January 2013

Molecular Technologies in the Science and Policy of Florida Largemouth Bass Micropterus floridanus Management in Florida

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Molecular Technologies in the Science and Policy of Florida Largemouth Bass *Micropterus floridanus* Management in Florida

by

Joshua C. Sakmar

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science School of Geosciences College of Arts and Sciences University of South Florida

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Date of Approval:
November 8, 2013

Keywords: Centrarchid, microsatellite, parentage, unnatural selection, genetics

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Dedication

To Kristen, for her love, kindness, encouragement and patience. To Mom, Dad and Liz, for their support and for teaching me to follow my dreams. To Lilly, for being by my side through many hours of writing. To the men who, in their presence or spirit, taught me how to fish.
Acknowledgments

This thesis would not have been possible without the support and assistance of staff at the Florida Fish and Wildlife Conservation Commission. I would like to sincerely thank Rick Stout, Mike Matthews, Brandon Barthel and Jon Fury for allowing me the opportunity to be involved with this work.
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Abstract

Advances in molecular technologies have provided conservation biologist with the opportunity to quantify the genetic structure of a population and, in turn develop management guidelines and policies aimed at preserving the genetic diversity of fish stocks challenged by human activities. This thesis examines the status of genetics as applied to the management of freshwater fisheries by state natural resource agencies with a purpose of understanding the keys to a successful genetics program. An online survey was used to investigate the breadth of molecular marker application to freshwater fisheries management by state natural resource departments. Seven questions were posed to 50 state agencies addressing species of concern, type of genetic resources used, type of molecular marker used, and management concerns. Genetics was listed as a concern in the management of 18 freshwater fish families representing 70 distinct species, with Salmonid species the most frequently reported (20%). A majority of agencies rely on outside resources to perform genetics testing (65%). The most common analysis technique used by state agencies was microsatellite DNA analysis (35%) and the most frequently reported management concerns were genetic stock identification and management boundaries (23%). The application of a specific molecular technology to a conservation question was addressed by investigating the mechanisms of unnatural selection in the form of a study of trait
heritability. Microsatellite parentage analysis was used to reconstruct familial relationships of juvenile Florida bass (*Micropterus floridanus*) displaying variable traits of growth and aggressiveness in a culture setting. Differences in the parentage of high growth and aggression (HGA) and baseline growth and aggression (BGA) offspring showed that certain parent-pairings contribute disproportionally to certain size classes and levels of aggression. These results suggest that the selective pressures of recreational harvest may negatively impact the fitness of wild fish stocks. Overall, this work provides natural resource managers with the basic information required to successfully develop and employ strategies aimed at preserving the genetic integrity of freshwater fisheries.
Chapter 1:

General Introduction

The maintenance of genetic diversity is understood as fundamental to the ability of species to adapt to short-term environmental change (natural or anthropogenic) and to permit long-term evolutionary success (King et al., 2007; Saura & Faria, 2011). As this concept has gained appreciation by natural resource managers, fisheries biologists have placed an emphasis on understanding the potential effects of harvest on the genetics and sustainability of wild fish stocks. Where harvest selects according to certain desirable life history traits, such as in commercial and recreational fishing, it is likely that with time, some undesirable changes will be observed in the exploited population (Allendorf & Hard, 2009). While the response of a species to this “unnatural” selection is readily theorized by extending the principles of heredity and evolution, its underlying biological mechanisms have only recently become accessible to scientific inquiry. Advances in molecular technologies have provided conservation biologist with the opportunity to quantify the genetic structure of a population and, in turn develop management guidelines and policies aimed at preserving the genetic diversity of fish stocks challenged by human activities.
The capability of fisheries managers to successfully develop and employ molecular technologies is often a critical factor in determining the success or failure of genetics based conservation strategies. As these technologies continue to evolve, biologists are increasingly presented with a number of analysis options. When combined with a wide array of institutional needs, constraints and conservation problems, the choices in technology can and frequently do, present hurdles in the science to policy pathway (Sagarin et al., 2009). The Florida Fish and Wildlife Conservation Commission (FWC) recognizes the importance of preserving the genetic integrity of recreationally fished species and has endeavored to craft sound management strategies and policies based on molecular technology. Through these policies, the FWC has developed a model pathway for successful integration of the genetic concerns of scientists and stakeholders. This thesis takes advantage of that pathway by partnering with the FWC to investigate the role of molecular technologies in the management of freshwater fisheries, specifically the Florida largemouth bass (*Micropterus floridanus*).

The overall objective of this thesis is to examine the status of genetics as applied to the management of freshwater fisheries by state natural resource agencies with a purpose of understanding the keys to a successful genetics program. A brief overview of the evolution of genetics as a discipline of fisheries science will be provided by way of general introduction. This will begin by reviewing the roles unnatural selection and commercial harvest have played in developing genetics management programs for stocks of Pacific salmon.
(Oncorhynchus spp.). The introduction will continue by discussing the impacts of angling and anthropogenic habitat modifications from a recreational viewpoint through an example of the Florida bass in Lake Apopka, Florida. The chapter will close by presenting the purpose and general hypotheses of this thesis.

Chapter 2 will address the current state of molecular technologies in the policy and management of fisheries from the perspective of institutional application. This is done by reviewing analysis options and discussing their relevance to management scenarios. Chapter 2 will include the results of a survey investigating the application of molecular technologies by state natural resource agencies to the management of freshwater fisheries. These results will be discussed in the context of black bass (Micropterus spp.) management in Florida. The work of Chapter 2 is my own, but the research was undertaken as part of a collaborative effort with the FWC. FWC staff developed and distributed the survey online through agency resources. I conducted all analysis and writing for this chapter in preparation for submission to Fisheries. Survey results were presented at the 143rd annual meeting of the American Fisheries Society in Little Rock, AR (Sakmar, Matthews & Stout, 2013).

Chapter 3 will investigate the application of a specific molecular technology to the conservation of Florida bass by the FWC. Here, microsatellite DNA parentage analysis is used to reconstruct familial relationships of juvenile Florida bass displaying variable life history traits in a culture setting. This chapter is designed to provide fisheries biologists with knowledge towards a better understanding of the heritability of trophy traits within fish populations and
developing appropriate conservation strategies. The work presented in this chapter was performed in conjunction with FWC staff at the Florida Bass Conservation (FBCC) in Webster FL, and the Fish and Wildlife Research Institute (FWRI), St. Petersburg FL. Trained FBCC staff performed all tissue and physiological data collection. I conducted microsatellite parentage analysis and subsequent data analysis with assistance from FWRI staff. I completed this chapter’s writing in preparation for submission to *The North American Journal of Fisheries Management*.

**Unnatural selection in fisheries**

For thousands of years, humans have unknowingly relied on the genetic principles of inheritance by selectively breeding the most productive plants and animals towards increasing the occurrence of desired phenotypes in offspring. While the results of this artificial selection could be somewhat calculated, it was not until Darwin (1859) put forth his theory of speciation by natural selection, that we began to understand the underlying mechanisms of adaptation and evolutionary change. The central tenet to this theory is that given a natural population and environment, where a physical trait provides reproductive advantage to the individual, the corresponding genotype will increase in frequency of occurrence in successive generations. Darwin (1868) further applied this principle to human induced, artificial environments where man intentionally selects for desired traits in domesticated plants and animals. Here, it was shown
that as nature has the ability to be purposeful in determining the physical expression of a population’s genetic framework, so to do humans.

While these concepts gave an elegant explanation of the biological diversity of the “natural” world, and mankind’s capacity to harness it directly, Darwin put little effort into combining the principles towards explaining the indirect effects of human selection on wild populations in the form of harvest. This “unnatural” selection, which describes the unintended consequences of harvest exploitation, generally acts in contrast to the long-term forces of natural selection by reducing the frequency of phenotypes advantageous to the animal and valued by humans (Allendorf & Hard, 2009). Darwin’s apparent oversight of unnatural selection may have been intentional in light of his already paradigm altering work. Where his theories of evolution were considered radical for the time, they may have been considered all together revolutionary in crossing the boundary between scientific and non-scientific knowledge by challenging the interests of economies based on natural resource exploitation.

The lack of scientific attention to and appreciation of the genetic consequences of unnatural selection has persisted until relatively recently. While recognition that harvest of wild animals can produce evolutionary shift is a generally accepted concept, few studies have been able to document with clarity the population level responses to exploitative selection. Where these responses have been shown, they are often in the form of changes in life-history traits reacting to commercial exploitation, such as with fisheries (Allendorf, England, Luikart, Ritchie, & Ryman, 2008; Heino & Godo, 2002; Law, 2000). Commercial
fishing may deliberately select according to traits for a number of economic reasons. In many situations, the harvest of larger individuals reduces operation cost while meeting demands of the consumer (Walters & Martell, 2004). As individuals displaying the most desirable traits related to yield (growth rate, length and fecundity) are removed from the stock, less desirable individuals are left contributing to successive generations. The commonly observed phenotypic effects of this unnatural selection are shifts towards lower maturing ages and/or sizes in exploited populations (Kuparinen & Merilä, 2007; Ricker, 1981).

Decreasing age and size at maturation can have cascading effects on the fitness of wild populations when presented with environmental challenges, as shown with Pacific salmon of the United States. Salmon have a deep and frequently dynamic history as an integral component to the identity of people in the Pacific Northwest. As Native Americans established a culture based on the predictability and abundance of seasonal runs, so too did European immigrants during development of the region in the 1850’s. The ability to anticipate timing and approximate size of annual salmon was not lost on settlers, and fishermen were quick to recognize the economic potential of this seemingly endless natural resource. Market opportunity combined with the advent of efficient commercial fishing and preservation methods, led to increased salmon harvest and the development of an industry (Chapman, 1986; Lackey, 1999). The sheer numbers of fish left the impression that the maintenance of salmon would be an easy chore.
With growth and development of the Pacific Northwest came habitat modification and increased fishing pressure. Pollution, hydrology altered by dams and channelization, water diversion for agriculture and increased turbidity due to logging had adverse effects on migration and spawning (Lackey, 2003). Though commercial fishing continued, as early as 1902, there was an understanding that patterns of extensive exploitation could have strong environmental influence on salmon stocks. “The salmon will certainly deteriorate in size if medium and larger sizes are taken for the markets and only the smaller with a few of the medium are allowed to breed” (Rutter, 1902, p.134). While the consequences of unnatural selection and habitat degradation had begun to be appreciated by the scientific community, implementing sustainable management strategies through policy was often rejected. With scientists having little ability to differentiate stocks or provide direct evidence of the effects of harvest, economic interests typically dominated discussions of management. As catch rates began to decrease, commercial and recreational anglers battled for and often received relatively high harvest limits (Taylor, 2001). At their peak in the 1930’s, North American salmon fishing was in some cases estimated to harvest 80-90% of individuals within certain populations (Hankin & Healey, 1986; Hard et al., 2008; Heard, 1991).

This general pattern of growth and exploitation continued so that by the 1980’s, the influence of decades of exploitative fishing combined with anthropogenic modifications to native habitat (physical and biological) led to many Pacific salmon stocks existing in critical condition (Huntington, Nehlsen, & Bowers, 1996; Nehlsen, 1997). If the salmon and their habitat continued to
diminish, it was likely that available management options would similarly diminish or altogether disappear. Fisheries managers were aware of the importance of diversity in the face of environmental challenge and looked to the new technologies of molecular markers to aid in their understanding of the mechanisms of unnatural selection. The first widespread applications of genetic data to analyze fisheries were instrumental in delineating specific salmon stocks for management (Grant, Milner, Krasnowski, & Utter, 1980; Milner, Teel, Utter, & Winans, 1985). As the ability of molecular technologies was revealed, scientists were quick to apply them to questions of population dynamics. Subsequent studies have contributed to a number of policy based conservation efforts for Pacific salmon, helping fisheries managers decode the mechanisms of unnatural selection by shedding light on the impacts of hatchery-bred fish on native populations (Verspoor, 1988), the evolutionary history of stocks (Murata, Takasaki, Saitoh, Tachida, & Okada, 1996), levels of stock diversity (Gustafson et al., 2007) and the potential impacts of climate change (Crozier et al., 2008).

With advances in molecular markers and their application to management strategies, the conservation of Pacific salmon has become defined by the role of genetics in science and policy (Lackey, 2003). These systems have proven invaluable by providing fisheries managers with the ability to quantify the impacts of anthropogenic influence on native populations. With the consequences of man’s actions being clearly shown, administrators and stakeholders have been given the evidence needed to craft functional catch regulations. Though the interjection of this scientific knowledge into the policy of salmon conservation has
not always been a smooth process, there has been headway made in recovery. The abundance of many natural stocks has remained stable or increased in the past decade with specific risks from harvest improving considerably (Ford, 2011).

This story of salmon in the Pacific Northwest is not unique with similar accounts of commercial exploitation affecting the evolution and fitness of fisheries being found for the Atlantic cod (*Gadus morhua*) (Swain, Sinclair, & Hanson, 2007), Orange roughy (*Hoplostethus atlanticus*) (Smith, Francis, & McVeagh, 1991), and European plaice (*Pleuronectes platessa*) (Rijnsdorp, 1993). As evidence mounts for the effects of unsustainable harvest in commercial fisheries, researchers have begun to focus on the potential for comparable reactions to recreational angling. In North America, an increasing concern for natural resource managers is the genetic integrity of black bass. Known to be strong fighters with an aggressive nature, this family of fish has long been a target for the recreational angler. In 2006, black bass attracted more than 10 million anglers, who spent more than $5 billion on travel and equipment (Aiken, 2009). Needless to say, the bass is big business and encompasses the interests of a variety of stakeholders. At the heart of the bass industry and many bass fishermen, is a strain prized for its reputation of reaching trophy size and being a highly aggressive and skilled fighter, the Florida largemouth bass.
The Florida largemouth bass in Florida

The Florida largemouth bass’s place as the freshwater fish of the state of Florida is well deserved. In 2006, Florida bass anglers spent more than 14 million days fishing, generating approximately $1.25 billion in economic impact for local communities and supporting roughly 12,000 jobs (U.S. Fish and Wildlife Service, 2006). The opportunity to catch trophy Florida bass in one of the state’s 7,700 lakes has a long history of being a huge draw for resident fishermen and tourists. Anglers and scientists alike have known for more than a century that bass have the capacity to reach very large sizes in Florida (Henshall, 1881). This observed growth potential for Florida bass was first attributed to state’s subtropical environments, fertile waters, and long growing season (Chew, 1974). Likewise, the observed growth potential for the human population of the state could arguably be attributed to similar factors. The Florida of today shows little semblance to the frontier time observations of Henshall (1881) when the population was approximately 270,000 (U.S Census Bureau, 2002). As of 2012, more than 19 million people call Florida home, making it the fourth largest state by population in the United States (U.S. Census Bureau, 2012).

