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Patterns and Drivers of Introgression in Louisiana's Largemouth Bass (*Micropterus salmoides*) Stocks

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**PATTERNS AND DRIVERS OF INTROGRESSION IN
LOUISIANA'S LARGEMOUTH BASS (MICROPTERUS
SALMOIDES) STOCKS**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Colleen Elizabeth Walsh
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ABSTRACT

In the southeastern U.S., populations of the popular sportfish Largemouth Bass (*Micropterus salmoides*; LMB) are often stocked with Florida Largemouth Bass (*Micropterus salmoides floridanus*; FLMB) to develop FLMB ancestry in systems north of its native peninsular Florida range, with the ultimate goal of creating trophy fisheries. Stock enhancement through introgression of FLMB ancestry can increase maximum potential size and growth in receiving populations, potentially through hybrid vigor of intergrade subspecies. We collected 60 fish and habitat data from six Louisiana lakes from 2018-2020 (N=360), quantified level of introgression of FLMB with fragment analysis at 12 microsatellite loci confirmed for subspecific identification, and assigned a percent FLMB ancestry to each sample fish with program STRUCTURE, with 20 replicates at K=2. A detrended correspondence analysis (DCA) was used to reduce the dimensionality of the lake habitat data, and linear components were modeled with beta regression to relate physicochemical variables to average percent Florida ancestry by lake. We then used generalized linear models (GLMs) to examine the effects of percent Florida ancestry and measured physicochemical variables on growth, mean length at age, W_r and log-length. DCA2, DCA3, and DCA4 were significant in the beta regression model with average percent Florida ancestry, suggesting a positive relationship between Florida ancestry and highly vegetated lakes with increased turbidity. W_r and mean length at age 1 were also influenced by percent Florida ancestry. However, addition of percent Florida ancestry resulted in an increase in weight and W_r only to a certain point, at which point the quadratic of this relationship became negative, indicating fish with intermediate levels of Florida ancestry were more robust, suggesting possible hybrid advantage. The relationship between length with Florida ancestry changed over age. In early ages, it was more advantageous to be an intermediate hybrid, however

as a fish aged, greater lengths were associated with “pure” (highest percentages of FLMB ancestry) FLMB. This overall relationship was dampened when physicochemical variables were included, with hybrids possessing 75% FLMB ancestry exceeding pure FLMB in length, indicating habitat may have also played a significant role in determining growth and condition of LMB in Louisiana.

CHAPTER 1. INTRODUCTION

Largemouth Bass (*Micropterus salmoides*; LMB) is considered one of the most desired sportfishes in North America and has been transplanted across the continent to create and enhance recreational fisheries (Tidwell et al. 2002). In the southeastern United States, LMB are the priority sportfish and have been extensively managed and studied (Buynak et al. 1999; Chen et al. 2003; Myers and Allen 2005; Hansen et al. 2015; Long et al. 2015; Tingley III et al. 2019). Because of the socioeconomic importance of a healthy recreational fishery, angler satisfaction is primarily a driving force in these management decisions and research objectives (Buynak and Mitchell 1999; Chen et al. 2003). Often the primary goal in LMB management is to increase and maintain high growth rates and create a trophy fishery through stocking efforts, habitat management, and creel limits (Buynak and Mitchell 1999; O'Rourke 2009; Canfield Jr. et al. 2013; Dotson et al. 2013).

Increasing the growth rate of LMB is dependent on several environmental factors and their variability, such as temperature (Diana 1984; McCauley and Kilgour 1990), aquatic vegetation community composition and coverage (Savino and Stein 1982; Durocher et al. 1984; Colle et al. 1989; Miranda and Pugh 1997; Pothoven et al. 1999; Valley and Bremigan 2002; Johnson et al. 2014), adequate forage availability (Timmons et al. 1980; Keast and Eadie 1985; Storck 1986; Allen et al. 1999), and water quality conditions, including turbidity (Gent et al. 1995; Shoup and Wahl 2009; Ferrari et al. 2014), salinity (Tebo Jr. and McCoy 1964; Meador and Kelso 1990; Norris et al. 2010), nutrient concentrations such as chlorophyll-a (Greene and Maceina 2000), and environmental toxins (Inendino et al. 2005). Management of waterbodies for LMB tends to focus on aquatic vegetation control, maintaining adequate forage populations, and supplemental stocking of LMB subspecies to enhance the population growth potential.

Fisheries managers have often stocked fishes outside their native ranges to enhance sport fishing opportunities or alter genetic composition of existing stocks (Buckmeier et al. 2003). Across the United States, stocking has been used to increase opportunities for sportfishing for many fishes, including Black Crappie (*Pomoxis annularis*; Parkos III et al. 2019), Walleye (*Sander vitreus*; Jennings and Philipp 1992), Channel Catfish (*Ictalurus punctatus*; Santucci, JR. et al. 1994), and several salmonid species, such as Brown Trout (*Salmo trutta*; Wills 2006), Brook Trout (*Salvelinus fontinalis*; Kennedy et al. 2018), and Atlantic Salmon (*Salmo salar*; Orciari et al. 1994). In addition to sportfish stocking, Golden Shiner (*Notemigonus crysoleucas*; Hickman and Kilambi 1974) and Threadfin Shad (*Dorosoma petenense*; Maceina and Sammons 2015) have also been stocked to ensure adequate forage for piscivorous sportfishes. Largemouth Bass are commonly the focus of most warmwater fishery programs, and extensive stocking programs across the country have proven to increase fish populations and enhance fisheries production (Buynak and Mitchell 1999; Hoxmeier and Wahl 2002; Hoffman and Bettoli 2005; Diana and Wahl 2008; Mesing et al. 2008). However, since identification of the Florida Largemouth Bass [*Micropterus salmoides floridianus*, FLMB; Bailey and Hubbs (1949)] subspecies, most stocking programs in the southeastern U.S. have placed greater emphasis on stocking and enhancing LMB fisheries through the introduction of Florida Bass genes.

Florida Largemouth Bass are endemic to peninsular Florida south and east of the Suwannee River drainage (Philipp et al. 1983), with an intergrade zone in northern portions of the range with the Northern Largemouth Bass (*Micropterus salmoides salmoides*, NLMB). The native range of the Northern Largemouth Bass is more extensive and stretches from the Great Lakes and St. Lawrence River to the Mississippi River drainage and the Gulf of Mexico and is the endemic species of LMB in Louisiana (Page and Burr 2011). These two subspecies are

similar in appearance with only a few meristic differences, such as counts of lateral line scales and pyloric caecae (Zolczynski, JR. and Davies 1976). However, it is difficult to distinguish the two without genetic methods because of hybridization within the natural intergrade zone and in manipulated stocks (Philipp et al. 1983) and the considerable natural variation in morphology among individuals (e.g., Hall et al. 2018).

The shift to stocking FLMB over NLMB in southeastern lakes and reservoirs is based on evidence from Florida and Texas suggesting greater maximum size in adult FLMB (Inman et al. 1977; Maceina et al. 1988; Crawford et al. 2002; Myers and Allen 2005). Increased growth rate and maximum size have been historically linked to a longer growing season in their native range (Bailey and Hubbs 1949). Consequently, this increased growth is not seen in all locations where FLMB are stocked (Clugston 1964; Zolczynski, JR. and Davies 1976; Philipp and Whitt 1991). Several studies have reported increased growth in hybrid fish, suggesting the possibility of hybrid vigor (Inman et al. 1977; Maceina et al. 1988; Kleinsasser 1990; Neal and Noble 2002). It is suspected that differences in growth rates and maximum size seen in FLMB stocked outside its native range are related to differences in environmental factors, such as temperature, vegetation type and density (Fields et al. 1987; Koppelman et al. 1988; Colle et al. 1989; Myers and Allen 2005), and prey availability (Keast and Eadie 1985; Hoffman and Bettoli 2005; Slaughter IV et al. 2008). However, the specific factors influencing observed differences in FLMB growth in stocked populations remain unidentified.

Introgression of FLMB genes into native northern bass populations has been achieved in several southeastern states, including Oklahoma (Gilliland and Whitaker 1989; Gelwick et al. 1995; Acy 2017), Texas (Buckmeier et al. 2003; Ray et al. 2012), Alabama (Dunham et al. 1992), Tennessee (Hargrove et al. 2019), and Arkansas (Johnson and Fulton 1999 & 2004;

Lamothe and Johnson 2013), although there are concerns that introduction of FLMB ancestry into non-native systems can result in stock impairment and possible outbreeding depression (Philipp 1991; Fries et al. 2002; Philipp et al. 2002; Cooke et al. 2005; Garlock et al. 2019). There is also debate concerning the relative importance of factors such as increased stocking size, increased survival of FLMB and hybrids (Maceina et al. 1988; Neal and Noble 2002; Peterson et al. 2017) and warmer temperatures (Gilliland and Whitaker 1989) on FLMB introgression success.

The Louisiana Department of Wildlife and Fisheries (LDWF) has stocked 115 million FLMB over 220 waterbodies since 1982 to promote introgression of Florida alleles into native bass populations and enhance growth and harvest of trophy fish. Historically, subspecific identification was determined through analyses of isocitrate dehydrogenase (IDH, EC 1.1.1.42) and aspartate aminotransferase (AAT, EC 2.6.1.1) allozymes with fixed allele differences between NLMB and FLMB. However, these analyses were only useful for estimating proportions of northern, Florida, and hybrid individuals, either F_1 or F_x , in these waterbodies, and the percentage of FLMB ancestry present in these populations were unknown. Further, misidentification of F_x individuals became more problematic after many years of stocking and interbreeding among hybrid individuals (unpublished data).

Microsatellite analyses can provide a much more accurate and detailed assessment of FLMB introgression in stocked populations (Lutz-Carillo et al. 2006; Barthel et al. 2010). Microsatellites are repeated, non-coding sequences that can be used to detect polymorphic differences between subspecies. They provide more detail than previous allozyme analysis because of the increased number of loci used for identification (Lutz-Carillo et al. 2006). I used 12 optimized diagnostic primers to determine the current level of FLMB introgression in six

Louisiana waterbodies that have varied histories of stocking frequency and density. I also collected environmental data from these lakes as well as estimates of bass length-at-age to investigate potential factors influencing growth of Largemouth Bass across the state. Overall objectives of the project were to determine the: 1) level of current level of introgression of in the six lake systems based on both allozymes and microsatellites; 2) habitat characteristics that impact the success and introgression of Florida Largemouth Bass in these lakes; and 3) the association between growth and condition characteristics in relation to the proportion of FLMB ancestry.

CHAPTER 2. INTROGRESSION

2.1. INTRODUCTION

Largemouth Bass (*Micropterus salmoides*; LMB) is considered one of the most sought-after sportfish in North America and consequently is a top management priority for many state fisheries agencies focused on producing trophy fish and satisfying angler desires (Buynak et al. 1999; Chen et al. 2003; Myers and Allen 2005; Hansen et al. 2015; Long et al. 2015; Tingley III et al. 2019). Management of LMB often involves manipulation of stocks through supplemental stocking (Boxrucker 1986; Maceina et al. 1988), habitat management (particularly vegetation alteration) (Pothoven et al. 1999; Bonvechio and Bonvechio 2006), and creel and size limits (Dotson et al. 2013; Miranda et al. 2017). One of the primary goals of LMB management programs across the U.S. is to increase, and subsequently maintain, high growth rates to produce the largest fish a system can sustain (Buynak and Mitchell 1999).

Since identification of the Florida Largemouth Bass (*Micropterus salmoides florianus*, FLMB) subspecies by Bailey and Hubbs (1949), most state fisheries agencies in the southeastern U.S. have developed stocking programs to introduce and maintain FLMB genes in systems north of its native range (Buckmeier et al. 2005). FLMB are endemic to peninsular Florida south and east of the Suwannee River drainage (Philipp et al. 1983), with populations intergrading with Northern Largemouth Bass (*Micropterus salmoides salmoides*, NLMB) in northern portions of the range. The native range of the Northern Largemouth Bass stretches from the Great Lakes and St. Lawrence River to the Mississippi River drainage and the Gulf of Mexico (Page and Burr 2011). These two subspecies are similar in appearance with only a few meristic differences, such as the number of lateral line scales and pyloric caecae (Zolczynski, JR. and Davies 1976).

However, considerable natural variation in morphology among largemouth bass (e.g., Hall et al. 2018) and subspecific hybridization make visual identification of the genetic identity of individual fish problematic in the intergrade zone and in stocked populations elsewhere (Philipp et al. 1983).

