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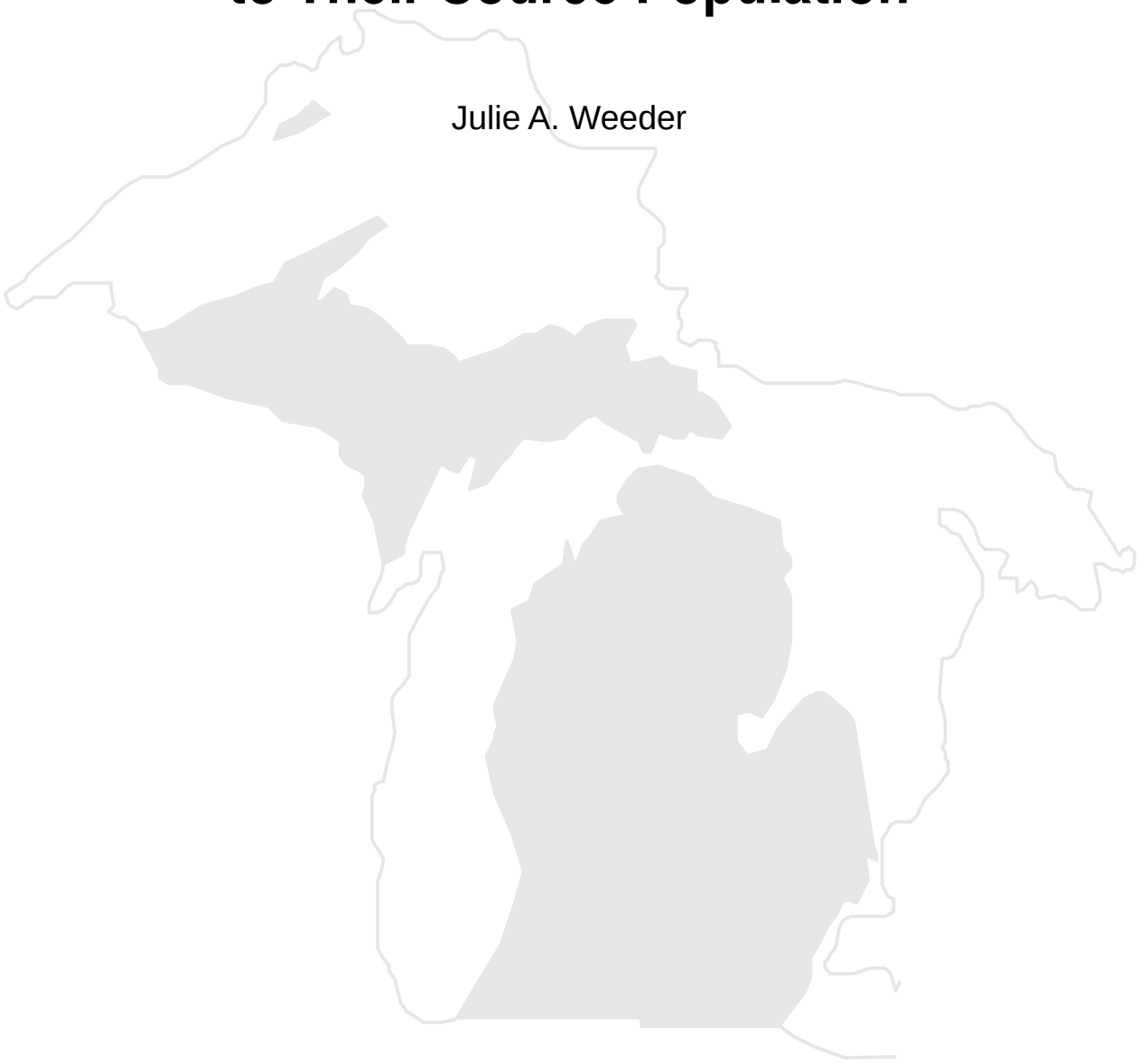
Julie A. Weeder



FISHERIES DIVISION
RESEARCH REPORT

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A GENETIC COMPARISON OF LAKE MICHIGAN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) TO THEIR SOURCE POPULATION

By

Julie Anne Weeder

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ABSTRACT

A GENETIC COMPARISON OF LAKE MICHIGAN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) TO THEIR SOURCE POPULATION

By

Julie Anne Weeder

To determine whether genetic drift has impacted the genetic diversity of Lake Michigan chinook salmon since their transfer from Washington's Green River in the late 1960's, I surveyed the allozyme variation of Lake Michigan chinook at 18 loci that were variable in a 1980's survey of Green River chinook salmon. The genetic diversity of Lake Michigan chinook salmon was consistently less than that of their Green River conspecifics (2.17 vs. 2.56 alleles per locus, 17% of variable loci monomorphic in Lake Michigan fish). Lake Michigan chinook salmon were more closely related to Green River chinook than to those of a tributary of Washington's Toutle River, a purported source population. The average yearly variance effective population size (N_e) of Lake Michigan chinook from 1967 to 1995 was 378 individuals. This is less than 1% of the estimated average census size, indicating that genetic drift has impacted the Lake Michigan population.

ACKNOWLEDGEMENTS

I would like to acknowledge my major professor, Dr. John Epifanio, for his inspiration and guidance throughout the development of my Master's research. I would like to thank my committee members, Dr. Thomas Coon and Dr. Donald Hall, for their fresh perspectives and valuable advice. My faculty advisors' enthusiasm toward research was a constant motivation.

The Michigan Department of Natural Resources (MDNR) and Michigan State University provided funding for this research project, and MDNR staff, notably Jan Sapak and Kelley Smith, were important for its implementation. Anne Marshall of the Washington Department of Fish and Wildlife has provided consistent encouragement and technical support throughout my research, and made it possible for me to obtain the most accurate data by inviting me out to her laboratory.

The resort owners, charter boat captains, private fishermen and agency personnel who helped me obtain tissue samples were important to the success of this project, and I greatly appreciate their help. Eric Stockinger, Dan Kennedy and Dan Gaebel provided critical assistance in the development of this project and learned with me - thanks, I couldn't have done it without you. To my fellow graduate students who became close

friends, I haven't had such fulfilling personal and professional relationships before, and I hope that you are an example of what I can look forward to in my career.

To my husband, Dave, I can't say how much you have helped me through this important personal and professional period in my life. I can't imagine doing this without you.

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CHAPTER ONE

The Genetic Diversity of Lake Michigan and Green River Chinook Salmon

Introduction

Chinook salmon (Oncorhynchus tshawytscha) were first successfully introduced into the Laurentian Great Lakes in 1967 to improve the sport fishery and to control populations of the invasive alewife (Alosa pseudoharengus) (Michigan Department of Natural Resources [MDNR] 1974). Approximately one million fertilized eggs were shipped to Michigan from Washington's indigenous Green River chinook population for each of three years in the late 1960's (1966-68). In 1969, the first mature cohort was successfully spawned in captivity and Michigan has since been self-sufficient in chinook salmon egg production. Descendants of the three groups of transferred embryos were ultimately stocked throughout the Great Lakes by the MDNR and other state and federal agencies, and Great Lakes chinook salmon numbers have since been augmented by an artificial propagation program. The Great Lakes chinook salmon program has by many accounts been successful: by 1986, the standing stock in Lake Michigan alone approached 40 million pounds, and the lakewide harvest by recreational anglers approached 1 million pounds (over 600,000 fish; Francis 1996).

In 1988, however, large numbers of dead chinook salmon washed up on the eastern shores of Lake Michigan, and the number of adults migrating up tributaries to spawn declined precipitously (Johnson and Hnath 1991). These losses were attributed to bacterial kidney disease (BKD). Whether BKD was the only factor in these mortalities is still debated, but no other direct causes of death have been identified. The population has not

fully recovered from this crash; from 1989 to 1994, returns to rivers and sport harvest have remained at less than 50% of pre-1988 levels (MDNR, unpublished data).

The dramatic fluctuations in Great Lakes chinook salmon populations in recent years has prompted a closer examination of the dynamics of this population. Genetic drift, or random change in allelic frequencies, has caused a loss of genetic diversity in other managed salmonid populations (e.g. Gharrett and Thomason 1987). To address the possibility of such a genetic loss in Lake Michigan chinook salmon, an understanding of the composition and structure of genetic variability in the Great Lake population(s) is necessary.

Genetic diversity is a useful index of the health and stability of populations; low levels of diversity within a population have been linked to reduced disease resistance (e.g. in rainbow trout, Ferguson and Drahushchak 1990), slower development, reduced size-at-age, higher mortality, and reduced fertility (e.g. Smith and Chesser 1981, Meffe and Carroll 1994). Furthermore, genetic variation within populations is a basic requirement for adaptation and the long-term persistence of the population in a changing environment (Soulé 1980, 1987). Thus, management for the future success of Great Lakes chinook salmon requires an understanding of the amount and structure of genetic variation in the population, in addition to an examination of the processes responsible for its present state.

I used allozymes as markers in order to determine whether Lake Michigan chinook salmon show evidence of population subdivision and genetic drift. I surveyed the allozyme variation of over two hundred chinook salmon from Lake Michigan, and I addressed two main issues. First, I tested for population subdivision of Lake Michigan chinook salmon using a null hypothesis of panmixia. The alternative hypothesis is that Lake Michigan chinook may have created river-specific or regional subpopulations due to their tendency to spawn in “natal” streams, which could reinforce lineage differences over time. Evidence for

such reproduction in the Great Lakes has existed almost since chinook were introduced (Carl 1982, Keller et al. 1990, Hesse 1994). Feral spawning may account for 20 to 50% of chinook salmon production in Lake Michigan alone (Carl 1982, Hesse 1994).

Despite substantial feral recruitment, hatchery-reared fish continue to play a prominent role in the persistence and management of Great Lakes chinook salmon. Stocking of such fingerlings is still extensive: 70% of the age-0 chinook salmon in Lake Michigan were probably stocked (Hesse 1994 and Carl 1982). Although highly variable, on average 4 million fingerlings (±454 S.E.) have been stocked into Lake Michigan from 1976 to 1987 [range 687,000 (1968) to 7.7 million (1984); MDNR unpublished data]. This estimate includes fish stocked by other states. Some of these fingerlings were stocked by other states bordering Lake Michigan, but because all chinook stocked into the Great Lakes were obtained from Michigan's hatchery system, all of these fish are descendants of the same gene pool of 3 million embryos from the 1960's. The MDNR stocks the majority of these fingerlings (although other Illinois, Wisconsin and Indiana have also participated in stocking), and although Michigan-stocked broodstock have been taken intermittently from other Michigan rivers, the vast majority of fingerlings stocked by the MDNR originate from less than 2,500 brood fish captured each year from the Little Manistee River (MDNR unpublished data). The stocking of millions of fish which represent the gene pool of only this one tributary could serve to maintain panmictic conditions lakewide, even if regional genetic differences would normally result from feral spawning in "natal" streams.

