2023

Characterizing Genetic Diversity and Testing for Fisheries Induced Evolution in Southeastern Kansas Populations of Bluegill (*Lepomis macrochirus*)

Morganne C. Borsh
University of Northern Iowa

Characterizing genetic diversity and testing for fisheries induced evolution in southeastern Kansas populations of bluegill (*Lepomis macrochirus*)

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree of Master of Science

Morganne C. Borsh

University of Northern Iowa

May 2023

Abstract

Bluegill (*Lepomis macrochirus*) is a popular and versatile sport fish with wide distribution. Fisheries management of the species is generally nebulous due to the additive role of environmental factors, genetics, social interactions, and alternative reproductive tactics in determining bluegill growth. Size structure could be further influenced by angling pressure: large parental males are more susceptible to harvest than smaller cuckolders. The systematic removal of large individuals through angling can result in stunted populations via fisheries induced evolution (FIE). In southeastern Kansas, bluegill from popularly fished impoundments are projected to have smaller sizes compared to isolated or inaccessible impoundments from the same region. The goal of this study is to characterize the genetic diversity of Kansas bluegill from 10 southeastern populations and detect potential fisheries-induced evolution suspected from long-term harvest. A total of 100 individuals were sampled from ten small impoundments in southeast Kansas. Five impoundments were identified as having high angling pressure and five as low angling pressure. All individuals were genotyped using genome-wide single nucleotide polymorphisms (SNPs) collected by restriction-site associated DNA sequencing (RADseq). We were not able to find evidence of FIE, although analyses are limited by the lack of a reference genome. Most of the genetic variation occurs within Bluegill populations, rather than between them. We could not detect any genetic differences between fished and unfished populations. Findings suggest the observed changes in size structure are likely a function of plastic or environmentally driven genetics.

This Study by: Morganne C. Borsh

Entitled: Characterizing genetic diversity and testing for fisheries induced evolution in southeastern Kansas populations of bluegill *(Lepomis macrochirus*)

has been approved as meeting the thesis requirements for the

Degree of Master of Science

Date	Dr. Peter B. Berendzen, Chair, Thesis Committee
Date	Dr. Mark Myers, Thesis Committee Member
Date	Dr. Ai Wen, Thesis Committee Member
Date	Ben Neely, Thesis Committee Member
Date	Dr. Gabriela Olivares, Interim Dean, Graduate College

Dedication

I would like to dedicate this paper to both my grandmothers, Donna Vogel and Lola Woods, who raised me to be who I am today. Although I can no longer thank them in person, I owe every opportunity for success and education to them. I miss you both.

Acknowledgements

Funding for this project was provided by the Kansas Department of Wildlife and Parks (KDWP) and a Graduate Research Award for Student Projects (GRASP) from the College of Humanities, Arts, and Sciences. I am especially grateful for Ben Neely and all the KDWP staff that helped collect data, answered my *many* questions, and initiated this project. Special thanks to Drs. Ai Wen and Mark Myers for their support and advisement throughout this process (not to mention keeping me accountable and motivated). I would also like to thank my cohort for their sympathetic understanding, camaraderie, and review help--I wish you all the best with your own thesis process! Additionally, I would like to particularly thank Jon Luiken for all his help and support through the arduous bioinformatics learning process. I would only have a fraction of the skillset I have now without your continued support. Lastly, I would like to thank my graduate advisor Pete for first encouraging me to stick around for a graduate thesis, understanding my sometimes odd work patterns, and pulling me into his family. I could not have accomplished so much without you all!

Table of Contents

List of Tables **vii**

List of Figures **viii**

Chapter 1: Introduction **1**

Bluegill Ecology and Life History 2

Fisheries Induced Evolution 6

Chapter 2: Methods **9**

Study System and Sampling 9

RADseq Library Preparation and Sequencing 11

Data Assembly and SNP Discovery 12

Genetic Diversity and Structure Analyses 13

Phenotypic Association Studies 15

Chapter 3: Genetic Diversity Results **16**

Diversity Statistics 16

Population Structure 22

Chapter 4: Genome Wide Association Studies (GWAS) Results **26**

Chapter 5: Discussion **30**

Population Structure 30

Fisheries Induced Evolution 32

Phenotypic Association Studies 34

Chapter 6: Conclusions **36**

References **37**

List of Tables

Table 1 *Southeast Kansas impoundments information* 10

Table 2 *Genetic diversity estimates* 17

Table 3 *Analysis of Molecular Variance (AMOVA)* 22

List of Figures

Figure 1 *A bluegill sunfish* 2
Figure 2 *Alternative Reproductive Tactics (ARTs) in Bluegill* 6
Figure 3 *Ten bluegill sample sites across southeastern Kansas* 9
Figure 4 *Genetic Relatedness Matrix for all individuals* 19
Figure 5 *Genetic relatedness matrix for all female bluegill* 20
Figure 6 *GRM for all males* 21
Figure 7 *FastStructure Barplots* 23
Figure 8 *Bayesian population clustering of all individuals* 24
Figure 9 *Bayesian population clustering of all females* 25
Figure 10 *Bayesian population clustering of all males* 25
Figure 11 *Manhattan plots for all individuals* 26
Figure 12 *Manhattan plots for all females* 27
Figure 13 *Manhattan plots for all males* 28
Figure 14 *Manhattan plots for all males at age three* 29
Figure 15 *Manhattan plots for all males at maturity* 29

Chapter 1: Introduction

Bluegill (*Lepomis macrochirus*) are a common, widely distributed sport fish with nebulous management strategies (Figure 1). Reductions in large individuals available for harvest have decreased the quality of bluegill fisheries across much of their range (Olson and Cunningham 1989; Beard and Kampa 1999; Rypel et al. 2016). Size-based management strategies struggle to identify causes of size reduction due to the additive role of environmental factors, genetic inheritance, social interactions, and sex-specific evolutionary processes, in determining bluegill growth. Population size structure is further influenced by angling pressure (Conover and Munch 2002; Olsen et al. 2004; Walsh et al. 2006). The systematic removal of larger individuals may genetically alter the population, resulting in Fisheries Induced Evolution (FIE) and causing stunted populations. Stunted populations are common and considered a major management problem within the U.S. (Swingle and Smith 1941; Beard et al. 1997; Kuparinen and Merila 2007). Despite difficulties in management, few studies have been done to understand the genetic diversity of bluegill in conjunction with phenotypic changes. This study seeks to fill this knowledge gap by characterizing the genetic diversity of several bluegill populations in southeastern Kansas, assessing potential genetic changes derived from angling pressure, and identifying potential genes associated with bluegill growth.

Figure 1
A bluegill sunfish (Lepomis macrochirus). Bluegill illustration by Maynard Reece from Iowa Fish and Fishing.

Bluegill Ecology and Life History

Bluegill are a popular freshwater sport fish found in both lotic and lentic water bodies (Page and Burr 2011). Their native range extends from Canada to northern Mexico, but they have been introduced in several other countries for fishing purposes (Welcomme 1988; Burgess and Franz 1989; Maezono and Miyashita 2003; Ma et al. 2003; Kawamura et al. 2006; Page and Burr 2011). Bluegill are a species of sunfish belonging to the family Centrarchidae. They generally average 7.5 inches (19.1 cm) in length, have a maximum recorded weight of just under 5 pounds (2.2 kg), and have an average lifespan of 5-8 years (USFWS n.d.). Bluegill are prolific colony breeders that spawn when water temperatures rise between 18 and 26 degrees Celsius (USFWS n.d.). This usually occurs from late spring to early summer, although multiple spawning periods can occur within a spawning season (USFWS n.d). During this time, large males construct shallow nests for females to deposit their eggs. Females may select nests based on location and male size; however, males solely guard the eggs once deposited.

Bluegill growth is highly plastic and determined by both conditional and genetic influences. Several environmental conditions have been shown to influence bluegill growth, including lake morphometry, water conditions, food availability, species interactions, and exploitation (Tomcko and Pierce 1997; Tomcko and Pierce 2001). Other environmental factors, including Secchi depth, maximum depth, temperature, and total alkalinity, have been found to be significant contributors to bluegill growth (Tomcko and Pierce 1999; Porath and Hurley 2005). Other notable factors include the abundance of aquatic vegetation (Trebitz et al. 1997), invertebrate densities (Paukert et al. 2002), and spawning date (Santucci and Wahl 2003).

Bluegill growth is further affected by species interactions and is density dependent (Gerking 1962; Wiener and Hanneman 1982; Mittelbach 1988; Osenberg et al. 1988; Belk 1992; Neely et al. 2020). Both interactions with competitors and predators can cause shifts in size structure (Michaletz 2020). Increased competitors result in less food availability and skew population size structure towards smaller individuals (Mittelbach 1988; Osenberg et al. 1988). This over-abundance of competitors can stem from a lack of predators; however, an overabundance in predators can similarly shift bluegill populations' size structure towards small individuals (Werner et al. 1983; Mittelbach 1988; Osenberg et al. 1988, 1994; Belk and Hales 1993; Snow and Staggs 1994). In addition, the size-selective nature of harvest has been historically cited as a cause for smaller size structures in bluegill populations (Drake et al. 1997; Michaletz 2020).

As a result of environmental and social interactions, stunted populations, or those with predominantly small, slow-growing individuals, are common and considered a

major management problem within the United States (Swingle and Smith 1941; Beard et al. 1997; Kuparinen and Merila 2007). In an attempt to improve bluegill population size structure, several different, site-specific management strategies have been attempted. Historically, these have included the elimination or addition of predator species (depending on if bluegill densities are too low or high, respectively), supplemental feeding, and fishing regulations (Porath and Hurley 2005; Allyon et al. 2018; Neely et al. 2018). These practices are met with varying levels of success. Overall, management strategies that focus on single factors that influence bluegill growth have proven ineffective in changing bluegill size structure (Beard et al. 1997; Schneider and Lockwood 1997).

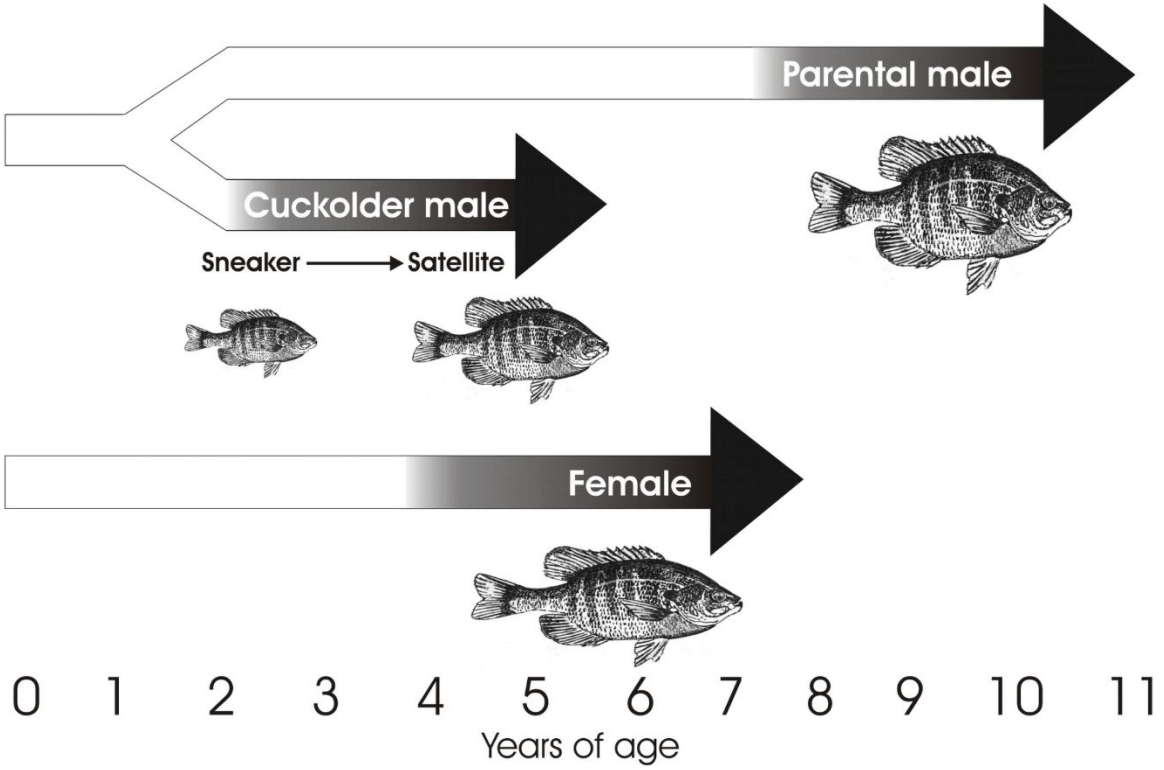
Genetic factors affecting growth may be further convoluted by sex-specific evolutionary processes. For example, Aday et al. (2006) noted that, while female bluegill growth was predominantly determined by resource availability, male bluegill growth was chiefly determined by social interactions regardless of resource level. This social structure is established by males' alternative reproductive tactics (ARTs). Male bluegill possess two complex, plastic, alternative life histories with differing survivorship (Aday et al. 2003): parental or cuckold reproductive strategies (Figure 2) (Gross 1982). Parentals act as nest-guarders and can fertilize an average of 78% of eggs, which they then provide the sole-parental care for (Philipp & Gross, 1994; Fu et al., 2001; Neff, 2001; Neff and Lister 2006). Cuckolders provide no parental care and use a parasitic tactic to steal fertilizations from parental males by sneaking into nests (sneakers), or mimicking females when they are older (satellites) (Gross 1982; Drake et al. 1997; Ehlinger et al. 1997).

