IDENTIFICATION OF *Micropterus salmoides floridanus* POPULATIONS IN BARROW PIT PONDS USING CELLULOSE ACETATE ELECTROPHORESIS

By

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This document is dedicated to my wife Samantha for her constant love and support.

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Barrow pit ponds in Florida are an attractive potential source for fish that could be utilized in stocking programs. However, implementing such fish management programs on a large scale in Florida could be problematic because of concerns about adversely affecting the genetics of the Florida largemouth bass (*Micropterus salmoides floridanus*) with indiscriminant stocking of intergrade or pure northern largemouth bass (*M. s. salmoides*). A cellulose acetate electrophoresis method (a relatively easy and inexpensive method) was used to differentiate known *M. s. floridanus* from known intergrade largemouth bass obtained from a Florida Fish and Wildlife Conservation Commission hatchery. Known diagnostic alleles were identified at the *sAAT-B** and *sIDHP** loci. The cellulose acetate electrophoresis method was then used to identify Florida and intergrade largemouth bass populations in barrow pit ponds in Florida. Highway barrow pit ponds in Orange and Sumter Counties had largemouth bass populations for which 100% of the alleles at two diagnostic loci were specific for *M. s. floridanus*. Barrow pit

ponds in Alachua and Jackson Counties were found to have intergrade populations. Two barrow pit ponds in St. Johns County had largemouth bass populations for which 100% of the alleles at two diagnostic loci were specific for *M. s. floridanus*. But, two other barrow pit ponds in St. Johns County had low frequencies (0.03 in Pond F, 0.04 in Pond B at *sAAT-B**, and 0.02 in Pond F at *sIDHP**) of northern alleles.

CHAPTER 1
INTRODUCTION

Bailey and Hubbs (1949) first described the Florida largemouth bass (*Micropterus salmoides floridanus*) as a separate subspecies in 1949 using taxonomic features (e.g., scale counts). They described the natural range of the northern largemouth bass (*Micropterus salmoides salmoides*) as north and west of the Choctawhatchee River and Apalachicola River drainages in Florida, Alabama, and Georgia, and north and east of the Savannah River drainage in South Carolina. The Florida subspecies was thought to reside in peninsular Florida to the south and east of the Suwannee River drainage, including the St. Johns River system. The area between these two regions was found to contain intergrade (genes from both subspecies) fish populations.

Philipp et al. (1983), using genetic techniques, redefined the geographic boundaries of the two subspecies and the extent of the intergrade zone in the 1980s. They found that the range of the Florida subspecies was similar to that originally proposed by Bailey and Hubbs (1949), but the intergrade zone had extended greatly to include the east coast north to Maryland, as well as most of Alabama and Mississippi. They further concluded that the expansion of the intergrade zone was due primarily to the stocking of Florida largemouth bass by state fish and wildlife agencies (Philipp et al. 1983).

Since the late 1990s, there have been concerns that stocking wild and hatchery-reared largemouth bass may be adversely affecting the genetics of native Florida and northern largemouth bass populations (Philipp et al. 1981, Fields et al. 1987, Philip 1991, Philipp and Whitt 1991, Maceina et al. 1992). During 1999, 2000, and 2001, Florida

experienced major drought conditions. Due to the prolonged drought, many lakes in north Florida completely dried or experienced extremely low water levels. This caused a loss/reduction of largemouth bass populations and a decline in fishing effort. This has lead some individuals to propose that adult or juvenile largemouth bass be stocked to provide immediate relief (stocking for economic mitigation) to local fisheries (Harris Chain of Lakes Restoration Council [HCOLRC] 2004). Highway barrow pit ponds or other types of pit ponds such as quarry ponds are a potential source for large fish in Florida. Fish stocked from barrow pit ponds can provide the larger largemouth bass that cannot be raised in large numbers in fish hatcheries and could reduce the demands on hatcheries for advanced-fingerling largemouth bass. Barrow pit ponds could also provide sufficient numbers of largemouth bass to ultimately allow for more water bodies in Florida to be stocked when needed. However, implementing such stocking programs on a large scale in Florida could be problematic because the Florida Fish and Wildlife Conservation Commission (FFWCC) has concerns about adversely altering the genetics of pure Florida largemouth bass populations with stocking of intergrade or pure northern fish.

The objectives of this project were (1) to assess if the cellulose acetate electrophoresis method (a relatively easy and inexpensive technique) could be used to differentiate known *Micropterus salmoides floridanus* from known intergrade largemouth bass, and (2) use cellulose acetate electrophoresis to determine the sub-specific genetic status of Florida and intergrade largemouth bass populations from barrow pit ponds in Florida.

CHAPTER 2
METHODS

Allozyme electrophoresis (cellulose acetate electrophoresis method) analysis was performed on tissue samples obtained from the FFWCC to determine the specific allele genotypes of known Florida largemouth bass and known intergrade largemouth bass. An allele is the form of a gene located at a specific locus. Loci are specific locations on a chromosome where genes reside. A genotype is the actual alleles present at the locus. The Florida and northern subspecies of largemouth bass are fixed for different alleles at two loci; those loci being *sIDHP** (Isocitrate dehydrogenase) and *sAAT-B** (Aspartate aminotransferase). Consequently, the two subspecies can be distinguished by use of electrophoretic techniques based on the fact that the Florida largemouth bass is fixed for the *sIDHP*3* allele and a combination of the *sAAT-B*3* and **4* alleles, whereas the northern largemouth bass is fixed for the *sIDHP*1* allele and a combination of the *sAAT-B*1* and **2* alleles (Philipp et al. 1983). (Nomenclature follows that of Shaklee et al. 1990) By comparing the known genotypes of the Florida and intergrade largemouth bass to the genotypes obtained from the liver samples taken from largemouth bass collected from the barrow pit ponds, it should be possible to assess the allele frequencies of the largemouth bass populations in each pond.

Largemouth bass from 16 barrow pit ponds and one limerock quarry pond were collected by boat electrofishing using a 5000-Watt AC generator, a Smithroot model VI-A pulsator, and a bow mounted cathode probe to supply the electrical output to the water. One person operated the boat and pulsator, while one to two individuals netted fish from

the bow of the boat. The sampling goal was to obtain 50 largemouth bass from each pond. A sample size of 50 fish was chosen as an adequate sample size based upon time in the field, effort involved in sampling, relative size of the pond, and past studies (Philipp et al. 1983, Dunham et al. 1992, Gelwick et al. 1995, Forshage and Fries 1995). However, it was not always possible to collect 50 fish due to environmental conditions.