I next determined if there has been genetic change in the chinook salmon population since its Great Lakes introduction by testing the null hypothesis that the allelic frequencies of Lake Michigan and the source population, that of Washington's Green River, are not significantly different. No genetic data was collected from this chinook salmon population

when it was sampled in the late 1960's. Instead, I used allelic data from the Green River population which was collected in the 1980's as a surrogate for the allelic frequencies of the Green River population from 1966-68. There is a substantial indigenous run on this river every year, and the population is also supplemented with hatchery-reared juveniles immediately derived from this run. The Green River frequencies were based on a total of 400 fish (average 353 fish per locus) which were randomly collected, 100 each year, in 1981, 1987, 1988 and 1990 (Anne Marshall, personal communication [per. comm.]). The three later samples were of returning adult spawners, while the sample from 1981 was a sample of hatchery-reared juveniles from the 1980 brood year (Anne Marshall, per. comm.). There are several properties of this population which make it reasonable to assume that these allelic frequencies were representative of late 1960's Green River fish. First, the risk of stochastic changes in allelic frequencies due to low population size is low, because the Green River breeding population is large: it has ranged from 5,000 to 10,050 and averaged 7,600 from 1987 through 1991 (Washington Department of Fish and Wildlife (WDFW) 1993). Second, much of the breeding in the Green River population occurs in the wild and therefore is not subject to potentially detrimental husbandry practices which could have caused genetic change during the past 20 years. Third, there were no significant differences between the allelic frequencies of the four sampling years. Thus, this population was temporally stable during the 1980's, which increases the likelihood that this population was also temporally stable in previous years. Finally, the Green River population has not been subjected to any natural disasters or dramatic human intervention since Michigan's fish were transferred which could cause population-level genetic changes due to such factors as bottlenecks.

If there were significant differences between the Lake Michigan and Green River populations, this would indicate a change in allelic frequencies and thus heterozygosity since

1966. Two factors in the Michigan chinook salmon management program could have caused such changes. Early genetic bottlenecks could have resulted in founder effects, which would reduce the genetic variability of the first and subsequent Michigan generations. In addition, chinook salmon in Lake Michigan (and other Great Lakes) have always been bred and raised using methods which can erode a population's genetic variability over time. One or both of these factors could be critically important to the future genetic management of this species. Documentation of the genetic history and current status of Lake Michigan's chinook salmon population can provide insight into the role each of these factors may have played in any changes in allelic frequencies of Lake Michigan chinook salmon.

Methods

Tissue collection

I and collaborators collected 213 chinook salmon (184 adults, 29 age-0) from six Lake Michigan tributaries (Figure 1; Table 1). We sampled rivers that had large spawning runs and were likely to include feral-origin fish, and included the greatest possible range of geographic locations given sampling limitations (Table 1). We obtained adult tissue from recreational anglers on shore and on charter boats, and from MDNR and Fish and Wildlife Service personnel. Finally, we collected juveniles from the Muskegon River in the Spring of 1996, because sampling of adults the previous autumn was impossible (Table 1). Sample sizes (N = 13 - 46) varied due to differences in availability of suitable tissue (Table 1). We excised and individually tagged skeletal muscle, eye and liver tissues plus vertebral samples from each fish, placed them immediately on wet or dry ice, and transported within 36 hours to the genetics laboratory at Michigan State University. Upon receipt, tissue samples were

frozen at -20°C for future electrophoretic analysis, which was completed within 8 months of receiving tissues.

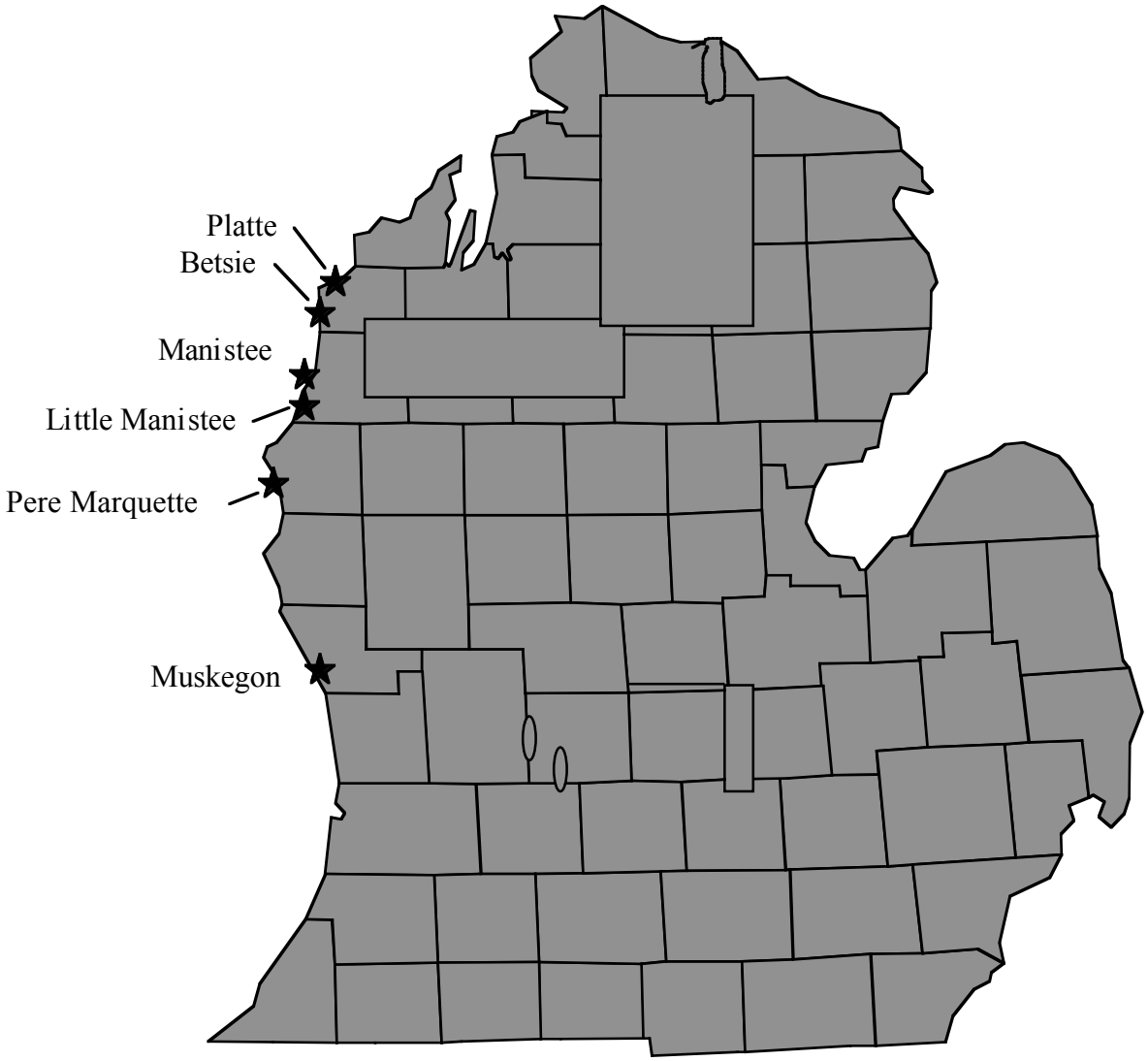

Figure 1: Lake Michigan sampling locations

Table 1: Rivers that were sampled in 1995-1996 for this study. Various evidence for feral recruitment is provided for each river where appropriate.

River	Sample size	Age	Collected in	Feral recruitment documented?
Betsie	46	Adult	Autumn 1995	yes^
Little Manistee	60	Adult	Autumn 1995	yes?
Manistee	13	Adult	Autumn 1995	yes*
Muskegon	28	Juvenile	Spring 1996	yes~*^
Platte	43	Adult	Autumn 1995	yes*
Pere Marquette	22	Adult	Autumn 1995	yes*

*Carl (1982) recovered juveniles before the stocking of hatchery-reared fish
^Large run with no recent history of stocking
~Juveniles recovered by MDNR before the stocking of hatchery-reared fish (MDNR unpublished data)
Seelbach (1985) recovered juveniles before the stocking of hatchery-reared fish

Oxytetracycline (OTC) mark detection

The MDNR marked all stocked chinook salmon fingerlings with oxytetracycline from 1990 to 1993 (Hesse 1994). When this chemical is fed to young fish, a mark is created which is retained throughout the fish's life. This mark can be detected with ultraviolet light (Weber and Ridgeway 1962). I determined whether the tributary samples included any feral fish by examining vertebrae from each individual for this mark. I and collaborators thawed, cleaned, and sectioned (a 10 mm middle cross section excised) vertebrae from each fish, and I illuminated the chemical mark with ultraviolet light (Weber and Ridgeway 1962). Fish exhibiting an OTC mark were of hatchery origin, while those without a mark were considered feral.

Protein Electrophoresis

Collaborators and myself prepared the tissues according to the methods of Aebersold et al. (1987) with minor modification. We used a Teflon grinding pestle to homogenize a ~0.5 g sample of each tissue from each fish in an equal volume of buffer (0.1 M tris HCl, pH

= 7.0), centrifuged the homogenates at 15,000 X gravity at 4°C for 12 minutes, and subjected the final supernatants to vertical or horizontal starch gel electrophoresis using conditions described in Table 2. We sliced and histochemically stained the gels for a suite of 14 enzymes encoded by 18 loci. The WDFW previously identified these loci as polymorphic in the Green River population. We conducted electrophoresis on the Lake Michigan samples in both the MSU and WDFW laboratories to assure comparable allelic designations and genotypic interpretations.

Table 2: Enzyme systems, loci, tissues, and electrophoretic conditions for chinook salmon protein electrophoresis.

