00	176.75	206.43	225.48	230.31
	Overall	**161.04 y**	**177.45 y**	**205.00 y**	**219.65 z**	**220.36 z**

In addition, over the entire study, both the F1 hybrid (*M. floridanus x M. salmoides*) and South Pasture populations were significantly larger ($p \leq 0.05$) than the ASFH population in terms of length. As shown in Table 9, F1 hybrids (*M. floridanus x M. salmoides*) exhibited the greatest average increase in length with a gain of 97.39 mm. Non-significant differences between populations of Florida Bass were evident as ASFH gained an average of 77.51 mm and South Pasture gained an average of 59.32 mm from the initial stocking until the fourth seine. Percentages of overall length increase followed the same pattern.

Table 9: Least squares means (±SE) total length (mm) values from initial stocking and seines. Statistical comparisons were conducted among genetic groups at each seine sampling timepoint (rows). Values within each row with different letters are significantly different ($p < 0.05$). Percent value represents percentage change between the prior sample date. Values in parentheses indicate the sample size of fish.

Sample	Population		
	ASFH	F1 hybrid[1]	South Pasture
Initial Stocking 12/12/19	130.79 ± 2.75 x (120)	160.67 ± 2.75 y (120)	161.04 ± 2.75 y (120)
First Seine 2/25/20	148.96 ± 3.20 x 13.89% (91)	184.58 ± 2.95 y 14.88% (107)	177.45 ± 3.18 y 10.19% (92)
Second Seine 4/21/20	173.86 ± 3.11 x 16.72% (40)	215.24 ± 2.82 y 16.61% (42)	205.00 ± 5.25 y 15.53% (37)
Third Seine 6/15/20	203.31 ± 2.70 x 16.94% (83)	249.35 ± 2.68 y 15.85% (84)	219.81 ± 2.75 z 7.22% (80)
Fourth Seine 8/11/20	208.30 ± 3.29 x 2.45% (56)	258.06 ± 3.35 y 3.49% (54)	220.36 ± 3.32 z 0.25% (55)

[1](*M. floridanus x M. salmoides*)

Linear regression lines/statistics as shown in Table 10 and Figure 5 display that ASFH and F1 hybrids (*M. floridanus x M. salmoides*) had nearly identical slopes: 0.058 and 0.057. Both were found to be significantly greater ($p \leq 0.05$), than the South Pasture population, with a slope of 0.040. Thus, over time ASFH and F1 hybrids (*M. floridanus x M. salmoides*) experienced greater increases in length than the South Pasture population.

Table 10: Length (mm) log_{10} transformed regression statistics for each population. Values are as follows: slope (b_1), intercept (b_0), regression variance (MSE), coefficient of determination (r^2), and number of fish collected (N).

Statistic	Population		
	ASFH	F1 hybrid[1]	South Pasture
b_1	0.058	0.057	0.040
b_0	2.791	2.149	2.162
MSE	0.0045	0.0026	0.0061
r^2	0.617	0.719	0.362
N	390	407	384

[1](*M. floridanus x M. salmoides*)

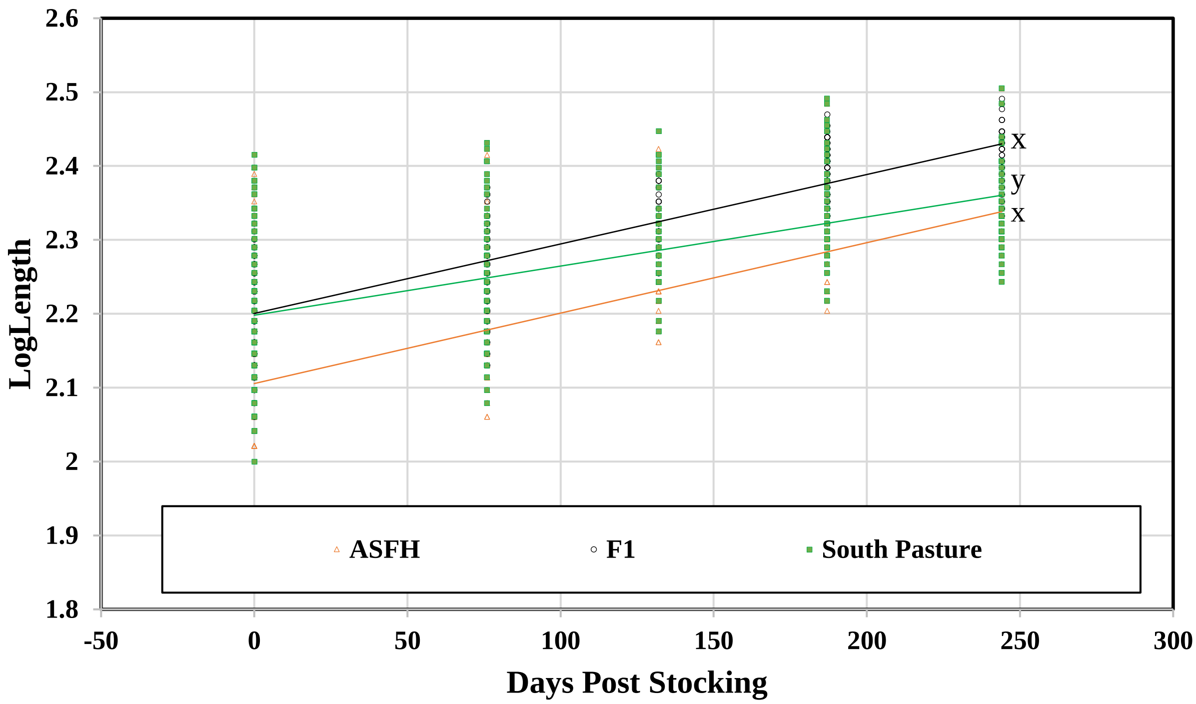


Figure 5: Length (mm) log_{10} transformed over time for each population of fish. Lines with different letters were significantly different ($p < 0.05$).

Weight Among Populations

During the study period, mean weights (g) among ponds per population of fish were consistent. Similar to length measurements, ASFH populations were significantly smaller ($p \leq 0.05$), than South Pasture and F1 hybrids (*M. floridanus x M. salmoides*) during the initial stocking. However, after the initial stocking, all populations manifested significantly different weights from each other ($p \leq 0.05$) at each sampling time point, with F1 hybrids (*M. floridanus x M. salmoides*) maintaining the heaviest weights when compared to ASFH and South Pasture populations. Mean total weights (g) between ponds over time are shown in Table 11. Standard errors are shown in Appendix 2.