Similar to the Pacific Northwest, this explosion of growth and associated development has presented numerous challenges to the maintenance of valuable freshwater fisheries. Attempts to drain floodplains through canals and locks have drastically altered Florida’s hydrology from its original state. Consider this with the large amount of water diverted for agriculture, high levels of run-off
and subsequent pollution, and it is no surprise that fisheries managers frequently cite water quality and policy as one of the largest pressures on black bass populations (Florida Fish and Wildlife Conservation Commission [FWC], 2011).

Though the scientific community has long been aware of the potential impact of these anthropogenic modifications on sportfish, it was often the case that management action was not taken until catch rates decreased and local economies were affected. This is can be seen in the history Lake Apopka, a once premiere bass fishery,

Prior to development, Lake Apopka was the second largest lake in the state (21,400 ha), with clear-water and abundant submerged aquatic vegetation. Extensive macrophyte coverage sequestered nutrients, stabilized sediments and provided cover for young fish. Correspondingly, the lake was known as a highly reputable sport fishery and produced a number of record largemouth bass (Lowe, Battoe, Coveney, & Stites, 1999; Schelske et al., 2005). In the first half of the 20th century, fish camps and lodges flourished around the lake, providing a source of income for local residents and enjoyment for tourists. Local fishermen were quoted as saying, “the fishing is so good and the water is so clear, you can pick the particular bass you want to catch. It’s the best freshwater fishing in the United States.” (Burgess, 1964, p14). As with all of Florida’s lakes at the time, the bass of Apopka were not subject to harvest regulations. The seemingly endless supply of trophy bass did not warrant the state agency in charge of such activities (then the Florida Game and Freshwater Fish Commission) to draft management
rules. Anglers and commercial netters were free to keep as many fish as they liked.

Figure 1.1. Group of people posing with “a day’s catch of bass”. Reproduced with permission of the State Archives of Florida (State Archives of Florida, 2013).

As the communities of Apopka grew, pressures on the lake system gradually increased. The agricultural run-off and point source pollution associated with surrounding development was precariously balanced by the ability of the lake’s aquatic vegetation to absorbed nutrients (Lowe et al., 1999; Schelske et al., 2005). In the mean time, trophy Florida bass fishing continued to be good with little action being taken to regulate harvest. A decline in Lake Apopka’s water quality was first noticed in 1947 with the recording of seasonal algal blooms. In the same year, a category 4 hurricane travelled across the central part of the state. It was reported that the storm spun off several tornadoes and a portion of the lake’s submerged vegetation was uprooted, with sediments being distributed through the water column (Burgess, 1964). Within weeks, extensive algal blooms were recorded in conjunction with extensive fish kills. It is proposed that the loss of macrophyte coverage combined with an increase in
available nutrients through suspended sediment was enough to push the lake to a eutrophic state (Scheffer, Carpenter, Foley, Folke, & Walker, 2001; Schelske & Brezonik, 1992).

This onset of eutrophication was rapid and resulted in a change in the lake’s fish community. Populations of apex predators such as the Florida bass decreased and were replaced with rough species such as the planktivorous Gizzard shad (*Dorosoma cepedianum*) (Clugston, 1963). As the bass disappeared, fish camps began to close with the loss of non-resident anglers. By the 1980’s, the progressive decrease in water quality gained Lake Apopka notoriety as Florida’s most polluted large lake (Saint John’s River Water Management District, 2012). During this time action had been taken to limit recreational catches, though with little effect. The once thriving Florida bass population had already been reduced to negligible levels (Carpenter, Foley, Folke, & Walker, 2001; Schelske & Brezonik, 1992).

Though extreme, the story of lake Apopka is typical of many bass fisheries within the state of Florida. Through decades of anthropogenic influence, state fisheries have continued to be targeted by the angler. In situations of environmental challenge, it is the ability of a population to adapt that frequently determines its success or failure. As with commercial fisheries, recreational anglers typically target individuals displaying trophy traits (high growth rate and length) (Arlinghaus, 2005). Where these individuals are removed from the stock, less desirable individuals are left contributing to successive generations. Should these traits be heritable within a population and give advantage when presented
with environmental challenges, a decrease in their occurrence would adversely affect fitness (Allendorf & Hard, 2009). For *M. floridanus*, whose angling reputation is defined by trophy traits, decades of unregulated recreational fishing pressure may have created just that scenario. The state of Florida recognizes the need understand unnatural selection and has moved to protect and maintain the genetic diversity of endemic fish species. As such, the FWC has placed emphasis on the role of molecular markers in the management of Florida black bass (FWC, 2011; Tringali et al., 2007). The agency has taken two important steps in this direction: (1) identifying/developing genetic markers and applying them to bass conservation (particularly the genetic testing of brood fish), and (2) enabling fishery managers to develop and implement the rules and practices necessary for conservation of Florida’s black bass populations.

The work of the FWC calls into question the status of molecular marker use in recreational fisheries. As these technologies continue to evolve and decrease in cost, it can be expected that fisheries scientists, policy makers and stakeholders will increasingly be presented with and rely upon a broad range of analysis options (Hauser & Seeb, 2008). It is important for these groups to understand the limitations of molecular technologies as applied to topics of management. This is particularly true in a diverse field such as freshwater fisheries. Well-developed programs such as that of the FWC, offer managers a guide towards future projects and an understanding of the role of molecular technologies in decoding the mechanisms of unnatural selection.
Purpose and general hypothesis

The purpose of this thesis is to explore the capacity of state natural resource agencies to gather genetic knowledge and the ways in which such knowledge is applied to management questions. Chapter 2 provides a brief review of the molecular technologies most frequently used in fisheries management. This is followed by the presentation of an online survey, which assesses the breadth of molecular marker application to the management of freshwater fisheries by state natural resource agencies. Results of this survey are discussed in the context of a black bass management in Florida. Chapter 3 will present a study investigating the heritability of physiological and behavioral traits in a population of black bass. Microsatellite parentage analysis is used to reconstruct familial relationships for cultured, juvenile Florida bass exhibiting variable traits of growth and aggressiveness. It is hypothesized that individuals displaying high levels of growth and aggression will be represented by significantly fewer parent-pairs when compared to the relationships of the their respective cohort. The result of this work will provide fisheries biologists with insight into the occurrence of trophy traits and mechanisms of unnatural selection associated with the species. Chapter 4 will summarize these findings in terms of management implications and will present opportunities for future research. Overall, this thesis will attempt summarize and provide direction towards the application of molecular technology in policy associated with recreational fisheries management.
Chapter 2:
The use of molecular technology in freshwater finfish policy and management by state natural resource departments.

Abstract

As the science of molecular technologies has expanded, conservation biologists are increasingly presented with a wide range of genetic analysis options. Within fisheries, a lack of consensus as to the abilities of these technologies has led to their generally slow and uneven integration into the strategic plans of many organizations. This work took three approaches to exploring the ability of state natural resource agencies to gather genetic knowledge and the ways in which such knowledge is applied to decisions of management. First, a brief review of molecular markers addressed their historical applications and respective limitations. Second, an online survey was used to investigate the breadth of molecular marker application to freshwater fisheries management by state natural resource agencies. Seven questions were posed to 50 state agencies addressing species of concern, type of genetic resources used, type of molecular marker used, and management concerns. Genetics was listed as a concern in the management of 18 freshwater fish
families representing 70 distinct species, with Salmonid species the most frequently reported (20%). A majority of agencies rely on outside resources to perform genetics testing (65%). The most common analysis technique used by state agencies was microsatellite DNA analysis (35%) and the most frequently reported management concerns were genetic stock identification and management boundaries (23%). Finally, a discussion incorporated themes of the review and survey in the context of the Florida Fish and Wildlife Commission’s (FWC) approach to the genetic management of black bass (*Micropterus spp.*) in Florida. Here, it was concluded that certain topics should be addressed towards incorporating genetics into a management strategy; (1) the cost/benefit of developing genetic capabilities (2) the limitations of specific genetic markers, and (3) the application of markers to questions of management.

**Introduction**

The successful integration of science into policy is often one the most challenging aspects of developing conservation strategy (Quevauviller et al., 2005). This can be seen during the last three decades of fisheries management when the role of genetics has become greatly emphasized. Advances in molecular technologies have allowed scientists and natural resource managers to decode the mechanisms of unnatural selection and establish conservation strategies aimed at maintaining the genetic diversity of many imperiled fish stocks (Allendorf, Hohenlohe, & Luikart, 2010; Araki & Schmid, 2010; Hauser &
Seeb, 2008; Sagarin et al., 2009). As these technologies continue to evolve and decrease in cost, it can be expected that fisheries scientists, policy makers and stakeholders will increasingly be presented with and rely upon a broad range of analysis options (Hauser & Seeb, 2008). Though the application of genetic marker analysis to conservation issues has shed light on many biological processes, the labyrinth of rapidly developing technologies and institutional needs has the potential to make the already complex practice of policy making even more complicated (Sagarin et al., 2009). This work investigates the status of molecular marker use by state natural resource agencies in the management of freshwater fisheries by reviewing analysis options and current applications.

Within fisheries, extensive and often emotional debates have centered on the role of genetics in stock assessment. Without consensus among managers, scientific guidance interjected into policy becomes suspect. The lack of consensus has led to a generally slow and uneven integration of molecular technologies into the strategic plans of many organizations (Hauser & Seeb, 2008). This can be seen in efforts to protect endemic salmon (*Oncorhynchus spp.*) in the Pacific Northwest, where stock differentiation is essential to management. It is likely more than coincidence that the maintenance of genetic diversity in native salmon populations was seen as a priority with the rise of molecular analysis in the 1970’s (Grant, Milner, Krasnowski, & Utter, 1980; Milner, Teel, Utter, & Winans, 1985). As fisheries scientists began to understand the workings of genetic variation, they were eager to employ new technology to species of concern. This was occasionally done without considering the
applicability to and shortcomings of the specific questions at hand (Ferguson, 1995; Hauser & Seeb, 2008). The resulting real or perceived disagreements among geneticists frequently led to confusion for fisheries managers. With each new leap in technology came promises of the “holy grail” marker, which would answer all of management’s questions. As new technologies revealed independent strengths and weaknesses, skepticism of claims for their potential eventually followed. (Ruckelshaus, Levin, Johnson, & Kareiva, 2002).

Though the successful application of molecular tools to fisheries management has often been challenged, certain recreational programs have taken advantage of the opportunities to gain knowledge. This can been seen with black basses (*Micropterus* spp.) in Florida and Texas, where both states have taken important steps towards creating policy centered on the genetic integrity of fish populations. The first of these steps was identifying and developing genetic markers relevant to specific management questions. Where technological advances presented themselves, they were vetted with the consideration of involved parties towards integration into existing conservation strategies. The second step was enabling scientists, administrators and stakeholders to craft and implement the rules necessary for conservation. Where consensus had been achieved on techniques and questions, the policy of management followed. The results of these efforts are genetic management programs with practical application to achieving the long-term conservation specific black bass species.
Prior to an investigation of the role genetics plays in the policy and management of freshwater fisheries, it is important to have an understanding of the molecular tools available. Contemporary conservation genetics offers a variety of molecular markers and analysis techniques to natural resource managers investigating population dynamics and unnatural selection (Allendorf et al., 2010; DeYoung & Honeycutt, 2005; Saura & Faria, 2011). Fisheries managers typically focus on marker systems providing robust information on the genetic diversity of natural and stocked populations. Recreational fisheries conservation programs are frequently concerned with the genetic components of species identification (Teletchea, 2009), genetic stock identification and management boundaries (Barthel et al., 2010), post release assessment of stocked fish (Bert et al., 2007; Pouder, Trippel, & Dotson, 2010), brood stock development (Porak, Barthel, & Philipp, 2007; Tringali et al., 2007), and conservation issues such as diversity levels (Austin et al., 2012; Coltman, 2008) and population size/vital rate estimation (Luikart, Ryman, Tallmon, Schwartz, & Allendorf, 2010). Where questions of these topics are posed, geneticists seek to identify molecular markers that display high levels of variability and follow predictable rules of inheritance and selection in shaping their distribution.

This search for the “perfect” genetic technique to meet the concerns of fisheries managers led to the development of four workhorse marker systems; enzymatic protein (allozyme) electrophoresis, mitochondrial DNA sequences (mtDNA), microsatellites (µSATs), and most recently single nucleotide polymorphisms (SNPs) (Hauser & Seeb, 2008; Ward, 2000). While the use of
these systems as research tools has provided valuable insight into the genetic diversity of fisheries, they have individual strengths, weaknesses and limitations. One of the first genetic techniques widely applied to fisheries research was allozyme analysis. This method takes advantage of the allelic variations of proteins produced by a single gene locus. The amino acid differences between allelic forms of enzymes reflect changes in the underlying DNA sequence and cannot be considered a direct assessment of DNA itself. Depending on the nature of the amino acid changes, the resulting proteins may migrate at different rates when run through a starch gel subjected to an electrical field (electrophoresis). These migration rates are used to quantify genetic variation and distinguish among genetic units at population and higher species levels (Liu & Cordes, 2004).

The pioneering work of Sick (1961) used protein electrophoresis to successfully describe hemoglobin variants in whiting (*Merlangius merlangus*) and Atlantic cod (*Gadus morhua*). Subsequent exploration of allozyme potential and refinement of statistical methods led to a proliferation of genetic studies for fish and other animals. These studies proved useful in examining patterns of geographic variation and relationships among populations and species (Allendorf & Phelps, 1981). Enzymatic protein analysis was quickly and extensively applied to the study of Pacific salmon stocks. While the anadromous nature of these fish posed significant challenges in the use of physical tags to study mixed populations, molecular markers provided biologists with the opportunity to obtain reliable contribution estimates of associated stocks (Fournier, Beacham, Riddell, 1995).
& Busack, 1984; Milner, Teel, Utter, & Winans, 1985b). The technique was also used to gain an understanding of the survival and influence of stocked fish on the genetic variability of native populations (Stahl, 1983; Waples, 1991). With the success of these applications in salmon, allozymes became the dominant marker used in early studies of fisheries genetics.