Enzyme	Enzyme number	Locus	Tissue analyzed	Buffer system
Adenosine deaminase	3.5.4.4	*ADA-1**	Muscle	TG
		*ADA-2**	Muscle	TG
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Muscle	TC
			Liver	CAME
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B2**	Muscle	TG
		*GPI-A**	Muscle	TG
Glutathione reductase	1.6.4.2	*GR**	Muscle	TC
			Eye	CAME
Isocitrate dehydrogenase (NADP+)	1.1.1.42	*sIDHp-1**	Muscle	TC
			Eye, Liver	CAME
		*sIDHp-2**	Muscle	TC
			Eye	CAME
L-Lactate dehydrogenase	1.1.1.27	*LDH-C**	Eye	CAME
Malate dehydrogenase	1.1.1.37	*sMDH-B1,2**	Muscle	TC
			Eye	CAME
Malic enzyme (NADP+)	1.1.1.40	*sMEP-1**	Muscle	TC
Mannose-6-phosphate isomerase	5.3.1.8	*MPI-1**	Muscle	TG
Phosphoglycerate kinase	2.7.2.3	*PGK-2**	Eye, Liver	CAME
Proline dipeptidase	3.4.13.9	*PEPD-2**	Muscle	TG
Tripeptide aminopeptidase	3.4.-.-.	*PEPB-1**	Muscle	TC, TG
Peptidase (PepLT)	3.4.11-13*	*PEP-LT**	Muscle	TC, TG
Superoxide dismutase	1.15.1.1	*sSOD-1**	Muscle	TG
			Liver	TC

Data analysis

Interpretation of protein banding patterns followed existing WDFW models or rules recommended by May (1992) and Buth (1990). I calculated allele and genotype frequencies, plus descriptive diversity statistics, with the statistical program BIOSYS-1 (Swofford and Selander 1981). I calculated these statistics for the following groupings: for the samples from each drainage system, for the pooled population (which included all Lake Michigan fish), and for Green River data obtained from WDFW. I initially treated each isolocus as two distinct loci and used a procedure by Waples (1988) to estimate maximum-likelihood allele frequencies for these "loci". Based on these results, I ultimately assigned all the variation to the second 'locus' and treated the first as monomorphic. I treated these isoloci the same way in the Green River population, as per WDFW methods (Anne Marshall, per. comm.).

Deviation from panmictic conditions

I employed six approaches to test for deviation from panmictic conditions in the watersheds and in the Lake Michigan population.

1. I directly compared a variety of descriptive statistics between the tributaries to detect differences in specific diversity indices. I tested our results against Hardy-Weinberg expectations 2. within each drainage system and 3. for the Lake Michigan population as a whole with the log-likelihood ratio test (Sokal and Rohlf 1995) using the statistical program GENES IN POPULATIONS (May 1992). 4. I investigated genetic diversity among populations by testing a null hypothesis of allele frequency homogeneity among tributaries. To test this hypothesis, I calculated a population-by-allele (R X C) contingency table for each locus using a procedure in GENES IN POPULATIONS (May 1992). 5. I estimated

average gene flow between drainages by calculating Wright's fixation index (F_{ST}) (1965, 1978) with BIOSYS-1 (Swofford and Selander 1981). 6. To depict genetic relationships among drainages, I calculated Nei's (1978) unbiased genetic distance (D) between tributaries. These D values were based on 17 common loci; I excluded the mAAT-1* locus due to low sample sizes in some tributaries. I used these distances to construct a dendrogram using the Unweighted Pair Group Method (UPGMA).

Genetic comparison of the Lake Michigan and Green River populations

The tributaries were pooled to create one Lake Michigan population with an average of 186 fish per locus. I used two methods to test for differences in the genetic variability of Lake Michigan and Green River fish. 1. I quantified any increases or decreases in the frequency of specific alleles in the Lake Michigan fish as compared to the Green River population. 2. If there had been no change in the Lake Michigan fish since 1966, there would be little genetic distance between these fish and their Green River source stock. To test this hypothesis, I calculated the genetic distance (D) between these two populations and illustrated the genetic relationships with a dendrogram, which included for comparison a population of more distantly related, Columbia river-derived Cowlitz hatchery fish (WDFW, unpublished data).

Results

Deviation from panmictic conditions in watersheds

1. I detected more than one allele in 15 of the 18 loci examined (Table 3). There was little range in variability estimates across watersheds (Table 3); mean heterozygosity values ranged from 0.195 (±0.051 S.E.) to 0.216 (±0.057 S.E.), and on average there were

1.85 alleles per locus. The percent of polymorphic loci was more variable; in particular, the Manistee and Pere Marquette populations were considerably less polymorphic than the others (55.6 and 61.1 vs. overall mean of others 75).

2. I tested the observed allelic distributions against Hardy-Weinberg expectations within individual watersheds. Multiple tests of the same hypothesis increase the probability of a Type I error. To compensate for this error, I evaluated all G-statistics according to an adjusted p (p*), where the original $\propto$ was arbitrarily set at 0.05 (Rice 1989). None of the loci in any separate drainage deviated significantly from the expected Hardy-Weinberg distributions under this adjusted significance level.

3. Similarly, I compared the Lake Michigan population to Hardy-Weinberg predictions. Only the PEP-B1* locus deviated from these predictions when compared to p*. Although the MPI* locus did not deviate significantly from our expectations when compared to p*, it showed a statistically significant deficiency of heterozygotes under the less stringent p value. In contrast, PEP-B1* showed significantly more heterozygotes than expected.

4. I tested for heterogeneity in allele frequencies across tributaries with a contingency table analysis. PEPB-1* allele frequencies were slightly more heterogeneous than others, although there was no significant heterogeneity at the $p < 0.1$ level for any loci, or for the mean of all loci. Similarly, overall heterogeneity considering all loci but PEPB-1* was also low. All populations except that of the Manistee River showed more heterozygotes at the PEP-B1* locus than expected, but in only three of the seven comparisons was the excess significant at the 0.05 level (Appendix A).

5. F_{ST}, a fixation index which is a measure of genetic differentiation of subpopulations within a larger population (Wright 1965, 1978), ranged from 0.006 (MPI*) to 0.057 (mAAT-1*) (Table 4). Because PEP-B1* is a sex-linked locus, F_{ST} values for this

locus are misleading. When this locus was excluded, the mean F_{ST} value was 0.026 (Table 4).

6. There was no measurable genetic distance between any watersheds except for that between the Little Manistee and the Manistee, and that between these two and the Betsie River (Figure 2; Appendix B).

Genetic comparison of Lake Michigan and Green River populations

1. I hypothesized that Lake Michigan and Green River chinook salmon would have similar genetic profiles. The mean number of alleles per locus for the Lake Michigan population was substantially less than that of Green River chinook (2.17 vs. 2.56). Nearly 17% of the loci variable in Green river fish were no longer variable in the Lake Michigan population (Table 3). Specifically, three loci were fixed in Lake Michigan stocks, two of which were strongly polymorphic in Green River stocks, where this is arbitrarily defined as when the less frequent allele occurred at 2% or greater (Table 3).

2. There was genetic distance (D = 0.00072) between Lake Michigan chinook salmon and the Green River population (Figure 3; Appendix B). D measures the extent of gene differences between two populations, and this low number is weak evidence against the null hypothesis of similar genetic profiles for Lake Michigan and Green River fish. The distance between these two recently separated populations is much less than that between the Cowlitz hatchery and the Lake Michigan/Green River cluster (D = 0.03046; Figure 3, Appendix B).

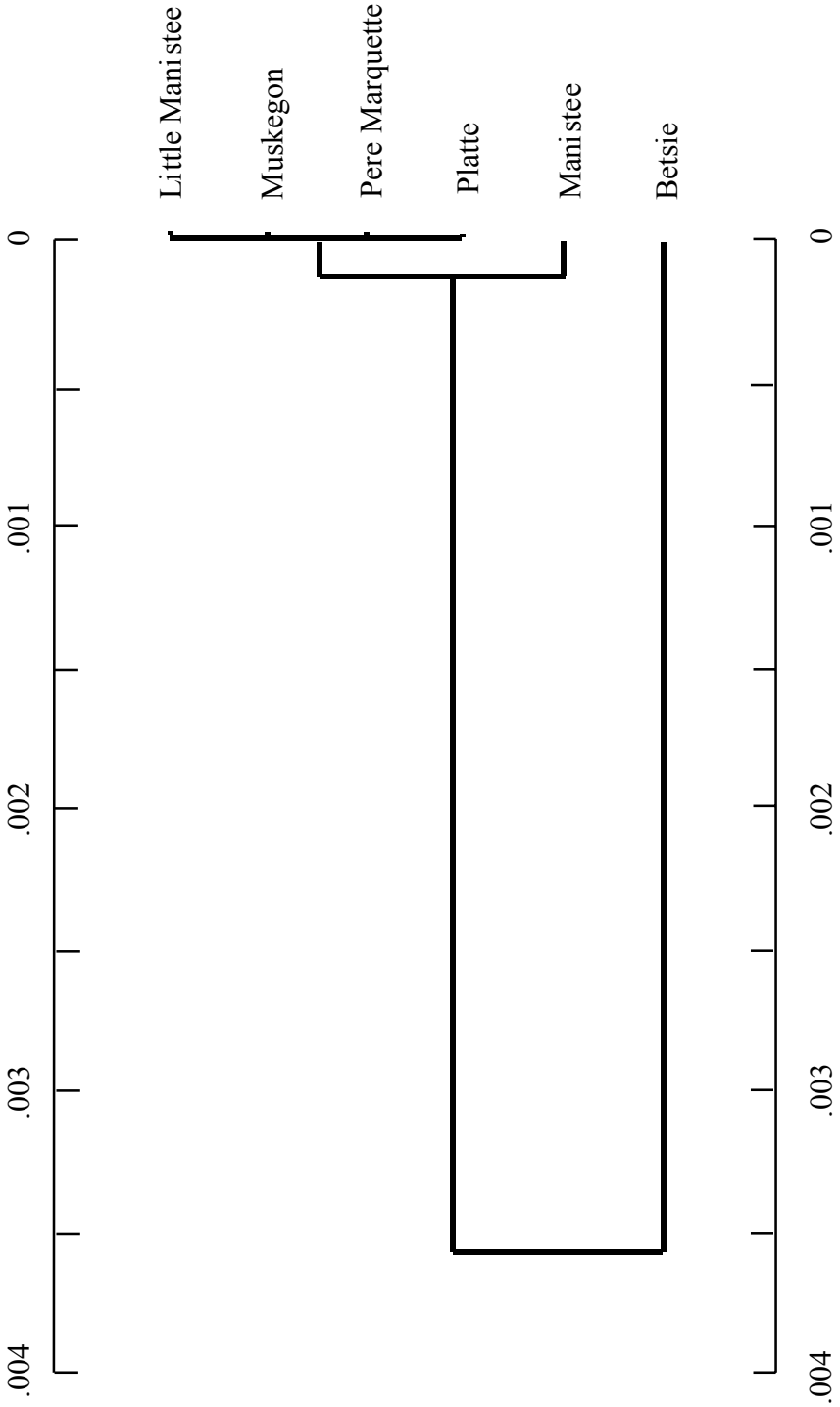

Figure 2: Relatedness of chinook salmon from six Lake Michigan tributaries, based on the Unweighted Pair Group Method and Nei's (1978) unbiased genetic distance.