Table 11: Mean weight (g) between populations and ponds. Statistical comparisons were conducted among genetic groups at each seine sampling timepoint (columns). Bolded values within each column with different letters are significantly different ($p < 0.05$).

Population	Pond	Mean weight (g)				
		Initial Stocking	Seine 1	Seine 2	Seine 3	Seine 4
ASFH	1	35.10	51.00	58.08	90.64	84.53
	2	51.00	82.17	67.00	103.77	114.21
	3	28.90	42.14	81.09	114.61	90.54
	4	17.27	31.00	64.25	90.59	107.07
	Overall	**33.07 x**	**52.12 x**	**67.43 x**	**99.94 x**	**98.98 x**
F1	1	44.13	85.29	112.17	182.19	196.07
	2	45.67	94.48	152.67	210.00	228.54
	3	54.33	91.88	133.80	207.52	193.31
	4	52.63	108.14	156.91	232.84	250.91
	Overall	**49.19 y**	**95.17 y**	**143.50 y**	**207.54 y**	**214.24 y**
South Pasture	1	54.90	85.46	116.00	124.30	100.57
	2	55.73	86.11	111.55	118.11	109.46
	3	70.40	90.30	129.40	146.95	141.67
	4	49.27	86.10	119.86	143.57	155.38
	Overall	**57.58 y**	**86.85 z**	**119.03 z**	**133.55 z**	**127.58 z**

The South Pasture population also maintained significantly heavier ($p \leq 0.05$) weights than the ASFH population over the course of the study. As shown in Table 12, F1 hybrids exhibited the greatest average increase in weight with a gain of 165.05 g over the course of the experiment. Non-significant differences between the populations of Florida Bass were evident, as South Pasture gained an average of 70.00 g and ASFH gained an average of 65.91 g (335.54%) from the initial stocking to the fourth and final seine. However, ASFH had a greater overall percentage increase in weight gain (199.30%) than South Pasture (121.57%).

Table 12: Least squares means (±SE) weight (g) values from initial stocking and seines. Statistical comparisons were conducted among genetic groups at each seine sampling timepoint (rows). Values within each row with different letters are significantly different ($p < 0.05$). Percent value represents percentage change between the prior sample date. Values in parentheses indicate sample size of fish.

Sample	Population		
	ASFH	F1 hybrid[1]	South Pasture
Initial Stocking 12/12/19	33.07 ± 3.44 x (120)	49.19 ± 3.44 y (120)	57.58 ± 3.44 y (120)
First Seine 2/25/20	52.12 ± 5.69 x 57.61% (91)	95.17 ± 7.21 y 93.47% (107)	86.85 ± 7.68 z 50.83% (102)
Second Seine 4/21/20	67.43 ± 7.39 x 29.37% (40)	143.50 ± 6.57 y 50.78% (42)	119.03 ± 10.00 z 37.05% (37)
Third Seine 6/15/20	99.94 ± 5.95 x 48.21% (83)	207.54 ± 5.92 y 44.64% (84)	133.55 ± 6.06 z 12.20% (80)
Fourth Seine 8/11/20	98.98 ± 7.69 x -0.96% (56)	214.24 ± 7.83 y 3.23% (54)	127.58 ± 7.76 z -4.47% (55)

[1](*M. floridanus x M. salmoides*)

Linear regression lines/statistics, as shown in Table 13 and Figure 6, display that all populations of bass had significantly different ($p \leq 0.05$) slopes. Therefore, F1 hybrids experienced the greatest increase in weight over time with a slope of 0.184. Differences between Florida Bass were present as well as the ASFH population had a significantly greater ($p \leq 0.05$) slope (0.162) than the South Pasture population (0.115).

Table 13: Weight (g) $\log_{10}$ transformed regression statistics for each population. Values are as follows: slope (b_1), intercept (b_0), regression variance (MSE), coefficient of determination (r^2), and number of fish collected (N).

Statistic	Population		
	ASFH	F1 hybrid[1]	South Pasture
b_1	0.162	0.184	0.115
b_0	1.262	1.511	1.582
MSE	0.0478	0.0365	0.0653
r^2	0.542	0.655	0.302
N	390	407	384

[1](*M. floridanus x M. salmoides*)

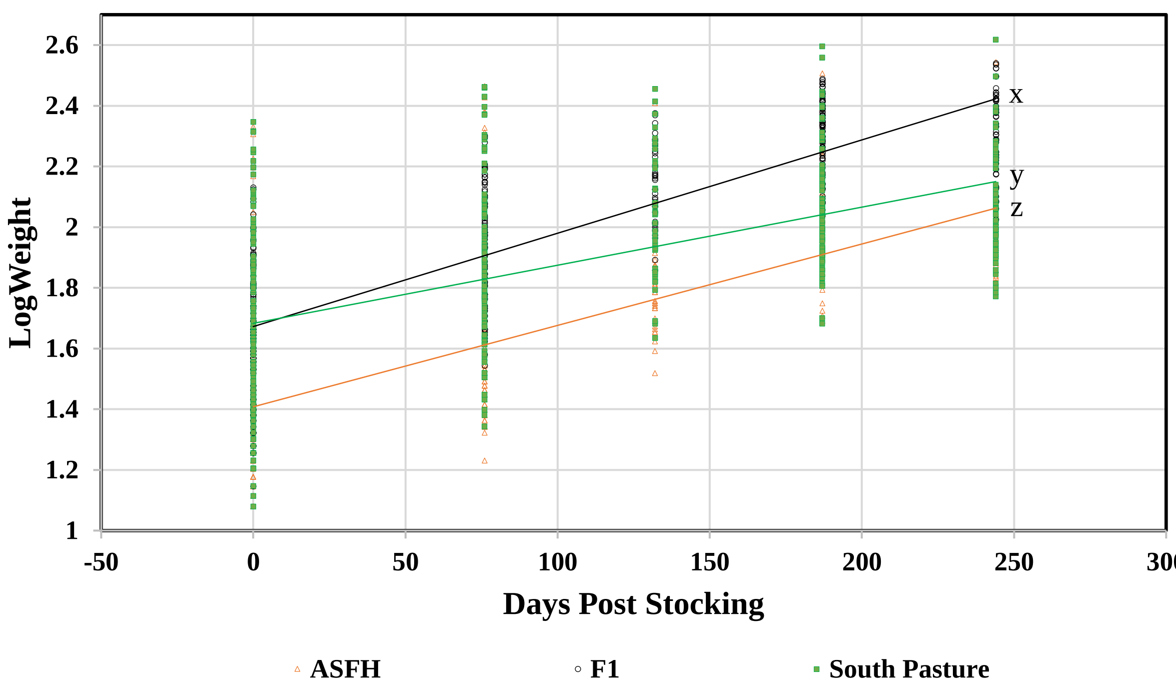


Figure 6: Weight (g) $\log_{10}$ transformed over time for each population of fish. Lines with different letters were significantly different ($p < 0.05$).

