Life history, growth, and genetic diversity of the spotted gar *Lepisosteus oculatus* from peripheral and core populations

by

Solomon R. David

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Doctoral Committee:

      Professor Michael J. Wiley, Chair
      Professor James S. Diana
      Professor Barry M. OConnor
      Adjunct Associate Research Scientist Edward S. Rutherford

Spotted Gar illustration by Solomon R. David, age 12

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To my parents and sisters

Ignatius and Esther, Rachel and Sarah

Without their love and support I would not have made it this far

To my grandparents

Rangappa and Wazirbai Yesudas, Jambiah and Kanthamma David

For inspiring me to learn more about the natural world around us

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Abstract

I studied the ecology and biogeography of the spotted gar (*Lepisosteus oculatus*) from core and Great Lakes Region peripheral populations. Peripheral populations occupy the edge of a species' range and are considered to be important in terms of a species' ecology, biogeography, evolution, and conservation. Peripheral populations often persist under different environmental conditions from the species' core populations, and may exhibit adaptations to potentially "harsher" marginal environments. In this study I used common garden experiments, life history analyses, and phylogeography (based on mitochondrial DNA) to address the overall hypothesis that spotted gars from peripheral, Great Lakes Basin populations exhibit distinct life history characteristics and patterns of genetic diversity in comparison to spotted gars from core populations.

In common garden laboratory experiments young-of-year spotted gars from peripheral populations exhibited significantly faster growth rates (0.09 cm/day, 0.26 g/day) than core populations (0.04 cm/day, 0.11 g/day, suggesting countergradient variation in growth. Life history analysis based on length-at-age data from 5 field populations (2 peripheral, 3 core) and incorporating thermal opportunity for growth (degree days above 18 °C) indicated significantly higher growth rate in spotted gars fromperipheral (1.23 mm/degree day) compared to core populations (0.22 mm/degree day). Catch-curve analyses of the same populations indicated annual mortality rate (A) was lower in peripheral ($A = 0.41$) compared to core populations (0.56). Analysis of mitochondrial DNA from core and peripheral populations indicated genetic diversity

(haplotype diversity, H) was highest in the Mississippi River Basin ($H = 0.80$), lowest in the Great Lakes Basin ($H = 0.00$, single haplotype), and most divergent in the western Gulf Coast Basin ($H = 0.70$, no haplotypes shared with other basins). Overall, the Great Lakes Basin population was shown to be a unique component of the species, and is adapted to life at higher latitudes with shorter growing seasons. As a useful case study, my work can inform gar conservation strategies and lead to a better general understanding of the evolution and maintenance of vertebrate life history patterns and genetic diversity.

Chapter 1

Introduction

Overview

The loss of biodiversity is a global crisis threatening all major habitats at multiple

geographical and ecological scales (Convention on Biological Diversity 2008). Loss of

even local species populations can have cascading effects, influencing entire ecosystems

and disrupting important ecosystem services (Garner et al. 2005, Hooper et al. 2005,

Helfman 2007). Furthermore, the relationship between biodiversity and ecosystem

services is mainly a function of the size of local populations, not just overall existence of

species themselves (Luck et al. 2003). Therefore conserving distinct local populations

(population diversity, Luck et al. 2003) is an essential part of conservation of

biodiversity.

Peripheral or "fringe" populations occupy the edge of a species' range

(ecologically, geographically, or both) and are considered to be exceptionally important

in terms of a species' ecology, biogeography, evolution, and conservation (Scudder 1989,

Lesica and Allendorf 1995, Latta 2003). Peripheral populations often persist under

different environmental conditions from the species' central or "core" populations, and

therefore may exhibit different genetic and morphological adaptations to potentially

"harsher" environments (Yakimowski and Eckert 2007). Due to small size,

fragmentation, or complete disjunction, many peripheral populations may experience low

recolonization potential, and therefore may be more susceptible to environmental

perturbations as well as extinction (Lesica and Allendorf 1995, Channell et al. 2000, Wisely et al. 2004). Peripheral populations also often experience very low gene flow and high degrees of genetic drift, leading to further divergence from core populations (Jones et al. 2001, Lammi et al. 2001, Johannesson and Andre 2006).

Because of differing environmental conditions related to geographical factors such as latitude, populations may also exhibit different reaction norms (Yamahira et al. 2007) which in turn affect various life history characteristics such as size and age at maturity, growth rate, or fecundity (Power and McKinley 1997, Munch et al. 2003, Heibo et al. 2005, Slaughter et al. 2008). Such latitudinal variation in life history characteristics have been observed in a diversity of taxa including plants (Yakimowski and Eckert 2007), mammals (Kyle and Strobeck 2002), reptiles (Wilson and Cooke 2004), invertebrates (Lee et al. 1998, Lardies et al. 2004), and fishes (Kynard 1997, Yamahira and Conover 2002, Foster and Vincent 2004). Coupled with genetic drift and low gene flow, these latitudinal variations in life history characteristics may contribute to divergence between peripheral and core populations. For all these reasons it is believed that speciation is likely to often take place in peripheral populations, making them evolutionarily important (Lesica and Allendorf 1995). Conserving peripheral populations is therefore a unique and integral component of conserving global biodiversity (Lammi et al. 2001, Johannesson and Andre 2006).

Freshwater systems are believed to be experiencing declines in biodiversity at a rate even greater than we observe in most terrestrial systems (Dudgeon et al. 2006), yet freshwater conservation priorities lag further behind those of terrestrial systems (Brooks et al. 2006). Considered the "sumps" and "receivers" of industrial and domestic wastes

and other land-use effluents, freshwater systems are exceptionally vulnerable to anthropogenic influence, often resulting in habitat loss, species range reduction or fragmentation, and higher susceptibility to exotic species invasion (Allendorf 1988, Moyle and Williams 1990, Bruton 1995, Dudgeon et al. 2006). Conservation and proper management of biodiversity in freshwater ecosystems must be a priority in order to maintain ecosystem services to humans, proper ecosystem function, and evolutionary potential (Helfman 2007).

Among the diversity of taxa inhabiting freshwater systems, fishes are the most familiar and can also serve as effective indicators of ecosystem health (Helfman 2007, United States Environmental Protection Agency 2007). In terms of biodiversity loss, approximately 3,600 of 10,250 known freshwater fish species (35%) are considered imperiled or threatened (Nelson 1994, Stiassny 1999), with approximately 95-170 species already extinct (Helfman 2007). Primary reasons for the extinction and imperilment of freshwater fishes are habitat alteration and exotic species invasions, with 95% of extinctions having occurred in the past 50 years (Harrison and Stiassny 1999). Previous studies have shown that in aquatic systems, species at higher trophic levels are at higher risk and are more frequently lost than those at lower trophic levels, in part because of their relatively small population sizes (Lande 1993, Petchey et al. 2004). Piscivorous fishes, therefore, may be particularly vulnerable amidst the ongoing biodiversity crisis. Furthermore, non-game piscivorous species (e.g. gars, Lepisosteidae; bowfin, *Amia calva*) may be even more at risk due to their poorly-studied ecology, perceived low economic value, and the higher priority given to propagation and management of game

species (centrarchids, percids, esocids); the latter often leading to the destruction of both non-game individuals and habitat (Scarnecchia 1992).

To further explore and better understand these issues, I studied the ecology and biogeography of the spotted gar (*Lepisosteus oculatus*) from core and peripheral populations. Although relatively common in the lower Mississippi River drainage and other areas of the southern United States, the spotted gar is poorly studied and its ecology and status are comparatively unknown in the Great Lakes basin. The spotted gar is a species of greatest conservation need (SGCN, Michigan Department of Natural Resources 2005) in the state of Michigan, and there have been no previous studies focusing on the species within the state. The spotted gar is a native top-level predator (primarily piscivorous), preferring clear vegetated waters, particularly wetlands and floodplain habitat of lakes and large rivers (Suttkus 1963, Trautman 1981, Page and Burr 1991). These characteristics suggest the species is an important component of native food webs, and may be threatened, or in some cases has completely disappeared, due to the degradation and loss of vegetated aquatic habitat in its range (Trautman 1981, Carman 2002). Because of its specific habitat preferences, the spotted gar may also serve as an environmental indicator of aquatic ecosystem health (USEPA 2007).

The Great Lakes population of spotted gars is also disjunct from the southern US population, with the species arriving in the Great Lakes region approximately 8,000 years ago (Bailey and Smith 1981, Hocutt and Wiley 1986, Hubbs et al. 2004). The Great Lakes population is geographically peripheral, and given the latitudinal distance from the southern US population, likely ecologically peripheral as well. The peripheral Great

Lakes population of spotted gars therefore provides the opportunity to compare intraspecific variation in life history, ecology, and biogeography between populations.

Gars (family Lepisosteidae) in general have a reputation amongst anglers for consuming game fishes and are generally considered "trash fish" (Netsch and Witt 1962, Goodyear 1967). When caught, these fishes are often killed (usually by breaking their backs) or severely damaged (by breaking their elongate snouts) and thrown back into the water (Scarnecchia 1992). A better understanding of gar ecology can inform more effective conservation plans, including public awareness, and will further benefit the species by increasing angler awareness of the ecosystem services of the species. For example, by consuming smaller individuals, gars can help prevent stunting of game fish populations, which contributes to larger individuals among game species (Becker 1983, Scarnecchia 1992).

Goals and methods

The overarching goal of this study was to better understand a very poorly-studied, much-maligned yet important native species at the edge of its range; and in doing so provide support for the development of effective strategies for conservation and management of ecologically sensitive peripheral populations central to the biodiversity of freshwater ecosystems. My dissertation research investigated variation in life history characteristics as well as factors influencing the genetic diversity and biogeography of spotted gars from the Great Lakes region and southern US populations. My overall research hypothesis is that spotted gars from the peripheral population segment exhibit different life history characteristics and genetic diversity than spotted gars from the core

population segment. Peripheral populations of species have a high adaptive significance to the overall species, and differences in life history characteristics and genetic diversity in peripheral populations may be indicative of adaptation to ecologically marginal environments (Soulé 1973, Scudder 1989, Lesica and Allendorf 1995). I addressed this hypothesis using three complementary studies. I used common garden laboratory experiments to compare growth rates of young-of-year fish between core and peripheral populations of spotted gars, and to determine whether potential variation in growth rate might be explained by countergradient variation theory (Conover et al. 2009; Chapter 2). I used field sampling, laboratory aging techniques, and meta-analysis to investigate potential differences in life history variables (e.g. mean age, mean length, length-at-age, mortality) among five populations of spotted gars from core and peripheral population segments, and to determine if variation in life history characteristics could be explained by environmental factors strongly influenced by different latitudes (Chapter 3). I used analysis of mitochondrial DNA (mtDNA) to determine differences in genetic diversity among spotted gar populations, and concepts from phylogeography (Avise et al. 1987) and historical biogeography to determine if population genetic structure would reflect geographic position of core and peripheral populations of spotted gars (Chapter 4). Finally, I synthesized the results of my research chapters and reiterated the importance of peripheral populations of species in the context of my findings; I also suggested directions for future study on lepisosteids (Chapter 5).

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Chapter 2

Countergradient variation in growth of the spotted gar *Lepisosteus oculatus* from core and peripheral populations

<u>Introduction</u>

The loss of biodiversity is a global crisis threatening all major habitats and ecological scales (Convention on Biological Diversity 2008). Loss of even local species populations can have cascading effects, influencing entire ecosystems and disrupting important ecosystem services (Garner et al. 2005, Hooper et al. 2005, Helfman 2007). Furthermore, the relationship between species and ecosystem services is mainly a function of the size of local populations, not just overall existence of species themselves (Luck et al. 2003). Therefore conserving distinct local populations (population diversity, Luck et al. 2003) is an essential part of the conservation of biodiversity.

Peripheral or "fringe" populations occupy the edge of a species' range and are considered to be exceptionally important in terms of a species' ecology, biogeography, evolution, and conservation (Scudder 1989, Lesica and Allendorf 1995, Latta 2003). Peripheral populations often persist under different environmental conditions from the species' central or "core" populations, and therefore may exhibit different genetic and phenotypic adaptations to potentially "harsher" environments (Yakimowski and Eckert 2007). Due to small size, fragmentation, or complete disjunction, many peripheral populations have low recolonization potential, and therefore may be more susceptible to environmental perturbations as well as extinction (Lesica and Allendorf 1995, Channell

et al. 2000, Wisely et al. 2004). Peripheral populations also often experience very low gene flow and high degrees of genetic drift, leading to further divergence from core populations (Jones et al. 2001, Lammi et al. 2001, Johannesson and Andre 2006).

Because of differing environmental conditions related to geographical factors such as latitude, populations may also exhibit different reaction norms which in turn affect various life history characteristics such as size and age at maturity, growth rate, or fecundity (Stearns and Koella 1986, Berrigan and Koella 1994, Power and McKinley 1997, Munch et al. 2003, Heibo et al. 2005, Slaughter et al. 2008). Such latitudinal variation in life history characteristics has been observed in many different taxa including plants (Yakimowski and Eckert 2007), mammals (Kyle and Strobeck 2002), reptiles (Wilson and Cooke 2004), invertebrates (Lee et al. 1998, Lardies et al. 2004), and fishes (Kynard 1997, Yamahira and Conover 2002, Foster and Vincent 2004). Coupled with genetic drift and low gene flow, these latitudinal variations in life history characteristics may contribute to evolutionary divergence between peripheral and core populations. For all these reasons it is believed that speciation is likely to often take place in peripheral populations, making them evolutionarily important (Lesica and Allendorf 1995). Conserving peripheral populations is therefore a unique and integral component of conserving global biodiversity (Lammi et al. 2001, Johannesson and Andre 2006).

The length of growing season, characterized by warmer temperatures, varies at different latitudes, contributing to differing growth rates among populations (Slaughter et al. 2004). Variation in growth rate or capacity for growth in a species due to differences in latitude may provide evidence for countergradient variation (CnGV, Conover 1990). Countergradient compensatory variation occurs when the average effects of genetic and

environmental influences oppose each other across an environmental gradient (Conover and Schultz 1995). Countergradient variation theory suggests that species populations at higher latitudes with shorter growing seasons have a higher capacity for growth than individuals from populations at lower latitudes (Conover and Present 1990, Yamahira and Conover 2002). Higher growth capacity at higher latitudes would contribute to increased overwinter survival and may result in relatively similar-sized individuals from high latitude populations and lower latitude populations at the end of the growing season (Hurst 2007, see Conover et al. 2009 for full review of CnGV).

Countergradient variation has been identified in a number of freshwater and marine fishes such as striped bass *Morone saxatilis*, mummichog *Fundulus heteroclitus*, American shad *Alosa sapidissima* (Conover 1990), lake sturgeon *Acipenser fulvescens* (Power and McKinley 1997), and Atlantic cod *Gadus morhua* (Marcil et al. 2006), but not all fishes exhibit this trait. Furthermore, tradeoffs with higher capacity for growth may occur in the form of reduced swimming ability and higher risk of predation (Billerbeck et al. 2001, Conover et al. 2005). Countergradient variation in growth may therefore result in both genetic and morphological differences between peripheral and core populations, further illustrating the conservation value of peripheral populations.

Although relatively common in the lower Mississippi River drainage and other areas of the southern United States, the spotted gar *Lepisosteus oculatus* is poorly studied and its ecology and status are comparatively unknown in the Great Lakes basin. The spotted gar is a species of greatest conservation need (Michigan Department of Natural Resources 2005) in the state of Michigan, and there have been no previous studies focusing on the species within the state. The spotted gar is a native top-level predator

(primarily piscivorous), preferring clear vegetated waters, particularly wetlands and floodplain habitat of lakes and large rivers (Suttkus 1963, Trautman 1981, Page and Burr 1991). The species is an important component of native food webs, and may be threatened, or in some cases has completely disappeared, due to the degradation and loss of habitat in its range (Trautman 1981, Carman 2002). Because of its specific habitat preferences, the spotted gar may also serve as an environmental indicator of aquatic ecosystem health (USEPA 2007).

The Great Lakes population of spotted gars represents the northern edge of the species range, and is also completely disjunct from the southern US population (Figure 2.1, Page and Burr 1991). The species dates back to the early Eocene (48-55 mya, Wiley 1976, Grande 2010) but arrived in the Great Lakes region relatively recently, approximately 8,000 years ago, when water temperatures began to rise following the Wisconsinan Glaciation (Bailey and Smith 1981, Hubbs et al. 2004). Spotted gars in the Great Lakes region are separated by a large latitudinal distance from the core population (approximately 1,231 km between population centers), and length of growing season is significantly shorter, approximately 111 days (Great Lakes region) compared to 229 days (southern US, NOAA National Climate Data Center 2011). Because of the large latitudinal distance and differences in length of growing season, variations in population life history characteristics such as growth rate may be evident. The Great Lakes population of spotted gars therefore provides a unique opportunity to investigate peripheral versus core population differences and adaptation.

Countergradient variation, or more generally, latitudinal variation, has not been studied in gars; the disjunct distribution and primitive ancestry of the spotted gar makes it

a unique model species for investigation of this phenomenon. To explore potential differences in core and peripheral gar populations in the context of countergradient variation theory, I compared growth rates for the first growing season between core and peripheral populations of the spotted gar. My primary objective was to investigate differences in life history patterns, specifically growth rate in the first growing season, between the Great Lakes (peripheral) and southern United States populations (core) of spotted gars using common garden experiments. My second objective was to determine whether any potential variation in growth rate might be explained by countergradient variation theory. I hypothesized that spotted gars from the peripheral population would exhibit a faster growth rate and higher capacity for growth at all temperatures than spotted gars from the core population. And further, I hypothesized that this variation in growth rate between populations is evidence of countergradient variation in growth of spotted gars.

Methods

Spotted gars were acquired from two major sources to represent the core and peripheral populations. Core population representatives were collected via colleagues at Nicholls State University (Thibodaux, LA) in late spring 2009 from several localities in southwestern Louisiana using experimental gill nets, and peripheral population representatives were acquired from several inland lakes in southern Michigan. Fish from Louisiana were the progeny of wild-caught individuals from 2 localities in the Barataria estuary system (Bayou Chevreuil and Golden Ranch) and 1 locality in the Terrebone estuary system (Chacahoula Swamp) collected in March-April 2009. Individuals from the core populations were intermixed in order to reduce potential genetic bias from a

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single locality, and the same was done for individuals from peripheral populations. Adult fish from all core populations were maintained together in an indoor tank, and spawning was induced at 21 °C using Ovaprim™ (Syndel Laboratories) injections at a concentration of 2.0 mL/kg body weight. Ovaprim™ was introduced via intramuscular injection near the anterior base of the dorsal fin, and spawning occurred within 24-48 hrs of injection. Viable embryos from this spawning event were then collected from the tank and approximately 150 specimens were shipped overnight to the University of Michigan.

Adult peripheral population representatives were collected in late spring (May) 2009 from five different inland lake localities in southern Michigan using a boom electrofishing boat. Marble and East Long lakes are part of the St. Joseph River watershed, and Round, Carpenter, and Sugarloaf lakes are part of the Grand River watershed. Adults from peripheral populations were maintained together in an indoor tank similar to that of core population fish. Spawning was similarly induced using Ovaprim™ but was not as successful, therefore several adult fish were stripped of milt and eggs to create embryos (approximately 200 specimens). Core population gars will be referred to as LA fish and peripheral population gars as MI fish from henceforth.

Embryos from both populations were raised in separate 38 L aquaria using aeration and daily 50% water changes to maintain water quality. A 25-watt heater was used to maintain consistent temperature (21-23 °C) during the incubation period as well as post-hatch. Sac-fry and free-swimming larvae were maintained in multiple aquaria separated into core or peripheral populations. Once larvae were zooplanktivorous, they were further separated into 3 aquaria per population to better maintain water quality. Zooplanktivorous larvae were first fed small *Daphnia* sp, and then larger *Artemia* adults.

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Larvae were fed 2-3 times daily to maintain a constant supply of food. Larvae from both populations were fed small (3.0 cm) fathead minnows *Pimephales promelas* upon converting to piscivory. Larvae were further separated roughly based on size into 3 aquaria per population to reduce cannibalism. To estimate early life growth rates during the period from 1-100 days after hatch (DAH) preceding experiment 1, 30 individuals from each population were randomly selected weekly for measurements of length (0.1 cm) and weight (0.1 g). Mean growth rates ($cm \cdot d^{-1}$ and $g \cdot d^{-1}$) were then calculated for each population. Once juvenile gars were regularly feeding on medium-sized (4.5-6.0 cm, size range used in experiments) fathead minnows, individuals were randomly selected from each population and placed into experimental aquariums. All selected individuals were acclimated to experimental aquariums for 4-5 days prior to the start of experiment 1. Excess individuals were maintained in separate aquaria (based on population) as replacements if needed and for experiment 2.

Experiment 1

Twenty 75 L aquaria were used for housing YOY spotted gars from both populations (N = 30 fish from each population). Each aquarium was divided equally into three compartments using thin fiberglass screening, which allowed passage of water, but not other gars or feeder minnows. Each individual compartment housed one gar (3 gars per aquarium, total of 60 gars). Each aquarium also contained an air pump-operated sponge filter to maintain water quality and a 50-watt heater to maintain consistent temperature of 22-24 °C. Temperature range was selected based on mean temperatures experienced during the growing season by both populations (Redmond 1964, Echelle and

Riggs 1972, Simon and Wallus 1989, Simon and Tyberghein 1991, personal

observation). To further maintain water quality, 50% of the water was changed weekly

for each tank, with waste material removed via siphon. Overhead fluorescent lights on

electronic timers were used to maintain a consistent 12-hour photoperiod during the

experiment. Individual spotted gars were fed fathead minnows ad libitum for the

duration of the experiment, 62 days for LA fish and 63 days for MI fish. To accomplish

ad libitum feeding, a small group of minnows (approximately 5.0-7.0 g total mass) was

consistently maintained in each experimental compartment; consumed minnows were

replaced and dead minnows were removed to prevent deterioration of water quality.

Individual gars were removed from compartments to measure length and weight

weekly as well as at the beginning and end of the experimental period. Mean length and

weight were used to determine increase in growth and growth rate ($cm \cdot d^{-1}$ and $g \cdot d^{-1}$) over

the experimental period. One-way analysis of variance (ANOVA) was used to test for

significant differences in initial and end mean length and weight for both populations.

Analysis of covariance (ANCOVA), with population and DAH as fixed factors, was used

to determine significant differences in growth rates between populations, if any. I

assumed a linear model for growth during the experimental period of development for

both populations of spotted gars. Increase in length and weight for each population was

plotted versus time (DAH or days of experiment) and analyzed using linear regression to

generate growth models. Length-weight relationships were also analyzed with ANOVA

and used as a proxy for comparing energy storage between populations.

Experiment 2

To investigate potential differences in growth rate between populations at different temperatures, spotted gars from both populations were divided into three temperature groups; 16 °C, 23 °C, and 30 °C, for a total of six groups (one peripheral group and one core group per temperature treatment). Each group was comprised of six spotted gars for a total of 36 gars in the experiment. Fish were randomly selected from both experiment 1 as well as excess individuals, and were all reared under the same temperature (23 °C) and feeding (ad libitum) regime for at least 30 days prior to beginning the experiment.

Each group of gars was placed in a 190 L fiberglass tank containing a stand pipe connected to a large recirculating system for constant water filtration. Temperature was maintained using 75-watt heaters in the control and warm treatment group tanks, and was monitored daily. All groups were acclimated to respective temperature treatments for at least 7 days prior to beginning the experiment. Spotted gars in all tanks were given unlimited ration of fathead minnows, and photoperiod was maintained at 12 hours light/dark. Within each tank individual fish were identified by a single fin clip from the right/left pectoral fin, right/left pelvic fin, anal fin, or no fin clip. Marked fins were re-clipped as necessary (due to fin regeneration) on measurement days over the course of the experiment. Length and weight of all fish were measured at the beginning of the experiment as well as weekly for five weeks. Total duration of the experiment was 42 days.

Mean length and weight were determined for both populations in each treatment weekly, and growth rate was calculated as in experiment 1. Length-weight relationships

were also calculated and analyzed for each temperature treatment and used as a proxy for energy storage similarly to experiment 1. Due to limitations in replication because of low numbers of available fish and tanks (only 1 replicate of 6 fish for each population per temperature treatment), primarily descriptive statistics were used to analyze experiment 2.

In addition to descriptive statistics, ANOVA tests were run using each fish as a replicate (N = 6 replicates per population in each treatment) to further investigate differences in growth rate and length-weight relationships between populations at each temperature. ANCOVA with temperature and population as fixed factors was performed for analysis of growth rate.

All statistical analyses were carried out using JMP SAS (2001) software with significance levels set at $\alpha = 0.05$.

Results

Eggs from both populations hatched 6-7 days after fertilization. Hatching success was 70-80% for both populations, and newly hatched larvae were approximately 1.0 cm in length and weighed approximately 0.5 g. Larval gars consumed their yolk sacs 6-7 DAH and began feeding on *Daphnia* and *Artemia*. Juveniles from both populations began eating small fathead minnows 35-40 DAH; 30 fish from each population were then randomly selected and moved into experimental tanks for acclimation.

Growth rates in length and weight during early life were significantly higher (ANCOVA, p <0.05) for LA spotted gars than MI spotted gars held at 23 ºC (Figures 2.2 and 2.3). Length and weight regression models explained 96-99% of variation in the

data. Although both groups of fish were of similar age when switching to piscivory and acclimating to experimental aquaria, 1-way ANOVA tests indicated MI fish were significantly smaller than LA fish at the beginning of experiment 1 (Table 2.1). One-way ANOVA tests indicated that end length and weight of MI fish, however, were significantly higher than end length and weight of LA fish. ANCOVA tests also indicated that growth rates of MI gars were significantly greater than those of LA gars. Linear regression analyses generated models of growth rates for both populations and explained 97-99% of variation in the data (Figures 2.4 and 2.5).

Length-weight relationships were compared using one-way ANOVA at the beginning and end of experiment 1; ANCOVA was used to compare rate of change in length-weight relationships during the course of experiment 1. At the beginning of experiment 1, MI fish had a significantly lower weight at a given length than LA fish. By the end of experiment 1, however, MI fish had a significantly higher weight at length than LA fish. Linear regression analysis and ANCOVA indicated that change in weight-length ratios was significantly different between MI (higher rate) and LA fish (lower rate) over the course of experiment 1 (Figure 2.6).

In experiment 2, both populations responded differently to temperature treatments (Table 2.2, Figure 2.7). Fish from both populations at 16 °C exhibited very low increases in length (MI fish = 0.02 cm, LA fish = 0.10 cm) and decreased in weight (MI fish = -1.18 g, LA fish = -0.38 g) during the 42-day period. Clipped fins (used to identify individual fish) did not regenerate on any individuals in either cool treatment, and consumption of fathead minnows was very low compared to other temperature treatments. Fish in the 23 °C and 30 °C treatments frequently required re-clipping of

marked fins, as well as much more frequent replacement of fathead minnows. MI fish at

23 °C and 30 °C experienced larger mean increase in growth and growth rate (weight)

compared to LA fish. One-way ANOVA tests comparing growth rates among all

temperature treatments indicated that both populations experienced lowest growth rates at

16 °C, higher growth rates at 23 °C, and highest growth rates at 30 °C (Figure 2.8).

Comparing growth rates within populations at different temperatures, MI fish

experienced significantly higher growth in length from 16 °C to 23 °C, but not from 23

°C to 30 °C. LA fish experienced significantly higher growth in length among all three

temperature treatments. MI fish experienced significantly higher growth in weight across

all temperature treatments, while LA fish experienced significantly higher growth in

weight from 16 °C to 23 °C, but not from 23 °C to 30 °C.   The 16 °C treatment may

have been near the point at which growth ceases in both populations of spotted gars.