Between parentals, size is a major competitive advantage: larger individuals fertilize more eggs, and get preferable nesting sites. Younger parentals will consequently allocate resources towards growth, rather than reproduction, and mature later (Aday et al. 2006). Functionally, older and larger parentals can cause juvenile males to delay maturation (Jennings et al. 1997; Aday et al. 2003). In removing larger and more mature individuals (altering the population's social structure), parentals can mature sooner at smaller sizes. Cuckholders, which do not compete via size, allocate resources towards reproduction (Beard et al. 1997; Jennings et al. 1997). Although both reproductive strategies offer similar growth rates, parentals generally mature later and can reach larger sizes, while cuckholders reach maturity more rapidly at smaller sizes (Gross 1982).

Parentals overall display lower survivorship. At maturity, parentals are considered more susceptible to angling pressure during spawning season due to high visibility in nesting sites and aggression when guarding nests (Beard et al. 1997). Moreover, progeny from parentals display lower survivorship compared to progeny from cuckholders; cuckold fry have higher conversion efficiency and are less likely to forage in risky habitats, ultimately having higher fitness (Balon 1986; Neff 2004; Lister and Neff 2006). Lower survivorship in parentals is balanced by female preference for larger, dominant males, and their ability to fertilize a majority of nested eggs (Drake et al. 1997).

Figure 2

Alternative Reproductive Tactics (ARTs) in Bluegill, borrowed from Partridge et al (2016).. Parentals are considered a fixed mechanism, while cuckolder's are plastic, switching from sneakers to satellite (or female mimics) as they grow in years of age, based on Gross and Charnov (1980) but ages may differ by population (Dominey 1980).



Fisheries Induced Evolution

The lack of life history data for male bluegills and how these strategies are affected by fishing is a key knowledge gap. Parental bluegill's decreased survivorship and angling susceptibility put populations at risk for Fisheries Induced Evolution (FIE) (Kuparinen and Merila 2007). Fisheries Induced Evolution is the rapid evolution of a population in response to strong selective pressures from fishing. Fisheries Induced Evolution has been documented in numerous ecological contexts and is now a widely accepted concept in fisheries management (Stockwell et al. 2003; Conover et al. 2006; Hoxmeier at al. 2009). Fisheries Induced Evolution can lead to undesired phenotypic changes in a population, resulting from the prolonged harvest of a specific demographic

(Enberg et al. 2010; Heino et al. 2015). Slow growing, late-maturing species are particularly vulnerable to FIE as increased mortality favors earlier sexual maturation at smaller sizes and an increased reproductive output (Roff 1992; Kuparinen and Merila 2007). Increased mortality from fishing includes population changes such as alterations in growth rate (Conover and Munch 2002), maturation schedules (Diana 1987; Drake et al. 1997; Olsen et al 2004), fecundity (Healey 1978; Baccante and Reid 1988), and larval survival (Walsh et al. 2006). In bluegill populations, increased mortality from fishing would remove large parentals and favor smaller cuckolders, leading to FIE. This has been well-documented in bluegill since 1981, where length- and age-frequency distributions shifted toward smaller sizes and younger ages, and mean age and life spans decreased to values below recommended ranges in Wisconsin lakes (Goede and Cobble 1981).

Despite the documented role of FIE in bluegill management, no genomic studies have been conducted to confirm fixed genetic determination of male ARTs. Bluegill ARTs can be a function of conditional mechanisms that change throughout life (plastic), fixed tactics that are inherited polymorphisms, conditional switches determined before maturation, or a combination of the two (fixed) (Gross 1996; Gross and Repka 1998; Taborsky 1998; Piche et al. 2008; Taborsky et al. 2008; Neff and Svensson 2013). Due to the lack of a reference genome, most studies have focused on differential gene expression to identify genes associated with these tactics (Fraser et al. 2014; Schunter et al. 2014; Stiver et al. 2015; Partridge et al. 2016). At present, studies lack clarity in the dominant driving mechanisms for bluegill ARTs, although it is generally noted that parental males is a fixed mechanism, while cuckolders are plastic (switching from sneakers to satellites, Figure 2) (Gross and Charnov 1980; Partridge et al. 2016). Neff and Lister report that life

histories are likely conditional and not determined by a genetic polymorphism (2006). Alternatively, Kuparinen and Merila suggest 20-30% of variation in life-history traits are inherited for the species and assert that both mechanisms play a role, a finding supported by Aday et al. (2007; 2008). Assessing life-history associated genetic variation could provide evidence for fixed genetic polymorphisms and help managers better understand FIE. Ultimately, understanding whether population changes are based on plastic or fixed genetic mechanisms is key for successful management (Kuparinen and Merila 2007).

The Kansas Department of Wildlife and Parks (KDWP) has observed a decline in large bluegill individuals harvested from popularly fished impoundments. They have not seen a similar decrease in large individuals from impoundments that are difficult to reach or inaccessible, presumably having lower fishing pressure (KDWP personal communication). KDWP managers suspect that, in heavily fished impoundments, anglers are systematically removing larger bluegill, resulting in stunted, genetically altered populations. To understand the driving factors in altering Kansas bluegill populations, finclip samples were collected from ten localities and individuals were genotyped using genome-wide SNP data collected via Restriction-site Activated DNA Sequencing (RADseq). Through a population genomics approach, this study seeks to characterize the genetic diversity of bluegill within and between impoundments, determine if there are phenotypically associated genes in which FIE could act on, and assess if FIE has occurred in these populations. Understanding these fine-scale genetic factors can better inform what components are influencing Kansas bluegill's growth, assess the effects of angling, and help improve stocking practices.

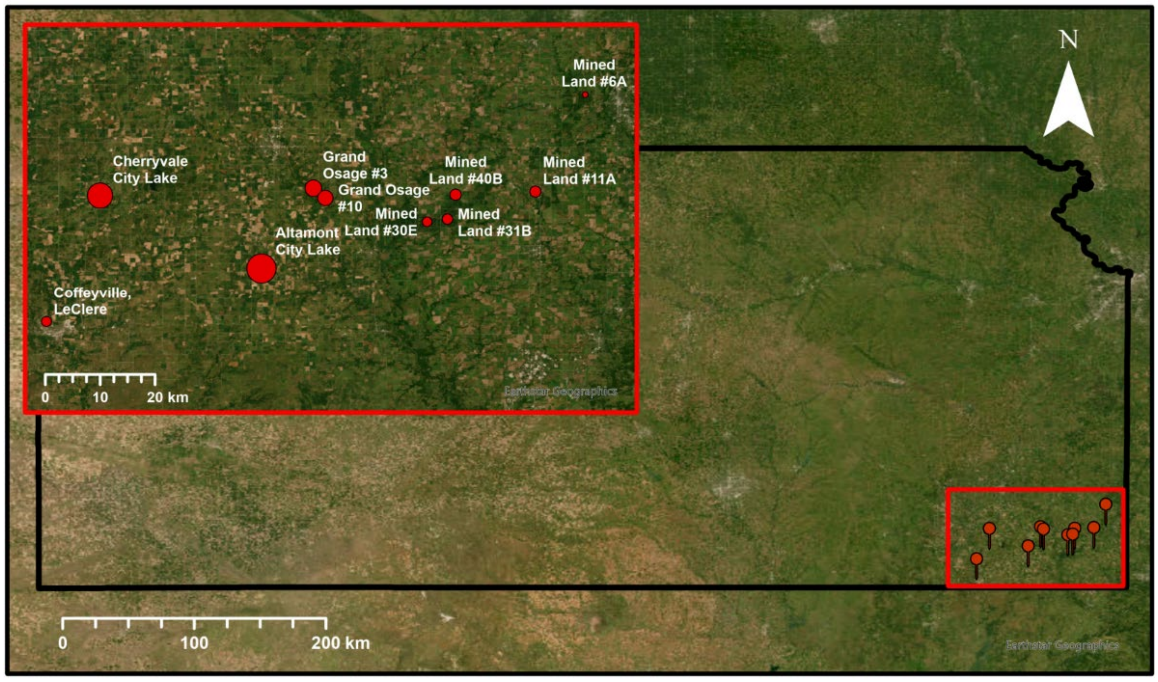
Chapter 2: Methods

Study System and Sampling

Bluegill were sampled from ten small impoundments (3.7-22.0 acres) across southeast Kansas (Figure 3). Five of the impoundments were popular fishing localities with high angling pressure ("Fished") and the other five impoundments had low angling pressure ("Unfished") (Kansas Department of Wildlife and Parks). All impoundments were man-made in the 1900s and established as either drainages or remnants from mining operations. Although they were initially stocked, impoundments have received no further regulation, including no further stocking, creel limits, or length limits. Lake details are summarized in Table 1.

Figure 3

Ten bluegill sample sites across southeastern Kansas, represented by the red pins and circles. Map insert also denotes the proportional size of locality.



At each locality, shoreline electrofishing was used to collect 10 whole individuals (100 in total) between March 1 and 15, 2021. For each individual, sex, mass, gonad mass, maturity, and total length were recorded. Finclip samples were taken from each individual for genetic sampling and stored in 99% ethanol. Gonad size as a percentage of overall mass was used to calculate Gonadosomatic Index (GSI) for each individual $(\frac{gonad\ mass}{total\ mass}\ X\ 100)$. Age was estimated from the whole view of the sagittal otolith using 10x magnification. Both sex and maturity were determined if ovaries or testes were visible and developed, respectively.

Table 1

Southeast Kansas impoundments, including projected angling pressure, size in acres, and last known stocking. Grand Osage localities have no record of stocking, instead their purchase date is recorded.

Locality	Abbreviation	Angling Pressure	Acres	Last Stocked
Altamont City Lake	ALCA	Fished	22.0	1979
Coffeyville-LeClere	CFCL	Fished	7.0	1977
Cherryvale City Lake	CHCL	Fished	18.9	1991
Mined Land 6A	MO6A	Fished	3.7	1951
Mined Land 11A	M11A	Fished	7.9	1951
Grand Osage 3	GOWA3	Unfished	12.2	1941*
Grand Osage 10	GOWA10	Unfished	11.2	1941*
Mined Land 40B	M40B	Unfished	8.0	1981
Mined Land 30E	M30E	Unfished	6.7	1981
Mined Land 31B	M31B	Unfished	7.3	1981

RADseq Library Preparation and Sequencing

Finclips were used for Restriction-site Activated DNA Sequencing (RADseq) to generate indexed, genetic libraries of individuals. This protocol was developed by Etter et al. (2011), and modified by Gamble et al. (2015) and Luiken et al. (2021). DNA was extracted from each finclip using a Qiagen DNeasy DNA Extraction Kit. Sample DNA was eluted to 200 μL, then further diluted to 22-60 μg/mL based on nanodrop concentrations. Approximately 1 μg of DNA was digested with the high-fidelity SbfI restriction enzyme (New England Biolabs) at 37°C for two hours, revealing a sequence overhang. Ten unique P1 adapters (molecular ID's) were then ligated to the cut site for each individual. The uniquely labeled individuals were pooled according to location, forming 10 libraries. These were then sheared using cyclic sonication (Diagenode Bioruptor). Sheared libraries were then size selected for 400-600 bp fragments using an electro-elution Pippin Prep system and internal standards (Sage Science, Beverly, MA).

Size-selected libraries were dA-tailed and purified with a Qiagen Reaction Cleanup Kit, following Rohland and Reich's protocol (2012). Unique P2 Illumina barcodes were ligated to each library. Libraries were amplified via PCR, cleaned, and size selected a second time using Qiagen's GeneRead Size Selection Kit. To prepare for sequencing, libraries were quantified, pooled, and size-verified using an Agilent Bioanalyzer Assay (Agilent, Santa Clara, CA). Pooled libraries were then sequenced on the NovaSeq 6000 Platform at the Iowa Institute of Human Genetics (University of Iowa Division), using 150 bp paired-end reads.

Data Assembly and SNP Discovery

Single Nucleotide Polymorphisms were identified and genotyped using Stacks v.2.62, including *process_radtags, denovo_map.pl,* and *populations* scripts (Catchen et al. 2013). Raw sequence reads were demultiplexed and quality filtered via the *process_radtags* script. Reads were trimmed to 133 base pairs to remove the P2 adapter sequence and low-quality bases. Additionally, two mismatches were allowed in the adapter sequence. Files were concatenated by individual.

As no reference genome was available for *L. macrochirus*, the Stacks v2.62 *denovo_map.pl* pipeline was used to generate a consensus sequence catalog and align loci (Catchen et al. 2013). The minimum number of identical raw reads required to create a stack (-*m*) was set to 4, and the maximum number of mismatches between loci when processing a single individual (-*M*) was set to 3. For catalog construction, a maximum of two mismatches were allowed between loci (-*n*) (Catchen et al. 2013; Paris et al. 2017).

Single Nucleotide Polymorphism (SNP) discovery and the resulting datasets were generated using the *populations* script. Following Cerca et al. (2020), *populations* was run independently for each locality to better detect individuals with a high degree of missingness. Percentage of missing data was identified (--*missing-indv*) and those with greater than 10% missingness were removed from the dataset (Danecek et al. 2011; Cerca et al. 2020). All remaining individuals with sufficient read quality (92 individuals) were then recombined and *populations* was re-run. As follows, three SNP datasets, grouped by impoundment, were created for downstream analyses: i) all individuals (92), ii) all males (35), and iii) all females (35).