Relative Weights Among Strains

Similar to lengths (mm) and weights (g), Relative weights (Wr) among ponds per population of fish were relatively uniform over time. At the initial stocking, relative weights among populations were all significantly different ($p \leq 0.05$), with ASFH having the highest mean relative weight at 107.70 and F1 hybrids with the lowest at 90.26. However, by the first seine, F1 hybrids achieved a significantly higher ($p \leq 0.05$) mean relative weight than the other two populations and remained at this level through the fourth seine. Mean relative weights (Wr) between ponds over time are shown in Table 14. Standard errors are shown in Appendix 3.

Table 14: Mean relative weights (Wr) between populations and ponds. Statistical comparisons were conducted among genetic groups at each seine sampling timepoint (columns). Bolded values within each column with different letters are significantly different ($p < 0.05$).

Population	Pond	Mean relative weight (Wr)				
		Initial Stocking	Seine 1	Seine 2	Seine 3	Seine 4
ASFH	1	107.52	105.00	95.82	90.76	82.71
	2	112.37	112.83	101.78	91.22	83.22
	3	106.96	104.68	101.87	90.74	82.31
	4	103.96	114.11	107.14	91.42	86.99
	Overall	**107.70 x**	**109.29 x**	**100.94 x**	**91.03 x**	**83.81 x**
F1	1	90.04	114.49	99.94	96.36	89.58
	2	88.46	117.75	112.32	99.39	90.87
	3	92.31	111.28	108.75	98.65	88.37
	4	90.23	120.27	109.52	96.64	89.31
	Overall	**90.26 y**	**116.07 y**	**109.00 y**	**97.81 y**	**89.48 y**
South Pasture	1	105.65	108.69	101.74	89.86	82.34
	2	101.35	114.61	102.57	92.53	87.17
	3	94.22	103.15	98.42	90.36	83.00
	4	96.66	111.77	102.60	90.48	91.10
	Overall	**99.47 z**	**110.00 x**	**101.25 x**	**90.78 x**	**86.17 x**

From the first seine until the end of the study, both Florida Bass populations' relative weights were not considered to be significantly different ($p \leq 0.05$) from one another. As shown in Table

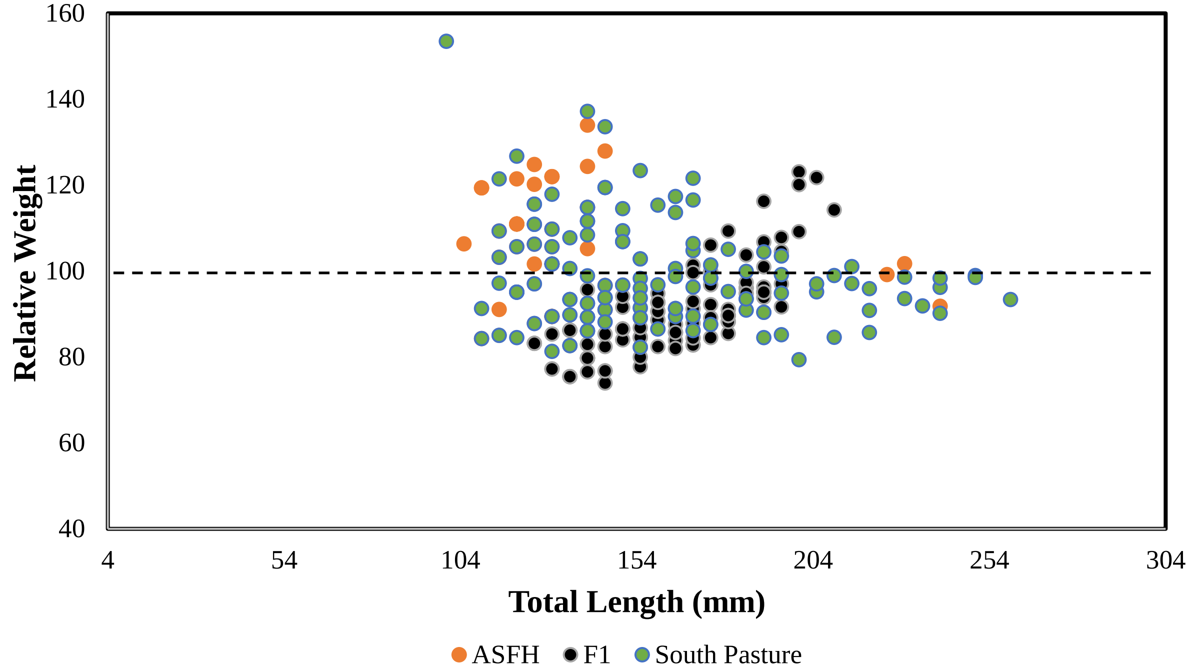
15, relative weights for all populations increased after the initial stocking but decreased in the growth periods thereafter. Bass fry were found in all study ponds following the second seine; therefore, spawning may have impacted the condition of the fish.

Table 15: Least squares means (±SE) relative weight (Wr) values from initial stocking and seines. Statistical comparisons were conducted among genetic groups at each seine sampling timepoint (rows). Values within each row with different letters are significantly different ($p < 0.05$). Percent value represents percentage change between the prior sample date. Values in parentheses indicate sample size of fish.