Discussion

I hypothesized that spotted gars from two disjunct population segments would

exhibit latitudinal compensation in growth similar to several other fish species (Conover

et al. 2009), and that under common environment conditions, fish from higher latitude

would grow faster than those from lower latitude. My experiments showed that in a

common environment simulating periods within the first growing season (experiment 1:

T = 23 ºC, duration approximately 60 days, 95-155 DAH; experiment 2: T = 16, 23, or 30

ºC, duration = 42 days), peripheral population spotted gars had a significantly higher

growth rate than core population spotted gars, suggesting that important genetic and

physiological differences exist between the two major population segments. Although

lack of replication limited the extent of our statistical analyses in experiment 2, results clearly suggest that MI spotted gars maintained a higher growth rate than core population spotted gars even at warmer temperatures, and that both populations had similar thermal minima for growth. These results strongly support evidence for CnGV in growth rate in spotted gars.

As in Atlantic silversides, the model species used to investigate CnGV in growth by Conover and Present (1990) (see also Conover 1992, Present and Conover 1992, Munch and Conover 2002), spotted gars begin spawning at approximately the same temperature (23 ºC) but later in the year with increasing latitude (Redmond 1964, Holt 1973, Trautman 1981, Becker 1983, Snedden 1999). Conover et al. (1990) also noted that later initiation of spawning and earlier onset of winter resulted in a much shorter growing season at higher latitudes. Although the length of growing season decreases as latitude increases, mean size at the end of first growing season does not decrease for several populations of fish species with increasing latitude (Conover et al. 2009). Therefore populations of these species at higher latitudes are able to compensate for shorter growing seasons by evolving faster growth rates than lower-latitude populations (Conover 1992).

These differences in growth rate may be indicative of other potentially interesting eco-evolutionary dynamics between core and peripheral populations of spotted gars (explored in chapter 3) such as differences in life history patterns, as well as morphological and genetic variation. From an evolutionary ecology perspective, my results suggest that a rapid adaptation in growth rate has occurred even in relatively slowly-evolving fishes such as gars (Wiley 1976, Conover et al. 2009, Grande 2010,

Carlson et al. 2011). The spotted gar, a warmwater species, entered the Great Lakes region via connections to the Mississippi River drainage (southern refugium) following the last glaciation no more than 8,000 years ago (Bailey and Smith 1981, Hocutt and Wiley 1986). Therefore adaptation of growth rate to length of growing season was relatively recent. Similarly, Mach et al. (2011) showed that in Atlantic silversides, another species expanding northward from a single southern refugium post-glaciation, regional adaptation (e.g. CnGV) and phenotypic patterns developed relatively recently. Using Pacific salmonids, Carlson et al. (2011) showed that shifts in body size due to selection over even a single generation can have large and lasting evolutionary impacts on both species and ecosystems.

The scope of my study was limited to two major populations (core and peripheral) of spotted gars; including more populations in future experiments may provide a better picture of gradient in growth rate with increasing latitude. Despite this limitation, my study populations did represent a natural break in the distribution of spotted gars, in that the species is completely disjunct between the Great Lakes and Mississippi River basins (Page and Burr 1991), therefore my population comparisons are realistic if not comprehensive. The core population does span a greater latitudinal range than the peripheral population (approximately 1550 km compared to 220 km), therefore growth rate comparisons among fish from multiple core populations are recommended. Detailed measurements of early life stages preceding piscivory (e.g. zooplanktivorous stage) were not made in my experiments, however, growth rates during these early stages were higher in core than peripheral populations. Once the piscivorous stage was reached, growth rate was higher in peripheral than core populations. Mittelbach and Persson

(1998) found that growth rates of piscivorous fishes (including gars) greatly increased between zooplanktivorous and piscivrous stages. Although growth rates were significantly different between populations at early life stages, differences in growth rate were comparatively much greater when gars switched to piscivory. Experiments comparing fish at pre-piscivorous stages may better elucidate early life stage differences between core and peripheral populations.

Although CnGV has been observed in a diversity of ectotherms, most frequently in fishes, it has not been previously observed in gars. Furthermore, our study is the first to use common garden experiments to test for latitudinal variation in a non-teleost fish; an under-studied group in such investigations, because of their typically late maturation and long generation time (Ferrara 2001), as well as high energy requirements (Alfaro et al. 2008) compared to teleosts in similar studies (Conover and Present 1990, Schultz et al. 1996, Arendt and Wilson 1997, Power and McKinley 1997, Conover et al. 2009, Baumann and Conover 2010). My results suggest that CnGV may exist in other evolutionarily and economically significant non-teleost species (i.e. lungfishes, sturgeons, alligator gar).

Countergradient variation in growth of spotted gars may also have implications in the context of climate change and range expansion. Using the weak latitudinal temperature gradient of the Pacific silversides *Atherinops affinis* as a proxy for the gradual effects of climate change, Baumann and Conover (2010) showed that two species, Atlantic and Pacific silversides, each experiencing very different latitudinal temperature gradients, still exhibited CnGV in growth. Their study indicated that ectotherms have evolved growth adaptations to even weak climate gradients, and that a

pole-ward migration of genotypes will be a likely result of an increasingly warmer climate. As a warmwater species exhibiting CnGV, spotted gars would likely successfully increase their range northward even with gradual increases in temperature.

Previous studies have shown that in aquatic systems, species at higher trophic levels are at higher risk and are more frequently lost than those at lower trophic levels, in part because of their relatively small population sizes (Lande 1993, Petchey et al. 2004). Piscivorous fishes, therefore, may be particularly vulnerable amidst the ongoing biodiversity crisis. Furthermore, non-game piscivorous species (e.g. gars, Lepisosteidae; bowfin, *Amia calva*) may be even more at risk due to their poorly-studied ecology, perceived low economic value, and the higher priority given to propagation and management of game species (centrarchids, percids, esocids); the latter often leading to the destruction of both non-game individuals and habitat (Scarnecchia 1992). My study provides evidence of unique characteristics of the peripheral population of spotted gars, and provides more evidence for the general argument that understanding and protecting peripheral populations should be a key component of our programs to conserve natural biodiversity.

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Table 2.1. Mean length (cm) and weight (g) at initiation and completion of experiment 1, along with total growth (Final-Initial), growth rate (cm·day^{-1}, g·day^{-1}), and descriptive statistics for LA and MI populations of spotted gars (N=30 fish per population). Experimental durations were 62 (LA) and 63 (MI) days.

Population	Michigan			Louisiana		
	Mean	Variance	St Dev	Mean	Variance	St Dev
Initial Length	14.29	3.41	1.85	15.56	2.40	1.55
Final Length	20.06	2.70	1.64	18.24	2.11	1.45
Total Growth	5.77			2.68		
Growth Rate	0.09			0.04		
Initial Weight	7.50	9.07	3.01	10.74	10.33	3.21
Final Weight	24.09	46.80	6.84	17.53	16.93	4.12
Total Growth	16.59			6.79		
Growth Rate	0.26			0.11		

Table 2.2. Mean length (cm) and weight (g) at initiation and completion of experiment 2, along with total growth (Final-Initial), growth rate (cm·day^{-1}, g·day^{-1}), and descriptive statistics for LA and MI populations of spotted gars at 3 different temperature treatments (N = 6 fish per population in each treatment). Experimental duration was 42 days.

Experimental Temperature (°C)		Michigan			Louisiana		
		Length					
		Mean	Variance	St Dev	Mean	Variance	St Dev
16	Initial Length	21.07	1.05	1.03	19.17	1.59	1.26
	Final Length	21.08	0.95	0.97	19.27	1.87	1.37
	Total Growth	0.02			0.10		
	Growth Rate	< 0.01			< 0.01		
23	Initial Length	20.75	0.67	0.82	19.85	7.30	2.70
	Final Length	23.52	1.26	1.12	21.33	7.37	2.72
	Total Growth	2.77			1.48		
	Growth Rate	0.07			0.04		
30	Initial Length	22.60	6.86	2.62	20.72	0.90	0.95
	Final Length	25.50	2.96	1.72	23.15	1.24	1.11
	Total Growth	2.90			2.43		
	Growth Rate	0.07			0.06		
		Weight					
		Mean	Variance	St Dev	Mean	Variance	St Dev
16	Initial Weight	27.73	22.41	4.73	21.77	24.25	4.92
	Final Weight	26.55	18.58	4.31	21.38	25.16	5.02
	Total Growth	-1.18			-0.38		
	Growth Rate	-0.03			-0.01		
23	Initial Weight	24.63	10.61	3.26	23.73	96.48	9.82
	Final Weight	37.12	44.87	6.70	30.05	195.19	13.97
	Total Growth	12.48			6.32		
	Growth Rate	0.30			0.15		
30	Initial Weight	32.50	134.49	11.60	25.27	20.06	4.48
	Final Weight	51.97	108.97	10.44	36.32	41.93	6.48
	Total Growth	19.47			11.05		
	Growth Rate	0.46			0.26		
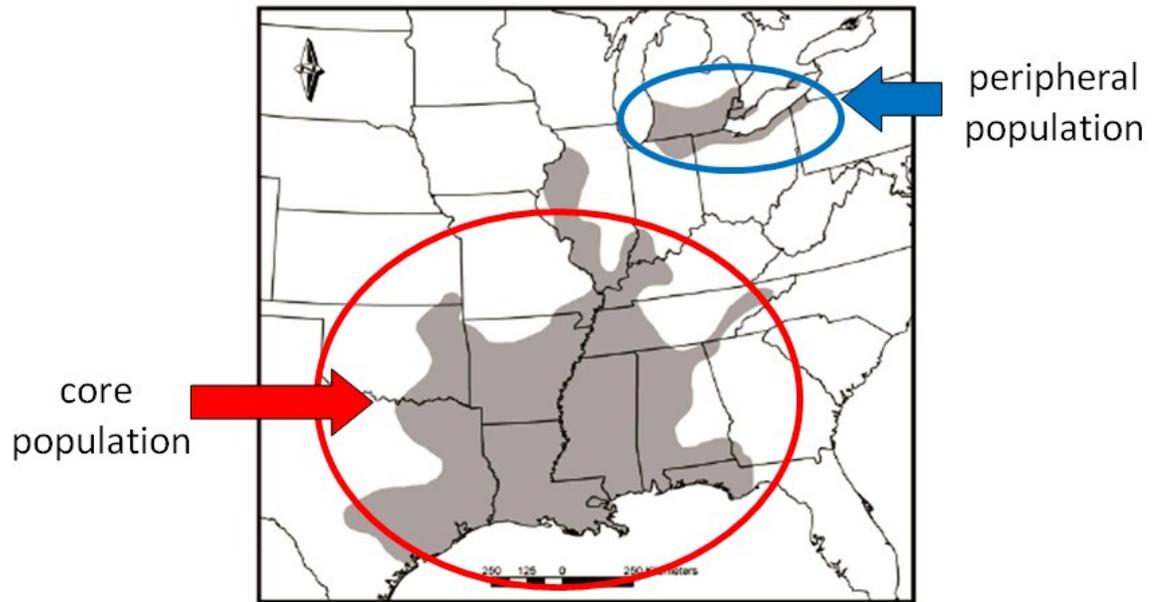
Figure 2.1. Distribution of core and peripheral populations of the spotted gar *Lepisosteus oculatus*. Note disjunction between populations. Modified from Page and Burr (1991).
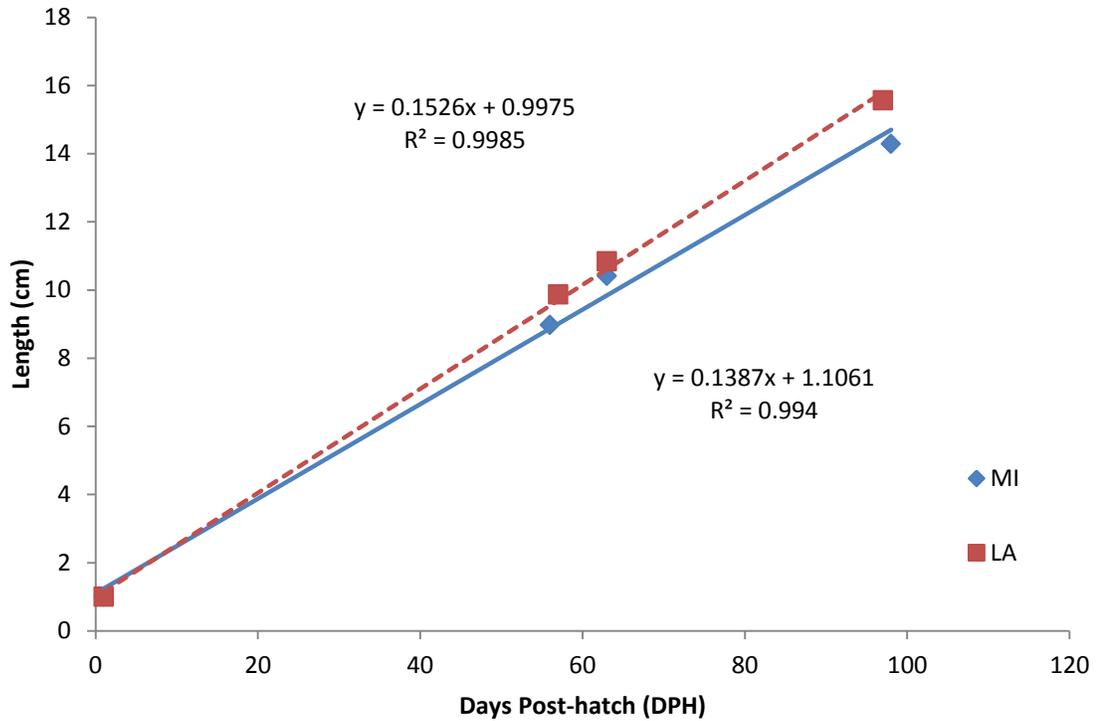
Figure 2.2. Comparison of early life stage length at age (period prior to start of experiment 1) of LA and MI populations of spotted gars held at 23 ºC (N = 30 fish per population). Larval fish from both populations hatched at approximately 1.0 cm. Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.
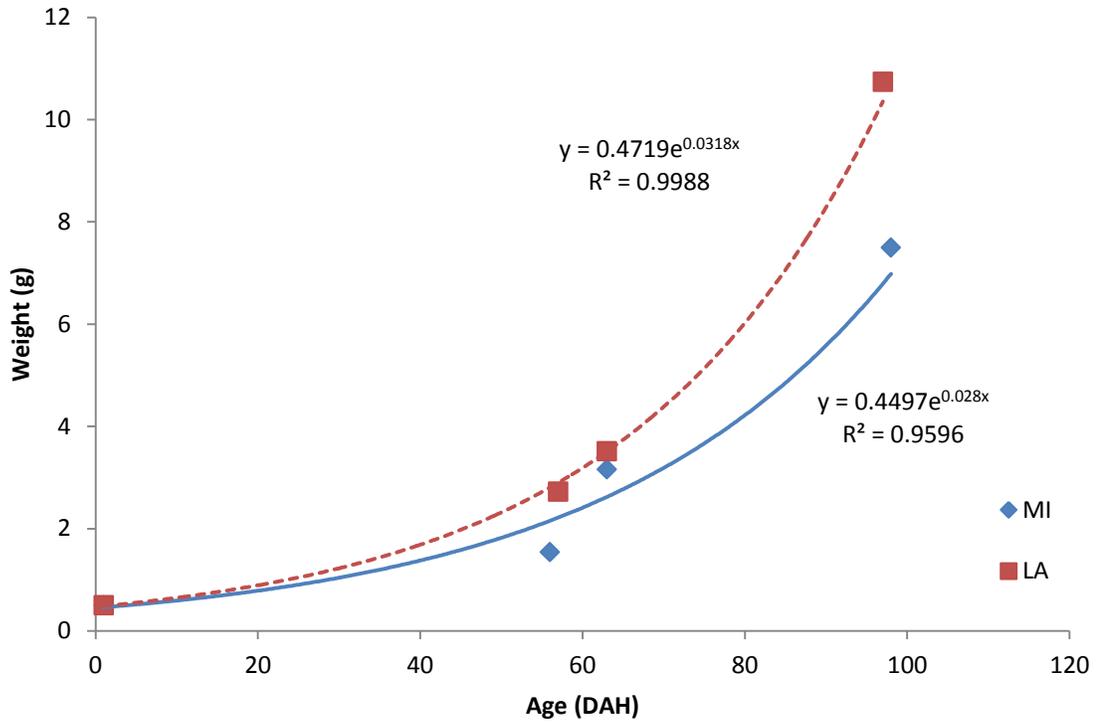
Figure 2.3. Comparison of early life stage weight at age (period prior to start of experiment 1) of LA and MI populations of spotted gars held at 23 ºC (N = 30 fish per population). Larval fish from both populations hatched at approximately 0.5 g. Exponential regression models (dashed = LA, solid = MI) and R^2 values were also calculated.
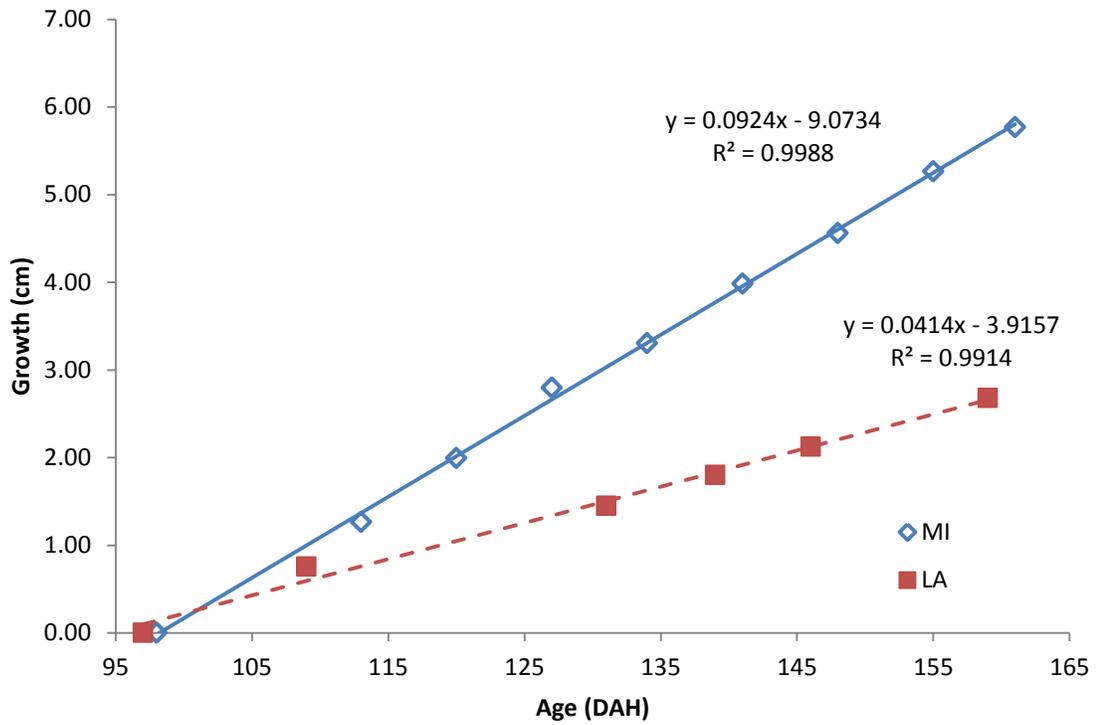
Figure 2.4. Increase in length over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.
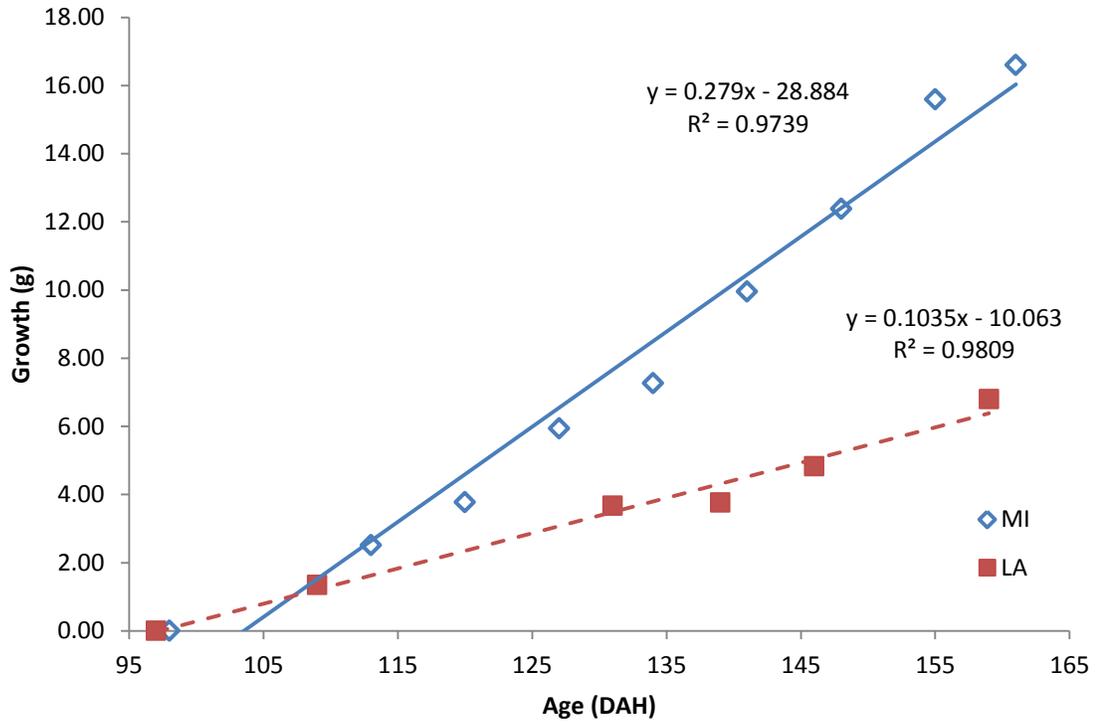
Figure 2.5. Increase in weight over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.
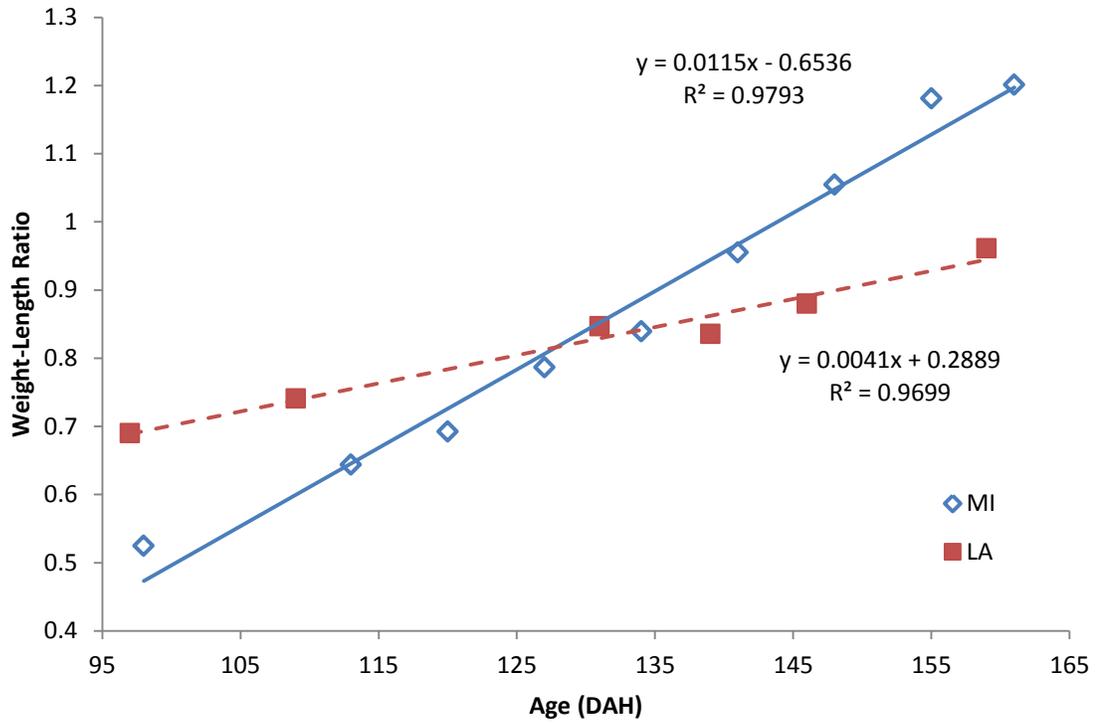
Figure 2.6. Mean weight-length ratios over time for LA and MI populations of spotted gars held at 23 ºC in experiment 1 (N = 30 fish per population). Linear regression models (dashed = LA, solid = MI) and R^2 values were also calculated.

A. 16 °C
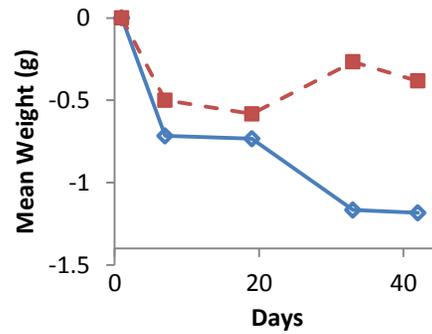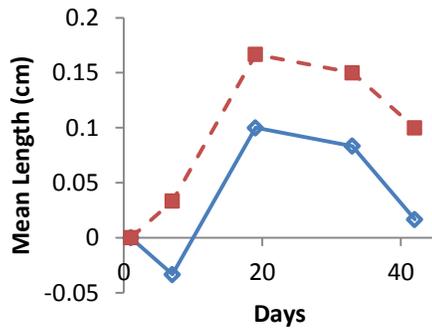
B. 23 °C
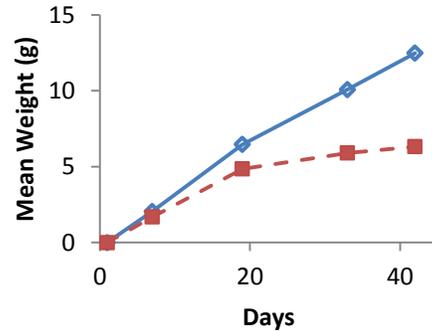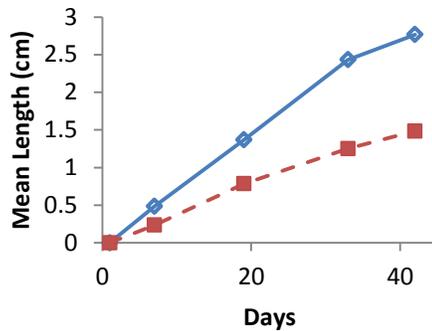
C. 30 °C
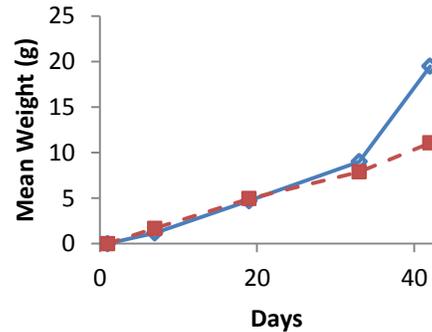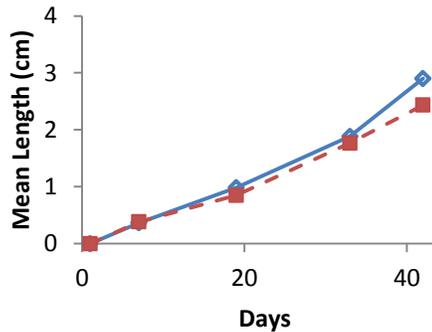
Figure 2.7. Changes in mean length and weight for MI (solid line) and LA (dashed line) populations of spotted gars at 3 temperature treatments (A = 16 °C, B = 23 °C, C = 30 °C; N = 6 fish per population in each treatment) in experiment 2 (experimental duration = 42 days).

A.