While the extension of Sick’s methods provided new opportunities to understand the role of genetics in fish and wildlife management, protein electrophoresis is not without its pitfalls. Issues are encountered with quality tissue collection as genotyping often depends on invasive biopsy procedures that endanger the survival of the animal. Many situations in fisheries management depend on the successful return of an individual either to a natural setting for further study or incorporation into a breeding program (Carmichael, Williamson, Schmidt, & Morizot, 1986). Where this is the desired outcome, protein electrophoresis may offer excessive risk. Additionally, some changes in DNA sequences are masked at the protein level, reducing detectable variation. This lack of variability in protein may belie actual differences in nucleotide sequences during electrophoresis (Liu & Cordes, 2004). Combined with the relatively low number of loci often employed in allozyme analysis (Allendorf & Seeb, 2000), the resulting low statistical power due to a lack of variability and invasive biopsy procedures were cause for a continued search for markers with more diagnostic precision.

With the work of Brown, George, & Wilson (1979) fisheries managers were offered the first molecular DNA marker with distinct advantages over
enzymatic proteins; mitochondrial DNA sequences (mtDNA). Unlike protein electrophoresis where the products of specific DNA sequences are used to quantify genetic variation, mtDNA analysis is based directly on nucleotide arrangements. Mitochondrial DNA is extranuclear and generally thought to be inherited asexually as a single maternal locus (Giles, Blanc, Cann, & Wallace, 1980). This non-mendelian mode of inheritance allows for a specific theoretical genealogical history of the individual whose molecular record has not been altered by the effects of sexual nuclear DNA recombination (Avise et al., 1987). As with allozymes, mtDNA is isolated from individual tissue samples and typically run through electrophoretic gels. While mtDNA may be isolated from any tissue, initial studies found that best results were often obtained from 50-100g samples of internal organs, such as the liver or kidney (Brown, 1980). Early use of the marker was limited in the same fashion as enzymatic proteins with the destructive procedure of tissue collection precluding its application by many field biologists. It was not until the development of the polymerase chain reaction (PCR) to amplify specific DNA target sequences, that mtDNA was able to provide genetic information from blood and nondestructive tissue samples, making it the new method of choice by many fisheries geneticists (Taberlet, Waits, & Luikart, 1999).

A distinct advantage of mtDNA over previous techniques is the marker’s high rate of evolution when compared to nuclear DNA (Kocher et al., 1989). Different regions of the mitochondrial genome display a wide array of mutation rates, making the molecule ideal for inter- and intra-species comparisons. The
molecular variation of mtDNA allowed for studies of intraspecific phylogeny, bringing to light patterns of variation resulting from gene flow between fish populations (Avise, 2000; Johnson, Magee, & Hodge, 2001). These patterns have been used to identify geographic regions, which showed similarities of endemism and provided a tool for the creation of genetically distinct management units in a number of species including Pacific salmon and Largemouth bass (Moritz, 1994; Nedbal & Philipp, 1994). Mitochondrial markers have also been popular among aquaculturists where they have been used to identify and develop brood stock and investigate the genetic diversity between hatchery and native stocks (Billington & Hebert, 1991; Grewe & Hebert, 1988).

Though initially seen as a more powerful tool than allozymes, the use of mitochondrial markers has revealed certain limitations and evidence of exceptions to the previously established theories of mtDNA inheritance. While understood that the population structures derived from mtDNA are limited as they reflect the nuclear genome via a single maternally inherited loci (Birky, Fuerst, & Maruyama, 1989), it has been documented that small amounts of paternal influence may occur within certain species (Guo, Liu, & Liu, 2006; Hoarau, Holla, Lescasse, Stam, & Olsen, 2002; Magoulas & Zouros, 1993). With the mechanism behind this action not fully understood, it is difficult to gauge its influence on evolution. The potential for biparental inheritance of mitochondrial DNA has caused some to challenge the validity of previous applications and call for attention to be focused on discerning its frequency and persistence in wild populations (Rokas, Ladoukakis, & Zouros, 2003; White, Wolff, Pierson,
Gemmell, 2008). Additional work has found inconsistent relationships between taxa when comparing mtDNA and their respective nuclear genomes (Ballard & Whitlock, 2004; Hurst & Jiggins, 2005), suggesting that a singular focus on mitochondrial analysis may not reference a larger and important genomic portion of the evolutionary history of the organism in question. Consequently, it is proposed that the mitochondrial analysis not be relied upon as the sole marker used to characterize population dynamics (Rubinoff, 2006).

Microsatellites (µSATs) have seen increasing use in conservation genetics and are now considered fundamental markers in many fisheries management programs (Guichoux et al., 2011). Though the existence of these markers has long been known and intriguing (Hamada, Petrino, & Kakunaga, 1982), their application to studies of population dynamics was not immediate. Microsatellites offer advantage over mtDNA in that they are highly variable non-coding sequences of nuclear DNA subject to known patterns of Mendelian inheritance through biparental contribution (Hansen, Kenchington, & Nielsen, 2001). Compared with allozyme markers, which in many species do not exhibit more than two or three alleles, µSATs consist of multiple repeat sequences, often having more than 10 alleles per locus (Goldstein & Pollock, 1997). Though the use of multiple loci reduces the influence of genotype error and mutations, and increases statistical power in assignment, the large amount of information provided initially required time-consuming analysis that hampered the widespread use of microsatellites. It was not until the development of advanced technologies including automated fluorescent sequencers, imaging systems and
statistical methods that the power of µSATs could be harnessed to answer questions of genetic diversity (Hansen et al., 2001; O’Connell & Wright, 1997; O’reilly & Wright, 1995).

One of the most practical advantages of µSATs when compared to other molecular techniques is that only small amounts of DNA are required to perform analysis. This makes it possible to perform nonlethal sampling and analyze older archival samples with very small amounts of highly degraded DNA (Hutchinson, Carvalho, & Rogers, 1999). Additionally, the large amount of information provided by the multilocus genotypes of individuals allows for the probability of assignment to a population. A number of assignments can be made when the individual genotype is compared to either known or unknown baseline populations (Hansen et al., 2001). The ease of sampling, generally high levels of variability, and advances in analysis techniques makes microsatellite markers ideal for studies of population genetic structure, genetic relatedness, genetic migration and population size (Chambers & MacAvoy, 2000; DeYoung & Honeycutt, 2005; Jones, Small, Paczolt, & Ratterman, 2010). For instance, µSATs have been used to describe the genetic structure and diversity of natural fish populations such as Chinook salmon (Banks, Rashbrook, Calavetta, Dean, & Hedgecock, 2000) and Red drum (Chapman, Ball, & Mash, 2002). Microsatellites have also seen wide usage in aquaculture for post-release assessment and brood stock development (Austin et al., 2012; Eldridge, Bacigalupi, Adelman, Miller, & Kapuscinski, 2002).
Though a valuable tool in the molecular study of fisheries, µSATs can present certain handicaps in their application. First among these, the high labor and cost associated with developing species-specific markers often inhibits their deployment by state agencies partitioning limited resources (Zane, Bargelloni, & Patarnello, 2002). Additionally, the high mutation rate of microsatellites can sometimes lead to mismatches between parents and offspring during assignment. Where this occurs in studies such as the post-release assessment of stocked fish, sample genotypes not corresponding to known brood stock could be mistakenly labeled as wild, thereby decreasing the overall estimate of the stocked population. Among the largest concerns is the high potential of microsatellites to exhibit non-amplifying (null) alleles and genotyping error associated with scoring bias of the investigator (Broquet, Manard, & Petit, 2007; Dewoody, Nason, & Hipkins, 2006; Hauser & Seeb, 2008). These concerns are particularly problematic when comparing data among laboratories and require standardization among collaborators. Notwithstanding the potential issues associated with microsatellite marker usage, µSATs remain the dominant mode of analysis in both fisheries studies and the broader field of conservation genetics (Guichoux et al., 2011).

Single nucleotide polymorphism (SNPs) analysis is the newest class of genetic technology to see relatively wide application in solving questions of fisheries genetics. These markers represent mutations at single base positions and are the most common type of genetic variability in most species’ genomes (Morin, Luikart, & Wayne, 2004). The incorporation SNPs in both coding and
non-coding regions of the genome has the potential to provide a wealth of information pertaining to variation within and between populations (DeYoung & Honeycutt, 2005). Like microsatellites, the existence of SNPs has been well characterized but received little attention due to the difficulty in genotyping the high number of samples needed for analysis. It was not until the application of advanced technology and statistical methods in the late 1990s, that SNPs became a focal point in nuclear marker development (Liu & Cordes, 2004).

While still in their infancy as applied to fisheries genetics, SNPs have shown several advantages when compared to previous methods of analysis. Like µSATs, SNPs can be derived from nondestructive and degraded tissue samples (Morin & McCarthy, 2007). Unlike microsatellite, which can be sometimes difficult to find in certain species and whose loci suffer from variable mutation patterns, SNPs generally follow simple bi-allelic mutation models (substitutions involve either two pyrimidines C/T or two purines A/G) (Vignal, Milan, SanCristobal, & Eggen, 2002). Because of this simplicity in mutation and their general abundance within a genome, SNPs are more amenable to automation and show lower rates of genotyping error than microsatellites (Morin & McCarthy, 2007). Hauser, Baird, Hilborn, Seeb, & Seeb (2011) showed the advantage of SNPs over µSATs in parentage assignments of offspring in wild Sockeye salmon (*Oncorynchus nerka*). While their use is not widespread, SNPs have shown promise in determining the population structure and genetic relatedness of a number of commercially important fish species including Atlantic cod (Moen et
al., 2008) and Chinook salmon (Schwenke, Rhydderch, Ford, Marshall, & Park, 2006).

The advantages of SNPs in population studies do not come without some cost. Where the bi-allelic nature of these markers provides for a more accurate method of analysis, their simplicity may limit application in studies of parentage and relatedness. To gain a power similar to that of microsatellites, often 2-5 times the number of SNPs loci are required (Glaubitz, Rhodes, & DeWoody, 2003). This can increase the computing time of some statistical packages rendering explicit reconstruction of population dynamics difficult (Hauser et al., 2011). Additionally, while SNPs display low levels of variability per loci, they still require the development of reference sequences from model organisms. Where these are not available, they must be established prior to the start of a population study (Jones et al., 2010). For the moment, the relevance of SNPs to fisheries is still under review with a limited understanding of their full function and application to specific questions. The expanded use of this marker will likely rely on unlocking their potential through additional software packages and modes of statistical analysis.

Beyond the four markers discussed in this work, fisheries managers have a number of other genetic tools at their disposal. Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), expressed tag sequences (ESTs), major histocompatibility complex (MHC) markers, sex chromosome markers and direct nucleotide sequencing have all seen use in aquaculture and fisheries
(reviewed by DeYoung & Honeycutt, 2005; Liu & Cordes, 2004). As these technologies have become more accessible, natural resource managers are becoming increasingly reliant on various sources of molecular data when creating conservation policy. This is particularly true in the realm of freshwater fisheries, where a broad range of species pose unique management challenges.

Towards understanding the complex role of genetics in fisheries policy, this study explores the application of genetic technologies by state natural resource departments with an emphasis on an established genetics program in Florida. An online survey was used to determine the ability of states to gather genetic data and determine how this data is applied to species of concern. Specifically, the survey questions the degree to which molecular technologies are applied to individual species as categorized by family, with an emphasis on black bass. Additionally, the degree to which natural agencies have developed molecular programs is addressed by questioning the in-house capabilities of management programs. Survey results are discussed in the context of the Florida Fish and Wildlife Conservation Commission’s (FWC) genetic conservation program for the Florida bass (\textit{M. floridanus}).

\textbf{Methods}

An online survey was used to assess the ability of state natural resource departments to gather knowledge of genetics related to freshwater fisheries and determine how such knowledge is employed. In March of 2012, two
representatives per resource agency were chosen at random from the American Fisheries Society (AFS) membership list and sent an introductory email originating from an FWC account. This email was meant to describe the study objectives and to confirm participation. Additionally, in an attempt to reach the most suitable survey participants per state (those with most knowledge of their respective genetics programs), representatives were asked to submit contact information for the most qualified individuals. Responses from the introductory email were used to compile a final contact list. Individuals on the final contact list were sent an email reminder of the upcoming survey and a request for final confirmation of participation.

The survey was conducted using SurveyMonkey (SurveyMonkey Inc., Palo Alto, California) with access granted via email Web link. Individual questions were generated from a review of pertinent literature and with assistance from FWC staff at the Florida Bass Conservation Center (FBCC) and the Fish and Wildlife Research Institute (FWRI). Before being distributed, the survey was reviewed and approved by the FWC’s Division of Freshwater Fisheries.

The survey instrument was a brief questionnaire, with seven simple questions and write-in sections for individuals to provide additional information when necessary (Table 2.1.). Questions one and two asked participants to identify their respective agency and whether or not their agency considers genetics during fisheries management decision-making or policy creation. The next three questions used multiple-choice responses to identify genetics analysis
resource type (in-house or outside resources), analysis techniques, and topics of concern in management and policy. Question six offered an open-ended response and asked participants to list freshwater fish species for which their agency incorporates genetics in management. Finally, question seven allowed participants to paste links or citations to documents regarding their agency’s use of genetics in management and policy.

Table 2.1. Questions posed to state natural resource agencies during an online survey conducted in March of 2013.

