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Genetic Variation Among Geographically Disparate Yellow Perch Broodstock Populations

Chandler Brooke Givens
Virginia Commonwealth University

GENETIC VARIATION AMONG GEOGRAPHICALLY DISPARATE YELLOW PERCH BROODSTOCK POPULATIONS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

By

CHANDLER BROOKE GIVENS

Bachelor of Science

Virginia Tech, Blacksburg, Virginia

2004

Director: BONNIE L. BROWN, Ph.D.

Associate Professor, Department of Biology

Virginia Commonwealth University

Richmond, Virginia

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Abstract

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As a prelude to strain selection for domestication and future marker assisted selection, genetic variation revealed by microsatellite DNA was evaluated in yellow perch, *Perca flavescens*, from four wild North American populations collected in 2003-2004 (Maine, ME; New York, NY; North Carolina, NC; and Pennsylvania, PA), and two captive populations (Michigan, MI; Ohio, OH). For the loci examined, levels of heterozygosity ranged from H_e = 0.04 to 0.88, genetic differentiation was highly significant among all population pairs, and effective migration ranged from low (N_em = 0.3) to high (N_em = 4.5). Deviation from Hardy-Weinberg equilibrium was regularly observed indicating significant departures from random mating. Instantaneous measures of inbreeding within

these populations ranged from near zero to moderate (median $F = 0.16$) and overall inbreeding levels averaged $F_{IS} = 0.18$. Estimates of genetic diversity, Φ_{ST}, and genetic distance were highest between Michigan and all other populations and lowest between New York and Ohio. Genetic differentiation among populations did not correlate with geographic distance. Overall, the patterns of variation exhibited by the captive (Michigan and Ohio) populations were similar to patterns exhibited by the other allegedly wild populations, indicating that the spawning and management practices to date have not significantly reduced levels of genetic variation. The manuscript presented in this thesis is formatted for the journal *Aquaculture Research* and has been submitted to that journal for publication.

Genetic variation among geographically disparate yellow perch broodstock populations

Chandler B. Givens[1], Li Li[1], Hanping Wang[2], and Bonnie L. Brown[1]

[1] Ecological Genetics Laboratory, Virginia Commonwealth University, Richmond, Virginia 23284-2012, USA

[2] Ohio State University Centers, 1864 Shyville Road, Piketon , Ohio 45661, USA

Correspondence: B.L. Brown. Tel.: +804-828-1562; Fax: +804-828-0503; BLBROWN@vcu.edu

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ABSTRACT

As a prelude to strain selection for domestication and future marker assisted selection, genetic variation revealed by microsatellite DNA was evaluated in yellow perch, *Perca flavescens*, from four wild North American populations collected in 2003-2004 (Maine, ME; New York, NY; North Carolina, NC; and Pennsylvania, PA), and two captive populations (Michigan, MI; Ohio, OH). For the loci examined, levels of heterozygosity ranged from H_e = 0.04 to 0.88, genetic differentiation was highly significant among all population pairs, and effective migration ranged from low (N_em = 0.3) to high (N_em = 4.5). Deviation from Hardy-Weinberg

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INTRODUCTION

The yellow perch, *Perca flavescens*, (Mitchill 1814) is an ecologically significant component of many North American freshwater food webs including lakes, ponds, creeks, and rivers. Yellow perch have a native distribution throughout the Nearctic ecozone from South Carolina to Nova Scotia, westward throughout the Great Lakes region and the Mississippi Valley, and northward to the Red River Basin (Nelson 1976). Yellow perch are carnivorous and feed on a wide variety of animals such as zooplankton, insect larvae, crayfish, and small fishes (Hildebrand and Schroeder 1928). They are common prey to top predators such as the walleye, northern pike, muskellunge, lake trout and are also consumed by herring gulls and diving ducks (Herman et al. 1964). Dramatic reductions in population sizes have been underway since approximately 1990 in most areas of the continental US (Manci 2001, Figure 1) attributed primarily to predation, unusual weather, starvation, competition with other organisms that feed on plankton, novel parasites, and interference from exotic organisms, such as zebra mussels in the Great Lakes. Complicating the potential anthropogenic effects on survival, yellow perch populations have been observed in relatively unimpacted, native environments to undergo regular cyclic oscillations that are thought to be precipitated by a combination of demographic factors, intraspecific competition and cannibalism, and predator-prey dynamics (Sanderson et al. 1999). In contrast to reductions observed in native populations, yellow perch have been introduced to a large number of watersheds in the western U.S. and have become established in most areas where they were introduced (Coots 1956). The most common impact of these introduced populations is competition for food (Coots 1956) and predation on young native fishes (Echo 1955).

A number of population genetic studies have been reported for *Perca flavescens*. Most recently, Leclerc et al. (2000) performed a comparison of microsatellite and mtDNA studies of genetic variability. They reported that the genetic variability determined by microsatellite typing was significantly higher than the variability inferred by mtDNA. Miller (2003) used microsatellites to determine the genetic structure of yellow perch in Lake Michigan and found appreciable polymorphism at the microsatellite loci, whereas prior studies of allozymes and mtDNA had shown little genetic variation (Miller 2003). Newly developed yellow perch microsatellite loci should be useful for fine scale analysis of population structure, for assessing hatchery effects, and ultimately for marker assisted selection for traits of economic interest.