The shift to stocking FLMB over NLMB in southeastern lakes and reservoirs is based on evidence from Florida and Texas (Inman et al. 1977; Maceina et al. 1988; Crawford et al. 2002; Myers and Allen 2005) suggesting greater maximum size in adult FLMB. Increased growth rate and maximum size have been historically linked to a longer growing season in their native range (Bailey and Hubbs 1949). Consequently, this increased growth is not seen in all locations where FLMB are stocked (Clugston 1964; Zolczynski, JR. and Davies 1976; Philipp and Whitt 1991). Several studies have reported increased growth in hybrid fish, suggesting the possibility of hybrid vigor (Inman et al. 1977; Maceina et al. 1988; Kleinsasser 1990; Neal and Noble 2002). It is suspected that differences in growth rates and maximum size seen in FLMB stocked outside its native range are related to differences in environmental factors, such as temperature, vegetation type and density (Fields et al. 1987; Koppelman et al. 1988; Colle et al. 1989; Myers and Allen 2005), and prey availability (Keast and Eadie 1985; Hoffman and Bettoli 2005; Slaughter IV et al. 2008). However, few studies have provided conclusive evidence on the specific factors influencing the observed differences in FLMB growth in stocked populations.

Introgression of FLMB genes into native northern bass populations has been achieved in several southeastern states, including Oklahoma (Gilliland and Whitaker 1989; Gelwick et al. 1995; Acy 2017), Texas (Buckmeier et al. 2003; Ray et al. 2012), Alabama (Dunham et al. 1992), Tennessee (Hargrove et al. 2019), and Arkansas (Johnson and Fulton 1999 & 2004; Lamothe and Johnson 2013), although there are concerns that introduction of FLMB genes into

non-native systems can result in stock impairment and possible outbreeding depression (Philipp 1991; Fries et al. 2002, Philipp et al. 2002; Cooke et al. 2005; Garlock et al. 2019). There is also debate concerning the relative importance of factors such as increased stocking size, increased survival of FLMB and hybrids (Maceina et al. 1988; Neal and Noble 2002; Peterson et al. 2017) and warmer temperatures (Gilliland and Whitaker 1989) on FLMB introgression success.

The Louisiana Department of Wildlife and Fisheries (LDWF) has stocked 115 million FLMB in over 220 waterbodies since 1982 to promote introgression of Florida alleles into native bass populations and enhance growth and harvest of large fish. Historically, subspecific identification was determined through analyses of isocitrate dehydrogenase (IDH, EC 1.1.1.42) and aspartate aminotransferase (AAT, EC 2.6.1.1) allozymes with fixed allele differences between NLMB and FLMB. However, these analyses were only useful for estimating proportions of northern, Florida, and hybrid individuals in these waterbodies, and the percentage of FLMB ancestry present in these populations were unknown. Further, misidentification of F_x individuals became more problematic after many years of stocking and interbreeding among hybrid individuals or backcrossing with parental stock (unpublished data).

Microsatellite analyses can provide a much more accurate and detailed assessment of FLMB introgression in stocked populations (Lutz-Carillo et al. 2006; Barthel et al. 2010). Microsatellites are repeated, non-coding sequences that can be used to detect polymorphic differences between subspecies. They provide more detail than previous allozyme analysis because of the increased number of loci used for identification and greater allelic variation (Lutz-Carillo et al. 2006). I used 12 optimized diagnostic primers to determine the current level of FLMB introgression in six Louisiana waterbodies that have varied histories of stocking frequency and density. I also collected environmental data from these lakes to investigate

potential factors influencing introgression and persistence of Florida Largemouth Bass across the state. I hypothesized that: 1) percent FLMB ancestry differed significantly across LMB populations in Louisiana; 2) persistence of FLMB ancestry and introgression of FLMB were related to the number of stocking events; 3) measured physicochemical characteristics also influenced the success of FLMB introgression.

2.2. METHODS

2.2.1. HABITAT SAMPLING

Eight Louisiana lakes located across the state were selected for this study in consultation with the Louisiana Department of Wildlife and Fisheries (LDWF). Bundick Lake was later removed because of a summer 2019 drawdown that limited boat access, and Chicot Lake was eliminated because of sampling limitations resulting from the COVID-19 pandemic. The remaining six lakes (Lake Cataouatche, Lake D'Arbonne, Grand Bayou Reservoir, Poverty Point Reservoir, Lake Rodemacher, and Lake St. John; Figure 2.1) varied in size and stocking intensity (Table 2.1).

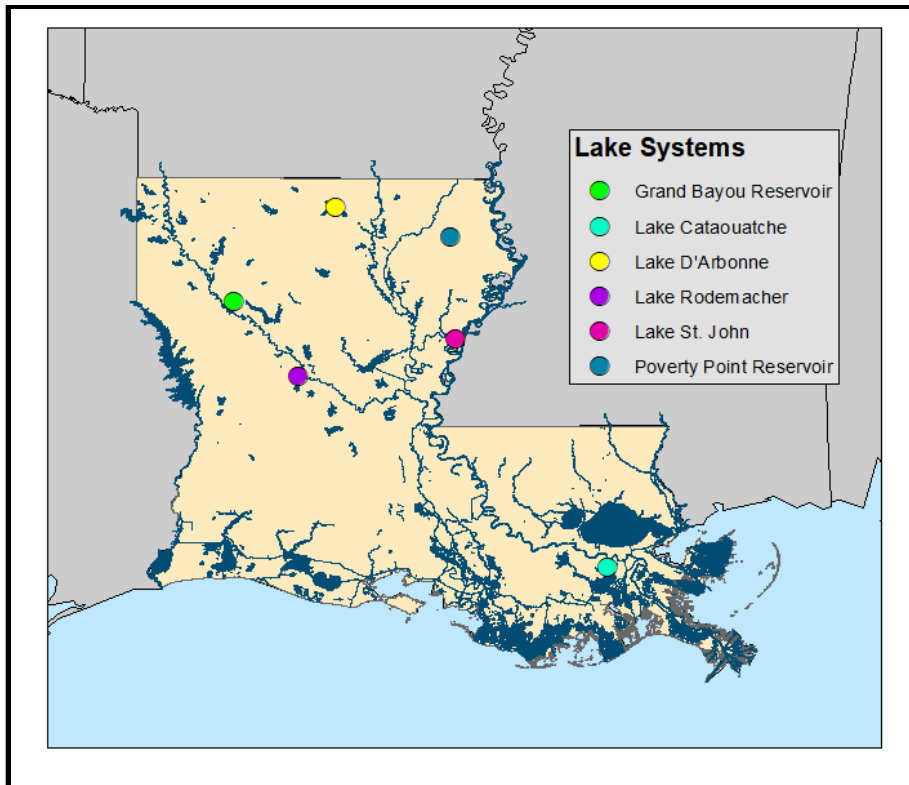


Figure 2.1. Location of six study lakes in Louisiana. Largemouth Bass were collected by boat electrofishing in 2018-20 and habitat characteristics, such as a vegetation coverage and shoreline modification, were sampled once per lake in the summer of 2019.

Table 2.1. Morphometric and stocking characteristics of six study lakes in Louisiana.

Water Body	Size (ha)	Average depth (m)	Maximum depth (m)	Fish stocked in 2018	Total fish stocked since 1985	Total times stocked
Lake Cataouatche	3755.5	1.83		1,389,000 fry	1,441,529	8
Lake D'Arbonne	6171.5	2.59	9.14	300,003 fingerlings	3,216,766	21
Grand Bayou Reservoir	1092.7	3.05	8.99	31,175 fingerlings	3,540,141	22
Poverty Point Reservoir	1127.1	2.26	8.53	20,206 fingerlings	2,270,709	18
Lake Rodemacher	1242.4	2.74	7.62	0	2,101,440	17
Lake St. John	849.8	3.66	8.53	32,119 fingerlings	283,207	13

The shoreline of each lake was digitized with QGIS (QGIS Development Team 2009) and a 500-m² grid was overlaid to determine sampling sites (1000 m² was used for Lake D'Arbonne due to its size and shoreline complexity). All grids that contained over 100 m of shoreline were numbered and the total number of grids was divided equally into quadrants. To determine exact sampling locations, 40% of the grids in each quadrant were randomly selected with a random-number generator and coordinates were taken at the start of the grid along the shoreline moving clockwise around the lake. The total number of sampling sites for each lake varied with lake size and ranged from 16 (Lake St. John) to 30 (Lake D'Arbonne). Sampling was designed to provide an overall assessment of lake habitat and not specifically target LMB habitat.

All lake habitats were sampled during Summer 2019 to ensure peak vegetation abundance for the season. A total of seven locations were sampled at each site with a random interval of boat travel time (30, 60, 90, 120 seconds) between each location. At each location, I recorded vegetation type and visually estimated density as well as distance from the perimeter of the vegetation bed to the shoreline and percent cover of submerged, floating, and/or emergent vegetation. Vertical profiles were taken at the middle of each lake as well as at any significant points of interest, such as noticeable changes in water color or appearance and overflow dams or other water control structures. Depth, dissolved oxygen, temperature, specific conductance, and turbidity were recorded with a multiprobe meter (ProDSS, YSI, Inc., Yellow Springs, OH). Water samples were collected in 1L bottles randomly at approximately a quarter of all sample sites and immediately placed on ice. Upon return to the lab, water samples were filtered through 47-mm glass microfiber filters for determination of chlorophyll-a concentrations. The habitat and

physicochemical measurements were collected to describe conditions at the lake scale, not at a meso- or microhabitat scale that could be directly compared with LMB collected in those areas.

2.2.2. FISH SAMPLING

From 2018-2020, a sample of 60 Largemouth Bass was electrofished from each lake with the assistance of LDWF personnel based on previous successful LMB sampling events. Sampling date varied across lakes due to availability of crew members. Collected fish were immediately placed on ice in the field and processed in the lab within 48-72 hours of sampling. Sex, total length (TL, nearest mm) and weight (W, nearest g) measurements were recorded for each fish. Livers were removed, immediately placed on ice, and then stored at -20C for genetic analysis. Livers were also collected from bass populations in Yucatan Lake and Finch Lake, as well as broodstock from Booker Fowler hatchery (total n=120), which served as parental stock populations for baseline genetic comparisons.

2.2.3. GENETIC SAMPLING

I used both allozyme and microsatellite analyses to assess the relative efficacy of these methods for determining bass genetic identity. For the allozyme analyses, approximately 1 g of tissue was removed from each liver and homogenized in distilled water. The homogenate was then used in horizontal starch gel electrophoresis at a constant voltage for 12 hours in a tris-citrate (TC) buffer. Histochemical stains described by Shaw and Prasad (1970) were used to identify band patterns for isocitrate dehydrogenase (IDH, EC 1.1.42) and aspartate aminotransferase (AAT, EC 2.6.1.1), which were used because their fixed allele differences permitted identification of NLMB, FLMB, and at least F₁ hybrids (Shaw and Prasad 1970).

A total of 12 optimized primers were used in the microsatellite analyses of bass genetic identity. These primers were Lar7, Lma10, Mdo3, Mdo6, Mdo7, MiSaTPW011, MiSaTPW028, MiSaTPW055, MiSaTPW068, MiSaTPW090, MiSaTPW112, and MiSaTPW117 (Lutz-Carrillo et al. 2006; Lutz-Carrillo et al. 2008). Of these 12 primers, 3 were definitive (fixed differences between NLMB and FLMB), with the remaining 9 containing some shared alleles. Primers were split into three matrices and a fluorescent M13 tail of either 6-FAM, VIC, NED, or PET was assigned to each. These tails labeled primers when used in a multicomponent mix for fragment analysis. For each fish, DNA was extracted from approximately 25 mg of liver tissue with the QIAGEN DNEasy Blood and Tissue Kit. Polymerase chain reaction (PCR) was performed across all fish for all 12 primers (Appendix A), with corresponding M13 tail added to each reaction. PCR products were then pooled by lake and by matrix for fragment analysis. Fragment analysis was performed on each pool with the Applied Biosystems 3130xl Genetic Analyzer at the LSU Genomics Facility. Outputs of each run were downloaded and manipulated with the software Geneious (Geneious Prime 2019.2.1). Peak calls were made for each microsatellite and results were then exported, where every locus had a 6-digit number that corresponded to the alleles present in each individual fish at each locus. Loci that failed to amplify were given a code of 000000.