<table>
<thead>
<tr>
<th>Question</th>
<th>Response type</th>
</tr>
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<tbody>
<tr>
<td>1. Which state agency do you represent?</td>
<td>Open response</td>
</tr>
<tr>
<td>2. Does your agency consider genetics during fisheries management decision-making or policy creation?</td>
<td>Yes or no</td>
</tr>
<tr>
<td><strong>Multiple choice questions (please indicate all that apply)</strong></td>
<td></td>
</tr>
<tr>
<td>3. Does your agency conduct fisheries related genetics testing in-house or use outside resources (ie: universities or private facilities)?</td>
<td>In-house Outside resources Both None</td>
</tr>
<tr>
<td>4. What genetic tools does your agency rely on for fisheries management decisions or policy creation?</td>
<td>mtDNA sequences Microsatellite markers Protein electrophoresis Single nucleotide polymorphism (SNPs) None Other: open response</td>
</tr>
<tr>
<td>5. What topics best address your management or policy concerns involving the use of genetics in fisheries?</td>
<td>Species ID/hybrid studies Genetic stock ID/management boundaries Conservation status (ie: diversity levels, inbreeding) Mark-recapture studies (ie: population size and vital rate estimation) Post-release assessment of stocked fish Broodstock development/screening None Other: open ended response</td>
</tr>
<tr>
<td>6. Please list freshwater species for which your agency involves genetics in management decisions or policy creation</td>
<td>Open ended response</td>
</tr>
<tr>
<td>7. This box provides an opportunity to paste links or references to documents regarding your agency’s use of genetics in fisheries management and policy</td>
<td>Open ended response</td>
</tr>
</tbody>
</table>
The final survey was distributed in April of 2012 with an allowed response time of three weeks. Statistical results were not significant due to small sample sizes. Basic frequency tables are used to describe results and were created using Microsoft Excel® 2007 for Windows.

**Results**

A total of 33 responses were received from state agencies, giving a total response rate of 66%. Figure 2.1 shows a United States map indicating state agencies that responded to the online survey. All responding states reported the use of genetics in making fisheries management and policy decisions. Genetics was listed as a concern in management and policy decisions for 18 freshwater fish families representing 70 distinct species. Salmonids were the most frequently reported family and accounted for 20% of total species responses. Percids followed with 15.5% and then Centrarchids with 12% of total responses. The remaining 15 families accounted for 52.5% of reported freshwater fish species. Of the 34 responding states, 13 (38%) report genetic management of black bass species (*M. salmoides, M. floridanus and M. cataractae*). The percent of total responses to the question of which freshwater fish species state agencies involve genetics in management decisions and policy creation are categorized by family and shown in Figure 2.2.
Figure 2.1. United States map of state agencies which responded to an online survey conducted in March of 2013. The 33 responding states are indicated in gray.

Figure 2.2. Frequency histogram showing percent of total responses to the question of which freshwater fish species state agencies involve genetics in management decisions and policy creation ($N=34$). The 70 distinct species listed are categorized by 18 freshwater fish families.

As shown in Figure 2.3, when asked whether state natural resource agencies rely on in-house, outside, or a combination of resources, more than half (65%) indicated the use of outside resources. This was also the case for those states working with black bass (69%). States reporting the use of both in-house
and outside resources accounted for 24% of the total responses for all freshwater fish. In a similar fashion, 23% of those agencies concerned with black bass rely on both types of resources to collect genetic data. Three states (8%) rely on in-house resources to complete genetics tasks with Florida being the only state to do so with black bass. New Hampshire was the single respondent to list “none” in answer to the question of resource type. The number of responses to the question of resource type used for all freshwater species and those states reporting work with black bass are shown in Figure 2.3.

![Figure 2.3](image)

**Figure 2.3.** Frequency histogram showing number of responses to the question genetic resource type used by state natural resource agencies in management decisions and policy creation for all freshwater fish species and black bass alone (N=34; N=13). Responses included outside resources alone, both outside and in house resources, in-house resources alone, and none. Black=all species; Grey=black bass.

The most common response to the question of analysis technique for all freshwater fish species and for those states working with black bass was the use of microsatellite markers (35 and 38% respectively). Roughly a quarter (26%) of all freshwater fish species reported by state agencies are subject to mtDNA analysis. For all freshwater fishes, allozymes ranked third with 17% of responses.
Mitochondrial DNA and allozyme analysis received an equal share of the responses for the black basses (24%). Single nucleotide polymorphisms accounted for 10% of the overall species responses with Virginia being the only state to employ these markers in conjunction with work on black bass. Responses listed as “other” included amplified fragment length polymorphisms (AFLPs), restriction-site associated DNA sequencing (RAD), PCR disease detection techniques, and MHC analysis. The number of responses to the question of genetic analysis techniques used by state natural resource agencies in management decisions and policy creation for all freshwater fish species and black bass alone is shown in Figure 2.4.

As shown in Figure 2.5, the most frequently reported management concern of state natural resource agencies for all freshwater fish species was genetic stock identification and management boundaries (23%). This was also the case for those states reporting work with black bass (23%). Conservation status (20%) and brood stock development (17.5%) were the second and third most frequently observed responses for all freshwater fish. This is similar to the concerns of states working with black bass where these categories each accounted for 16.5% of responses. The remaining five potential responses for all freshwater species and black bass can be seen in Figure 2.5.
Figure 2.4. Frequency histogram showing number of responses to the question genetic analysis techniques used by state natural resource agencies in management decisions and policy creation for all freshwater fish species and black bass alone (N=34; N=13). Responses include microsatellites (μSATs), mitochondrial DNA (mtDNA), protein electrophoresis (allozymes), single nucleotide polymorphisms (SNPs), other and none. Black=all species; Grey=black bass.

Figure 2.5. Frequency histogram showing number of responses to the question genetic management concerns of state natural resource agencies in management decisions and policy creation for all freshwater fish species and black bass alone (N=34; N=13). Responses include genetic stock identification and management boundaries (GMU), conservation status (CST), brood stock development (BSD), species identification and hybrid studies (SID), post-release assessments (PRA), mark-recapture studies (MRS), other and none. Black=all species; Grey=black bass.
Discussion

The lack of complete response to this survey means that it is not representative of all state natural resource agency genetics programs. In particular, the application of molecular tools to the conservation of black bass is under-represented, as states with known programs (ie: California, Illinois and Mississippi) did not submit responses. Though not a true gauge of the use of genetics by state fisheries managers, this work does achieve its goals by giving an impression of the variety of approaches used to address concerns of genetic integrity.

With over 70 freshwater fish species reported, it is clear that state agencies have broadly considered genetics in management activities. Salmonids were the most frequently observed response to the question of species of concern. This is of little surprise as the application of molecular markers to fisheries was first tested on this family in the 1980’s (Grant et al., 1980; Milner et al., 1985a). Percids, which include walleye (*Sander vitreus*), sauger (*Sander canadenis*), a variety of perch (*Perca spp.*) and darters (*Ammocrypta* and *Etheostoma spp.*), were the second most frequently observed response. Centrarchids, of which the black bass are members, were the third most reported freshwater fish family. Within this group, the Northern (*M. salmoides*), Florida (*M. floridanus*) and Shoal (*M. cataractae*) basses have management programs based on genetics. Florida also listed these three species as concerns.
Specific to the type of capabilities, most states rely on outside resources in the form of universities or private labs to conduct genetics related tasks. The purchase of expensive genotyping equipment is frequently not justifiable for agencies where high throughput of analysis is not a priority. For these organizations, the occasional use of outside resources is an effective alternative to lab development (Liu & Cordes, 2004). Those states that have developed in-house capabilities (Alaska, South Carolina and Florida), are typically applying markers to a number of species and populations. Florida for instance, uses genetic markers to assess a variety of fish and wildlife populations including the Florida mottled duck (*Anas fulvigula fulvigula*), Florida manatee (*Trichechus manatus latirostris*), sheepshead (*Archosargus probatocephalus*), Atlantic tarpon (*Megalops atlanticus*), red drum (*Sciaenops ocellatus*) and common snook (*Centropomus undecimalis*). Florida also takes advantage of partnerships with outside resources to collect molecular data. Specific to black bass, the FWC has relied on a number of previously published studies and the work of universities to develop management guidelines and rules aimed at maintaining genetic diversity (Austin et al., 2012; Barthel et al., 2010; Philipp, Childers, & Whitt, 1983).

Microsatellite accounted for 35% of responses to the question of analysis technique and were the mode of analysis most relied upon by state natural resource agencies. This is consistent with trends in marker use for fisheries and aquaculture publications (Guichoux et al., 2011; Liu & Cordes, 2004). Many state agencies reported the use of multiple marker systems in developing fisheries policy. While the FWC currently depends upon microsatellites to
conduct genetics tasks, rules pertaining to Florida’s black bass do reflect historical use of a variety of molecular technologies. Allozymes were used by Philipp et al. (1983) to evaluate and confirm populations of intergrade Largemouth bass (*M. salmoides* X *M. floridanus* hybrids) within the north-central part of the state. Below this zone, scientists expected bass populations to consist of individuals displaying endemic *M. floridanus* genotypes. Subsequent allozyme analysis of stocks expected to maintain pure *M. floridanus* genes showed that non-native Largemouth bass alleles had progressed below the intergrade zone. This was attributed not to natural mechanisms, but to decades of unregulated state and private stocking activities (FWC unpublished data).

As a result, the FWC, in conjunction with members of the Illinois Natural History Survey and the Texas Parks and Wildlife Department, began a statewide genetics study to assess the geographic distribution of pure Florida bass and inter-specific hybrid populations. Barthel et al. (2010) relied on allozymes, microsatellites, and mitochondrial DNA to investigate the genetic population structure among populations of Largemouth bass, Florida bass and their hybrids in 48 lakes across Florida. The use of nuclear and mitochondrial markers produced somewhat different results in attempting to differentiate genetic stocks. Allozyme genotypes alone did not resolve into well-defined groups and mtDNA markers failed to detect introgression throughout much of the known intergrade zone. It was microsatellites alone or the combination of all nuclear genotypes that provided enough resolving power to differentiate genetic structure among four regional groups within Florida. Beyond the ability to answer questions
related to management, this study showed the importance of understanding the capabilities of molecular technologies. As suggested by Rubinoff (2006), where individual markers may be inadequate for addressing a specific concern, combined marker systems can increase the power of analysis.

The work of Barthel et al. (2010) occurred in conjunction with the development of specific policy aimed at maintaining the genetic diversity of Florida’s endemic fish populations. In 2004, the FWC’s Genetic Policy for the Release of Finfishes in Florida (GPRFF) (Tringali et al., 2007) was crafted to serve as the basis for incorporating genetic concerns into rules, permits and special activities by restricting the introduction or transfer of all non-native fish species beyond known stock boundaries. The concerns of the GPRFF are similar to those most frequently listed by state natural resource agencies in considering the genetics of fisheries; genetic stock identification and management boundaries. This was also the most frequently reported management concern of state agencies working with black bass. Genetic stock identification towards the development of management units for Salmonids was among the first topics addressed in the early applications of molecular markers to fisheries analysis (Grant et al., 1980; Milner et al., 1985). In order to devise strategies to protect genetic diversity, it is crucial that biologists first understand the extent to which it occurs and the processes which sustain it (Moritz, 2002). For fisheries managers, this often entails determining patterns of association between populations and the development of units by which they can be managed. Indeed, the investigation of genetic stock structure has been a critical first step towards the
conservation of a number of freshwater fish species (Gatt, Fraser, Liskauskas, & Ferguson, 2002; Krabbenhof, Rohde, Leibman, & Quattro, 2008; Milner et al., 2003; Palsboll, Berube, & Allendorf, 2007; Powers, Mayden, & Etnier, 2004).

For black bass in Florida, studies have pointed to unregulated stocking activities as a major challenge to the stock structure of endemic populations. As such, the GPRFF was crafted to regulate all activities that involve the intentional or unintentional release of cultured finfish into state waters, including those activities conducted by the FWC. Initially, the state was considered a single management unit where the translocation of non-native Largemouth bass genes into the native range of Florida bass was prohibited through FWC stocking activities. In order to achieve this, it was necessary for FWC geneticists to address the fourth most frequently reported concern listed by state natural resource agencies, species identification. Where many fish populations have long since been described at the species level through meristics, the distance between M. floridanus and M. salmoides has only recently been characterized. Philipp et al. (1983) used protein electrophoresis to estimate the genetic composition of black bass samples from numerous regions throughout Florida. This work provided a basis for early efforts of the FWC to maintain pure M. floridanus broodstock in its hatchery system. Concordant with the concerns of translocation, a decision was made to genetically screen each brood fish being spawned at the FWC’s main production facility via the available method of protein electrophoresis.
Though the allozyme method described by Philipp et al. (1983) was useful in describing population level dynamics, FWC biologists understood it did not provide the power required for testing the taxonomic status of individual fish, as necessitated by stocking regulations of the GPRFF. In response, geneticists at the FWRI turned to existing in-house microsatellite programs for red drum and common snook to develop a suite of 18 new microsatellite DNA specific to Florida bass (Seyoum et al., 2013). The development of these markers met not only the taxonomic standards of geneticists, but also the sampling concerns of hatchery staff. As noted by Carmichael et al. (1986), the procedures associated with tissue collection for allozyme analysis can often jeopardize the survival of the animal in question. For hatchery biologists, this risk is especially high when considering the expense of collecting and maintaining brood stock. In considering this risk, the application of allozyme analysis to private hatcheries would have likely been met with much criticism. By developing new microsatellite markers using existing in-house capabilities, FWC biologists had the tools necessary to investigate genetic concerns on a broad level.

With marker development, the FWC was prepared to address the agency related concerns of the GPRFF. Standard operating procedures for Florida bass brood stock collection were established at the FWCs main production facility, the FBCC. Newly caught wild brood fish are tagged for individual identification with a small portion of fin tissue removed for microsatellite analysis, ensuring it is of pure *M. floridanus* lineage. Permanent records are kept including the tag number, gender, and spawning history of each brood bass, allowing for
subsequent studies of offspring. As of July 2012, a total of 1,058 FWC brood fish had been submitted for confirmation of lineage. Of those fish, 33 were identified as hybrids and not added to brood stock populations (FWC, 2012; FWC, 2013).

The concern for introduction of non-native bass alleles through stocking activities of private hatcheries was addressed when the FWC passed Rule 68-5.002 (r) Florida Administrative Code (F.A.C.) to list Northern Largemouth Bass and hybrids of Largemouth Bass as a Conditional Non-native Species in Florida, prohibiting possession and transport in the state without a permit. This rule led FWC biologists to work with private in-state facilities to develop certification and authentication procedures similar those found at the FBCC. To date, FWC biologists have tagged and collected fin-clips from 209 brood fish from private hatcheries, of which 84 were determined to be hybrids and removed from the spawning stock (FWC, 2012; FWC, 2013).