B.
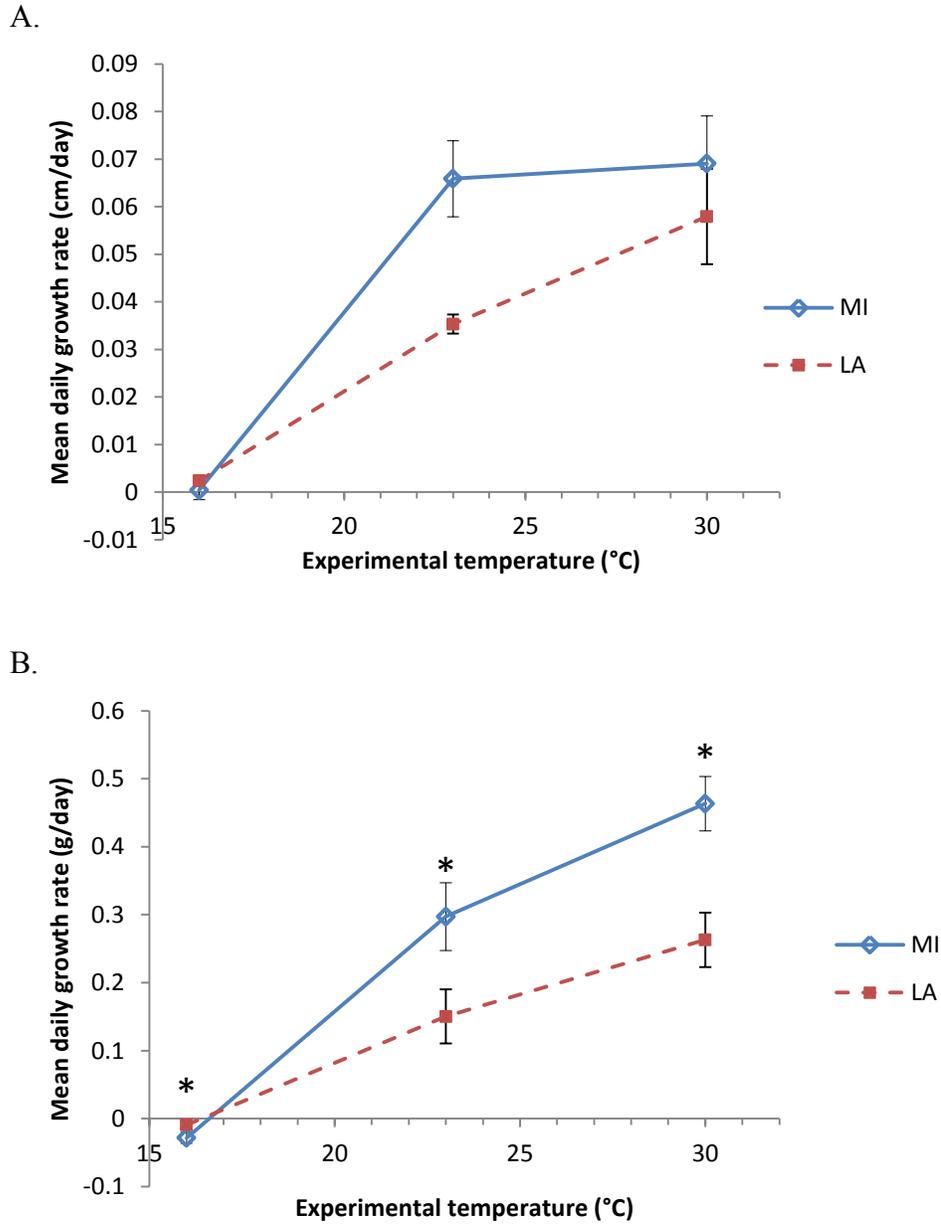
Figure 2.8. Mean daily growth rates for length (A) and weight (B) of LA and MI populations of spotted gars at three temperature treatments (16 °C, 23 °C, 30 °C; N = 6 fish per population in each treatment) in experiment 2 (experimental duration = 42 days). Error bars indicate ±1 standard error, * indicates significant difference between populations at temperature treatment.

Chapter 3

Variation in life history patterns of the spotted gar *Lepisosteus oculatus* from core and peripheral populations

Introduction

Peripheral or "fringe" populations occupy the edge of a species' range and are considered to be exceptionally important in terms of a species' ecology, biogeography, evolution, and conservation (Scudder 1989, Lesica and Allendorf 1995, Latta 2003). Peripheral populations often persist under different environmental conditions from the species' central or "core" populations, and therefore may exhibit different genetic and phenotypic adaptations to potentially "harsher" environments (Yakimowski and Eckert 2007). Due to small size, fragmentation, or complete disjunction, many peripheral populations may experience low or non-existent recolonization potential, and therefore may be more susceptible to environmental perturbations as well as extinction (Lesica and Allendorf 1995, Channell and Lomolino 2000, Wisely et al. 2004). Peripheral populations also often experience very low gene flow and high degrees of genetic drift, leading to further divergence from core populations (Jones et al. 2001, Lammi et al. 2001, Johannesson and Andre 2006).

Because of differing environmental conditions related to geographical factors such as latitude, peripheral populations may also exhibit differences in life history characteristics such as size at age, age at maturity, growth rate, and mortality rate in comparison to core populations (Power and McKinley 1997, Munch et al. 2003, Charnov

and Gillooly 2004, Heibo et al. 2005, Slaughter et al. 2008). Such latitudinal variation in life history characteristics has been observed in a diversity of taxa including plants (Yakimowski and Eckert 2007), mammals (Kyle and Strobeck 2002), reptiles (Wilson and Cooke 2004), invertebrates (Lee et al. 1998, Lardies et al. 2004), and fishes (Kynard 1997, Yamahira and Conover 2002, Foster and Vincent 2004). Coupled with genetic drift and low gene flow, these latitudinal variations in life history characteristics may further contribute to divergence between peripheral and core populations. For all these reasons it is believed that speciation is likely to often take place in peripheral populations, making them evolutionarily significant (Lesica and Allendorf 1995). Conserving peripheral populations is therefore a unique and integral component of conserving global biodiversity (Lammi et al. 2001, Johannesson and Andre 2006).

Among ectotherms such as fishes, factors associated with temperature have been shown to have particularly strong influence on variation in life history characteristics, especially over wide latitudinal ranges (Atkinson and Sibly 1997, Yamahira and Conover 2002). The length of growing season, characterized by warmer temperatures and often designated as "thermal opportunity for growth" (TOG; Power and McKinley 1997), varies at different latitudes, contributing to differing growth rates among populations (Slaughter et al. 2004). Stillwell (2010) stated that environmental and ecological variables that vary with latitude, such as temperature and seasonality, are of primary interest in describing life history patterns such as variation in body size.

The Great Lakes population segment of the spotted gar *Lepisosteus oculatus* represents the northern edge of the species range, and is also completely disjunct from the large southern US population segment (Page and Burr 2011). The peripheral population

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segment is comprised of northwestern inland lake populations (primarily found in southern Michigan) and northeastern populations in several isolated bays of Lake Erie (Trautman 1981, Carman 2002, Page and Burr 2011). The species dates back to the early Eocene (48-55 mya; Wiley 1976, Grande 2010) but arrived in the Great Lakes region relatively recently, approximately 8,000 years ago following the Wisconsinan Glaciation (Bailey and Smith 1981, Hubbs et al. 2004). Spotted gars in the Great Lakes region are separated by a large latitudinal distance from the core population (approximately 1,231 km between population centers), and length of growing season is significantly shorter, approximately 111 days (Great Lakes region) compared to 229 days (southern US, NOAA National Climate Data Center 2011). Because of the large latitudinal distance and differences in length of growing season, variations in population life history characteristics such as growth rate, size and age at maturity, and mortality might be expected. The Great Lakes population of spotted gars therefore provides an interesting opportunity to investigate patterns in life history of peripheral versus core populations within a species with a wide latitudinal range.

My primary objective in this chapter was to describe any potential differences in life history pattern between peripheral and core population segments of spotted gars, as well as among component populations of those segments, with respect to common demographic variables (e.g. mean age, mean length, length-at-age). Secondary objectives include exploring whether potential variation in life history patterns might be related to patterns in latitudinal eco-physiological variation, and to consider the extent to which local adaptation by peripheral populations may have occurred. I hypothesize that peripheral populations of spotted gars will exhibit variation in life history characteristics

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in comparison to core populations. More specifically, I hypothesize that spotted gars from peripheral populations have a greater lifespan and larger mean size than those from core populations. And further, I hypothesized that variation in life history characteristics could be explained by factors (e.g. temperature, thermal opportunity for growth) strongly influenced by different latitudes.

Methods

Study Species

The spotted gar is a moderately long-lived fish (maximum reported age = 18 years), with males reaching maturity in 1-3 years and females in 1-5 years (Redmond 1964, Love 2004). Onset of spawning takes place at similar water temperatures (usually 19-22 °C) in late spring through early summer throughout the species' distribution, regardless of latitude (Redmond 1964, Holt 1973, Trautman 1981, Snedden 1999). Spotted gars, like all gar species, are polyandrous, with several male fish spawning in a group with 1-2 female fish. Young-of-the-year (YOY) spotted gars can reach over 30 cm by the end of the first growing season, before growth slows or is completely suspended during winter months (Redmond 1964, Holt 1973, Snedden 1999). Experimental results (see chapter 2) suggest that spotted gars suspend or resume positive growth at approximately 15-18 °C. Since maturity is reached in 1 or more years, only somatic growth is experienced in the first growing season.

Study Populations

Spotted gars were collected from multiple localities within both peripheral and core population segments for life history analyses (Figure 3.1). Peripheral population fish came from several inland lakes in southern Michigan and from Rondeau Bay, Lake Erie, in Canada. Southern Michigan localities from which spotted gars were collected were characterized as small to medium-sized (average surface area 2.82 km^2, mean maximum depth 15.21 m) glacial lakes, consisting of headwater (only outflow), inline (both inflow and outflow), and disconnected (neither inflow nor outflow) lake connectivity designations (see Appendices 3.1 and 3.2 for lake and catch data). These lakes typically consisted of well-defined areas of prominent submerged aquatic vegetation and clear water (Michigan Department of Natural Resources unpublished data). Both vegetated and non-vegetated sites were sampled in each lake, and spotted gars were only found in areas with submerged aquatic vegetation. Rondeau Bay (approximately 31 km^2) is a shallow coastal wetland in the northern portion of the central basin of Lake Erie. The bay is characterized by abundant submerged aquatic vegetation, clear water, and is relatively shallow (less than 3 m; Glass et al. 2011).

Core population spotted gars were collected from three localities: Mingo Swamp, Missouri, Lake Seminole, Georgia, and Bayou Chevreuil, Louisiana. Two core population localities (Mingo Swamp and Bayou Chevreuil) were from within the Mississippi River drainage basin, and one (Lake Seminole) from the Apalachicola River drainage basin (southeastern Gulf Coast). Mingo Swamp (14.16 km^2 surface water) is located in the Mingo National Wildlife Refuge, Missouri, and is characterized by surrounding bottomland hardwood forest and waters with frequently fluctuating turbidity

and depth, as the swamp is managed primarily for migratory waterfowl (Redmond 1964, USFWS 2009). Bayou Chevreuil (approximately 25 km long, 40 m wide; 1.00 km^2; Google Earth 2012) is located in the Barataria Estuary, and is the primary riverine connection draining the upper northwest portion of the estuary into Lac des Allemands. Bayou Chevreuil is characterized by surrounding cypress-tupelo swamps and bottomland hardwood forests, abundant submerged and floating aquatic vegetation, and turbid to "tea-colored" waters (USACE 2004, Fontenot 2006). Lake Seminole (132 km^2, mean depth 3 m, max depth 11 m) is a reservoir located at the confluence of the Flint and Chattahoochee rivers, with outflow into the Apalachicola River (USACE 1996, Ferrara 2001) and is characterized by flooded timber and abundant submerged aquatic vegetation (approximately 70% coverage of *Hydrilla*; Florida Fish and Wildlife Conservation Commission 2012).

Data Collection

Spotted gars from Michigan inland lakes were collected from late spring-early fall (2008-2010) using pulsed DC boat electrofishing. Additional peripheral population data were included from fyke net sampling of spotted gars from Rondeau Bay, Lake Erie during May-June 2007 (Glass, unpublished data). Core population fish from Bayou Chevreuil, LA were collected during spring (2009-2010) using experimental gill nets. Additional core population data (length, age, and sex) for spotted gars from Mingo Swamp, Missouri (Redmond 1964) and Lake Seminole, Georgia (Ferrara 2001) were also included in analyses. Redmond (1964) primarily used experimental gill nets and rotenone for collection of spotted gars from spring-early fall 1962-1963, and Ferrara

(2001) used pulsed DC electrofishing for collecting spotted gars on December 15, 1999,

March 8, 2000, and June 8, 2000. Although sampling period differed somewhat among

populations, all sampling encompassed the spawning period (spring-summer); Ferrara

(2001) found that based on opaque band formation in otoliths, annuli formed in late

spring to early summer for spotted gars, longnose gars *Lepisosteus osseus*, and alligator

gars *Atractosteus spatula*, therefore I assumed sampling period did not greatly influence

our aging estimates.

Peripheral population fish were designated as MI-p for Michigan fish and LE-p

for Lake Erie fish. Core population fish were designated as MO-c for the Mingo Swamp,

Missouri population, GA-c for the Lake Seminole, Georgia population, and LA-c for the

Bayou Chevreuil, Louisiana population. The "peripheral population segment" refers to

both MI-p and LE-p, and the "core population segment" refers to MO-c, GA-c, and LA-c.

Life history analyses consisted of identifying and comparing length, age, and sex

characteristics of core and peripheral population spotted gars. I did not include weight or

condition comparisons in my analyses due to high variability in these characteristics in

gars, usually associated with differences in sampling period, particularly during spawning

season (Love 2004, Smith 2008, McGrath and Hilton 2011, Glass et al. 2011). Because

of potential bias in sampling periods, I considered length and age comparisons among

populations to be more accurate descriptors of population variability. Individuals (MI-p,

LA-c) were measured (total length, mm) upon capture, and then maintained in holding

tanks for spawning (offspring used for other studies) or immediately euthanized using

MS-222 or clove oil. LE-p fish were released after length, weight, and age (pectoral fin

ray clip) data were collected (Glass et al. 2011). LA-c population individuals were

euthanized and then dissected by colleagues at Nicholls State University for identification

of sex based on gonads and gonad release pathways (vasa efferentia in males, oviducts in

females) following the methods of Ferrara and Irwin (2001). MI-p individuals were

euthanized then placed on ice for later dissection. Sex of MI-p spotted gars was also

determined using dissection following the methods of Ferrara and Irwin (2001). Due to

the threatened status of the spotted gar in Canada, LE-p fish were not sacrificed and

therefore sex determination was not possible (Glass et al. 2011).

Otoliths and branchiostegal rays were removed from MI-p spotted gars for aging

following the methods of Redmond (1964) and Holt (1973). Age of individual Michigan

fish was determined by two independent readers for both otoliths and branchiostegal rays.

Otoliths were sectioned using a progression of coarse to fine sand paper and then viewed

under a microscope. Branchiostegal rays were cleaned of excess flesh by hot water bath

and then viewed in water under a microscope (see Redmond 1964 and Holt 1973 for

further details on aging of gar otoliths and branchiostegal rays). Age values were then

compared among structures (2 otoliths, 2 branchiostegal rays per fish) to determine a

single age value for each fish as well as compare accuracy of age estimation between

otoliths and branchiostegal rays. Otoliths were removed from LA-c spotted gars by

colleagues at Nicholls State University and sent to the University of Michigan for aging.

Aging of LA-c otoliths was performed by the same readers as MI-p fish and followed the

same methodology. GA-c spotted gars were aged using otoliths by Ferrara (2001). MO-

c fish were aged by Redmond (1964) using branchiostegal rays and the resulting

methodology serves as the current standard for aging of gar branchiostegal rays.

Redmond (1964) collected a small number of older spotted gars that could not be

accurately aged, one of which was estimated to be at least 18 years old. Due to

inconclusive aging of these older fish, Redmond (1964) limited his analyses to fish age 8

and younger, and these are the data included in my MO-c analyses as well. The "age 18"

individual from Redmond (1964), however, remains the referenced (Love 2004, Murie et

al. 2009, Glass et al. 2011) maximum age reported for the species, therefore I include age

18 from that study in my discussion.

Aging of LE-p fish was performed using pectoral fin ray sectioning, a non-lethal

technique (Glass et al. 2011). To test the validity of this aging technique, Glass et al.

(2011) collaborated with me to age 10 MI-p fish using otoliths, branchiostegal rays, and

pectoral fin rays (Table 3.1). Multiple readers, including the authors, compared all 3

types of structures and were in acceptable agreement based on indices of precision (Den

Haas and Mandrak 2004). Glass et al. (2011) considered pectoral ray sectioning to be a

valid aging technique if the index of precision was below 0.29 (Den Haas and Mandrak

2004). Index of precision was calculated using the following equation:

$$\text{Index of precision} = \frac{(\text{annuli counted on structure} - \text{assigned age})}{\text{assigned age}}$$

Assigned age was based on the first reader's estimate from the branchiostegal ray. Based

on comparisons of the three aging structures among 10 MI-p fish, the combined average

index of precision was 0.14 for otoliths, 0.11 for pectoral rays, and 0.03 for

branchiostegal rays (Glass et al. 2011). All methods were considered valid, with

branchiostegal rays the most precise and otoliths the least precise. Additionally, Glass et

al. (2011) cautioned that pectoral ray sectioning may underestimate age in older

specimens, as annuli became more crowded in older fish (Table 3.1). Further validation

comparing aging techniques for all structures (otoliths, branchiostegal rays, pectoral fin

rays) was done among the authors (Ferrara 2001, Glass et al. 2011) and myself at workshops specifically focusing on gar aging methodology (Lepisosteid Fish Research and Management Committee Meeting, January 2009; 3rd International Meeting on Lepisosteid and Research Management, May 2010). By analyzing both otoliths and branchiostegal rays, as well as participating in pectoral fin ray analysis, I was able to successfully compare my age data with datasets from other studies (Redmond 1964, Ferrara 2001, Glass unpublished data).

Latitude for each population locality was estimated from GIS maps (Google Earth data 2012) to the nearest 0.25 degrees. Because daily water temperature was not available for all localities, I used mean annual air temperature estimated from 30-year (1971-2000) climate norms from weather stations nearest to sampling localities (Power and McKinley 1997, National Climatic Data Center 2011, Atmospheric Environment Service 2012). Water surface temperatures have been shown to have strong correlations with short and long-term air temperatures (McCombie 1959, Shuter et al. 1983, Livingstone and Dokulil 2001) and mean annual air temperature has been used as a proxy for water temperature in similar studies (Beamesderfer and North 1995, Power and McKinley 1997, Hoxmeier et al. 2009). Power and McKinley (1997) stated that in comparison of growth rates among populations from different latitudes it is important to incorporate a measure of opportunity for growth in the analyses. I used thermal opportunity for growth (TOG) as a measure of growing season/seasonality for the different study populations; TOG was taken from 30-year climate norms (1971-2000) and estimated to be the mean annual sum of (cooling) degree days greater than 18 °C at (the weather station nearest) each sampling locality (Power and McKinley 1997, Climatic

Data Center 2011). This estimate of TOG is essentially the average number of degree

days per year (over a 30-year period) with temperatures above 18 °C. Degree days were

calculated as follows:

degree days (for the day) = mean temperature (for the day) – 18 °C

For example, a mean daily temperature of 25 °C would equal 7 degree days; degree days

were then summed for entire year, and an annual mean was calculated for 30 years

(Climatic Data Center 2011). Based on experimental data (see chapter 2), 18 °C is near

the temperature range where spotted gars greatly reduce or cease feeding activity.

Statistical Analyses

Komolgorov-Smirnov test for normality was used to determine if $\log_{10}$

transformation of the data was necessary. Analysis of variance (ANOVA) was used to

analyze and compare length and age distributions among populations and between sexes

among populations. ANOVA was also used to test for significant correlations between

age and length for each population. Analysis of covariance (ANCOVA) was used to test

for differences in length-at-age relationships between population segments, among

component populations, and between sexes within populations. Length-at-age models

were constructed directly from mean length of all individuals belonging to a given age

class for each population; no back-calculation of length-at-age was estimated due to

limited availability and potential high variability (in back-calculation) of different aging

structures for study populations. Slope of the length-at-age regression was used as a

proxy for growth rate, with significant differences in slope (significant difference in

population x age interaction factor) among regression models considered to be

differences in growth rate among populations. Higher slope value indicated higher growth rate and vice versa. Significant difference in the population factor of ANCOVA was considered to be significant difference in length at age among populations. For the remaining analyses, data for both sexes for each population were pooled to allow for comparisons with LE-p (for which sex data was unavailable) as well as between the overall peripheral and core population segments.

In addition to ANCOVA, I used the following derivation of the von Bertalanffy growth (VBGM) equation to model and compare growth rates among populations:

$$L_t = L_\infty - (L_\infty - L_0)*e^{(-kt)}$$

Where L_t is length at time t, L_∞ is the mean asymptotic length, L_0 is length at hatch (length at time zero), and k is the growth rate coefficient (Love 2001, Hart and Chute 2009). L_0 was set at 50 mm based on mean size at hatch of spotted gar fry (see chapter 2). Parameters L_∞ and k were estimated using non-linear regression (SPSS version 19, 2011). Significant differences in growth models among populations were determined by comparing the 95% confidence intervals for the von Bertalanffy growth parameters (Love 2001) as well as ANCOVA.

Total instantaneous mortality (Z) was estimated from catch-curve analysis using linear regression plotting natural log (catch) versus age (Ricker 1975). The slope of the regression line fit to the descending right portion of the catch-curve (Ricker 1975) was estimated as total instantaneous mortality (Z). Annual mortality (A) was then estimated from the equation:

$$A = 1-e^{(-Z)}$$

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Catch-curves were plotted separately for each population (male and female catch data pooled) and also combined as peripheral and core population segments. I excluded older age classes with consecutive catches of only 1 fish per class to avoid the influence of outliers on mortality estimates. Only significant correlations based on catch-curve regressions were used for determining mortality estimates. ANCOVA was used to determine if mortality rates were significantly different between core and peripheral population segments as well as among component populations.

In order to identify significant relationships between latitude and environmental variables, mean annual air temperature and TOG were regressed against latitude. Significant positive or negative correlations between latitude and environmental variables were considered to indicate environmental gradients potentially influencing spotted gar populations. In order to explore potential influence of environmental gradients on spotted gar populations, regression analysis was used plotting life history characteristics that showed significant variation among component populations (mean and maximum length, mean and maximum age, mortality) versus latitude, mean annual air temperatures (°C), and thermal opportunity for growth (TOG, degree days > 18 °C). In order to test for the effect of length of growing season, mean length for each age was divided by TOG to determine growth (mm) per degree day at age for each population. Mean "TOG-corrected" growth rate (mm/degree day) was then determined for each population and compared using ANOVA to test for significant differences in growth rates based on seasonality. TOG-corrected growth rate therefore accounts for length of growing season in a given year. Mean TOG-corrected growth rates were regressed with latitude and tested using ANOVA to identify significant correlations between growth rate and latitude

in spotted gars. TOG-corrected growth rate was also compared to the mortality model based on core and peripheral population segments to identify a potential countergradient between the two life history variables. All statistical tests were conducted with α set at 0.05.

Comparisons of populations in the results section are presented in the following order: 1- core versus peripheral population segments, 2- comparisons within the peripheral population segment, 3- comparisons between sexes within the peripheral population segment, 4- comparisons within the core population segment, 5- comparisons between sexes within the core population segment, and 6- relevant individual population and cross-population segment comparisons.

Results

A total of 36 fish from MI-p and 49 fish from LA-c were measured, sexed, and aged for this study; sample numbers for populations from other sources and studies as well as geographic and climate information are included in Table 3.2. Descriptive statistics for overall sample length and age by sex for each population are found in Table 3.3. Pooled length and age data for both sexes (in order to compare with LE-p) for each population as well as overall core and peripheral population segments are found in Table 3.4.

Mean length and age for overall population sample distributions varied significantly between core and peripheral population segments, as well among some individual populations and between sexes of some individual populations (Tables 3.5 and 3.6). Comparing overall sample distributions, ANOVA indicated that LE-p fish were

longer than MI-p fish, with no significant difference in age. Within MI-p, paired-sample t-tests indicated that females were larger and older than males (based on overall sample means). ANCOVA indicated that females were larger at age than males, although growth rate was not significantly different between sexes. Comparing overall sample distributions among populations in the core segment, all component populations were significantly different from each other in overall mean length and mean age. Overall mean length progressed largest to smallest from LA-c to GA-c to MO-c, and overall mean age progressed oldest to youngest from GA-c to LA-c to MO-c. Comparing sexes within the core segment populations, MO-c males and females were not significantly different in overall mean length or age, however ANCOVA indicated that growth rates were different between sexes, with females growing faster than males (Figure 3.2). GA-c males and females were also significantly different in overall mean length and age, with females larger and older than males. Growth rates were also significantly different between sexes for GA-c, with females larger at age and also growing faster than males. Overall mean length of LA-c females was greater than that of LA-c males, however mean age was not different between the sexes. LA-c females were larger at age than males, but growth rates were not significantly different. Complete cross-population segments pair-wise comparisons of overall mean length and age are found in Tables 3.5 and 3.6.

Estimation and comparison of growth rates among populations had different results from different analyses (length-at-age regression, von Bertalanffy growth models, TOG-corrected length-at-age regression); some results of which were ostensibly contradictory, however, TOG-corrected regression was the only analysis that accounted for length of growing season. ANCOVA indicated significant differences in length-at-

age regressions between population segments and among component populations of spotted gars (Table 3.7, Figures 3.3 and 3.4). Growth rate (slope), but not length at age (population factor), was significantly different between core and peripheral population segments, with core population fish growing faster than peripheral population fish. Length-at-age and growth rate did not significantly differ between MI-p and LE-p. Within the core population segment, MO-c was larger at age than GA-c, and had a slower growth rate than LA-c. GA-c had both a slower growth rate and was smaller at age than LA-c spotted gars. With the exception of growth rate comparison to MO-c, GA-c was consistently smaller at age and had a slower growth rate compared to all other study populations. Complete ANCOVA pair-wise comparisons of length-at-age and growth rates among spotted gar populations are found in Table 3.7.

Von Bertalanffy growth parameters (asymptotic length, L_∞, and growth coefficient, k; estimated from length and age data pooled for both sexes for each population) and growth curves were compared using 95% confidence intervals which indicated no significant differences between peripheral and core populations; however, significant variation was observed among individual populations both within and across segments (Table 3.8, Figures 3.5-3.7). Both MI-p and LE-p had larger asymptotic lengths than GA-c; and LA-c had a significantly higher growth coefficient than GA-c. The wide range in confidence intervals for LA-c growth parameters may have been a result of the weak (but significant) correlation between length and age for the population.

Mortality estimates (instantaneous, Z, and annual, A; tested using ANCOVA) based on catch-curve regression analysis were significantly different between peripheral ($Z = 0.53$) and core population segments ($Z = 0.82$); there were also significant

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differences between some component populations within the major segments (Table 3.9, Figure 3.8). LE-p and GA-c both experienced significantly higher mortality rates (0.63 and 0.76, respectively) than MO-c (0.32); all other comparisons were not significant.

Regression analysis of environmental variables (mean annual temperature, latitude, TOG) versus life history variables (mean and maximum length and age, mortality) based on individual populations revealed no significant correlations. However, regression analysis comparing environmental variables indicated significant negative correlations between TOG and latitude, and also negative correlations between mean annual temperature and latitude. Regression of TOG and latitude indicated over a six-fold decrease in TOG from northernmost (424 degree days) to southernmost localities (2773 degree days; Figure 3.9). Correlations of the environmental variables suggested latitudinal gradients in seasonality and temperature among spotted gar population localities (Table 3.10, Figure 3.9).