2.2.4. DATA ANALYSIS

Results from fragment analysis were imported and processed in Microchecker (Van Oosterhout et al. 2004). Checked and corrected data were then analyzed with FSTAT (Goudet 1995) to provide summary statistics, including allelic richness, number of alleles per locus, and expected and observed homozygosity for each of the nine populations, including the three

parental stock populations, which provided baselines for the remaining six study populations. Introgression of Florida Bass was then inferred with the program STRUCTURE 2.3.4 (Pritchard et al. 2000), which used Bayesian clustering to identify populations and subpopulations among related individuals. User inputs included the number of populations ($K = 1-10$; 20 replications), 100,000 burn-in steps, and 100,000 Markov chain Monte Carlo iterations. To determine the most appropriate number of populations, STRUCTURE HARVESTER (Earl and vonHoldt 2012) was implemented to evaluate model likelihood and the best K based on the Evanno method (Evanno et al. 2005). Percent Northern and Florida ancestry (q) was assigned to each fish based on the proportion of ancestry from the Florida cluster. For the purpose of comparing results of the microsatellite analysis to those from allozymes, fish that possessed $\leq 10\%$ Florida ancestry were assigned as pure NLMB and those that possessed $\geq 90\%$ Florida ancestry were assigned as pure FLMB, with remaining individuals assigned as hybrids. These cut-off values were determined from the parental stock populations. However, this subspecific identity was only used in this comparison and percent FLMB ancestry for each fish was used in any subsequent analyses. Lastly, CLUMPAK (Kopelman et al. 2015) was used to create bar plots of individual ancestry proportions (q) for each fish and population.

I performed beta regression and logistic generalized linear models in Program R (R Core Team, Vienna, Austria) with functions from package “betareg” (Cribari-Neto and Zeileis 2010) and base Program R, respectively, to determine if there were significant differences in Florida ancestry among lakes. Similarly, beta regression was used to determine if percent Florida ancestry could be predicted by total number of times each waterbody was stocked. To reduce the dimensionality of the physicochemical data and fully describe the effects of habitat on percent Florida ancestry, multivariate analysis was performed with principal components analysis (PCA),

detrended component analysis (DCA), and non-metric multidimensional scaling (NMDS). The DCA was chosen as the primary form of analysis due to highly clustered data seen with the PCA and the higher values of the Wisconsin double standardization seen with NMDS, with values never dropping below 0.2. This DCA allowed for the habitat data to be reduced to detrended components (DCs) that could then be modeled against percent Florida ancestry to determine effects of each component based on simple linear, logistic generalized linear models, and beta regressions, with the model selected for inference based on the lowest AIC score.

2.3. RESULTS

A total of 480 fish was sampled during the course of the study, including parental stock populations. Genetic results yielded two distinct ancestry populations, based on STRUCTURE runs with $K=2$ as the best output (Figure 2.2). The greatest average percentage of FLMB ancestry was found in Poverty Point Reservoir (50.7%), whereas Lake Cataouatche had the greatest average percentage of NLMB ancestry (86.6%; Figure 2.3). Allelic richness was 7.33 ± 1.06 , while observed and expected heterozygosities were 0.62 ± 0.06 and 0.72 ± 0.04 , respectively (Table). Results of the first beta regression determined there was a significant difference in percent FLMB and NLMB ancestry within lakes ($AIC = -244$, $X^2 = 120.9$, $p < 0.01$), with all lakes differing from the overall grand mean of percent Florida ancestry with the exception of Poverty Point Reservoir. Results of the second beta regression determined there was also a significant difference in percent Florida ancestry in relation to the number of times the water body was stocked (parameter estimate = 0.079, SE = 0.011, $p < 0.01$; $AIC = -177$, $X^2 = 46.13$, $p < 0.01$).

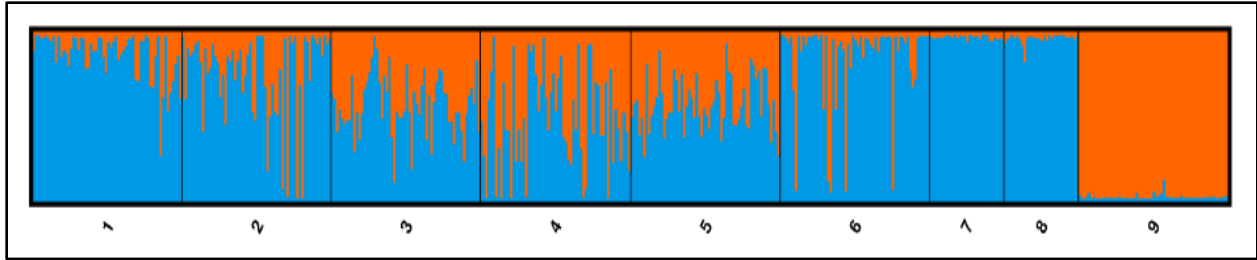


Figure 2.2. Results of STRUCTURE runs with $K = 2$. Each vertical line represents a fish, with FLMB ancestry indicated in orange, and NLMB ancestry indicated in blue. The populations shown are Lake Cataouatche (1), Lake D'Arbonne (2), Grand Bayou Reservoir (3), Poverty Point Reservoir (4), Lake Rodemacher (5), Lake St. John (6), and the three parental stock populations, Finch Lake (7) and Yucatan Lake (8) for NLMB, and Booker Fowler Hatchery (9) for FLMB.

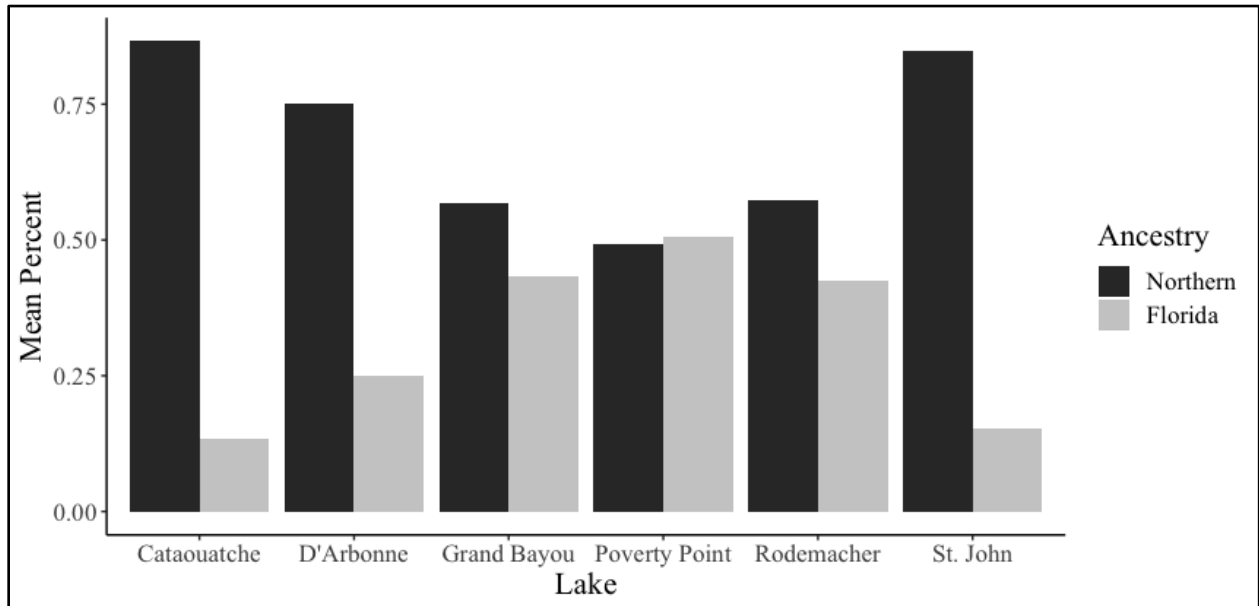


Figure 2.3. Mean percent of Northern and Florida ancestry of Largemouth Bass from six Louisiana lakes sampled in 2018-2020 determined with fragment analysis.

Overall, allozymes overestimated the number of NLMB in each water body and vastly underestimated the number of hybrid fish (Table 2.2). Results of the DCA for the six study lakes produced four linear combinations that reduced the dimensionality of the physicochemical data and provided scores to model against average percent Florida ancestry for each lake (Table 2.3). The first linear combination described lakes that had high levels of floating and submerged

vegetation, low levels of chlorophyll-a, and large littoral zones with vegetated shorelines featuring little anthropogenic development, such as houses or buildings. The second linear component characterized lakes that also had significant amounts of floating vegetation, however these lakes had considerably more shoreline development, less shoreline vegetation, and high turbidity. Lakes with higher amounts of emergent vegetation and highly developed shorelines that included buildings, vegetation, and falling timber were described by the third linear combination. Lastly, the fourth linear component described lakes with higher turbidity, an abundance of emergent and floating vegetation, and less submerged vegetation.

Table 2.2. Comparison of subspecific identification results from both allozymes and microsatellite analyses for Largemouth Bass sampled in Louisiana in 2018-20.

Lake	Allozyme Results			Microsatellite Results		
	Northern	Florida	Hybrid	Northern	Florida	Hybrid
Cataouatche	44	1	15	31	0	29
D'Arbonne	42	3	15	23	4	33
Grand Bayou	21	11	28	2	0	58
Poverty Point	20	12	28	8	8	44
Rodemacher	22	7	31	1	0	59
St. John	51	5	4	43	4	13

Table 2.3. Standardized variable scores for the four linear combinations (DCAs) retained by detrended component analysis on habitat and water quality data. Interpretable variables for each DCA are bold.

Variable	DCA1	DCA2	DCA3	DCA4
Cataouatche	1.38	-3.41	6.83	-1.85
D'Arbonne	-1.03	-0.87	3.24	-1.04
Grand Bayou	0.66	-1.52	-0.01	-1.08
Poverty Point	-3.13	0.49	-0.43	0.02
Rodemacher	0.64	1.59	2.52	2.56
St. John	-2.03	1.39	-1.24	-0.40
Month	-0.42	-0.03	0.34	-0.29
Littoral zone distance	1.55	-1.53	-0.49	-1.28
Percent floating vegetation	1.17	2.10	-0.97	-1.82
Percent emergent vegetation	0.16	-0.31	-1.36	1.42
Percent submerged vegetation	1.77	-1.34	0.06	1.19
Housing	-1.91	0.92	-0.17	-0.73
Building	-1.68	1.40	4.58	0.58
Open landscape	0.49	-0.57	0.77	-0.07
Timber standing	0.66	0.27	-0.24	0.14
Falling timber	-0.51	0.88	5.22	0.77
Open – no vegetation	-1.21	1.28	-0.11	-0.35
Vegetation	0.81	-1.97	1.34	-0.93
Developed	-2.22	0.79	1.02	-0.57

(table cont'd)

Variable	DCA1	DCA2	DCA3	DCA4
Chlorophyll-a	-1.78	0.76	-0.60	-0.34
Average depth	-0.79	0.26	0.22	-0.31
Average specific conductance	0.55	-0.86	0.96	-0.92
Average dissolved oxygen	-0.01	-0.38	1.18	-0.42
Average temperature	-0.17	-0.16	0.74	-0.33
Average turbidity	0.37	1.34	1.57	1.12
Average percent Florida ancestry	-0.49	0.50	0.41	0.27

Based on AIC scores from the analyses based on linear habitat combinations and average percent Florida ancestry, beta regression was the best fit model (Table 2.4). In this model, three of the four linear components were significant in explaining percent Florida ancestry (DCA2, $p < 0.01$; DC3, $p = 0.01$; DCA4, $p < 0.01$). DCA2 had a negative relationship with percent Florida ancestry (parameter estimate = 0.697, SE = 0.102, $p < 0.01$; Figure 2.4), whereas DCA3 and DCA4 had positive relationships (parameter estimate = -0.334, SE = 0.081, $p = 0.01$; parameter estimate = -0.330, SE = 0.091, $p < 0.01$; Figures 2.5 and 2.6).

Table 2.4. Summary of models used to regress percent Florida ancestry against DCA scores. Significant p-values ($p < 0.05$) are bold.