In addition to its ecological importance, the yellow perch is a popular food item, a common public aquarium fish, a popular recreational angling resource, and it supports commercial fisheries in Lake Michigan, Lake Eerie, and Lake Huron (Malison 2000). The yellow perch has a mild taste and firm flesh with low fat and phospholipid content, making it appealing to both consumers and restaurant industries and providing for a long-shelf life, resistance to freezer damage, and minimal problems with off-flavor and cooking odors (Malison 2000). Despite a dramatic decline of yellow perch populations in the Great Lakes from 1950 to 1970, market demand for the fish remained high, illustrating strong consumer preferences for this particular seafood product (Malison 2000, Manci 2001). Today, yellow perch have a high market value compared to catfish, trout, and other freshwater species that are successfully aquacultured or have significant aquaculture potential (Malison 2000). The average yellow perch fillet retail value in 2002 was $12/lb in the U.S (Kentucky State University 2003); as

compared to $5/lb retail for catfish (Kentucky State University 2003) and $8 to $12/kg for fresh tilapia fillet (Lutz, et al. 2003).

Because of the sustained high demand (despite the reduction in domestic supplies of yellow perch) and due to concern over micro-contaminant levels in Great Lakes fishes, there has been a tremendous increase in the interest in yellow perch aquaculture (Malison 2000). However, despite the recent technical advancements in yellow perch aquaculture methods (Manci 2001), this species still is considered in most areas as an "alternate aquaculture species." As part of the effort to enhance aquaculture production of yellow perch, Ohio State University has undertaken a program to improve aquaculture production traits for yellow perch. One component of our broodstock selection efforts entailed examination of genetic diversity in six geographically disparate stocks of yellow perch. The objectives of our study were to locate and optimize a large number of polymorphic microsatellites within the yellow perch genome, to utilize these molecular markers to conduct a population genetic analysis among relevant broodstock groups collected from a geographically broad range of native populations, and to utilize estimates of molecular genetic variation to complement data being collected on differential performance of these same geographic strains.

MATERIALS AND METHODS

Sample collection

Similarly aged adult yellow perch were collected live from wild populations in Maine (ME, Sebasticook River; n = 96), North Carolina (NC, Perquimans River, n = 62), New York (NY, Erie Canal; n = 76), Pennsylvania (PA, Lake Wallenpaupack; n = 97), and from captive

populations held in Michigan (MI, F_1 of an original sample collected from Saginaw Bay; n = 88) and at Ohio State University (OH, F_x originally from Lake Erie; n = 73). Non-lethal biopsy (fin clip) specimens were preserved immediately in 70% ethanol.

Microsatellite identification and optimization

For each specimen, DNA was extracted from 50 mg of tissue according to the methods outlined by Waters et al. (2000). A microsatellite-enriched library was prepared according to the methods outlined by Li et al. (in review). Briefly, a mixture of 5 μg of total nucleic acid pooled from several yellow perch specimens was restriction digested with *Sau*3AI, ligated to linkers, and hybridized to a cocktail of biotinylated tandem repeat oligonucleotides [$(AAC)_{11}$, $(GAAT)_{10}$, $(ACAT)_{11}$, $(AAAG)_{11}$, $(GTA)_{15}$ and $(AAT)_{15}$]. Coupled molecules were separated from non-repeat sequences using avidin, PCR repaired, and TA-cloned with the TOPO™ vector (Invitrogen). Approximately 700 colonies with inserts were picked and subjected to PCR using M13 primers. Appropriately sized amplicons (500-1200 bp) were sequenced in both directions resulting in a suite of 200 microsatellite-containing sequences. Of those, 30 loci produced amplicons displaying at least 4 different alleles. Twelve polymorphic loci were selected for further analysis (Table 1) in combination with 8 previously published loci Leclerc et al. (2000), Kapuscinski and Miller (2000), Borer et al. (1999), and Wirth et al. (1999). Ultimately, seventeen loci yielded sufficient data for analysis of the six broodstock populations (Table 2).

Broodstock genotyping

All primer sets were modified as described by Boutin et al. (2001) with the addition of a unique sequence to the 5′ end of one of each pair (referred to hereafter as modified primer) as shown in

Table 1. Each 6 μL PCR reaction contained 1 μL of template, 0.4 μL 0.67 μM modified primer, 0.2 μL 10 μM unmodified primer, 0.2 μL 10 μM labeled primer (5′-CAGTCGGGCGTCATCA-3′ labeled with FAM, TET or HEX), 1 μL H_2O, 0.2 μL 4mM spermidine, and 3 μL of JumpStart Red Taq (Sigma–Aldrich). PCR was performed using MJ Research PTC100 thermal cyclers to cycle through the following steps: 3 min denaturation at 95°C, followed by 20 sec at 94°C, 30 sec annealing at the appropriate temperature (Table 1), and 2 min extension at 72°C. These three steps, repeated 40 times, were followed by a final 3 min extension step at 72°C. The 5′-modified primers allowed use of the third fluorescently labeled primer in PCR, which facilitated pooling of PCR reactions and automated detection and genotyping using a BaseStation 51™ DNA fragment analyzer (MJ Research). Each lane of each ultra thin gel contained a 70-400 base pair ROX-labeled molecular marker (BioVentures). Genotypes were automatically scored using Cartographer®.

Statistical tests

To calculate allele frequencies and genotypic proportions, GENEPOP Version 3.4 (Raymond and Rousset 1995) was used. Linkage disequilibrium was tested with the probability test using a Markov chain method (Guo and Thompson 1992) and global tests were performed across all populations with Fisher's method. Pairwise genetic differentiation among populations was calculated using exact tests for each locus. The significance of deviation from Hardy-Weinberg expectations was examined with exact *P*-values that were estimated using a Markov chain method, and where significant deviations occurred, tests for heterozygote excess and heterozygote deficiency for each locus were conducted. All Markov chain runs consisted of

1000 dememorization steps, 100 batches, and 1000 iterations. In each instance where multiple independent tests were performed, significance levels (α) were revised by Bonferroni correction (Rice 1989). Multilocus inbreeding estimates, originally described by Ayres and Balding (1992) and subsequently illustrated by Dyer (2005) to be useful in consideration of inbreeding in wild populations, were examined in each of the six yellow perch samples. The distribution of inbreeding coefficients, F, was plotted to compare estimated levels of inbreeding.