Once collected, the fish were either transported live to the laboratory or processed in the field. At the laboratory, the fish were put in an ice-water bath until movement stopped. Liver tissue was extracted by making an incision through the abdominal cavity, locating the liver, removing a portion of the liver, and placing it into a cryogenic vial. The samples were then placed into a –70 C freezer until analyzed. For ponds that were long distances from the laboratory, the tissue removal process was performed in the field. The field procedure was identical to that of the laboratory except that samples were stored in a cooler of dry ice until returned to the laboratory and stored in a -70 C freezer until analyzed.

Liver tissues were manually ground in Eppendorf tubes that contained 100-200µg of a grinding solution. Tris-Glycin was diluted with deionized (DI) water to a ratio of 1:9 and used as the grinding solution for samples that were to be stained for asportate aminotransferase (*sAAT-B**). Nicotinamide adenine dinucleotide phosphate (NADP) was used as the grinding solution for samples that were to be stained for isocitrate dehydrogenase (*sIDHP**) (Sigma-Aldrich, Cat No: 2934.90.3900). While grinding tissues, the tubes were placed into ice to prevent enzyme degradation due to heat. Once homogenized, samples were centrifuged (Qualitron, Cat No: DW-41) for approximately 1 to 2 minutes. After centrifugation, 10-µl aliquots of the resulting supernatant were added

to individual wells of the sample loading plate (Helena Laboratories, Cat. No. 4096, Beaumont, TX). Samples were transferred from the wells of the sample plate onto a cellulose acetate gel (Helena Laboratories, Cat. No. 3033, Titan III, 76 mm x 76 mm, Beaumont, TX) with the use of a Helena Super Z-12 applicator (Helena Laboratories, Cat. No. 4090, Beaumont, TX). Prior to sample application, the cellulose acetate gels were soaked in a buffer solution (Tris-Glycin diluted 1:9 with DI water) for at least 20 minutes (Prepared according to Hebert and Beaton 1993).

Tissues from fish with known allele genotypes (i.e., obtained from FFWCC) were placed into the first two wells of every gel and used as benchmarks for comparison with fish of unknown genetic makeup. The gels were then placed onto wicks in an electrophoresis tank with Tris-Glycin diluted 1:9 with DI water used as a buffering solution (Hebert and Beaton 1993). Electrophoresis was performed at room temperature at 200 volts for 15 to 20 minutes (Figure 1). After the appropriate amount of time (15 to 20 minutes), the gels were removed and histochemically stained with solutions specific for the diagnostic enzymes (*sIDHP** and *sAAT-B**) according to Hebert and Beaton (1993). Enzymes were resolved with stain solutions, producing banding patterns that were used to determine the expressed genotype. Once the gel had stained sufficiently (4-8 min) to resolve the enzyme, the stain was rinsed from the gel with tap water. The gel was then scored (banding patterns observed to distinguish the expressed genotype) by being compared to the known largemouth bass benchmarks. All samples were stained for both diagnostic loci (*sIDHP** and *sAAT-B**).

When scored, alleles were given a numerical designation according to their migration speed; those alleles migrating farther anodally from the origin (from negative

to positive) were given a greater numerical designation, following Philipp et al. (1983). Based on the banding patterns observed, the genotype of each fish was determined. For *sIDHP**, banding patterns for *M. s. floridanus* alleles are homozygous and further from the origin. For *M. s. salmoides*, alleles are homozygous and closer to the origin, while first generation (F_1) intergrades are heterozygous and show banding patterns of both previously described locations. For *sAAT-B**, banding patterns for *M. s. floridanus* are homozygous at the 3 location, or homozygous at the 4 location, or heterozygous at the 3 and 4 locations. For *M. s. salmoides*, the banding patterns are homozygous at the 1 location, or homozygous at the 2 location, or heterozygous at the 1 and 2 locations. Since aspartate aminotransferase is a heterodimeric isozyme some intergrades display a third intermediate banding pattern (Phillip et. al 1983). First generation intergrades will have some combination of *M. s. floridanus* and *M. s. salmoides* alleles. It is important to note that second generation (F_2) and later generatons (F_x) of intergrades have the potential to backcross, in which a percentage of the fish sampled will show banding patterns that will indicate that they are *M. s. floridanus* or *M. s. salmoides*, when in fact they are intergrades. For this reason, electrophoresis data is used primarily to assess allele frequencies within a population and not individual fish.

The frequency of each expressed genotype was calculated by summing the number of alleles at a particular loci and then dividing by the number of total alleles. Allele frequencies were calculated for each barrow pit pond largemouth bass population by summing the frequency of homozygous alleles and one-half the frequency of the heterozygous alleles. For both the *sIDHP** and *sAAT-B** alleles, the following general equations were used:

1. $f(A) = f(AA)+1/2f(Aa)$
2. $f(a) = f(aa)+1/2f(Aa)$
3. $f(A)+f(a) = 1.0$

In equation (1), $f(A)$ is the frequency of a particular allele, $f(AA)$ is the frequency of the alleles that are homozygous for that allele, and $f(Aa)$ is the frequency of the alleles that are heterozygous for *A* and *a*. For example, the frequency of the allele *sAAT-B*3* was calculated by summing the homozygous alleles (*B*3B*3*) and one half of the heterozygous alleles (*B*3B*4*).

In equation (2), $f(a)$ is the frequency of a second allele, $f(aa)$ is the frequency of the alleles that are homozygous for *a*, and $f(Aa)$ is the frequency of the alleles that are heterozygous for *A* and *a*. For example, the frequency of the allele *sAAT-B*4* was calculated by summing the homozygous alleles (*B*4B*4*) and one half of the heterozygous alleles (*B*3B*4*).

In equation (3), $f(A)$ is the frequency of *A* and $f(a)$ is the frequency of *a*. The sum of all the alleles at a particular locus will equal 1.0. For example, at the *sAAT-B** locus, the total of all the *B*3* alleles and all the *B*4* alleles will equal 1.0.

Following this pattern of summing the homozygous alleles with one half of all heterozygous alleles, allele frequencies were calculated for each the diagnostic loci (*sAAT-B*1*, *sAAT-B*2*, *sAAT-B*3*, *sAAT-B*4*, *sIDHP*1*, and s*IDHP*3*). Allele frequencies were then grouped together for those specific for *M. s. floridanus* (*sAAT-B*3*, *sAAT-B*4*, and *sIDHP*3*) and those specific for *M. s. salmoides* (*sAAT-B*1*, *sAAT-B*2*, and *sIDHP*1*).

Seventeen pit ponds were sampled for this study (Table 1, Figure 2). They were chosen based upon their geographic potential to contain *M. s. floridanus*, *M. s. salmoides*, or intergrade populations. The ponds ranged in size from 0.4 to 11 ha.