Model	AIC	Degrees of Freedom	DC1 p-value	DC2 p-value	DC3 p-value	DC4 p-value
Linear	-891.34	6	< 0.17	< 0.01	< 0.09	< 0.01
Logistic	716.45	5	< 0.79	< 0.02	< 0.67	< 0.08
Beta	-955.94	6	< 0.10	< 0.01	< 0.01	< 0.01

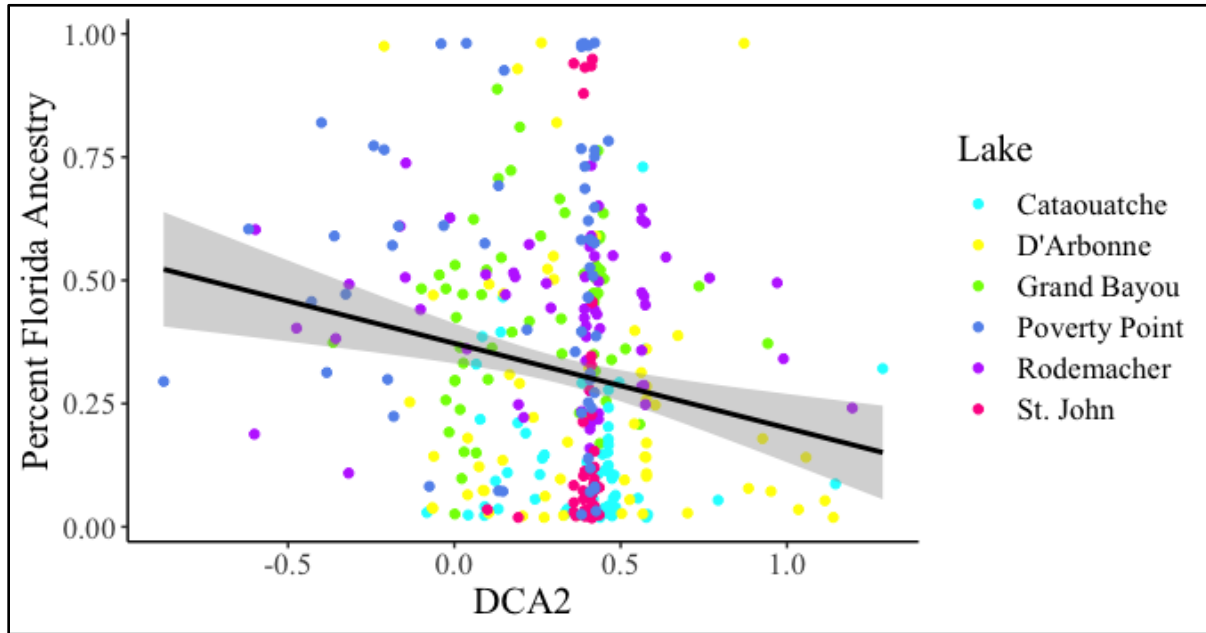


Figure 2.4. Relationship between linear component DCA2 obtained from detrended component analysis and percent Florida Largemouth Bass ancestry.

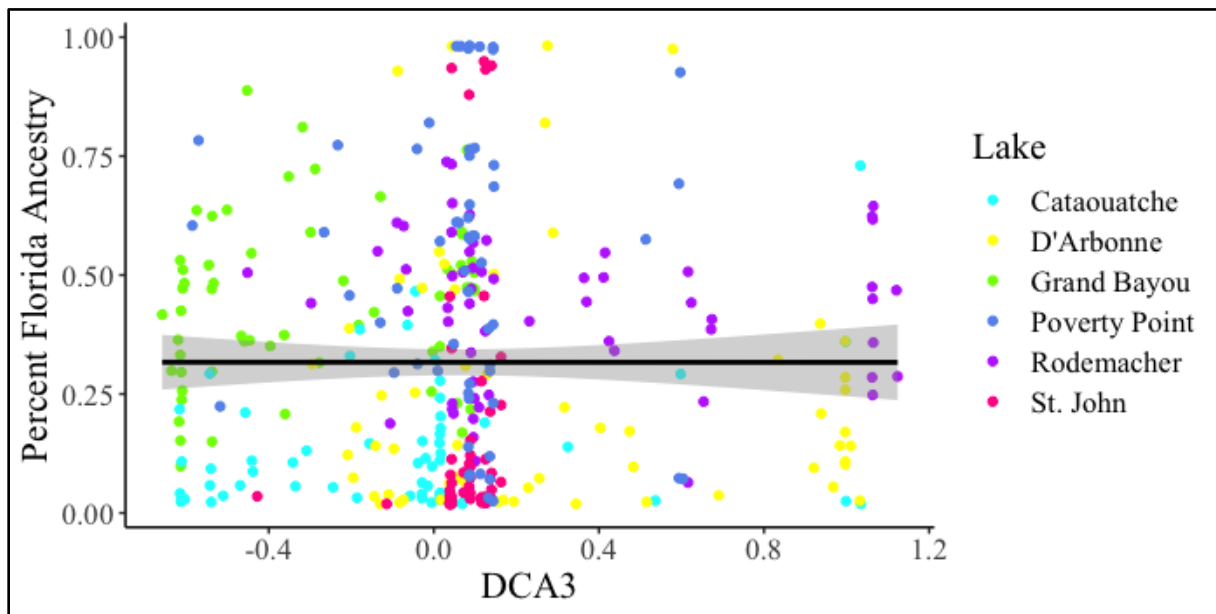


Figure 2.5. Relationship between linear component DCA3 obtained from detrended component analysis and percent Florida Largemouth Bass ancestry.

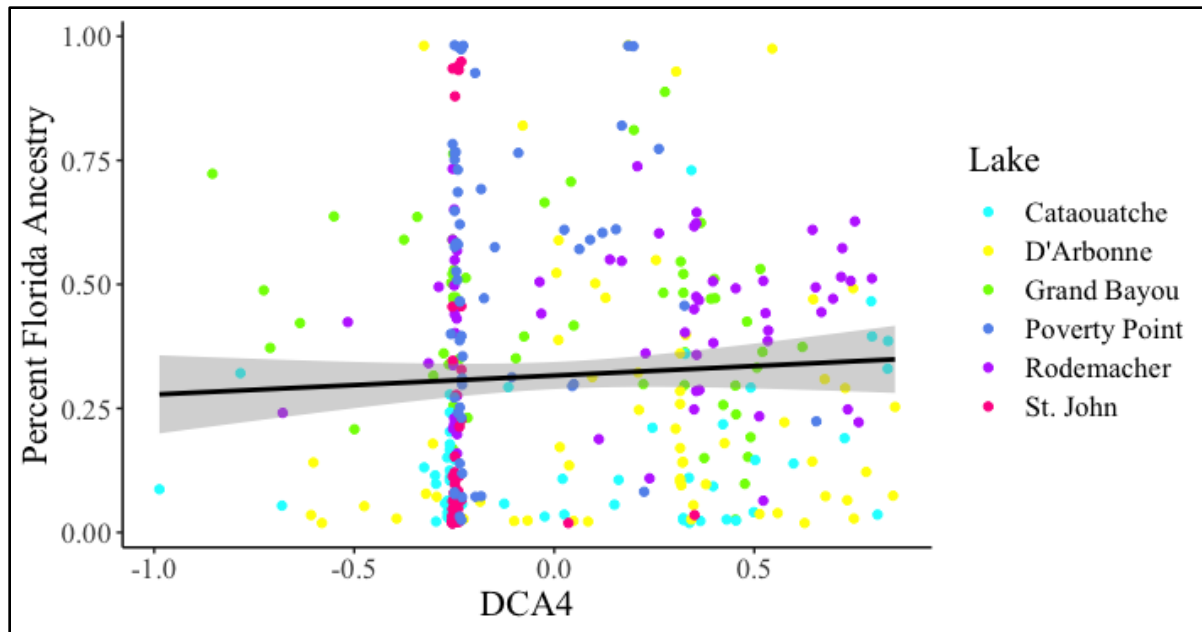


Figure 2.6. Relationship between linear component DCA4 obtained from detrended component analysis and percent Florida Largemouth Bass ancestry.

2.4. DISCUSSION

As expected, because of the increased number of assayed loci, microsatellite analyses allowed for increased accuracy in subspecific identification relative to allozymes (Corujo et al. 2004). Allozyme results also tended to overestimate observed homozygosity in a population (Degen et al. 1999; Delmotte et al. 2002), as seen with in my data, with higher numbers of NLMB identified than hybrids with allozymes compared to microsatellites. Historic data may have underestimated the level of introgression for F_x individuals and the overall amount of Florida ancestry in sampled populations. Allozyme results from a previous study in Louisiana (Fries 2010) follow the same trend as my results, with the highest number of fish identified as NLMB, and smaller estimates of hybrid and FLMB. One issue that will arise when comparing data from contemporary and future genetic studies of LMB to historic data is the reduced ability to correctly identify hybrid individuals with allozyme analyses as the number of F_x individuals increased. It is possible that conclusions concerning the success of FLMB introductions in the

past may not agree with conclusions based on the genetic identity now available with microsatellite data.

Overall, observed heterozygosities tended to be lower than expected, with the exception of three loci. However, both observed and expected heterozygosities were higher than in previous studies (Lutz-Carrillo et al. 2004; Lutz-Carrillo et al. 2006), suggesting that bass in Louisiana are not experiencing any declines in genetic diversity.

Percent Florida ancestry differed significantly across populations and those differences were related to the number of stocking events and several lake physicochemical characteristics. Successful introduction of non-native species is often related to propagule pressure, or the number of individuals released into a system a given time (Ruesink 2005; Von Holle and Simberloff 2005), with higher numbers of individuals in an initial release leading to higher chances of successful establishment (Memmott et al. 2004; Lockwood et al. 2005). In freshwater systems, propagule pressure has shown to be a driver of success in non-native species (Marchetti et al. 2004; Korsu and Huusko 2009; Woodford et al 2013). My results indicate the number of times FLMB were introduced, rather than the overall number, is related to the percent of Florida ancestry present, suggesting increased introductions heighten propagule pressure and allow for more successful establishment and maintenance of high-FLMB ancestry individuals.

Linear components DCA2, DC3, and DCA4 were significant in explaining the level of Florida ancestry in Louisiana lakes. DCA2 was negatively associated with percent Florida ancestry, indicating that more suitable habitat for FLMB was found in lakes with low turbidity, large littoral zones, little floating vegetation but considerable submerged vegetation, and highly vegetated shorelines with little to no development (in particular buildings). When waters are highly turbid, LMB forage less and are less selective in choosing prey items, often choosing less

energetically beneficial prey (Shoup and Wahl 2009) and exhibiting decreased piscivory (Huenemann et al. 2012). Piscivorous FLMB may be able to survive better in waters that are less turbid due to increased foraging opportunities. Submerged vegetation provides refugia from predation for smaller bass (Savino and Stein 1982; Durocher et al. 1984; Stahr and Shoup 2015), spawning habitat in the littoral zone (Shirley and Andrews 1977), and suitable foraging grounds for bass along the edges of aquatic macrophyte beds (Arocena 2007). Increased vegetation (up to a point) may thus promote FLMB survival and more optimal foraging conditions. Lakes with larger littoral zones and vegetated yet undeveloped shorelines allow for increased nesting opportunities for LMB, as bass have shown avoidance of developed areas for nest selection (Reed and Pereira 2009). Increased prevalence of FLMB in Louisiana lakes may also be attributed to increased nesting opportunities provided by these types of lakes. Although vegetation is positively related to FLMB, increased vegetation coverage and complexity can lead to decreases in piscivory (Sammons and Maceina 2006), increased size at which bass become piscivorous (Cailteaux et. al 1996), and decreased year-class strength (Nagid et al. 2015). Thus, it is important to ensure that vegetation is managed at an appropriate level in order to maintain high survival and subsequent growth of bass stocks (Valley and Bremigan 2002).

DCA3 described turbid lakes that had highly developed shorelines with buildings, woody debris and vegetation in the littoral zone, and was positively related to the presence of Florida ancestry. Although contrary to DCA2, higher turbidity associated with this component, in addition to structurally complex littoral zones, may have been related to reduced predation on stocked FLMB fingerlings (Lunt and Smee 2015). The source of turbidity (i.e. anthropogenic) could also impact the difference in association between these components (Collin and Hart 2014; Nieman 2019; Giacomazzo et al. 2020). It has been reported that growth of larger bass declines

when there are increased levels of shoreline infrastructure (Gaeta et al. 2011). Consequently, this positive association may be related to the abundance of small fish in my samples, and a relative paucity of larger fish older than 3+ in most lakes. It would be interesting to obtain larger samples of these older age classes and investigate whether the patterns of FLMB introgression in larger fish mirror my results. These lakes also were characterized by shorelines with fallen timber, contributing to shoreline habitat complexity and structure. LMB have shown a positive relationship with coarse woody habitat (CWH) in regard to shoreline development (Weis and Sass 2011) and exhibit lower consumption rates of less desired prey (Ahrenstorff et al. 2009). Fallen timber likely provides excellent low-density habitat for foraging and increased survival of FLMB.

Presence of Florida ancestry was positively related to DCA4, describing turbid lakes that had smaller littoral zones comprised primarily of submerged and emergent vegetation. This trend in vegetation is consistent with DCA 2 and 3, again suggesting the importance of littoral structural complexity in the foraging ecology of sub-adult and adult foraging FLMB, and perhaps the benefit of vegetation and turbidity to survival of stocked age-0 individuals (Nohner 2017; Looby et al. 2021). Few studies have focused on the habitat preferences between these two subspecies; however, because of the similarities between lakes in Louisiana and those in peninsular Florida, the endemic range of FLMB, it is likely that FLMB would respond the same to NLMB in these conditions.