Population genetic structure was examined using Φ_{ST} calculated by AMOVA (Excoffier et al. 1992) as implemented by the population genetic software GENO (Dyer 2005) and by estimating F-statistics with GENEPOP (Weir and Cockerham 1984; Wright 1946). F_{IS} was calculated across all populations and within each population. F_{ST} was calculated across all populations and for each population pair (Cockerham 1973; Weir and Cockerham 1984). As a further indication of population structure, inbreeding coefficients were determined using GENO. Finally, Nei's standard genetic distance (D_S; Nei 1987) was calculated for each population pair using MICROSAT Version 1.5d (Minch 1997) and PHYLIP phylogenetic software (Felsenstein 1993) was used to obtain a neighbor-joining tree (Saitou and Nei 1987) based on D_S-values.

Isolation by distance was estimated using map distances (km) between each pair of populations and the relationship between genetic distance (D_S) and geographic distance was tested across all populations with Mantel's (1967) general regression test as implemented by the population genetic software GENO (Dyer 2005). To further evaluate historical gene flow, effective migration rate (N_em) was computed using the standard relationship of N_em to F_{ST} (Wright 1946) and with GENEPOP using private allele frequencies (Barton and Slatkin 1986; Slatkin 1985). The latter method relied on the expectation that private alleles reach high

frequencies in populations when the migration rate is low enough to prevent homogenizing effects of out breeding.

RESULTS

Within sample genetic variation

Across all populations, a total of 223 alleles were detected at the 17 loci analyzed, 37% of which occurred at a frequency of 5% or lower. The average total number of alleles per locus (Å) ranged from a low of Å = 3 for locus P2 to a high of Å = 22 alleles for P4, with a mean of Å = 7. The frequency of private alleles was relatively low and averaged 5% across all population samples tested. A number of populations showed significant deviations from Hardy-Weinberg equilibrium (Table 2). In all cases except MI, the broodstock populations were characterized by significant heterozygote deficits ($P < 0.0001$) indicating departures from random mating. A few cases of apparent Wahlund Effects were observed in Michigan, New York, and North Carolina (Figure 2), especially at loci P2, and P4, YP17, YP30 and YP32. Although linkage disequilibrium over all loci was not prevalent, there were few incidences of significant linkage disequilibrium in these populations. For example, of 171 comparisons, 4 instances of linkage were observed in PA, 3 in OH, 2 each in MI and NY, one in NC, and none were observed in ME. Three of these twelve significant values involved YP17 and YP32, indicating a chance that these two loci are proximal to one another. Four instances included locus P6 and three included locus YP7, indicating a possibility of null alleles at these two loci. Single-locus inbreeding estimates, F_{IS}, ranged from a low of -0.55 (MI : YP30) to a high of 0.90 (MI : YP79) and averaged approximately 0.20 for all populations except MI (where average F_{IS} was 0.01). Multi-locus

inbreeding coefficients, F, showed distinct distributions (Figure 4) that differed significantly between MI and the remaining five populations ($P < 0.0001$).

Among sample genetic variation

For the loci tested, allele frequencies were not homogeneous among samples, indicating significant genetic differentiation among all population pairs (each comparison $\chi^2 = \infty$, $P = 0.0000$). The overall Φ_{ST} value for the North American *P. flavescens* populations sampled in this study (0.242, $P < 0.0001$), indicated that a large proportion of detectable genetic variation was found among the populations rather than within them; approximately 24.2% of the genetic diversity. Indeed, the highest observed value was between Maine and Michigan ($\Phi_{ST} = 0.490$; $P < 0.0001$). A number of populations exhibited very little divergence (Table 3); the lowest observed was between Ohio and New York ($\Phi_{ST} = -0.052$; $P = 0.999$). Genetic subdivision, estimated by F_{ST}, yielded similar results, ranging between a low of 0.05 between Ohio and New York and a high of 0.44 between Maine and Michigan (Table 3). Pairwise estimates of genetic distance (D_S) among populations of *P. flavescens* ranged from 0.13 between New York and Ohio to 1.15 between Michigan and Maine (Table 4 and Figure 3).

Across the range of the sampled populations tested, there was no significant relationship detected between genetic distance (D_S) and geographic distance ($Z = 1917$, $P = 0.639$). Pairwise estimates of effective migration rate (N_em) among populations using F_{ST} values (Table 4) ranged from a low of 0.3 migrants/generation detected between the Maine and Michigan populations to high gene flow between Ohio and New York ($N_em = 4.5$).

RELEVANCE

Yellow perch exhibit relatively low levels of microsatellite polymorphism

The relatedness and genetic distance patterns observed in this study are consistent with previous studies. The average total number of alleles per locus observed was similar to prior published studies for *P. flavescens*; Miller (2003) observed similar numbers of alleles (mean Å = 8.7, range 3.2-19.1), and Leclerc et al. (2000) recorded Å = 7.5 (range 2-18). The range of heterozygosity observed in this study (H_o = 0.04 to 0.88) was broader than the 0.21 to 0.86 range observed by Miller (2003) and the 0.25 to 0.82 range observed by Leclerc et al. (2000) likely due to the larger sample sizes, larger number of populations surveyed, and the increased geographic range covered in the current study. Virtually the same level of population differentiation was recorded by Miller (2003) for eighty samples collected from two native spawning yellow perch populations of Lake Michigan. Miller (2003) also found similarly close genetic relationships between *P. flavescens* populations located in large lakes and river systems of the Lake Michigan and Green Bay regions. This implies the levels of variation detected are likely an accurate reflection of the range of genetic variation in *P. flavescens*.