With a solution to the problem of non-native largemouth bass introductions into the natural range of the Florida bass, FWC fisheries managers turned their attention to further refinement of genetic management units. Similar to concerns of scientists working with Salmonids, where intraspecific outbreeding has the potential to reduce the fitness of wild populations (Edmands, 2007; Ryman, Utter, & Laikre, 1995), FWC biologists proposed to manage state stocking efforts according to the genetic structure of bass populations across Florida. Though at the time, little work had been conducted to establish the effects of intraspecific outbreeding on Florida bass populations, evidence did suggest the potential for changes in life history traits (Rogers, Allen, & Porak, 2006). Considering this and
the recent efforts to mitigate previous introductions of non-native bass alleles, FWC managers took a precautionary approach to the topic of intrapecific hybridization. The work of Barthel et al. (2010) resolved the genetic structure of four regional Florida bass populations within the state. Based on these results and United States Geological Survey (USGS) Hydrologic Unit Boundaries, the FWC defined four GMUs for populations of Florida bass and their hybrids in Florida. This led to the establishment of GMU specific brood stock within the FWC hatchery system, with the stocking of fish not occurring across unit boundaries except in special circumstances.

For FWC biologists, brood stock development is a growing priority. This topic was the third most frequently reported genetic concern by all state agencies in the survey. Specific to black bass, this concern ranked third and equal to that of post-release assessment. Beyond the rules laid out by the GPRFF, the FWC has placed considerable emphasis on the genetic contributions of brood stock to offspring and their subsequent interactions with wild populations. The maintenance of fish within a hatchery system has the potential to exert selective pressures in successive generations. In these situations, fish of an extended hatchery lineage may exhibit phenotypes advantageous to hatchery conditions and deleterious in the wild (Araki & Schmid, 2010; Berejikian, 1995). To avoid this scenario, the FBCC uses only wild-type adults for production (Lorenzen, 2005). Furthermore, FWC biologists have collaborated with University of Florida staff to investigate brood stock population size in respect to the genetic variability of offspring. The loss of genetic variability from stocking fish representing few
families and the resulting decline in phenotypic and physiological traits is a concern for many natural resource agencies (Hitoshi, Cooper, & Blouin, 2009; Lynch & O'Hely, 2001). In the case of the FBCC, FWC scientists used the existing microsatellite framework to analyze offspring and propose an effective population size for brood stock (Austin et al., 2012). Hatchery biologists use this as a guide to the development of spawning regimes for seasonal production.

Since the development of the GPRFF, the FWC has continued to take advantage of microsatellite markers specific to *M. floridanus*, and the in-house capability to analyze tissue samples. With the recently developed Black Bass Management Plan (BBMP), the agency has laid out specific action items designed to address the conservation status of Florida black bass. Among the concerns stated in this document are continued studies of the genetic variability of endemic black bass populations. With a framework of genetic technologies in place, the FWC is well positioned to address the conservation of Florida’s native fisheries.

**Conclusion**

It is clear that molecular technologies have been broadly applied to the management of freshwater fisheries by state natural resource agencies. Results of this work have shown that certain topics should be considered in the development of any genetics policy or management strategy. When addressing questions of fisheries management, it is important for scientists to consider all the
resources at their disposal. For those agencies that do not have a need for high sample throughput, it may be of benefit to explore partnerships with other institutions or private facilities. In the case of Florida, where a number of species are concerned, the capabilities of an in-house lab meet the needs of most projects though collaboration does benefit certain situations. It is also important that fisheries biologists understand the limitations of molecular marker systems when posed with specific conservation issues. As shown with Barthel et al. (2010), the ability to increase the power of analysis through combined marker systems provided results that would have been overlooked by a single system.

As fisheries biologists move forward with the use of molecular markers, lessons learned from previous applications can guide projects to their successful completion. With the development of marker specific statistical software packages, geneticists have the capability to give answers to long-standing questions of population dynamics and unnatural selection. For the FWC, a number of on-going studies show the importance of maintaining a genetics toolbox. Scientists are currently using microsatellite markers to address the impacts of bed fishing on native bass populations, determining whether poststocking survival of bass can be increased by altering culture techniques, and the occurrence of trophy traits within bass populations. For Florida’s freshwater fisheries, early genetics policies have set the structure on which future work can progress. This is shown in the BBMP, which represents a comprehensive and long-term commitment to the genetic integrity of endemic Florida bass.
Chapter 3:

Microsatellite parentage analysis of cultured juvenile Florida largemouth bass *Micropterus floridanus* displaying variable traits of growth and aggression.

Abstract

Understanding the heritability of traits within sportfish populations is a requisite for understanding the impacts of unnatural selection in the form of angler harvest. This study investigates the heritability of physiological and behavioral characteristics in juvenile Florida largemouth bass *Micropterus floridanus*. Microsatellite parentage analysis was used to reconstruct familial relationships of Florida bass displaying variable traits of growth and aggression in a culture setting. Age-0 juveniles were segregated into two groups according to size and randomly sampled; baseline growth and aggression group (BGA; \( N = 250 \)) and high growth and aggression group (HGA; \( N = 250 \)). Ten microsatellite loci in four multiplexes were used for assignment of offspring to 119 potential wild-type brooders (males \( N = 47 \), females \( N = 72 \)). Parentage was successfully assigned at a rate of 78%. Offspring of the BGA group represented significantly more parents (44 unique parents; 31 pairings) than offspring of the HGA group.
(25 unique parents; 14 pairings). There was a significant difference of the top three parent-pairs according to contribution rank between groups (BGA=48%; HGA=90%). This was due to a majority of the HGA group (83%) being represented by a single-pair (P22/P25). The pair showed a significantly reduced contribution to the BGA group (7%). A difference was observed in the display of aggression between the two groups (BGA N=1; HGA N=29). A majority of aggressive fish resulted from the P22/P25 pairing (N=21). Additionally, aggressive fish displayed significantly higher levels of fitness than non-aggressive fish as described by Fulton’s condition factor. This study agrees with previous works and suggests that traits of growth and aggression can be predicted by familial relationship and may be heritable within wild populations of Florida bass. Results show that the influence of angler-induced evolution should be taken into consideration when planning management strategies for recreational fisheries.

Introduction

The maintenance of genetic diversity is fundamental to the ability of species to adapt to short-term environmental change (natural or anthropogenic) and to permit long-term evolutionary success (King et al., 2007; Saura & Faria, 2011). With this in mind, advanced fisheries conservation programs often set goals to augment natural populations and develop a self-sustainable local wild stock by emphasizing genetic variability (Hitoshi, Cooper, & Blouin, 2007; Lynch
These programs are frequently concerned with the fitness of local populations as affected by artificial selection, introgression of genetic material and outbreeding depression (Araguas, Sanz, Pla, & Garcia-Marín, 2004; S. Cooke, Kassler, & Philipp, 2001; Hansen, Ruzzante, Nielsen, & Mensberg, 2001).

One of the most popular tools for use in conservation is the production and release of hatchery reared fish into the wild. While hatcheries have been widely relied upon in management efforts, the effect of captive bred fish on native populations has long been debated (Miller, 1958; Moyle, 1976; Needham & Slater, 1944). Araki and Schmid (2010) summarized 266 peer-reviewed papers published in the last 50 years related to the ecology and genetics of hatchery stocks and their effects on stock enhancement. The 131 studies of genetic diversity and fitness yielded consistent topics to address when relying on hatchery fish to meet conservation goals. Of these topics, reproductive capacity, allele variability and heterozygosity as influenced by broodstock numbers were found to most influence the fitness of wild populations. The review of Araki and Schmid (2010) shows a need to avoid the selective effects (intentional or otherwise) often associated with captive breeding schemes. The knowledge gathered concerning the influence of hatchery fish on native stocks has led progressive management agencies to develop breeding policies which focus on enhancing relative fitness characteristics to improve disease resistance, survival and recruitment by maintaining or increasing the allelic diversity of stocked and
wild populations (Austin et al., 2012; Fries, Hutson, & Warren, 1996; Lynch & O'Hely, 2001; Tringali & Bert, 1998; Tringali et al., 2007).

This need to maintain genetic integrity of hatchery fish and wild populations poses a paradox for recreational fisheries managers when faced with angler-induced unnatural selection in natural populations. It is widely accepted that unnatural selection is a causative agent in the phenotypic evolution of commercial fish stocks. (Allendorf, England, Luikart, Ritchie, & Ryman, 2008; Heino & Dieckmann, 2008; Jorgensen et al., 2007; Kuparinen & Merila, 2007; Law, 2000, 2007; Policansky, 1993; Stenseth & Dunlop, 2009). The indirect effects of human-induced selection can alter the structure and function of a concerned population by modifying species-specific life history traits and physical characteristics. As individuals displaying the most desirable phenotypes related to yield (growth rate, length and fecundity) are often targeted for harvest, less desirable individuals are left contributing to successive generations. With time, this directional selection leads to a potential increase in the frequency of less desirable alleles, yielding a decrease in the fitness of wild populations and altering ecosystem interactions. (Allendorf & Hard, 2009; Enberg, Jorgensen, & Mangel, 2010; Law, 2000). Resulting population level shifts such as early maturity and smaller body size at maturation are difficult to reverse and have been attributed to the decline of regional commercial fisheries and global fish stocks (Cooke & Cowx, 2006; Hard et al., 2008; N.W. Kendall, Hard, & Quinn, 2009; Olsen et al., 2005; Sharpe, Wandera, & Chapman, 2012; Ward & Myers, 2005).
It reasons that a similar effect would result from selection due to pressures of recreational fishing. The annual exploitation rates (fraction of fish removed from the population) of recreational fisheries can range from < 10% to > 80% and thus have the potential to be of comparable dimensions to commercial exploitation (Allen, Miranda, & Brock, 1998; Lewin, Arlinghaus, & Mehner, 2006). Anglers frequently select individuals with respect to species, size class or behavioral traits (Lewin et al., 2006). Trophy and common recreational anglers often target the largest individuals exhibiting high growth rates and high vulnerability to angling (aggressiveness) (Arlinghaus & Mehner, 2003; Bryan & Larkin, 1972; Petering, Isbell, & Miller, 1995; Philipp et al., 2009; Radomski, 2003). In these cases, removing the larger, more aggressive individuals from a population may allow smaller, less aggressive individuals to perpetuate with greater success (Drake, Claussen, Philipp, & Pereira, 1997; Lewin et al., 2006; Philipp et al., 2009). As with commercial fisheries, this selection for size-related and behavioral characteristics has the potential to cause evolutionary changes in physical and life history traits of the target sport fish population and on the ecosystem as a whole (Cooke & Cowx, 2006; Lewin et al., 2006). This leads to the paradox of recreational fisheries management: using available tools to construct a program to target restoration of fisheries altered by human induced selection while maintaining the genetic diversity of the wild population.

Though the selective effects of recreational angling have shown great potential to influence fisheries, little effort has been devoted to understanding the dynamics of phenotypic display within sportfish populations. This is especially
true for the black basses (*Micropterus* spp.). The Florida largemouth bass (*M. floridanus*) is a subspecies of black bass and is closely related to the northern largemouth bass (*M. salmoides*) (Bailey & Hubbs, 1949). Black bass are one of the most sought after freshwater sport fish in Florida, annually generating $1.25 billion for the state's economy and supporting approximately 12,000 jobs (U.S. Department of the Interior & Commerce, 2006). The Florida largemouth bass is genetically unique and endemic to peninsular Florida with a native range extending south and east of the Suwannee River drainage basin. Above the Suwannee is a zone of northern and Florida bass hybrids (Bailey & Hubbs, 1949; Philipp, Childers, & Whitt, 1981, 1983). *M. floridanus* is a particularly prized black bass strain as it has a reputation for exhibiting traits of increased growth and fighting ability when compared to its northern cousin. This has made the subspecies highly desirable for stocking and use in hybrid production programs throughout the world.

The influence of angler-induced selection on largemouth bass populations has until recently received little attention. Catchability or vulnerability to angling is generally thought to be a product of an individual's general level of aggression (Bryan & Larkin, 1972). Though a number of early evaluations report a difference in catchability between individual bass (Anderson & Heman, 1969; Bennett, 1954; Martin, 1958), few works have investigated vulnerability to angling as a heritable trait. Burkett, Mankin, Lewis, Childers, & Philipp (1986) demonstrate that recapture of largemouth bass in Ridge Lake, Illinois, is not a random phenomenon. The authors report contribution of 0 and 6 capture-frequency
categories to chi-square statistics as 26.38% and 61.76% respectively. This suggests variability in individual vulnerability to angling with Burkett et al. (1986) proposing further research into the potential of heritability and selective breeding for the trait. Garrett (2002) used selective breeding of *M. salmoides* to determine if angling vulnerability has a predictable, heritable component. A random sample of wild stock was subject to angling pressure at Heart of the Hills Research Station, Kerr County, Texas. Fish caught three or more times (vulnerable) were separated from fish that had not been caught (wary). Spawning was conducted through two generations of the separated populations followed again by introduced angling. F$_2$ fish bred for high vulnerability were likely to be caught multiple times more than were those bred for wariness, suggesting the trait is predictable. Philipp et al. (2009) continued investigations of catchability variation in *M. salmoides* with a long-term selection experiment in Ridge Lake, Illinois. The authors used methodology similar to Garrett (2002) to produce and sample three generations of high- and low-vulnerability largemouth bass. The study calculated a realized heritability of 0.146 ($r^2 = 0.995$) for F$_3$ offspring, indicating that vulnerability of largemouth bass to angling is a heritable trait.

As largemouth bass are generally not cultured for food in North America, studies of selective breeding and the heritability of growth within the species are rare. While small scale commercial fish farms have reported success in selection for growth (see Tiger bass and Gorilla bass) scholarly publications tend to focus on differences between the northern and Florida subspecies. Kleinsasser, Williamson, & Whiteside (1990) evaluated the growth of *M. salmoides* (N X N),
M. floridanus (F X F), and their reciprocal F₁ hybrids (F X N and N X F) in ponds at the San Marcos National Fish Hatchery and Technology Center and Texas State University, Texas. F X N crosses were significantly heavier than other crosses at the end of the study. F X F crosses were significantly shorter, weighed less and were in poorer condition than all other crosses. Garret (2002) suggests differences in observed growth rates may be attributed to catchability. Lower vulnerability individuals attain a longer lifespan, increasing growth potential. While these studies investigate the potential for growth, they do not address the heritability of the trait within subspecies populations. The only reported investigation of selective breeding for growth in M. salmoides is offered by Shengjie et al. (2009). The authors evaluated the growth through three generations of two families exhibiting increased growth. Results show improved daily growth rates (length and weight) of 25.32% and 23.42% when compared to a control and suggest that individual growth rate can be improved with selection.