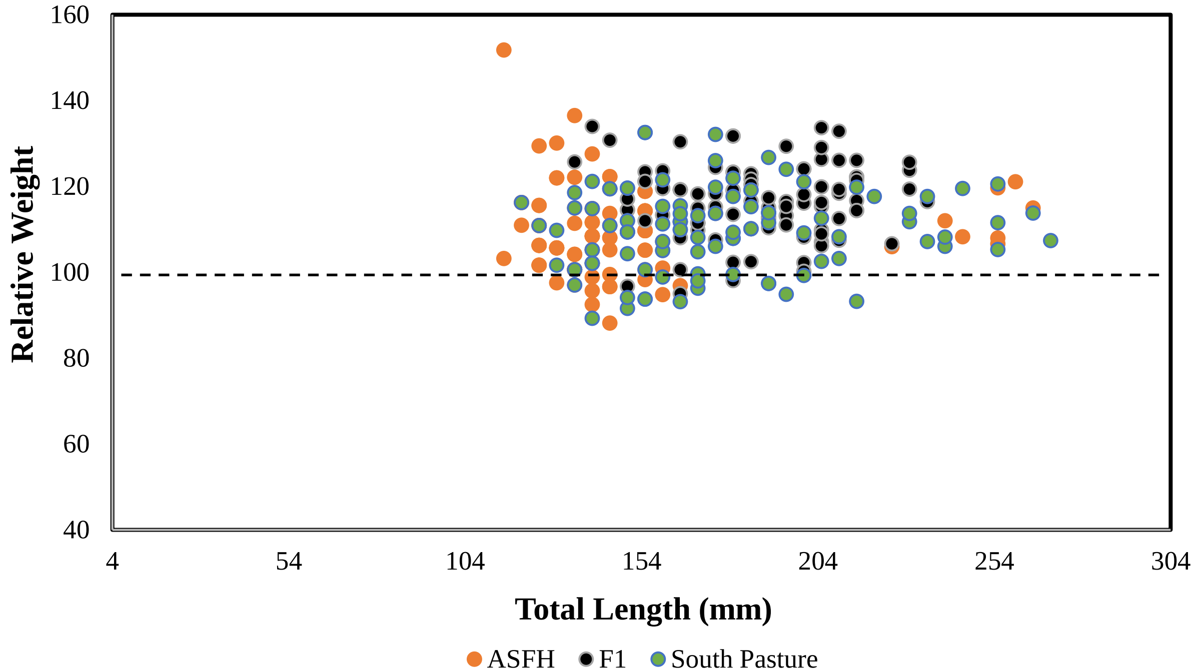
Sample	Population		
	ASFH	F1 hybrid[1]	South Pasture
Initial Stocking 12/12/20	107.70 ± 1.14 x (120)	90.26 ± 1.14 y (120)	99.47 ± 1.14 z (120)
First Seine 2/25/20	109.29 ± 0.97 x 1.48% (91)	116.07 ± 0.89 y 28.60% (107)	110.00 ± 0.96 x 10.59% (102)
Second Seine 4/21/20	100.94 ± 1.56 x -7.64% (40)	109.00 ± 1.52 y -6.09% (42)	101.25 ± 1.62 x -7.95% (37)
Third Seine 6/15/20	91.03 ± 0.67 x -9.82% (83)	97.81 ± 0.67 y -10.27% (84)	90.78 ± 0.68 x -10.34% (80)
Fourth Seine 8/11/20	83.81 ± 0.75 x -7.93% (56)	89.48 ± 0.76 y -9.31% (54)	86.17 ± 0.75 x -5.08% (55)

[1](*M. floridanus x M. salmoides*)

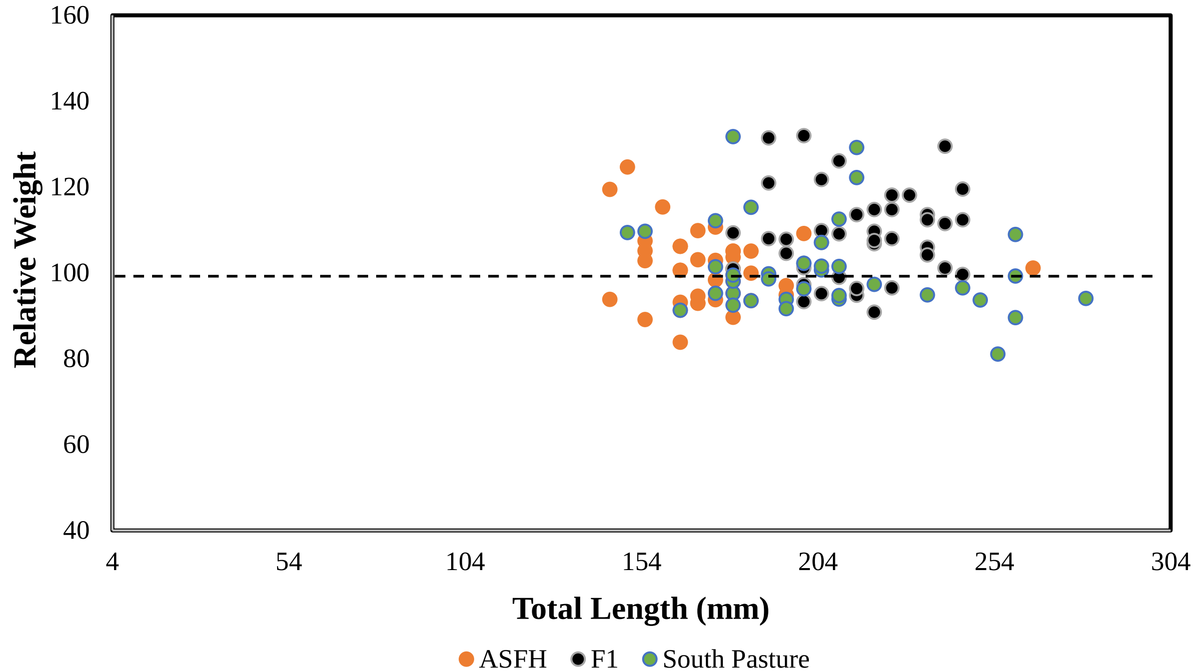
Relative weight distributions of all fish sampled per stocking and seine visually show the initial increase and subsequent decrease of relative weights. Fish above the reference line at 100 represent fish in the top 25% of condition. Initial stocking and seines 1-4 are reflected in Figure 7 (A-E), respectively.