For each dataset, flags were used to remove missing data, remove artifactual nucleotide sites, and minimize linkage disequilibrium. Only loci in a minimum of one population (*-p*) and shared by a minimum of 80% of all individuals (*-r*) were retained. Alleles occuring at a frequency of less than 5% (*--min-maf*) and individuals with an observed heterozygosity greater than 50% due to paralogous or multilocus contigs (*--max-obs-het*) were removed. Only the first SNP mined per locus was considered to reduce the non-random association of alleles at different loci (linkage disequilibrium) (*--write-single-snp*) (Catchen et al. 2011; Catchen et al. 2013; Gargiulo et al. 2021). All SNPs were called with respect to all individuals across populations (*--min-samples-overallI*). Supplementary output options were additionally used to generate files for bioinformatic analyses or conversion in the software package PGDSpider 2.1.1.5 (Lischer and Excoffier 2012).

Genetic Diversity and Structure Analyses

To measure genetic diversity within and between the ten impoundments, several diversity statistics were produced. Expected and observed heterozygosity, nucleotide diversity, inbreeding coefficients, and pairwise F_{ST} values were calculated using the Stacks v2.62 *populations* pipeline (Rochette and Catchen 2017). Effective population (N_e) was estimated using the linkage-disequilibrium method (Hill 1981), as implemented in NeEstimator v2.1 (Do et al. 2014). Additionally, loci were assessed to identify loci under selection using BayeScan v2.1 (Foll and Gaggiotti 2008).

To determine if the variation for each dataset was best explained within or between groups, an Analysis of Molecular Variance (AMOVA) test was performed using

the *Arlequin* v3.5 (Excoffier and Lischer 2010). To visualize genetic distance and genetic clusters, a Discriminant Analysis of Principal Components (DAPC) was done and membership probability was further visualized. The DAPCs and genotype composition plots were generated using the *adegene*t v2.1.7 package in R v4.2.0 (Jombart 2008; Jombart et al. 2010; Jombart and Ahmed 2011; R Core Team 2022). Each dataset was cross validated 500 times with 100 replicates using the maximum number of principal components (PCs). From the cross-validation, the recommended PCs with the lowest Root Mean Square Error (RMSE) were retained for each analysis. To optimally describe differences between groups and individuals, all Discriminant Analyses (DAs) were retained. For the overall dataset, 20 PCs demonstrated the highest means and were retained. The resulting recommended DAs and PCAs were retained and further used in the *adegenet* R package (2022.07.1+554) to generate assignment plots for each dataset.

Because DAPCs transform the data using Principal Component Analysis (PCA), some information can be lost if not all Principal Components are retained (Jombart 2013). To support the transformed data without identifying populations *a priori*, a K-means Bayesian assignment test using fastStructure was conducted (Python v.3.10.10) to determine genetic clusters (K) (Raj et al. 2014). Cluster values (n +1) were assessed for each dataset and 500 cross-validation (--cv=500) test runs were done. The *chooseK.py* script was then used to recommend the model complexity and maximum marginal likelihood, which reflect the likely number of subpopulations best needed to describe the diversity within the entire dataset (Raj et al. 2014). The largest optimal clustering value was selected and used for the *district.py* v2.3 script to output admixture plots showing membership probability (Chhatre 2018).

Phenotypic Association Studies

For the Genome-Wide Association Studies (GWAS), datasets for all individuals, females, and males were considered. Males were further divided into mature males, and age three males (mix of mature and immature individuals) to better assess potential markers associated with different male ARTs. Age three males were selected because they had the highest sample size of age classes at an age where cuckolds should be mature and parentals may not. For these five datasets, GWAS analyses were conducted for angling pressure (fished vs. unfished), total length, mass, gonad mass, GSI, sex, age, and maturity. Files were prepared and analyzed using Plink v.1.9 (Purcell and Chang 2015). The standard case/control association analysis was done and association files were imported into RStudio. Manhattan plots were generated using the R package *qqman* and Due to the high amounts of retained SNPs, the authors recommended p-value of 5e-08 was used to restrict significance of markers (Turner 2018).

Chapter 3: Genetic Diversity Results

Diversity Statistics

After filtering, 41,889 loci and 17,446 variant sites were retained for genotyping across all 92 individuals. For females, 41,709 loci with 17,494 variant sites were retained. Across males, 43,717 loci consisting of 17,494 variant sites were retained. Averages across datasets for variant loci were consistent, ranging from 17,446-17,494. For all sites in all datasets, the observed heterozygosity (H_O) was consistently higher than the expected heterozygosity (H_E). Although expected heterozygosity was comparable between males and females, males had higher observed heterozygosity across populations (Table 2). Cherryvale males had the highest observed heterozygosity, particularly in comparison to the expected, with a 0.154 difference. The average inbreeding coefficient (F_{IS}) for all individuals was <0.000. The inbreeding coefficients for males and females averaged to 0.006, despite differing measurements between individual populations. Across all individuals and for the separate males and females datasets, Mined Land 31B consistently demonstrated an inbreeding coefficient <0.000 (Table 2). Effective population size estimates for all populations and datasets ranged from 62 to infinity.

Table 2

Genetic diversity estimates for (A) All individuals, (B) Females only (C) Males only datasets, including the number of individuals (N), average number of individuals per locus (Avg.N/locus), observed heterozygosity (H_O), expected heterozygosity (H_E), the inbreeding coefficient (F_{IS}), and the corresponding standard error for each (SE). Asterisks (*) denotes fished populations with high angling pressure.

Site ID	N	Avg. *N*/locus (SE)	Variant loci	H_O (SE)	H_E (SE)	F_{IS} (SE)
(A) All samples						
Mined_6A*	10	9.7 (0.005)	17448	0.255 (0.002)	0.250 (0.001)	0.020 (0.005)
Mined_11A*	10	9.6 (0.006)	17448	0.269 (0.002)	0.266 (0.001)	0.026 (0.006)
Mined_40B	10	9.7 (0.005)	17448	0.278 (0.001)	0.275 (0.001)	0.030 (0.005)
Mined 31B	9	7.7 (0.011)	17432	0.348 (0.002)	0.276 (0.001)	-0.114 (0.011)
Mined_30E	10	9.4 (0.007)	17448	0.303 (0.002)	0.264 (0.001)	-0.055 (0.007)
GrandOsage_10	9	8.2 (0.009)	17447	0.275 (0.002)	0.262 (0.001)	0.013 (0.009)
GrandOsage_3	8	6.2 (0.010)	17446	0.309 (0.002)	0.299 (0.001)	0.045 (0.010)
Altamont*	9	8.6 (0.006)	17448	0.286 (0.002)	0.268 (0.001)	-0.004 (0.006)
Cherryvale*	9	7.9 (0.009)	17448	0.307 (0.002)	0.281 (0.001)	-0.016 (0.009)
Coffeyville*	8	7.0 (0.009)	17447	0.281 (0.002)	0.262 (0.001)	0.004 (0.009)
Average	**92**	**8.4**	**17446**	**0.291**	**0.270**	**-0.005**
Site ID	**N**	**Avg. *N*/locus (SE)**	**Variant loci**	**H_O (SE)**	**H_E (SE)**	**F_{IS} (SE)**
(B) Females only						
Mined_6A*	0	--	--	--	--	--
Mined_11A*	4	3.8 (0.003)	17610	0.252 (0.002)	0.233 (0.001)	0.034 (0.003)
Mined_40B	6	5.8 (0.004)	17611	0.264 (0.002)	0.259 (0.001)	0.042 (0.004)
Mined 31B	4	3.4 (0.006)	17497	0.333 (0.002)	0.246 (0.001)	-0.072 (0.006)
Mined_30E	3	2.9 (0.003)	17604	0.267 (0.002)	0.220 (0.001)	0.002 (0.003)
GrandOsage_10	2	1.8 (0.003)	17134	0.276 (0.003)	0.192 (0.002)	-0.004 (0.003)
GrandOsage_3	5	4.2 (0.007)	17575	0.318 (0.002)	0.289 (0.001)	0.034 (0.007)
Altamont*	5	4.7 (0.004)	17611	0.276 (0.002)	0.246 (0.001)	-0.002 (0.004)
Cherryvale*	4	3.6 (0.005)	17596	0.288 (0.002)	0.250 (0.001)	0.011 (0.005)
Coffeyville*	2	1.8 (0.003)	17208	0.270 (0.003)	0.193 (0.002)	0.009 (0.003)
Average	**35**	**3.6**	**17494**	**0.283**	**0.236**	**0.006**

Site ID	N	Avg. *N*/locus (SE)	Variant loci	H_O (SE)	H_E (SE)	F_{IS} (SE)
(C) Males only						
Mined_6A*	8	7.8 (0.005)	17610	0.262 (0.002)	0.261 (0.001)	0.037 (0.005)
Mined_11A*	5	4.8 (0.004)	17611	0.277 (0.002)	0.263 (0.001)	0.032 (0.004)
Mined_40B	4	3.8 (0.003)	17497	0.286 (0.002)	0.264 (0.001)	0.034 (0.003)
Mined 31B	4	3.5 (0.006)	17604	0.361 (0.002)	0.265 (0.001)	-0.085 (0.006)
Mined_30E	3	2.8 (0.003)	17134	0.300 (0.002)	0.240 (0.001)	-0.013 (0.003)
GrandOsage_10	4	3.6 (0.006)	17575	0.276 (0.002)	0.244 (0.002)	0.024 (0.006)
GrandOsage_3	0	--	--	--	--	--
Altamont*	4	3.8 (0.003)	17611	0.288 (0.002)	0.259 (0.001)	0.020 (0.003)
Cherryvale*	1	1.0 (0.000)	17596	0.308 (0.004)	0.154 (0.002)	0.000 (0.000)
Coffeyville*	2	1.8 (0.003)	17208	0.288 (0.003)	0.204 (0.002)	0.008 (0.003)
Average	**35**	**3.7**	**17494**	**0.294**	**0.239**	**0.006**

Pairwise F_{ST} values indicated low genetic differentiation, ranging from 0.034 to 0.086 for all individuals. Female differentiation ranged from 0.062 to 0.202. Females from Grand Osage #10 were the most dissimilar from all other populations, with F_{ST} values ranging from 0.100-0.202. Males showed the highest degree of differentiation, with average F_{ST} values ranging from 0.063 to 0.235; Cherryvale and Coffeyville populations differed the most. The genetic relatedness matrix summarizes these comparisons by individual, displaying slight genetic differentiation for both the Grand Osage populations (GOWA10 and GOWA3). Pairwise F_{ST} values and GRM visuals show Mined land sites 30E and 31B consistently have a higher degree of similarity than with other sites (Figures 4-6).

Figure 4

Genetic Relatedness Matrix for all individuals across all populations. Relatedness is indicated by heat: increasing relatedness is indicated by warmer temperatures and decreased relatedness by cooler temperatures.

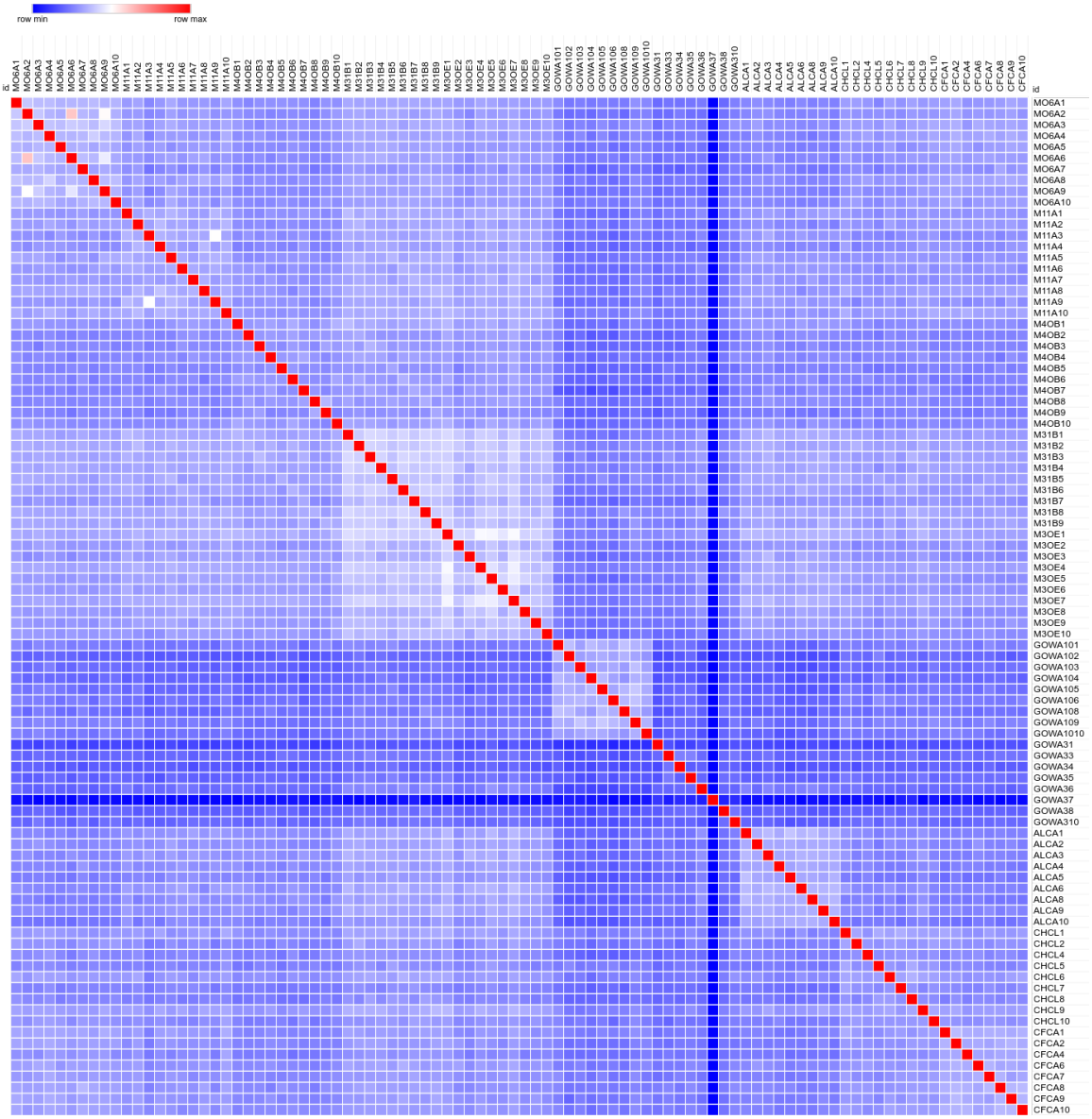


Figure 5

Genetic relatedness matrix for all female bluegill. Relatedness is indicated by heat: increasing relatedness is indicated by warmer temperatures and decreased relatedness by cooler temperatures.