2.5. CONCLUSION

To minimize costs and maximize outcome of stocking programs, it necessary to target populations in which introgression has been proven successful and manipulate habitats that are

not as conducive for FLMB survival. My analyses indicate that productive lakes with appropriate amounts of vegetative cover and CWH should be the primary focus for stocking efforts, and habitat manipulation should focus on littoral habitat complexity where necessary to improve survival of stocked fingerlings as well as foraging opportunities for larger individuals.

CHAPTER 3. AGE AND GROWTH

3.1. INTRODUCTION

Largemouth Bass (*Micropterus salmoides*; LMB) is considered one of the most sought-after sportfish in North America and consequently is a top management priority for many state fisheries agencies focused on producing trophy fish and satisfying angler desires (Buynak et al. 1999; Chen et al. 2003; Myers and Allen 2005; Hansen et al. 2015; Long et al. 2015; Tingley III et al. 2019). Management of LMB often involves manipulation of stocks through supplemental stocking (Boxrucker 1986; Maceina et al. 1988), habitat management (particularly vegetation alteration) (Pothoven et al. 1999; Bonvechio and Bonvechio 2006), and creel and size limits (Dotson et al. 2013; Miranda et al. 2017). One of the primary goals of LMB management programs across the U.S. is to increase, and subsequently maintain, high growth rates to produce the largest fish a system can sustain (Buynak and Mitchell 1999).

Since identification of the Florida Largemouth Bass (*Micropterus salmoides floridianus*, FLMB) subspecies by Bailey and Hubbs (1949), most state fisheries agencies in the southeastern U.S. have developed stocking programs to introduce and maintain FLMB genes in systems outside of its native range (Buckmeier et al. 2005). FLMB are endemic to peninsular Florida south and east of the Suwannee River drainage (Philipp et al. 1983), with populations intergrading with Northern Largemouth Bass (*Micropterus salmoides salmoides*, NLMB) in northern portions of the range. The native range of the Northern Largemouth Bass stretches from the Great Lakes and St. Lawrence River to the Mississippi River drainage and the Gulf of Mexico (Page and Burr 2011). These two subspecies are similar in appearance with only a few meristic differences, such as the number of lateral line scales and pyloric caecae (Zolczynski, JR.

and Davies 1976). However, despite these differences, considerable natural variation in morphology among largemouth bass (e.g., Hall et al. 2018), and subspecific hybridization makes visual identification of individual fish problematic in the intergrade zone and in stocked populations (Philipp et al. 1983).

The shift to stocking FLMB over NLMB in southeastern lakes and reservoirs is based on evidence from Florida and Texas (Inman et al. 1977; Maceina et al. 1988; Crawford et al. 2002; Myers and Allen 2005) suggesting greater maximum size in adult FLMB. Increased growth rate and maximum size have been historically linked to a longer growing season in their native range (Bailey and Hubbs 1949). However, this increased growth is not seen in all locations where FLMB are stocked (Clugston 1964; Zolczynski, JR. and Davies 1976; Philipp and Whitt 1991). Several studies have reported increased growth in hybrid fish, suggesting the possibility of hybrid vigor (Inman et al. 1977; Maceina et al. 1988; Kleinsasser 1990; Neal and Noble 2002). It is suspected that differences in growth rates and maximum size seen in FLMB stocked outside its native range are related to differences in environmental factors, such as temperature and vegetation type and density (Fields et al. 1987; Koppelman et al. 1988; Colle et al. 1989; Myers and Allen 2005) and prey availability (Keast and Eadie 1985; Hoffman and Bettoli 2005; Slaughter IV et al. 2008). However, few studies have provided conclusive evidence on the specific factors influencing the observed differences in FLMB growth in stocked populations.

Introgression of FLMB genes into native northern bass populations has been achieved in several southeastern states, including Oklahoma (Gilliland and Whitaker 1989; Gelwick et al. 1995; Acy 2017), Texas (Buckmeier et al. 2003; Ray et al. 2012), Alabama (Dunham et al. 1992), Tennessee (Hargrove et al. 2019a), and Arkansas (Johnson and Fulton 1999 & 2004, Lamothe and Johnson 2013). However, there are still concerns that introduction of FLMB genes

into non-native systems can result in stock impairment and possible outbreeding depression (Philipp 1991; Fries et al. 2002, Philipp et al. 2002; Cooke et al. 2005; Garlock et al. 2019). There is also debate concerning the relative importance of factors such as increased stocking size, increased survival of FLMB and hybrids (Maceina et al. 1988; Neal and Noble 2002; Peterson et al. 2017) and warmer temperatures (Gilliland and Whitaker 1989) on FLMB introgression success.

The Louisiana Department of Wildlife and Fisheries (LDWF) has stocked 115 million FLMB in over 220 waterbodies since 1982 to promote introgression of Florida alleles into native bass populations and enhance growth and harvest of trophy fish. Historically, subspecific identification was determined through analyses of isocitrate dehydrogenase (IDH, EC 1.1.1.42) and aspartate aminotransferase (AAT, EC 2.6.1.1) allozymes with fixed allele differences between NLMB and FLMB. However, these analyses were only useful for estimating proportions of northern, Florida, and hybrid individuals in these waterbodies, and the percentage of FLMB ancestry present in these populations were unknown. Further, misidentification of F_x individuals became more problematic after many years of stocking and interbreeding among hybrid individuals or backcrossing to parental fish (unpublished data).

Microsatellite analyses can provide a much more accurate and detailed assessment of FLMB introgression in stocked populations (Lutz-Carillo et al. 2006; Barthel et al. 2010). Microsatellites are repeated, non-coding sequences that can be used to detect polymorphic differences between subspecies. They provide more detail than previous allozyme analysis because of the increased number of loci used for identification (Lutz-Carillo et al. 2006). I used 12 optimized diagnostic primers to determine the current level of FLMB introgression in six Louisiana waterbodies that have varied histories of stocking frequency and density. I also

collected environmental data from these lakes to investigate introgression and persistence of Florida Largemouth Bass ancestry as well as estimates of bass length-at-age to investigate potential factors influencing growth of Largemouth Bass across the state. I hypothesized that: 1) percent Florida ancestry differs significantly across LMB populations in Louisiana; 2) higher percentages of Florida ancestry would lead to increases in growth and condition for LMB in Louisiana; 3) growth and length-weight relationships would be impacted by measured physicochemical characteristics.

3.2. METHODS

3.2.1. HABITAT SAMPLING

Eight Louisiana lakes located across the state were selected for this study in consultation with the Louisiana Department of Wildlife and Fisheries (LDWF). Bundick Lake was later removed because of a summer 2019 drawdown that limited boat access, and Chicot Lake was eliminated because of sampling limitations resulting from the COVID-19 pandemic. The remaining six lakes (Lake Cataouatche, Lake D'Arbonne, Grand Bayou Reservoir, Poverty Point Reservoir, Lake Rodemacher, and Lake St. John) varied in size and stocking intensity.

The shoreline of each lake was digitized with QGIS (QGIS Development Team 2009) and a 500-m² grid was overlaid to determine sampling sites (1000 m² was used for Lake D'Arbonne due to its size and shoreline complexity). All grids that contained over 100 m of shoreline were numbered and the total number of grids was divided equally into quadrants. To determine exact sampling locations, 40% of the grids in each quadrant were randomly selected with a random-number generator and coordinates were taken at the start of the grid along the

shoreline moving clockwise around the lake. The total number of sampling sites for each lake varied with lake size, and ranged from 16 (Lake St. John) to 30 (Lake D'Arbonne).

All lake habitats were sampled during Summer 2019 to ensure peak vegetation abundance for the season. A total of seven locations were sampled at each site with a random interval of boat travel time (30, 60, 90, 120 seconds) between each location. At each location, I recorded vegetation type and density as well as distance from the perimeter of the vegetation bed to the shoreline and percent cover of submerged, floating, and/or emergent vegetation. Vertical profiles were taken at the middle of each lake as well as at any significant points of interest, such as drastic changes in water color or appearance and overflow dams or other water control structures. Depth, dissolved oxygen, temperature, specific conductance, and turbidity were recorded with a multiprobe meter (ProDSS, YSI, Inc., Yellow Springs, OH). Water samples were collected in 1L bottles randomly at approximately a quarter of all sample sites and immediately placed on ice. Upon return to the lab, water samples were filtered through 47-mm glass microfiber filters to measure chlorophyll-a concentrations.

3.2.2. FISH SAMPLING

From 2018-2020, a sample of 60 Largemouth Bass was electrofished from each lake with the assistance of LDWF personnel. Sampling date varied across lakes due to availability of crew members. Collected fish were immediately placed on ice in the field and processed in the lab within 48-72 hours of sampling. Sex, total length (TL, nearest mm) and weight (W, nearest g) measurements were recorded for each fish. Livers were removed, immediately placed on ice, and then stored at -4 C for genetic analysis. Livers were also collected from bass populations in

Yucatan Lake and Finch Lake, as well as broodstock from Booker Fowler hatchery (total n=120), which served as parental stock populations for baseline genetic comparisons.

Sagittal otoliths were removed from fish that were at least 110 mm in length and each pair of otoliths was placed in a scintillation vial for drying. After at least one month of drying, one otolith was ground down to the focus from the posterior or anterior sides with a Dremel tool (Robert Bosch Tool Corporation, Racine, WI). The remaining half of the otolith was mounted with super glue (Loctite Super Glue) to a microscope slide and was then further ground down with the Dremel tool and finely sanded with 700 grain sandpaper to less than 2 mm in thickness. Slides were coated with a small amount of immersion oil B to allow for increased clarity and viewed with an Accu-Scope 3000-LED Series light microscope at 10X magnification. A scaled ocular micrometer was used to measure the distance from the focus to the edge of the last annulus and the distance from the focus to the edge of the otolith (Maccina 1988).

3.2.3. GENETIC SAMPLING

A total of 12 optimized primers were used in the microsatellite analyses of bass genetic identity. These primers were Lar7, Lma10, Mdo3, Mdo6, Mdo7, MiSaTPW011, MiSaTPW028, MiSaTPW055, MiSaTPW068, MiSaTPW090, MiSaTPW112, and MiSaTPW117 (Lutz-Carrillo et al. 2006; Lutz-Carrillo et al. 2008). Of these 12 primers, 3 were definitive (fixed differences between NLMB and FLMB), with the remaining 9 containing some shared alleles. Primers were split into three matrices and a fluorescent M13 tail of either 6-FAM, VIC, NED, or PET was assigned to each. These tails labeled primers when used in a multicomponent mix for fragment analysis. For each fish, DNA was extracted from approximately 25 mg of liver tissue with the QIAGEN DNEasy Blood and Tissue Kit. Polymerase chain reaction (PCR) was

performed across all fish for all 12 primers (Appendix A), with corresponding M13 tail added to each reaction. PCR products were then pooled by lake and by matrix for fragment analysis. Fragment analysis was performed on each pool with the Applied Biosystems 3130xl Genetic Analyzer at the LSU Genomics Facility. Outputs of each run were downloaded and manipulated with the software Geneious (Geneious Prime 2019.2.1). Peak calls were made for each microsatellite and results were then exported, where every locus had a 6-digit number that corresponded to the alleles present in each individual fish at each locus. Loci that failed to amplify were given a code of 000000.

3.2.4. DATA ANALYSIS

Results from fragment analysis were imported and processed in Microchecker (Van Oosterhout et al. 2004). Checked and corrected data were then analyzed with FSTAT (Goudet 1995) to provide summary statistics, including allelic richness, number of alleles per locus, and expected and observed homozygosity for each of the nine populations, including the three parental stock populations, which provided baselines for the remaining six study populations. Introgression of Florida Bass was then inferred with the program STRUCTURE 2.3.4 (Pritchard et al. 2000), which used Bayesian clustering to identify populations and subpopulations among related individuals. User inputs included the number of populations ($K = 1-10$; 20 replications), 100,000 burn-in steps, and 100,000 Markov chain Monte Carlo iterations. To determine the most appropriate number of populations, STRUCTURE HARVESTER (Earl and vonHoldt 2012) was implemented to evaluate model likelihood and the best K based on the Evanno method (Evanno et al. 2005). Percent Northern and Florida ancestry (q) was assigned to each fish based on the proportion of ancestry from the Florida cluster. For the purpose of comparing the results of the

microsatellite analysis to those from allozymes, fish that possessed $\leq 10\%$ Florida ancestry were assigned as pure NLMB and those that possessed $\geq 90\%$ Florida ancestry were assigned as pure FLMB, with remaining individuals assigned as hybrids. These cut-off values were determined from the parental stock populations. However, this subspecific identity was only used in this comparison and percent FLMB ancestry for each fish was used in any subsequent analyses. Lastly, CLUMPAK (Kopelman et al. 2015) was used to create bar plots of individual ancestry proportions (q) for each fish and population.