Nine Orange County barrow pit ponds (Table 1, Figure 2) were used in this study. These ponds were constructed and are maintained by the Orlando Expressway Authority (OEA). They range in area from 0.4 to 5.6 ha. There are no records to indicate that the pits were intentionally stocked (personal communication with OEA personnel). The pits, however, are not fenced, and they are located alongside State Highway 417. Although the barrow pit areas are patrolled by the Florida Highway Patrol and individuals are not allowed to stop, individuals could stop and put fish into these ponds. Two study ponds were located in Sumter County (Table 1, Figure 2). These are relatively older (>20 years) barrow pit ponds and are 1.8 and 1.6 ha in area. The pits are fenced, gated, and locked by the Florida Department of Transportation (FDOT). The ponds were not intentionally stocked by FDOT. The Orange County and Sumter County barrow pit ponds were selected because these waters represent pits that were likely to contain *M. s. floridanus* populations because they are within the natural range of *M. s. floridanus* described by Philipp et al. (1983).

The Alachua County pit pond (Table 1, Figure 2) that was selected for this study is fenced, gated, and locked by Gainesville Regional Utilities (GRU). It is 11 ha in area. This pond receives minimal fishing pressure by GRU employees during an annual fishing tournament that the company conducts. This pit was chosen for its potential to have an intergrade population because it lies on the border between the ranges of *M. s. floridanus*

and its intergrade (Philip et al. 1983) and employees of GRU are known to have released fish from local waters into this pond.

The four ponds in St. Johns County (Table 1, Figure 2) were constructed in 1999 on the border between the intergrade and *M. s. floridanus* ranges defined by Philipp et al. (1983). The ponds range from 0.4 to 4.4 ha. The St. Johns County barrow pits are gated and locked by the FDOT. Access and fishing are not permitted. The St. Johns County ponds were chosen because the ponds were stocked with fish from other barrow pit ponds located in the intergrade zone and *M. s. floridanus'* range. The four ponds in St. Johns County were stocked in the fall of 2000 with bluegill (*Lepomis macrochirus*) and in the spring of 2001 with adult largemouth bass. The fish were obtained from barrow pit ponds located in three peninsular Florida counties (Orange County, Sumter County, and Alachua County). Bluegill were stocked at a rate of 50 fish per ha and largemouth bass were stocked at a rate of 20 fish per ha (personal communication, Mark Hoyer, Florida LAKEWATCH).

The Jackson County barrow pit pond (Table 1, Figure 2) differs from the other ponds in morphology. It is a deep, steep-sided, 10.9-ha dolomite quarry. It is on the property of Dolomite, Inc. and is fenced, gated, and locked. This pond is located within the intergrade zone (Philipp et al. 1983) and was chosen to determine if largemouth bass in a barrow pit pond would express both northern and Florida alleles.

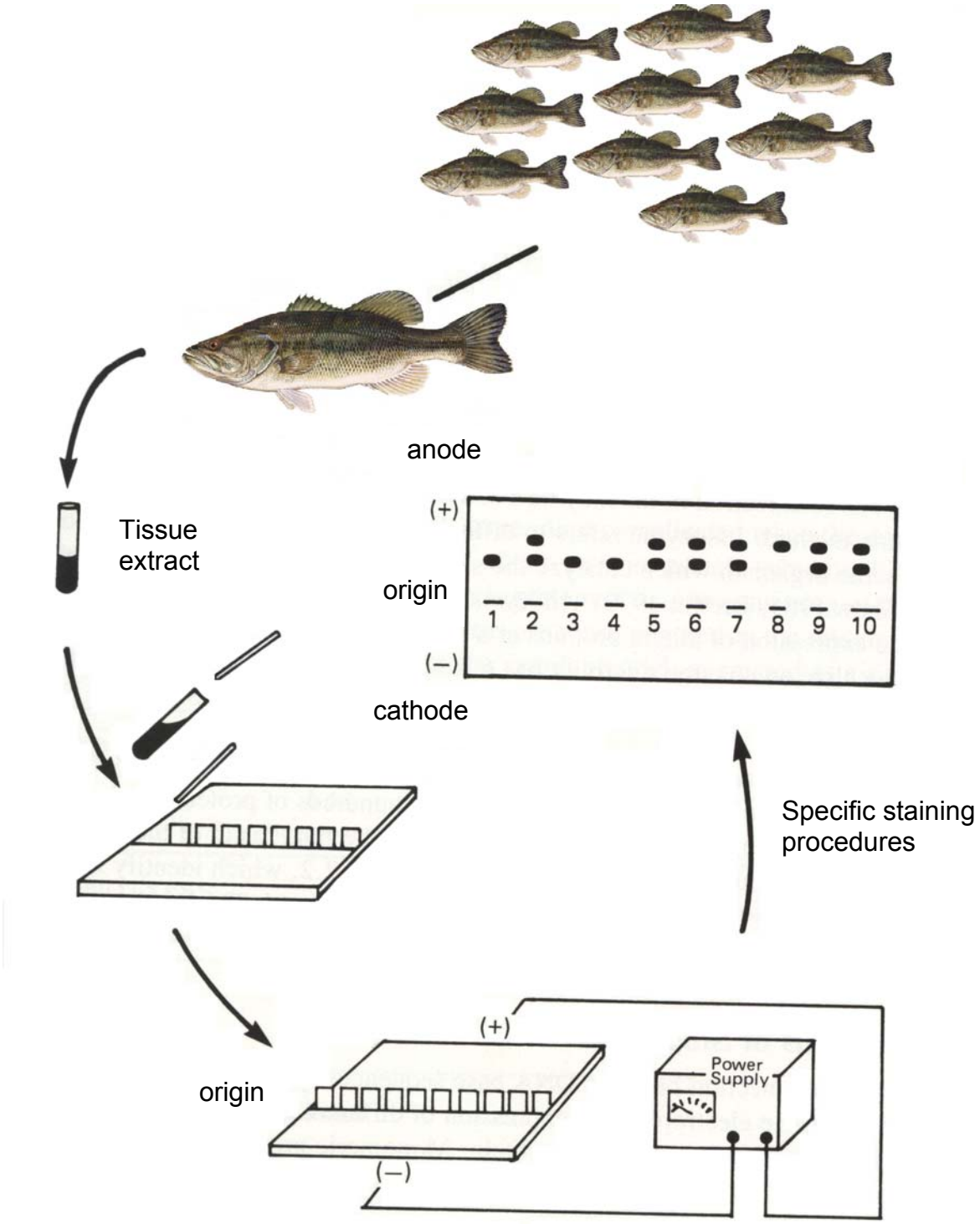

Figure 1. A general diagram of the electrophoresis process. (Modified from Ryman and Utter, Population Genetics & Fishery Management, University of Washington, 1987)