When thermal opportunity for growth was incorporated into length-at-age regression models, significant differences in growth rate, a reversal of those observed when TOG was not incorporated, were observed between peripheral and core population segments (Table 3.11, Figure 3.10). ANOVA indicated that peripheral and core population segment (TOG-corrected) growth rates were significantly different, with peripheral population spotted gars growing faster (relative to length of growing season) than core population spotted gars; these results were a reversal of those observed for growth rate comparisons that did not incorporate TOG. When comparing component populations, LE-p had the shortest TOG (424 degree days) and highest growth rate (1.44 mm/degree day), followed by MI-p (second shortest TOG, second highest growth rate),

then MO-c, and finally LA-c and GA-c (both of which did not significantly differ from each other in growth rate). Mean annual (TOG-corrected) growth rate was significantly positively correlated with latitude, and indicated a countergradient between growth rate and thermal opportunity for growth (Figure 3.11). Mortality (Z), based on peripheral and core population segment models (component population data pooled), suggested a negative correlation with latitude and also indicated a countergradient with TOG-corrected growth rate; regression lines for mortality and growth rate crossed at approximately 40 °N (Figure 3.12).

Discussion

I hypothesized that spotted gars from two major population segments (core and peripheral) would exhibit variation in life history traits such as length-at-age, VBGM parameters, and mortality rates. I also hypothesized that variation in life history traits between the two population segments as well as among individual populations could be related to latitudinal gradients in environmental variables such as mean annual temperature and thermal opportunity for growth. My study showed that spotted gars from peripheral and core population segments exhibited variation in life history traits such as growth rate and mortality, and component populations from each segment also exhibited interpopulation variation. Additionally, a portion of this variation could be explained by differences in length of growing season at different latitudes, although non-latitudinal influences likely played a substantial role in interpopulation variation as well. Thermal opportunity for growth (TOG) and mean annual temperature significantly decreased with increasing latitude, indicating a clinal gradient from northern to southern

populations. Von Bertalanffy growth models indicated that growth rates were not significantly different between peripheral and core population segments nor among the majority of component populations (small asymptotic length in GA-c relative to MI-p and LE-p was the only exception), however TOG-corrected growth models indicated significantly higher growth rates in northern peripheral populations compared to southern core populations. Mortality rate was lower for peripheral segment fish than core segment fish, which may be a form of compensatory mortality in higher-latitude populations of spotted gars (Beamesderfer and North 1995). These results suggest potential latitudinal variation in the form of countergradient variation in growth and mortality exists among populations of spotted gars (Conover et al. 2009).

Some of my results supported other studies that investigated life history trends in spotted gars, other lepisosteids, and other ectotherms, such as greater mean size, mean age, and size-at-age in higher-latitude populations. Previous studies on other populations of spotted gars, primarily from core populations, also suggest variation in life history traits from southern to more northern populations. Love (2001, 2004) compared spotted gar populations from Lake Ponchartrain, Louisiana, and Mingo Swamp, Missouri (Redmond 1964), and found that gars from the more northern population achieved a greater maximum age (approximately 18 years in MO compared to 10 years in LA). Similarly to Redmond (1964), a population of spotted gars in Kentucky had a maximum reported age of 15 years (Holt 1973). Other studies of spotted gar populations from more similar latitudes have also observed variation in age, suggesting other influences on interpopulation age structure of spotted gars. Ferrara (2001) found that maximum and mean ages for spotted gars from Lake Seminole, GA, were 10 and 5 years, respectively,

whereas Smith (2008) found maximum and mean ages in the Barataria Estuary System, LA, to be 6 and 3 years, respectively. In my study, overall mean age of spotted gars from the peripheral population segment (6.10 years) was greater than that of the lower-latitude core population segment (4.53 years), however variation in age among individual populations within the core segment did not fully support a latitudinal trend. Mean age increased from LA-c to GA-c, but decreased between GA-c to MO-c; maximum reported age, however, did increase from LA-c (7 years) to GA-c (10 years) to MO-c (approximately 18 years; Redmond 1964). Similar to the potential trend in lifespan and increasing latitude that I observed in spotted gars, Munch and Salinas (2009) showed that many other ectotherms demonstrated increasing lifespan as latitude increased, and that this correlation was primarily associated with temperature. With effects of temperature removed from their models, however, substantial intraspecific variation remained, suggesting that localized factors were also important influences on lifespan within species (Munch and Salinas 2009). Given my results, variation in lifespan may be more greatly influenced by latitude and temperature among more geographically distant populations (such as peripheral and core segments, or MI-p/LE-p and GA-c/LA-c), and more influenced by local factors among geographically closer populations (GA-c, LA-c). Additionally, the converse of Munch and Salinas' (2009) results suggests that mortality may decrease with increasing latitude; my results indicated that peripheral segment spotted gars had a significantly lower mortality rate than core segment spotted gars. Sampling of additional populations would better inform potential latitudinal variation in lifespan and mortality in spotted gars.

Glass et al. (2011, also this study) found the age range of spotted gars in Rondeau Bay, Lake Erie (LE-p) to be 3-10 years, a much lower maximum age than that of Michigan inland lake spotted gars (16 years); however overall mean age between the two peripheral populations was not significantly different. Additionally, use of different aging techniques may have underestimated the age of older fish. Glass et al. (2011) cautioned the use of pectoral ray sectioning in aging older fish; in the comparative analysis of three aging structures (otoliths, branchiostegal rays, pectoral fin rays), pectoral fin rays, when in disagreement with the accepted age, underestimated age by 1-2 years. Although different aging techniques may have contributed to differing maximum age estimates, differences (although not as great) in maximum age among similarly proximal populations in the core segment such as GA-c (max age 10 years) and LA-c (max age 7 years) did exist. Additional comparisons to other peripheral populations of spotted gars (such as northern Indiana or Lake Erie populations in Pennsylvania) may result in a better understanding of geographic variation in age structure.

My study compared mean length, age, and length-at-age between sexes among populations and found results similar to other studies on spotted gars and other lepisosteids. Female spotted gars from Michigan were significantly longer and older than males (overall mean length and weight), and were also longer at age than males. Love (2002, 2004) observed similar patterns in spotted gars, and the same trends have been observed in other lepisosteids such as the Florida gar *L. platyrhincus* (Murie et al. 2009), longnose gar (Johnson and Noltie 1997, McGrath and Hilton 2011), and shortnose gar *L. platostomus* (Ladonski 1998). In contrast to some core populations (GA-c, MO-c) and

other studies (Redmond 1964, Love 2004), however, Michigan fish did not differ in growth rate between sexes.

Mortality estimates were greater for core population fish than peripheral population fish, and when regressed against latitude, suggested an inverse relationship which may have been due to several factors, including potential compensatory mortality (Allen et al. 1998). Predation pressure is higher for southern populations than northern populations and may contribute to differences in mortality between population segments. Natural predators of gars such as alligators *Alligator mississpiensis* are abundant in southern states whereas adult spotted gars generally lack natural predators in northern states (Love 2001, Murie et al. 2009). Higher metabolic costs associated with higher temperatures at lower latitudes may also increase mortality in southern populations (Atkinson and Sibley 1997). Density-dependent factors may also affect mortality rates by means of limited food and other resources in southern populations, as spotted gars are considered much more abundant at lower latitudes than northern latitudes (Suttkus 1963, Trautman 1981). Allen et al. (1998) noted that compensatory mortality is a form of density-dependent population regulation, and that populations at higher density (such as core spotted gars) may be more regulated by density-dependent factors, whereas populations at lower density (such as peripheral spotted gars) may be more regulated by density-independent factors. Beamesderfer and North (1995) also noted that natural mortality rate was negatively correlated with increasing latitude in largemouth bass *Micropterus salmoides* and smallmouth bass *M. dolomieu* populations, and that lower mortality may compensate for decreased productivity of populations at higher latitudes. Sample size of older individuals may also have affected my mortality estimates;

Redmond (1964) excluded several older (age > 8 years) individuals from his analysis that he felt could not be accurately aged, including a spotted gar at least 18 years of age. My MI-p sample size was low overall (N = 36 fish), however, I did collect four individuals over 10 years old; additional sampling of larger age classes would provide a better estimate of mortality for both core and peripheral population segments.

Von Bertalanffy growth models indicated that growth rates for spotted gars from peripheral and core population segments, as well as their individual component populations, were not significantly different (the only exception being asymptotic length of GA-c compared to peripheral populations). Although VBGM indicated growth rates were similar among populations of spotted gars, the length of growing season, based on thermal opportunity for growth, decreased six-fold from southern latitudes to northern latitudes, therefore compensatory mechanisms may be contributing to the similar growth rates (Conover 2009). Conover (2009) suggested that similar phenotypes within a species at different latitudes (and different lengths of growing season) may be indicative of "cryptic" countergradient variation in growth. When CnGV exactly compensates for environmental influence on phenotypically plastic traits, the result would be no identifiable change in phenotypes across latitudes. In the case of spotted gar populations, statistically identical VBGM growth models may fit this compensatory phenomenon.

When thermal opportunity for growth was incorporated into length-at-age models, differences between core and peripheral population segments were even more distinct, with peripheral population fish exhibiting significantly higher growth rate than core population fish; this was a reversal of results from length-at-age comparisons that did not account for TOG among populations. Although length of growing season is considerably

shorter (based on TOG) at northern latitudes, peripheral population spotted gars were still of similar length at age in comparison to core population fish, and exhibited higher growth rates. These results suggest a countergradient in growth rate versus TOG may exist among populations of spotted gars. These seemingly contradictory results in growth rate between core and peripheral population segments (compared to results for models that did not account for TOG) reflect the importance of TOG (length of growing season) in comparisons of growth rate across latitudes (Conover 1990, Power and McKinley 1997). Power and McKinley (1997) found that lake sturgeon from higher latitudes (and therefore smaller TOG) were smaller in length, weight, and condition factor than those from lower latitudes, however, when TOG was incorporated into their models, growth rates were shown to be higher at higher latitudes than at lower latitudes. Although mean size at end of growing season of lake sturgeon was still smaller for higher latitude fish than lower latitude fish, Power and McKinley (1997) showed that countergradient variation in growth partially compensated for shorter growing season. My regression models for length-at-age suggest that CnGV may fully compensate for the reduced TOG at higher latitudes, since peripheral population spotted gars were (statistically) the same length at age as core population spotted gars.

Differences in TOG between peripheral populations may also have significant impacts on growth rate; TOG for Lake Erie was estimated to be 146 degree-days (26 %) less than Michigan inland lakes, yet mean growth rate was significantly different between the two peripheral populations (growth rate LE-p > MI-p). Baumann and Conover (2011) showed that CnGV evolved in the Pacific silversides *Atherinops affinis* even across small gradients in seasonality, and suggested that CnGV may be the prevalent adaptive

mechanism across spatial temperature gradients. In comparison to the pattern observed by Baumann and Conover (2011) and between peripheral populations, TOG for GA-c was 222 degree-days (8 %) less than LA-c and growth rates between the two core populations were not significantly different; this result suggests that differences in TOG at lower latitudes, where length of growing season proportionally much longer than at higher latitudes, may have less impact on growth rate. Study of additional spotted gar populations from similar latitudes within core and peripheral segments may indicate whether small differences in TOG have greater influence on growth rate among higher or among lower latitudes.

Other abiotic and biotic factors such as prey availability and habitat type may have had a stronger influence on growth rate than TOG (comprised of mean annual temperature, seasonality, and latitude) at more localized scales or similar latitudes. Wagner et al. (2007) showed that temperature was not the only major influence on regional trends in mean length-at-age of fishes from Michigan and Wisconsin inland lakes, and that among-lake differences accounted for a large portion of variation in mean length-at-age trends.   Keeley et al. (2006) demonstrated that different habitat types, such as lentic and lotic environments, had a significant influence on phenotype of rainbow trout *Oncorhynchus mykiss.*

Ostrand et al. (2004) showed that both vegetation density and prey type affected foraging success and behavior in spotted gars, therefore these factors likely varied and influenced growth in my study populations. Lagler et al. (1942) found that gizzard shad *Dorosoma cepedianum* and other forage fishes were the primary components of gars' diets (longnose, shortnose, and spotted gars) in southern Indiana, whereas Snedden et al.

(1999) and Robertson et al. (2008) found crayfish to be the main component of spotted gar diets in Louisiana and Texas, respectively. Slaughter et al. (2008) showed that in a common environment, prey availability and density-dependent factors had a greater influence on growth of largemouth bass compared to latitudinal origin of populations and associated capacity for growth. Hoxmeier et al. (2009) showed that in 23 populations of bluegill *Lepomis macrochirus* across several latitudes, after temperature, prey availability and water clarity were the most important factors influencing growth rates. Yamahira et al. (2007) also suggested that growth rates (based on medaka, *Oryzias latipes*) are influenced by seasonal environmental fluctuations other than temperature, and that differences in prey availability may have relatively stronger influences on growth rate at higher latitudes than lower latitudes due to the shorter growing season.

Some results could have been confounded by sampling error in collection and sex identification of MI-p spotted gars as I found no young females (less than 5 years old) nor small individuals (less than 550 mm). Sex of spotted gars is not positively identifiable without internal examination of gonad release pathways (Ferrara and Irwin 2001), and I was limited in the number of individuals that could be sacrificed due to the protected status of the species in Michigan (Michigan Department of Natural Resources 2006). Time of sampling differed among populations, however, most populations were sampled just before and/or during their spawning seasons regardless of latitude, therefore differences in length at age due to time of collection should be minimal. I only used electrofishing for collecting spotted gars in MI as opposed to other (sometimes multiple) methods (gill nets, electrofishing, fyke nets) used for other populations (LE-p, MO-c, LA-c); electrofishing still allowed me to collect a wide distribution of lengths and ages of

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spotted gars (smaller individuals may pass through fyke and gill nets), but utilizing other gear-types may better increase sample size in future work. Sample sizes for other studies (Redmond 1964, Ferrara 2001, Love 2001 and 2004) were larger than that of my study, therefore maximum age and size may be even greater than our estimates for MI populations. Larger sample sizes may also provide better estimates of mortality, as some component populations did not show significant correlations in mortality regression models, although combined component population catch data did result in significant correlations for core and peripheral segments. My sample of Louisiana spotted gars was also relatively small (49 fish) and showed a weak (but still significant) correlation between length and age; a larger sample size may provide a more robust relationship between length and age as well as less variance in VBGM parameter estimates (LA-c had the largest confidence intervals. Munch and Salinas (2009) found that body size did not have a strong influence on lifespan, but that temperature was a more important factor in explaining latitudinal variation in longevity; spotted gars at much lower latitudes may therefore show a weaker relationship between size and age than those at higher latitudes.

Biogeography and genotype may also have played a role in some of the interpopulation variation in spotted gars. All samples except for GA-c came from populations that are presently or were at one time connected with the Mississippi River drainage basin (Bailey and Smith 1981, Hocutt and Wiley 1976). GA-c spotted gars came from the Apalachicola River drainage basin on the eastern Gulf Coast; in a study of gar phylogeography, Spiorski (2011) found that spotted gars from the eastern Gulf Coast region, particularly the Apalachicola River basin had a genetic makeup more similar to the Florida gar than other spotted gar populations from the Mississippi River basin.

Although considered the sister species to the spotted gar, the Florida gar reaches a smaller maximum size, has a lower growth rate, and longer lifespan than the spotted gar (Murie et al. 2009). In my study GA-c spotted gars were consistently different from most other spotted gar populations, having a smaller size at age and lower growth rate. Additional comparisons to populations outside the Mississippi River basin as well as genetic analyses may better inform interpopulation differences in spotted gars as well as closely related species (see chapter 4).

My study has found significant variations in life history characteristics between core and peripheral population segments of spotted gars, as well as interesting inter-population variations within both the core and peripheral regions. Significant variations among populations within core and peripheral segments suggest that additional local-scale factors that were not explored in my study, such as habitat type and prey availability, as well as potential genetic variation, likely play a role in spotted gar population diversity. The peripheral population of spotted gars was shown to be a distinct component of the overall species. Understanding its unique characteristics will undoubtedly be important in terms of conservation planning, and holds additional value in its potential as a case study that can help us better understand the evolution and maintenance of vertebrate life history patterns.

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Table 3.1. Comparison of estimated age for 10 MI-p spotted gars collected fall 2008 using 3 aging structures (pectoral rays, branchiostegal rays, otoliths). Ages in years were determined by two separate readers. Index of precision is shown in parentheses for each structure, with age based on branchiostegal reader 1 taken as the standard, therefore index of precision is zero for all branchiostegal reader 1 ages. Mean index of precision for each structure was 0.03 for branchiostegal rays, 0.11 for pectoral rays, and 0.14 for otoliths. All structures were determined to be valid aging methods as index of precision scores were all less than 0.29 (Den Haas and Mandrak 2004). Table modified from Glass et al. (2011).

Fish Number	Length (mm)	Sex	Pectoral ray reader 1	Pectoral ray reader 2	Branchiostegal reader 1	Branchiostegal reader 2	Otolith reader 1	Otolith reader 2
118	545	male	4 (0.2)	4 (0.2)	5	5 (0)	7 (0.4)	8 (0.6)
120	620	female	7 (0)	7 (0)	7	7 (0)	7 (0)	8 (0.14)
121	550	female	4 (0.2)	5 (0)	5	5 (0)	5 (0)	5 (0)
122	504	male	3 (0)	3 (0)	3	2 (0.33)	3 (0)	4 (0.33)
123	480	male	2 (0)	2 (0)	2	2 (0)	3 (0.5)	3 (0.5)
124	650	female	6 (0.14)	7 (0)	7	7 (0)	7 (0)	8 (0.14)
125	405	male	2 (1)	1 (0)	1	1 (0)	1 (0)	1 (0)
127	520	male	4 (0)	4 (0)	4	4 (0)	4 (0)	4 (0)
128	759	female	12 (0.14)	14 (0)	14	14 (0)	14 (0)	14 (0)
130	685	female	7 (0.13)	7 (0.13)	8	8 (0)	9 (0.13)	9 (0.13)

Table 3.2. List of spotted gar population data used in life history analyses. Includes environmental variables data for latitude (°North), mean annual air temperature from weather station nearest sampling locality (°C), and thermal opportunity for growth (TOG) as mean annual sum of degree days > 18 °C from weather station nearest sampling locality.

Population	Code	Locality	Source	Year	Population Segment	N
Michigan	MI-p	Michigan inland lakes	this study	2008-2010	peripheral	36
Lake Erie	LE-p	Rondeau Bay, Lake Erie, Canada	Glass, unpublished data	2007	peripheral	78
Missouri	MO-c	Mingo Swamp, Missouri	Redmond 1964	1962-1963	core	100
Georgia	GA-c	Lake Seminole, Georgia	Ferrara 2001	1999-2000	core	194
Louisiana	LA-c	Bayou Chevreuil, Louisiana	this study	2009-2010	core	49

Table 3.2. Extended. List of spotted gar population data used in life history analyses. Includes environmental variables data for latitude (°North), mean annual air temperature from weather station nearest sampling locality (°C), and thermal opportunity for growth (TOG) as mean annual sum of degree days > 18 °C from weather station nearest sampling locality.

Population	Code	Latitude	Mean Temperature	TOG
Michigan	MI-p	42.00	9	570
Lake Erie	LE-p	42.25	10	424
Missouri	MO-c	37.00	14	1639
Georgia	GA-c	30.75	20	2551
Louisiana	LA-c	29.75	20	2773

Table 3.3. Descriptive statistics for length (mm) and age (years) entire (overall) sample distributions of spotted gar populations used in life history analyses. Data is presented by sex for all populations except LE-p. Peripheral and core population segment data are indicated in bold.

LENGTH

Population	Sex	N	Mean	Min	Max	Med	StDev	StE
MI-p	male	27	547	405	735	545	73.24	14.09
	female	9	675	550	785	685	74.31	24.77
LE-p	combined	78	605	515	748	581	62.87	7.12
Peripheral		**114**	**597**	**405**	**785**	**580**	**73.78**	**6.91**
MO-c	male	54	484	259	551	500	106.05	14.43
	female	46	551	274	787	424	150.29	22.16
GA-c	male	101	455	239	580	458	64.68	6.44
	female	93	528	251	726	540	97.97	10.16
LA-c	male	25	543	455	660	531	49.89	9.98
	female	24	613	501	745	607	68.26	13.93
Core		**343**	**487**	**239**	**787**	**503**	**108.85**	**5.88**

AGE

Population	Sex	N	Mean	Min	Max	Med	StDev	StE
MI-p	male	27	6	1	16	5	3.34	0.64
	female	9	9	5	14	8	2.69	0.90
LE-p	combined	78	6	3	10	6	1.60	0.18
Peripheral		**114**	**6**	**1**	**16**	**6**	**2.33**	**0.22**
MO-c	male	54	3	1	8	3	1.83	0.25
	female	46	3	1	8	2	2.03	0.30
GA-c	male	101	5	1	9	5	1.62	0.16
	female	93	6	2	10	6	1.78	0.19
LA-c	male	25	4	2	7	4	1.16	0.23
	female	24	5	3	7	4	1.25	0.26
Core		**343**	**5**	**1**	**10**	**5**	**2.01**	**0.11**

Table 3.4. Descriptive statistics for length (mm) and age (years) for entire (overall) sample distributions of spotted gar populations used in life history analyses. Data for both sexes were pooled to allow for comparisons with LE-p. Peripheral and core population segment data are indicated in bold.

LENGTH

Population	N	Mean	Min	Max	Med	StDev	StE
MI-p	36	579	405	785	571	91.70	15.28
LE-p	78	605	515	748	581	62.87	7.12
Peripheral	**114**	**597**	**405**	**785**	**580**	**73.78**	**6.91**
MO-c	100	437	259	787	462	127.67	12.77
GA-c	194	490	239	726	494	89.94	6.46
LA-c	49	577	455	745	557	68.75	9.82
Core	**343**	**487**	**239**	**787**	**503**	**108.85**	**5.88**

AGE

Population	N	Mean	Min	Max	Med	StDev	StE
MI-p	36	6	1	16	6	3.45	0.58
LE-p	78	6	3	10	6	1.58	0.18
Peripheral	**114**	**6**	**1**	**16**	**6**	**2.33**	**0.22**
MO-c	100	3	1	8	3	1.92	0.19
GA-c	194	5	1	10	5	1.72	0.12
LA-c	49	4	2	7	4	1.32	0.18
Core	**343**	**5**	**1**	**10**	**5**	**2.01**	**0.11**

Table 3.5. Matrix of pair-wise ANOVA comparisons for overall mean age and length of peripheral and core populations of spotted gars by sex. Above diagonal = mean length comparisons, below diagonal = mean age comparisons. Significant differences between pairs are designated with "+" and non-significant comparisons designated with "-".

Population	MI-p male	MI-p female	LE-p	MO-c male	MO-c female	GA-c male	GA-c female	LA-c male	LA-c female
MI-p male		+	+	+	+	+	-	-	+
MI-p female	+		-	+	+	+	+	+	-
LE-p	-	+		+	+	+	+	+	-
MO-c male	+	+	+		-	-	+	+	+
MO-c female	+	+	+	-		+	+	+	+
GA-c male	-	+	+	+	+		+	+	+
GA-c female	-	+	-	+	+	+		-	+
LA-c male	+	+	+	-	-	+	+		+
LA-c female	-	+	+	+	+	-	+	-	

Table 3.6. Matrix of pair-wise ANOVA comparisons for overall mean age and length of peripheral and core populations of spotted gars. Above diagonal = mean length comparisons, below diagonal = mean age comparisons. Age and length data were pooled for both sexes for all populations to allow for comparison with LE-p. Significant differences between pairs are designated with "+" and non-significant comparisons designated with "-".

Population	MI-p	LE-p	MO-c	GA-c	LA-c
MI-p		+	+	+	-
LE-p	-		+	+	+
MO-c	+	+		+	+
GA-c	+	+	+		+
LA-c	+	+	+	+	

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Table 3.7. Matrix of pair-wise ANCOVA for length-at-age and growth rate of peripheral and core populations of spotted gars. Above diagonal = length at age comparisons, below diagonal = growth rate. Significant differences between pairs are designated with "+" and non-significant comparisons designated with "-".

Population	MI-p	LE-p	MO-c	GA-c	LA-c	PERI	CORE
MI-p		-	-	+	-	-	-
LE-p	-		-	+	-	-	+
MO-c	+	+		+	-	-	-
GA-c	+	+	-		+	+	-
LA-c	-	-	+	+		-	+
PERI	-	-	+	+	-		-
CORE	+	-	-	-	-	+	

Table 3.8. Von Bertalanffy growth model (VBGM) parameters for core and peripheral populations of spotted gars. Parameters were compared using 95% confidence intervals ("upper and lower CI").

Asymptotic Length

Population	L_∞ (mm)	Std. Error	lower CI	upper CI	N	Range (years)
MI-p	777	29.77	712	842	36	1-16
LE-p	872	53.07	744	1004	78	3-10
Peripheral	780	31.20	712	848	114	1-16
MO-c	704	18.17	659	748	100	1-8
GA-c	629	15.78	593	656	194	1-10
LA-c	781	64.41	615	947	50	2-8
Core	681	18.68	638	724	344	1-10

Growth Coefficient

Population	k	Std. Error	lower CI	upper CI	N	Range (years)
MI-p	0.32	0.04	0.24	0.40	36	1-16
LE-p	0.39	0.05	0.27	0.51	78	3-10
Peripheral	0.34	0.04	0.25	0.43	114	1-16
MO-c	0.32	0.02	0.26	0.37	100	1-8
GA-c	0.27	0.02	0.22	0.31	194	1-10
LA-c	0.61	0.10	0.36	0.87	50	2-8
Core	0.30	0.03	0.25	0.36	344	1-10

Table 3.9. Instantaneous (Z), annual (A), and percent annual (A%) mortality estimates and coefficient of determination for core and peripheral populations of spotted gars. Mortality was estimated for both sexes combined using catch-curve analysis (Ricker 1975). Correlations were significant (indicated in bold) for LE-p, Peripheral, MO-c, GA-c, and Core populations. ANCOVA indicated that mortality rates were significantly different between peripheral and core populations as well as between LE-p and MO-c and between GA-c and MO-c.

Population	Z	R-squared	A	A%
MI-p	0.19	0.60	0.17	17.30
LE-p	**0.63**	**0.89**	**0.47**	**46.74**
Peripheral	**0.53**	**0.96**	**0.41**	**41.14**
MO-c	**0.32**	**0.91**	**0.27**	**27.39**
GA-c	**0.76**	**0.92**	**0.53**	**53.23**
LA-c	0.49	0.87	0.39	38.74
Core	**0.82**	**0.95**	**0.56**	**55.96**

Table 3.10. Summary table of variables for all study populations. Variables and units are as follows: TOG = thermal opportunity for growth, degree days above 18 °C; Mean Temp = mean air temperature of locality (degrees °C); LAT = latitude °North of locality; L_∞ = asymptotic length parameter of von Bertalanffy growth model; k = growth coefficient parameter of von Bertalanffy growth model; Mean Length = overall mean length (mm) for population; Mean Age = overall mean age (years) for population; Max Length = max age (years) for population; Z = instantaneous mortality estimate for population; A% = percent annual mortality estimate for population.

Population	TOG	Mean Temp	LAT	L_∞	k	Mean Length	Mean Age	Max Length	Max Age	Z	A%
MI-p	570	9	42.00	777	0.32	579	6	785	16	0.07	7
LE-p	424	10	42.25	872	0.39	605	6	748	10	0.77	54
MO-c	1639	14	37.00	704	0.32	437	3	787	8	0.32	27
GA-c	2551	20	30.75	629	0.27	490	5	726	10	0.76	53
LA-c	2773	20	29.75	781	0.61	577	4	745	7	0.29	25

Table 3.11. Matrix of pair-wise ANOVA for TOG-corrected growth rate and difference in degree days for peripheral and core populations of spotted gars. Above diagonal = absolute value of difference in degree days between populations, below diagonal = mean growth rate with TOG incorporated (mm/degree day). Significant differences between population pairs are designated in bold. Growth rates were significantly different among all populations except between GA-c and LA-c. Mean TOG-corrected growth rate for each population are as follows: LE-p (1.44 mm/dd), MI-p (1.06 mm/dd), MO-c (0.32 mm/dd), GA-c (0.19 mm/dd), and LA-c (0.20 mm/dd).