Both growth and aggressiveness have been shown to influence first year recruitment of M. salmoides. Angling may select against more aggressive individuals that provide better parental care to their offspring, as in the case of male nest guarding in bass (Cooke, Suski, Ostrand, Wahl, & Philipp, 2007; Philipp et al., 2009). Should selection against increased growth occur, first year recruitment of largemouth bass may be negatively influenced. Ludsin and DeVries (1997) showed a positive correlation between size and overwinter recruitment of bass in southern ponds. The authors attribute larger size to the onset of comparatively early piscivory. This led to elevated fall lipid accumulation.
and higher overwinter success in larger fish when compared to their smaller counterparts. Miranda & Hubbard (1994) document mortality of age-0 bass being size dependent, with smaller fish experiencing higher mortality. Five length groups of juvenile *M. salmoides* were stocked into experimental ponds with and without predators. Fish in the lower length groups had a gradually lower survival rate than larger fish in the presence of predators. This led to the author’s suggestion of increased growth being an advantage for juvenile recruitment in situations of predation.

Understanding the heritability of traits within sportfish populations is a requisite for understanding the impacts of unnatural selection in the form of angler harvest (Philipp et al., 2009). The purpose of this study was to investigate the hypothesis that physiological and behavioral traits are heritable within populations of Florida bass as has previously been suggested (Garrett, 2002; Philipp et al., 2009; Shengjie et al., 2009). Microsatellite parentage analysis was used to reconstruct familial relationships for cultured, juvenile Florida bass exhibiting variable traits of growth and aggressiveness. Specifically, it was expected that individuals displaying high levels of growth and aggression (HGA) would be represented by significantly fewer parent-pairs when compared to the relationships of the their respective cohort, the baseline growth and aggression group (BGA). Where this occurs, it can be assumed certain parent-pairs are predisposed to producing offspring displaying certain traits. In gaining an understand of the occurrence of these traits, it is possible to make assumptions
as to their heritability within natural populations and evaluate potential management options from the perspective of unnatural selection.

**Methods**

This study used animals produced during the fall of 2012 at the FWC’s Florida Bass Conservation Center (FBCC) in Webster, Florida. Sample individuals were selected from a population designated for general production, for which procedures are reviewed in the following sections. Bass fingerlings underwent a period of feed training to convert their diets from zooplankton to a pellet. Sampling and data collection took place post feed training, with FWC personnel performing fish grading, euthanasia and tissue collection. Methodologies for parentage assignment, rearing and grading were performed according to FWC standard procedures. The student performed all activities related to microsatellite DNA and final data analyses.

**Breeding design and rearing.** FWC personnel conducted the collection and subsequent genotyping of brood stock per standard FWC protocol. Wild adult Florida bass were collected between 2007 and 2013 from lakes within the St. John’s River-Kissimmee genetic management unit (GMU). All brood stock were implanted with a 12 mm, 125 KHZ glass Passive Integrative Transponder (PIT) tag (Biomark, Inc. Boise, Idaho) for individual identification and fin clipped (≈ 1mm² of dorsal fin tissue) for microsatellite DNA genotyping and confirmation
as pure *M. floridanus*. Individual PIT tags numbers were listed as ten-digit codes and used to identify brood stock contributions to study offspring in parentage analysis. Post analysis, the ten digit tag numbers were transformed to three-digit parent IDs (P##) as reported herein. Unique parent-pairs are designated as combined parent IDs in a P##/P## format. Adult bass sex determination was attempted by observing size (bass >3.63 kg were assumed female) or observing the milting of mature males and/or catheterization using a 2 mm glass tube.

Towards induction of an out-of-season October spawn, brood stock underwent a three-month period of exaggerated temperature and photoperiod manipulation to simulate winter- spring temperatures and day length over a 90 day period. This spawning method is standard procedure for the FBCC.

Natural spawning took place in October of 2012 via two 24 (l) X 2.5 (w) X 1 m (h) flow through, concrete raceways: R3 and R4. R3 was populated with 24 and 31, R-4 with 23 and 41 putative males and females respectively. Twenty 51 X 56 cm Spawntex® mats (Pentair Aquatic Eco-Systems Inc., Apopka, Florida) were placed in each raceway to serve as a spawning substrate. Mats were checked each morning for breeding activity with spawned mats being immediately transferred to a 9.1 (l) X 0.8 (w) X 0.6 m (h), 15.1 L/min flow through incubation tank. The study population was produced from 26 spawns (R-3, N=16; R-4, N=10) over the course of three days. Eggs received a 100 mg/L hydrogen peroxide (35% PEROX-AID®, Western Chemical Inc., Ferndale, Washington) treatment twice daily, for a period of three days, to prevent outbreak of winter fungus (*Saprolegnia spp.*) (Matthews, Sakmar, & Trippel, 2012). Free-
swimming larvae (~3 days post-hatch) were pooled to meet desired stocking compliments of ≤ 197,600/ha (80,000/ac) and transferred to three fertilized ponds. Bass fry fed on natural zooplankton populations in ponds with fingerling harvest occurring 27 days after stocking (fingerlings of 35 to 40 mm TL). Monitored afternoon outdoor pond temperatures at the FBCC ranged from 32°C in early October to 20°C in mid November.

Fingerlings were harvested in December of 2012 and stocked into two 9.1 (l) X 0.8 (w) X 0.6 m (h) raceways at a density of 6g fish/L. Feed training began the day of harvest per standard FBCC protocol. This consisted of introducing cultured premium grade *Artemia salina* (Brine Shrimp Direct, Ogden, Utah) and Otohime C1 marine fish larval feed (Reed Mariculture Inc., Campbell, California) every half hour for the initial 72 hours of training. Following this was a gradual transition to feedings every two hours of Richloam bass diet #15 (Nelson and Sons Inc., Murray, Utah), the staple diet through the remainder of production.

**Sampling.** Sampling was conducted in February of 2013 with a study population of 34,003 feed-trained fish. Pelleted diet was withheld from the population for the 24 hours prior to sampling. Random selection (*N*=250) was performed on the general population to represent the BGA group with selected individuals placed in a 9.1 (l) X 0.8 (w) X 0.6 m (h) holding tank. The study population (minus 250 BGA samples) was then passed through an adjustable vertical grader with bars set so as to capture those individuals observed to be significantly larger than the study population mean (HGA group). Of the 726 fish
captured by this method, 250 were randomly selected to represent the HGA group and held in a similar manner as BGA samples.

Physiological data was collected the day of grading. Prior to data collection, sample individuals were anesthetized in 25 fish batches by introduction to a lethal dose (250 mg/L) of Tricaine-S (Tricaine methanesulfonate, Western Chemical Inc., Ferndale, Washington) (Summerfelt & Smith, 1990). Sampling began once fish had lost equilibrium and ceased ventilation (~ 2 min). All BGA and HGA sample fish had total length (TL) recorded to the nearest mm, and weights recorded to the nearest 0.1g. These were used to calculate Fulton’s condition factor according to the following equation:

\[ K_{TL} = \frac{100,000W}{L^3} \]

where \( W = \) weight in grams, and \( L = \) total length in millimeters (Lagler, 1956)

The contents of individual stomachs were examined to determine feeding behavior. Those individuals whose stomachs contained identifiable fish remnants were deemed aggressive (Hecht & Appelbaum, 2009). All other individuals were deemed non-aggressive. Tissue samples for microsatellite DNA analysis were collected and stored individually in a 95% ethanol solution and refrigerated (-81°C) until time of DNA extraction.

**Microsatellite DNA analysis.** Microsatellites are relatively small (1-6 base pairs) sequences of non-coding nuclear DNA subject to known patterns of Mendelian inheritance through biparental contribution (Hansen, Kenchington, & Nielsen, 2001). These markers are highly variable and abundantly distributed
across genomes, making them ideal for studies of population genetic structure, genetic relatedness, genetic migration and population size (Chambers & MacAvoy, 2000; DeYoung & Honeycutt, 2005; Adam G Jones, Small, Paczolt, & Ratterman, 2010). A complete review of the general protocol associated with microsatellite development and genotyping is provided by Selkoe and Toonen (2006). In summary, loci-specific flanking regions of DNA identify a µSAT for isolation. Short stretches of primer DNA are tagged with fluorescent dye and bind to flanking regions, guiding µSAT amplification with the polymerase chain reaction (PCR). Variation in amplified µSAT allele lengths are standardized and distinguished by a high-resolution gel electrophoresis sequencer. Sequencing software converts raw data from banding patterns into a plot with peaks corresponding to the width and intensity of each band. Peak position along the x-axis represents µSAT allele scores used and is used for comparison of individual samples. For the current work, µSATS were assayed in multiplexes, where the coamplification of two or more loci was performed in a single reaction.

This study followed protocol for microsatellite DNA amplification and scoring as described by Tringali et al. (2011). Briefly, isolation of genomic DNA from fin-clip tissues took place at the FBCC using the PUREGENE DNA Purification Kit (Gentra Systems, Inc., Minneapolis, Minnesota) in accordance with the manufacturer's directions. Amplification and scoring took place at the FWRI, St. Petersburg, Florida. Using a reaction profile of 94C for 2 min, 35 cycles at (94C for 30 s, 58C for 30 s, 72C for 30 s) and 72C for 7 min, microsatellite loci were assayed in 25-µL PCR reactions seeded with 50-100 ng
of genomic DNA. Ten loci were intended for use in genetic screening (Msa-05, Msa-06, Msa-10, Msa-17, Msa-22, Msa-24, Msa-27, Msa-28, Msa-29 and Msa-32) (Seyoum et al., 2013). Loci were arranged in four multiplex PCRs (MP1, MP2, MP3 and, MP4; Table 3.1) as described by (Tringali et al., 2010).

Fragments were sized using GeneScan-500 ROX size standard and visualized on an ABI 3130 genetic analyzer (Applied Biosystems, Inc., Grand Island, New York). Raw genotype data was evaluated and processed with GENEMAPPER software v3.7 (Applied Biosystems, Inc., Grand Island, New York).

Table 3.1. Multiplex, microsatellite loci, primer sequences, fluorescent labels (NED=black, HEX=green, FAM=blue) and Genebank Accession numbers for parentage assignment of *M. floridanus*.

<table>
<thead>
<tr>
<th>Multiplex</th>
<th>Locus</th>
<th>Primer Sequence (5’&gt;3’)</th>
<th>Label</th>
<th>Genbank Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP1</td>
<td>Msa-06</td>
<td>F:GACAGTGCAACCAGGGCAAG</td>
<td>NED</td>
<td>EU180168</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:ATCTCGAGGAGATTCTAGAGGATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Msa-29</td>
<td>F:CGTTCTCTGAAAATGTTTCACTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:ATACAATTTTCTCATATTGCTCTTGTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP2</td>
<td>Msa-05</td>
<td>F:CGTCACCTCAGCCTCTGATT</td>
<td>HEX</td>
<td>EU180167</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:TCAGCAGCAACCAAAACAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Msa-17</td>
<td>F:AGGTGGAGGAGAGCGTAGAGCA</td>
<td>NED</td>
<td>EU180175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:ACGATGAGCCTGTTGGAGCTGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Msa-24</td>
<td>F:CAGGCCTTCCCCCATCTTCCC</td>
<td>FAM</td>
<td>EU180163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:TTGGGACGGGGAGAGGAGAGTAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP3</td>
<td>Msa-10</td>
<td>F:ATCCCTCTCCTCCTCTCCTAT</td>
<td>FAM</td>
<td>EU180171</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R:AAACTGTTTGAATATACTGTTCAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Msa-22</td>
<td>F:CCGAGCAGGAGGAGAGGAGGCAAG</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>R:ACTTTATGCTGAAAGACGAGTAC</td>
<td>HEX</td>
<td>EU180177</td>
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<tr>
<td>MP4</td>
<td>Msa-27</td>
<td>F:CTTCAGTTTAGAGGTTGAG</td>
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<tr>
<td></td>
<td></td>
<td>R:ATGCAGCCTAAAATGATCCAC</td>
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<tr>
<td></td>
<td>Msa-28</td>
<td>F:CTTTATGTTTCTGTTTTTAGCATA</td>
<td>FAM</td>
<td>GU085831</td>
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<td></td>
<td>R:CTTTGTCAGCCTCCTGACTCTCCT</td>
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<tr>
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<td>Msa-32</td>
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<tr>
<td></td>
<td></td>
<td>R:AGGTCACTGACTGACTTGCAC</td>
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</table>

**Parentage assignment.** A number of statistical methods and software packages are available for use in molecular maker based parentage assignment.
(A.G. Jones & Ardren, 2003). For microsatellite parentage assignment, the potential for genotyping error, null alleles and mutations often necessitates the use of statistical approaches to resolve unassignable offspring. This study used exclusion and categorical likelihood approaches to assign parents to offspring with 95% confidence. The exclusion approach relies on incompatibilities between parents and offspring to eliminate all but one parent pair from a complete sample of all possible parents for each offspring within a population. Where complete exclusion of specific parent-offspring hypothesis is not possible, categorical allocation can assign progeny to non-excluded parents based on likelihood. This method selects the most likely parental pair from a pool of non-excluded parents, allocating for some degree of transmission error (A.G. Jones & Ardren, 2003).

CERVUS v3.0 (Field Genetics Ltd., London, UK) software was used to perform both exclusion and categorical parentage assignments (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 2002). To perform complete exclusion assignments, CERVUS v.3.0 compared all possible combinations of known parent genotypes to the genotypes of offspring. Where single parent-pair matches occurred, they were deemed the true parents. In cases where true parents could not be established due to mismatched genotypes or multiple parent-pair matches, CERVUS v3.0 performed categorical parentage assignment. To do so, the software uses the likelihood-based approach to statistically distinguish non-excluded candidate parents by capturing two sources of information: 1) frequency of offspring alleles or alleles possible from candidate parents and 2) whether candidate parent is heterozygote or homozygous. Allele
frequencies, number of candidate parents, proportion of candidate parents sampled, completeness of genetic typing and estimated frequency of typing error are used to run multiple simulations of parentage assignment and confidence of assignment. Analysis is carried out with both simulated and real genotypes. Possible typing errors are taken into account when developing likelihood ratios with overall ratios calculated by multiplying likelihoods at each locus. Overall likelihood ratios are expressed as LOD scores (natural log of the overall likelihood ratio) with high positive LOD scores being the most likely candidate based on available information (procedure detailed by Kalinowski, Taper, & Marshall, 2007). The software compares the distribution of LOD scores for tests in which the most likely parent is the true parent with scores for tests in which the most likely candidate is not the true parent. Confidence of assignment is defined as the proportion of all candidate parents with LOD scores exceeding a given score that is the true parent. Any candidate parent with a LOD score exceeding this value is assigned parentage with 95% confidence (Field Genetics Ltd., 2012; Marshall et al., 2002).