I ran beta regression and logistic generalized linear models in Program R (R Core Team, Vienna, Austria) with functions from package “betareg” (Cribari-Neto and Zeileis 2010) and base Program R, respectively, to determine if there were significant differences in Florida gene prevalence among lakes. Similarly, beta regression was used to determine if percent Florida ancestry could be predicted by total number of times each waterbody was stocked. Total length and weight for all bass were \log_{10} -transformed and a linear model following a Gaussian distribution was created that included a set of additional explanatory variables selected based on results of a previous study in Louisiana examining introgression of FLMB (Fries 2010). These variables were percent Florida ancestry, the quadratic of percent Florida ancestry, sex, average depth, average dissolved oxygen (DO), average distance from the edge of the vegetation bed to shoreline (VB distance), average chlorophyll-a, and average turbidity. A null model without additional variables was created and tested against the complex model for determination of statistical significance and explanatory ability based on root mean square error (RMSE), Pearson’s Chi-square/degree of freedom fit statistic ($\hat{\chi}$), likelihood ratio test (LRT), and Akaike’s Information Criterion (AIC).

To evaluate fish condition, Relative weight (W_r) was calculated for each fish as the individual weight divided by the standard weight for that fish, multiplied by 100. The standard weight was calculated by the log-transformed standard weight-length equation:

$$W_s = 10^{(-5.528 + 3.273 \times \log L)}$$

where a is the intercept and b is the slope of the log-weight and log-length regression equation, with values from Henson (1991) for LMB. This equation is only applicable to bass greater than 150 mm, thus fish smaller than 150 mm were excluded from all W_r analyses. A total of eight models were created in Program R: a linear model, a linear mixed model with a random lake effect, three generalized linear models (GLM) that followed a Poisson (log link), Gamma (inverse link), and negative binomial (log link) distribution, and three generalized linear mixed models (GLMM; package “lme4”, Bates et al. 2015) that followed the same distributions and a random lake effect. Each model included the same set of additional explanatory variables used in the length-weight model, and the best fitting model was selected based on RMSE and $\hat{\epsilon}$. A null model of the best fit model was then created and compared to the complex model by RMSE, LRT, and AIC and the final model was selected.

The Fraser-Lee method of back-calculation was used to estimate length at the most recent annulus for each fish:

$$L_i = \frac{L_c - a}{S_c} S_i + a$$

where L_c is the length of the fish at capture, S_c is the radius of the otolith at capture, S_i is the radius of the otolith at the most recent annulus, and a is the intercept of the regression of L_c and S_c (Schramm, Jr. et al. 1992, Kaller et al. in press). Due to limited sample sizes of fish, growth was only modeled for fish ages 1-3. Growth was initially modelled with von Bertalanffy, Gompertz, logistic, and Richards growth models, and a second set of models that also included a

random lake effect (PROC NLIN and NLMIXED, SAS, vers. 9.4, SAS Institute, Inc., Cary, NC). The logistic growth model without a random lake effect produced the smallest RMSE, and the additional variables used in the previous analyses were added to the logistic growth model separately to assess their effects on growth rate, k . Variables had to be modeled separately and not together as in previous analyses due to the complexity of the logistic growth equation and limited observations for parameterization. Models with additional variables were evaluated by RMSE to select the best model for growth of age 1-3 bass.

Exploratory data analysis indicated an interaction between length and percent Florida ancestry that changed over bass age of a population. Models compared in investigating this interaction were a GLMM with an identity link and normal distribution, a GLMM with a log link and Poisson distribution, a GLMM with a log link and a negative binomial distribution, and a GLMM with an inverse link and Gamma distribution, all with a random effect of lake (PROC GLIMMIX, SAS, vers. 9.4). The model with a (\hat{c}) closest to 1 was selected as the best model. Additional combinations of four physicochemical variables, depth, DOD, VB distance, and chlorophyll-a, were then introduced to attempt to increase the explanatory power of the subsequent model and then were evaluated by AIC.

3.3. RESULTS

A total of 480 fish were sampled during the course of the study, including parental stock populations. Genetic results yielded two distinct ancestry populations, based on STRUCTURE runs with $K=2$ as the best output. The greatest average percentage of FLMB ancestry was found in Poverty Point Reservoir (50.7%), whereas Lake Cataouatche had the greatest average percentage of NLMB ancestry (86.6%).

3.3.1. LENGTH-WEIGHT

A linear model following Gaussian distribution with additional physicochemical variables was the best model for explaining the relationship between log-weight and log-length (Figure 3.1). This model was selected over the null model based on AIC and RMSE (AIC = -1258, RMSE = 0.41; AIC_{null} = -1148, RMSE_{null} = 0.48). Significant parameters included in this model were percent Florida ancestry and its quadratic, Julian day, DOD, VB distance, and chlorophyll-a. Increases in percent Florida ancestry led to increases in weight (parameter estimate = 0.10, SE = 0.03, $p < 0.01$), up to a certain point at which the quadratic became negative (parameter estimate = -0.09, SE = 0.04, $p = 0.01$) and any additional Florida ancestry did not lead to increased weight. Fish sampled later in the year (larger Julian day), had decreased weight (parameter estimate = -0.0002, SE = 0.0001, $p = 0.049$). Increased DOD resulted in decreased weight (parameter estimate = -0.004, SE = 0.002, $p = 0.04$), whereas increased VB distance (parameter estimate = 0.002, SE = 0.0005, $p < 0.01$) and chlorophyll-a (parameter estimate = 0.003, SE = 0.001, $p < 0.01$) resulted in increased weight.

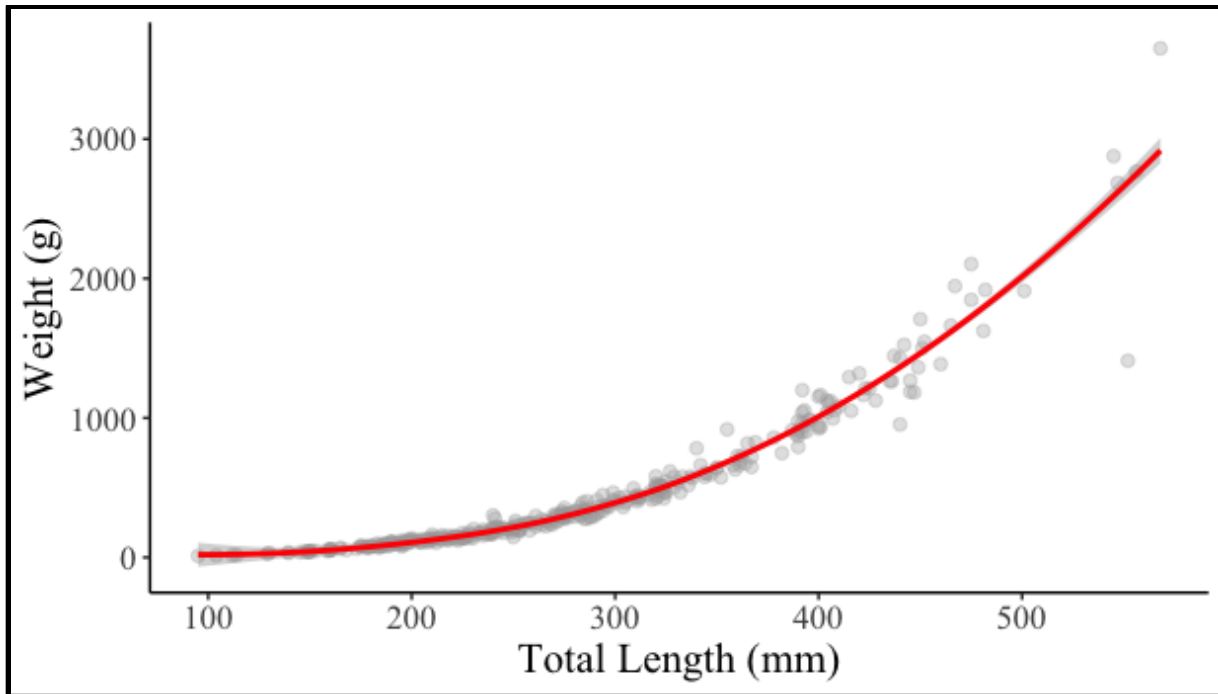


Figure 3.1. Total length (mm) and weight (g) relationship for Largemouth Bass in six Louisiana lakes sampled from 2018-2020.

3.3.2. RELATIVE WEIGHT

A generalized linear model with a Poisson distribution was selected as the best model to explain the effect of the measured physicochemical variables on W_r of bass in Louisiana.

Although GLMMs produced better fit statistics, singular boundary issues were detected with these models, and the models were excluded (Table 3.1). The full model with the additional explanatory variables was selected over the null model based on a significant difference in chi-square deviance ($X^2= 176.30, p < 0.01$). Of the additional variables included in the best model, percent Florida ancestry and its quadratic, DOD, VB distance, and average chlorophyll-a were significant, which was identical to the length-weight model, with the exception of Julian day.

Increased percent Florida ancestry resulted in an increase in W_r (parameter estimate = 0.28, SE = 0.08, $p < 0.01$); however, the negative quadratic term (parameter estimate = -0.26, SE = 0.08, $p < 0.01$) indicated that high percentages of Florida ancestry resulted in no additional increase, or

even a decrease in W_r . Increases in VB distance (parameter estimate = 0.004, SE = 0.001, $p < 0.01$) and average chlorophyll-a (parameter estimate = 0.007, SE = 0.001, $p < 0.01$) also resulted in an increase in W_r , whereas increased DOD resulted in a decrease in W_r (parameter estimate = -0.01, SE = 0.005, $p = 0.01$).

Table 3.1. Root mean square error (RMSE) and Pearson’s Chi-square/degree of freedom fit statistic (\hat{c}) estimates for relative weight (W_r) models for Largemouth Bass sampled in Louisiana in 2018-20. Models include a linear model (LM), a linear mixed model (LMM), generalized linear models following a Poisson (GLM_p), Gamma (GLM_g), and negative binomial (GLM_nb) distribution, and generalized linear mixed models following the same distributions (GLMM_p, GLMM_g, GLMM_nb). The model selected for further analysis with additional variables is in bold.

Model	RMSE	\hat{c}
LM	9.723	97.05
LMM	9.723	97.63
GLM_p	0.950	0.926
GLM_g	0.094	0.009
GLM_nb	0.950	0.926
GLMM_p	0.950	0.929
GLMM_g	0.094	0.009
GLMM_nb	0.950	0.932

3.3.3. AGE AND GROWTH

A logistic growth model without a random effect of lake was the best fitting model to explain age 1-3 bass growth, compared to von Bertalanffy, Richards, and Gompertz growth models with and without a random lake effect. Based on RMSE, the null logistic model was chosen as the best model, with addition of percent Florida ancestry and its quadratic producing

models with the highest RMSE (Table 3.2). The null model produced a growth curve typical for bass growth ages 1-3 in Louisiana (Figure 3.2; Kaller et al. in press).

Table 3.2. Root mean square error (RMSE) estimates for logistic growth models for age 1-3 Largemouth Bass in Louisiana. Models include a null model without any additional variables and a model for each additional variable included in the model as an effect on k, growth rate. The best model selected is in bold.