Population	LE-p	MI-p	MO-c	GA-c	LA-c
LE-p		146	1215	2127	2349
MI-p	**0.38**		1069	1981	2203
MO-c	**1.12**	**0.74**		912	1134
GA-c	**1.25**	**0.87**	**0.13**		222
LA-c	**1.24**	**0.86**	**0.12**	0.01	
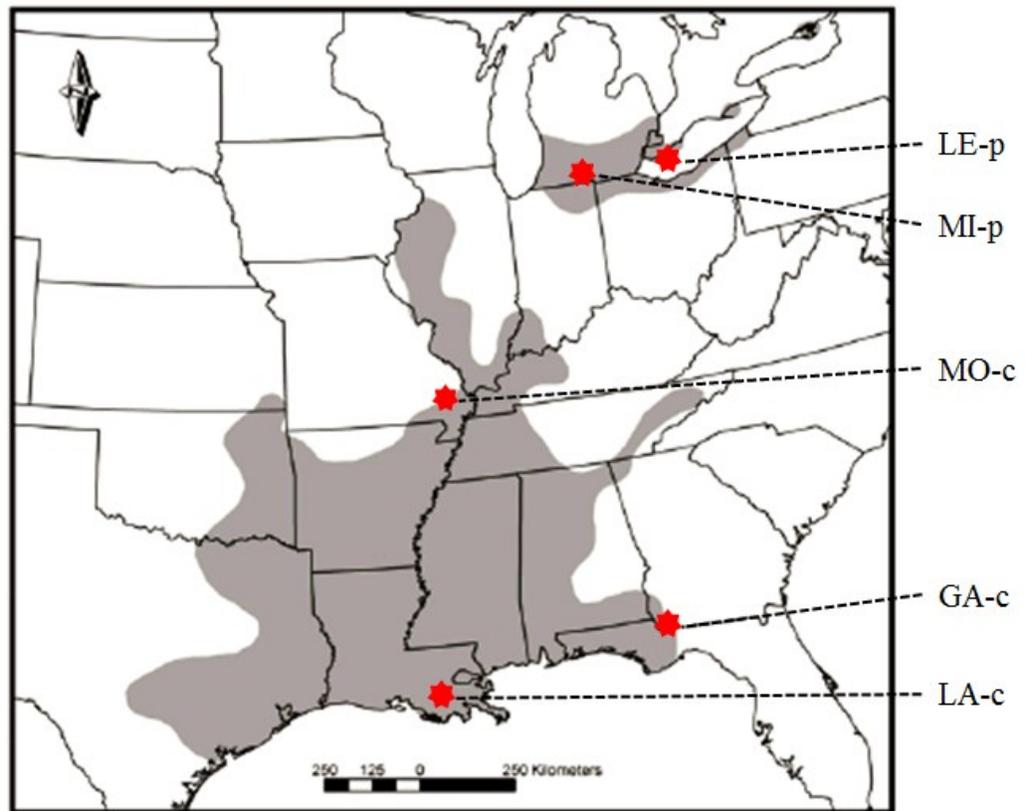
Figure 3.1. Map of spotted gar localities used in core versus peripheral population analyses. Peripheral populations consisted of 2 localities (Michigan inland lakes, MI-p; and Rondeau Bay Lake Erie, LE-p); Core populations consisted of 3 localities (Mingo Swamp, Missouri, MO-c; Lake Seminole, Georgia, GA-c; and Bayou Chevreuil, Louisiana, LA-c). Original population data sources are as follows: MO-c, Redmond (1964); GA-c, Ferrara (2001); LE-p, Glass (unpublished data), MI-p and LA-c, this study. Range distribution map modified from Page and Burr (1991).
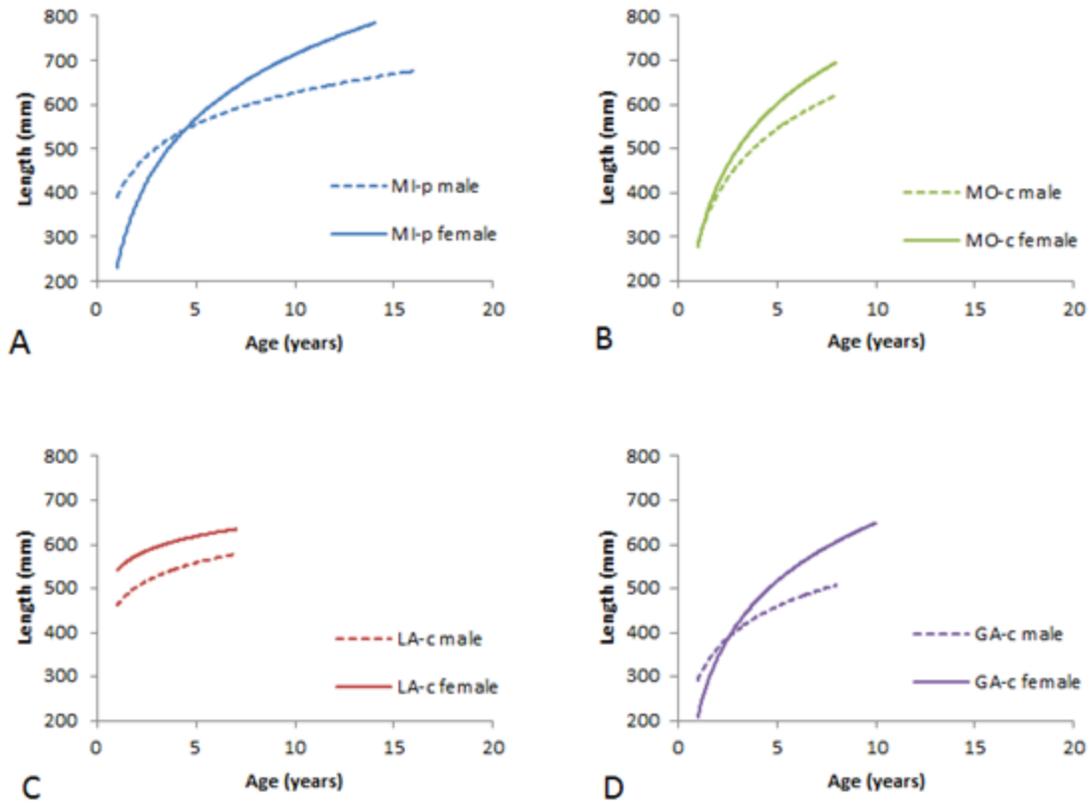
Figure 3.2. Length at age regressions by sex for peripheral and core populations of spotted gars. ANCOVA was used to determine significant differences in length at age and growth rate between sexes; significant difference in slope indicated different growth rates, significant difference in male/female effect indicated different length at age. Dashed lines represent male fish, solid lines represent female fish. A. ANCOVA indicated that MI-p females were larger at age than males, but growth rates were not significantly different. B. ANCOVA indicated that MO-c length at age was the same for males and females, but females had a faster growth rate. C. ANCOVA indicated that females were larger at age than males, but growth rates were not significantly different. D. ANCOVA indicated that females were larger at age than males and also had a faster growth rate.

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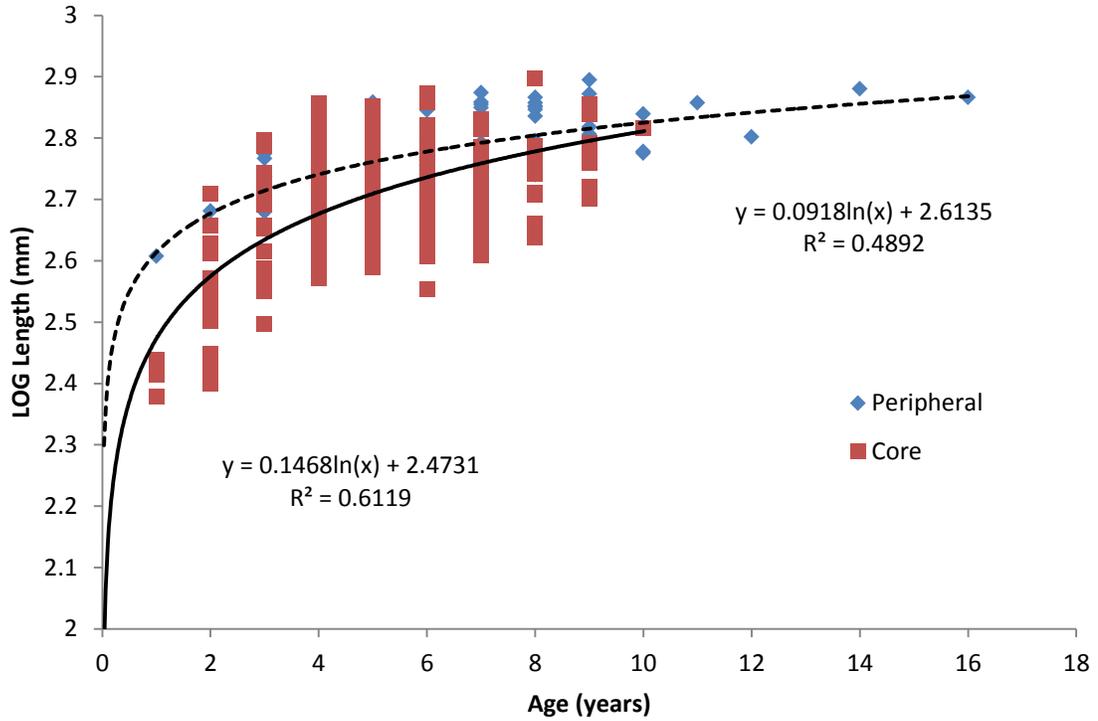
Figure 3.3. Length at age regressions for peripheral and core population segments of spotted gars. Dashed line represents peripheral population (MI-p, LE-p) regression model, solid line represents core population (MO-c, GA-c, LA-c) regression model. ANCOVA indicated that slopes (growth rate) were significantly different between core and peripheral populations, with core population fish growing faster than peripheral population fish. Overall length-at-age between the two populations was not significantly different (based on population factor comparison in ANCOVA).
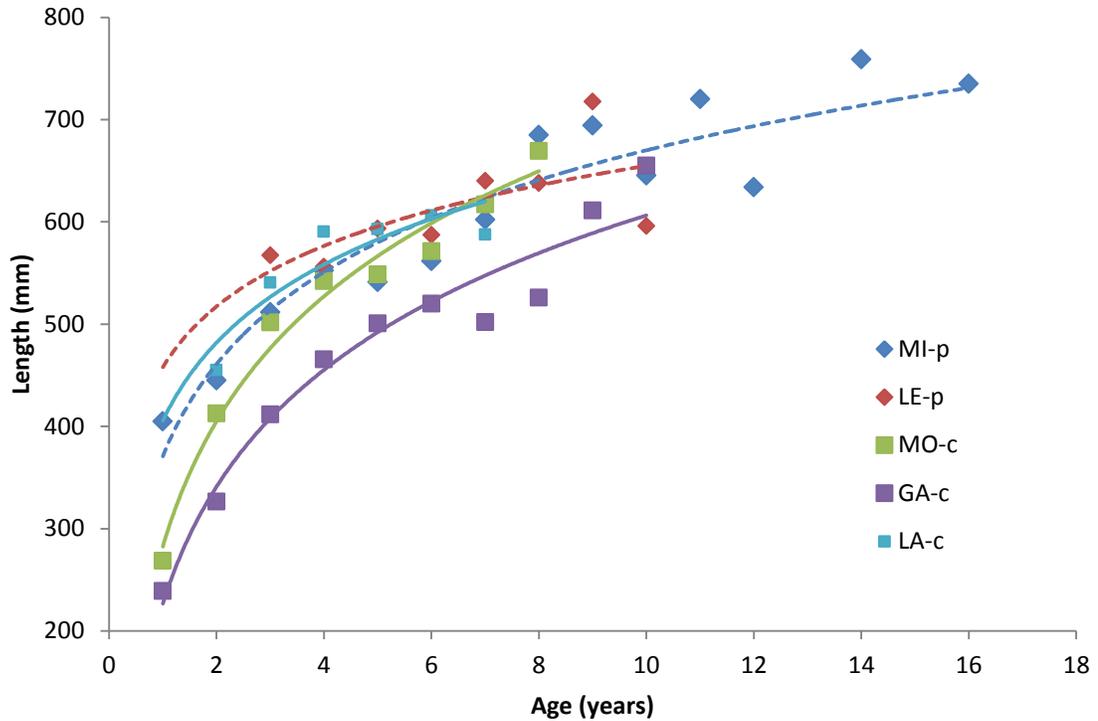
Figure 3.4. Length at age regressions for peripheral and core populations of spotted gars. Dashed lines represent peripheral population models (MI-p, LE-p), solid lines represent core population models (MO-c, GA-c, LA-c). ANCOVA indicated that length at age and growth rates were not significantly different between MI-p and LE-p. Within the core population segment, MO-c was larger at age than GA-c, and had a slower growth rate than LA-c. GA-c had a slower growth rate and was smaller at age than LA-c. Across population segments, MI-p was larger at age than GA-c, and had a slower growth rate than MO-c and GA-c. LE-p was larger at age than GA-c, and had a slower growth rate than GA-c and MO-c.
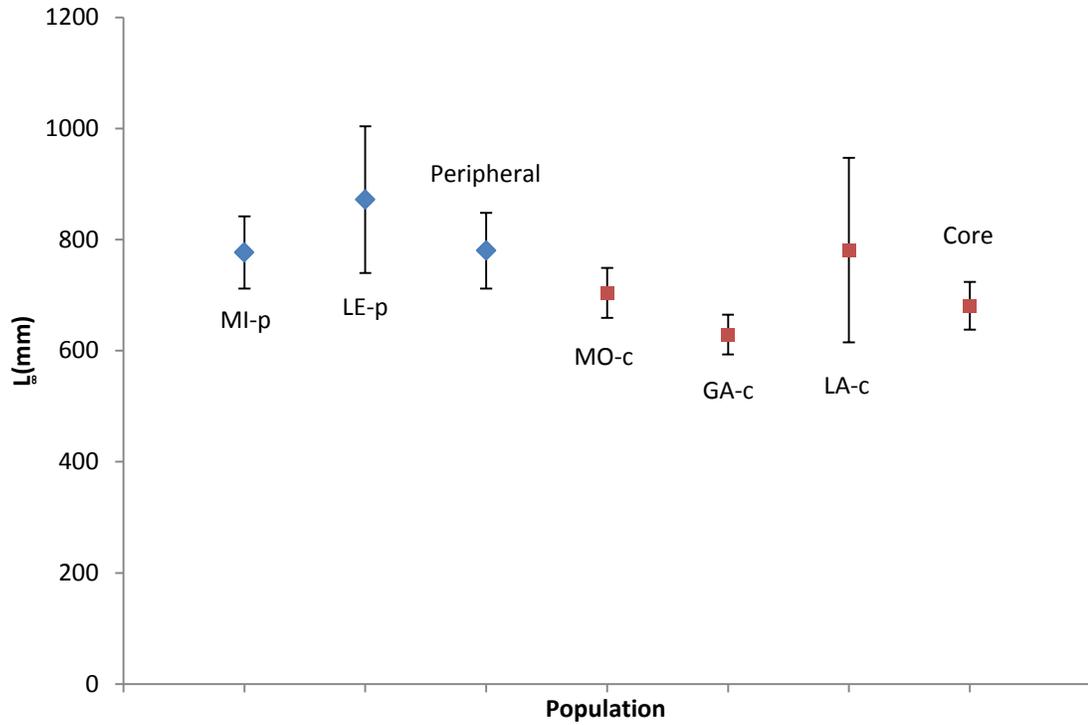
Figure 3.5. Von Bertalanffy growth parameter L_∞, asymptotic length, for core and peripheral populations of spotted gars. Bars represent 95% confidence intervals. MI-p and LE-p had larger asymptotic length than GA-c; all other comparisons were not significantly different.
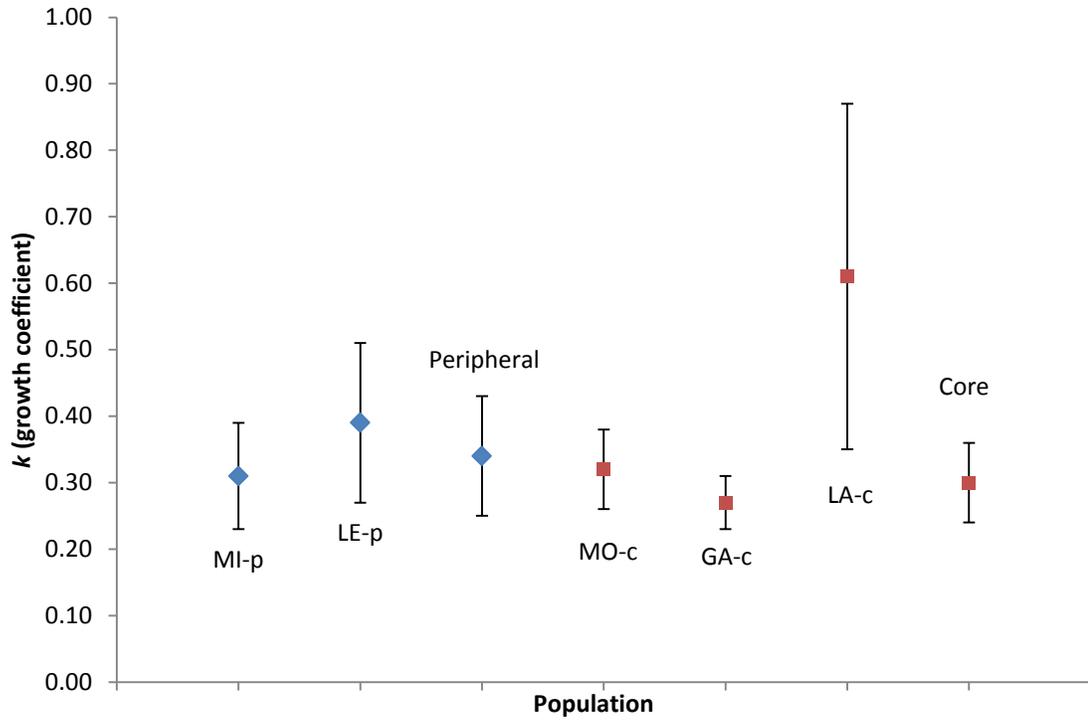
Figure 3.6. Von Bertalanffy growth parameter k, coefficient of growth, for core and peripheral populations of spotted gars. Bars represent 95% confidence intervals. LA-c had a significantly higher growth coefficient than GA-c, all other comparisons were not significant.
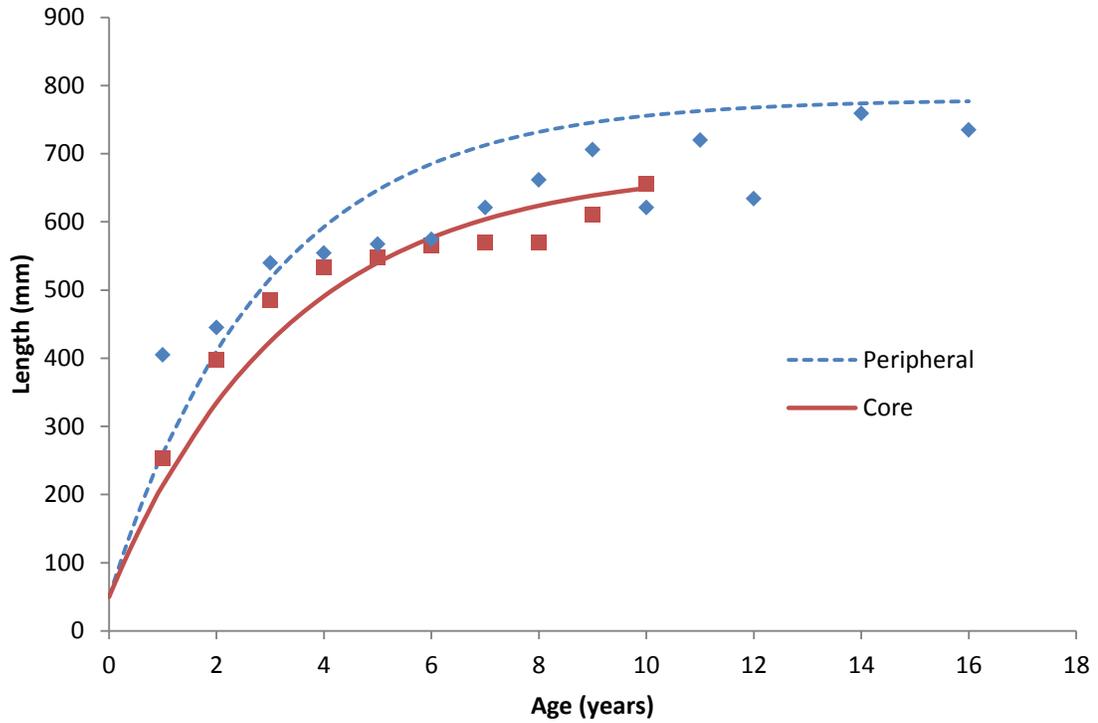
Figure 3.7. Von Bertalanffy growth curves for core and peripheral population segments of spotted gars. Growth models represent pooled data for both males and females of each population and combined as overall core and peripheral population models. Blue diamonds represent mean length-at-age for peripheral population segment, red squares represent mean length-at-age for core population segment. Comparison of 95% confidence intervals as well as ANCOVA indicated that growth models were not significantly different between the two population segments.
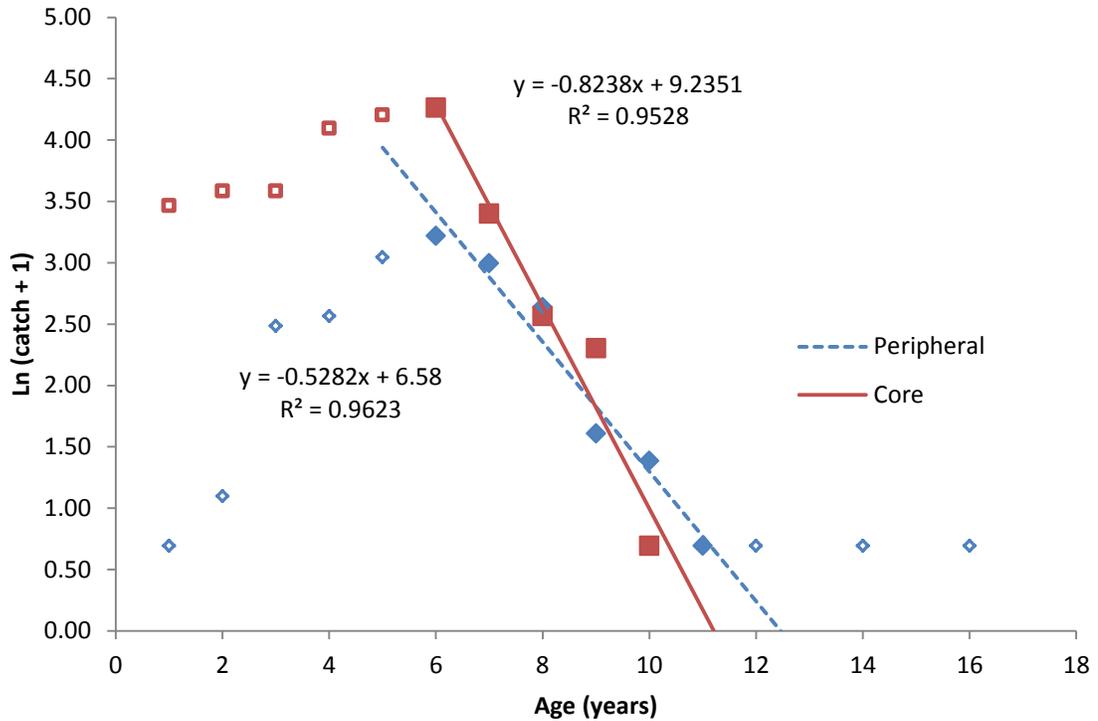
Figure 3.8. Catch curve regressions of ln(catch + 1) as a function of age (both sexes combined) for core and peripheral population segments of spotted gars (blue diamonds, dashed line = peripheral population; red squares, solid line = core population). Solid markers represent catch data used in regression models, open markers represent catch data for age classes not included in regression models, but as reference for catch variation in datasets. LE-p and MI-p catch data were combined for Peripheral regression (R^2 = 0.96); MO-c, GA-c, and LA-c data were combined for Core regression (R^2 = 0.95). ANCOVA indicated that mortality estimates between peripheral and core populations were significantly different.
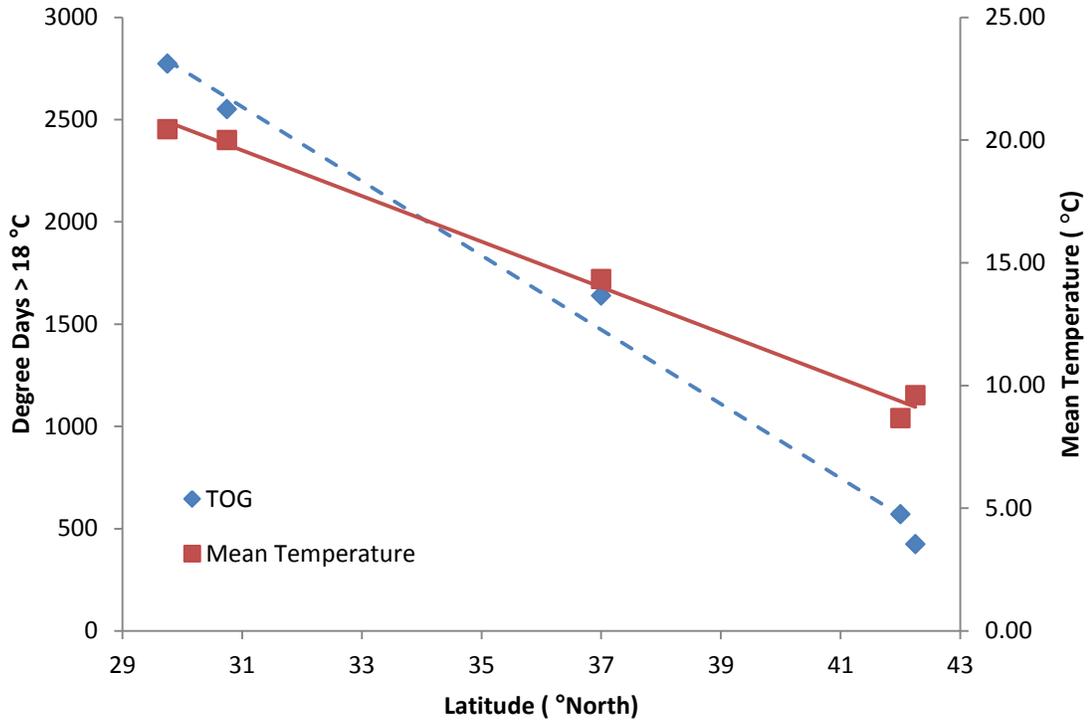
Figure 3.9. Thermal opportunity for growth (TOG, degree days > 18 °C) and mean annual temperature (°C) versus latitude for core and peripheral populations of spotted gars. ANOVA indicated significant negative correlations between TOG and latitude as well as between mean annual temperature and latitude.
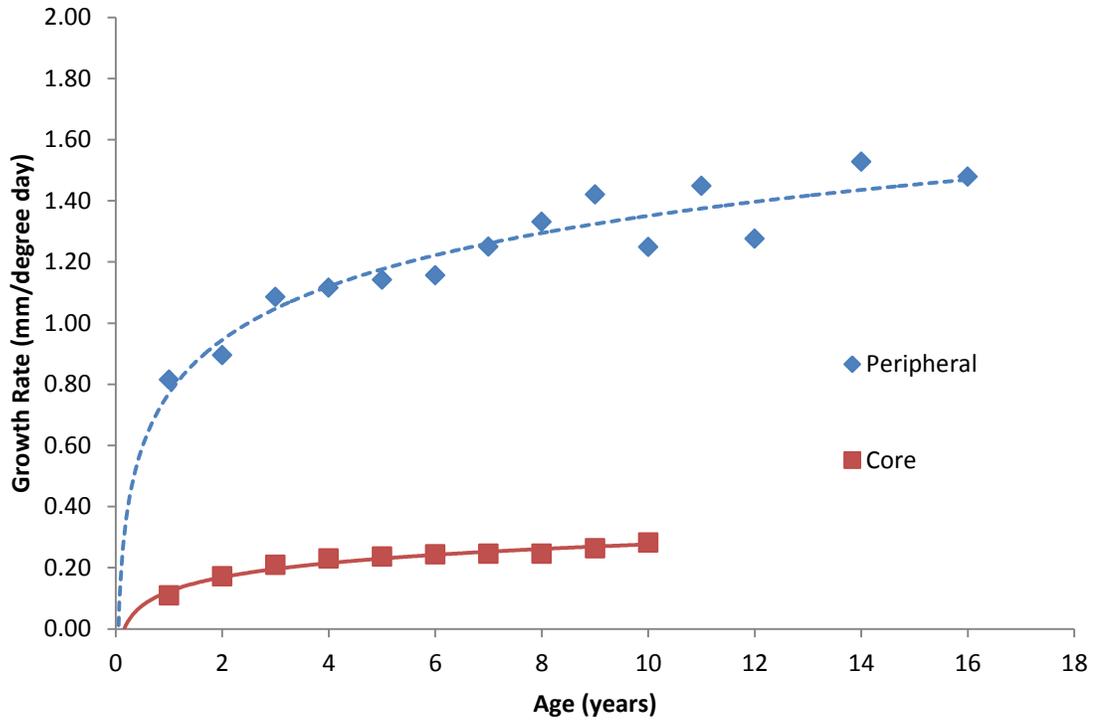
Figure 3.10. TOG-corrected growth rates (mm/degree day) for peripheral and core population segments of spotted gars. Dashed line represents peripheral population model (MI-p, LE-p), solid line represents core population model (MO-c, GA-c, LA-c). ANOVA indicated that mean growth rates for population segments were significantly different. When TOG was incorporated into analysis, peripheral population spotted gars (1.23 mm/degree day) were shown to grow faster than core population spotted gars (0.22 mm/degree day).
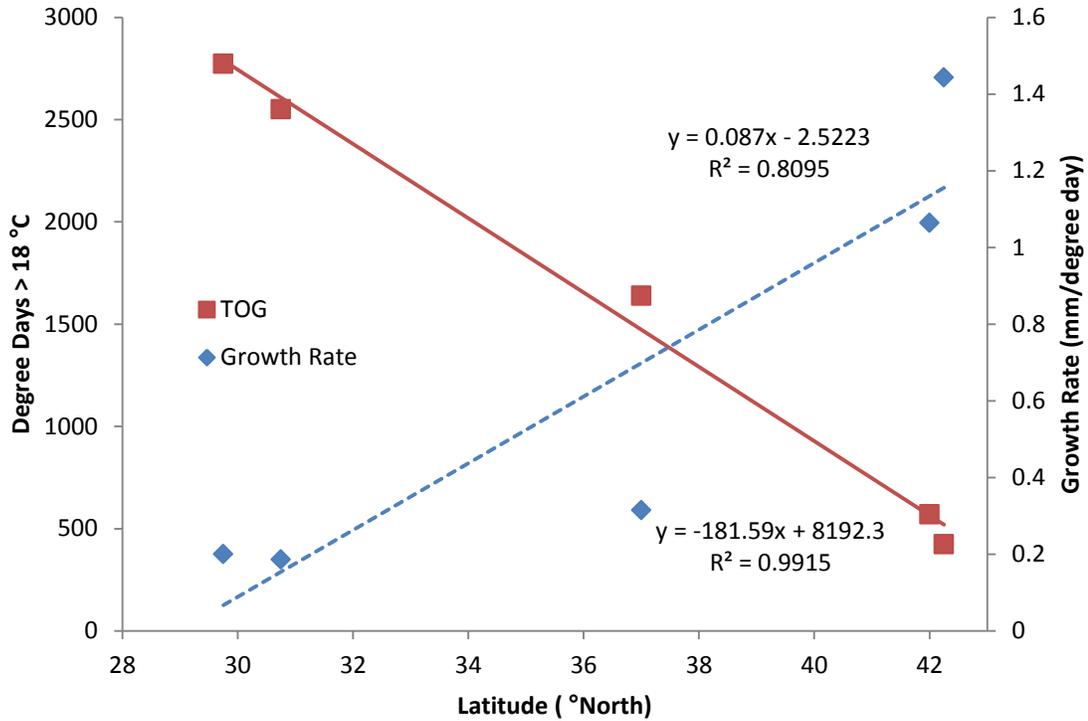
Figure 3.11. Thermal opportunity for growth (degree days > 18 °C) and mean TOG-corrected growth rate (mm/degree day) for core and peripheral populations of spotted gars versus latitude. Mean TOG-corrected growth rate was significantly positively correlated with latitude, and indicated a countergradient between growth rate and thermal opportunity for growth in spotted gars.
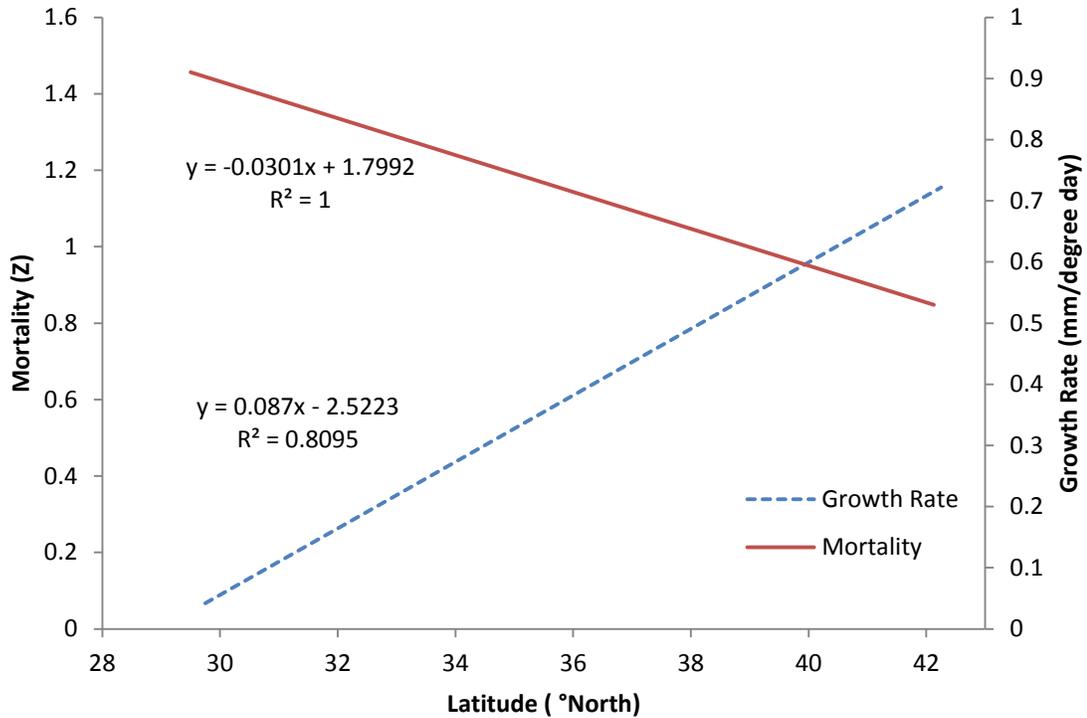
Figure 3.12. Mean TOG-corrected growth rate (mm/degree day) and mean instantaneous mortality rate (Z) for core and peripheral populations of spotted gars versus latitude. Mortality model was based on core and peripheral population segment rates only, and suggested a countergradient between mortality rate and latitude as well as potential compensatory mortality between higher and lower latitudes.