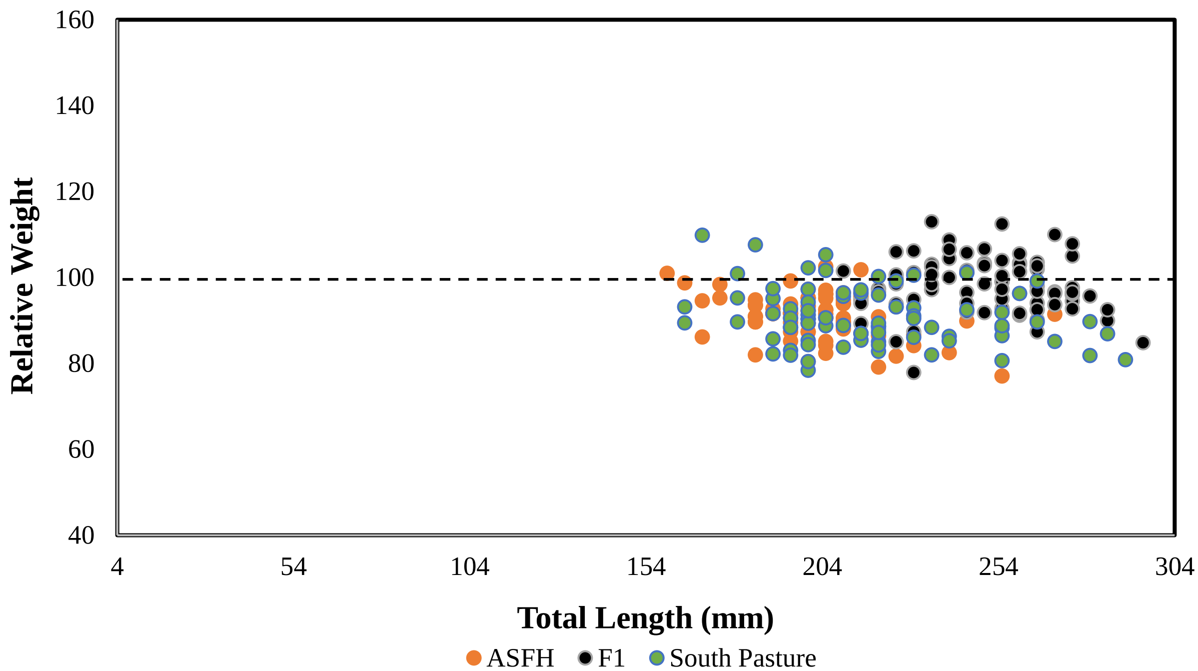
(A) Relative weights (Wr) between populations at the initial stocking.



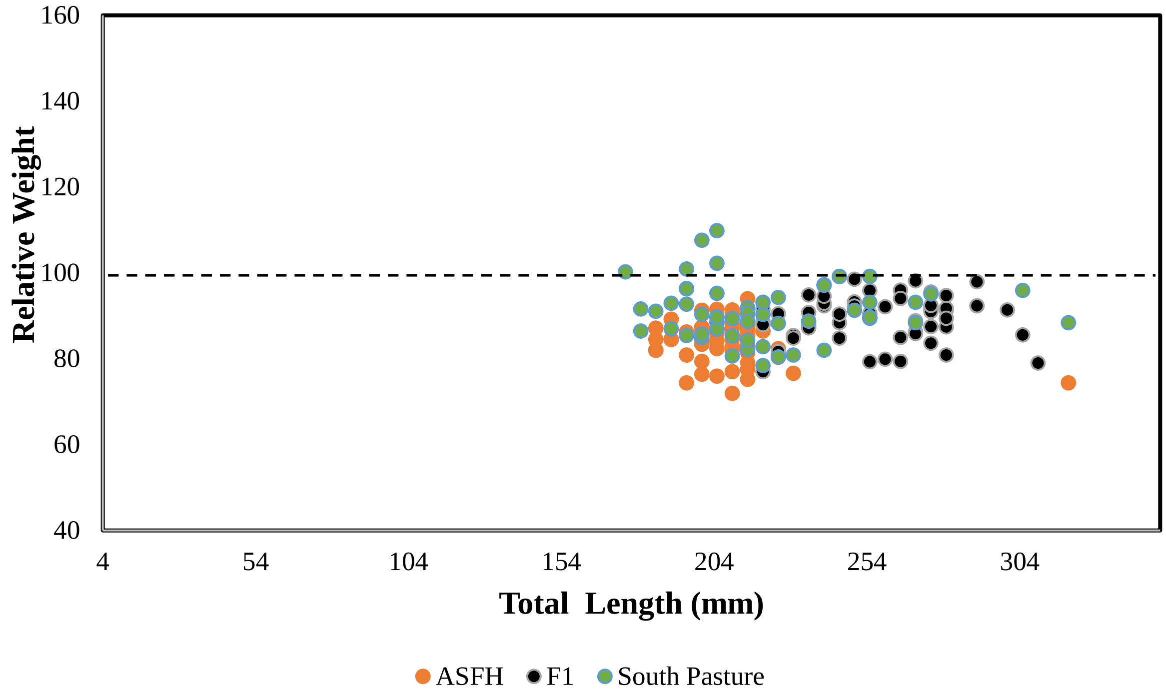
(B) Relative weights (Wr) between populations at the first seine.



(C) Relative weights (Wr) between populations at the second seine.



(D) Relative weights (Wr) between populations at the third seine.



(E) Relative weights (Wr) between populations at the fourth seine.

Figure 7: Relative weights (Wr) between populations during sample periods. The reference line at a Wr of 100 represents the condition of a fish in the top 25% of individuals.

Total Biomass and Growth Rates

During the initial stocking of the study ponds, there were no significant differences ($p \leq 0.05$) in the overall biomass (g) among ponds. Following the third seine there remained no significant differences ($p \leq 0.05$) in overall biomass (g) among ponds. However, at the initial stocking, the South Pasture population of fish was significantly greater ($p \leq 0.05$) in biomass than the ASFH population. At the third seine, the F1 hybrid population was significantly greater ($p \leq 0.05$) than both the ASFH and South Pasture populations. Mean weights (g) decreased for ASFH and South Pasture following the third seine, therefore their total biomass would be less if considering the fourth seine. Initial stocking and third seine population biomasses are shown in Table 16.

Table 16: Initial stocking and third seine total pond biomass (g). Values within each total row or column without a letter in common are significantly different (p < 0.05) based on a single factor ANOVA with a mixed procedure and Tukey's HSD post hoc test.

		Population biomass (g)			
	Pond	ASFH	F1 Hybrid[1]	South Pasture	Total (g)
Initial	1	1053	1324	1647	4024 x
	2	1530	1370	1672	4572 x
	3	867	1630	2112	4609 x
	4	518	1579	1478	3575 x
	Total (g)	3968 x	5903 xy	6909 y	Total (g)
Third	1	1994	3826	2486	8306 x
	2	2698	4410	2244	9352 x
	3	2063	4773	2939	9775 x
	4	1484	4424	3015	8923 x
	Total (g)	8239 x	17433 y	10684 x	

[1](*M. floridanus x M. salmoides*)

Absolute growth rates (g/day) were calculated beginning with the initial stocking and ending with the fourth seine. Overall, the F1 hybrids (*M. floridanus x M. salmoides*) had the greatest absolute growth rate with 0.69 g/day, which was significantly higher ($p \leq 0.05$) than both Florida Bass populations. Absolute growth rates between populations are shown in Table 17.

Table 17: Least squares means (±SE) absolute growth rate (g/day) between populations. Values were subjected to a single-factor ANOVA with a mixed procedure and Tukey's HSD post-hoc tests. Values within each row without a letter in common are significantly different (p < 0.05).