Model	RMSE
Logistic null	37
Logistic + Average DOD	38.4
Logistic + Average chlorophyll-a	39.6
Logistic + Average depth	40.2
Logistic + Average VB distance	47.1
Logistic + Sex	48.6
Logistic + Percent Florida ancestry	50.7
Logistic + (Percent Florida ancestry) ²	52.2

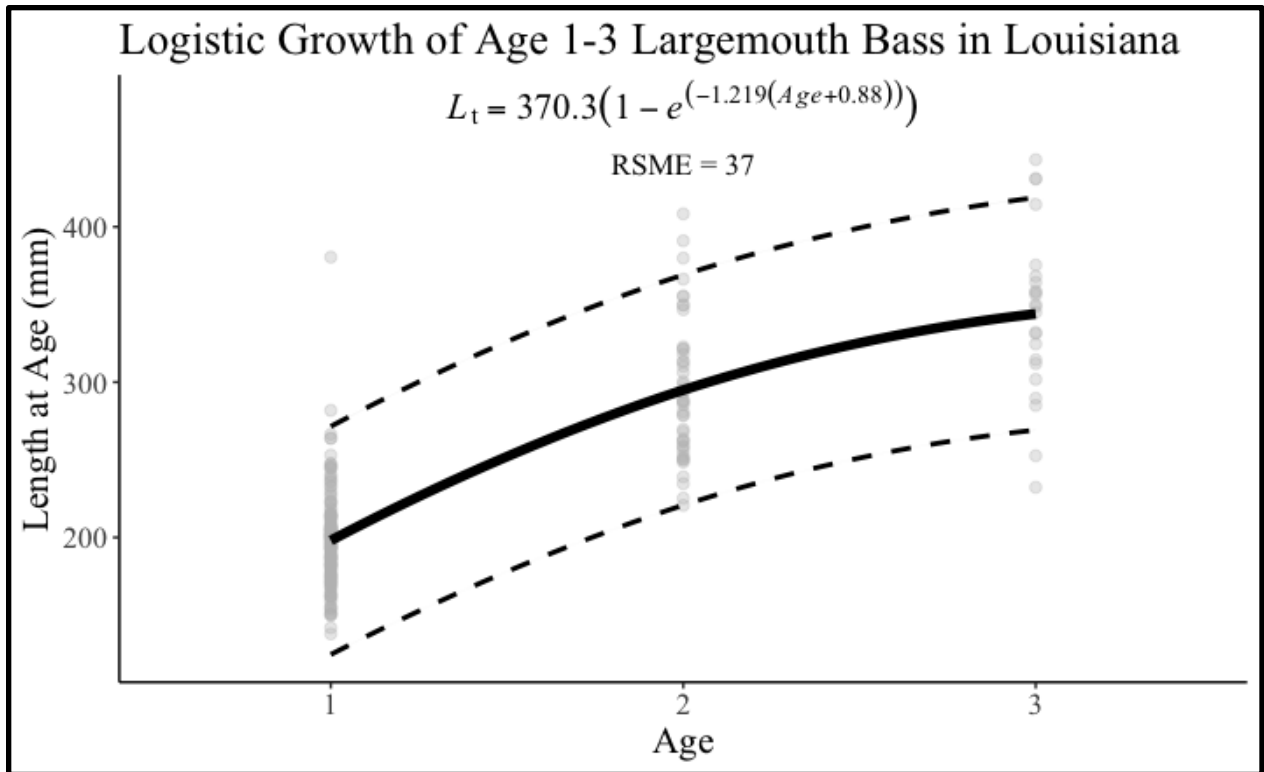


Figure 3.2. Logistic growth curve for Largemouth Bass ages 1-3 from six lakes in Louisiana sampled in 2018-20. Growth was modeled statewide because of a non-significant addition of a lake random effect.

A GLMM with a log link, Poisson distribution, and random lake effect was selected as the best fitting model for explaining the differential effects of percent Florida ancestry on total length at across ages 1-3. A total of 16 models were created with different combinations of additional explanatory variables (Table 3.3). However, the base model without additional explanatory variables was still selected the more informative model (AIC = 213).

Table 3.3. The top 6 models used to explain the relationship between length, age, and percent Florida Largemouth Bass ancestry and physicochemical variables for fish sampled in Louisiana from 2018-20. The models are named by the additional variables added to the base model.

Model	AIC	Δ AIC	AIC weight
Base Model	213	0	0.583
Depth	214	1	0.353
DOD	219	6	0.029
Chlorophyll-a	220	7	0.018
Depth DOD	221	8	0.011
VB distance	222	9	0.01

Results suggested a slight length advantage for individuals with approximately 50% FLMB ancestry at age 1, however, by age 3, the greatest lengths were attained by individuals with the highest percentages of FLMB ancestry, followed by high percentages of NLMB ancestry (Figure 3.3). The response surface changed with the addition of measured habitat variables, with the greatest lengths at age 3 exhibited by individuals with about 25% or 75% FLMB ancestry (Figure 3.4). Longer and older fish were also found more likely to be male hybrids (parameter estimate = 0.27, SE = 0.08; parameter estimate = -0.07, SE = 0.13).

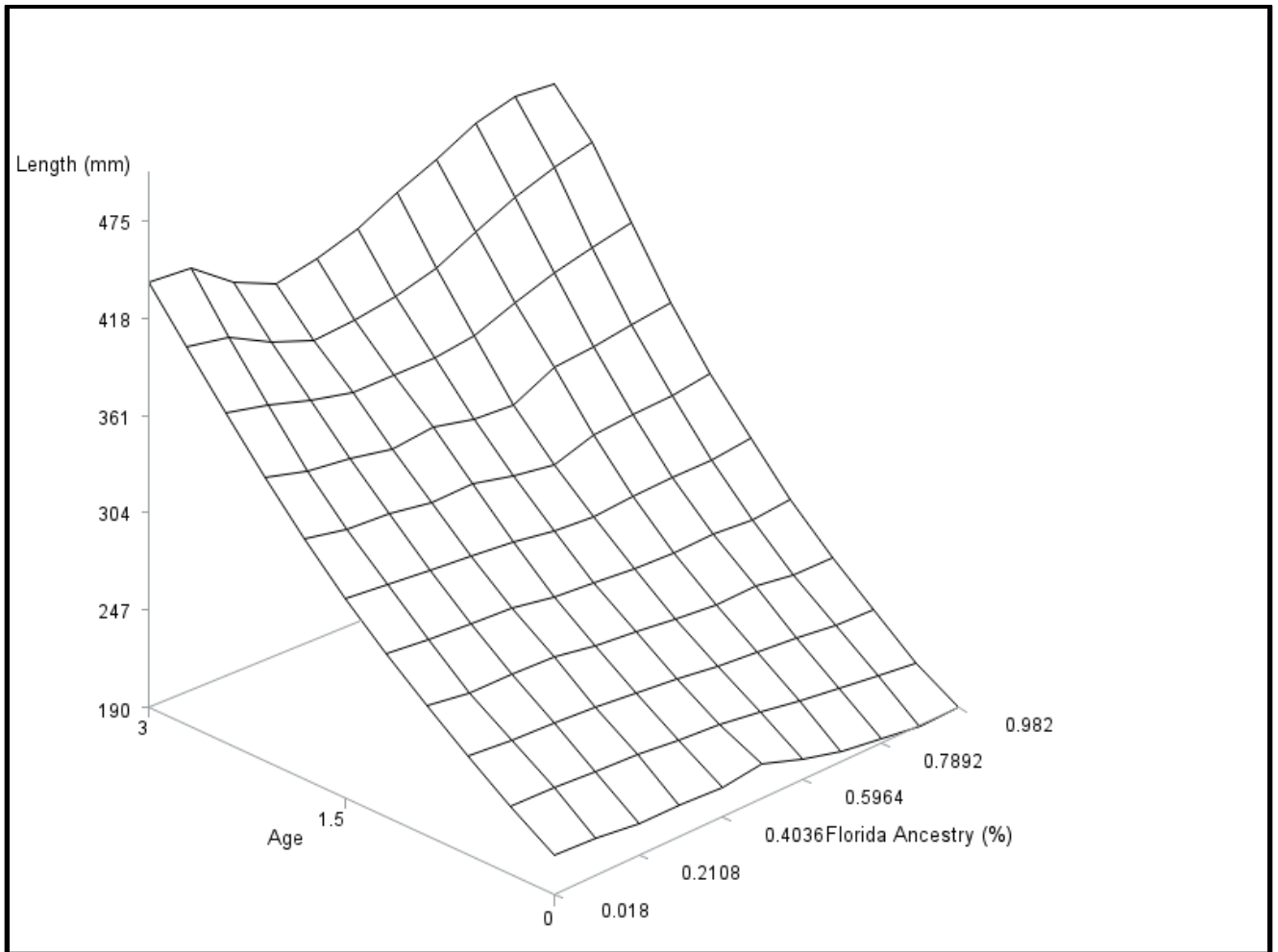


Figure 3.3. The effect of percent Florida ancestry on total length (mm) across 1-3 age Largemouth Bass sampled in Louisiana in 2018-20. The surface illustrates the generalized linear mixed model (GLMM) explaining interaction with percent Florida ancestry and length that changes with age, without additional explanatory variables.

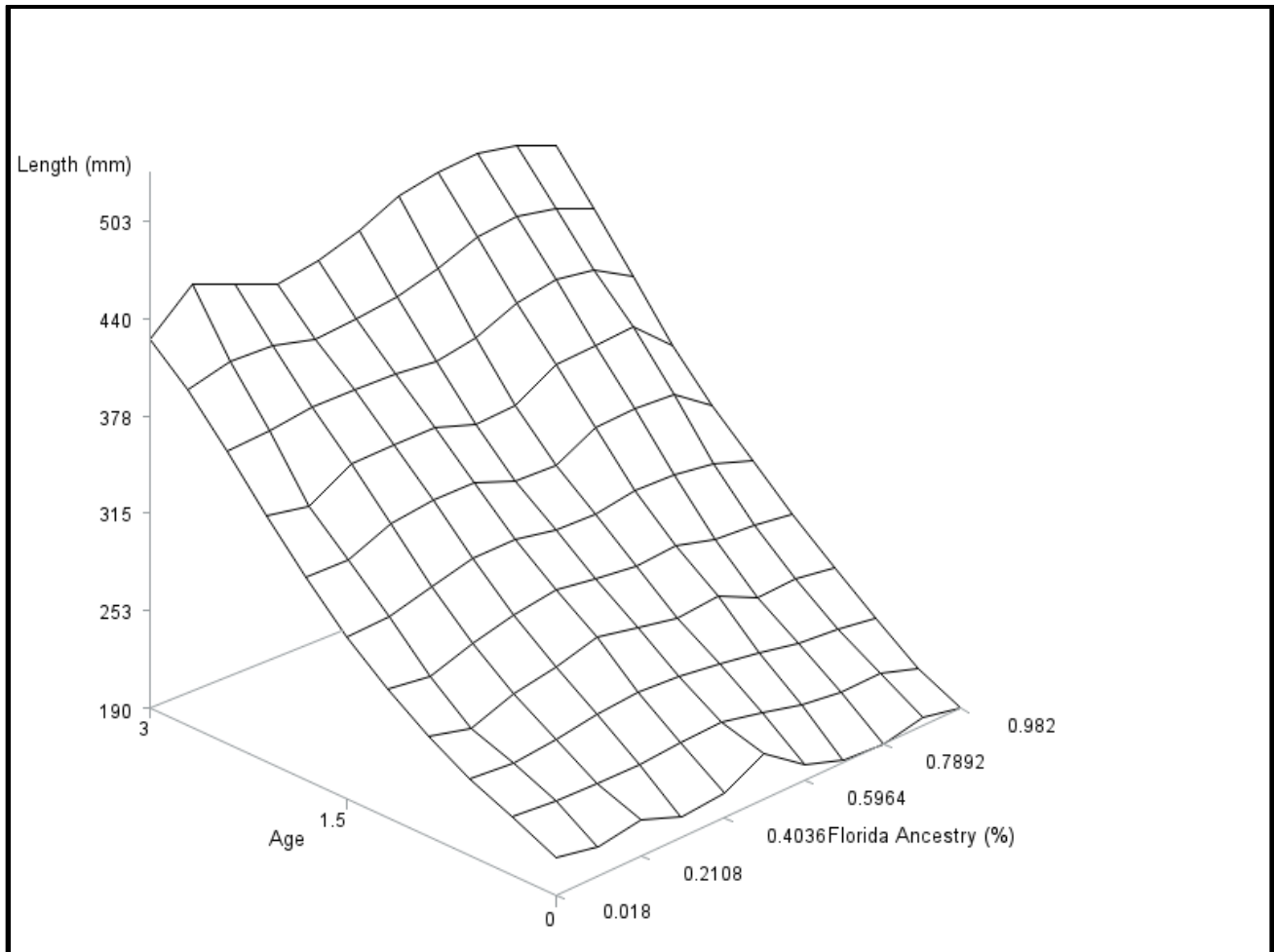


Figure 3.4. The effect of percent Florida ancestry on total length (mm) across 1-3 age Largemouth Bass sampled in Louisiana in 2018-20. The surface illustrates the generalized linear mixed model (GLMM) explaining interaction with percent Florida ancestry and length that changes with age, with additional explanatory variables.

3.4. DISCUSSION

My results indicate that FLMB ancestry plays an influential role in growth and condition characteristics for bass in Louisiana. Differences in length-weight relationships among NLMB, FLMB, and their hybrids have been reported historically, however these differences have not been directly related to genetic composition. Furthermore, there have been conflicting results as to whether Florida ancestry actually enhances or depresses growth. Kleinsasser et al. (1990) and Maceina and Murphy (1988) both reported lower length-weight increases and smaller maximum

size in pure FLMB compared to NLMB and their hybrids. However, trophy fishing opportunities in Texas and Arkansas have increased following FLMB supplemental stocking (Myers and Allen 2005; Lamothe and Johnson 2013 but see Hargrove et al. 2019b), and of 251 trophy bass sampled in Oklahoma, 93% had high or intermediate levels of Florida ancestry, with the largest and most robust individuals identified as either pure FLMB or hybrids (Horton and Gilliland 1993). Our length-weight analysis indicated that the increasing percent Florida ancestry increased weight, but only to a certain point at which the quadratic of the relationship became negative, suggesting a possible hybrid advantage (Neal and Noble 2002) for fish in Louisiana. This relationship was particularly evident when measured physicochemical variables were included in the model of length at age.