Appendix 3.1. Michigan inland lakes sampled for total of 36 adult spotted gars used in life history analyses, summer 2008-2010.

Lake	County	Lake Type	Surface Area (hectares)	Max Depth (meters)
Marble Lake	Branch	inline	297.80	18.29
Lake Pleasant	Hillsdale	headwater	302.00	12.80
Round Lake	Hillsdale	inline	31.46	15.24
Boot Lake	Hillsdale	headwater	28.56	12.19
Long Lake	Kalamazoo	headwater	202.90	17.37
Van Auken Lake	Van Buren	inline	102.20	18.29
Mean			160.82	15.70

Appendix 3.2. List of Michigan inland lakes sampled, catch (number of fish), and mean length (mm) for spotted gars collected using boat electrofishing during late spring-early fall 2008-2010. Of 31 inland lakes sampled, spotted gars were collected in 19. Lakes were initially selected based on historical catch data from the Michigan Department of Natural Resources.

Water Body	County	Catch	Mean Length
Lake Allegan	Allegan		
Saddle Lake	Allegan	8	606
Coldwater Lake	Branch	1	554
East Long Lake	Branch	27	548
Loon Lake	Branch	5	561
Marble Lake	Branch	50	573
Duck Lake	Calhoun		
Bass Lake	Hillsdale	5	562
Baw Beese Lake	Hillsdale	1	583
Bear Lake	Hillsdale		
Boot Lake	Hillsdale	4	517
Carpenter Lake	Hillsdale		
Hemlock Lake	Hillsdale		
Lake Pleasant	Hillsdale	14	599
Round Lake	Hillsdale		
Olcott Lake	Jackson	3	438
Wolf Lake	Jackson	2	581
Little Sugarloaf Lake	Kalamazoo	3	664
Long Lake	Kalamazoo	5	608
Sugarloaf Lake	Kalamazoo	3	532
Mona Lake	Muskegon	1	355
Muskegon Lake	Muskegon		
Brooks Lake	Newaygo		
Hess Lake	Newaygo		
Pigeon Lake	Ottawa		
Duck Lake	Van Buren	1	650
Saddle Lake	Van Buren	8	606
Van Auken Lake	Van Buren	14	605
Ford Lake	Washtenaw		
Sugarloaf Lake	Washtenaw	3	579
Belleville Lake	Wayne		
		Total = 158	Mean = 564

Chapter 4

Genetic variation and biogeography of the spotted gar *Lepisosteus oculatus* from core and peripheral populations

<u>Introduction</u>

Although relatively common in the lower Mississippi River drainage and other areas of the southern United States, the spotted gar *Lepisosteus oculatus* is poorly studied and its ecology and status are comparatively unknown in the Great Lakes basin. The Great Lakes population of spotted gars represents the northern edge of the species range, and is completely disjunct from the southern US population (Page and Burr 2011). Because little is known about the status of this peripheral population of spotted gars, the species has varying levels of protection in the region, being listed as threatened in Canada (COSEWIC 2005), endangered in Ohio and Pennsylvania (Ohio Department of Natural Resources 2010, Pennsylvania Code 2011), and considered a species of greatest conservation need in the state of Michigan (Michigan Department of Natural Resources 2006). The spotted gar is a native top-level predator (primarily piscivorous), preferring clear vegetated waters, particularly wetlands and floodplain habitat of lakes and large rivers (Suttkus 1963, Trautman 1981, Page and Burr 2011). The species is an important component of native food webs, and may be threatened, or in some cases has completely disappeared, due to the degradation and loss of habitat in its range (Trautman 1981, Carman 2002). Because of its specific habitat preferences, the spotted gar may also serve as an environmental indicator of aquatic ecosystem health (USEPA 2007).

The spotted gar dates back to the early Eocene (48-55 mya, Wiley 1976, Grande 2010) but arrived in the Great Lakes region relatively recently, approximately 8,000 years ago, when water temperatures began to rise following the Wisconsinan Glaciation (Bailey and Smith 1981, Hocutt and Wiley 1986, Hubbs et al. 2004). Based on previous (primarily morphologically-based) phylogenetic analyses of fossil and recent species, gars are believed to have changed relatively little over time (Wiley 1976, Inoue et al. 2003, Grande 2010, Amores et al. 2011), especially when compared to teleosts. Despite their unique ancestral lineage, few studies have focused on the biogeography of gars, and even fewer have investigated phylogeographic patterns (spatial distributions of genealogies, Avise et al. 1987) of extant lepisosteid species (Wiley 1963, Barrientos-Villalobos and Monteros 2008, Grande 2010, Sipiorski 2011). The ancient lineage, wide latitudinal range, and complete disjunction between core and peripheral populations of the spotted gar make it a unique species in which to explore phylogeographic patterns. The relative young age of the Great Lakes ichthyofauna (approximately 8,000-12,000 years), including peripheral populations of the spotted gar, also presents an opportunity to compare potential genetic variation in an ancient lineage between geologically young (Great Lakes) and old (Mississippi River and Gulf Coast) aquatic systems (Bailey and Smith 1981, Hocutt and Wiley 1986, Bernatchez and Wilson 1998, Hubbs et al. 2004). Understanding phylogeographic patterns of peripheral populations can further elucidate species dispersal abilities, genetic diversity, and vulnerability to extinction, and therefore also inform conservation strategies (Avise 2009).

The objectives of this study were to identify and explore the genotypic relationships, based on mitochondrial DNA (mtDNA) analyses, between and among

populations of spotted gars from both core and peripheral populations. Additionally, my

goal was to use concepts from phylogeography (coalescent theory; Avise 2000) and

historical biogeography (dispersal and vicariance; Mayden 1988) to interpret current

molecular genetic relationships among populations of spotted gars. I hypothesized that

population genetic structure based on mtDNA analyses would reflect geographic position

of core and peripheral populations of spotted gars. More specifically, I hypothesized that

peripheral population spotted gars would exhibit comparatively low genetic diversity,

influenced by both disjunction (lack of gene flow) from the core population and founder

effects associated with recent colonization into a new environment (colonization of the

Great Lakes region from Mississippi River refugia). Additionally, I hypothesized that

genetic distance among populations would reflect geographic distance among

populations, with proximal populations more similar than distal populations (isolation by

distance, IBD; Wright 1942, Jenkins et al. 2010).

Methods

Mitochondrial DNA Analyses

Mitochondrial DNA has several characteristics that make it highly suitable for

analyses of intra- and interspecies relationships in comparison to nuclear DNA, primarily

its non-recombining nature and comparatively fast rate of evolution (see Avise et al.1987,

Avise 2000 for full review of mtDNA in molecular analyses). Additionally, recent

molecular analysis of gar phylogenetics suggests that mtDNA may provide better

resolution of intraspecies relationships compared to nuclear loci (Wright et al. *in press*).

In molecular genetic studies such as this investigation, large sample size from a given

population is not as important as in "more traditional" ecological surveys; this is primarily due to the non-recombining nature of mtDNA, and therefore individuals (as opposed to populations or species) can be justifiably considered as operational taxonomic units, with each individual providing its own large sample of data (Avise et al. 1987, Avise 2000). Three mtDNA loci (cytochrome oxidase subunit I, *COI*; cytochrome oxidase subunit II, *COII*; and 16S rRNA, *16S*) with varying evolutionary rates were used in this study to provide a more robust concatenated dataset for estimation of genetic diversity. *16S* has been shown to be relatively slower in evolutionary rate than *COI* and *COII* (Kocher and Stepien 1997). Several studies have used the control region or "d-loop" of mtDNA for analyses due to its quickly evolving nature compared to other mtDNA loci (Kocher and Stepien 1997); however it has recently come into question in terms of underestimating population structure in some species, therefore analysis of more conservatively evolving loci has been suggested (Bradman et al. 2011)

Specimen Collection & Study Regions

Spotted gars were collected from multiple localities for molecular phylogenetic analyses (Table 4.1, Figure 4.1). Samples from peripheral population fish were taken from two Michigan inland lakes (Loon Lake, Branch County, and Lake Pleasant, Hillsdale County; N = 5 fish) and Rondeau Bay, Lake Erie (N = 1 fish). Core population samples were taken from Horseshoe Lake, Illinois (N = 5 fish), Bayou Chevreuil, Louisiana (N = 6 fish), and Choke Canyon Reservoir, Texas (N = 5 fish). For comparison to an out-group, and in this case a sister species, Florida gar (*Lepisosteus platyrhincus*) samples were included from three localities (Lake Okeechobee, Florida,

Caloosahatchee River, Florida, and Everglades Conservation Area, Florida; N = 3 fish).

Spotted gar samples were coded by population as follows: MI-p = Michigan, LE-p =

Lake Erie, IL-c = Illinois, LA-c = Louisiana, and TX-c = Texas. Florida gar samples

from three localities (FLG1, Lake Okeechobee, FL; FLG2, Caloosahatchee River, FL;

and FLG3, Everglades Conservation Area, FL) were included in analyses as a single

population, FLG. Multiple sampling methods were used to collect fishes. Boat

electrofishing was used to collect MI-p and TX-c fish, fyke nets for LE-p fish,

experimental gill nets for LA-c fish, and dip-nets for IL-c fish and Florida gars.

The distribution of the spotted gar was divided into 4 major regions for this study:

the Great Lakes, Mississippi River drainage, western Gulf Coast, and eastern Gulf Coast

regions. Regional divisions were determined based on arbitrary combinations of regions

from zoogeographic studies of Hocutt and Wiley (1986) and phylogeographic studies of

lepisosteids by Sipiorski (2011). Study populations were assigned to regions as follows:

MI-p and LE-p to the Great Lakes region, IL-c and LA-c to the Mississippi River

drainage region, TX-c to the western Gulf Coast region, and FLG to the eastern Gulf

Coast region (Figure 4.2).

Genetic Comparisons

Approximately 1.0 cm^2 fin clips were taken from all fish and stored in 95%

ethanol for use in DNA preparations. Preserved tissues were used to extract DNA using

Qiagen DNeasy Tissue Extraction Kits (QIAGEN, Valencia, CA). Portions of the

mitochondrial genes for cytochrome oxidase subunit I (*COI*), cytochrome oxidase subunit

II (*COII*), and 16S rRNA (*16S*) were PCR amplified using previously published primer

sequences and cycling conditions (Normark et al. 1991, Palumbi 1996, Ward et al. 2005).

Amplified PCR products were prepared for sequencing by 1:5 dilution with distilled

water, and all sequencing was performed at the University of Michigan DNA Sequencing

Core, using the forward and reverse PCR primers. LE-p sequence data was taken from

GenBank (accession #EU524699); this data was part of the "Barcode of Life Project"

(BOLD, Hubert et al. 2008) and only *COI* information was available for comparisons.

Gene sequences and chromatograms were analyzed using Sequencher 4.8 (Gene

Codes Corporation, Ann Arbor, MI, U.S.A.) and were manually aligned using the

program Se-Al v.2.0a11 Carbon (Rambaut 1996), which was also used to evaluate the

presence of haplotype variation in spotted gar samples. The program PAUP* 4.0b10

(Swofford 2003) was used to generate matrices of uncorrected p-distances to serve as a

measure of genetic differentiation and variation between and within core and peripheral

populations. These measures were also derived from data sets containing sequence

information for *L. platyrhincus* (in which peripheral and core *L. oculatus* were treated as

both a single population and individual populations), to offer an indication of these values

for interspecific comparisons of closely related gar species.

Haplotype diversity (*H*) was calculated for all genes, populations, and combined

for both species using the following formula:

$$H = \frac{N}{N-1}\left(1 - \sum_i x_i^2\right)$$

Where N is the sample size and x_i is the relative haplotype frequency for each sample.

Haplotype diversity was used to compare variation among populations as well as across

species. Additionally, analysis of molecular variance (AMOVA, Excoffier et al. 1992)

and F_{st} values (a measure of population differentiation) were used to further evaluate

genetic variation within and among core and peripheral populations (ARLEQUIN 3.5, Excoffier et al. 2010). Pairwise F_{st} values take into account both haplotype frequency and sequence divergence between haplotypes.

Regression analysis was used to identify significant correlations between genetic distance (F_{st} values) and geographic distance among spotted gar populations, indicating potential isolation by distance (IBD) effects (Wright 1942, Jenkins et al. 2010). Geographic distance (km) was estimated from Euclidean distances between population localities from GIS data (Google Earth 2011). Correlations were based on all pairwise combinations of genetic distance ($F_{st}/(1-F_{st})$) and regressed against geographic distances among populations.

Results

Genetic Comparisons

The total genetic data set consisted of 1919 base positions, with fairly evenly distributed contributions from the three loci sampled (*16S* = 608 bp, *COI* = 685 bp, *COII* = 626 bp). All three loci sampled showed different levels of variation among loci as well as within and among *L. oculatus* populations. A single *16S* haplotype was observed for all *L. oculatus*, and a single *16S* haplotype for *L. platyrhincus*. Due to this homogeneity, the *16S* data was excluded from individual interpopulational gene distance analyses (which would have been zero in all cases), with the exception of a genetic distance comparison with *L. platyrhincus* (uncorrected p-distance = 1.09%). These results indicated that *16S* sequences from *L. platyrhincus* differed from *L. oculatus* to a lesser degree than that observed for the other two loci sampled.

Variation between spotted gar samples was greater in the *COI* and *COII*, with three and four unique haplotypes observed, respectively. A single *COI* haplotype was observed in all TX-c individuals, in which a single pyrimidine transition (T→C) was found at base position 291. This transition was also found in one LA-c individual (LA SpG 2736), which also showed a single purine transition (A→G) at base position 634. This haplotype was unique to this individual. Only *COI* information was available for the LE-p sample, and sequence data was identical to that of MI-p specimens. A single *COII* haplotype (Haplotype *COII*-b) was observed in all MI-p individuals, with a single base substitution (T→C) at position 248; this haplotype was also shared with one LA-c individual and two IL-c individuals. Core populations consisted of 2-3 haplotypes in each component population, with two haplotypes observed in IL-c, and 3 in LA-c and TX-c. One TX-c individual showed a unique haplotype from all others with multiple base substitutions (T→C at position 248, G→A at positions 119 and 218, G→A at position 53; Table 4.2, Figures 4.3 and 4.4).

Concatenated results for all loci revealed 7 haplotypes for *L. oculatus* and also 3 unique haplotypes for *L. platyrhincus* (Tables 4.2 and 4.3, Figures 4.5 and 4.6). Of the 7 *L. oculatus* haplotypes, 3 were unique (each occurred in only one individual); these singletons occurred in two TX-c fish and one LA-c fish. Haplotype A was the most common (38% of individuals) and widespread haplotype and occurred MI-p, IL-c, and LA-c populations, but not in TX-c. Haplotype B was second most common (19%) and only occurred in IL-c and LA-c populations. Haplotype C was found only in LA-c, and Haplotypes D, F, and G were all unique to TX-c.

All MI-p individuals shared the same haplotype (haplotype diversity, H = 0.00) for individual loci and concatenated results. LA-c was the most diverse population (H = 0.80) with 4 haplotypes (A, B, C, E), followed by TX-c (H = 0.70) with 3 haplotypes (D, F, G). Haplotype data were also combined to compare core and peripheral populations (peripheral population was only represented by MI-p except for *COI*, which included LE-p) resulting in zero haplotype diversity for the peripheral population and 0.98 for the core population. Concatenated results for FLG indicated 3 unique haplotypes from the 3 different populations with a haplotype diversity value of 1.00 (Table 4.3).

Average genetic distance (uncorrected p-distance) between core and peripheral populations was very low (0.09%), over an order of magnitude less than that seen between *L. oculatus* and *L. platyrhincus* (1.50%). Three different *COII* haplotypes were observed for FLG samples, and identical haplotypes for *16S* and *COI* (Tables 4.3 and 4.4).

AMOVA tests indicated that significant variation occurred between core and peripheral populations of spotted gars, as well as within and among component populations (Table 4.5). Amount of variation explained by comparison of peripheral versus all core populations (MI-p vs. IL-c, LA-c, TX-c combined) was only 14.42%, with 34.77% of variation coming from comparisons among (core) populations, and 50.81% of variation from within populations.   Pairwise comparisons based on F_{st} values indicated that MI-p was significantly different from LA-c and TX-c populations, but not from IL-c. TX-c was significantly different from MI-p and IL-c, but not LA-c. In comparing peripheral versus core populations (MI-p vs. IL-c, LA-c, TX-c combined), the peripheral population was significantly different from the core population. When comparing each

individual population to all population data combined, only TX-c was significantly different (Table 4.6).

Genetic distance ($F_{st}/(1-F_{st})$) was significantly correlated ($r^2 = 0.70$, $p < 0.05$) with geographic distance (km) between populations suggesting a pattern of isolation by distance (IBD; Figure 4.7).

Discussion

Spotted gars from peripheral and core populations exhibited low but significant genetic variation based on analyses of 3 mitochondrial loci. Among spotted gar populations, seven unique haplotypes were identified (data for all 3 loci combined), which reflected potential interpopulation-level genetic structuring. The spotted gar and its sister species, the Florida gar, exhibited low levels of variation in genetic comparisons, however interspecies variation was over an order of magnitude larger than intraspecies variation. Interspecies variation (1.50% between *L. oculatus* and *L. platyrhincus*) was similar to that reported among other lepisosteids such as the alligator gar *Atractosteus spatula* and Cuban gar *A. tristoechus* where genetic distances (uncorrected p-distance) between species were low (1.21%) compared to several other fishes (Barrientos-Villalobos and Monteros 2008, Borden and Krebs 2009).

My results support and contribute additional resolution to previous theories related to lepisosteiform biogeography which primarily focused on larger-scale patterns of distribution. Wiley (1976) used vicariance biogeography with track (distribution) analysis of fossil and recent gars and determined that both extant genera of Lepisosteidae (*Lepisosteus* and *Atractosteus*) had a Pangean distribution. Wiley (1976) also suggested

that a vicariance event producing the two genera likely occurred before the breakup of Pangea. Based on track analysis of the "*oculatus* species group", comprised of *L. oculatus*, *L. platyrhincus*, and the fossil European species *L. fimbriatus*, the vicariance event that split the common ancestor of the European and North American species took place in the early Eocene, making the *L. oculatus-L. platyrhincus* pair the most derived species group within *Lepisosteus* (Wiley 1976). Grande (2010) added several additional fossil species to historical biogeographical analyses of Lepisosteiformes and observed similar patterns to Wiley (1976), as well as support for long-standing sympatry among extant gar species in the eastern United States.

Colonization by extant gars in the Great Lakes region is believed to be relatively recent compared to the age of the family in North America (Bailey and Smith 1981, Hocutt and Wiley 1986, Grande 2010). Several biogeographic studies based on vicariance and dispersal have indicated that spotted gars entered into the Great Lakes region from Mississippi River refugia after waters warmed following the Wisconsinan Glaciation (approximately 8,000 years ago; Bailey and Smith 1981, Hocutt and Wiley 1986, Mandrak and Crossman 1992, Hubbs et al. 2004). Furthermore, Mandrak and Crossman (1992) suggested that spotted gars entered the Great Lakes region (and progressed to southwestern Ontario) specifically through the Chicago and Michigan Lower Peninsula glacial outlets (a shorter connection to Lake Erie via the Fort Wayne outlet is believed to have been too cold for the species to use for dispersal). Results from my analyses of haplotype diversity and IBD support these theories of dispersal of spotted gars into the Great Lakes region. I found MI-p and LE-p fish to have identical haplotypes (comparing *COI* data), and my isolation by distance regression model showed

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greater similarity between peripheral and proximal core populations (IL-c, LA-c; Mississippi River drainage) compared to more distal core populations (TX-c; western Gulf Coast drainage).

Genetic structuring among populations of spotted gars also supports previous phylogeographic studies of North American fishes, particularly those comparing species from glaciated and non-glaciated regions (Bermingham and Avise 1986, Bernatchez and Wilson 1998). Bernatchez and Wilson (1998) found that genetic diversity was lower among populations of species in previously glaciated regions compared to that of populations of the same species in non-glaciated regions, and that this pattern occurred across a large diversity of fishes (Bermingham and Avise 1986). My results similarly indicated that spotted gar populations from the most recently deglaciated localities (MI-p and LE-p) had lower genetic diversity than those from non-glaciated localities (LA-c and TX-c).