Growth Period	Population		
	ASFH	F1 Hybrid[1]	South Pasture
Initial Stocking to Fourth Seine	0.27 ± 0.05 x	0.69 ± 0.05 y	0.28 ± 0.05 x

[1](*M. floridanus x M. salmoides*)

When comparing growth rates between study ponds, the highest growth rate was from Pond 4 with 0.54 g/day, whereas the lowest growth rate was from Pond 1 with 0.34 g/day, and this difference was found to be significant ($p \leq 0.05$). Table 18 shows absolute growth rates between ponds.

Table 18: Least squares means (±SE) absolute growth rate (g/day) between ponds. Values were subjected to a single-factor ANOVA with a mixed procedure and Tukey's HSD post-hoc tests. Values within each row without a letter in common are significantly different ($p < 0.05$).

Growth Period	Pond			
	1	2	3	4
Initial Stocking to Fourth Seine	0.34 ± 0.14 x	0.41 ± 0.14 xy	0.37 ± 0.14 xy	0.54 ± 0.14 y

Differences between Sex

During the third seine, 10 fish from each pond were collected and sexed, resulting in a total sample consisting of 21 female and 19 male fish. The following parameters were calculated for each population: least squares means (±SE) length (mm), weight (g), relative weight (Wr), and absolute growth rate (g/day). For the ASFH population, no significant differences ($p \leq 0.05$) between sex were found across all parameters. However, F1 hybrid (*M. floridanus x M. salmoides*) females exhibited significantly greater ($p \leq 0.05$) relative weights and growth rates than males. South Pasture females were also found to have significantly greater ($p \leq 0.05$) lengths and weights than males as reflected in Table 19.

Table 19: Least squares means (±SE) total length (mm), weight (g), relative weight (Wr), and absolute growth rate (g/day) between sex for each population. Values within each row without a letter in common are significantly different ($p < 0.05$).

Population	LSM (±SE)	Sex	
		Male	Female
ASFH	Length (mm)	167.57 ± 12.53 x	171.92 ± 15.45 x
	Weight (g)	73.77 ± 11.71 x	74.80 ± 17.44 x
	Wr	105.91 ± 2.28 x	103.01 ± 3.32 x
	Growth Rate (g/day)	0.26 ± 0.03 x	0.30 ± 0.05 x
F1	Length (mm)	196.35 ± 6.10 x	205.67 ± 4.90 x
	Weight (g)	99.63 ± 10.40 x	134.22 ± 8.52 x
	Wr	100.66 ± 2.06 x	105.67 ± 1.33 y
	Growth Rate (g/day)	0.37 ± 0.06 x	0.75 ± 0.05 y
South Pasture	Length(mm)	179.04 ± 18.88 x	227.73 ± 17.94 y
	Weight (g)	70.66 ± 35.32 x	166.96 ± 33.68 y
	Wr	100.76 ± 2.40 x	96.72 ± 1.69 x
	Growth Rate (g/day)	0.29 ± 0.05 x	0.44 ± 0.05 x

Discussion

The goal of this study was to investigate early growth genetic differences within the Florida Bass species, as well as in comparison to an F1 hybrid bass (*M. floridanus x M. salmoides*). This study served to provide an updated analysis, as previous studies lacked or utilized outdated genetic techniques to confirm the genetic purity of fish. Previous studies also failed to compare growth differences between different populations of the same species of bass. This research is relevant as hatcheries continually aim to produce the fastest and largest growing individuals. Currently, the F1 hybrid cross has gained this reputation. Using this garnered knowledge, hatchery managers can better produce fish to satisfy fisheries biologists and anglers alike by selecting individuals with more desirable growth characteristics to use in their cross.

For the current study, a common-garden experimental design was used to reduce the effect of environmental variation between ponds from skewing the data to favor a population. Still, the overall environmental parameters of the ponds likely contributed to significant effects on the growth of fish. Previous studies stated optimal temperatures for bass growth ranged from 26º C to 28º C (Tidwell et al., 2003; Díaz et al., 2007). These temperatures were reached in late-May of the current study; however, it is apparent the greatest increases in length and weight occurred prior to May (Figure 3, Table 9, Table 12). A larger abundance of forage fish available during the beginning of the study, could have skewed these growth results. Also, as temperatures grew closer to the thermal maximums of the fish (>30º C), they caused a greater expenditure of energy towards respiration processes, and likely resulted in decreases in growth (Stuber et al., 1982; Fields et al., 1987).

All ponds contained characteristically soft water, with hardness and alkalinity values below 55 ppm over the course of the study, but all ponds were observed to successfully maintain algal blooms from March-August (Table 6). Pond 4 had the highest secchi depth (lowest algal bloom) over the course of the study, but also maintained the highest growth rate per pond across all populations of bass (Table 7, Table 18). Typically, the most biologically productive pond is expected to be highly fertile (low secchi depth). One variable present at Pond 4 and not at the others was shade from a nearby tree line during the afternoon of the day. This shade line on the pond may have allowed bass a place to hide and ambush prey, allowing them to more effectively feed. Also, the increased clarity of Pond 4 may have simply aided the visual hunting strategies of the bass. It is worth noting the total biomass of the ASFH population initially stocked into Pond 4 was less than other ponds, which may have led to the inflation of its growth rate (Table 16, Table 18). Overall, in the current study it was evident the algal bloom was not the limiting factor in bass growth.

When measured, dissolved oxygen (DO) concentrations averaged ~8 ppm in all study ponds (Figure 4). Aeration was used in the ponds to minimize DO variations during nighttime hours. Morning and afternoon DO measurements rarely varied by more than 3 ppm, when measurements at both times of day were recorded. Stewart et al. (1967) observed in a laboratory study that largemouth bass fingerlings growth increased near linearly until DOs reached ~8 ppm. Although our fish were larger in size, it can likely be concluded the fish experienced near optimal dissolved oxygen concentrations to promote growth.

In the current study, length differences over time were found between both Florida Bass populations. While the ASFH population started nearly 30 mm shorter than the South Pasture population, ASFH finished only approximately 12 mm shorter. ASFH had a greater mean length increase (mm), and percent increase than the South Pasture population. This is surprising as the ASFH population likely faced increased competition from the larger South Pasture and F1 hybrid bass (*M. floridanus x M. salmoides*), as it was the smallest population during the initial stocking (Table 8, Table 9). It was also clear in our study that the F1 hybrid (*M. floridanus x M. salmoides*) experienced superior growth in length in comparison to both Florida Bass populations with respect to overall increase (mm) and percentage increase. This differs from Inman et al. (1977), where there were no significant growth differences in length between Florida Bass and the F1 hybrid bass (*M. floridanus x M. salmoides*) after a growout of nearly 3 years. However, Figure 5 shows that the rate of increase in the length of the F1 hybrid (*M. floridanus x M. salmoides*) was nearly identical to the ASFH population. In another study, Philipp and Whitt (1991) found significant differences in length between Florida Bass and both reciprocal F1 hybrids after two years of growout. Still, the current study found greater differences in mean length between Florida Bass and an F1 hybrid (*M. floridanus x M. salmoides*) than Philipp and Whitt (1991) with younger, one-year old fish.