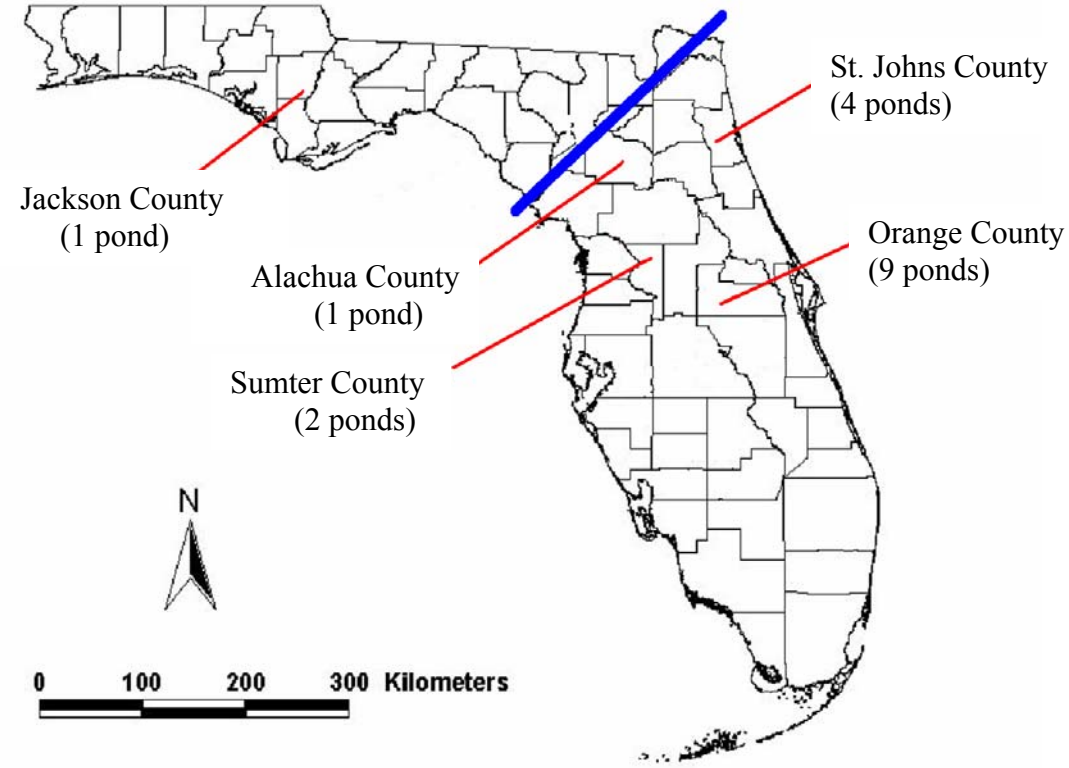

Figure 2. Location of highway barrow pits sampled in Florida. The dark line indicates the approximate line of distinction between the *Micropterus salmoides floridanus'* range and the intergrade zone. Based on Phillipp et. al (1983) and Bailey and Hubbs (1949).

Table 1. Location and size of barrow pit ponds sampled in Florida.

Water Body	Latitude	Longitude	Size (hectares)
St. Johns County Pond B	N 29° 50.754'	W 081° 21.805'	1.0
St. Johns County Pond D	N 29° 51.181'	W 081° 21.434'	0.8
St. Johns County Pond E	N 29° 51.687'	W 081° 20.851'	0.4
St. Johns County Pond F	N 29° 51.288'	W 081° 20.468'	4.4
Orange County Pond 1	N 28° 33.934'	W 081° 11.538'	3.3
Orange County Pond 5	N 28° 24.236'	W 081° 14.330'	1.1
Orange County Pond 7	N 28° 22.258'	W 081° 18.363'	5.6
Orange County Pond 8	N 28° 22.221'	W 081° 18.528'	1.6
Orange County Pond 9	N 28° 22.040'	W 081° 19.849'	1.5
Orange County Pond 10	N 28° 22.022'	W 081° 19.162'	4.2
Orange County Pond 11	N 28° 22.676'	W 081° 16.785'	0.4
Orange County Pond 12	N 28° 22.814'	W 081° 16.426'	0.4
Orange County Pond 13	N 28° 23.500'	W 081° 15.247'	0.6
Sumter County East Pond	N 28° 52.273'	W 082° 05.494'	1.8
Sumter County West Pond	N 28° 52.574'	W 082° 06.174'	1.6
Alachua County Pond	N 29° 45.522'	W 082° 24.016'	11.0
Jackson County Pond	N 30° 39.281'	W 085° 09.907'	10.9

CHAPTER 3
RESULTS AND DISCUSSION

Numerous researchers have used starch gel electrophoresis to describe differences in the electrophoretic mobility of isozymes from tissues of Florida largemouth bass and northern largemouth bass (Philipp et al. 1983; Maceina et al. 1988; Dunham et al. 1992; Bulak et al. 1995). By observing the mobility of isozymes, specific alleles in largemouth bass can be determined. Philipp et al. (1983) described alleles that were diagnostic of the Florida (s*AAT-B*3*, s*AAT-B*4*, and *sIDHP*3*) and northern largemouth bass (s*AAT-B*1*, s*AAT-B*2*, and *sIDHP*1*).

Cellulose acetate electrophoresis is a similar method for genetic analysis that also has the potential to distinguish Florida largemouth bass from northern largemouth bass, but this technique has not been evaluated for largemouth bass prior to this study. Cellulose acetate electrophoresis differs from the starch gel electrophoresis process used in previous largemouth bass studies by use of a different medium. The cellulose acetate requires much shorter run times (10 to 20 minutes versus 1 hour or longer), and limited gel preparation, and is comparatively simple (Hebert and Beaton 1983). These aspects make it ideal for assessing the genetic make-up of largemouth bass.

Six largemouth bass were obtained from FFWCC's Richloam hatchery. Total length of the fish ranged from 296 to 591 mm. Of the six fish, three were brood fish that had previously been certified to be *M. s. floridanus* by starch gel electrophoresis using liver tissue taken from a biopsy. The other three were known intergrades because they

were offspring (F_x) of known intergrades and known Florida largemouth bass (Personal communication, Richard Krause, Florida Fish and Wildlife Conservation Commission).