Genotypic divergence in spotted gars may be related to geographic isolation, recent colonization, and founder effects. The Texas population of spotted gars is the southern-most population in my study, and from a locality not included on many current distribution maps for the species (Hendrickson and Cohen 2010, NatureServe 2011, Page and Burr 2011). The Texas population also occurs in a separate regional watershed unit from all other populations investigated in my study, with TX-c belonging to the western Gulf Coast, and all other populations connected with the Mississippi River regional watershed (either presently or historically), therefore geographic isolation between the two major watershed units may have facilitated divergence by genetic drift (Kawamura et al. 2009). Sipiorski (2011) found that variation in mtDNA (control region, or "D-loop")

of spotted gars was greater between the eastern Gulf Coast watershed and Mississippi River regional watershed populations than among several populations within the Mississippi River watershed. Bermingham and Avise (1986) also noted similar patterns of interpopulation variation between eastern Gulf Coast and Mississippi River watershed regions among *Lepomis spp*. and *Amia calva*. Bernatchez and Wilson (1998) showed that populations of fishes from western Gulf drainages were more divergent (among populations) than those from eastern Gulf drainages. The Texas population of spotted gars consisted of 3 unique haplotypes not found in other study populations, and mutations (based on number of base substitutions in individual loci) were greater in TX-c than other populations, supporting higher levels of divergence in TX-c from other populations (Avise 2009). According to coalescent theory, rarer haplotypes are likely more recently derived, and older haplotypes (more ancestral) should be more widespread than younger haplotypes (Templeton 1998, Avise 2000, Barrientos-Villalobos and Monteros 2008). TX-c possessed multiple rare haplotypes (3 unique to TX-c) compared to other populations, and therefore may be the most derived of the spotted gar populations in this study. Haplotype A was shared by the most individuals in this study and widespread over 3/4 of study populations, therefore it may be the most ancestral haplotype (Avise 2009).

Important historic geological events, particularly the Pleistocene glaciations, may also have influenced population structuring in spotted gars. Bernatchez and Wilson (1998) showed that glaciation events greatly influenced the genetic structuring of northern populations of many fishes in North America. Spotted gars entered into the Great Lakes region from Mississippi River refugia (Hocutt and Wiley 1986), and would therefore be expected to share some genetic similarity with Mississippi River drainage

populations (Bailey and Smith 1981, Bernatchez and Wilson 1998). I found that among

7 unique haplotypes for spotted gars, Michigan individuals, representing the peripheral

population, all shared the same haplotype (Haplotype A). This haplotype was not unique

to Michigan fish, but also shared with core population fish (IL-c and LA-c) from the

Mississippi River drainage. Spotted gars from core populations in the Mississippi River

drainage also had other haplotypes not found in any Michigan individuals (Haplotypes B,

C, E). The singular but shared (with IL-c and LA-c) haplotype found in MI fish suggests

very low genetic diversity in the peripheral population of spotted gars, and given the time

period since the most recent glacial recession (~8,000 years), also is consistent with a

relatively recent colonization by the species into the Great Lakes region (Bailey and

Smith 1981, Hubbs et al. 2004). Low genetic diversity coupled with shared haplotype(s)

also provides evidence for bottleneck effects, more specifically founder effects in the

peripheral population by the Mississippi River drainage core population (Hamner et al.

2007). Base substitution (mutation) in the *COII* haplotype shared by MI-p fish

(Haplotype *COII*-b) also suggests a more derived population than other Mississippi River

drainage fish (IL-c and LA-c). Analysis of only the *COI* gene, which allowed me to

include data for the Lake Erie population of spotted gars, also provided evidence for low

genetic diversity in peripheral populations as well as recent colonization from Mississippi

River refugia in that sequence data was identical for MI-p and LE-p populations (Welsh

et al. 2008, Borden and Krebs 2009).

Significant positive correlation between genetic distance and geographic distance

also indicated isolation by distance effects among spotted gar populations. Michigan fish

had genetic distances significantly different from the two most geographically distant

populations, LA-c and TX-c, but were not significantly different from the geographically close population in Illinois. Although comparisons within the peripheral population were limited, *COI* sequence data indicated that MI-p and LE-p haplotypes were identical; furthermore, MI-p samples came from two Michigan inland lakes approximately 32 km apart (with no connection) and all genetic data were identical among individuals. Mandrak and Crossman (1992) showed that spotted gars likely colonized Lake Erie localities (post-glaciation) by way of connections through the southern lower peninsula of Michigan; given their findings and my results for isolation by distance and limited comparison of genetic data with LE-p, there is high likelihood that genetic diversity would be very similar or identical between the two peripheral populations. Analyses of shared haplotypes in Mississippi River drainage and Great Lakes drainage fish also indicated a continuum of greater to lesser haplotype diversity from LA-c to MI-p. Bernatchez and Wilson (1998) also noted similar clinal patterns in genetic diversity from lower to higher latitudes in several North American fishes with broad ranges. Additional sampling of core and peripheral populations may further elucidate potential latitudinal variation in genetic diversity of spotted gars.

Texas fish were not significantly different from LA-c fish, but were significantly different from IL-c and MI-p fish. Texas fish were likely more divergent from other populations primarily due to genetic drift following geographic (regional watershed) isolation, as TX-c would have been less affected by the most recent glacial events than MI-p fish (Hocutt and Wiley 1986). Michigan fish were likely significantly different due to low genetic diversity from recent colonization and founder effects following the last glaciation of the Great Lakes region (Bailey and Smith 1981, Hocutt and Wiley 1986,

Bernatchez and Wilson 1998, Avise 2009). Kuwarama et al. (2009) found that genetic

diversity in bluegill sunfish *Lepomis macrochirus* was much lower in populations found

in previously glaciated regions compared to those from unglaciated regions, and Welsh et

al. (2009) noted that low genetic diversity in several populations of lake sturgeon

Acipenser fulvescens in the Great Lakes region was likely due to relatively recent

colonization events.

Alternatively to founder effects and recent colonization, low genetic diversity in

the Great Lakes Basin population could reflect selection for the most suitable or adaptive

genotype for ecologically harsher, high-latitude environments with shorter growing

seasons. Scudder (1989) stated that selection in ecologically peripheral environments

favors adaptation to a diversity of density-independent factors (as opposed to density-

dependent factors in core environments) as well as colonization ability. Other genotypes

may have been present when spotted gars initially entered the Great Lakes Basin, but

may have been selected against (and therefore eliminated) in the ecologically harsher

peripheral environment (Scudder 1989). Low genetic diversity in ecologically peripheral

versus core populations of species has been observed in several other studies supporting

the adaptive significance of peripheral populations (see Scudder 1989 for review).

Identification and analysis of additional peripheral populations of spotted gars may

further elucidate the relationship between selection and adaptation in ecologically

marginal environments.

Significant genetic differences among populations of spotted gars reflect both

vicariance and dispersal mechanisms, both of which have been shown to play roles in

intraspecies variation among broadly distributed North American ichthyofauna

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(Bernatchez and Wilson 1998, Borden and Krebs 2009, Kuwarama et al. 2009).

Variation between MI-p and other populations is likely associated with glacial recession followed by colonization from Mississippi refugia (dispersal), and eventual disjunction from the core population by vicariance, the specific event(s) of which are still unknown (Bailey and Smith 1981, Bernatchez and Wilson 1998). As noted by Kuwarama et al. (2009), recent phylogeographic studies have shown that events shaping current distribution and diversity of fishes are more complex than previously thought, when influences on distributions were characterized as either vicariance or dispersal mechanisms (Berendzen et al. 2003). The population structuring of spotted gars examined in this study suggests a similarly complex combination of mechanisms influencing diversity and distribution of the species.

Comparisons of genetic distances (uncorrected p-distance) between spotted gar populations and the sister species *L. platyrhincus* indicated interspecies variation was over an order of magnitude greater than intraspecies variation. Previous analysis based on cytochrome b (*cyt b*) and *COI* genes by Barrientos-Villalobos and Monteros (2008) showed that *L. oculatus* and *L. platyrhincus* differed by only 0.55% (based on uncorrected p-distance). My analyses based on 3 loci indicate an overall genetic distance of 1.05% between species. Genetic distance between the sister species may vary depending on the geography of the populations being compared. Sipiorski (2011) found that spotted gars sampled from the Apalachicola River in western Florida (eastern Gulf Coast region), possessed a haplotype (based on mtDNA control region analysis) that grouped more closely with the Florida gar than spotted gars from other regions. The Apalachicola River is considered to be within a potential hybridization zone as the range

of both species overlap in the panhandle region of Florida (Becker 1983, Page and Burr 2011). I did not sample spotted gars from the eastern Gulf Coast region, and Florida gars in my analyses had genetic distances over an order of magnitude larger between species than within either species. Although sample size of *L. platyrhincus* was very small (N = 3 fish from 3 localities), it should be noted that 3 different haplotypes were observed from the 3 localities, suggesting potential genetic structuring among much more geographically close populations. Further investigation of genetic diversity in this introgression zone may reveal higher-resolution patterns of variation between the two closely-related species.

Barrientos-Villalobos and Monteros (2008) and Sipiorski (2011) are currently the only other studies that focused on the phylogeography of lepisosteids, the former investigating population structure in the tropical gar *Atractosteus tropicus* in Central America and the latter in all five North American lepisosteids. Several methodologies and findings compare and contrast between the current study and that of Sipiorski (2011). For example, I included the Florida gar as an out-group in genetic analyses, whereas Sipiorski (2011) combined the two species; given my findings of large genetic distance between the species relative to within *L. oculatus*, combination of the two species may have influenced haplotype diversity and AMOVA results. Sipiorski (2011) sampled more localities (8) than the current study (4), however, total number of individuals analyzed was similar (23 individuals compared to 21 in the current study). Furthermore, the majority of different localities (6/8) analyzed by Sipiorski (2011) could be generalized (using my regional geographic divisions) to the Mississippi River drainage region, with the other major region being the eastern Gulf Coast. Comparatively, I

analyzed populations from 3 major regions, 2 (Great Lakes and western Gulf Coast) of which were not investigated by Sipiorski (2011). Combining the findings of both studies probably creates the most complete current picture of spotted gar population diversity, with a majority of haplotype diversity found within the Mississippi River drainage region, and high levels of divergence in both the western and eastern Gulf Coast regions. The Great Lakes region showed very low haplotype diversity, but several factors suggest strong influence from the more genetically diverse Mississippi River drainage region. The peripheral population showed genetic similarity (shared haplotype) to Mississippi River drainage populations (IL-c, LA-c), but not to the western Gulf Coast population (TX-c). The genetic similarity between Great Lakes drainage and Mississippi River drainage regions likely reflects former connection and recent colonization via Mississippi River glacial refugia (Hocutt and Wiley 1976, Mandrak and Crossman 1992, Bernatchez and Wilson 1998). Recent colonization followed by disjunction from Mississippi River refugia would contribute to founder effects on the Great Lakes population, and subsequent low genetic diversity. Eastern Gulf Coast populations are also likely to be more closely related to *L. platyrhincus* than spotted gars from other regions.

My study was limited to comparisons primarily among 4 populations of spotted gars, with 3 from the core population and one from the peripheral population. *COI* analyses included an additional peripheral population (LE-p), but only very limited comparisons were possible. Including additional populations from both core and peripheral populations would provide further insight to haplotype diversity both within and among populations. Given the recent colonization and geographic isolation of the peripheral population, however, genetic diversity would still likely be low even including

additional populations. Data from my MI-p analysis supports this in that samples for MI-p analyses came from two "sub-populations", 2 fish from Loon Lake (Branch County, MI) and 3 fish from Lake Pleasant (Hillsdale County, MI), and resulting sequence data for all individuals was identical. Inclusion of the LE-p population in *COI* analyses also suggests that haplotype diversity among peripheral populations would still be very low. Results from my isolation by distance analysis further support probable low genetic diversity throughout peripheral populations in that geographically close populations were shown to be more genetically similar to each other than to geographically distant populations. Including additional populations from the western Gulf Coast region would help elucidate differences and divergence within that region as well as between western Gulf Coast and the Mississippi River watershed regions. Inclusion of populations from the eastern Gulf Coast populations would provide similar comparisons to a region not included in this study (aside from the interspecies comparisons to *L. platyrhincus*), as well as provide insight to divergence among core populations at similar latitudes. Additional genetic data in the form of nuclear genes and microsatellite markers may also add further resolution to intraspecies variation based on slower and faster-evolving genetic compounds, respectively.

Other analyses may further elucidate relationships and variation among core and peripheral populations of spotted gars and closely related species. Life history analysis (chapter 2 this study), common garden experiments (chapter 1 this study), habitat use modeling, and morphological analyses may be useful in uncovering patterns of divergence and local adaptation among populations in different geographic regions. Pope and Wilde (2003) found a significantly high degree of variation in spotted gar mass-

length relationships among 49 reservoirs throughout the state of Texas. In my study Texas spotted gars were the most divergent population in terms of haplotype diversity, and might therefore be considered a "genetically" peripheral population; life history, genetic, and habitat analyses of additional Texas populations may clarify patterns of variation among spotted gars from the western Gulf Coast and other regions. Bernatchez and Wilson (1998) found that populations of species from previously glaciated regions may have different morphologies (morphotypes) than those from unglaciated regions. Lesica and Allendorf (1995) also noted that morphological characters are expected to diverge more rapidly in peripherally isolated populations. Spotted gars from peripheral and core populations may also differ morphologically, as individuals from peripheral populations appear to have more elongate caudal peduncles than those from core populations (personal observation, Figure 4.8). Morphologically, only a single diagnostic, presence or absence of bony plates on the isthmus, separates spotted gars from Florida gars, therefore a combination of genetic and morphological analyses may provide further insight into divergence or similarities within and between species (Trautman 1981, Grande 2010, Page and Burr 2011).

From a conservation perspective, phylogeographic studies can be important in identifying evolutionarily significant units (ESUs) such as distinct population segments (Ryder 1986, Bernatchez and Wilson 1998). Peripheral populations of species often experience very low gene flow and high degrees of genetic drift, leading to divergence from core populations (Jones et al. 2001, Lammi et al. 2001). Additionally, populations of species with very low genetic diversity have been shown to be much more vulnerable to perturbations such as habitat loss, invasive species, and overfishing (Garcia de Leaniz

et al. 2007). Peripheral populations of spotted gars in this study were found to share a single haplotype, therefore exhibiting extremely low genetic diversity; furthermore, the peripheral population is completely disjunct from the core population, therefore gene flow is likely non-existent. Spotted gars are currently listed as threatened and therefore protected throughout their range in Canada (COSEWIC 2005, Glass et al. 2011), but are only listed as a "species of greatest conservation need" in Michigan, where a large portion of the peripheral population resides in inland lakes (Carman 2002, Hubbs et al. 2004, Page and Burr 2011). Spotted gars are dependent on aquatic vegetation for multiple life stages, and loss of habitat is believed to be the largest threat to peripheral populations of the species (Trautman 1981, Carman 2002, COSEWIC 2005). Loss of essential habitat coupled with very low genetic diversity make peripheral populations of spotted gars highly susceptible to local extinction, which has already been recorded in localities within Ohio and Michigan (Trautman 1981, Carman 2002, David unpublished data). Additional investigations into habitat use, abundance, and effective population size are recommended to protect potentially vulnerable peripheral populations of spotted gars, and therefore contribute to the conservation of local biodiversity.

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Table 4.1. Specimen details for spotted and Florida gars included in analyses.

Species	Population	Population Code	Individual Code	Locality	N
Lepisosteus oculatus	Michigan	MI-p	SpG118	Loon Lake, Michigan, USA	3
Lepisosteus oculatus	Michigan	MI-p	SpG120	Loon Lake, Michigan, USA	
Lepisosteus oculatus	Michigan	MI-p	SpG123	Loon Lake, Michigan, USA	
Lepisosteus oculatus	Michigan	MI-p	SpG125	Lake Pleasant, Michigan, USA	2
Lepisosteus oculatus	Michigan	MI-p	SpG130	Lake Pleasant, Michigan, USA	
Lepisosteus oculatus	Lake Erie	LE-p	LE SpG	Rondeau Bay, Lake Erie, Canada	1
Lepisosteus oculatus	Illinois	IL-c	IL SpG1	Horseshoe Lake, Illinois, USA	5
Lepisosteus oculatus	Illinois	IL-c	IL SpG2	Horseshoe Lake, Illinois, USA	
Lepisosteus oculatus	Illinois	IL-c	IL SpG3	Horseshoe Lake, Illinois, USA	
Lepisosteus oculatus	Illinois	IL-c	IL SpG4	Horseshoe Lake, Illinois, USA	
Lepisosteus oculatus	Illinois	IL-c	IL SpG5	Horseshoe Lake, Illinois, USA	
Lepisosteus oculatus	Louisiana	LA-c	LA SpG2730	Bayou Chevruil, Louisiana, USA	6
Lepisosteus oculatus	Louisiana	LA-c	LA SpG2731	Bayou Chevruil, Louisiana, USA	
Lepisosteus oculatus	Louisiana	LA-c	LA SpG2732	Bayou Chevruil, Louisiana, USA	
Lepisosteus oculatus	Louisiana	LA-c	LA SpG2733	Bayou Chevruil, Louisiana, USA	
Lepisosteus oculatus	Louisiana	LA-c	LA SpG2734	Bayou Chevruil, Louisiana, USA	
Lepisosteus oculatus	Louisiana	LA-c	LA SpG2736	Bayou Chevruil, Louisiana, USA	

Table 4.1. Extended. Specimen details for spotted and Florida gars included in analyses.

Species	Population	Population Code	Individual Code	Locality	N
Lepisosteus oculatus	Texas	TX-c	Tx SpG8164	Choke Canyon Reservoir, Texas, USA	5
Lepisosteus oculatus	Texas	TX-c	Tx SpG8165	Choke Canyon Reservoir, Texas, USA	
Lepisosteus oculatus	Texas	TX-c	Tx SpG8169	Choke Canyon Reservoir, Texas, USA	
Lepisosteus oculatus	Texas	TX-c	Tx SpG8455	Choke Canyon Reservoir, Texas, USA	
Lepisosteus oculatus	Texas	TX-c	Tx SpG8456	Choke Canyon Reservoir, Texas, USA	
Lepisosteus platyrhincus	Florida	FLG (1)	FLG SRD 18	Lake Okeechobee, Florida, USA	1
Lepisosteus platyrhincus	Florida	FLG (2)	FLG SRD 19	Caloosahatchee River, Ft Meyers, Florida, USA	1
Lepisosteus platyrhincus	Florida	FLG (3)	FLG SRD 21	Everglades Conservation Area, Florida, USA	1

Table 4.2. Haplotypes for each individual spotted and Florida gar by individual and combined mtDNA loci. Alphabetized haplotype identification indicates level of mutations (base substitutions), with "a" and "A" having no base substitutions, and those following (b, c, B, C, D, etc.) having cumulative base substitutions.

Population	Population Code	Individual Code	*16S* Haplotype	*COI* Haplotype	*COII* Haplotype	Combined Haplotype
Michigan	MI-p	SpG118	a	a	B	A
Michigan	MI-p	SpG120	a	a	B	A
Michigan	MI-p	SpG123	a	a	B	A
Michigan	MI-p	SpG125	a	a	B	A
Michigan	MI-p	SpG130	a	a	B	A
Lake Erie	LE-p	LE SpG	-	a	-	-
Illinois	IL-c	IL SpG1	a	a	A	B
Illinois	IL-c	IL SpG2	a	a	B	A
Illinois	IL-c	IL SpG3	a	a	A	B
Illinois	IL-c	IL SpG4	a	a	B	A
Illinois	IL-c	IL SpG5	a	a	A	B
Louisiana	LA-c	LA SpG2730	a	a	A	B
Louisiana	LA-c	LA SpG2731	a	a	B	A
Louisiana	LA-c	LA SpG2732	a	a	C	C
Louisiana	LA-c	LA SpG2733	a	a	C	C
Louisiana	LA-c	LA SpG2734	a	a	C	C
Louisiana	LA-c	LA SpG2736	a	c	A	E
Texas	TX-c	Tx SpG8164	a	b	A	D
Texas	TX-c	Tx SpG8165	a	b	A	D
Texas	TX-c	Tx SpG8169	a	b	A	D
Texas	TX-c	Tx SpG8455	a	b	D	G
Texas	TX-c	Tx SpG8456	a	b	C	F
Florida	FLG (1)	FLG SRD 18	FLG-a	FLG-a	FLG-a	FLG-A
Florida	FLG (2)	FLG SRD 19	FLG-a	FLG-a	FLG-b	FLG-B
Florida	FLG (3)	FLG SRD 21	FLG-a	FLG-a	FLG-c	FLG-C

Table 4.3. Haplotype diversity of individual and combined mtDNA loci for study populations of spotted gars and Florida gars. Number in parenthesis indicates inclusion of LE-p individual sequence data. N = number of individuals, followed by number of haplotypes observed for each locus (*16S, COI, COII*, Combined). H = haplotype diversity calculated for individual and combined loci.

Population	N	*16S*	*COI*	*COII*	Combined	H (*16S*)	H (*COI*)	H (*COII*)	H (Combined)
MI-p	5	1	1	1	1	0.00	0.00	0.00	0.00
LE-p	1	-	1	-	-	-	0.00	-	-
IL-c	5	1	1	2	2	0.00	0.00	0.60	0.60
LA-c	6	1	2	3	4	0.00	0.03	0.73	0.80
TX-c	5	1	1	3	3	0.00	0.00	0.70	0.70
PERI	5 (6)	1	1	1	1	0.00	0.00	0.00	0.00
CORE	16	1	3	4	7	0.00	0.48	0.63	0.98
Total	21 (22)	1	3	4	7	0.00	0.39	0.66	0.81
FLG	3	1	1	3	3	0.00	0.00	1.00	1.00

Table 4.4. Matrix of genetic distances (uncorrected p-distance shown as percent) among study populations of spotted gars and Florida gars. Values above and below diagonal are identical.

Population	MI-p	IL-c	LA-c	TX-c	FLG-1	FLG-2	FLG-3
MI-p		0.03	0.11	0.14	1.44	1.38	1.68
IL-c	0.03		0.10	0.10	1.40	1.34	1.64
LA-c	0.11	0.10		0.13	1.47	1.41	1.71
TX-c	0.14	0.10	0.13		1.46	1.40	1.70
FLG-1	1.44	1.40	1.47	1.46		0.12	0.24
FLG-2	1.38	1.34	1.41	1.40	0.12		0.36
FLG-3	1.68	1.64	1.71	1.70	0.24	0.36	

Table 4.5. Results of analysis of molecular variance (AMOVA) run in Arlequin 3.5 (Excoffier et al. 2010) comparing peripheral and core populations of spotted gars. AMOVA compared peripheral (MI-p) versus core (IL-c, LA-c, TX-c combined) populations. MI-p was significantly different from the core population, however, a large portion of variation remained within groups.

Source of Variation	d.f.	sum of squares	variance components	percentage of variation
Among Groups	1	3.56	0.15	14.42
Among pop within groups	2	4.96	0.37	34.77
Within populations	17	9.10	0.54	50.81
Total	20	17.62	1.05	
Fixation Index, Fst	0.49	p < 0.0001		

Table 4.6. Matrix of pairwise genetic distances (F_{st} values below diagonal, significance values above diagonal) for study populations of spotted gars, as well as comparisons with core populations (combined) and all populations (all data combined).