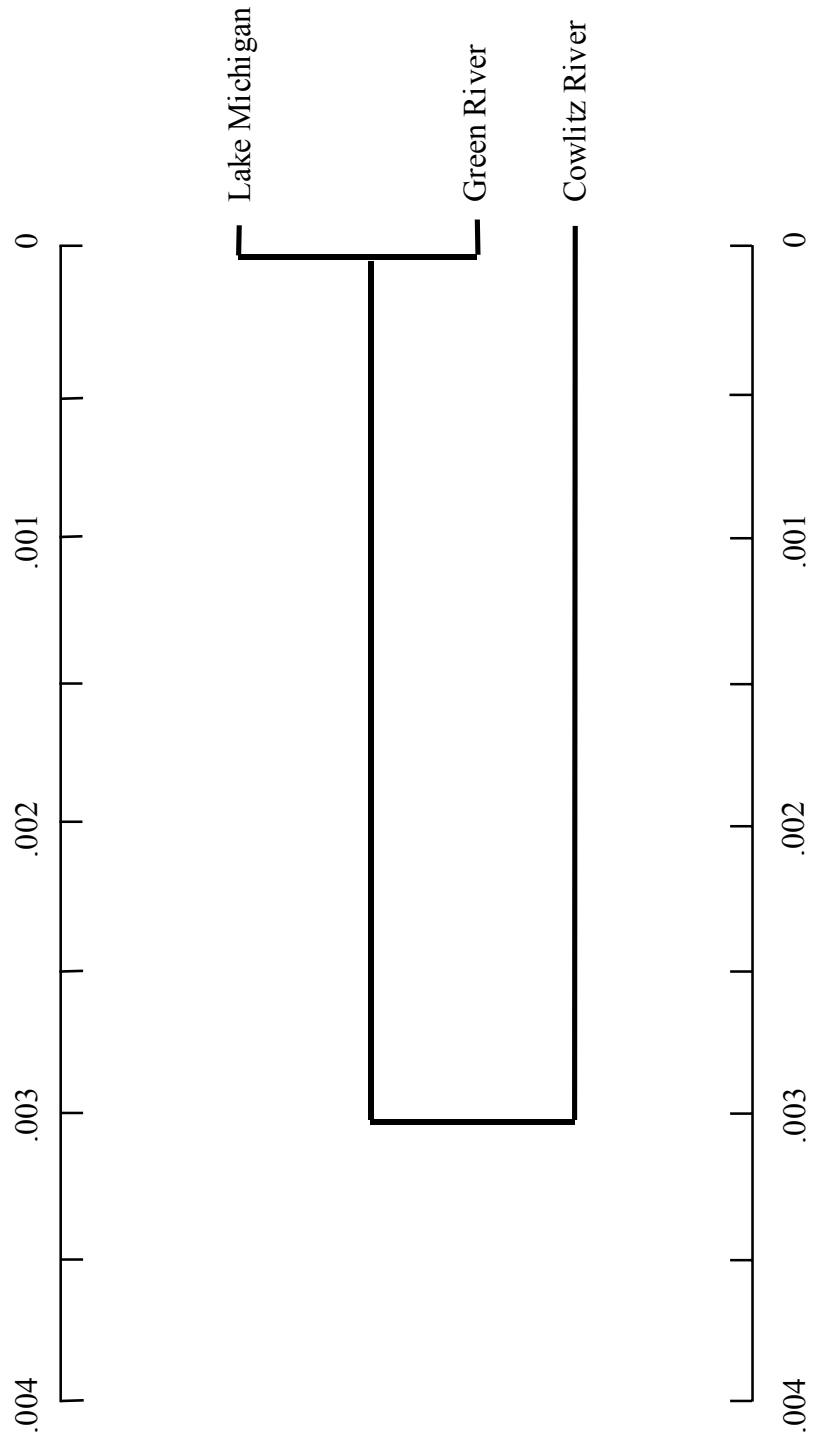

Figure 3: Relatedness of chinook salmon from Lake Michigan and two Washington drainages. "Green River" refers to the source stock, a Puget Sound strain, and "Cowlitz River" is an outgroup from the Columbia River basin. Calculations are based on the Unweighted Pair Group Method and Nei's (1978) unbiased genetic distance.

Table 3: Variable loci allelic frequencies and deviation from Hardy-Weinberg equilibrium for individual watersheds, the Michigan pooled population, and the Green River population.

Locus, allele and statistic	River Populations							
	Betsie River	Little Manistee	Manistee River	Muskegon River	Platte River	Pere Marquette	Lake Michigan	Green River
*sAAT-2**	44	58	12	21	35	16	186	397
**100*	0.875	0.897	0.750	0.857	0.800	0.875	0.858	0.949
**85*	0.125	0.103	0.250	0.143	0.200	0.125	0.142	0.050
*mAAT-1**	45	58	12	4	35	16	170	299
**-100*	0.789	0.784	0.667	1.000	0.771	0.844	0.785	0.844
**-77*	0.033	0.043	0.042	0.000	0.057	0.063	0.044	0.037
**-104*	0.178	0.172	0.292	0.000	0.171	0.094	0.171	0.119
*ADA-1**	46	57	11	20	34	17	185	397
**100*	0.957	0.974	1.000	0.950	1.000	0.971	0.973	0.975
**83*	0.043	0.026	0.000	0.050	0.000	0.029	0.027	0.025
*ADA-2**	44	57	13	23	33	16	186	299
**100*	0.943	0.939	1.000	0.978	0.939	1.000	0.954	0.952
**105*	0.057	0.061	0.000	0.022	0.061	0.000	0.046	0.048
**112*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
*GPI-B2**	36	48	9	23	34	15	165	298
**100*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.983
**60*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
*GPI-A**	41	54	12	23	33	16	179	398
**100*	1.000	0.991	1.000	1.000	0.985	1.000	0.994	0.933
**105*	0.000	0.009	0.000	0.000	0.015	0.000	0.006	0.006
**93*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
*GR**	46	60	13	27	41	22	209	398
**100*	0.989	1.000	1.000	0.981	0.976	1.000	0.990	0.994
**85*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004
**110*	0.011	0.000	0.000	0.019	0.024	0.000	0.010	0.003
*sIDHP-1**	46	60	12	24	36	19	197	298
**100*	0.946	0.917	0.750	0.875	0.861	0.816	0.888	0.909
**74*	0.000	0.000	0.000	0.042	0.000	0.000	0.005	0.000
**142*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
**94*	0.000	0.000	0.042	0.000	0.000	0.026	0.005	0.003
**129*	0.043	0.083	0.208	0.083	0.139	0.158	0.099	0.084
**136*	0.011	0.000	0.000	0.000	0.000	0.000	0.003	0.000

Locus, allele and statistic	River Populations							
	Betsie River	Little Manistee	Manistee River	Muskegon River	Platte River	Pere Marquette	Lake Michigan	Green River
*sIDHP-2**	46	60	12	24	40	20	202	299
**100*	0.967	0.967	1.000	0.958	0.962	1.000	0.970	0.958
**127*	0.033	0.033	0.000	0.042	0.038	0.000	0.030	0.015
**50*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027
*LDH-5**	35	60	12	23	38	15	183	395
**100*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995
**90*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
*sMDH-B2**	46	60	13	28	43	21	211	398
**100*	0.967	1.000	0.962	0.964	0.965	0.952	0.974	0.995
**121*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
**70*	0.033	0.000	0.038	0.036	0.035	0.048	0.026	0.005
*sMEP-1**	39	51	9	19	5	9	132	299
**100*	0.487	0.451	0.222	0.342	0.400	0.278	0.417	0.400
**92*	0.487	0.539	0.667	0.605	0.600	0.722	0.557	0.567
**105*	0.026	0.010	0.111	0.053	0.000	0.000	0.027	0.033
*MPI**	36	53	10	23	31	14	167	397
**100*	0.639	0.717	0.650	0.674	0.597	0.643	0.662	0.695
**109*	0.361	0.283	0.350	0.326	0.403	0.357	0.338	0.304
**95*	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ($p<0.05$)	0.001
*PGK-2**	10	51	3	27	34	13	138	390
**100*	0.500	0.706	0.833	0.704	0.794	0.731	0.717	0.646
**90*	0.500	0.294	0.167	0.296	0.206	0.269	0.283	0.354
*PEPD-2**	42	59	11	22	34	17	185	299
**100*	0.917	0.881	0.955	0.909	0.926	0.941	0.911	0.877
**107*	0.083	0.119	0.045	0.091	0.074	0.059	0.089	0.123
*PEPB-1**	45	60	13	23	34	17	192	398
**100*	0.489	0.450	0.462	0.587	0.338	0.412	0.453	0.533
**130*	0.511	0.550	0.538	0.413	0.662	0.588	0.547	0.467
**-350*	0.000	0.000	0.000	0.000	0.000	0.000	0.000 ($p<0.05$)	0.000
*PEP-LT**	42	57	12	22	34	14	181	299
**100*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972
**110*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028

Locus, allele and statistic	River Populations							
	Betsie River	Little Manistee	Manistee River	Muskegon River	Platte River	Pere Marquette	Lake Michigan	Green River
*sSOD-1**	46	60	13	23	35	17	194	397
**-100*	0.652	0.608	0.654	0.652	0.600	0.618	0.626	0.604
**-260*	0.348	0.342	0.269	0.283	0.314	0.353	0.327	0.354
**580*	0.000	0.050	0.077	0.065	0.086	0.029	0.046	0.042
Mean number alleles/locus	1.9	1.9	1.8	1.9	1.9	1.8	2.17	2.56
(S.E.)	.2	.2	.2	.2	.1	.2		
*Percent of loci polymorphic***	0.778	0.722	0.556	0.722	0.778	0.611	0.833	100
Heterozygosity direct count	0.213	0.200	0.216	0.195	0.209	0.170	0.205	-
standard error	0.051	0.054	0.057	0.051	0.050	0.044	0.049	-

***a locus was considered polymorphic i f more than one allele was detected*

'-'isoloci presented as two loci with allelic frequencies estimated using a maximum-likelihood approach reported by Waples (1989).