The known *M. s. floridanus* fish expressed *M. s. floridanus* specific alleles using cellulose acetate electrophoresis (Table 2, Figures 3 and 4). The known intergrade fish expressed alleles that are diagnostic for both the *M. s. floridanus* and the *M. s. salmoides* (Table 2, Figures 3 and 4). Since the intergrade fish were not first generation offspring their banding patterns indicate that backcrosses have likely occurred, this is the reason that fish number 2 and 3 appear to be *M. s. salmoides* (Table 2, Figures 3 and 4). These results demonstrate that the cellulose acetate electrophoresis method can be used to distinguish the diagnostic alleles of northern and Florida largemouth bass.

The cellulose acetate electrophoresis results of the known fish were used as benchmarks to assess the genetic make-up of largemouth bass from the barrow pit ponds. A total of 592 largemouth bass, ranging in total length from 92 to 497 mm, were collected from barrow pit ponds. Catch per unit effort (CPUE) in the ponds ranged from 0.24 to 3.75 fish/min (Table 3). Frequency of the *M. s. floridanus* specific alleles in the barrow pit pond populations ranged from 0.25 to 1.00 at the *sAAT-B** locus and 0.48 to 1.00 at the *sIDHP** locus. Measured frequency of *M. s. salmoides* specific alleles in the barrow pit pond populations ranged from 0.00 to 0.75 at the *sAAT-B** loci and 0.00 to 0.52 at the *sIDHP** loci (Table 5).

In the nine Orange County barrow pit ponds and two Sumter County barrow pit ponds (the southern most pit ponds), a total of 291 largemouth bass were sampled and 100% of the alleles at both the *sAAT-B** and *sIDHP** loci were identified as being specific for *M. s. floridanus* (Tables 4, 5, and 6). Largemouth bass from these waters

could, therefore, be stocked into other Florida waters with little concern for possible negative genetic impacts to the native *M. s. floridanus* populations.

In the Alachua County barrow pit pond, 50 fish were sampled, but only 68% of the alleles at the *sAAT-B** locus and 48% of the alleles at the *sIDHP** locus were identified as being specific for *M. s. floridanus* (Tables 4, 5, and 6). These findings clearly show that this barrow pit pond, which is located near the historic intergrade zone, does not support a pure *M. s. floridanus* population. The GRU pit pond, therefore, would not be suitable for obtaining largemouth bass to stock into water bodies that are in the *M. s. floridanus* range. However, this pit would be a suitable site from which to obtain fish for stocking into areas that are outside of the *M. s. floridanus* range.

In two of the St. Johns County ponds (D and E), 50 fish were sampled. In each pond, 100% of the alleles at both the *sAAT-B** and *sIDHP** loci were identified as being specific for *M. s. floridanus* (Tables 4, 5, and 6). At the two other St. Johns County ponds (B and F), 50 fish were also sampled (Tables 4, 5, and 6). In pond B, 96% of the alleles at the *sAAT-B** locus and 100% of the alleles at the *sIDHP** locus were identified as being specific for *M. s. floridanus.* Two of the sampled fish from this pond were homozygous for *sAAT-B*2*, but both were homozygous for *sIDHP*3*. At pond F, 97% of the alleles at the *sAAT-B** locus and 98% of the alleles at the *sIDHP** locus were identified as being specific for *M. s. floridanus.* One fish was homozygous for *sAAT-B*2*, but homozygous for *sIDHP*3*. One fish was heterozygous for *sAAT-B*2* and *B*4*, but was homozygous for *sIDHP*3*. Another fish was homozygous for *sIDHP*1*, but was homozygous for *sAAT-B*4*. These results show that largemouth bass in two of the St. Johns County ponds (B and F) had northern alleles.

The presence of northern alleles in largemouth bass from two of the St. Johns County barrow pit ponds (B and F) were likely due to the introduction of largemouth bass from the Alachua County pond (a pond later found to have intergrades) during the initial stocking of these ponds in 2000 and 2001 (Tables 4 and 5). The St. Johns County barrow pit ponds with *M. s. floridanus* populations (D and E), were stocked in 2000 and 2001 from the southern most ponds which have *M. s. floridanus* populations (Tables 4 and 5). Consequently, the results from these ponds show that barrow pit ponds can be stocked with Florida largemouth bass to develop a pure *M. s. floridanus* population. St. Johns County Pond B and F indicate that if an intergrade population is used to obtain fish for stocking (ie., Alachua County Pond), northern alleles can be detected in the new populations using cellulose acetate electrophoresis.

In the Jackson County barrow pit pond, 51 largemouth bass were sampled, but only 25% of the alleles at the *sAAT-B** locus were identified as being specific for *M. s. floridanus*. Fifty-three percent of the alleles at the *sIDHP** locus were identified as being specific for *M. s. floridanus* (Tables 4, 5, and 6). The largemouth bass population in this pond contained the lowest percentage of *M. s. floridanus* specific alleles at the *sAAT-B** locus and the second lowest percentage of *M. s. floridanus* specific alleles at the *sIDHP** locus, which should be expected because this pond is the farthest from the pure *M. s. floridanus* range. This also suggests that using cellulose acetate electrophoresis does find allele frequency in accordance to the historical ranges of the subspecies of largemouth bass.

There, however, may be another reason for the occurrence of a low frequency of *M. s. floridanus* alleles in largemouth bass from the Jackson County limestone quarry pond.

This pond presents a different situation than the other barrow pit ponds sampled during this study because it could discharge into Rock Creek, which is a tributary to the Chipola River. More importantly, topographical maps and water level data, from the United States Geological Survey (USGS) monitoring station near Marianna, Florida, indicate that during flood events, the Chipola River floods Rock Creek causing it to flow into the quarry. The pit is also within 0.5 mile of the Chipola River. Because the Chipola River contains shoal bass (*Micropterus coosae*), there is a possibility that shoal bass have entered this Jackson County pit pond.

Some of the fish sampled from the Jackson County quarry pond could have been shoal bass. Known shoal bass were not obtained during this study to compare with the other fish, so the allele frequencies for this pond may not represent a typical intergrade largemouth bass population for that region of Florida. Consequently, any largemouth bass stocking program, in this area of panhandle Florida, would need to consider the potential impact of stocking not only pure Florida largemouth bass, but intergrade, northern largemouth bass, or even shoal bass.

Table 2. Genotypes of the individual largemouth bass obtained from the Florida Fish and Wildlife Conservation Commission.