Similarly, weight differences over time were also found between Florida Bass populations. While the ASFH population had the lowest initial and overall increase in mean weight (g) over the study period, its percentage increase was greater than the South Pasture population (Table 12). Figure 6 shows the increased growth that the ASFH population demonstrated relative to the South Pasture population. Again, the F1 hybrid (*M. floridanus x M. salmoides*) was still superior to both Florida Bass populations in terms of weight gain. This result coincides with Kleinsasser et al. (1990) who observed F1 hybrid bass (*M. floridanus x M. salmoides*) reaching greater mean weights than Florida Bass in Texas ponds. The previous study used two-year old fish, whereas the current study utilized fish that were approximately 1-year old. Similar to the length increases, many previous studies failed to identify significant differences between reciprocal F1 hybrids and Florida Bass, or if they did, it was during an extended growout period (Zolczynski & Davies 1976; Inman et al. 1977; Williamson & Carmichael 1990). Potentially the addition of more forage throughout the current study/shorter study period allowed fish more feeding opportunities, whereas in previous studies, the forage base of the ponds dissipated over time. The increased feeding opportunities may have translated to increased growth, causing differences between populations to become more obviously evident.

Relative weights of all populations followed the same general trend over the study period. All populations' relative weights increased from the initial stocking until the first seine, likely due to the initial abundance of available forage. Following the first seine until the end of the study, the relative weights of all populations continually decreased, as shown in Figure 7. One plausible explanation for why this occurred was due to spawning impacts. Juvenile bass were found in each study pond following the second seine that occurred on April 6th, confirming that a successful spawn did occur. Prior to this seine, no juvenile fish were observed. Therefore, female fish were likely carrying eggs during the first seine on February 25th, inflating their relative weights at this sample period, and causing a decrease thereafter as they likely spawned sometime in March. Similarly, Kleinsasser et al. (1990) observed a decline in relative weights from initial to final samples over his year-long growout (Dec.-Jan.). Perhaps in the past study and current study, forage became depleted over time, and supplemental stockings were not enough as rising temperatures increased the metabolism of fish. Also, the forage may have become more efficient at evading the bass over time or outgrew the gape width of the bass. In the current study, large numbers of 7.62-12.7 cm Bluegills (*L. macrochirus*) were observed during seine samples, too large for most bass to consume.

Among bass populations, ASFH had a significantly greater mean relative weight at the beginning of the study compared to South Pasture and the F1 hybrid bass (*M. floridanus x M.*

salmoides; Table 15). This was likely due to ASFH fish stocked into the study ponds that originated from extra bass held in tanks at the North Auburn Polebarn facility. These fish were fed live forage bi-weekly and were likely in better condition than the other populations of bass, which were sourced from growout ponds where they had to hunt for their food. At this same period of time, the F1 hybrid bass (*M. floridanus x M.* salmoides) had significantly lower mean relative weights than both Florida Bass populations. In contrast, the F1 hybrid (*M. floridanus x M. salmoides*) maintained the highest mean relative weight from the first seine until the end of the study, whereas the two Florida populations were not significantly different from one another. Kleinsasser et al. (1990) found similar results with a F1 hybrid bass (*M. floridanus x M.* salmoides) having a final relative weight significantly higher than Florida Bass in their study. Florida Bass had higher initial relative weights when compared to F1 hybrid bass (*M. floridanus x M. salmoides*) (F1: 92; FL: 93), and final relative weights between strains of bass (F1: 90; FL: 82) were similar in direct comparison to the current study (F1: 89; FL: ~85). Maceina et al. (1988a) displayed similar results, as F1 hybrids (cross unknown) typically maintained higher relative weights than Florida Bass over a 3-year sample period. It is worth noting that the F1 hybrids in Maceina et al. (1988a) were naturally produced as a result of Florida Bass stocking into a small lake.

Ponds generally doubled their biomass levels from the beginning of the study to the third seine, with no significant differences observed among them (Table 16). However, fish in Pond 4 were found to have a significantly greater absolute growth rate (g/day) than Pond 1 (Table 17). As previously discussed, this was likely due to environmental parameters such as the availability of shade or lack of an algal bloom since all ponds were stocked equally with forage. In terms of populations, the F1 hybrid (*M. floridanus x M. salmoides*) clearly exhibited a larger total biomass by the third seine, whereas both Florida Bass populations were not significantly different from each other at this time (Table 18). Similarly, the overall absolute growth rate (g/day) for the F1 hybrid (*M. floridanus x M. salmoides*) was double either Florida Bass population, which were nearly identical to each other. Maceina et al. (1988b) found similar results with F1 hybrids (unknown cross) exhibiting significantly greater absolute growth rates (mm/d) than Florida Bass. Clugston et al. (1964) also found specific growth rate (%/day) differences between what he considered northern Largemouth Bass and Florida Bass, but the fish used in this study were not genetically confirmed.

Generally, female bass are valued for their greater overall size in comparison to their male counterparts. In the current study, the F1 hybrid (*M. floridanus x M. salmoides*) females had significantly greater growth rates and relative weights than males (Table 19). The South Pasture female population also had significantly greater lengths and weights than the males. As previously stated, successful spawning was observed to occur in all of the ponds, so the spawning process likely aided in increasing the weights of females and increasing the likelihood of observed sex differences. In general, the weight of eggs in females will be 10% or more of her body weight (Davis and Lock, 1997). Also, males typically do not eat during the spawning period causing a decline in their weight/condition.

Lorenzoni et al. (2002) found that females were bigger than males at all ages during his study, but this study made no comparisons within unique populations of bass themself. In fact, the current study is the first to identify growth related sex differences between pure Florida Bass populations. A sex/time interaction effect was also found for F1 hybrids (*M. floridanus x M.* salmoides), indicating it took a longer time for females to diverge from males, likely after spawns had occurred. The ASFH population also had no significant differences for all growth parameters between males and females, perhaps due to failure to reach sexual maturity during the study period.