Table 4: Inbreeding coefficents and contingency table analysis of all loci across watersheds. F-statistics are described in Wright(1965, 1978), where F_{IS} is the fixation index of individuals as compared to their subpopulation, F_{IT} is the fixation index of individuals relative to the total population and F_{ST} measures differentiation among subpopulations as compared to the total population.

Locus	F_{IS}	F_{IT}	F_{ST}	G statistic	D.F.	p
*sAAT-2**	-0.094	-0.072	0.020	5.53	5	**
*mAAT-1**	0.027	0.082	0.057	8.26	10	**
*ADA-1**	-0.042	-0.026	0.015	6.39	5	**
*ADA-2**	-0.059	-0.035	0.023	7.30	5	**
*GPI-3**	-0.013	-0.004	0.009	3.11	5	**
*GR**	-0.020	-0.009	0.011	5.36	10	**
*sIDHp-3**	-0.101	-0.066	0.031	28.54	40	**
*sIDHp-4**	0.081	0.093	0.013	4.307	5	**
*sMDH-B2**	-0.040	-0.033	0.007	7.663	10	**
*sMEP-1**	0.144	0.172	0.032	13.11	10	**
*MPI-1**	0.158	0.162	0.006	2.879	5	**
*PGK-2**	-0.076	-0.018	0.054	6.876	5	**
*PEPD-2**	0.083	0.090	0.008	2.463	5	**
*PEPB-1**	-0.262	-0.233	0.023	7.73	5	**
*sSOD-1**	-0.137	-0.131	0.005	12.62	10	**
Mean	-0.034	-0.015	0.019	122.03	140	**
Mean without PEP-B1*	0.004	0.029	0.026	114.30	130	**

** not significant at the $p < 0.1$ level

Discussion

Panmixia and Hardy-Weinberg equilibrium

I concluded that chinook salmon in Lake Michigan constitute one randomly breeding population that is in Hardy-Weinberg equilibrium, based on several lines of evidence.

1. Fish from different tributaries showed similar levels of genetic diversity and similar allelic profiles. River-specific differences in these measures could be evidence of meaningful genetic differences between tributaries, if different tributaries show different profiles. For example, if one river was notably less diverse than others, this could be attributed to a bottleneck only in that population, to different mating conditions there than in the other populations, or to other conditions specific to that river. Similarly, if there was a heterogeneous distribution of the alleles at a particular locus across different watersheds, this could indicate non-random mating among watersheds and perhaps that watersheds should be treated as different genetic groups.

All possible evidence of subdivision in the Lake Michigan population, however, can be attributed to simpler causes. Although the Manistee and Muskegon Rivers had considerably fewer polymorphic loci than the others (Table 3), however this is probably because their small sample sizes limited the potential for occurrence of less frequent alleles at some loci (Table 3).

2. No loci within rivers deviated from Hardy-Weinberg expectations, indicating the consistency of one gene pool across rivers.

3. There was no unexplained deviation from Hardy-Weinberg predictions within the Lake Michigan population, indicating that this population is functionally one gene pool. Because the PEP-B1* locus is sex-linked, it violates an assumption of the Hardy-Weinberg

test and this explains the deviation from Hardy-Weinberg at this locus within the Lake Michigan population.

4. Because there was no significant heterogeneity at any loci even with a liberal p* value (0.1), the test for heterogeneity did not provide any evidence against panmixia.

5. Although a few F_{ST} values were large (arbitrarily defined as ≥ 0.03), there was no trend toward large values which would indicate substantial subpopulation structure.

6. If a population is subdivided, it should show allelic differences that would be reflected in genetic distance (D). Although there were small D values between a few of the watersheds, most showed no distance from each other (Figure 2; Appendix B). I concluded that the distances were not statistically rigorous evidence against the hypothesized panmictic Lake Michigan population for three reasons. 1. If several branches have distance lengths as small as 0.004, the phylogeny is probably incorrect (Nei 1987), as such small genetic distances can probably be explained by experimental error. 2. The Betsie River population showed alleles which are either absent or rare in the other river populations (Table 3), which explains the distance between the Betsie and the Little Manistee/Manistee cluster. 3. The Manistee population is based on a very small number of fish (13), and its distance from the other populations is probably due to its small size, which limits the potential for occurrence of alleles.

Impacts of genetic drift

I found evidence of genetic drift in the Lake Michigan chinook salmon population. The genetic diversity of Lake Michigan chinook salmon was consistently lower than that of Green River chinook salmon; 17% of the loci that were variable in Green River fish were monomorphic in Lake Michigan fish, and this population had lost nine alleles present in the

Green River population. In addition, there was genetic distance between Lake Michigan and Green River stocks. This distance is likely due both to the losses described above, as well as to the increased frequency of several alleles in the Lake Michigan population, and to the occurrence of two rare alleles at the sIDHp-1* locus which were not found in the Green River population. These alleles were apparently retained by chance in Michigan's gene pool, but did not occur in the Green River allozyme survey of the 1980's.

Causes of drift in Michigan's chinook salmon program

Breeding practices

If there is a limited amount of genetic material early in a population's history, less common alleles may be lost and generally depressed levels of genetic variability can result from a genetic bottleneck effect (Nei et al. 1975). The results I have described are consistent with such a bottleneck between Green River sources and the first Michigan chinook salmon introductions, which would have resulted in the loss of genetic variability. Because it was common practice in the late 1960's to harvest large lots of eggs over only one or two days, to pool milt, and to allow high variance in female reproductive success, allelic variability was probably lost through genetic drift due to such factors (e.g. Gall 1987). From 1989 on, an average 5.8:1 sex ratio has been used at the Little Manistee Weir (MDNR unpublished data).

Similarly, deriving the founding embryonic pool from a relatively small number of parents was probably another cause of genetic drift and loss of allelic diversity (e.g. Simon et al. 1986). The initial yearly harvest of one million Green River eggs destined for Michigan could have involved as few as 200 females if the average female produced 5000 eggs (a conservative estimate of female fecundity; Healey 1991), provided that the breeding operation was of sufficient capacity. Even if a 1:1 sex ratio were used, a maximum of 400

parents would have contributed to Michigan's embryonic pool for each of three years if the fecundity of both sexes was maximized. This would represent only 5% of the estimated total source river yearly brood run. Because of these circumstances, the embryos that founded Michigan's population were probably not wholly representative of the Green River population's breeding gene pool and thus a genetic bottleneck probably occurred during the stock transfers.

An additional breeding factor, variance in female reproductive success, was likely an important early cause of genetic drift. It is common practice in Michigan and other states to combine the fertilized eggs from multiple broods into common egg trays in the hatchery. When broods are pooled in this way, the reproductive success of individual broods cannot be determined and is presumed to be approximately equal; however, as there is great variance in brood success, all females used in breeding do not make an equal contribution to the resulting offspring pool. Such variance in female contribution is amplified by the extreme fecundity of most salmonids, because large numbers of individuals from a single brood can dominate even very large pools of stocked fingerlings. Ultimately, the genetic contribution of the less successful females is lost. If the described methods were implemented early in the Great Lakes chinook salmon program, the genetic variability of these founding stocks was likely compromised.

Population bottlenecks and founder effects

Early Great Lakes chinook salmon populations were probably derived from low numbers of surviving fingerlings, due to mortality both in the hatchery and after stocking. Losses of fry in the hatchery, and additional mortality of stocked fingerlings, meant that founding adult populations constituted a fraction of the original embryonic pool. Two

tributaries of Lake Michigan (the Little Manistee and Muskegon Rivers) were stocked with a total of 714,000 fingerlings yearly during each of the first three years of planting (1967-69) (Parsons 1973). Thus, about 300,000 of the original 1,000,000 embryos likely died before stocking each year. The Little Manistee River was stocked with an average of 380,000 chinook fingerlings yearly. Post-stocking mortality cut the number of returning spawning adults at the LMW to about 2100 per year from 1970 to 1977 (MDNR, unpublished records). Although more than twice as many fingerlings were stocked each year for the next three years (1970-72), the initial low numbers of stocked fingerlings probably created a genetic bottleneck. Since stock transfers ended in 1968, Michigan has collected nearly all of its eggs from a fraction of this spawning population. Milt pooling, egg-lot pooling, and restricted harvest dates (about 3 to 4 days of entire spawning run, which usually lasted 3-4 weeks) are currently part of the Michigan egg-take procedure (MDNR, unpublished data) and were likely also practiced early in the program. Thus, early chinook salmon populations likely experienced a decrease in allelic variability each year due to random genetic drift resulting from these breeding and husbandry practices. Feral spawning runs appeared in trout streams soon after initial stocking (Keller et al. 1990). Because these were likely founded by small numbers of individuals, there were likely similar, if not more pronounced, founder effects in these feral populations.

Evidence from other salmonid populations

The genetic circumstances of Michigan's stock transfer on current chinook salmon populations are consistent with those documented in several other studies of Pacific salmon.

Pink salmon (*Oncorhynchus gorbuscha*) were accidentally introduced into the Laurentian Great Lakes in 1956. Upon comparison to the source population, Gharrett and

Thomason (1987) determined that these salmon have lost an average of 0.3 alleles per locus since introduction. These fish are entirely feral, and thus culture practices have not contributed to this loss. Instead, this loss of variability is attributed to repeated bottlenecks resulting from limited founding sizes and limited survival of early colonists.

Chinook salmon were introduced into New Zealand from California's Sacramento river at the turn of the century. 300,000 to 500,000 embryos were transferred each year from 1904 to 1907, and these four embryonic pools constituted the founding populations for all current New Zealand chinook salmon populations (Quinn et al. 1996). Because these populations have been self-sustaining since introduction, unlike Michigan stocks, New Zealand populations have suffered little effects of drift due to artificial breeding or an extensive culturing system. Nonetheless, these populations show less genetic diversity than the presumed source stocks, probably due to an early bottleneck at the time of transfer and to very small founding populations in many new Zealand tributaries which were subsequently colonized (Quinn et al. 1996).

Genetic analysis of other Great Lakes salmonids has documented the importance of the hatchery system to the success of these populations.