| Population	MI-p	IL-c	LA-c	TX-c	CORE	ALL
MI-p		0.16	0.01	0.01	0.00	0.05
IL-c	0.50		0.16	0.01	0.16	0.53
LA-c	0.46	0.18		0.07	0.50	0.26
TX-c	0.77	0.55	0.27		0.11	0.02
CORE	0.33	0.06	-0.02	0.12		0.68
ALL	0.20	-0.02	0.04	0.22	-0.03	
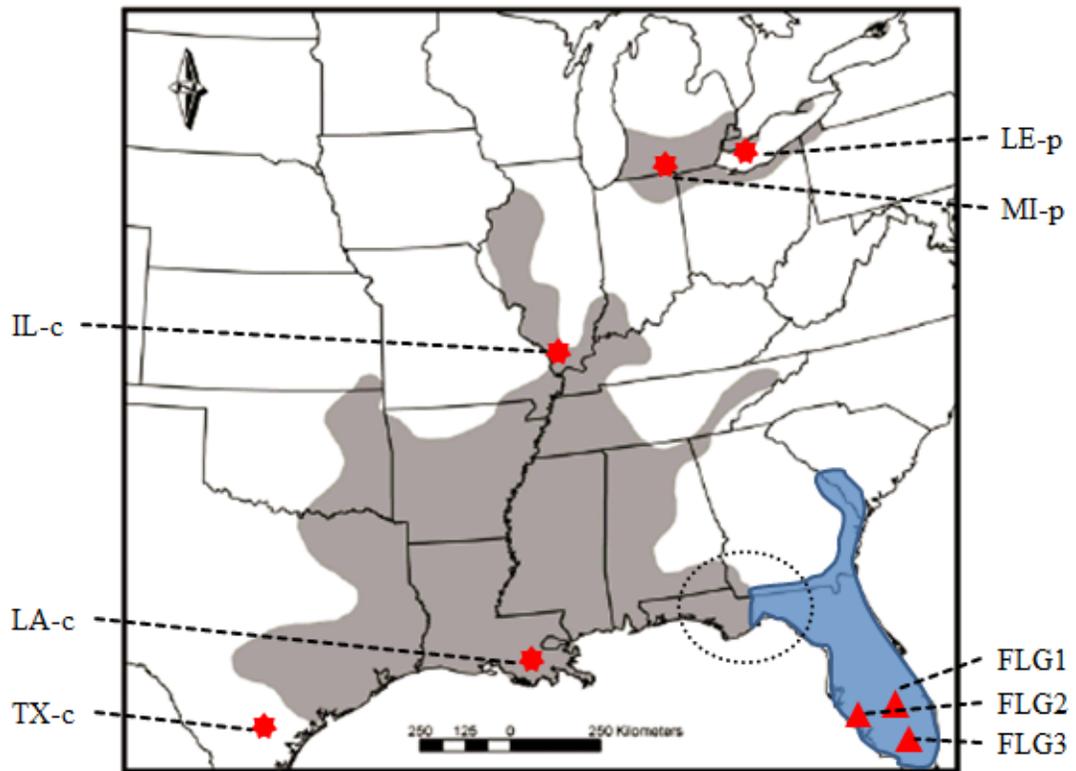
Figure 4.1. Collection sites (by population code) and range distribution for spotted (grey) and Florida (blue) gars used in genetic analyses. Spotted gar localities are as follows: Loon and Pleasant Lakes, Michigan (MI-p), Rondeau Bay, Lake Erie (LE-p), Horseshoe Lake, Illinois (IL-c), Bayou Chevreuil, Louisiana (LA-c), Choke Canyon Reservoir, Texas (TX-c). Florida gar localities are as follows: Lake Okeechobee, Florida (FLG1), Caloosahatchee River, Florida (FLG2), Everglades Conservation Area, Florida (FLG3). Note Texas population did not fall within current range distribution. Dashed circle indicates potential hybridization zone for spotted and Florida gars (Becker 1983, Page and Burr 2011, Sipiorski 2011). Map modified from Page and Burr 1991.
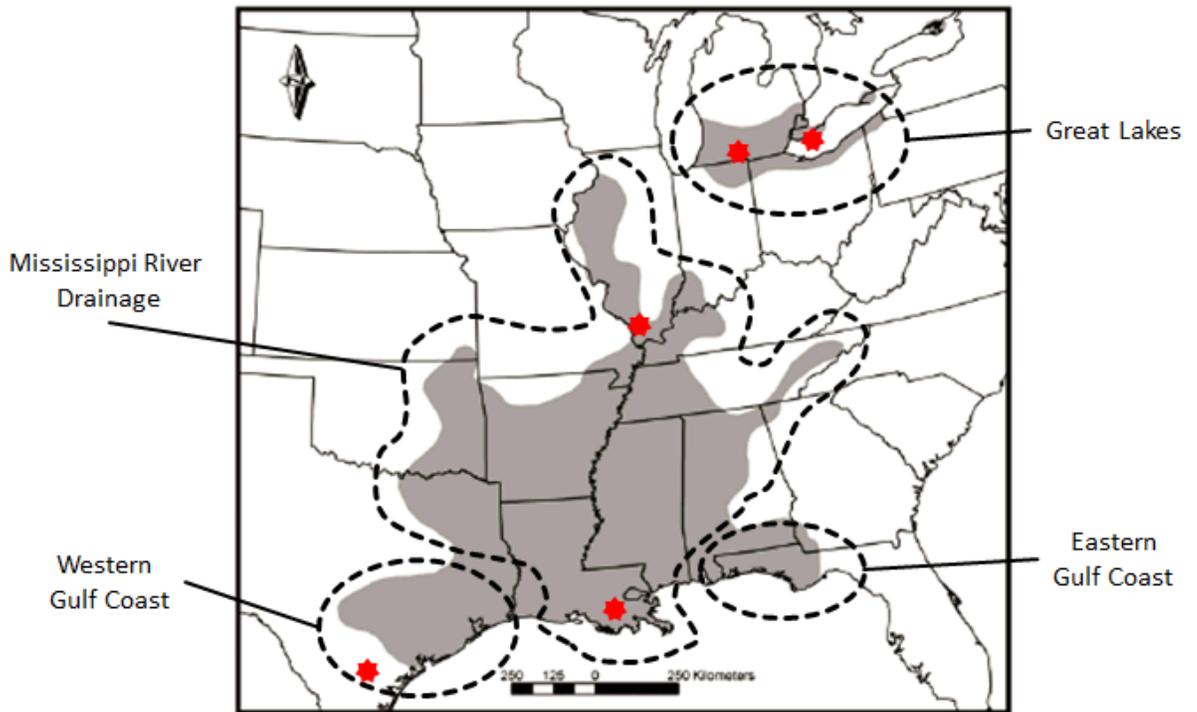
Figure 4.2. Range distribution of the spotted gar including geographic regions used in this study. Distribution was arbitrarily divided into four major regions based on zoogeographic studies of Hocutt and Wiley (1986) and lepisosteid phylogeography by Sipiorski (2011). Divisions consisted of the Great Lakes, Mississippi River drainage, Western Gulf Coast, and Eastern Gulf Coast regions. Spotted gar collection sites indicated by red stars. Distribution map modified from Page and Burr (1991).
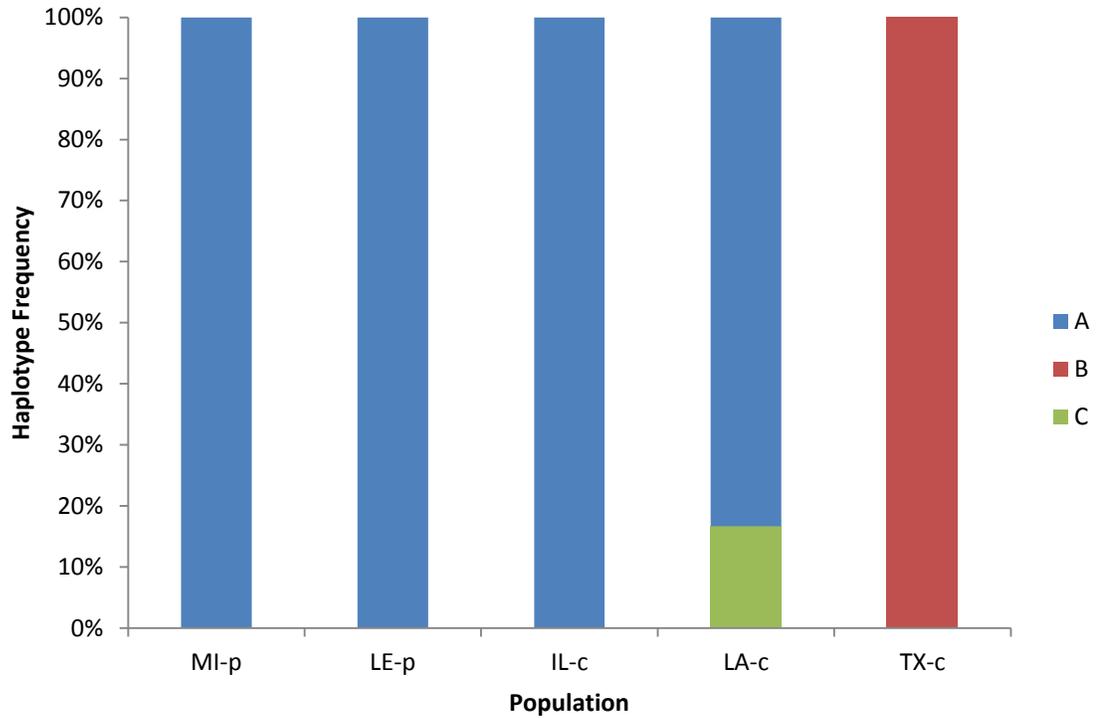
Figure 4.3. Relative haplotype frequency of *COI* for each study population of spotted gars. *COI* analysis included LE-p data, which were identical to MI-p and IL-c (A). All TX-c individuals possessed a haplotype unique to the population.
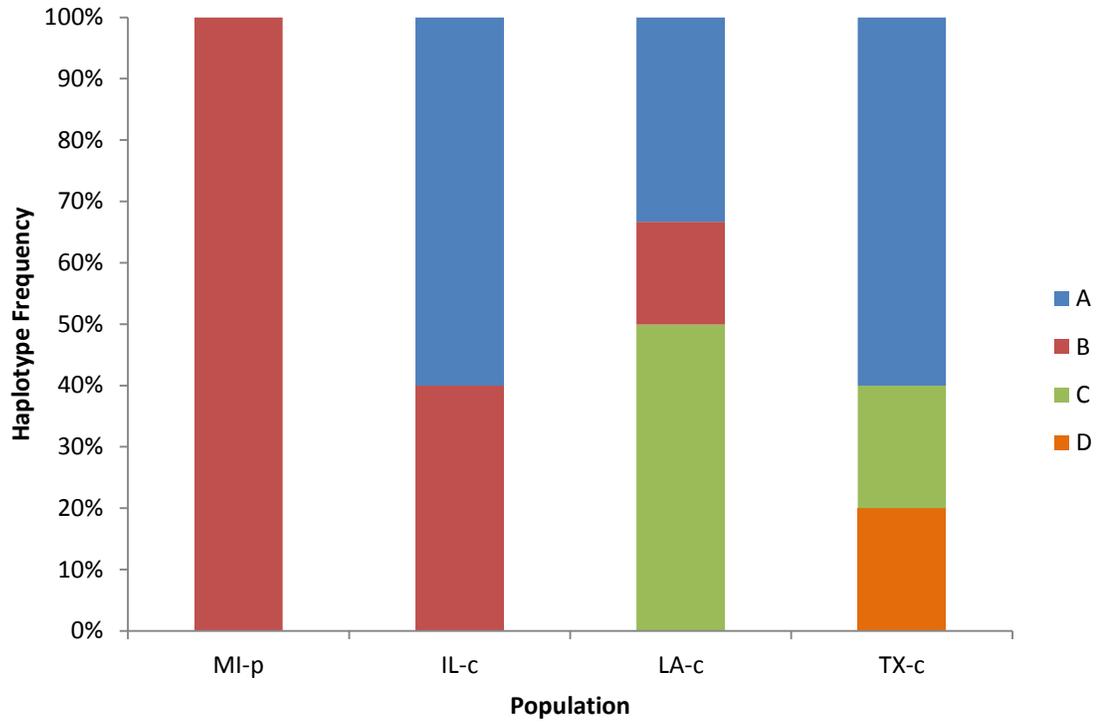
Figure 4.4. Relative haplotype frequency of *COII* for each study population of spotted gars. Note continuum of haplotype diversity from LA-c northward to MI-p. Also note unique haplotype "D" in TX-c.
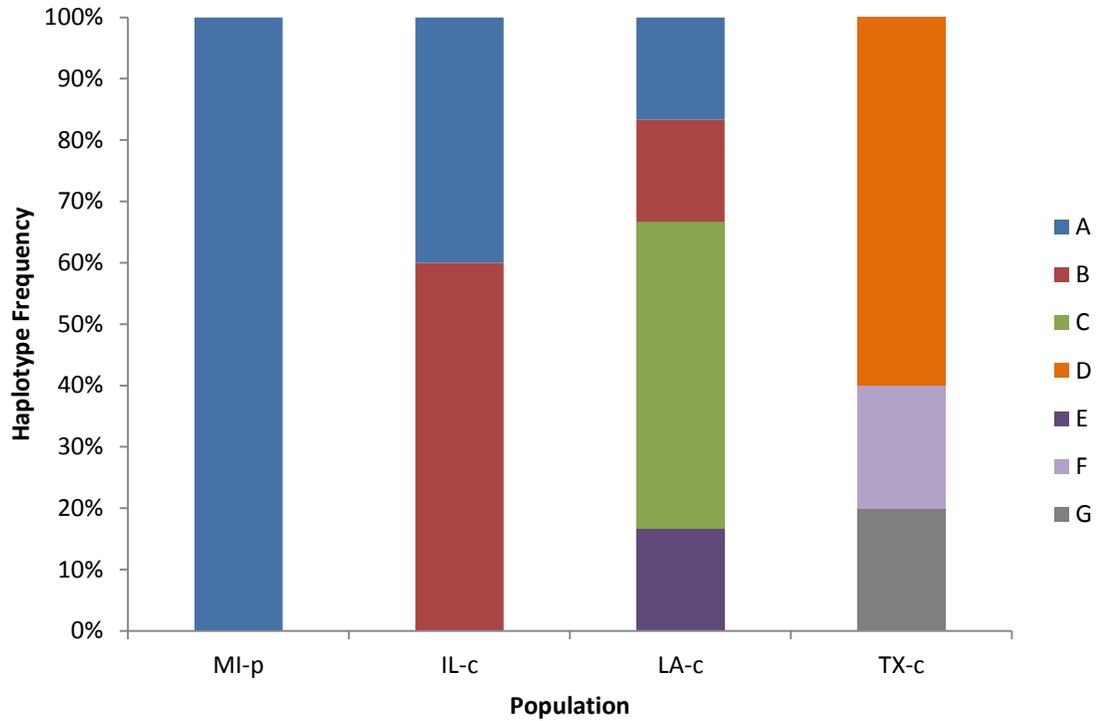
Figure 4.5. Relative haplotype frequency of all loci combined for each study population of spotted gars. Lowest haplotype diversity was observed in MI-p, with highest haplotype diversity observed in LA-c. TX-c possessed haplotypes unique to the population. Also note continuum of haplotypes and haplotype diversity from LA-c northward to MI-p.
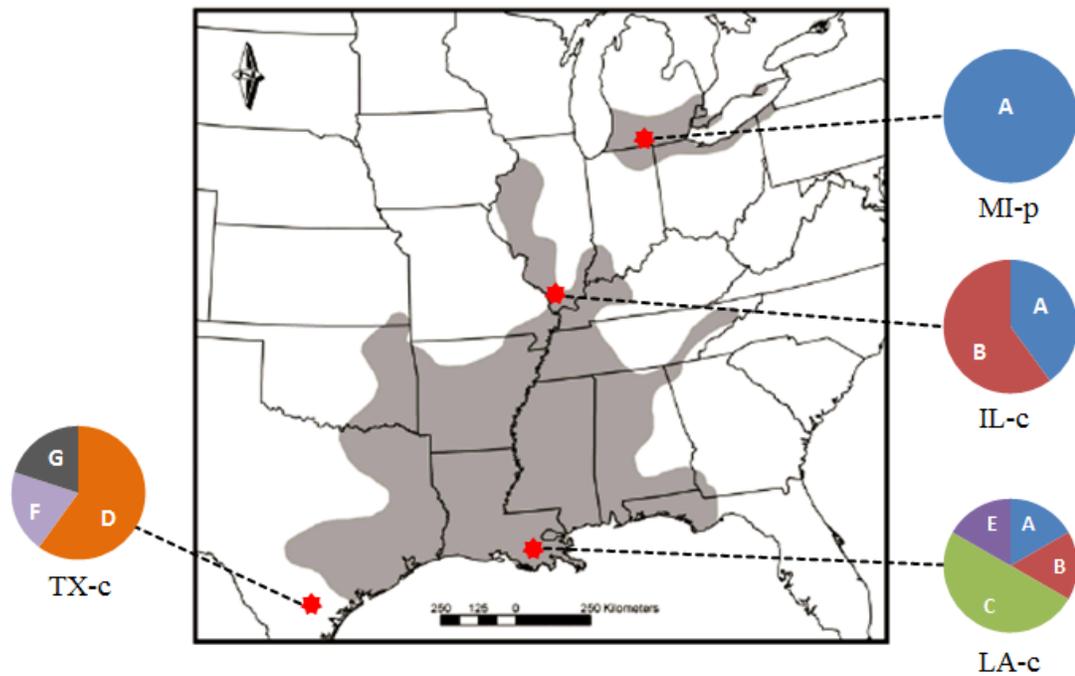
Figure 4.6. Relative haplotype frequency of all loci combined and relative geographic position for each study population of spotted gars. Lowest haplotype diversity was observed in MI-p, with highest haplotype diversity observed in LA-c. TX-c possessed haplotypes unique to the population. Also note continuum of haplotypes and haplotype diversity from LA-c northward to MI-p. Distribution map modified from Page and Burr 1991.
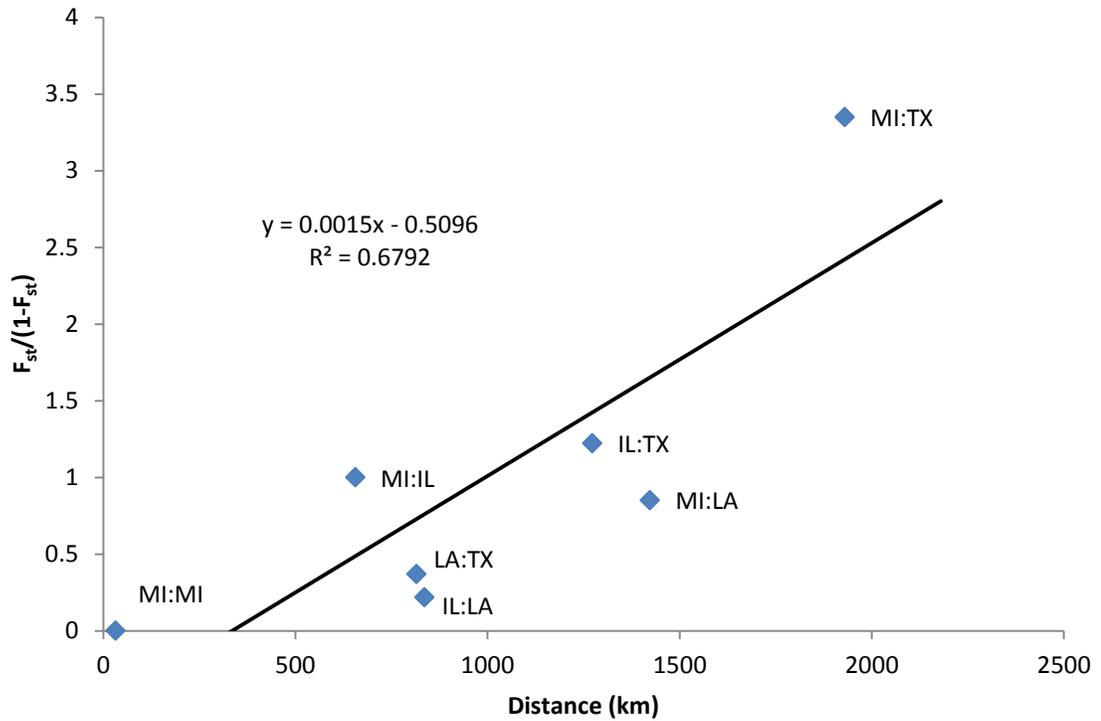
Figure 4.7. Pairwise geographic distance (km) versus genetic distance ($F_{st}/(1-F_{st})$) for spotted gar populations. ANOVA indicated significant positive correlation ($r^2 = 0.68$) between genetic distance and geographic distance, suggesting isolation by distance in spotted gars. "MI:MI" refers to genetic versus geographic distance for the two MI-p subpopulations used in analyses.
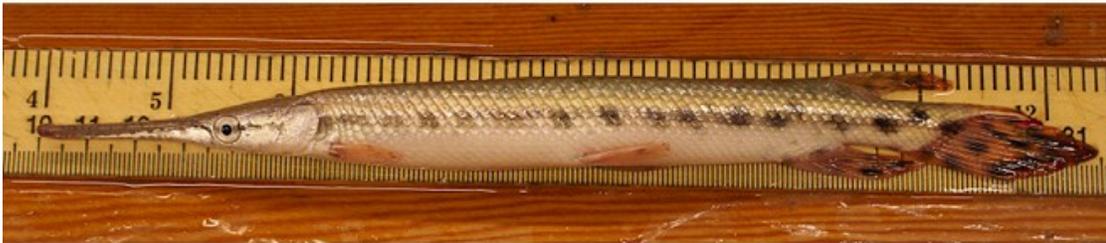
Figure 4.8. Comparison of adult and juvenile spotted gars from core and peripheral populations. Adult spotted gar from Michigan (top photo) compared to adult spotted gar from Louisiana (second photo); young of the year spotted gar from Michigan (third photo) compared to young of the year spotted gar from Louisiana (bottom photo). Note elongate morphology of caudal peduncle in peripheral population specimens compared to shorter and stouter caudal peduncle in core population specimens. Photos by David (2008, 2009).

Chapter 5

Conclusion

<u>Summary</u>

Peripheral populations of species often exist under different and "harsher" environmental conditions than core populations, and as a result may exhibit different life history characteristics. Further, due to their often small population size, geographic position, and low gene flow, peripheral populations may also exhibit variation in genetic diversity compared to core populations of species. All these factors, coupled with increased likelihood of genetic drift, suggest increased potential for local adaptation and speciation to occur in peripheral populations as they diverge from core populations. Due to low recolonization potential, peripheral populations are further susceptible to localized extinction in comparison to core populations. For all these reasons, conservation of peripheral populations of species is an integral part of conserving natural biodiversity.

My study used the spotted gar *Lepisosteus oculatus* as a model species to investigate variation among peripheral and core populations and found significant differences in life history traits as well as population genetic structuring between the two segments and among component populations. My findings suggest that peripheral population spotted gars have adapted to life at higher latitudes (shorter growing season) and exhibit very low genetic diversity, most likely due to founder effects, low gene flow, and population disjunction (from the core population segment).

Chapter 2 investigated the differences in growth rate between YOY spotted gars from core (Louisiana) and peripheral (Michigan) populations using common garden experiments. This investigation consisted of two experiments: experiment 1 observed spotted gars from both populations maintained at the same temperature (23 °C) for approximately 60 days with unlimited ration; experiment 2 explored growth in spotted gars from both populations at three different temperature treatments (16 °C, 23 °C, 30 °C) for 42 days with unlimited ration. Differences in growth rate observed between populations were then described in relation to countergradient variation theory. I found that spotted gars from the peripheral population grew significantly faster and to a larger size than spotted gars from the core population, and that observed differences in growth rate indicated countergradient variation in growth in spotted gars.

Chapter 3 explored differences in life history characteristics (e.g. size at age, mortality rate) among 5 populations of spotted gars from core and peripheral populations. I analyzed length and age data to model growth and mortality rates for core and peripheral population segments as well as component populations. Different modeling techniques yielded different results, however, only one model accounted for length of growing season (using thermal opportunity for growth, TOG) and therefore provided the most accurate comparison of growth rates between core and peripheral populations. Comparisons of population sample means and TOG-corrected growth rates indicated that spotted gars from the peripheral population segment were larger (length), older (higher mean age and maximum age), and had higher growth rates than spotted gars from the core population segment. Comparison of mortality rates between population segments also suggested potential compensatory mortality in spotted gars. My results indicated

that core and peripheral population segments have developed different life history characteristics and that these differences can be related (at least in part) to latitudinal variation in environmental factors such as length of growing season.

In chapter 4 I analyzed mitochondrial DNA (mtDNA) to explore differences in genetic structure among core and peripheral populations of spotted gars. I then used concepts from phylogeography and historical biogeography to describe variation in genetic structure among spotted gar populations relative to geographic position. Genetic diversity, based on analysis of haplotypes, was highest in the Mississippi River drainage, lowest in the Great Lakes drainage, and most divergent in the western Gulf Coast drainage. Genetic structure and low diversity in the Great Lakes drainage (peripheral population) was likely related to recent post-glacial colonization from Mississippi River refugia, founder effects, and lack of gene flow. Alternatively, low diversity observed in the peripheral population may reflect selection for the most adaptive genotype (to the harsher environment). High divergence in the western Gulf Coast population was likely associated with genetic drift and lack of gene flow from the Mississippi River drainage and comparatively minimal influence from Pleistocene glaciations (longer time for divergence). My results suggest that both the Great Lakes and western Gulf Coast populations could be considered peripheral populations, due to their phylogeographic characteristics, relative to the Mississippi River drainage populations. Due to extremely low genetic diversity and complete disjunction, the Great Lakes population of spotted gars may be the most vulnerable to local extinction.

<u>Synthesis</u>

Based on the results of my three research chapters (2-4), I concluded that spotted gars from core and peripheral populations exhibited variation in life history traits as well as genetic structure. The peripheral population segment was shown to be a distinct component of the overall species, exhibiting faster growth rate, longer lifespan, and very low genetic diversity. Spotted gars from the peripheral population appear to grow faster and larger than those from the core population, primarily during their first growing season. My common garden experiments demonstrated countergradient variation in growth in YOY spotted gars, and higher growth rates (in the peripheral population) in early life are also suggested from models in my life history analyses (chapter 3). Faster growth to larger size is particularly important in the first growing season at higher latitudes, as overwinter mortality in YOY fish has been shown to have a large impact on recruitment (Hurst 2007). After the first winter, many fishes are believed to have attained sizes large enough to reduce the impact of overwinter mortality, and therefore size may not play as important a role in older fish as in YOY individuals. Combining the results from chapters 2 and 3, I conclude that countergradient variation in growth is most observable in YOY spotted gars, when fast growth is most important for overwinter survival at higher latitudes. At yearling stages and beyond, differences in growth rate are less noticeable based on standard length-at-age analyses (length-at-age regression, VBGM), however, when length of growing season is accounted for, I have shown that peripheral population spotted gars exhibit a faster growth rate than core population spotted gars. Compensatory mortality may also exist among spotted gar populations; peripheral population fish had significantly lower mortality rates than core population

fish, however, larger sample sizes from component populations are likely necessary to determine if the mortality rates I observed are ecologically significant.

Genetic structure among core and peripheral populations reflected recent geological events as well as indicated possible additional peripheral populations for further study. The only haplotype observed in peripheral population spotted gars was also observed in populations from the Mississippi River drainage, reflecting former connection and origin from Mississippian refugia. The single Great Lakes drainage haplotype may also represent the genotype best adapted to the ecologically peripheral environment of the region and/or the most adaptive genotype of the species (Scudder 1989). The western Gulf Coast population of spotted gars consisted of haplotypes unique to the region; this level of divergence suggests that it may also be a peripheral population of the species. Combining results from chapter 3 and 4, I found that the population from Lake Seminole, Georgia, representative of the eastern Gulf Coast drainage, showed consistent differences from other core populations (e.g. size, growth rate); Sipiorski (2011) found that spotted gars from this region showed more genetic similarity to Florida gars *Lepisosteus platyrhincus* than spotted gars from the Mississippi River drainage. Given these results, the Mississippi River drainage may be the "true" core population segment, with peripheral populations based on genetic divergence and geologic separation in the Great Lakes, western Gulf Coast, and eastern Gulf Coast drainages. Further study of additional populations may further elucidate relationships among these potential population segments.

Spotted gars from peripheral populations exhibit very low genetic diversity and are completely disjunct from the core populations in the Mississippi River drainage and

southern United States. Populations of species with very low genetic diversity have been shown to be much more vulnerable to perturbations such as habitat loss, invasive species, and overfishing (Garcia de Leaniz et al. 2007). Spotted gars are currently listed as threatened and therefore protected throughout their range in Canada (COSEWIC 2005, Glass et al. 2011), but are only listed as a "species of greatest conservation need" in Michigan, where a large portion of the peripheral population resides in inland lakes (Carman 2002, Hubbs et al. 2004, Page and Burr 2011). Spotted gars are dependent on aquatic vegetation for multiple life stages, and loss of habitat is believed to be the largest threat to peripheral populations of the species (Trautman 1981, Carman 2002, COSEWIC 2005). Loss of essential habitat coupled with very low genetic diversity make peripheral populations of spotted gars highly susceptible to local extinction, which has already been recorded in localities within Ohio and Michigan (Trautman 1981, Carman 2002, David unpublished data). Additional investigations into habitat use, abundance, and effective population size are recommended to protect potentially vulnerable peripheral populations of spotted gars, and therefore contribute to the conservation of local biodiversity.

Latitudinal variation and potential countergradient variation in growth may also exist in other gar species. With the exception of the Cuban gar *Atractosteus tristoechus*, all extant gar species have relatively wide latitudinal distributions, therefore interpopulation variation associated with length of growing season is quite possible. Further study of latitudinal variation in life history traits may be important in conservation efforts for species such as the alligator gar *A. spatula*, which has been extirpated from much of its historical range and continues to be threatened by habitat loss, but is also an important food fish and game fish in its current distribution

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(Scarnecchia 1992, García de León 2001, Clay et al. 2011). The tropical gar *A. tropicus* has a range that extends from Mexico to Costa Rica, and is an important local food fish in many parts of its range (Barrientos-Villalobos and Monteros 2008); better understanding of latitudinal variation in growth of gars may lead to better production in aquaculture and restocking efforts for the species (Alfaro et al. 2008, Conover 2009). Additionally, better understanding of the implications of low genetic diversity, such as that observed in peripheral population spotted gars, may better inform aquaculture and conservation efforts of the threatened and highly endemic Cuban gar, which is relegated to a very small region in southwestern Cuba and the Isle of Pines (Comabella et al. 2010).

My dissertation research has shown that the peripheral population of spotted gars in the Great Lakes region is a unique component of the overall species, existing at the edge of the species range under conditions dramatically different (i.e. length of growing season) from those experienced by the species core populations. Lesica and Allendorf (1995) noted that peripheral populations of species often exist under harsher conditions and are more susceptible to local extinction than core populations. Further, peripheral populations of species often have disproportionately high conservation value because of their potential divergence and degree of local adaptation relative to population size and frequency (Lesica and Allendorf 1995). Luck et al. (2003) stated that the relationship between biodiversity and ecosystem services is mainly a function of local populations; peripheral populations of species may therefore play an integral role in unique environments compared to core populations. Long considered a "trash" or "rough" fish (and in many localities this classification persists), gars have been shown to be important components of local food webs, contributing to the balance of

game and forage fish populations (Becker 1983, Scarnecchia 1992). Gray et al. (2012) determined that sedimentary turbidity significantly negatively affected hatching success of spotted gars. Additionally, Wehrly et al. (2012) showed that (peripheral population) spotted gars were significant indicator species in the classification of Michigan inland lakes (specifically indicating small, warm, mesotrophic lakes) and may have value as indicators of ecosystem health and native macrophyte diversity. The Great Lakes Basin population of spotted gars was shown to be a unique component of the overall species, and I believe that my work can inform conservation strategies and help us to better understand the evolution and maintenance of vertebrate life history patterns and genetic diversity.

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