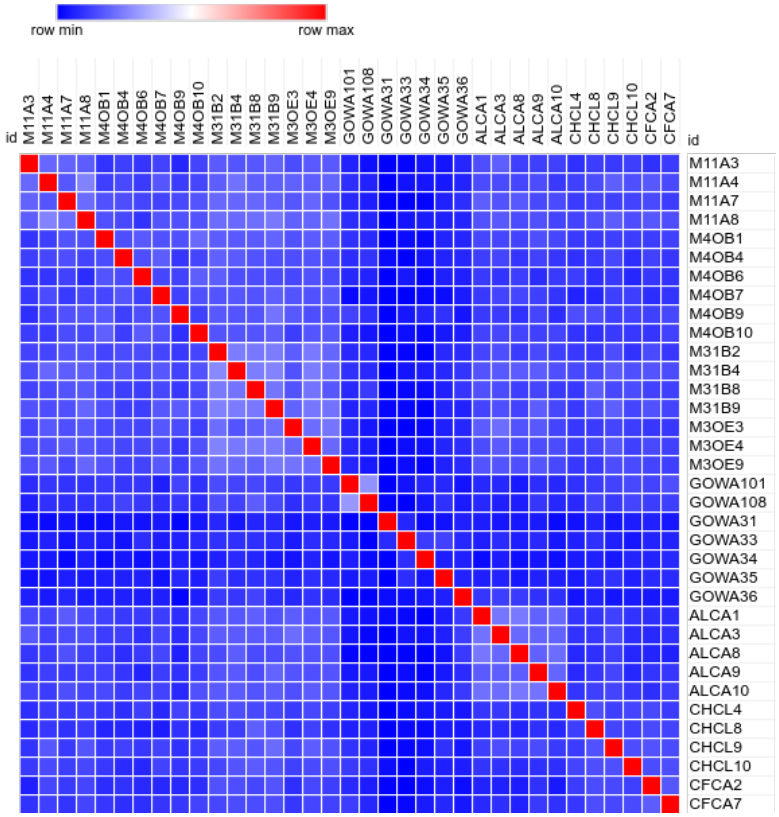
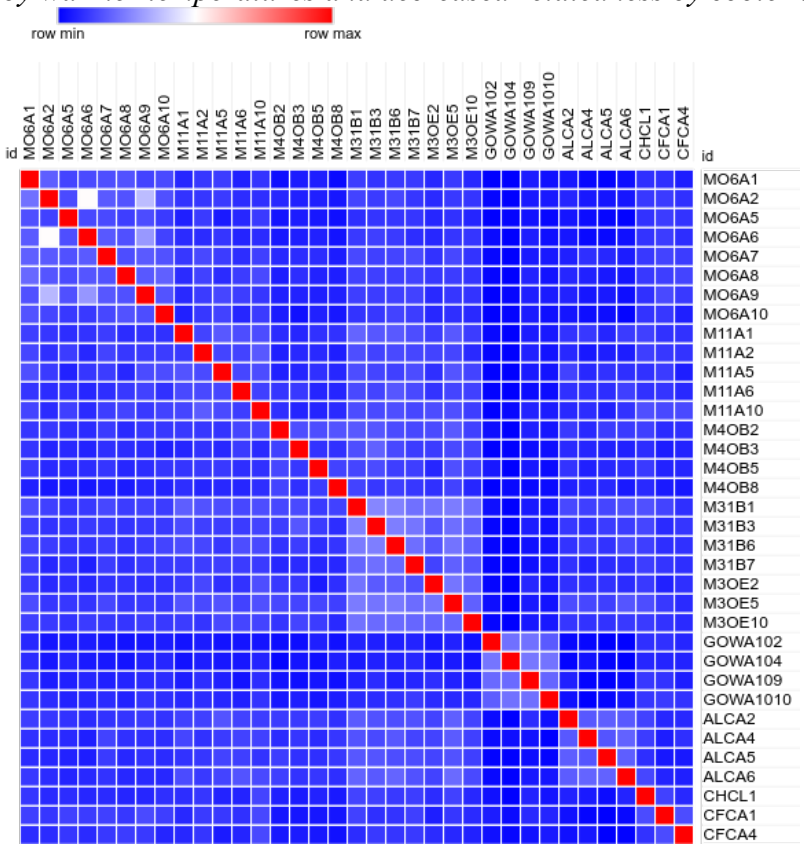


Figure 6

GRM for all males. Relatedness is indicated by heat: increasing relatedness is indicated by warmer temperatures and decreased relatedness by cooler temperatures.



Population Structure

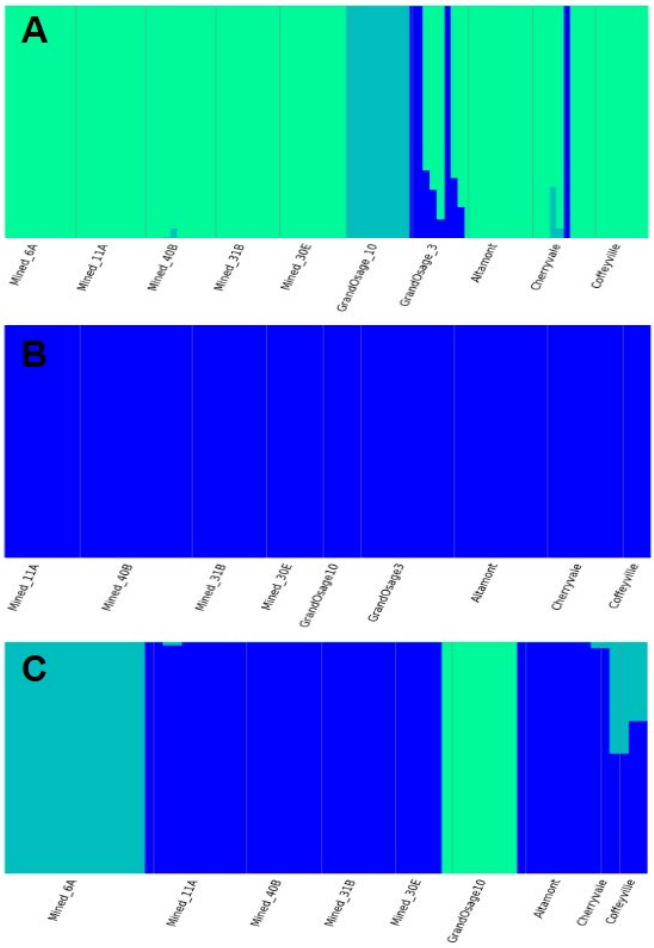
The AMOVA tests found the greatest source of variation exists among individuals within populations, across all datasets (>94%) (Table 3). The remaining variation was explained between populations. The Bayesian assignment test indicated the genetic variation for all individuals was best described with three genetic clusters (K = 3) (Figure 7A). For females, a clustering value of K = 1 was recommended (Figure 7B). The optimal clustering value for males as K= 3 (Figure 7C). Across all datasets, Grand Osage 10 was a unique cluster.

Table 3
Analysis of Molecular Variance (AMOVA) for (A) among and within populations for all individuals, (B) among and within populations for all females, and (C) among and within populations for all males.

Source of Variation	Variation (%)	d.f.	Sum of Squares	Variance components
(A) All individuals				
Among populations	5.20	9	274.60	0.83
Within Populations	94.80	174	2642.23	15.19
Total	100	183	2916.83	16.02
(B) All females				
Among populations	3.77	8	190.92	0.72
Within Populations	96.23	61	1119.41	18.35
Total	100	69	1310.33	19.07
(C) All males				
Among populations	5.53	8	11821.10	59.97
Within Populations	94.47	61	62497.33	1024.55
Total	100	69	74318.43	1084.52

Figure 7

FastStructure Barplots indicating admixture of (A) Bluegill across all individuals for 3 identified clusters (K=3), (B) across all females for 1 identified cluster (K=1), and (C) across all males for 3 identified clusters (K=3). No males were sampled for GOWA3 and no females for Mined_6A.



The DAPC cross-validation functions determined the number of PCs with the lowest RMSE for all individuals as 20. For females and males, counts with the lowest RMSE were both four. These counts were retained to assign clusters (Figures 8-10). There was a higher amount of genetic distinction between Grand Osage 10 and all other populations, for all datasets. Grand Osage 10 was identified as a unique cluster across

individuals and for males alone (no samples were retained for females); these results were consistent across analyses (Figures 8-10).

Figure 8

Bayesian population clustering of all individuals shown in an (A) DAPC where solid dots represent individuals, the inertia ellipses represent groups, and the solid black lines show the minimum spanning tree based on the squared distances between the groups; (B) An assignment plot showing likelihood of group membership by heat (red indicates high probability) for K=3; (C) a composition barplot based on prior grouping by locality, where each bar represents an individual.

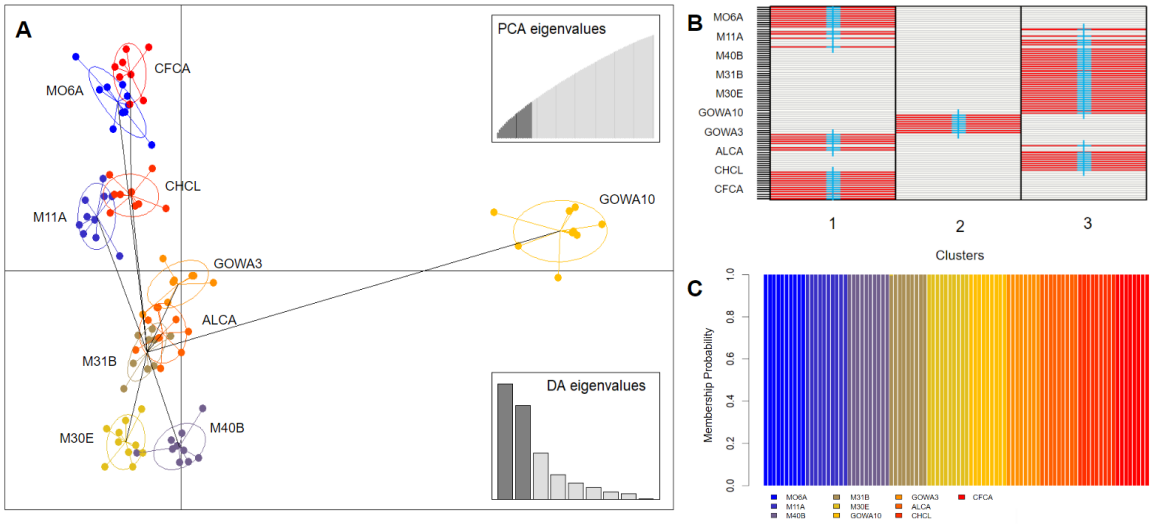


Figure 9

Bayesian population clustering of all females shown in an (A) DAPC where solid dots represent individuals, the inertia ellipses represent groups, and the solid black lines show the minimum spanning tree based on the squared distances between the groups; (B) An assignment plot showing likelihood of group membership by heat (red indicates high probability) for K=2; (C) an composition barplot based on prior grouping by locality, where each bar represents an individual.