When the categorical approach is used in microsatellite parentage analysis, it is important to quantify the efficiency of marker assignment with the probability to exclude random parents or parent pairs unrelated to offspring. In Cervus v3.0, this is referred to as the average non-exclusion probability ($P$) and is calculated for parent pairs across $n$ independently inherited loci ($l$) in the following manner:
If candidate parents are not typed for all loci, the actual probability of an unrelated candidate parent or candidate parent-pair matching the known offspring by chance may be higher than the individual non-exclusion probability calculated. For this study, a high number of genotyping errors (>50% of samples) were observed for Msa-29. This led to the exclusion of the loci as a marker for the study. The subsequent reduction in assignment power led to an inability to perform complete exclusion resulting from multiple parent-pair matches for 33 offspring. In these cases, an attempt was made to assign offspring with multiple parent matches to a single pair using principles of exclusion under the following assumptions: 1) raceway designations for parents was correct and 2) the control group represents all possible contributing parents. With these assumptions in hand, any parent pair containing individuals from different raceways or an individual not accounted for in the control group was eliminated. In cases where this method left one remaining parent-pair, non-exclusion probability was considered. Parent-pairs meeting a 95% confidence in non-exclusion probability were assigned as the true parents.

**Statistical Analysis.** For all data, normality was assessed with a one-sample Kolmogorov-Smirnov test and homogeneity of variance was assessed using Levene’s test. When comparisons were made between highly unbalanced sample sizes, larger groups ($N \geq 50$) had outliers removed and were sub-sampled.
using a random number generator (Excel: Mac 2008®, Microsoft, Redmond, Washington). Where assumptions of normality and equal variance were not met, data was transformed using a Box-Cox power transformation. Comparisons of mean total length for offspring in the HGA group were made using a one-way analysis of variance (ANOVA). For the one-way ANOVA, statistical differences between pairs were evaluated using the Tukey-Kramer honestly significant difference (HSD) test. Student’s t-test was used to compare mean lengths and $K_{TL}$ of aggressive and non-aggressive offspring in the HGA group. All tests and transformations were carried out using R v3.0 (R Foundation for Statistical Computing, Vienna, Austria) based modules provided by Wessa (2013). Values are reported as means (± SE) and the level of significance (α) used for all tests was 0.05.

Results

Of the 500 project samples, 390 (BGA $N=165$, HGA $N=225$) (Table 3.2) were assigned to a single parent pair with confidence ($p\geq0.05$). In spite of the high number of genotype errors, some offspring were assignable using elimination and statistical method. Offspring assigned with these methods displayed low parent pair non-exclusion probabilities (BGA=$1.19\times10^{-5}$, HGA=$8.47\times10^{-5}$). Twelve BGA offspring retuned multiple matches. Of these, six assignments were made using the described methodology related to dealing with multiple matches. The remaining six multiple matches remained unassigned for
the sample group. Categorical allocation via Cervus v3.0 was able to assign mismatch candidate pairs for three BGA offspring. These pairs had one mismatch each at either Msa-05 (N=2) or Msa-17 (N=1). The remaining 85 BGA mismatches (≥ 1 loci) were not included in final assignments. Twenty multiple match parings were observed for HGA offspring. Fourteen of these could be assigned to individual parent pairs using elimination. Cervus v3.0 software was unable to distinguish with confidence unique brood stock pairs for any of the remaining mismatches. Of the 19 unassigned mismatches within the HGA group, 17 were a result of genotype error (≥ 1 loci). Two pairings were deemed mismatches as a result of inappropriate raceway pairings (R-3 with R-4).

Grading captured 726 fish (2.1% of BGA population) to account for the HGA group. The total length (mean±SE; N=250) of HGA sample fish was 114.9±0.56mm, 28% greater than that of the BGA group (83.1±0.63). A much larger distinction was observed between groups according to mean weight with HGA fish (19.77±0.43g) being 263% heavier than BGA offspring (7.51±0.22). Though the groups contrast according to standard physical measurements, Student’s t-test showed no significant difference (p≤0.05) in condition (K) [BGA=1.25±0.01 (mean±SE) (N=250); HGA= 1.26±0.01 (N=250); P=0.33].

Stomach contents revealed one aggressive fish (<0.01% of offspring) in the BGA group and 29 aggressive fish (12%) in the HGA group. The 165 assigned offspring of the BGA group represent 44 unique parents involved in 31 pairings. Fewer broodstock contributed to the 225 assigned HGA offspring with 25 unique
parents involved in 14 pairings. A comparison of physical variables and parent assignments for BGA and HGA offspring is presented in Table 3.2.

Table 3.2. Comparison of physical variables and parent assignments for baseline growth and aggression (BGA) and high growth and aggression (HGA) offspring presented as mean (±SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>BGA</th>
<th>HGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Total length (mm)</td>
<td>250</td>
<td>83.0</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>250</td>
<td>7.51</td>
</tr>
<tr>
<td>Fulton's condition factor ($K_{TL}$)</td>
<td>250</td>
<td>1.25</td>
</tr>
<tr>
<td>Aggressive fish</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Assigned offspring</td>
<td>165</td>
<td>-</td>
</tr>
<tr>
<td>Unique parents</td>
<td>44</td>
<td>-</td>
</tr>
<tr>
<td>Parent pairs</td>
<td>31</td>
<td>-</td>
</tr>
</tbody>
</table>

BGA and HGA parent-pair contributions to offspring differed significantly between groups. The top three parent-pairs according to contribution rank were responsible for 48% of BGA offspring (P19/P39, $N=47$; P35/P11, $N=20$; P22/P25, $N=12$). The contribution activity of BGA parents contrasts markedly with that of the HGA parents. The top three HGA parent-pairs accounted for 90% of offspring within the group (P22/P25, $N=187$; P35/P11, $N=10$; P29/P45, $N=7$). Where P19/P39 is the top contributor to the BGA group, the pair is noticeably absent in the HGA offspring. Two BGA pairs which are represented in the HGA group, do so less authority (P35/P11, -8%; P29/P45, -2%). This is due to an overwhelming majority of HGA offspring resulting from the P22/P25 pairing, which saw a 75% increase in contribution from baseline, to account for 83% of the group. Parent-pair group contributions as rank, percent of group offspring.
and TL (mean±SE) are presented in Table 3.3, with percent of group offspring depicted graphically in Figure 3.1.

Table 3.3. Top three parent pair contributors presented according to rank, percent of group offspring (%), number of offspring and mean (±SE) total length (mm) for the baseline growth and aggression (BGA) and high growth and aggression groups (HGA).

<table>
<thead>
<tr>
<th>Parent-pair</th>
<th>BGA</th>
<th>HGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rank</td>
<td>%</td>
</tr>
<tr>
<td>P19/P39</td>
<td>1</td>
<td>28.5</td>
</tr>
<tr>
<td>P35/P11</td>
<td>2</td>
<td>12.1</td>
</tr>
<tr>
<td>P22/P25</td>
<td>3</td>
<td>7.3</td>
</tr>
<tr>
<td>P/29/P45</td>
<td>4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Figure 3.1 Pie chart showing percent parent-pair contributions to high growth and aggression (HGA) (N=14) and baseline growth and aggression (BGA) (N=31) offspring. Combined group top three rankings are shown in color (P19/P39=green, P35/P11=yellow, P22/P25=blue, P29/P45=red). Remaining ranks are shown in grey scale.

A pair-wise comparison of mean (±SE) TL for the top four parent-pair contributors and remaining assigned BGA (ReBGA; N=77) offspring was performed to identify disproportionate parent-pair contributions to length classes within the BGA group. To meet the ANOVA assumption of normality, total length data of BGA offspring required transformation (λ= -1.13). Additionally, as the ReBGA group consisted of a relatively high number of samples when compared
Figure 3.2. Length frequency histogram of contribution by parent-pairs in 3mm classes for baseline growth and aggression (BGA) \((N=165)\) and high growth and aggression (HGA) offspring \((N=225)\). Combined group top three rankings are shown in color (P19/P39=green, P35/P11=yellow, P22/P25=blue, P29/P45=red). Remaining ranks are shown in grey scale to other offspring groups, difficulty was encountered in meeting the assumption of equal variance with Levene’s test post-transformation. To remedy this, outliers were removed from the ReBGA group followed by sub-sampling \((N=50)\). A one-way ANOVA showed a significant difference between the transformed mean (±SE) TLs of the top four parent-pair contributors and remaining assigned offspring \((p<0.001; F\text{ ratio}=7.97)\). A Tukey-Kramer HSD pair-wise comparison identified the P22/P25 offspring as having a mean significantly different from all
other groups. P22/P25 offspring had a higher mean TL (97.3±1.02 mm) than other groups, suggesting a general contribution of larger fish, displaying faster growth than expected for the BGA group. The mean TL’s of P35/P11, P29/P45, P19/P39 and ReBGA did not differ significantly, implying similar growth patterns. Comparison of mean TL for the top four parent-pair contributors and the remainder of the BGA group are graphically illustrated by means of notched box plots in Figure 3.3. Results of Tukey-Kramer pair-wise comparisons are shown in Table 3.4 and depicted as 95% family-wise confidence levels in Figure 3.4.

![Figure 3.3. Notched box plots showing distributions of total length (mm) of top four parent-pair contributors and remaining baseline growth and aggression(BGA) group offspring prior to subsampling and transformation. Notch indicates median, box shoulders are the 25th and 75th percentiles and bars represent the 10th and 90th percentiles; circles are outliers.](image)

Thirty cannibalistic fish were identified overall (BGA, N=1; HGA N=29) with 27 of these being assigned to a single parent pair. At a near 30:1 ratio, the HGA group contained a markedly higher number of aggressive fish than did the BGA group. Of aggressive fish in the HGA group, parent pair contribution was observed by P22/P25 (N=21), P23/P32 (N=3), and P35/P11 (N=2). Both P22/P25 and P35/P11 are represented as top three contributors to all assigned
**Figure 3.4** Family-wise 95% confidence intervals for differences in mean transformed ($\lambda=-1.13$) levels of length of top four parent-pair contributors and remaining baseline growth and aggression (Re-BGA) offspring. If confidence interval for mean level of length does not include 0, statistical significance is implied.

**Table 3.4.** Results of Tukey-Kramer HSD pair-wise comparison of mean (±SE) transformed ($\lambda=-1.13$) total lengths of top four parent-pair contributors and remaining baseline growth and aggression (BGA) offspring. Remaining BGA represents a subsample ($N=50$) of remaining offspring. Significant values ($p\leq0.05$) are in boldface type.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean difference</th>
<th>Lower bound</th>
<th>Upper bound</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P22/P25-P19/P39</td>
<td>-1.336</td>
<td>-2.009</td>
<td>-0.664</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>P29/P45-P19/P39</td>
<td>-0.137</td>
<td>-0.893</td>
<td>0.62</td>
<td>0.987</td>
</tr>
<tr>
<td>P35/P11-P19/P39</td>
<td>-0.217</td>
<td>-0.772</td>
<td>0.338</td>
<td>0.817</td>
</tr>
<tr>
<td>Re-BGA-P19/P39</td>
<td>-0.087</td>
<td>-0.509</td>
<td>0.336</td>
<td>0.979</td>
</tr>
<tr>
<td>P29/P45-P22/P25</td>
<td>1.199</td>
<td>0.282</td>
<td>2.116</td>
<td>$0.004$</td>
</tr>
<tr>
<td>P35/P11-P22/P25</td>
<td>1.119</td>
<td>0.36</td>
<td>1.879</td>
<td>$0.001$</td>
</tr>
<tr>
<td>Re-BGA-P22/P25</td>
<td>1.249</td>
<td>0.581</td>
<td>1.918</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>P35/P11-P29/P45</td>
<td>-0.08</td>
<td>-0.915</td>
<td>0.755</td>
<td>0.999</td>
</tr>
<tr>
<td>Re-BGA-P29/P45</td>
<td>0.05</td>
<td>-0.703</td>
<td>0.803</td>
<td>1</td>
</tr>
<tr>
<td>Re-BGA-P35/P11</td>
<td>0.13</td>
<td>-0.42</td>
<td>0.68</td>
<td>0.966</td>
</tr>
</tbody>
</table>

HGA offspring. P23/P32 ranked 4th ($N=6$) in contribution to the group as a whole.

The single contribution to aggressive fish in the BGA group was by P17/P37. It should be noted that this pairing was absent as a contributor to any assigned HGA offspring. Other than a simple comparison of group observances, the single
aggressive fish found in BGA offspring yielded low statistical power and did not offer opportunity for further analysis.

Within the HGA group, a \( t \)-test showed significant difference (\( p \leq 0.05 \)) between the TL (mean±SE) of aggressive fish and non-aggressive. With a mean TL of 128.2±1.48 mm, aggressive fish were larger than their non-aggressive counterparts (113.1±0.49; \( P \leq 0.001 \)). Similar results were observed in a comparison of \( K_{TL} \) for HGA offspring as distinguished by level of aggression. The \( K_{TL} \) (mean±SE) value for aggressive fish (1.46±0.024) was significantly higher than that of non-aggressive offspring (1.24±0.006; \( P \leq 0.001 \)). Results of \( t \)-tests comparing mean total length’s and condition factors for HGA aggressive and non-aggressive fish are shown in Table 3.6 and illustrated graphically by means of notched box plots in Figures 3.5 and 3.6.