The levels of observed microsatellite polymorphism were somewhat greater than polymorphism observed for several other commercially produced aquaculture fishes. For example, wild populations of trout (*Salmo trutta)* typically exhibit Å = 4 to 19 with an average of Å = 9 (Was and Wenne 2002), striped bass, *Morone saxatilis*, average Å = 4 (Ross et al. 2004), catfish, *Ictalurus punctatus*, average Å = 4 (Tan et al. 1999), and sunfish, *Lepomis* spp., average Å = 5 (Neff et al. 1999). Conversely, yellow perch exhibit levels of polymorphism that are considerably lower than that observed for rainbow trout, *Oncorhynchus mykiss*, where Å = 17

(Nielsen et al. 1999) and tilapia, *Oreochromis niloticus*, populations, where it is common to detect an average of 10-20 alleles per locus (Fuerst, et al. 2000, Romana-Eguia et al. 2004, Hassanein and Gilbey 2005).

Departures from random mating

The current data indicate that non-random mating is common in extant yellow perch populations. Although Miller (2003) observed few deviations from Hardy-Weinberg in the samples taken from Lake Michigan, of ten microsatellites described, Leclerc et al. (2000) observed nine to exhibit heterozygote deficits. Our results for the MI group agreed with Miller (2003) in that we found this population to largely conform to Hardy-Weinberg expectations. It is notable that this sample originated from a relatively large population, with very little evidence of inbreeding (Figure 4). Conversely, the other groups we examined showed significant deviations from Hardy-Weinberg expectations at a majority of the loci tested (Table 2), similar to the observations of Leclerc et al. (2000). A number of ecological factors are likely contributors to non-random mating in yellow perch. These include high variation in effective population size (Shroyer and McComish 2000), unequal numbers of sexes (Shroyer and McComish 2000), the contemporary dramatic decline in spawning populations (Manci 2001), and regular oscillations in yellow perch population sizes (Sanderson et al. 1999). It is also likely that the patterns of genetic variance exhibited by these populations are a manifestation of anthropogenic impacts of supplementation or harvesting, which are expected to result in high levels of inbreeding. Only the Michigan sample exhibited population genetic results expected of a healthy wild population (conformation to HWE and both F and F_{IS} near zero). Of the remaining allegedly wild samples, Pennsylvania demonstrated lower levels of inbreeding ($F < 0.2$), the Maine group exhibited

moderate inbreeding (median F = 0.2), and inbreeding levels exhibited by the New York and North Carolina were high (median F = 0.3). Thus, based on the current analysis, inbreeding may be an issue of relevance not only in the case of captive populations, but in the dwindling native populations as well.

Ramifications of inbreeding for yellow perch broodstock management and selective breeding

The NY origin of the captive OH population was corroborated by the population parameters as well as the taxonomic analysis. This pair exhibited the lowest genetic distance (D_S = 0.13), the highest effective migration rate (N_em = 4.5), and the smallest Φ_{ST} (-0.05) and F_{ST} (0.05) values. Although the observed number of alleles was slightly lower for OH than for its founder NY, there was not a profound Founder Effect. Nevertheless, an obvious concern in this selective breeding program is the potential for excessive levels of inbreeding in crosses between these two groups. Indeed, one of the most significant problems in aquaculture and fisheries is the decline in productivity of broodstocks due to unintentional inbreeding. It has been suggested that breeding programs used by most fish farmers produce inbreeding rates of 3–5% per generation (Tave 1999). Because yellow perch have been propagated in both tanks and ponds at various locations with little or no genetic control since 1970's (Malison 2000 and Manci 2001), one might suspect this problem to be imminent in commercial scale perch production and selective breeding.

These high levels of inbreeding in newly created broodstock populations are of concern because they exceed the conventional cutoff of 0.125, indicative of first cousin mating (Tave 1993, 1999). Knowledge of inbreeding is extremely valuable to the current breeding program

because these estimates highlight the potential for unintentional inbreeding when crossing within and among broodstock populations. The molecular markers identified in this study are therefore being use to facilitate use of the simple marker-assisted breeding scheme known as walk back selection (Doyle and Herbinger 1994) to ensure that unintentional inbreeding is controlled. This strategy allows selection for growth, feed conversion efficiency, size at maturity, etc., without negatively affecting the genetic background of the broodstock.

Utility of microsatellite markers for selective breeding in yellow perch

Prior to this effort, no analysis had been made of the effects of broodstock management, particularly inbreeding, during initial stages of yellow perch domestication. The current analysis of microsatellite variation illustrates that the captive Ohio broodstock group (the group most likely to show unintentional inbreeding as a result of artificial selection) has not been significantly impacted and shows levels of genetic variation similar to, and in some cases greater than, the wild groups surveyed. Fortunately, levels of variation revealed by the current set of microsatellites are adequate for constructing molecular pedigrees and for estimating genetic relatedness among potential spawning pairs thereby avoiding unintentional inbreeding. Combining data for superior phenotypes with data for relatedness will be an effective foundation on which to base efforts designed to increase the economic value of aquacultured yellow perch. However, because of the relatively low numbers of alleles exhibited for yellow perch microsatellite loci, we now know that to create a reasonably high density genome map, additional higher polymorphism markers (e.g., AFLP, RAPD, SNP) will likely be necessary.

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Table 1: Details for twelve new microsatellite loci developed for genetic analysis of yellow perch, *Perca flavescens*, populations.