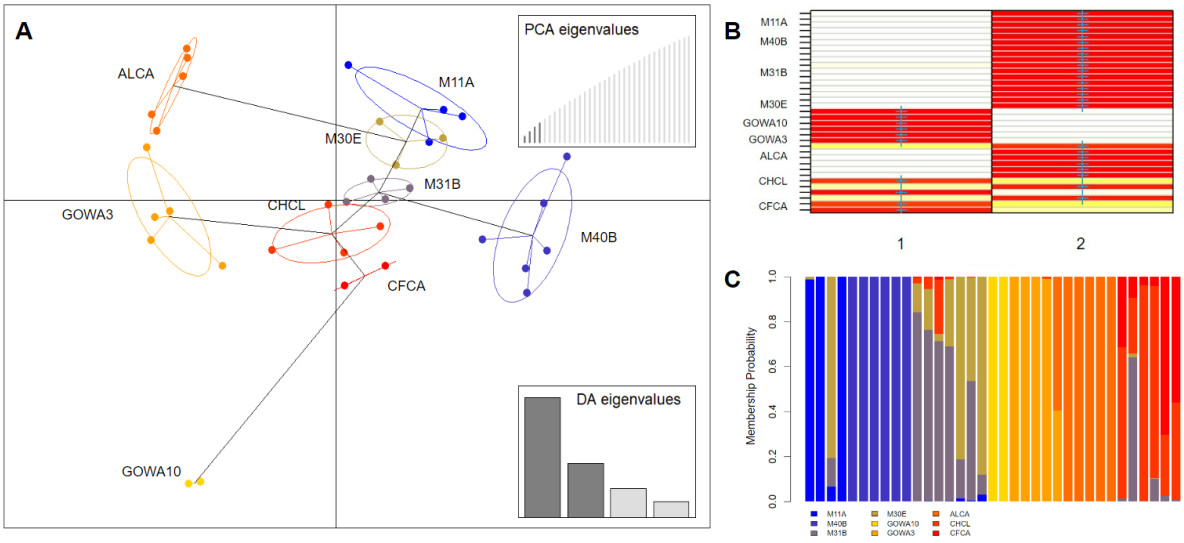
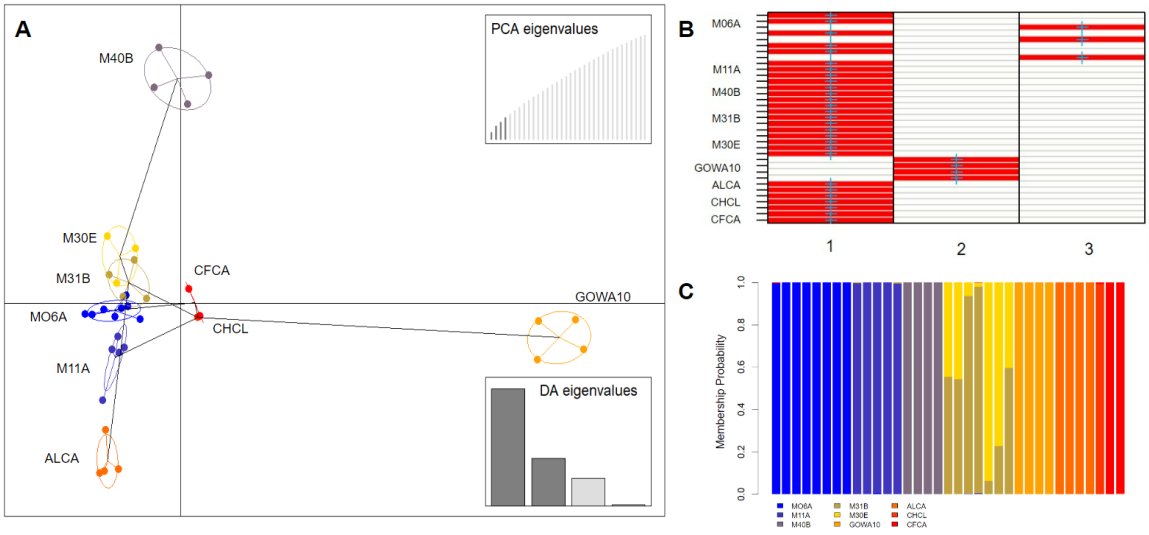


Figure 10

Bayesian population clustering of all males shown in an (A) DAPC where solid dots represent individuals, the inertia ellipses represent groups, and the solid black lines show the minimum spanning tree based on the squared distances between the groups; (B) An assignment plot showing likelihood of group membership by heat (red indicates high probability) for K=3; (C) a composition barplot based on prior grouping by locality, where each bar represents an individual.



Chapter 4: Genome Wide Association Studies (GWAS) Results

Of the five datasets for the phenotypic association studies (all individuals (*n*=92), females (*n*=35), males (*n*=35), males age three (*n*=17), and mature males (*n*=23)), only two phenotypes were associated with significant markers: angling pressure across all individuals and age for mature males. Of the angling pressure across all individuals, there were only two significant SNPs (Figure 11B). Age for mature males revealed many significant SNPs (Figure 15A). All other association tests did not detect significant markers with p-values above the -log10(5e-08) p-value (Figures 11-15).

Figure 11

Manhattan plots of the genome-wide association study analyses for all individuals. The solid red line indicates the cutoff p-value (-log(5e-08)); all markers above the cutoff indicate significant SNPs associated with the specified phenotype for all sampled bluegill. Differing colors are only for visualization.

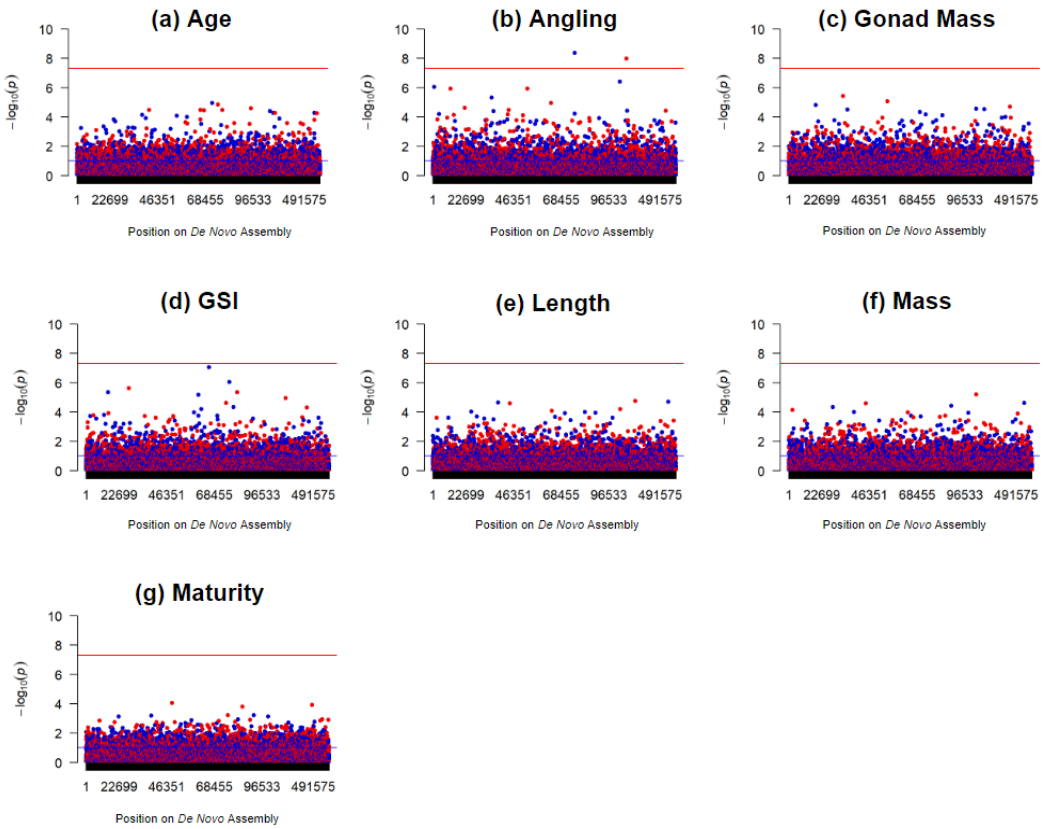


Figure 12

Manhattan plots of the genome-wide association study analyses for all females. The solid red line indicates the cutoff p-value (-log(5e-08)); all markers above the cutoff indicate significant SNPs associated with the specified phenotype for all females. Differing colors are only for visualization.

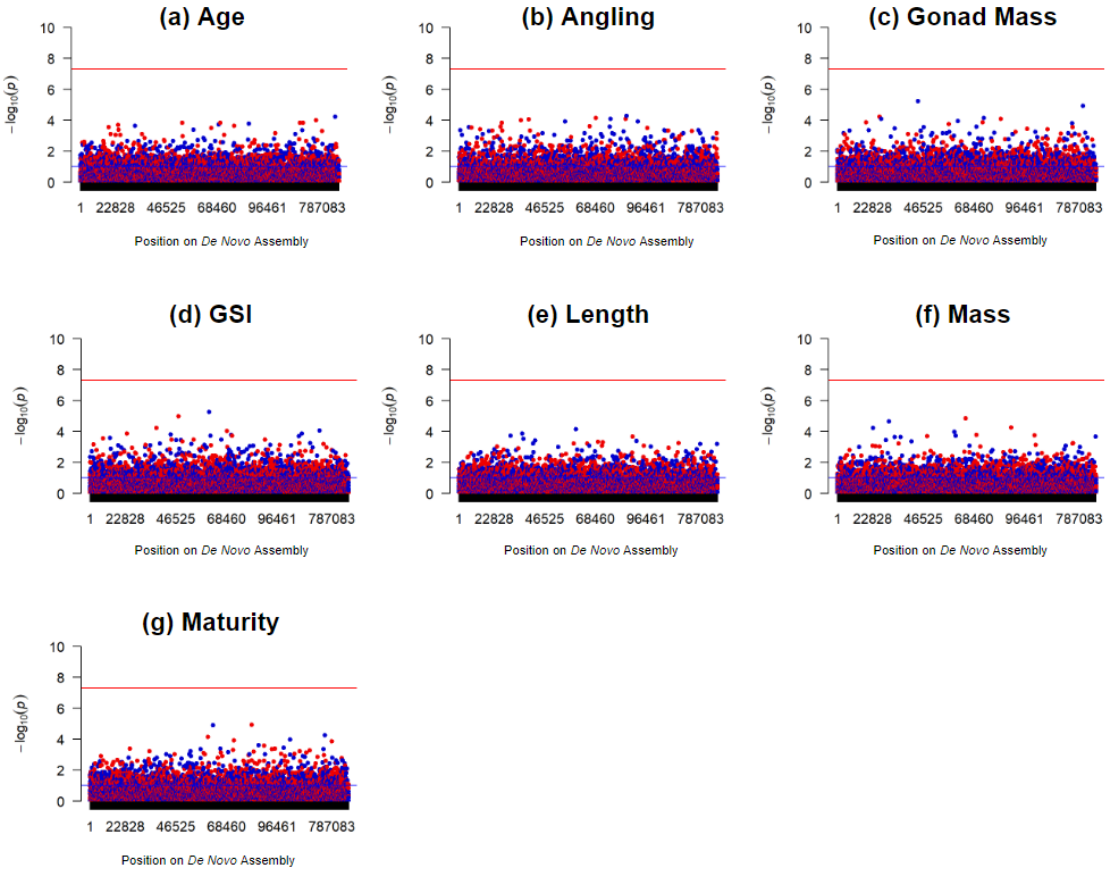


Figure 13

Manhattan plots of the genome-wide association study analyses for all males. The solid red line indicates the cutoff p-value (-log(5e-08)); all markers above the cutoff indicate significant SNPs associated with the specified phenotype for all males. Differing colors are only for visualization.

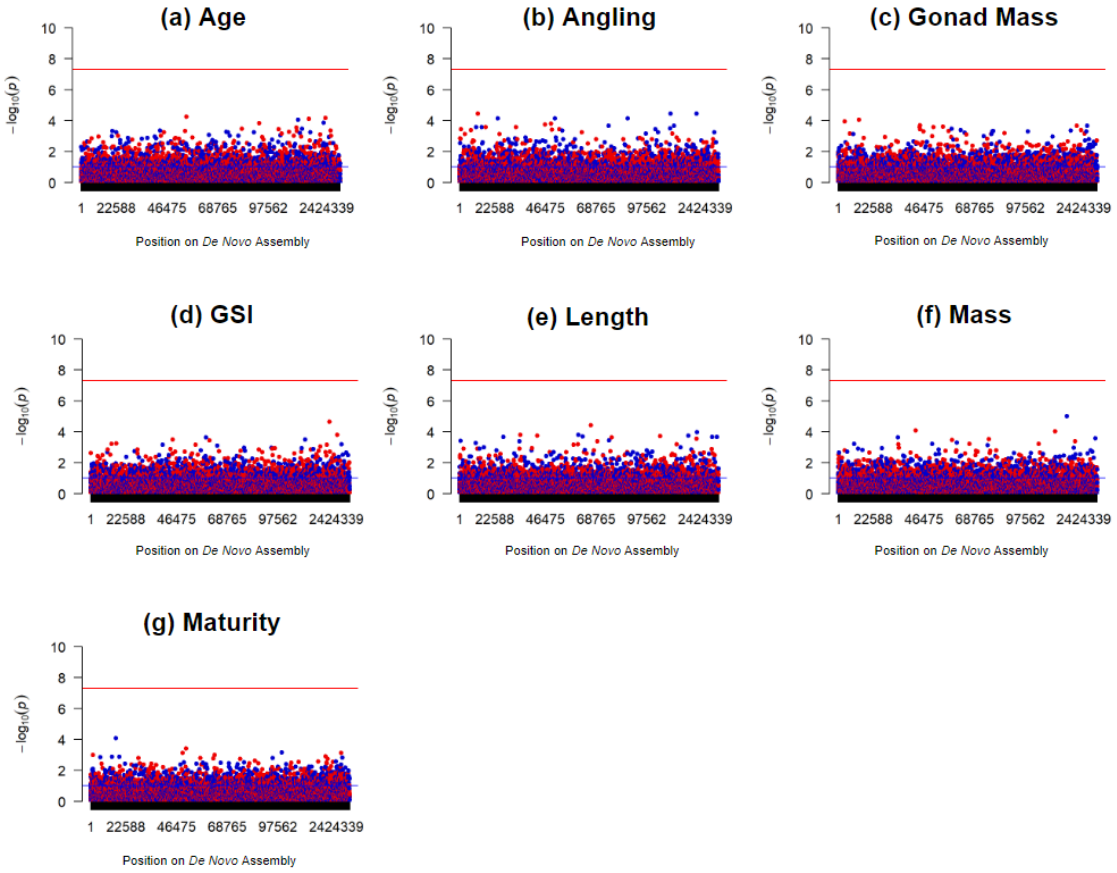


Figure 14

Manhattan plots of the genome-wide association study analyses for all males at age three. The solid red line indicates the cutoff p-value (-log(5e-08)); all markers above the cutoff indicate significant SNPs associated with the specified phenotype for all males age 3. Differing colors are only for visualization.