Known *Micropterus salmoides floridanus*

fish	*sAAT-B**	*sIDHP**
1	4/4	3/3
2	3/3	3/3
3	3/4	3/3

Known Intergrades

fish	*sAAT-B**	*sIDHP**
1	1/3	1/1
2	1/1	1/1
3	1/1	1/1

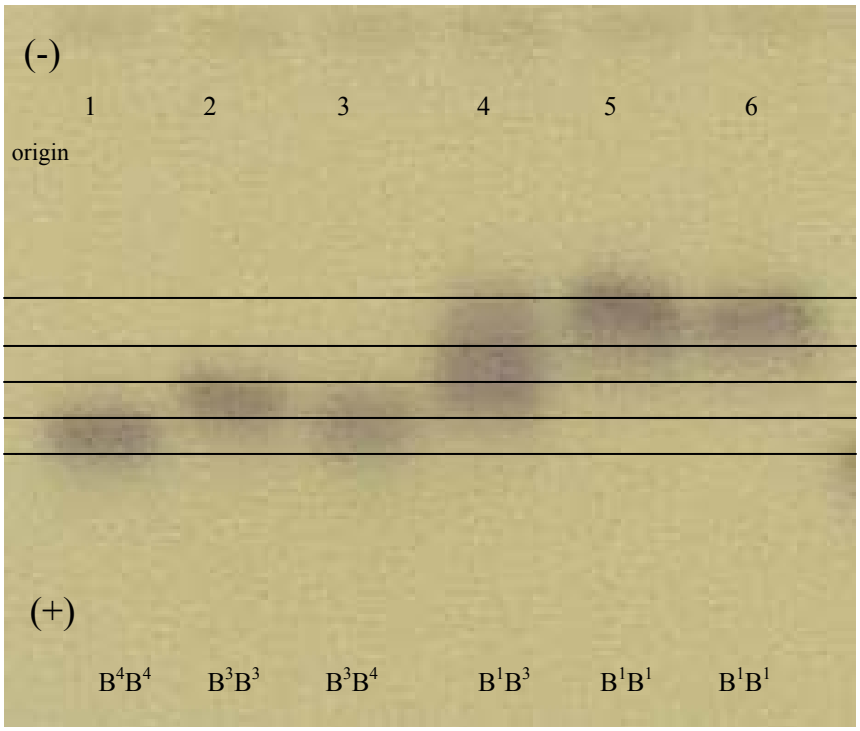

Figure 3. Allelic variants at the *sAAT-B** locus. Lanes 1,2, and 3 contain fish that are known to be *Micropterus salmoides floridanus*. Lanes 4, 5, and 6 contain fish that are known to be intergrades.

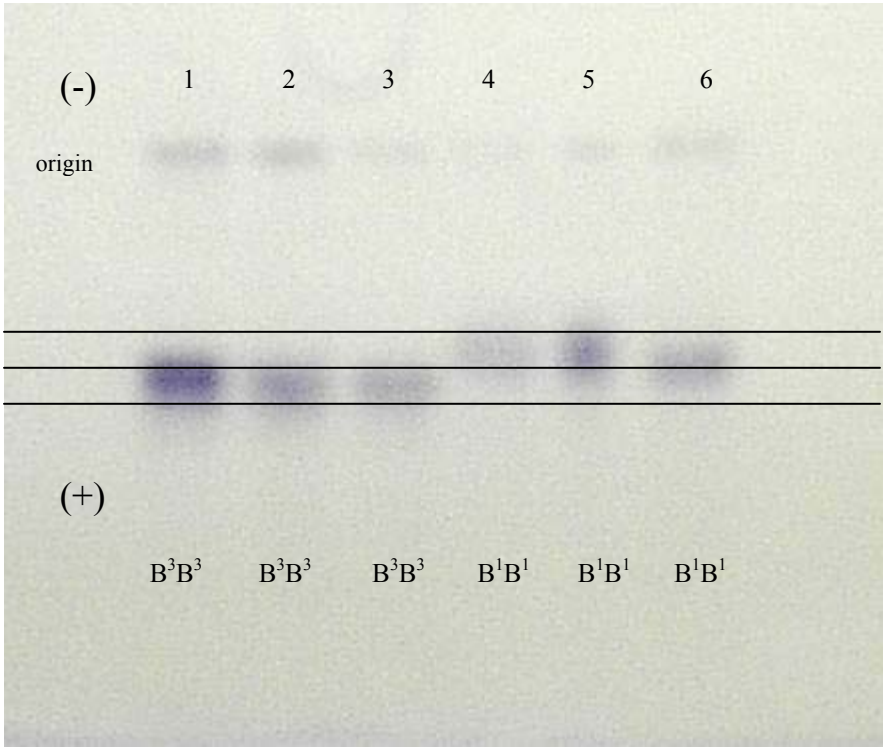

Figure 4. Allelic variants at the *sIDHP** locus. Lanes 1, 2, and 3 contain fish that are known to be *Micropterus salmoides floridanus*. Lanes 4,5, and 6 contain fish that are known to be intergrades.

Table 3 Number of largemouth bass caught, amount of electrofishing effort, and catch per unit effort of electrofishing measured as pedal time at each barrow pit pond.

Water Body	Catch	Electrofishing Effort		Catch Per Unit Effort	
	(# of fish)	(seconds)	(minutes)	(fish/minute)	(fish/10 min)
St. Johns County Pond B	116	3000	50.0	2.32	23.20
St. Johns County Pond D	68				
St. Johns County Pond E	75	1200	20.0	3.75	37.50
St. Johns County Pond F	56				
Orange County Pond 1	26	2030	33.8	0.77	7.68
Orange County Pond 5	27	4081	68.0	0.40	3.97
Orange County Pond 7	29	3520	58.7	0.49	4.94
Orange County Pond 8	7	1249	20.8	0.34	3.36
Orange County Pond 9	10	2513	41.9	0.24	2.39
Orange County Pond 10	32	3110	51.8	0.62	6.17
Orange County Pond 11	18	1697	28.3	0.64	6.36
Orange County Pond 12	6	1340	22.3	0.27	2.69
Orange County Pond 13	35	2324	38.7	0.90	9.04
Sumter County East Pond	50	2716	45.3	1.10	11.05
Sumter County West Pond	50	2647	44.1	1.13	11.33
Alachua County Pond 1	50	8049	134.2	0.37	3.73
Jackson County Pond 1	52	2884	48.1	1.08	10.82

Table 4. Allele frequencies of largemouth bass populations sampled from barrow pit ponds in Florida. N indicates number of fish sampled