Locus Name (Repeat)	GenBank Access. No.	Primer Sequences (5′ – 3′)	Anneal (°C)
YP1	Pending	ATTTTCCAGAAGATGAGAGAGACC	55
$(AAC)_3GAC(AAC)AC(AAC)_5$		CAGTCGGGCGTCATCATAGAGATGGTCTTCATAAAGTAGGAG	
YP6	Pending	CAGTCGGGCGTCATCAAAGTAGCAGATGTAAAAGAGCAAGAAA	57
$(AGT)_{10}$		GGGCAAGAGACAGAAAGCCAATA	
YP7	Pending	ATGTATTTCTGTCAACTGGCGG	55
$(AAC)_7AAT(AAC)_2$		CAGTCGGGCGTCATCAGAATGTGTCCTTATTTGCGTGG	
YP9-1	Pending	CAGTCGGGCGTCATCATTGAGCAGACAGGGCAGAGA	58
$(ACT)_9AAT(ACT)_2$		CCCGTTTCAACTCCACCACT	
YP13	Pending	GGCACCCAAACTACCACT	55
$(GTA)_{11}$		CAGTCGGGCGTCATCATCAAACAAGCCCCATACA	
YP16	Pending	CAGTCGGGCGTCATCAGTGTGTGGGTTACTGCTGGC	57
$(CATT)_9$		TCCCTCTCTCTCCCCTTTCA	
YP17	Pending	CAGTCGGGCGTCATCACAGCGTTTCCACAGTATTGACC	55
$(TAG)_{10}TANGTG(TAG)_2$		GGGTTTTACACTGTTGATGGGAT	
YP30	Pending	CAGTCGGGCGTCATCAACATCTATCTCACTTCATTTCACATT	55
$(TTCT)_6$		ACATCTTCCTCTTCTCAAACTCCT	
YP32	Pending	AACAGTTGGTGAGATGGGAATG	55
$ACTAC(ACT)_{12}$		CAGTCGGGCGTCATCAACAGGTGCCGAAGGAGGT	
YP66	Pending	CAGTCGGGCGTCATCACTGCTGATGAAGTGGACAA	55
$(TTCT)_{11}$		CATAGGGGTCAGGGCAAAC	
YP73	Pending	CAGTCGGGCGTCATCAGATGGGAGGAAATGGTGAGA	55
$(CAA)_{16}$		GAACGCCCAAGCCTGAAT	
YP79	Pending	CTCCAACAGTCAACAGGTAACA	55
$(ACAC)_2ACAAA(ACAC)_5$		CAGTCGGGCGTCATCATCCATTCCTTTACTGCTTTCTA	

Table 2: Microsatellite genetic variation in *Perca flavescens* from six North American populations categorized as the number of alleles observed (A), heterozygosity (observed and expected), and the *P*-values for exact tests of fit to Hardy-Weinberg equilibrium (HWE). Populations with missing data are denoted " -- ".

	Population						Mean
Locus P2	**ME**	**MI**	**NY**	**NC**	**OH**	**PA**	
A	10	7	12	16	14	2	11
Ho	0.58	0.75	0.86	0.94	0.75	0.91	0.80
He	0.70	0.71	0.88	0.83	0.84	0.87	0.80
HWE	0.0136	0.5417	0.7702	0.0568	0.1067	0.9836	
Locus P4							
A	29	--	18	23	16	21	21
Ho	0.58	--	0.64	0.88	0.90	0.63	0.72
He	0.87	--	0.91	0.94	0.92	0.88	0.90
HWE	0.0000	--	0.0000	0.0000	0.0219	0.0000	
Locus P5							
A	--	8	6	8	2	6	6
Ho	--	0.47	0.62	0.63	0.50	0.23	0.49
He	--	0.45	0.70	0.80	0.40	0.34	0.54
HWE	--	0.0146	0.2125	0.0895	1.0000	0.0000	
Locus P6							
A	5	10	13	14	13	15	12
Ho	0.27	0.68	0.73	0.51	0.83	0.56	0.60
He	0.25	0.59	0.81	0.68	0.87	0.75	0.66
HWE	0.8457	0.0459	0.0000	0.0286	0.0004	0.0000	
Locus P9							
A	--	9	12	10	11	18	12
Ho	--	0.73	0.29	0.55	0.86	0.64	0.61
He	--	0.76	0.81	0.88	0.86	0.85	0.83
HWE	--	0.0165	0.0000	0.0043	0.6077	0.0000	

Table 2 (continued)

	Population						Mean
Locus YP1	**ME**	**MI**	**NY**	**NC**	**OH**	**PA**	
A	7	3	6	8	4	9	6
Ho	0.40	0.04	0.31	0.07	0.18	0.72	0.29
He	0.67	0.04	0.70	0.41	0.46	0.80	0.52
HWE	0.0000	1.0000	0.0000	0.0000	0.0014	0.0009	
Locus YP6							
A	--	2	2	3	2	5	3
Ho	--	0.06	0.55	0.38	0.24	0.42	0.33
He	--	0.06	0.43	0.45	0.21	0.51	0.33
HWE	--	1.0000	0.0293	0.3243	1.0000	0.0000	
Locus YP7							
A	4	4	4	5	10	7	6
Ho	0.68	0.64	0.30	0.27	0.34	0.67	0.49
He	0.49	0.49	0.59	0.50	0.57	0.74	0.56
HWE	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	
Locus YP9-1							
A	5	6	5	7	4	5	5
Ho	0.28	0.91	0.20	0.31	0.78	0.54	0.50
He	0.24	0.63	0.26	0.32	0.71	0.56	0.45
HWE	0.5666	0.0000	0.0013	0.0982	0.0000	0.0000	
Locus YP13							
A	12	6	11	12	7	14	10
Ho	0.43	0.45	0.43	0.44	0.36	0.70	0.47
He	0.54	0.42	0.83	0.78	0.73	0.88	0.70
HWE	0.0015	0.9383	0.0000	0.0000	0.0000	0.0000	
Locus YP16							
A	4	3	2	3	3	5	3
Ho	0.42	0.20	0.51	0.54	0.39	0.51	0.43
He	0.56	0.26	0.50	0.58	0.44	0.47	0.47
HWE	0.0000	0.0671	1.0000	0.3978	0.3004	0.6979	