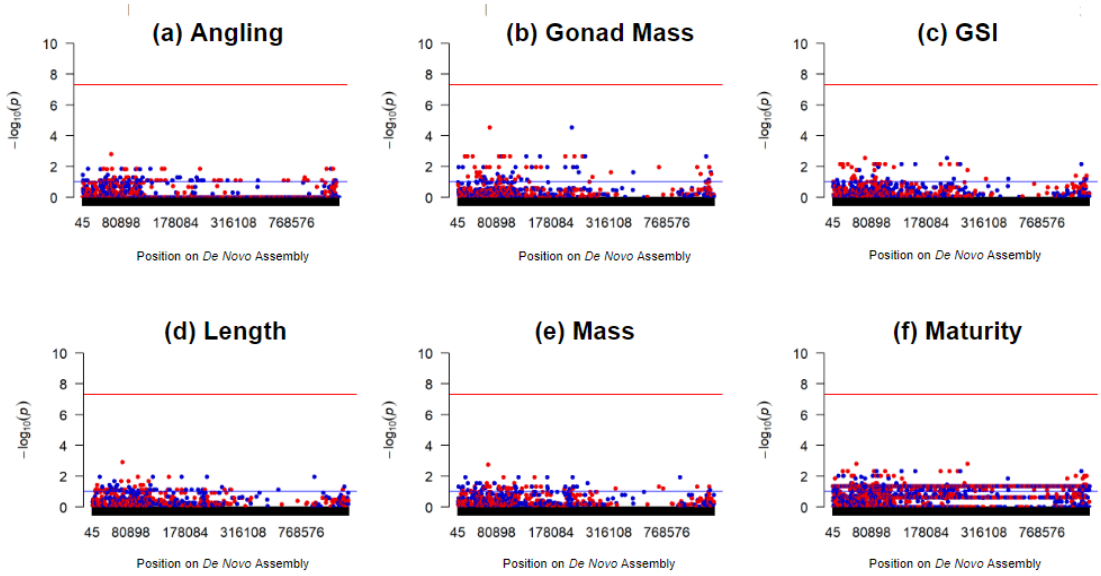
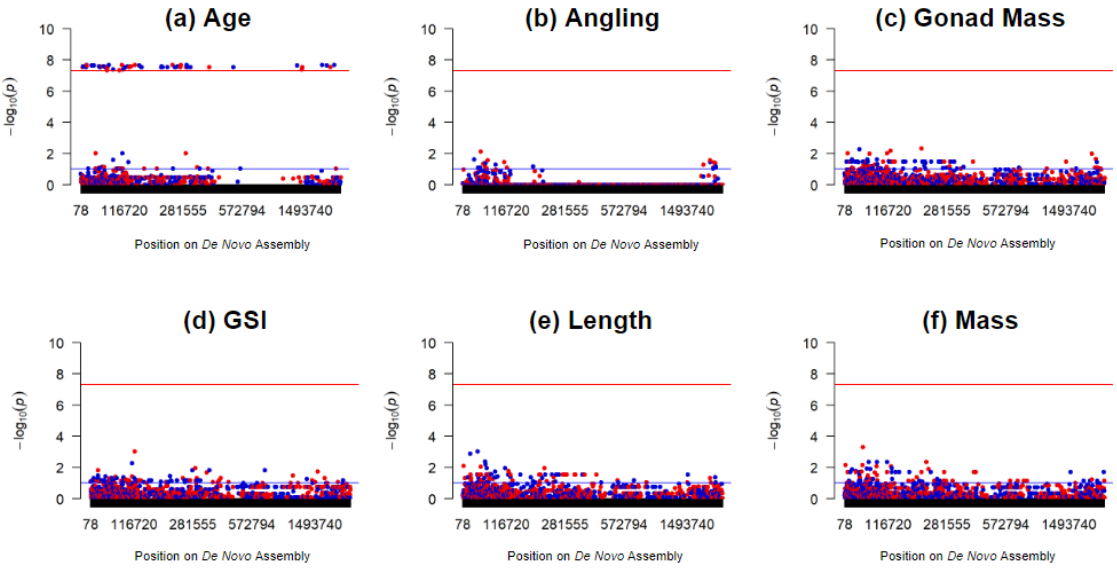


Figure 15

Manhattan plots of the genome-wide association study analyses for all males at age of maturity. The solid red line indicates the cutoff p-value (-log(5e-08)); all markers above the cutoff indicate significant SNPs associated with the specified phenotype for all males age 3. Differing colors are only for visualization.



Chapter 5: Discussion

Population Structure

Overall, the results of these analyses indicate little genetic differentiation between southeastern Kansas bluegill populations. Based on the AMOVA results, most of the variation was explained by variation among individuals within groups (94%) rather than among groups (6%), indicating little overall population structure. These results are supported by the GRMs, Bayesian assignment tests, and DAPCs which all showed high admixture among populations. Local bluegill populations were genetically diverse with high heterozygosity and little to no evidence of inbreeding (Table 2). These results indicate high admixture between populations, despite geographic separation. Of the small amount of variation observed among populations, Grand Osage 10 was the most unique and consistently was observed as a separate genetic cluster across analyses.

The lack of population structure is somewhat surprising given the stocking history of these systems and their geographic isolation. As closed systems, they would have little to no gene flow and the expected genetic signature should indicate relatively unique populations for each lake. However, the genetic signature indicated one large population, with the exception of Grand Osage 10. One possible explanation is that there is gene flow between populations, but this is unlikely given systems are closed. Gene flow could be artificially occurring from fishers releasing their catches from other lakes, but would not explain homogeneity between popularly fished lakes and inaccessible ones. Another source of gene flow could stem from dispersal of eggs from waterfowl, which is considered an often overlooked possibility (Lovas-Kiss et al. 2020; Green et al. 2023). Though no studies have assessed the potential of waterbird transport for bluegill

particularly, experimental trials have documented successful passage of the eggs of various carp species through the digestive tract of waterfowl (Lovas-Kiss et al. 2020). As few of the internally transported eggs reportedly survive (0.2%), this likely does not explain the high degree of genetic homogeneity between populations.

A second explanation is that time lag has a greater influence on genetics than contemporary fishing pressures. For example, longer time lags are associated with low dispersion, decreases in connectivity, and large effective population sizes (Epps and Keyghobadi 2015). After initial stocking there would be little to no connectivity and/or dispersion, while effective population sizes were large. These factors would contribute to low rates of genetic differentiation or a time lag in the landscape genomics of these bluegill. These findings are consistent with previous studies concerning bluegill growth rates in Kansas lakes, which indicated bluegill growth rates are significantly regulated by bluegill size structure, latitude, and relative abundance of Largemouth Bass (Neely et al. 2020). The evolutionary small amount of time, high degree of diversity, and the resultant large N_e are likely responsible for the low degree of variation among populations.

The exception to these findings was Grand Osage 10. Grand Osage 10 has no record of harvest and is one of the oldest systems among the study lakes. Grand Osage 10 is additionally inaccessible to the public, which would prevent translocation from anglers and is completely isolated from any watershed. As a result, this population would have had more time within a completely closed system to allow for genetic differentiation. Grand Osage 3, which shares similar characteristics, did not diverge as distinctly as Grand Osage 10. The admixture bar plots do indicate some degree of divergence for Grand Osage 3, but show marked admixture with the other populations that is not present

within Grand Osage 10. This could be a result of different initial stocking practices, which remain unknown, or could indicate gene flow via an aforementioned mechanism.

Fisheries Induced Evolution

Fisheries Induced Evolution is a commonly cited cause of stunted fish populations (Conover and Munch 2002; Olsen et al. 2004). Because fishing generally targets larger, more aggressive individuals, the corresponding harvest would select for maturation at younger ages and smaller sizes by reducing older and/or larger individuals (Law and Grey 1989; Ratner and Lande 2001; Conover and Munch 2002; Enberg et al. 2009). For example, in size-selective harvesting of Atlantic silversides, average body size was shown to decrease (Conover and Munch 2002). For guppies, increased predation selected for earlier maturation at smaller sizes (Olsen et al. 2004).

In this study, pairwise F_{ST} analyses, Bayescan, and structuring analyses all failed to detect a genetic signature indicating FIE. It is likely that not enough time has passed with the selective pressures from angling, a conclusion supported by similar studies. For example, in efforts to calculate the rate of FIE and the resulting changes in yield for any stock as a function of life-history parameters, Andersen and Brander (2009) found that the expected rate of evolutionary changes from fishing are generally slow compared to the direct effects of exploitation (overall effects of harvest on observable, plastic, phenotypes) (Andersen and Brander 2009; Kinnison et al. 2009; Audzijonyte et al. 2013; Hoxmeier et al. 2009). Other studies similarly report FIE plays only a minor role in recovery of stunted populations when compared with overfishing, magnitude of depletion, and natural mortality (Hutchings and Kuparinen 2019). These studies, in

conjunction with our findings, indicate that factors other than FIE are likely more important in driving any perceived phenotypic changes in these populations. These findings coincide with preliminary phenotypic data which do not evidence phenotypic differences between populations.

Although no genetic signature of FIE was detected within these populations, that does not mean FIE is not occurring, merely that historical factors have a greater influence over the population structures. Because several studies have documented the role of genetics in influencing bluegill growth, low selection pressures could still be present but not detectable. For example, in assessing genetic and environmental influences on bluegill, Aday et al. (2003), found that population source (genetics) did play a minor role in bluegill growth and maturation when all other environmental factors were kept similar. This coincides with another study, which reports that 20-30% of the variation in life-history traits, such as age and size at maturation, are heritable (Stokes and Law 2000; Law 2000). Given that there is heritable variation for bluegill growth, underlying polymorphisms could still be selected for or against.

Despite this possibility, the growing view of FIE highlights that its effects are often minor (Kinnison et al. 2009; Hutchings and Kuparinen 2019). For management, it may be more practical to concentrate on harvest limitations, such as size limits, bag limits, or spawning season restrictions. To produce larger bluegill, other management practices have included stocking with coppernose bluegill (*Lepomis macrochirus purpurascens*), a subspecies native to Florida. These species grow faster and take supplemental feed more readily, possibly reaching sizes larger than two pounds (American Sport Fish 2023).

Because this study encompasses a relatively small area, in the middle of the species range, a comprehensive study of genetic markers across the species range could help reveal fixed genetic polymorphisms related to growth. Although preliminary measures of phenotypic differences between fished and unfished populations were taken, this study lacks annual comparisons for each site. Expanding the study area and including year-to-year comparisons of both gene frequencies and phenotypic changes would likely clarify more overarching influences of bluegill growth and maturation (Marshall and Browman 2007; Browman et al. 2008; Kuparinen et al. 2009). Such studies could be more fruitful for detecting early FIE and untangling the driving factors influencing bluegill growth.

Phenotypic Association Studies

The phenotypic association studies did not reveal any significant markers associated with length, mass, gonad mass, or gonadosomatic index. The only association that yielded significant markers was age for mature males. Because the predominant differences in life histories assert that cuckolders mature earlier, these associations may reveal a genetic component for age at maturation between the alternative reproductive tactics. If there's genetically fixed components of the ARTs, this could help fisheries monitor social structures of different ARTs by assessing the proportion of parentals to cuckolds. Because size at maturation is positively related to size of adult male bluegills, it could also allow fisheries to stock parentals that mature later, and therefore at larger sizes (Hoxmeier et al. 2009).

Recent studies have looked at gene expression between male ARTs and several candidate genes have been associated with different bluegill spawning tactics. This includes *cyp19a1b* which is associated with testosterone levels and the expression of *crem* (Partridge et al. 2016). *Crem* plays a large role in spermatogenesis and modulating the hypothalamic-pituitary-gonadal (HPG) axis (Partridge et al. 2016). Spawning parentals expressed variations of *crem* at significantly higher levels than any other group (including non-spawning parentals). Although gene expression does not determine if parentals and cuckholders are polymorphic, it does provide evidence of some degree of genetic variation, linked to testosterone, spermatogenesis, and the HBG axis. Each of these have been correlated with gonad mass in other species and encourages further exploration in bluegill, particularly given that cuckolders' testes make up a larger proportion of their body mass than those of parentals (Burns et al. 2014; Dominey 1980).

To best explore the genetic component of size, understanding linked genes that are associated with sex and growth could help guide the management of stunted populations (Wang et al. 2010). With the presence of the newly published reference genome for bluegill (Ludt et al. 2023), further studies could help reveal the driving mechanisms of bluegill ARTs. Moreover, understanding the relationship between heritability and phenotypic factors such as age at maturity or GSI could help explain the relationship between size and ARTs. While studies utilizing the reference genome could help deepen understanding of ARTs, they may not directly relate to fastest growth rates or larger sizes that would be beneficial to fisheries management.

Chapter 6: Conclusions

The results of this study provide the first description of the genetic variation present in bluegill populations in Kansas and suggest that there are fixed genetic components potentially related to ARTs. Genetic diversity was high within populations; however, there was little genetic structure among populations in our study areas, suggesting that time lag has a greater influence on population structure than contemporary influences such as angling pressure. No differences between lakes with high and low angling pressure were found, and there was no evidence of fisheries-induced evolution. Further studies should consider combining data on changes in gene frequencies with phenotypic changes in maturation. Management should focus on harvest restrictions to reduce the direct effects of overexploitation on phenotypic changes, which would preclude any detectable genetic changes associated with FIE.

This study was additionally able to detect significant genetic markers associated with maturation in males, potentially identifying fixed polymorphisms for male ARTs. The ability to detect genetically fixed components of the ARTs would not only expand the limited genetic knowledge on bluegill, but could help with bluegill management by identifying males that will mature later, at larger sizes. Although the significant markers were not annotated, the recent publication of the bluegill reference genome (Ludt et al. 2023) will likely provide further insight on ARTs and the genetic component of growth rate. Future studies including paired maturation and genetics, with increased sample sizes, sex-specific studies, and the reference genome could further disentangle the complicated nature of bluegill growth.