Water Body	N	*sAAT-B*1*	*sAAT-B*2*	*sAAT-B*3*	*sAAT-B*4*	*sIDHP*1*	*sIDHP*3*
St. Johns County Pond B	50	0.00	0.04	0.59	0.37	0.00	1.00
St. Johns County Pond D	50	0.00	0.00	0.23	0.77	0.00	1.00
St. Johns County Pond E	50	0.00	0.00	0.65	0.35	0.00	1.00
St. Johns County Pond F	50	0.01	0.02	0.62	0.35	0.02	0.98
Orange County Pond 1	26	0.00	0.00	0.69	0.31	0.00	1.00
Orange County Pond 5	27	0.00	0.00	0.08	0.92	0.00	1.00
Orange County Pond 7	29	0.00	0.00	0.59	0.41	0.00	1.00
Orange County Pond 8	7	0.00	0.00	0.00	1.00	0.00	1.00
Orange County Pond 9	10	0.00	0.00	0.70	0.30	0.00	1.00
Orange County Pond 10	32	0.00	0.00	0.91	0.09	0.00	1.00
Orange County Pond 11	18	0.00	0.00	0.56	0.44	0.00	1.00
Orange County Pond 12	6	0.00	0.00	0.83	0.17	0.00	1.00
Orange County Pond 13	36	0.00	0.00	0.94	0.06	0.00	1.00
Sumter County East Pond	50	0.00	0.00	0.77	0.23	0.00	1.00
Sumter County West Pond	50	0.00	0.00	0.97	0.03	0.00	1.00
Alachua County Pond 1	50	0.02	0.30	0.36	0.32	0.52	0.48
Jackson County Pond 1	51	0.49	0.26	0.25	0.00	0.47	0.53

Table 5. Allele frequencies of largemouth bass populations sampled from barrow pit ponds in Florida grouped together as northern largemouth bass and Florida largemouth bass alleles. N indicates number of fish sampled.

		*sAAT-B**		*sIDHP**	
Water Body	N	northern	Florida	northern	Florida
St. Johns County Pond B	50	0.04	0.96	0.00	1.00
St. Johns County Pond D	50	0.00	1.00	0.00	1.00
St. Johns County Pond E	50	0.00	1.00	0.00	1.00
St. Johns County Pond F	50	0.03	0.97	0.02	0.98
Orange County Pond 1	26	0.00	1.00	0.00	1.00
Orange County Pond 5	27	0.00	1.00	0.00	1.00
Orange County Pond 7	29	0.00	1.00	0.00	1.00
Orange County Pond 8	7	0.00	1.00	0.00	1.00
Orange County Pond 9	10	0.00	1.00	0.00	1.00
Orange County Pond 10	32	0.00	1.00	0.00	1.00
Orange County Pond 11	18	0.00	1.00	0.00	1.00
Orange County Pond 12	6	0.00	1.00	0.00	1.00
Orange County Pond 13	36	0.00	1.00	0.00	1.00
Sumter County East Pond	50	0.00	1.00	0.00	1.00
Sumter County West Pond	50	0.00	1.00	0.00	1.00
Alachua County Pond 1	50	0.32	0.68	0.52	0.48
Jackson County Pond 1	51	0.75	0.25	0.47	0.53

Table 6. Genotype frequencies of largemouth bass populations sampled from barrow pit ponds in Florida. N indicates the number of fish sampled

Water Body		*sAAT-B**						*sIDHP**		
	N	1/1	1/2	2/2	3/3	3/4	4/4	1/1	1/3	3/3
St. Johns County Pond B	50	0	0	0.04	0.46	0.26	0.24	0	0	1
St. Johns County Pond D	50	0	0	0	0.16	0.14	0.7	0	0	1
St. Johns County Pond E	50	0	0	0	0.58	0.14	0.28	0	0	1
St. Johns County Pond F	50	0.01	0	0.02	0.61	0.02	0.34	0.02	0	0.98
Orange County Pond 1	26	0	0	0	0.65	0.08	0.27	0	0	1
Orange County Pond 5	27	0	0	0	0.04	0.07	0.89	0	0	1
Orange County Pond 7	29	0	0	0	0.59	0	0.41	0	0	1
Orange County Pond 8	7	0	0	0	0	0	1	0	0	1
Orange County Pond 9	10	0	0	0	0.70	0	0.30	0	0	1
Orange County Pond 10	32	0	0	0	0.91	0	0.09	0	0	1
Orange County Pond 11	18	0	0	0	0.56	0	0.44	0	0	1
Orange County Pond 12	6	0	0	0	0.83	0	0.17	0	0	1
Orange County Pond 13	36	0	0	0	0.94	0	0.06	0	0	1
Sumter County East Pond	50	0	0	0	0.68	0.18	0.14	0	0	1
Sumter County West Pond	50	0	0	0	0.96	0.02	0.02	0	0	1
Alachua County Pond 1	50	0.02	0	0.3	0.35	0.02	0.31	0.50	0.04	0.46
Jackson County Pond 1	52	0.42	0.15	0.18	0.25	0	0	0.47	0	0.53

CHAPTER 4
MANAGEMENT IMPLICATIONS

Florida largemouth bass and northern largemouth bass are routinely stocked by state fish and wildlife agencies and private individuals throughout the United States. In Florida, largemouth bass from as far away as Arkansas have been stocked (Porak, Florida Fish and Wildlife Conservation Commission, personal communication). Largemouth bass with northern alleles have been found in Florida waters well within the *M. s. floridanus* native range (Porak and Krause, Florida Fish and Wildlife Conservation Commission, personal communication).

Philipp (1991) proposed that the introduction of largemouth bass from one environment into a population of largemouth bass in another environment, followed by their subsequent interbreeding, will create a new intergrade population with decreased fitness compared to the original native population. Although Philipp's hypothesis has not been tested, FFWCC has concerns about the possible effects of stocking any northern largemouth bass or intergrade largemouth bass on the genetic integrity of *M. s. floridanus* in individual waters. However, FFWCC stocks hatchery-reared *M. s. floridanus* with variable success throughout Florida. Stocking has also been successful throughout the United States (Smith and Reeves 1986). Therefore, Philipp's hypothesis remains untested.

Largemouth bass have been stocked to supplement poor recruitment, expand the species range, and alter the genetic composition of existing populations to enhance fishing (Forshage and Fries 1995). The size of stocked fish is an important consideration

because survival tends to be lower for small fish and production costs increase with large fish (Loska 1982). Fingerling fish have typically been used to introduce largemouth bass into new and reclaimed waters (Keith 1986). Fingerling fish can have relatively low survivability and are generally stocked at high rates (100 fingerlings / acre) to increase the chance of success. Consequently, it has been proposed to use advanced (larger) fingerlings to increase survivability and reduce stocking rates. The cost and time of production, however, is higher and problems have been encountered in Florida with survivability once the hatchery-reared advanced fingerling largemouth bass are stocked due to a failure to convert from hatchery food to natural foods (Porak et al. 2002, Heidinger and Brooks 2002).