Table 2 (continued)

	Population						Mean
Locus YP17	**ME**	**MI**	**NY**	**NC**	**OH**	**PA**	
A	5	5	5	4	4	5	5
Ho	0.48	0.65	0.39	0.57	0.70	0.48	0.55
He	0.49	0.57	0.70	0.59	0.63	0.61	0.60
HWE	0.0000	0.0279	0.0000	0.7337	0.1012	0.0005	
Locus YP30							
A	4	5	4	3	3	5	4
Ho	0.68	0.82	0.79	0.76	0.87	0.97	0.81
He	0.66	0.53	0.57	0.59	0.65	0.66	0.61
HWE	0.0621	0.0000	0.0000	0.0131	0.0003	0.0000	
Locus YP32							
A	2	4	5	3	4	5	4
Ho	0.08	0.53	0.43	0.36	0.20	0.32	0.32
He	0.12	0.53	0.77	0.45	0.60	0.68	0.53
HWE	0.1263	0.7558	0.0000	0.0073	0.0000	0.0000	
Locus YP66							
A	5	3	5	6	5	5	5
Ho	0.21	0.13	0.33	0.56	0.29	0.47	0.33
He	0.44	0.15	0.79	0.80	0.73	0.67	0.60
HWE	0.0000	0.0266	0.0000	0.007	0.0000	0.0000	
Locus YP73							
A	6	4	6	4	4	5	5
Ho	0.08	0.09	0.07	0.22	0.27	0.04	0.13
He	0.70	0.32	0.58	0.74	0.56	0.35	0.54
HWE	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
Locus YP79							
A	6	3	7	3	3	5	5
Ho	0.36	0.03	0.58	0.76	0.54	0.51	0.46
He	0.50	0.25	0.64	0.56	0.65	0.47	0.51
HWE	0.0085	0.0000	0.0005	0.0000	0.0000	0.0243	

Table 3: Microsatellite genetic variation in *Perca flavescens* from six North American broodstock populations categorized as the F_{ST} value (above the diagonal) and estimates of divergence (Φ_{ST} values) are below the diagonal (P-values shown in parenthesis).

Population	ME	MI	NC	NY	OH	PA
ME		0.445	0.185	0.179	0.189	0.207
MI	0.490 (0.000)		0.345	0.220	0.164	0.338
NC	0.251 (0.000)	0.455 (0.000)		0.106	0.122	0.091
NY	0.159 (0.000)	0.315 (0.000)	0.125 (0.000)		0.052	0.091
OH	0.146 (0.000)	0.191 (0.000)	0.064 (0.000)	-0.051 (0.999)		0.154
PA	0.208 (0.000)	0.453 (0.000)	0.198 (0.000)	0.115 (0.000)	-0.006 (0.952)	

Table 4: Microsatellite genetic variation in *P. flavescens* from six North American broodstock populations categorized using effective migration rate and genetic distances. The pairwise estimates of genetic distance (D_S) among populations are found above the diagonal and the effective migration rate (N_em) using the F_{ST} value to estimate the migration rate is found below the diagonal.

Population	ME	MI	NC	NY	OH	PA
ME		1.15	0.36	0.37	0.40	0.45
MI	0.31		0.77	0.35	0.20	0.82
NC	1.10	0.47		0.28	0.31	0.41
NY	1.15	0.89	2.10		0.13	0.20
OH	1.07	1.27	1.80	4.51		0.40
PA	0.96	0.49	1.34	2.49	1.34	

Figure 1: Data from a portion of the Lake Michigan yellow perch spawning survey conducted by Wisconsin Department of Natural Resources. Graph prepared from data provided on http://www.dnr.state.wi.us/org/water/fhp/fish/lakemich/yellowperch.htm#yp%20project.