References

Aday, D. Derek, David P. Philipp, and David H. Wahl. 2006. “Sex-Specific Life History Patterns in Bluegill (Lepomis Macrochirus): Interacting Mechanisms Influence Individual Body Size.” Oecologia 147 (1): 31–38.

American Sport Fish. “Coppernose Bluegill.” 2023. American Sport Fish Hatchery. Accessed May 1, 2023. https://americansportfish.com/fish-stocking-2/coppernose-bluegill/.

Andersen, Ken H., and Keith Brander. 2009. “Expected Rate of Fisheries-Induced Evolution Is Slow.” Proceedings of the National Academy of Sciences 106 (28): 11657–60.

Audzijonyte, Asta, Anna Kuparinen, and Elizabeth A. Fulton. 2013. “How Fast Is Fisheries-induced Evolution? Quantitative Analysis of Modelling and Empirical Studies.” Evolutionary Applications 6 (4): 585–95.

Ayllón, Daniel, Steven F. Railsback, Ana Almodóvar, Graciela G. Nicola, Simone Vincenzi, Benigno Elvira, and Volker Grimm. 2018. “Eco-Evolutionary Responses to Recreational Fishing under Different Harvest Regulations.” Ecology and Evolution 8 (19): 9600–9613.

Baccante, Dominic A., and David M. Reid. 1988. “Fecundity Changes in Two Exploited Walleye Populations.” North American Journal of Fisheries Management 8 (2): 199–209. https://doi.org/10.1577/1548-8675(1988)008<0199:FCITEW>2.3.CO;2.

Balon, Eugene K. 1986. “Types of Feeding in the Ontogeny of Fishes and the Life-History Model.” In Contemporary Studies on Fish Feeding: The Proceedings of GUTSHOP ’84: Papers from the Fourth Workshop on Fish Food Habits Held at the Asilomar Conference Center, Pacific Grove, California, U.S.A., December 2–6, 1984, edited by Charles A. Simenstad and Gregor M. Cailliet, 11–24. Developments in Environmental Biology of Fishes. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-017-1158-6_1.

Beard JR, T. Douglas, Melissa T. Drake, James E. Breck, and Nancy A. Nate. 1997. “Effects of Simulated Angling Regulations on Stunting in Bluegill Populations.” North American Journal of Fisheries Management 17 (2): 525–32.

Beard Jr., T. Douglas, and Jeffrey M. Kampa. 1999. “Changes in Bluegill, Black Crappie, and Yellow Perch Populations in Wisconsin during 1967–1991.” North American Journal of Fisheries Management 19 (4): 1037–43. https://doi.org/10.1577/1548-8675(1999)019<1037:CIBBCA>2.0.CO;2.

Belk, Mark C., and L. Stanton Hales. 1993. “Predation-Induced Differences in Growth and Reproduction of Bluegills (Lepomis Macrochirus).” Copeia 1993 (4): 1034–44. https://doi.org/10.2307/1447081.

Bergeon Burns, Christine M., Kimberly A. Rosvall, Thomas P. Hahn, Gregory E. Demas, and Ellen D. Ketterson. 2014. “Examining Sources of Variation in HPG Axis Function among Individuals and Populations of the Dark-Eyed Junco.” Hormones and Behavior 65 (2): 179–87. https://doi.org/10.1016/j.yhbeh.2013.10.006.

Browman, Howard I., Richard Law, and C. Tara Marshall. 2008. “The Role of Fisheries-Induced Evolution.” Science 320 (5872): 47–50.

Burgess, George H., and Richard Franz. 1989. “Zoogeography of the Antillean Freshwater Fish Fauna.” Biogeography of the West Indies: Past, Present, and Future, 263–304.

Catchen, Julian, Paul A. Hohenlohe, Susan Bassham, Angel Amores, and William A. Cresko. 2013. “Stacks: An Analysis Tool Set for Population Genomics.” Molecular Ecology 22 (11): 3124–40. https://doi.org/10.1111/mec.12354.

Catchen, Julian M., Angel Amores, Paul Hohenlohe, William Cresko, and John H. Postlethwait. 2011. “Stacks: Building and Genotyping Loci de Novo from Short-Read Sequences.” G3: Genes| Genomes| Genetics 1 (3): 171–82.

Cerca, José, Marius F. Maurstad, Nicolas C. Rochette, Angel G. Rivera-Colón, Niraj Rayamajhi, Julian M. Catchen, and Torsten H. Struck. 2021. “Removing the Bad Apples: A Simple Bioinformatic Method to Improve Loci-Recovery in de Novo RADseq Data for Non-Model Organisms.” Methods in Ecology and Evolution 12 (5): 805–17. https://doi.org/10.1111/2041-210X.13562.

Chhatre, V. E. 2018. “Distruct v2. 3, A Modified Cluster Membership Plotting Script.”

Conover, D. O., L. M. Clarke, S. B. Munch, and G. N. Wagner. 2006. “Spatial and Temporal Scales of Adaptive Divergence in Marine Fishes and the Implications for

Conservation." Journal of Fish Biology 69 (sc): 21–47. https://doi.org/10.1111/j.1095-8649.2006.01274.x.

Conover, David O., and Stephan B. Munch. 2002. "Sustaining Fisheries Yields over Evolutionary Time Scales." Science 297 (5578): 94–96.

Danecek, Petr, Adam Auton, Goncalo Abecasis, Cornelis A. Albers, Eric Banks, Mark A. DePristo, Robert E. Handsaker, et al. 2011. "The Variant Call Format and VCFtools." Bioinformatics 27 (15): 2156–58. https://doi.org/10.1093/bioinformatics/btr330.

Derek Aday, D., David H. Wahl, and David P. Philipp. 2003a. "Assessing Population-Specific and Environmental Influences on Bluegill Life Histories: A Common Garden Approach." Ecology 84 (12): 3370–75.

Diana, James S. 1987. "Simulation of Mechanisms Causing Stunting in Northern Pike Populations." Transactions of the American Fisheries Society 116 (4): 612–17. https://doi.org/10.1577/1548-8659(1987)116<612:SOMCSI>2.0.CO;2.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. "NeEstimator v2: Re-Implementation of Software for the Estimation of Contemporary Effective Population Size (Ne) from Genetic Data." Molecular Ecology Resources 14 (1): 209–14. https://doi.org/10.1111/1755-0998.12157.

Dominey, Wallace J. 1980. "Female Mimicry in Male Bluegill Sunfish—a Genetic Polymorphism?" Nature 284 (5756): 546–48.

Drake, Melissa T., Julie E. Claussen, David P. Philipp, and Donald L. Pereira. 1997. "A Comparison of Bluegill Reproductive Strategies and Growth among Lakes with Different Fishing Intensities." North American Journal of Fisheries Management 17 (2): 496–507. https://doi.org/10.1577/1548-8675(1997)017<0496:ACOBRS>2.3.CO;2.

Ehlinger, Timothy J., Mart R. Gross, and David P. Philipp. 1997. "Morphological and Growth Rate Differences between Bluegill Males of Alternative Reproductive Life Histories." North American Journal of Fisheries Management 17 (2): 533–42. https://doi.org/10.1577/1548-8675(1997)017<0533:MAGRDB>2.3.CO;2.

Enberg, Katja, Christian Jørgensen, Erin S. Dunlop, Mikko Heino, and Ulf Dieckmann. 2009. “Implications of Fisheries-induced Evolution for Stock Rebuilding and Recovery.” Evolutionary Applications 2 (3): 394–414.

Enberg, Katja, Christian Jørgensen, and Marc Mangel. 2010. “Fishing-Induced Evolution and Changing Reproductive Ecology of Fish: The Evolution of Steepness.” Canadian Journal of Fisheries and Aquatic Sciences 67 (10): 1708–19.

Epps, Clinton W., and Nusha Keyghobadi. 2015. “Landscape Genetics in a Changing World: Disentangling Historical and Contemporary Influences and Inferring Change.” Molecular Ecology 24 (24): 6021–40. https://doi.org/10.1111/mec.13454.

Etter, Paul D., Susan Bassham, Paul A. Hohenlohe, Eric A. Johnson, and William A. Cresko. 2011. “SNP Discovery and Genotyping for Evolutionary Genetics Using RAD Sequencing.” Methods in Molecular Biology (Clifton, N.J.) 772: 157–78. https://doi.org/10.1007/978-1-61779-228-1_9.

Excoffier, Laurent, and Heidi E. L. Lischer. 2010. “Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows.” Molecular Ecology Resources 10 (3): 564–67. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Foll, Matthieu, and Oscar Gaggiotti. 2008. “A Genome-Scan Method to Identify Selected Loci Appropriate for Both Dominant and Codominant Markers: A Bayesian Perspective.” Genetics 180 (2): 977–93.

Fraser, Bonnie A., Ilana Janowitz, Margaret Thairu, Joseph Travis, and Kimberly A. Hughes. 2014. “Phenotypic and Genomic Plasticity of Alternative Male Reproductive Tactics in Sailfin Mollies.” Proceedings of the Royal Society B: Biological Sciences 281 (1781): 20132310.

Fu, P., B. D. Neff, and M. R. Gross. 2001. “Tactic-Specific Success in Sperm Competition.” Proceedings of the Royal Society B: Biological Sciences 268 (1472): 1105–12. https://doi.org/10.1098/rspb.2001.1625.

Gamble, Tony, Jessi Coryell, Tariq Ezaz, Joshua Lynch, Daniel P. Scantlebury, and David Zarkower. 2015. “Restriction Site-Associated DNA Sequencing (RAD-Seq) Reveals an Extraordinary Number of Transitions among Gecko Sex-Determining Systems.” Molecular Biology and Evolution 32 (5): 1296–1309.

Gargiulo, Roberta, Tiiu Kull, and Michael F. Fay. 2021. "Effective Double-Digest RAD Sequencing and Genotyping despite Large Genome Size." Molecular Ecology Resources 21 (4): 1037–55. https://doi.org/10.1111/1755-0998.13314.

Gerking, Shelby D. 1962. "Production and Food Utilization in a Population of Bluegill Sunfish." Ecological Monographs 32 (1): 31–78. https://doi.org/10.2307/1942360.

Goedde, Larry E., and Daniel W. Coble. 1981. "Effects of Angling on a Previously Fished and an Unfished Warmwater Fish Community in Two Wisconsin Lakes." Transactions of the American Fisheries Society 110 (5): 594–603. https://doi.org/10.1577/1548-8659(1981)110<594:EOAOAP>2.0.CO;2.

Green, Andy J., Ádám Lovas-Kiss, Chevonne Reynolds, Esther Sebastián-González, Giliandro G. Silva, Casper H. A. van Leeuwen, and David M. Wilkinson. 2023. "Dispersal of Aquatic and Terrestrial Organisms by Waterbirds: A Review of Current Knowledge and Future Priorities." Freshwater Biology 68 (2): 173–90. https://doi.org/10.1111/fwb.14038.

Gross, Mart R. 1982. "Sneakers, Satellites and Parentals: Polymorphic Mating Strategies in North American Sunfishes." Zeitschrift Für Tierpsychologie 60 (1): 1–26. https://doi.org/10.1111/j.1439-0310.1982.tb01073.x.

Gross, Mart R. 1996. "Alternative Reproductive Strategies and Tactics: Diversity within Sexes." Trends in Ecology & Evolution 11 (2): 92–98.

Gross, Mart R., and Joe Repka. 1998. "Stability with Inheritance in the Conditional Strategy." Journal of Theoretical Biology 192 (4): 445–53.

Healey, Michael, and Amber Heckelman. 1978. "Healey1978FecundityChangedWhitefish." Australian Road Research Board, July.

Heino, Mikko, Beatriz Diaz Pauli, and Ulf Dieckmann. 2015. "Fisheries-Induced Evolution." Annual Review of Ecology, Evolution, and Systematics 46: 461–80.

Hill, William G. 1981. "Estimation of Effective Population Size from Data on Linkage Disequilibrium." Genetical Research 38 (3): 209–16. https://doi.org/10.1017/S0016672300020553.

Hoxmeier, R. John H., D. Derek Aday, and David H. Wahl. 2009. "Examining Interpopulation Variation in Bluegill Growth Rates and Size Structure: Effects of

Harvest, Maturation, and Environmental Variables." Transactions of the American Fisheries Society 138 (2): 423–32.

Hutchings, Jeffrey A., and Anna Kuparinen. 2021. "Throwing down a Genomic Gauntlet on Fisheries-Induced Evolution." Proceedings of the National Academy of Sciences 118 (20): e2105319118.

Jennings, Martin J., Julie E. Claussen, and David P. Philipp. 1997. "Effect of Population Size Structure on Reproductive Investment of Male Bluegill." North American Journal of Fisheries Management 17 (2): 516–24. https://doi.org/10.1577/1548-8675(1997)017<0516:EOPSSO>2.3.CO;2.

Jombart, Thibaut. 2008. "Adegenet: A R Package for the Multivariate Analysis of Genetic Markers." Bioinformatics 24 (11): 1403–5. https://doi.org/10.1093/bioinformatics/btn129.

Jombart, Thibaut, and Ismaïl Ahmed. 2011. "Adegenet 1.3-1: New Tools for the Analysis of Genome-Wide SNP Data." Bioinformatics 27 (21): 3070–71. https://doi.org/10.1093/bioinformatics/btr521.