Overall, the F1 hybrid bass (*M. floridanus x M. salmoides*) experienced increased growth in comparison to both Florida Bass populations across nearly all growth parameters. For decades, state agencies have been pressured by anglers to stock pure Florida Bass in hopes to promote the growth of larger individuals. Florida Bass stockings have shown mixed results. In Tennessee's Lake Chickamauga, Florida Bass stockings have been shown to produce larger tournament-angled fish, as a result of their hybridization with native northern Largemouth Bass populations and subsequent production of F1 hybrid bass (Hargrove et al., 2020). Perhaps in other impoundments in northern Alabama and mid-south states with a high percentage of northern Largemouth Bass alleles, a similar stocking strategy could provide a positive impact (Gowan, 2015). However, in other stocking scenarios, juvenile Florida Bass are potentially easy prey for native Largemouth Bass populations, and do not contribute to the overall genetics of the fishery. In this situation, stocking an F1 hybrid bass (*M. floridanus x M. salmoides*) with an increased size and growth rate advantage over a Florida Bass, as observed in the current study, may increase the chances of survival and contribute to the size of fish in the population.

However, studies have found there is no observed heterosis or hybrid vigor in F1 hybrid bass (both reciprocal crosses) across multiple physiological factors (Williamson and Carmichael, 1986; Cooke et al., 2001; Philipp et al., 2002; Cooke and Philipp, 2006; Allen et al., 2009). In addition, much is still unknown about the generational impacts of stocking F1 hybrid bass (*M. floridanus x M. salmoides*), and if this observed increase in early growth is present in subsequent generations resulting from hybridization between F1 bass and the dominant/native population. In Goldberg et al. (2005), F2 generation hybrids suffered reduced fitness in comparison to F1 hybrids. In this respect, F1 hybrid bass (*M. floridanus x M. salmoides*) stockings would likely be treated as a put/take fishery, similar to trout stocking practices.

Observable early growth genetic differences between pure Florida Bass populations were also evident in the current study and hold strong implications toward the development of an improved F1 hybrid individual. By implementing a selective breeding approach, a hatchery could utilize a more fit Florida Bass population to create an improved F1 hybrid bass (*M. floridanus x M. salmoides*) contributing to the next generation of trophy bass management practices. It must be noted, the Florida Bass broodfish used for the study have been observed to have phenotypic differences and originate from differing pond environments. The South Pasture broodfish were observed to be shorter and stockier than typical ASFH broodfish (S. McNulty, personal communication). The South Pasture's native environment consists of a high alkalinity, nutrient rich, ~100-hectare lake (F. Langford, personal communication). This is in direct contrast to low alkalinity, 0.40-hectare hatchery ponds ASFH broodfish are held in. In addition, South Pasture broodfish have a larger variety of forage available to them including tilapia and other exotic species of fish inhabiting central Florida. South Pasture broodfish are estimated to derive from 10-15 generations of fish, whereas the ASFH line is nearly twice as old with 30 generations. Given these differences, perhaps the ASFH fish are more locally adapted to the Alabama environment allowing them to outperform the South Pasture fish in the current study. Still, more research is warranted in the area of early growth bass genetics, as this study was the first to encompass a growth comparison of two pure Florida Bass populations.

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Appendix

Appendix I: Standard error (±SE) of mean total length (mm) between populations and ponds during each sample period.

Population	Pond	Mean total length (mm)				
		Initial Stocking	Seine 1	Seine 2	Seine 3	Seine 4
ASFH	1	5.01	6.52	3.49	4.66	3.10
	2	7.76	9.38	2.90	4.49	8.37
	3	4.60	6.07	9.38	4.87	2.94
	4	1.54	2.14	6.23	3.37	2.01
	Overall	**2.77**	**3.53**	**3.11**	**2.31**	**2.48**
F1	1	3.73	4.16	2.79	3.90	6.17
	2	4.44	4.81	5.27	4.04	7.02
	3	4.08	4.39	6.01	4.22	4.74
	4	3.83	4.09	5.48	4.77	8.24
	Overall	**2.02**	**2.18**	**2.82**	**2.19**	**3.27**
South Pasture	1	6.35	7.89	12.69	7.91	7.55
	2	6.32	6.54	8.61	5.84	5.65
	3	7.40	7.88	11.81	7.39	9.67
	4	6.10	7.42	9.04	6.68	8.02
	Overall	**3.32**	**3.66**	**5.25**	**3.51**	**4.05**

Appendix II: Standard error (±SE) of mean weight (g) between populations and ponds during each sample period.

Population	Pond	Mean weight (g)				
		Initial Stocking	Seine 1	Seine 2	Seine 3	Seine 4
ASFH	1	6.12	10.58	4.30	6.54	4.04
	2	10.49	17.88	2.51	9.25	18.39
	3	9.98	11.37	18.02	9.71	4.20
	4	0.79	1.59	7.85	4.34	2.81
	Overall	**3.54**	**6.28**	**5.41**	**4.15**	**5.01**
F1	1	3.89	6.19	4.13	9.86	14.88
	2	5.56	8.92	12.86	11.27	19.76
	3	5.03	6.81	11.75	11.33	11.53
	4	3.83	7.81	12.32	12.50	23.57
	Overall	**2.44**	**3.78**	**6.57**	**5.85**	**8.83**
South Pasture	1	8.18	14.41	25.71	14.91	12.58
	2	7.13	10.93	16.32	11.56	10.31
	3	10.07	14.89	21.47	17.88	21.39
	4	7.12	13.73	33.15	15.29	20.47
	Overall	**4.12**	**6.57**	**10.00**	**7.57**	**8.91**

Appendix III: Standard error (±SE) of mean relative weights (Wr) between populations and ponds during each sample period.

Population	Pond	Mean relative weight (Wr)				
		Initial Stocking	Seine 1	Seine 2	Seine 3	Seine 4
ASFH	1	2.33	1.66	1.68	1.34	1.09
	2	2.19	1.85	2.59	1.03	1.38
	3	2.87	1.45	1.66	1.15	1.36
	4	2.04	2.45	4.01	0.88	1.02
	Overall	**1.21**	**1.04**	**1.31**	**0.56**	**0.64**
F1	1	1.47	1.82	2.36	1.57	1.51
	2	1.92	1.38	2.50	1.35	0.97
	3	1.78	1.16	3.37	1.42	1.25
	4	1.92	1.30	3.32	1.30	2.07
	Overall	**0.89**	**0.79**	**1.59**	**0.71**	**0.73**
South Pasture	1	1.76	2.08	4.17	1.31	1.37
	2	2.97	1.41	3.41	1.63	1.68
	3	3.04	2.10	2.98	1.46	1.91
	4	1.74	1.82	4.13	1.48	1.05
	Overall	**1.28**	**1.00**	**1.77**	**0.73**	**0.87**