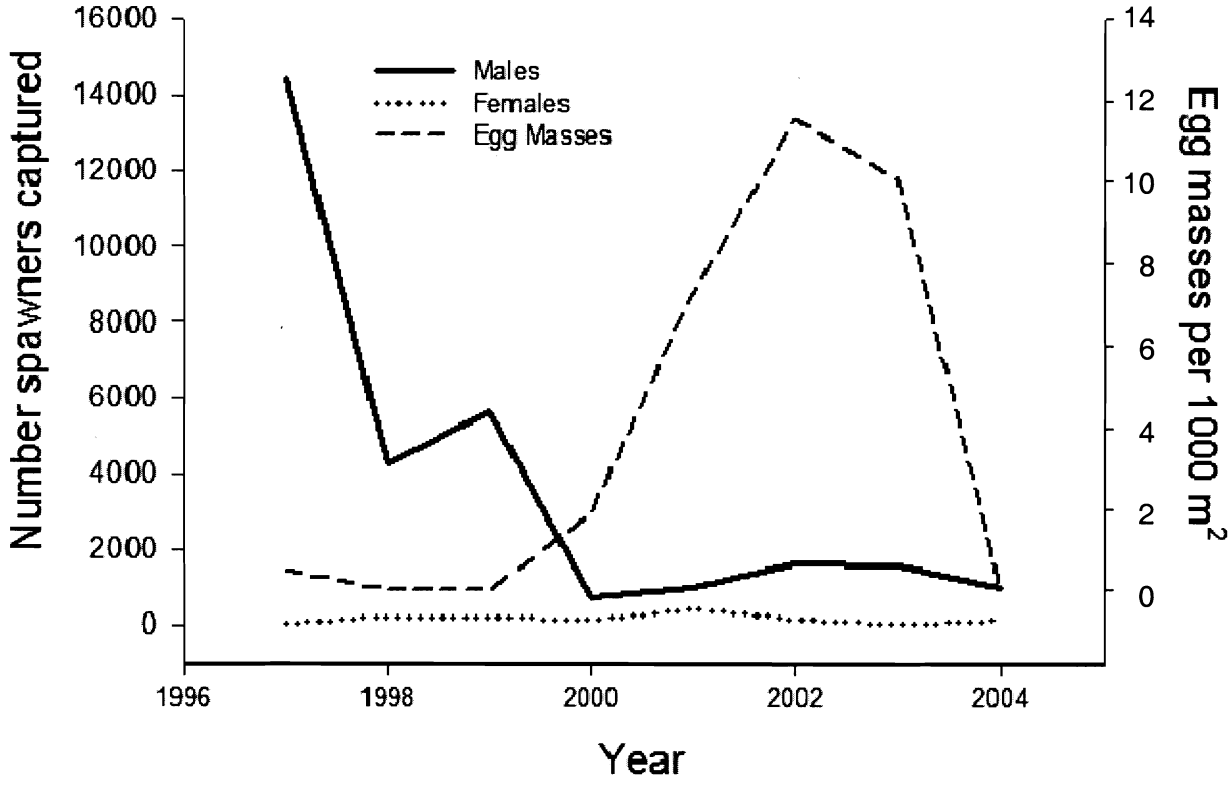


Figure 2: Wahlund Effect observed for locus P4 in the New York *P. flavescens* population. Similar effects were observed in the New York and North Carolina populations at several loci.

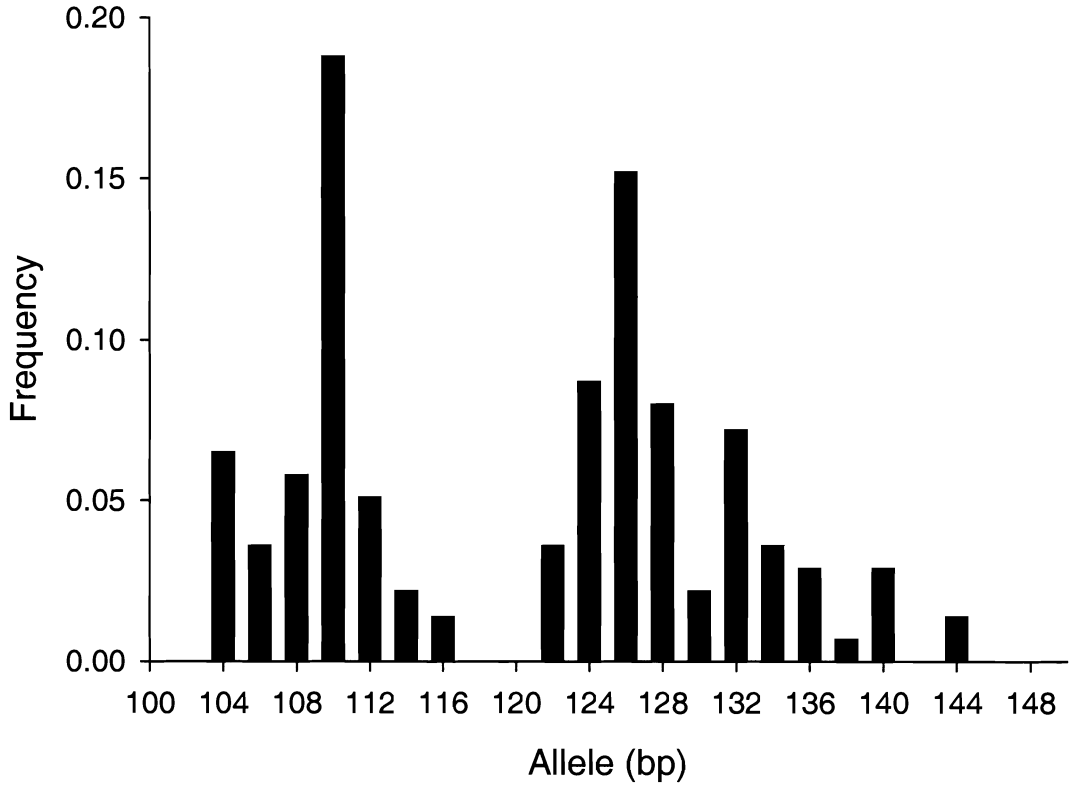


Figure 3: Neighbor-joining tree constructed from Nei's unbiased distance (D_S) values among six broodstock populations of *Perca flavescens*. Bootstrap values at the nodes indicate the number of unambiguous branches at that point out of 1000 resampling events.