Jombart, Thibaut, and Caitlin Collins. 2013. "A Tutorial for Discriminant Analysis of Principal Componenets (DAPC) Using Adegenet 1.3-6."

Jombart, Thibaut, Sébastien Devillard, and François Balloux. 2010. "Discriminant Analysis of Principal Components: A New Method for the Analysis of Genetically Structured Populations." BMC Genetics 11 (1): 94. https://doi.org/10.1186/1471-2156-11-94.

Kawamura, Kouichi, Ryuji Yonekura, Osamu Katano, Yoshinori Taniguchi, and Kenji Saitoh. 2006. "Origin and Dispersal of Bluegill Sunfish, Lepomis Macrochirus, in Japan and Korea." Molecular Ecology 15 (3): 613–21. https://doi.org/10.1111/j.1365-294X.2006.02823.x.

Kinnison, Michael T., Eric P. Palkovacs, Chris T. Darimont, Stephanie M. Carlson, Paul C. Paquet, and Christopher C. Wilmers. 2009. "Some Cautionary Notes on Fisheries Evolutionary Impact Assessments." Proceedings of the National Academy of Sciences 106 (41): E115–E115. https://doi.org/10.1073/pnas.09007871106.

Kuparinen, Anna, and Jeffrey A. Hutchings. 2019. “When Phenotypes Fail to Illuminate Underlying Genetic Processes in Fish and Fisheries Science.” ICES Journal of Marine Science 76 (4): 999–1006.

Kuparinen, Anna, Sakari Kuikka, and Juha Merilä. 2009. “Estimating Fisheries-Induced Selection: Traditional Gear Selectivity Research Meets Fisheries-Induced Evolution.” Evolutionary Applications 2 (2): 234–43. https://doi.org/10.1111/j.1752-4571.2009.00070.x.

Law, Richard. 2000. “Fishing, Selection, and Phenotypic Evolution.” ICES Journal of Marine Science 57 (3): 659–68.

Law, Richard, and David R. Grey. 1989. “Evolution of Yields from Populations with Age-Specific Cropping.” Evolutionary Ecology 3: 343–59.

Lischer, Heidi EL, and Laurent Excoffier. 2012. “PGDSpider: An Automated Data Conversion Tool for Connecting Population Genetics and Genomics Programs.” Bioinformatics 28 (2): 298–99.

Lister, Joanna S., and Bryan D. Neff. 2006. “Paternal Genetic Effects on Foraging Decision-Making Under the Risk of Predation.” Ethology 112 (10): 963–70. https://doi.org/10.1111/j.1439-0310.2006.01248.x.

Lovas-Kiss, Ádám, Orsolya Vincze, Viktor Löki, Felícia Pallér-Kapusi, Béla Halasi-Kovács, Gyula Kovács, Andy J. Green, and Balázs András Lukács. 2020. “Experimental Evidence of Dispersal of Invasive Cyprinid Eggs inside Migratory Waterfowl.” Proceedings of the National Academy of Sciences 117 (27): 15397–99. https://doi.org/10.1073/pnas.2004805117.

Luiken, Jon M., Tony Gamble, and Peter B. Berendzen. 2021. “Comparative Riverscape Genomics of the Rainbow Darter (Etheostoma Caeruleum) in Glaciated and Unglaciated Environments.” Ecology and Evolution 11 (24): 18305–18. https://doi.org/10.1002/ece3.8422.

Ma, X., X. Bangxi, W. Yindong, and W. Mingxue. 2003. “Intentionally Introduced and Transferred Fishes in China’s Inland Waters.”

Maezono, Yasunori, and Tadashi Miyashita. 2003. “Community-Level Impacts Induced by Introduced Largemouth Bass and Bluegill in Farm Ponds in Japan.” Biological Conservation 109 (1): 111–21. https://doi.org/10.1016/S0006-3207(02)00144-1.

Marshall, C. Tara, and Howard I. Browman. 2007. “Introduction.” Marine Ecology Progress Series 335: 249–52.

Michaletz, Paul H. 2020. “Interrelationships among Bluegill Demographics, Predator and Competitor Abundances and Impoundment Characteristics.” Journal of Freshwater Ecology 35 (1): 491–506.

Mittelbach, Gary G. 1988. “Competition among Refuging Sunfishes and Effects of Fish Density on Littoral Zone Invertebrates.” Ecology 69 (3): 614–23.

Neely, Ben C., Jeff D. Koch, Keith B. Gido, Connor J. Chance-Ossowski, and Elizabeth A. Renner. 2020. “Factors Influencing Bluegill Growth in Small Kansas Impoundments.” *Journal of Fish and Wildlife Management* 11 (1): 121–29.

Neely, Ben C., Jeff D. Koch, and Sean T. Lynott. 2018. “Effects of Gizzard Shad Reduction on Relative Abundance, Growth, and Body Condition of Bluegills and Largemouth Bass in a Small Kansas Impoundment.” North American Journal of Fisheries Management 38 (4): 856–66.

Neff, Bryan D. 2004. “Increased Performance of Offspring Sired by Parasitic Males in Bluegill Sunfish.” Behavioral Ecology 15 (2): 327–31. https://doi.org/10.1093/beheco/arh016.

Neff, Bryan D., and Erik I. Svensson. 2013. “Polyandry and Alternative Mating Tactics.” Philosophical Transactions of the Royal Society B: Biological Sciences 368 (1613): 20120045.

Olsen, Esben M., Mikko Heino, George R. Lilly, M. Joanne Morgan, John Brattey, Bruno Ernande, and Ulf Dieckmann. 2004. “Maturation Trends Indicative of Rapid Evolution Preceded the Collapse of Northern Cod.” Nature 428 (6986): 932–35. https://doi.org/10.1038/nature02430.

Olson, Donald E., and Paul K. Cunningham. 1989. “Sport-Fisheries Trends Shown by an Annual Minnesota Fishing Contest over a 58-Year Period.” North American Journal of Fisheries Management 9 (3): 287–97. https://doi.org/10.1577/1548-8675(1989)009<0287:SFTSBA>2.3.CO;2.

Osenberg, C. W., E. E. Werner, G. G. Mittelbach, and D. J. Hall. 1988. “Growth Patterns in Bluegill and Pumpkinseed Sunfish: Environmental Variation and the Importance

of Ontogenetic Niche Shifts." Canadian Journal of Fisheries and Aquatic Sciences 45: 17–26.

Osenberg, Craig W., Russell J. Schmitt, Sally J. Holbrook, Khalil E. Abu-Saba, and A. Russell Flegal. 1994. "Detection of Environmental Impacts: Natural Variability, Effect Size, and Power Analysis." Ecological Applications 4 (1): 16–30. https://doi.org/10.2307/1942111.

Page, Lawrence M., and Brooks M. Burr. 1991. A Field Guide to Freshwater Fishes: North America North of Mexico. Houghton Mifflin Harcourt.

Page, LawrenceM., and Brooks M. Burr. 2011. Peterson Field Guide to Freshwater Fishes of North America North of Mexico. Houghton Mifflin Harcourt.

Partridge, Charlyn G., Matthew D. MacManes, Rosemary Knapp, and Bryan D. Neff. 2016. "Brain Transcriptional Profiles of Male Alternative Reproductive Tactics and Females in Bluegill Sunfish." Edited by Hanping Wang. PLOS ONE 11 (12): e0167509. https://doi.org/10.1371/journal.pone.0167509.

Paukert, Craig P., David W. Willis, and Joel A. Klammer. 2002. "Effects of Predation and Environment on Quality of Yellow Perch and Bluegill Populations in Nebraska Sandhill Lakes." North American Journal of Fisheries Management 22 (1): 86–95. https://doi.org/10.1577/1548-8675(2002)022<0086:EOPAEO>2.0.CO;2.

Philipp, D. P., and M. R. Gross. 1994. "Genetic Evidence for Cuckoldry in Bluegill Lepomis Macrochirus." Molecular Ecology 3 (6): 563–69.

Piché, Jacinthe, Jeffrey A. Hutchings, and Wade Blanchard. 2008. "Genetic Variation in Threshold Reaction Norms for Alternative Reproductive Tactics in Male Atlantic Salmon, Salmo Salar." Proceedings of the Royal Society B: Biological Sciences 275 (1642): 1571–75.

Porath, Mark T., and Keith L. Hurley. 2005. "Effects of Waterbody Type and Management Actions on Bluegill Growth Rates." North American Journal of Fisheries Management 25 (3): 1041–50.

Raj, Anil, Matthew Stephens, and Jonathan K Pritchard. 2014. "FastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets." Genetics 197 (2): 573–89. https://doi.org/10.1534/genetics.114.164350.

Ratner, Susan, and Russell Lande. 2001. "Demographic and Evolutionary Responses to Selective Harvesting in Populations with Discrete Generations." Ecology 82 (11): 3093–3104.

Rochette, Nicolas C., and Julian M. Catchen. 2017. "Deriving Genotypes from RAD-Seq Short-Read Data Using Stacks." Nature Protocols 12 (12): 2640–59.

Roff, Derek. 1993. Evolution Of Life Histories: Theory and Analysis. Springer Science & Business Media.

Rohland, Nadin, and David Reich. 2012. "Cost-Effective, High-Throughput DNA Sequencing Libraries for Multiplexed Target Capture." Genome Research 22 (5): 939–46. https://doi.org/10.1101/gr.128124.111.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rypel, Andrew L., John Lyons, Joanna D. Tober Griffin, and Timothy D. Simonson. 2016. "Seventy-Year Retrospective on Size-Structure Changes in the Recreational Fisheries of Wisconsin." Fisheries 41 (5): 230–43. https://doi.org/10.1080/03632415.2016.1160894.

Schneider, James C. n.d. "EXPERIMENTAL MANAGEMENT OF STUNTED BLUEGILL LAKES," 76.

Schunter, Celia, Steven V. Vollmer, Enrique Macpherson, and Marta Pascual. 2014. "Transcriptome Analyses and Differential Gene Expression in a Non-Model Fish Species with Alternative Mating Tactics." BMC Genomics 15: 1–14.

Snow, Howard E., and Michael D. Staggs. 1994. Factors Related to Fish Growth in Northwestern Wisconsin Lakes. Vol. 162. Wisconsin Department of Natural Resources.

Stiver, Kelly A., Rayna M. Harris, Jeffrey P. Townsend, Hans A. Hofmann, and Suzanne H. Alonzo. 2015. "Neural Gene Expression Profiles and Androgen Levels Underlie Alternative Reproductive Tactics in the Ocellated Wrasse, Symphodus Ocellatus." Ethology 121 (2): 152–67. https://doi.org/10.1111/eth.12324.

Stockwell, Craig A., Andrew P. Hendry, and Michael T. Kinnison. 2003. "Contemporary Evolution Meets Conservation Biology." Trends in Ecology & Evolution 18 (2): 94–101. https://doi.org/10.1016/S0169-5347(02)00044-7.

Stokes, Kevin, and Richard Law. 2000. "Fishing as an Evolutionary Force." Marine Ecology Progress Series 208: 307–9.

Swingle, H. S., and E. V. Smith. 1941. "Managing Ponds for Fish Production." The Progressive Fish-Culturist 8 (53): 8–13. https://doi.org/10.1577/1548-8640(1941)8[8:MPFFP]2.0.CO;2.

Taborsky, Michael. 1998. "Sperm Competition in Fish:Bourgeois' Males and Parasitic Spawning." Trends in Ecology & Evolution 13 (6): 222–27.

Taborsky, Michael. 2008. "10· Alternative Reproductive Tactics in Fish." Alternative Reproductive Tactics an Integrative Approach 251.

Tomcko, Cynthia M., and Rodney B. Pierce. 1997. Bluegill Growth Rates in Minnesota. Minnesota Department of Natural Resources, Section of Fisheries.

Tomcko, Cynthia M., and Rodney B. Pierce. "The Relationship of Bluegill Growth, Lake Morphometry, and Water Quality in Minnesota." Transactions of the American Fisheries Society 130 (2): 317–21. https://doi.org/10.1577/1548-8659(2001)130<0317:TROBGL>2.0.CO;2.

Trebitz, Anett, Stephen Carpenter, Paul Cunningham, Brett Johnson, Richard Lillie, David Marshall, Thomas Martin, Richard Narf, Thomas Pellett, and Scott Stewart. 1997. "A Model of Bluegill-Largemouth Bass Interactions in Relation to Aquatic Vegetation and Its Management." Ecological Modelling 94 (2–3): 139–56.

United States Fish and Wildlife Service. "Bluegill (Lepomis Macrochirus) | U.S. Fish & Wildlife Service." 2015. FWS.Gov. December 1, 2015. https://www.fws.gov/species/bluegill-lepomis-macrochirus.

Wang, Wei-Ji, Han-Ping Wang, Hong Yao, Geoff K. Wallat, Laura G. Tiu, and Qing-Yin Wang. 2010. "A First Genetic Linkage Map of Bluegill Sunfish (Lepomis Macrochirus) Using AFLP Markers." Aquaculture International 18 (5): 825–35.

Welcomme, R. L. 1988. International Introductions of Inland Aquatic Species. Food & Agriculture Org.

Werner, Earl E., James F. Gilliam, Donald J. Hall, and Gary G. Mittelbach. 1983. “An Experimental Test of the Effects of Predation Risk on Habitat Use in Fish.” Ecology 64 (6): 1540–48.

Wiener, James G., and Wendy R. Hanneman. 1982. “Growth and Condition of Bluegills in Wisconsin Lakes: Effects of Population Density and Lake PH.” Transactions of the American Fisheries Society 111 (6): 761–67. https://doi.org/10.1577/1548-8659(1982)111<761:GACOBI>2.0.CO;2.