Similar to the length-weight model, Florida ancestry and its quadratic, and measured physicochemical variables significantly influenced W_r . Both percent Florida ancestry and its quadratic were significant, but with opposite effects, i.e., the presence of higher percentages of Florida ancestry increased W_r to a certain point, at which the relationship leveled off, with higher percentages of Florida ancestry failing to further increase W_r . As with the length-weight analyses, these results further suggest a possible hybrid advantage (Neal and Noble 2002). In Oklahoma and Texas, hybrids were found to have higher relative weights than NLMB (Inman et al. 1977; Wright and Wigtil 1980; Kleinsasser et al. 1990). Hybrid fish have also shown to be more robust relative to FLMB, exhibiting greater W_r at longer lengths despite similar growth (Neal and Noble 2002). Pure FLMB have shown to have lower W_r than both NLMB and hybrids, particularly at shorter lengths (Maceina and Murphy 1988; Kleinsasser et al. 1990). A previous study in Louisiana detected no differences in W_r among FLMB, NLMB, and their hybrids (Fries 2010), but this study was based on allozyme analyses, and it is likely the increased accuracy of

identity obtained with microsatellites permitted much finer resolution of genetic differences between individuals and a more robust assessment of FLMB introgression on W_r .

Additional physicochemical variables that were significant in the best fit model for length-weight and W_r were DOD, vegetation bed distance, and chlorophyll-a, to the same effect in both models. Julian day was also significant in the length-weight model, with fish sampled later in the year exhibiting lower weights than those sampled earlier in the year. Bass spawn between mid-February to mid-April in subtropical environments (Mesing and Wicker 1986). In preparation for spawning, female bass significantly increase in weight due to increased gonad production and drastically decline in weight following spawning (Brown and Murphy 2004). Thus, bass sampled later in the year after spawning were likely to have exhibited lower weights than those sampled during the pre-spawning period.

My results showed a positive relationship between vegetation bed distance and chlorophyll-a for both weight and W_r . Both of these measures indirectly assess the amount of vegetation in a lake. Aquatic vegetation coverage is important for evading predation and survival (Savino and Stein 1982; Durocher et al. 1984), increasing foraging opportunities on associated invertebrates (Arocena 2007), and providing spawning habitat in the littoral zone (Shirley and Andrews 1977). Greene and Maceina (2000) and Boucek et al. (2017) found bass with the best condition and growth in eutrophic systems with higher chlorophyll-a concentrations, suggesting that productive lakes support optimal bass growth. Although my results indicate a positive association with increased vegetation bed distance from shore, increased vegetation coverage and complexity can lead to decreases in piscivory (Sammons and Maceina 2006), increased size at which bass become piscivorous (Cailteaux et al. 1996), and decreased year-class strength (Nagid

et al. 2015). Thus, it is important to ensure that vegetation is managed at an appropriate low or intermediate level in order to maintain high growth of bass stocks (Valley and Bremigan 2002).

Results also indicated a negative influence of DOD on the length-weight relationship and W_r . Lakes with higher DOD indicate significant stratification, which is of particular concern in the summer months. Highly stratified lakes experience hypoxia (<2 mg/L) in the hypolimnion that can negatively impact aquatic organisms and plants. Under hypoxic conditions, LMB have been reported to exhibit decreased prey consumption (Brown et al. 2015; French and Wahl 2018), reduced growth potential (Budnik et al. 2021), and decreased year-class strength and predictability (Nagid et al. 2015). FLMB have also been shown to be less tolerant to hypoxic conditions compared to NLMB and their hybrids (Carmichael et al. 1988). Importantly, strongly stratified lakes reduce the habitat available not only to LMB, but the entire trophic web, and chronic hypoxia summer stratification may have significant impacts on LMB growth, condition, and survival. These effects may be exacerbated under a warming climate scenario in the coming decades (Woolway et al. 2021), reducing the number of mixing events (Woolway and Merchant 2019), and increasing the frequency and duration of hypoxic conditions (Butcher et al. 2015). These data highlight the importance of mitigating the effects of stratification in the future, particularly in Louisiana, where lake stratification is common (Moore 1950; Moore 1970; Hartung 1983). Lake aeration has been shown to help mediate the effects of summer hypoxic conditions in highly stratified lakes (Cowell et al. 1987; Kortmann et al. 1988) and can provide managers with a tool to prevent deleterious effects on bass growth.

Because of limited sample sizes of older fish, growth could only be modeled for bass ages 1-3. Growth of LMB over these age classes was not significantly affected by the percentage of Florida ancestry or any additional measured physicochemical variables, although addition of

Florida ancestry and its quadratic in logistic models resulted in the highest RMSE values. Several studies have reported superior growth of NLMB relative to FLMB and their hybrids in the first year (Zolczynski and Davies 1976; Inman et al. 1977; Kleinsasser et al. 1990; Lei et al. 2012). Increased growth that surpassed that of FLMB has also been reported in older NLMB (Isely et al. 1988; Philipp and Whitt 1991), although superior growth of hybrids relative to NLMB has also been reported in older fish (Inman et al. 1977; Horton and Gilliland 1993). Growth advantages of FLMB may not be apparent in the first few years of life but may actually result from increased longevity (Maceina et al. 1988; Neal and Noble 2002). Stocking location is also important in this regard, as introductions into systems that are substantially different from those in the native FLMB range may be less successful because of lowered survival (Zolczynski and Davies 1976; Slaughter IV et al. 2008). Although sampling younger fish has been shown to provide representative data for introgression studies (Dumont and Lutz-Carillo 2011), I expect results of the growth analyses would have been different had I been able to sample larger numbers of older (age 4+) individuals.

The relationship between length and percent Florida ancestry changed across ages. This relationship was examined by the composite model of length and percent FLMB ancestry that included DOD, depth, vegetation bed distance, and chlorophyll-a, and the model indicated it was only slightly beneficial for a fish to be an intermediate hybrid at year 1. However, by age 3, fish that possessed 75% FLMB ancestry were longer than pure FLMB and NLMB. Several previous reports have indicated NLMB were larger than FLMB and hybrids during the first year of growth (Zolczynski and Davies 1976; Inman et al. 1977; Maceina and Murphy 1988). However, as fish increased in age, growth advantages were evident in hybrid fish (Inman et al. 1977; Horton and Gilliland 1993). The finding that longer, older fish were more likely to be hybrids further

strengthens the idea of an advantage possessed by hybrids (Neal and Noble 2002). Importantly, addition of physicochemical parameters in the analyses of LMB length at age altered this relationship for Louisiana LMB stocks, suggesting a large role of habitat that can likely dampen or enhance growth advantages given by FLMB introgression.

3.5. CONCLUSION

To minimize costs and maximize outcome of stocking programs, it necessary to target populations in which introgression has been proven successful and manipulate habitats that are not as conducive for FLMB survival. My data along with other studies have shown that any growth and condition advantages provided by FLMB introgression may not compensate for less ideal environmental conditions (Slaughter IV et al. 2008). Shallower, productive lakes with appropriate amounts of vegetative cover should be the primary focus for stocking efforts, and management programs targeting vegetation management and summer aeration of severely stratified lakes may improve growth in selected systems. Supplemental stocking of forage fishes (particularly Threadfin Shad; Maceina and Sammons 2015) would encourage fast growth in all LMB stocks, increasing the likelihood of providing quality fishing overall and trophy fisheries in particularly suitable systems.

APPENDIX A. SUMMARY OF GENETIC PROTOCOL

Table A.1. Microsatellite primer sequences, annealing temperature (T_a), expected (H_E) and observed (H_O) heterozygosities, range in allele size (bp), and allelic richness (number of alleles). The primer name is listed by the sequence in forward and then reverse. The sequence for the primer M13 on the forward sequence is also included.

Locus	Primer sequence 5'-3'	T_a (°C)	H_E	H_O	Size (bp)	Allelic Richness
Lar7	GTGCTAATAAAGGCTACTGTC TGTTCCCTTAATTGTTTTGA	47	0.68	0.73	125-191	8.60
Lma10	GTCTGTAAGTGTGTTTGCTG GAAACCCGAACCTTGCTAG	57.7	0.78	0.45	127-149	7.83
Mdo3	AGGTGCTTTGCGCTACAAGT CTGCATGGCTGTTATGTTGG	46.2	0.76	0.67	126-144	5.63
Mdo6	TGAAATGTACGCCAGAGCAG TGTGTGGGTGTTTATGTGGG	55	0.39	0.32	166-172	1.70
Mdo7	TCAAACGCACCTTCACTGAC GTCACCTCCATCATGCTCCT	53	0.77	0.66	176-188	8.47
MiSaTPW011	CAACATGGACGCTACTAT CAACCATCACATGCTTCT	60	0.85	0.91	172-228	10.61
MiSaTPW028	TTGAGTTATTTATGCCATTA ACCGAAGCTCTAAGAGG	45	0.76	0.75	240-264	6.57
MiSaTPW055	ATCATGGTCTAAAAACTATT TTTTGAATGGAATTAAGT	50	0.74	0.51	120-148	6.18
MiSaTPW068	CAACTTTAATGCAAATACAGA CAGGCAGGCTTAAGTAA	55	0.91	0.96	174-250	15.20
MiSaTPW090	TGCCAGAGATCCTGAGCTAC CCACTTACCTGAATAACCAGAGACA	55	0.75	0.48	159-199	6.25
MiSaTPW112	GTCCACCAGAGACAACCTGCGT CTAGTGCGACCCAGAGAACG	60	0.40	0.34	164-196	1.70
MiSaTPW117	TGTGAAAGGCACAACACAGCCTGC ATCGACCTGCAGACCAGCAACACT	60	0.87	0.62	217-275	9.20
M13(-29)	CACGACGTTGTAAAACGAC					

Table A.2. Polymerase chain reaction (PCR) recipe used to amplify 12 loci for subspecific identification of Louisiana Largemouth Bass (*Micropterus salmoides*). Desired concentrations for specific reagents are listed in parentheses.

Reagent	Volume (μL)
dH ₂ O	4.20
Buffer	1.00
dNTP's	1.00
MgCl ₂	1.20
Forward primer (10 μM)	0.15
Reverse primer (10 μM)	0.30
DMSO	0.20
Betaine	0.40
Taq	0.25
M13	0.30
DNA (35 μM)	1.00

Table A.3. Polymerase chain reaction (PCR) thermocycler protocol used to amplify 12 loci for subspecific identification of Louisiana Largemouth Bass (*Micropterus salmoides*). The annealing temperature used is specific to each primer for a particular locus (See Table A.1.).

Step	Temperature (°C)	Duration (min)
1	94	2:00
2	95	0:30
3	Annealing temperature	0:30
4	72	0:45
5	Go to Step 2	35 cycles
6	72	10:00
7	10	Indefinite

APPENDIX B. SUMMARY OF PHYSICOCHEMICAL CHARACTERISTICS

Table B.1. Mean values of measured physicochemical variables from six study lakes in Louisiana, collected in the summer of 2019. VB distance is the distance from the edge of the vegetation bed to the shoreline.

Lake	Percent Emergent Vegetation	Percent Submerged Vegetation	Percent Floating Vegetation	VB Distance (m)	Chlorophyll-a ($\mu\text{g/L}$)	Depth (m)	Specific Conductance ($\mu\text{S/cm}$)	Dissolved Oxygen (mg/L)	Dissolved Oxygen Differential	Temperature ($^{\circ}\text{C}$)	Turbidity (NTU)
Cataouatche	0	28.51	3.63	50.83	15.3	0.65	0.35	7.48	1.18	31.38	8.32
D'Arbonne	24.16	5.84	2.23	8.55	32.47	3.59	0.07	8.66	7.44	40.28	8.19
Grand Bayou	30.73	11.73	6.37	20.44	13.14	1.63	0.05	5.49	4.15	27.67	6.32
Poverty Point	0.75	0.02	0	0.07	58.22	0.66	0.17	8.65	5.67	31.66	16.88
Rodemacher	31.58	12.57	18.61	10.22	6.54	1.50	0.10	6.30	1.16	30.81	41.40
St. John	36.50	0.76	8.13	6.70	58.00	2.44	0.22	4.69	11.70	29.00	6.57

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VITA

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