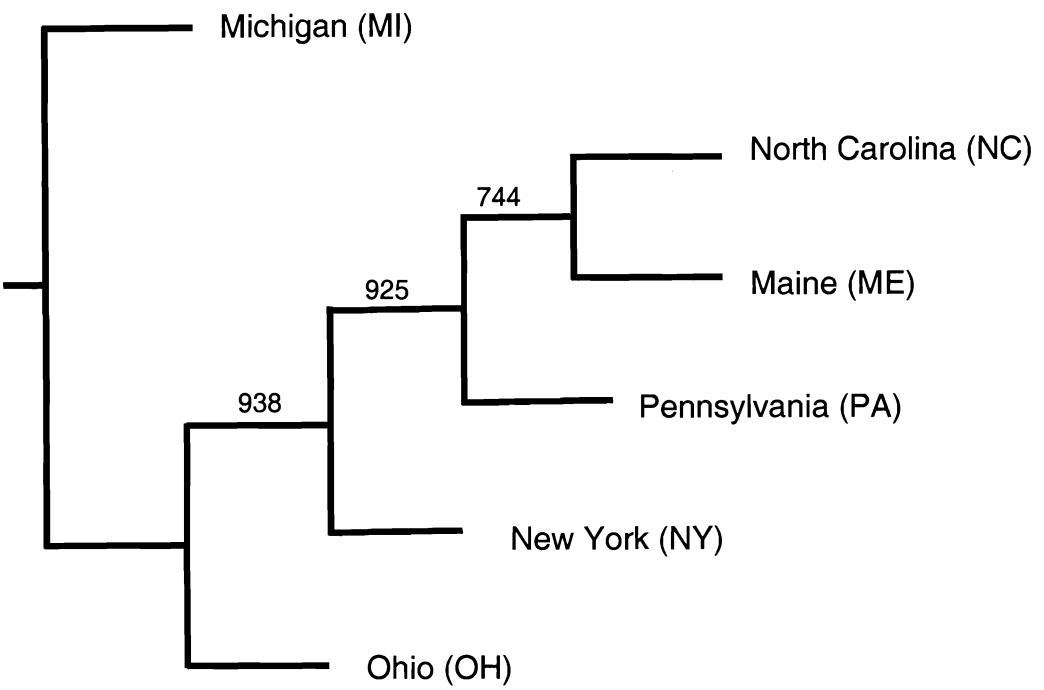
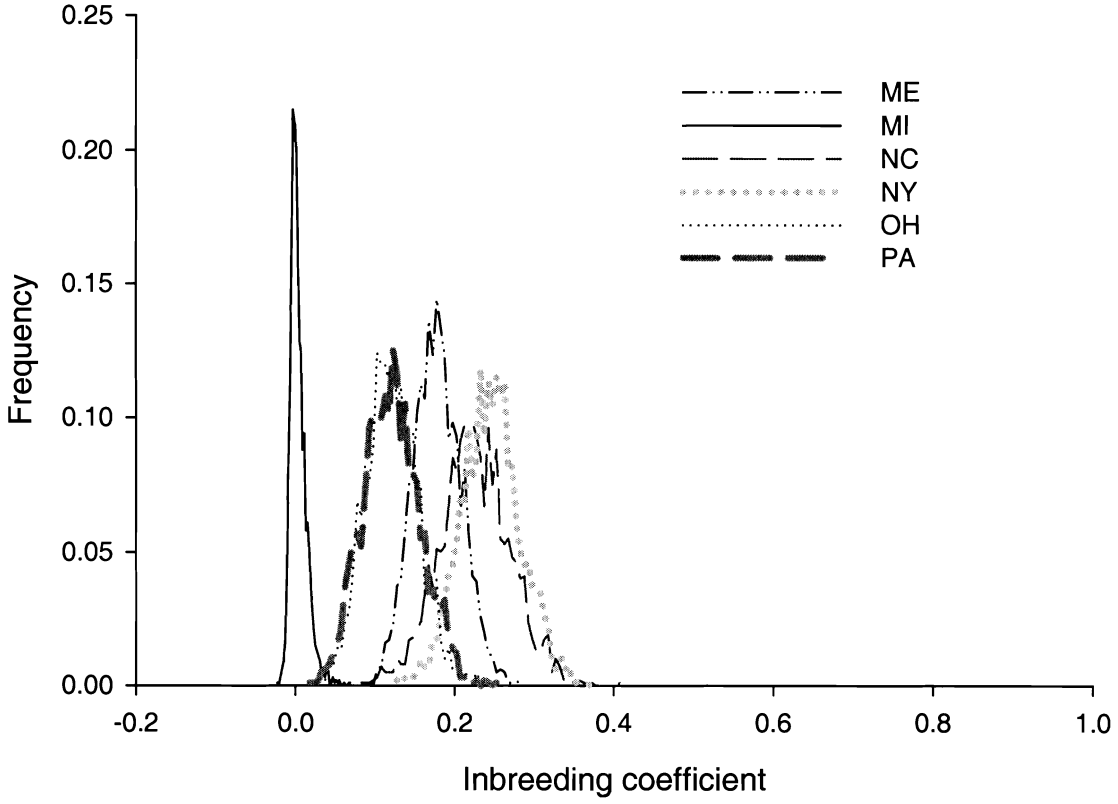


Figure 4: Distribution of inbreeding coefficients in six North America. *Perca flavescens* groups. Frequencies appear on the y-axis and inbreeding coefficient values, *F*, along the x-axis.



Vita

Chandler Brooke Givens was born on January 17, 1982 in Salem, Virginia. She attended South Salem Elementary School, and graduated from Salem High School in 2000. In May 2004, Chandler received her B.S. in Biology with a minor in Chemistry from Virginia Polytechnic Institute and State University in Blacksburg, Virginia. After college, she went straight into her Master's degree studies at Virginia Commonwealth University in Richmond, Virginia. While at VCU, she has worked as a Graduate Teaching Assistant for the Department of Biology. For her first year, she taught the Introductory Biology Laboratory for Biology majors and this past year, she has enjoyed teaching juniors and seniors in the Biotechniques class. She has found teaching to be quite rewarding and is considering it as a career in the future. After graduating from VCU, Chandler is engaged to be married in July 2006 to Matthew Kwarta and plans to begin her career working in a research laboratory, with the possibility of teaching as well.