Table 3.5. Results of \( t \)-test (\( p \leq 0.05 \)) comparing mean (±SE) total lengths (mm) and Fulton’s condition factor (\( K_{TL} \)) of aggressive (\( N = 29 \)) and non-aggressive (\( N = 221 \)) fish in the baseline growth and aggression (BGA) group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aggressive</th>
<th></th>
<th>Non-aggressive</th>
<th></th>
<th>Test Statistic</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Length (mm)</td>
<td>29</td>
<td>128.2±1.48</td>
<td>221</td>
<td>113.1±0.49</td>
<td>-9.71</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Condition factor (( K_{TL} ))</td>
<td>29</td>
<td>1.46±0.024</td>
<td>221</td>
<td>1.24±0.001</td>
<td>-9.04</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>
Figure 3.5. Notched box plots showing distributions of mean (±SE) total lengths (mm) for aggressive and non-aggressive fish of the HGA group. Notch indicates median, box shoulders are the 25th and 75th percentiles and bars represent the 10th and 90th percentiles; circles are outliers.

Figure 3.6. Notched box plots showing distributions of mean (±SE) Fulton’s condition factor ($K_{TL}$) for aggressive and non-aggressive fish of the HGA group. Notch indicates median, box shoulders are the 25th and 75th percentiles and bars represent the 10th and 90th percentiles; circles are outliers.
Discussion

The number of unassigned progeny was unexpected and a cause for investigation. An effort was made to identify potential causes for mismatch loci. This centered on three scenarios: 1) method errors in deriving offspring genotypes 2) method errors in deriving parent genotypes and 3) unaccounted for parents in spawning raceways. Towards the potential for method errors in deriving offspring genotypes, the 110 unassigned progeny were re-genotyped. New genotypes were then compared to original parent and offspring genotypes to identify discrepancies. These were noticed in 17 scores and led to the assignment of three offspring to known parents. While these assignments are evidence for methodological error within the study, their relative occurrence is low and within the expectations for such error when using microsatellite markers (<2%) (Selkoe & Toonen, 2006). As such, the scenario of widespread mismatches being the result of errors in the genotyping of offspring was eliminated.

Towards potential method errors in deriving the genotype of parents, allele scores generated in study analysis were compared to initial brood stock scores in GENEMAPPER v3.7 to assure accuracy of data input. Then, mismatch parent-pair scores and size standards were sub-sampled, re-genotyped and reviewed for initial scoring error. Of the 13 parents sub-sampled, one showed a change in score from its original genotype. Though this change was significant as it
occurred at three loci, a subsequent evaluation with CERVUS v3.0 yielded only four additional offspring assignments.

Finally, towards the scenario of unaccounted for parents, categorical assignment was performed with CERVUS v3.0 for unassigned study offspring against all SJR brood-stock used in production during the previous six years. This yielded an additional 18 potential parents according to nine loci. The large number of potential parents indicated that the scenario of unaccounted for parents could be possible though further refinement was required. To achieve this, COLONY v2.0.4.7 (O. R. Jones & Wang, 2010) was used to simulate genotype data from the known parent/offspring structure of this study. The program assumes a sample of individual genotypes taken from a large, randomly mating population. This sample is split into three subsamples: the offspring sample, candidate father sample and candidate mother sample. COLONY’s algorithm uses Mendelian rules to partition individuals into a number of clusters based on the likelihood of familial relationships. Familial clusters of highest likelihood are retained and used to reach solutions of full- and half-sibling relationships, maternal and paternal parentage assignments, and inferred genotypes for unknown individuals.

COLONY v2.0.4.7 was able to assign all study offspring by inferring six unknown parents in the original spawning population. CERVUS v3.0 was used to conduct an identity analysis to determine whether the six inferred genotypes matched any genotypes in the database of all FBCC brood fish. Results showed that four of the inferred genotypes matched previously genotyped brood fish not
meant to be in the October 2012 spawning group. While two of these matches are listed in brood stock records as existing, the remaining two are listed as deceased.

CERVUS v3.0 calculated the probabilities that an unrelated individual would have one of the four genotypes and the probabilities that siblings would share one of the four genotypes. Those values ranged from $2.01 \times 10^{-14}$ to $1.4 \times 10^{-9}$ and from $9.27 \times 10^{-6}$ to $1.30 \times 10^{-3}$, respectively. These low probabilities suggest the simulated genotypes are unlikely to be duplicated within either brood stock or wild populations and likely represent the true parents. Regardless of their current status as brood stock, the four inferred parents theoretically contributed to 86 originally unassigned offspring. The remaining two inferred parents are of genotypes not consistent with known FBCC brood stock and accounted for ten additional offspring matches. The high number of matches resulting from the scenario of unaccounted for parents suggests this as the most likely solution to the question of offspring mismatches. At the time of this writing, investigations as to the cause of this scenario are on going.

Notwithstanding the high number of unassignable offspring, this study is able to make statements concerning the relatedness of individual *M. floridanus* juveniles displaying variable traits of growth and aggression. Specifically, the contrast in parent-pair contributions to the BGA and HGA groups supports the hypothesis that certain pairings do contribute disproportionally to certain size classes. In the case of HGA offspring, the overwhelming majority of juveniles (83%) resulted from the P22/P25 pairing. By comparing this to the contribution of
P22/P25 in the BGA group (7%), it can be assumed, that with the confines of the current study, this particular pairing is predisposed to generating comparably faster growing progeny. In a similar fashion, comparing the contribution of the P19/P39 paring to both BGA (28%) and HGA (0%) groups, suggests this pairing retains some bias against the production of faster growing offspring. These statements are further bolstered by results of pair-wise comparisons of parent-pair contributors within the BGA group where P22/P25 offspring had a mean TL significantly higher than all other pairings.

Considering parentage assignments for BGA and HGA groups, the difference in TL between groups (28%) is similar to that found by Shengjie et al. (2009) and suggests growth as a heritable trait with populations of *Micropterus* spp. As discussed by Arlinghaus & Mehner (2003), recreational anglers often select individuals according to growth traits. Where mortalities are elevated due to the direct effect of this form of unnatural selection, indirect effects can result in fisheries-induced evolution of fish populations. Though examples of this are infrequent, existing work does show that recreational angling can alter the potential growth of wild fish stocks (Neala W Kendall & Quinn, 2011; Lewin et al., 2006; Nuhfer & Alexander, 1994). These examples are further supported by investigations of the effect of size specific harvest regulations on fish populations. Conover & Munch (2002) subjected exploited populations of Atlantic silversides (*Menidia menidia*) to variable levels of size-selective harvest over the course of four generations. The authors found that removal of the largest individuals resulted in selection for individuals exhibiting slow growth. The conclusion being
that harvest regulations can directly affect the display of phenotypes by a 
population. For the Florida bass, if growth is indeed heritable, decades of 
unregulated recreational angling may have caused a shift in the trait’s frequency 
of display within wild stocks.

With a majority of cannibalistic offspring assigned to the HGA group, one 
can expect the larger fish of this particular population to be the most aggressive. 
It can also be assumed that familial relationships predict the display of 
aggression as the greatest share of these fish resulted from the P22/P25 pairing. 
These findings are consistent with previous studies of the heritability of 
aggression within populations of largemouth bass (Burkett et al., 1986; Garrett, 
2002; Philipp et al., 2009). Though in the case of Garrett (2002), where results 
showed that differences in observed growth rates might be attributed to 
differences in catchability, this study suggests an inverse relationship. Overall, 
cannibalistic fish contributed to 12% of the HGA group, deeming the bulk of this 
group as non-aggressive. The chance of finding an aggressive fish is higher 
within the HGA group, but as growth is the dominant trait, it cannot be assumed 
that aggression drives growth. Hence, this study would state that differences in 
catchability might be attributed to differences in observed growth rates. This is a 
significant statement in light of unnatural selection. Where commercial fishing 
often directly selects individuals according to size, as dictated by gear type of the 
fisherman (Law, 2000; Mansueti, 1961), recreational fishing often directly selects 
according to catchability, as dictated by the behavior of the fish (Philipp et al., 
2009; Sutter et al., 2012; Uusi-Heikkila, Wolter, Klefoth, & Arlinghaus, 2008).
Garret (2002) suggests that removal of aggressive fish from a population may lead to a related decrease in the display of high growth rates. The current work suggests that the removal of aggressive fish may only yield a less aggressive population, one that still retains most of its growth associated traits.

This is not to say that the removal of the most aggressive fish from a population would not correspond to a negative affect on overall fitness. In this study, aggressive fish showed relatively higher level of fitness ($K_{TL}$) when compared to the remainder of their cohort, including those of the BGA group. For *M. salmoides*, the fitness of juveniles has been shown to directly impact first year recruitment and overwinter success (Ludsin & DeVries, 1997). High aggression may also affect the potential of an individual to contribute offspring to future generations. In selection experiments of vulnerability to angling, aggressive lineages of largemouth bass have consistently been shown as providing more intense and vigilant parental care than their less aggressive counterparts (Cooke et al., 2007; Sutter et al., 2012). In these instances, more aggressive parents provide better opportunity for offspring survival. With this in mind, results of the current investigation suggest that where the frequency in displays of aggression within a Florida bass population are decreased due to unnatural selection, one can expect a similar reduction in the recruitment of age-0 juveniles.

Though this study does provide evidence for the heritability of physiological and behavioral traits within Florida bass populations, the results do have certain limitations in application to the topic of unnatural selection. The current work relied on a single population of fish spawned over the course of
three days. This small snapshot of familial relationships in fish produced at the FBCC requires replication. Additionally, the display of traits in juveniles is not necessarily indicative of the display of traits in adults. Redpath, Cooke, Arlinghaus, Wahl, & Philipp (2009) contradict the results of this study in an investigation of aggression and related growth in pond reared *M. salmoides*. The authors found that at age-1, aggressive individuals achieved lower absolute growth than their less aggressive counterparts. In this case, it was assumed that the metabolic requirements of highly aggressive fish limit resource partitioning to somatic growth. When this is taken into consideration with the current study, it can be theorized that the correlation between aggression and growth in juvenile Florida bass may not be predictive of a similar relationship in adults. A long-term investigation of these life-history traits is necessary to fully understanding the effects of unnatural selection upon the species.

**Conclusion**

This study has shown evidence for the heritability of trophy related traits with populations of Florida largemouth bass. Though these results provide evidence for the effects of angler-induced evolution on wild stocks of Florida bass, the application of this knowledge in management and policy is not straightforward. The paradox of recreational fisheries management will require managers to walk a fine line in applying these findings to black bass in Florida. The traditional mode of supplemental stocking to augment a depleted population
may not be the most effective tool in restoring the trophy status of a bass population. An attempt to directly select for trophy traits in a hatchery setting may lead to an increase in the frequency of less desirable alleles. With this in mind, fisheries managers will likely need to implement strategies aimed at preserving genetic integrity through catch regulations. As shown by Conover and Munch (2002), size related harvest regulations can directly affect the display of traits by a population. As the FWC moves forward with policy aimed at preserving the genetic integrity of black bass, it will be necessary to address the influence of anglers on fish populations. With examples of the effects of unnatural selection specific to recreational fisheries, managers in Florida are in a position to protect the trophy status of the state’s native black bass.
Chapter 4:
General Conclusions

Molecular markers provide conservation biologists with an opportunity to decode the mechanisms of unnatural selection (Allendorf, Hohenlohe, & Luikart, 2010). As fisheries biologists move to develop management strategies based on genetic integrity, they will increasingly be presented with a broad range of analysis molecular technologies (Hauser & Seeb, 2008). The purpose of this thesis was to explore the range of molecular technologies in use by state natural resource agencies and draw conclusions from their application to freshwater fisheries management. In particular, the science and policy of a well-established genetic conservation program for the Florida bass (Micropterus floridanus) provides the opportunity to understand the influence of angler-induced selection on recreational fisheries.

In Chapter 2, an online survey was used to investigate the breadth of molecular marker application to freshwater fisheries management by state natural resource agencies. It was shown that state natural resource agencies do indeed incorporate a diverse array of freshwater fish species and molecular technologies into their management strategies. When the results of this survey were discussed in the context of the Florida Fish and Wildlife Conservation
Commission’s strategy to preserve the genetic integrity of the Florida bass, certain themes became clear towards developing a successful fisheries management program bases on molecular technologies; (1) the cost/benefit of developing genetic capabilities (2) the limitations of specific genetic markers, and (3) the relevance of markers to questions of management. Where these issues have been taken into consideration, as by the FWC, resulting policy is highly effective at attaining the goals of biologists, administrators and stakeholders alike.

Chapter 3 took an in-depth approach to investigating the role of molecular markers in managing for unnatural selection in the form of a study of trait heritability. Here, microsatellite parentage analysis was used to reconstruct familial relationships of juvenile Florida bass displaying variable traits of growth and aggressiveness in a culture setting. Differences in the parentage of high growth and aggression (HGA) and baseline growth and aggression (BGA) offspring showed that certain parent-pairings do contribute disproportionally to certain size classes and levels of aggression. These findings are consistent with previous studies of the heritability and aggression within populations of largemouth bass (Burkett, Mankin, Lewis, Childers, & Philipp, 1986; Garrett, 2002; Philipp et al., 2009). Results did contrast the current view that aggression drives growth (Garrett, 2002). Aggressive fish contributed to only a small percentage of the HGA group, deeming the bulk of this group as non-aggressive. The chance of finding an aggressive fish was higher within the HGA group, but as growth was the dominant trait, it cannot be assumed that aggression drives
growth. Hence, this study would state that differences in aggression might be attributed to differences in observed growth rates. This suggests that the selection of aggressive fish (as done in recreational fishing) may only yield a less aggressive population, one that still retains most of its growth associated traits. Though these findings do shed light on the heritability of trophy traits within populations of *M. salmoides*, the results do have certain limitations and require further investigation.

Towards this requirement, the FWC has committed to two studies based on this thesis. This first is designed to replicate results within additional populations of age-0 Florida bass. It is planned for three populations of production fish from the 2014 year class to be assessed in a fashion similar to the methods described in Chapter 3. Results of this work will be used to assess the need for future long-term study of the topic. Additionally, the populations from which samples were derived for this thesis have been incorporated into a head to head study of the sustainability of growth and aggression as traits through age-1. This is being done in six experimental ponds at the Florida Bass Conservation Center with results to be discussed in a doctorial dissertation. It is hoped that these additional studies provide fisheries managers with more information concerning the effects of unnatural selection on Florida bass.
References


Henshall, J.A. (1881). *Book of the black bass-comprising its complete scientific and life history together with a practical treatise on angling and fly fishing and a full description of tools, tackle and implements*. Cincinnati, OH: Robert Clarke & Company.