Native lake trout (Salvelinus namaycush) populations in Lake Ontario have been on the decline since the 1950's due to sea lamprey (Petromyzon marinus) predation, overfishing and the decline of suitable habitat. Efforts to re-establish self-sustaining populations have included the stocking of a variety of hatchery strains. A genetic evaluation of wild-born fry from Stony Island reef demonstrated that 67-90% of these fry were descended from the Seneca strain, even though only a small proportion of the hatchery fry stocked in previous years were of this strain (Grewe et al. 1994). In contrast, other strains that were stocked in great abundance were poorly represented in the wild-born fry. The authors concluded that

such great variation in the success of hatchery-stocked strains, variation which was independent of stocking densities, was indicative of differences in the suitability of different strains for re-establishment of Lake Ontario lake trout populations, and that the relative genetic contribution of different strains should influence stocking priorities for these strains (Grewe et al. 1994). The importance of stocking policies to the success of Lake Ontario lake trout is similar to the prominent role that culturing and stocking practices have played in the gene pool of Lake Michigan chinook salmon.

Rainbow trout (Oncorhynchus mykiss) were first introduced into Lake Superior from drainages of the Pacific Ocean during the late 1880's, and widespread feral reproduction has resulted in the naturalization of this species throughout Lake Superior (Krueger et al. 1994). Two strains of rainbow trout have been stocked throughout Minnesota tributaries since the 1960's, and since this time, the angler effort required per fish caught has increased dramatically. The potentially detrimental effects of interbreeding between hatchery-stocked fish and their naturalized conspecifics are one potential cause of this change in the fishery (Krueger et al. 1994), as wild and stocked stocks could be adapted to different environmental conditions. Krueger et al. (1994) compared 1. the allozyme variation of trout from different Lake Superior tributaries in Minnesota, and 2. the variation in these tributaries to that of the hatchery strains stocked throughout the area. In general, trout in heavily stocked streams were genetically similar to the hatchery strains they were stocked with, indicating that the stocked fish may have interbred with the wild populations. There were genetic differences among tributaries; however, the extent of such differences may have been diluted by extensive stocking of a particular strain (the "Michigan" strain) of hatchery fish throughout the area (Krueger et al. 1994). The authors recommend that such stocking of this or other hatchery strains should be stopped if genetic differences between tributaries are to be

maximized. The dilution of feral differences between tributaries due to the introgression of hatchery strain fish is also a possible explanation for the lack of tributary differences between Lake Michigan chinook salmon; this evidence emphasizes the potentially great genetic impacts of hatchery and stocking policies on feral populations of salmonids in the Great Lakes.

Management recommendations

Lake Michigan's chinook salmon population shows the effects of genetic drift. This drift has resulted in the loss of the population's genetic variability and was likely caused by specific husbandry practices, by founder effects and by early bottlenecks. While founder effects from historic events cannot be directly mitigated, current causes of diversity loss should be identified and their impacts reduced. Husbandry practices which allow high variance in parental reproductive success are known to have deleterious genetic impacts, and these impacts should be guarded against in breeding programs. I recommend that every effort be made to increase the number of males contributing to breeding in order to equalize the sex ratio in the fertilization system. Even modest improvements can give genetic benefits, and may not appreciably decrease the efficiency of breeding operations. In addition, a one-time experiment is needed wherein individual broods remain isolated throughout the rearing program and their success is tracked in order to quantify the extent of variance in brood success. If this variance is high, the culling of broods with particularly high survival rates could be a practical and effective method of reducing this variance and thus increasing the number of broods contributing to the pool of fish eventually stocked.

There is both historic documentation, and genetic evidence, that the Green River was the source population for chinook salmon in the Great Lakes. WDFW records, which

account for the three million embryos thought to be transferred from 1966 to 1968, indicate that the Green River was the only source population. This is contrary to previous accounts of possible transfer(s) from other drainages such as the Toutle River, a Columbia River strain (Keller et al. 1990) believed to have been a major source of Michigan's chinook salmon by many authors (e.g. Keller et al. 1990). I ruled out this possible source through a genetic comparison of this stock to the Green River and Lake Michigan populations (Figure 3), which demonstrated that Lake Michigan chinook are closely related to the Green River population, but more distantly related to Toutle River chinook salmon (Appendix B).

The transfer of additional chinook from elsewhere to the Great Lakes in order to bolster genetic diversity would seem to be a possible response to the loss of genetic diversity in Lake Michigan's chinook salmon. However, such a transfer could in fact make the situation worse. If the transferred fish were different enough from Great Lakes chinook, the success of their offspring could be reduced due to the combination of incompatible gene complexes, or outbreeding depression. However, if such supplementation were ever to occur, it is clear that the new fish should come from the Green River, so that the compatibility of the introduced and established populations would be maximized.

Effective population size (N_e) is a population genetics parameter that is useful for estimating the expected extent of drift impacts on a particular population, and for predicting the genetic impacts of particular demographic factors, such as those described above. The application of genetic-based N_e equations, such as Waple's (1989) temporal method or linkage disequilibrium (e.g. Bartley et al. 1992), to the genetic data described in this thesis salmon population would be a robust way to quantify genetic drift effects. I suggest the implementation of a management plan which minimizes drift, maximizes genetic diversity, and allows for genetic monitoring (perhaps with N_e) in order to maintain the Lake Michigan

chinook salmon gene pool. The genetic trends I documented in this population are extremely relevant to other fish populations in the Great Lakes as well, especially those where management policies play a prominent role in a population's life history and persistence.

Conclusions of this study of a recently founded, closed chinook salmon population

1. Small, non-representative founding populations probably caused an early bottleneck which restricted the genetic information available to founding populations. Efforts should be made to ensure that robust numbers of founding individuals, which are representative of the genetic variability of the source population, be used in fisheries introductions.

2. Several breeding practices have likely eroded Michigan's gene pool since inception of the chinook salmon program, and continued use of these methods will undermine any efforts to retain or restore the genetic variability of this population. The effects of artificial propagation programs on introduced (and native) stocks should be carefully considered, and a breeding plan designed to reduce the effects of genetic drift should be a priority in management of this and other fish species in the Great Lakes.

3. Michigan's hatchery system may have eased early bottlenecks through the use of consistently large breeding populations. Hatcheries can facilitate the maintenance of variability through well-designed breeding and husbandry plans. The future genetic sustainability and success of this and other fish populations is still very much affected by management policy.

CHAPTER TWO

An Application of the "Variance" Effective Population Size Method

Introduction

Chinook salmon (Oncorhynchus tshawytscha) were first successfully introduced into the Laurentian Great Lakes in 1967 to improve the sport fishery and to control populations of the invasive alewife (Alosa pseudoharengus) (Michigan Department of Natural Resources [MDNR] 1974). Approximately one million fertilized eggs were shipped to Michigan from Washington's indigenous Green River chinook population for each of three years in the late 1960's (1966-68). In 1969, the first mature cohort was successfully spawned in captivity and Michigan has since been self-sufficient in chinook salmon egg production. Descendants of the three groups of transferred embryos were ultimately stocked throughout the Great Lakes by the MDNR and other state and federal agencies, and Great Lakes chinook salmon numbers have since been augmented by an artificial propagation program. The Great Lakes chinook salmon program has by many accounts been successful: by 1986, the standing stock in Lake Michigan alone approached 40 million pounds, and the lakewide harvest by recreational anglers approached 1 million pounds (over 600,000 fish; Francis 1996).

In 1988, however, large numbers of dead chinook salmon washed up on the eastern shores of Lake Michigan, and the number of adults migrating up tributaries to spawn declined precipitously (Johnson and Hnath 1991). These losses were attributed to bacterial kidney disease (BKD). Whether BKD was the only factor in these mortalities is still debated, but no other direct causes of death have been identified. The population has not fully

recovered from this crash; from 1989 to 1994, returns to rivers and sport harvest have remained at less than 50% of pre-1988 levels (MDNR, unpublished data).

Although Great Lakes chinook salmon populations are largely supported through artificial propagation, feral reproduction in Great Lakes tributaries is widespread and supports a significant portion of chinook salmon production. Feral fish may constitute 20-30% of chinook salmon production in eastern Lake Michigan alone (Carl 1982 and Hesse 1994). In fact, many Michigan streams that were never stocked now support runs of feral-origin adult chinook salmon, which indicates that chinook can stray and colonize rivers with suitable spawning conditions (Carl 1982). Thus, each year, returns are likely to be a mix of recent strays and feral fish (Carl 1982). Hesse (1994) surveyed adult vertebrae for oxytetracycline, a chemical mark applied to all stocked fish, and estimated that 39-54% (±5%) of the three-year-old chinook salmon returning to two major Lake Michigan tributaries in 1992-93 were not of direct hatchery origin. Hesse (1994) concluded that these represented feral-born fish, potentially from “naturalized” populations.

Despite the significant contribution of feral reproduction to Great Lakes chinook salmon populations, Michigan’s hatchery system continues to play a prominent role in management. Although highly variable, on average 4 million fingerlings (±454 S.E.) have been stocked into Lake Michigan from 1976 to 1987 [range 687,000 (1968) to 7.7 million (1984); MDNR unpublished data]. By some estimates, 70% of the age-0 chinook salmon in Lake Michigan were probably of immediate hatchery origin (Carl 1982, Hesse 1994).

Dramatic fluctuations in the chinook salmon population over the last decade have prompted closer examination of the dynamics of this population. In Chapter 1 of this thesis, I tested the hypothesis that chinook salmon in different watersheds have genetically diverged in the 30 years since introduction, but concluded that Lake Michigan chinook salmon are in

fact operationally one “genetic” population. I also determined, through comparison to the source population, that Lake Michigan chinook salmon have lost genetic variability since their introduction (Chapter 1). Genetic diversity is a useful index of the health and stability of populations; low levels of diversity within a population have been linked to reduced disease resistance (e.g. in rainbow trout, Ferguson and Drahushchak 1990), slower development, reduced size-at-age, higher mortality, and reduced fertility (e.g. Smith and Chesser 1981, Meffe and Carroll 1994). Furthermore, genetic variation within populations is a basic requirement for adaptation and the long-term persistence of populations in changing environments (Soulé 1980, 1987). Thus, the long-term persistence of Great Lakes chinook requires an understanding of the amount and structure of genetic variation in the population, in addition to an examination of the processes responsible for its present state.