Sub-adult and/or adult largemouth bass may also be stocked to enhance fisheries and such a management program would provide angling opportunities much quicker than lakes stocked with fingerling fish (Buynak et al. 1999). The demand for stocking largemouth bass in Florida after the recent extreme drought is greater than the capacity of the FFWCC hatcheries. Supplemental sources of nonhatchery-reared subadult or adult fish could help meet the demand to stock with fish that could immediately help ailing fisheries. Because highway barrow pit ponds are abundant throughout Florida and many have existing largemouth bass populations, they present a possible source of unexploited fish. This study shows that these fish could be used in stocking programs if care is taken to match the genetic make-up of the source populations with the receiving waters. As new ponds are dug for the construction of highways, appropriate ratios of forage fish and largemouth bass could be stocked to produce, genetically-appropriate largemouth bass populations for relocation.

Stocking is a valuable tool for fisheries management. If the decision is made to stock and there are concerns about the genetic make-up of the fish that will be stocked, it would be prudent to conduct genetic tests to minimize the risk of genetic contamination. There are many types of genetic testing instruments and protocols; each with its own cost per sample. The cost, in this study, ranged from $1.00 to $2.00 per sample, not including cost of equipment or personnel. The cost per sample would decrease when more samples are analyzed. This study has shown that cellulose acetate electrophoresis can be used to determine the genetic makeup of populations of largemouth bass relatively easily and inexpensively before fish from those populations are transported and stocked elsewhere. In this study, the test fish were sacrificed, but cellulose acetate electrophoresis can also be used to identify the genetic composition of a fish by using a non-lethal biopsy needle to extract liver tissue. The cellulose acetate electrophoresis method, therefore, is a tool that fisheries managers can use to minimize the risk of genetic contamination while still effectively managing fish populations. New techniques are currently being developed using different types of DNA analysis. In the future, when these methods are developed, blood samples or fin clips could be used for analysis. But at this time, these methods are still in the developmental stage and their accuracy is being evaluated. These methods could make it easier to sample a population, but the cost will be much greater than that of electrophoresis.

No technique, however, can be 100% certain in identifying the genetic composition of an individual fish or the genetic purity of a fish population in an individual water body. Cellulose acetate electrophoresis is a reasonable identification method of the allele frequencies in a water body and large numbers of fish should not have to be sampled to

provide a minimal risk of genetic contamination. However, the greater the sample size, the less risk there is of genetic contamination. Walsh (2000) explains methods used to estimate the probability of drawing a sample in which all individuals show the same state, if individuals with unsampled (hidden) states actually exist in the population at some hypothetical frequency (e.g., 0.05). Using these methods, it can be estimated that when using a sample size of 50 (the sampling goal in this study), there is 92% confidence that hidden character states can be identified if they exist at 5.7% of the population or greater. In order to reject with 95% confidence that 5% of the individuals carry hidden character states, a sample of 59 individuals is necessary. The methods described by Walsh (2000) can be used to evaluate different sample sizes and realize the level of potential risk of genetic contamination that is present (Figure 5).

With simple planning, barrow pit ponds could easily be used as a source of fish for stocking largemouth bass into Florida lakes without causing concern for genetic contamination. This study shows good evidence that barrow pit ponds within the historical *M. s. floridanus* range, can be utilized for procuring fish that are pure *M. s. floridanus*. Fish from these barrow pit ponds could potentially be stocked into lakes throughout Florida that are in the *M. s. floridanus* range. This evidence also indicates that newly constructed barrow pit ponds can be stocked from other ponds that have pure *M. s. floridanus* populations to develop new pure strain *M. s. floridanus* populations. Barrow pit ponds that contain intergrade populations could be utilized in stocking programs outside of the *M. s. floridanus* range.

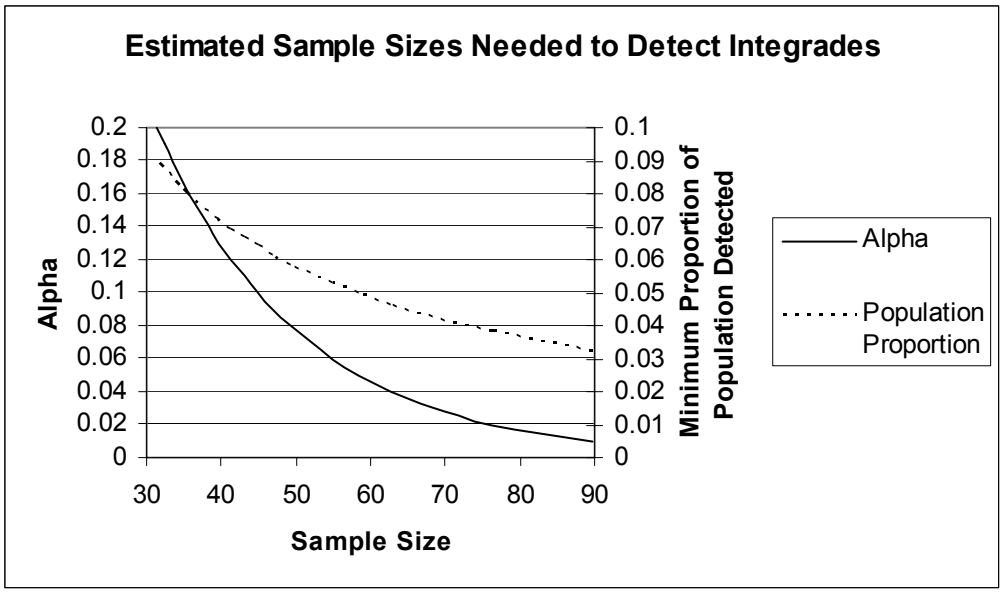

Figure 5 Estimated Sample Sizes needed to detect intergrades. Modified from Walsh (2000).

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BIOGRAPHICAL SKETCH

Jason Ryan Childress was born November 30, 1978, and raised in the small Oklahoma town of Wewoka. Jason grew up as an avid fisherman and enjoys the outdoors. He attended Wewoka Public Schools. Jason went on to receive a Bachelor of Science degree from East Central University in Ada, Oklahoma, where he majored in environmental health science and minored in biology. At East Central University, Jason was involved in research at EPA's R.S. Kerr groundwater research laboratory. This is where he realized that he wanted to pursue a graduate degree in aquatic research and management. In the summer of 2001, Jason began work on a master's degree at the University of Florida in the Department of Fisheries and Aquatic Sciences under Dr. Dan Canfield. Jason will receive a Master of Science degree in May 2004 and he plans to pursue a career in the research/management of aquatic resources.