The influence of artificial propagation on the genetic diversity of fish stocks has been well documented: propagated stocks tend to have lower levels of genetic variability than founder sources, as indicated by changed allele frequencies since the implementation of hatchery programs (e.g. Gharrett and Thomason 1987). The loss of allelic variability due to less heterozygous parents is possible solely as a consequence of genetic drift. A managed gene pool can be further compromised when it is subjected to concerted or inadvertent directional or stabilizing selection, such as selection against jack males, or selections for faster growing fish. The genetic diversity of Lake Michigan chinook salmon has been compromised by founder events and breeding and husbandry practices, and this could affect the long-term persistence of this population.

My primary objective was to estimate the extent of genetic drift and its effects on Lake Michigan chinook salmon. Effective population size (N_e) is a population genetics parameter used to estimate the potential impact of genetic drift on a population. When

compared to the actual number of breeders in a population (N_c), N_e is useful in detecting decreases in a population's genetic variability, or discrepancies between assumed and actual levels of allelic diversity. I used a variation of Waples' (1989) "variance method" as the basis for my estimate of N_e (Hedgecock et al. 1992). I estimated the variance between Lake Michigan and Green River chinook populations, the target population and its source population, and used this variance to estimate N_e and the effects of genetic drift. A similar approach was used by Hedgecock et al. (1992) for estimating the N_e of several captively bred shellfish and shrimp populations. Great Lakes managers need complete information in order to make informed decisions on issues that have genetic impacts; to this end, I used our effective population size estimates to make recommendations for effective management of the genetic diversity of chinook salmon and other Great Lakes salmonids.

Methods

The variance method measures changes in a population's allelic frequencies between two temporally distinct samples (S_o and S_t) to estimate N_e. Use of this method assumes that the alleles examined are selectively neutral and not subject to segregation, that mutation and migration are negligible, and that allele frequency estimates are unbiased. Because I did not have allele frequency data for Lake Michigan chinook salmon that was taken one or more generations apart, I substituted the frequencies from a sample taken from the founding population as time 0. This approach is valid when two additional assumptions are met. First, the allele frequency estimates from the surrogate time 0 population must accurately represent frequencies at the time of initial embryonic transfer (1966-1968). My second assumption was that differences in allele frequencies between the two groups are due to drift in the Green River population that occurred after the founding events.

I used the 1995 allelic data for Lake Michigan chinook salmon described in Chapter 1 as a sample at time t (S_t). I used data collected from the Green River to approximate the allele frequencies of Lake Michigan chinook at the time of introduction (S_o). These frequencies were based on a total of 400 fish (average 353 fish per locus) which were randomly collected, 100 each year, in 1981, 1987, 1988 and 1990 (Anne Marshall, personal communication [per. comm.]). The three later samples were of returning adult spawners, while the sample from 1981 was a sample of hatchery-reared juveniles from the 1980 brood year (Anne Marshall, per. comm.). I averaged all of the years together for the Green River allelic frequencies, thus these frequencies represent Green River fish during the 1980's. In my original survey of Lake Michigan chinook salmon (Chapter 1), I assayed loci that were variable in the Green River population in order to permit direct comparison between these two populations. I and collaborators conducted electrophoresis on the Lake Michigan samples in both the Michigan and WDFW laboratories, in order to ensure comparable allelic designations and genotypic interpretations of gel banding patterns.

Because the allele frequencies of the Green River Hatchery population were temporally stable over the four years sampled in the 1980's (Chapter 1), I concluded that the Green River population was probably stable between 1966 and 1985. Furthermore, I determined that the Green River population was probably the only source of Michigan's founding chinook salmon populations, because the genetic distance between the other purported source (the Toutle River, represented by its tributary the Cowlitz River) and Lake Michigan chinook salmon was quite large (Chapter 1).

I estimated N_e from Waples (1989):

$$F_k = [1/(K-1)] * [\Sigma[[(x_i - y_i)^2] / [(x_i + y_i) / 2]]$$

$$N_e = t / [\ 2[F_k - 1/(2\ S_o) - 1/(2\ S_t) + 1/N]\ ]$$

where F_k = the variance in allele frequency over t generations, S_o and S_t = the size of the sample taken at time 0 and time t respectively, t = the number of generations between S_o and S_t, N = the total breeding population size at the time of the initial sample, K = the number of segregating alleles and x_i and y_i = the allele frequencies of the Green River and Lake Michigan populations.

Although variable, the historical run on the Green River has averaged approximately 7,600 breeders (WDFW, 1993); therefore, I set the total source breeding population in the Green River (N) equal to 7,600. The Green River frequencies were based on an average of 353 fish per locus, so and I approximated S_o at 400. Because the number of Lake Michigan individuals sampled for each locus differed, I calculated the harmonic mean of all the sample sizes and weighted for the number of alleles in order to calculate S_t (Waples 1989). Similarly, because the number of segregating alleles (K) varied over loci, I calculated F_k for each locus and then calculated the weighted mean of the single-locus values (Waples 1989). The Lake Michigan data set included 23 alleles from 10 loci and was derived from an average of 218 fish (Chapter 1). The PEPB-1* and MPI* loci were excluded because they violated model assumptions (Chapter 1). All alleles which occurred at a frequency less than 0.02 in Lake Michigan or Washington were excluded to reduce bias due to rare allele effects (Waples 1989). I ultimately calculated F_k with 23 alleles.

Because chinook salmon life history includes overlapping generations, accurate estimation of t is difficult. As Lake Michigan spawners are mostly three and four years old

(MDNR unpublished data), I estimated that the average generation time for breeding Lake Michigan chinook salmon is 3.5 years. If I assumed that S_o is representative of a sample taken in 1967, $t \cong 8$, but under the assumption that S_o represents fish in 1985, $t \cong 3$. To evaluate the range of possible N_e values based on different assumptions, I calculated N_e based on $t \cong 3$ through 9. I also examined the effect of including and excluding juvenile fish (N = 28, Muskegon River) as a source of bias described by Waples (1989). Finally, I calculated the ratio of N_e to N_c, where N_c = 125,959, the average number of all 3 to 5 year old chinook salmon caught in Michigan waters of Lake Michigan, 1985-1994 (MDNR, unpublished records). I excluded the rare age-2 year class from N_c in order to avoid an underestimate of the N_e/N_c ratio which can result from an inflated N_c value.

Results

Estimates of Fk and N_e from variable t values and sample composition are summarized in Table 5. N_e estimates increased by a factor of 0.33 as t increased from 3 to 9. When juveniles were excluded, N_e estimates were 6-10% smaller and the mean Lake Michigan sample size across alleles (S_t) decreased from 218 to 196 (Table 5). N_e was lowest (139) when t = 3 and juveniles were included, and highest (425) when t = 9 and juveniles were excluded (Table 5). Under the most realistic conditions, where t = 8 and juveniles are excluded, N_e = 378.

Table 5: N_e as estimated with the variance method and as a proportion of N_c when t and sample composition are varied, where S_o = 353, t = the number of generations between samples, and juveniles were included or excluded.

			t													
			3		4		5		6		7		8		9	
Juveniles	F_k	Mean S_t	N_e	N_e/N_c	N_e	N_e/N_c	N_e	N_e/N_c	N_e	N_e/N_c	N_e	N_e/N_c	N_e	N_e/N_c	N_e	N_e/N_c
included	0.0148	218	140	0.001	186	0.001	233	0.002	280	0.002	326	0.003	373	0.003	420	0.003
excluded	0.0151	196	142	0.001	189	0.002	236	0.002	283	0.002	330	0.003	378	0.003	425	0.003

The ratio of N_e to N_c was never above 0.003, and was as low as 0.001. When t = 8 and juveniles were excluded, this ratio was 0.003 (Table 5). Thus, under the most realistic conditions, the effective breeding size of the Lake Michigan chinook salmon population was less than 1% of the estimated actual breeding population. In fact, any combination of t, S_o and age composition resulted in a N_e/N_c ratio less than 1% (Table 5).

Discussion

Lake Michigan's chinook population may be experiencing a greater amount of genetic drift than might be anticipated from such a demographically large population. Because the amount of drift is related to the effective population size, it is helpful to consider N_e rather than N_c when managing genetic diversity. I determined that there were an average of 374 effective breeding individuals in Lake Michigan per year according to the most realistic estimate, but that N_e could be as low as 139 depending on sample composition and various values of t and So. Because all of the t and So values included are historically possible, the effective breeding population size is probably much less than 1,000 individuals (or 500 breeding pairs), the minimum N_e required to maintain long-term genetic variation in an isolated population (Franklin 1980). I concluded that the effective size of Lake Michigan's chinook salmon population is, at best, only 42% of the minimum effective size recommended for long-term evolutionary stability, and at worst, well below the level required to overcome the effects of genetic drift on long-term allelic frequencies.

N_e/N_c of Lake Michigan chinook salmon

Despite a large-scale state breeding program which includes thousands of breeders per year, and despite substantial feral recruitment, the average effective breeding population

size of Lake Michigan's chinook salmon population since 1968 has been less than 1% of the estimated number of breeding adults, even under the most liberal conditions (See Table 5). Even if the estimates of N_c used are ten times too large, this ratio would still be less than 0.05, or 5% of the census size.

*Comparison to other N_e estimate*s

Salmonids

In order to put these N_e/N_c values into perspective, I compared them to ratios from several salmonid populations studied by Bartley et al. (1992) (Figure 4). These data were derived using the linkage disequilibrium estimate of N_e. As there are no published estimates of temporal N_e from populations of salmonids, I could not directly compare the estimate to one derived in an identical fashion from a similar species. Although the basis for and data used in these two N_e methods differ, both approaches rely on allozyme data, and both methods have been rigorously tested with theoretical models. In the absence of directly comparable temporal method N_e estimates, the linkage N_e estimates were useful for rough comparison of estimates.

"Hatchery born - Sacramento River" represents a heavily managed population (Figure 4). "Wild-born - Sacramento River" consists of wild-born fish from a historically wild population. Finally, "single-pair matings" is the N_e/N_c ratio based on the offspring of 17-20 single pair matings of rainbow trout (Oncorhynchus mykiss). The effectiveness of this breeding program is readily apparent, as the effective population size (38.5) is very close to the actual number of breeders used (N_c). In contrast, both the hatchery-bred and wild populations from the Sacramento River showed effective population sizes that were less than one tenth of the N_c value. The wild-born fish had a larger N_e/N_c ratio than those born in the

hatchery (0.044 vs. 0.013), although the difference was not statistically significant (Figure 4). This larger ratio could result from a more equal number of males and females breeding in the wild population, and/or less variance in the success of wild-born broods. "Hatchery and feral born - Lake Michigan" represents the N_e/N_c ratio of Lake Michigan chinook salmon as measured with the temporal method, where t = 8 and juveniles are excluded. This N_e/N_c ratio for Lake Michigan chinook salmon (0.003) is an order of magnitude smaller than that of even the hatchery-born fish from the Sacramento river.

N_e is much greater in breeding systems using only single-pair matings, such as that of the Shasta hatchery, than in populations where the genetic contributions of individuals are highly variable, such as in the hatchery-bred Sacramento river population (Figure 4; e.g. Simon et al. 1986 (large variance in family size) and Waples and Teel 1990 (modified sex ratio)). However, the N_e/N_c ratio of Lake Michigan's chinook salmon was less than one tenth of the ratio of hatchery-bred Sacramento river fish, a population which was propagated using similar breeding and rearing methodologies. This order of magnitude difference suggests that Lake Michigan chinook salmon have suffered sizable impacts of genetic drift as compared to their Sacramento River conspecifics.

Plants and Shellfish

A comparison to variance method N_e estimates for plant and shellfish species reveals even more dramatic differences. Hedgecock and Sly (1990) determined that the N_e/N_c ratio for two hatchery stocks of the Pacific oyster *(Crassostrea gigas*) ranged from 0.082 to 0.39. This ratio for populations of an annual plant, *Eichhornia paniculat*a, averaged 0.47 (Husband and Barrett 1992). While these species are taxonomically distant from salmonids, they share high fecundity with salmonids and thus are subject to similar genetic concerns, such as those

due to high variance in parental reproductive success (Hedgecock et al. 1992, Chapter 1). The fact that all three ratios are exponentially larger than that of Lake Michigan population supports the contention that the effective population size of Lake Michigan chinook salmon is very low.

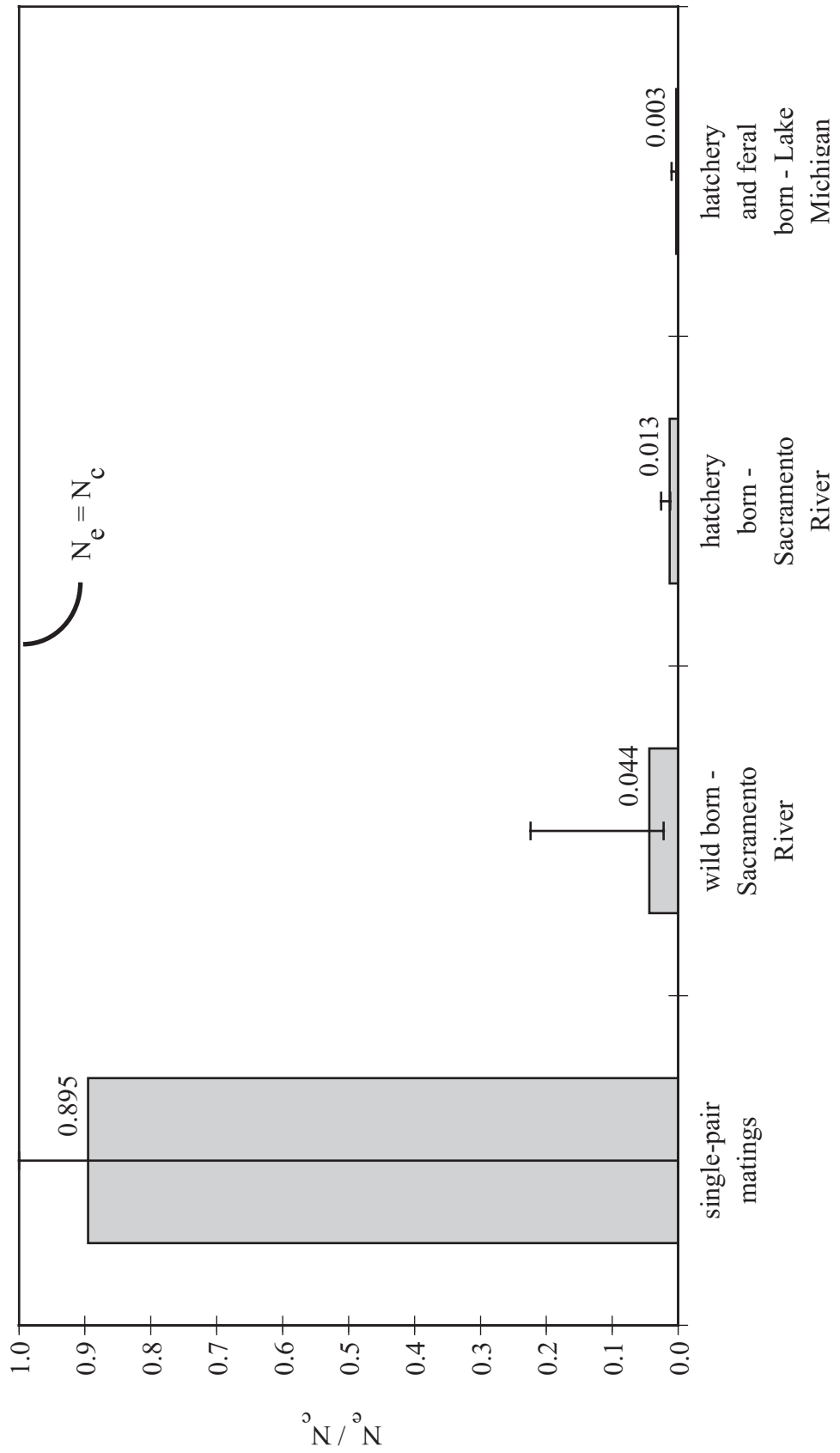

Figure 4: N_e/N_c and 95% confidence intervals for various salmonid populations, where N_e is computed with two genetic methods

Sources of potential bias

The N_c value I used is an estimate of the number of fish capable of breeding, and it is probably an overestimate because not all potential breeders actually breed. However, as this N_c includes only fish caught within Michigan waters of Lake Michigan, it underestimates the number of potential breeders. In addition, angling effort was probably not equal for all age classes and may be a source of bias. Overall, however, this is the best available estimate of the average number of breeding chinook salmon in Lake Michigan based on existing records.

My sample included a large number of individuals from six drainages. I determined that Lake Michigan chinook salmon had lost nine alleles that were present in the source population (Chapter 1); however, it is possible that the presence of these alleles went undetected due to sampling error. If this were the case, allelic differences between the temporal samples would be inflated, resulting in the underestimation of N_e. I ultimately concluded, however, that the absence of these alleles can be satisfactorily explained by genetic drift from several inferred population bottlenecks that occurred early in the chinook salmon program (Chapter 1). Even if one or two alleles were passed over in sampling, it is unlikely that the N_e values would change by an order of magnitude, and thus our conclusions would remain unchanged.

Conclusions

The effective population size of Lake Michigan chinook salmon was lower than recommended N_e values across all the variables I explored, indicating that the population is at high risk for loss of allelic variability. Lake Michigan chinook salmon have a particularly low effective population size (adjusted for census size) as compared to conspecific populations and other highly fecund plant and animal species, and are thus clearly subject to

detrimental drift effects. This low N_e could reflect historic bottlenecks early in the chinook salmon program, and it could also be indicative of genetic drift resulting from certain procedures used in Michigan's husbandry and culture protocol (Chapter 1).

Management recommendations

The current Lake Michigan chinook salmon breeding and rearing protocol is expected to decrease the genetic variability of Lake Michigan's chinook salmon, and I have demonstrated that this decrease has occurred (Chapter 1) and that genetic drift has caused this decrease. It is critical that managers consider the following recommendations (see Chapter 1 for additional background). First, the number of breeders used should remain consistently large, in order to avoid population bottlenecks and additional losses of uncommon alleles due to sampling error. Second, more males should be use on each breeding day, in order to equalize the breeding sex ratio. Finally, a study designed to quantify the extent of variance in female reproductive success should be carried out. All of these factors are expected to have particularly dramatic impacts on genetic variability, and the extent of such genetic drift effects should be explored in order to promote more effective genetic management.

APPENDIX A

Genotypic frequencies for variable loci, in individual watersheds and the Lake Michigan population.

POP	#	N	NAME
Pop	0	46	- Betsie River;
Pop	1	60	- Little Manistee;
Pop	2	13	- Big Manistee;
Pop	3	29	- Muskegon;
Pop	4	43	- Platte River;
Pop	5	22	- Pere Marquette;
Pop	6	213	- Lake Michigan;

Hardy Wienberg

pop 0 locus aat2
Expected value for smallest class was made equal to one

chi	-	1.210	df	-	1
G	-	1.577	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	33	11	0	0
expected	-	33.69	9.63	0.69	1.00

pop 0 locus maat1
Expected value for smallest class was made equal to one

chi	-	1.481	df	-	2			
G	-	1.645	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (22,23)	
observed	-	27	3	14	0	0	1	0
expected	-	28.01	2.37	12.62	0.05	0.53	1.42	1.00

pop 0 locus ada1
Expected value for smallest class was made equal to one

chi	-	1.008	df	-	1
G	-	0.182	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	42	4	0	0
expected	-	42.09	3.83	0.09	1.00

pop 0 locus ada2
Expected value for smallest class was made equal to one

chi	-	1.018	df	-	1
G	-	0.301	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	39	5	0	0
expected	-	39.14	4.72	0.14	1.00

pop 0 locus gr
Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1			
G	-	-0.011	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (13,33)	
observed	-	45	-	1	-	-	0	1
expected	-	45.01	-	0.99	-	-	0.01	1.00

pop 0 locus idh3

chi	-	0.019	df	-	1							
G	-	0.019	crVal	- 3.84								
genotype	-	11	12	13	14	15	16	17	18	19	22	23
	24	25	26	27	28	29	33	34	35	36	37	38
	39	44	45	46	47	48	49	55	56	57	58	59
	66	67	68	69	77	78	79	88	89	99 (19,88,89,99)		
observed	-	41	-	-	-	-	-	-	4	1	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	0	0	0	1	
expected	-	41.14	-	-	-	-	-	-	3.78	0.95	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	0.09	0.04	0.01	1.08	

pop 0 locus idh4

Expected value for smallest class was made equal to one

chi	-	1.003	df	-	1
G	-	0.101	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	43	3	0	0
expected	-	43.05	2.90	0.05	1.00

pop 0 locus mdh4

Expected value for smallest class was made equal to one

chi	-	1.003	df	-	1			
G	-	0.101	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	43	-	3	-	-	0	0
expected	-	43.05	-	2.90	-	-	0.05	1.00

pop 0 locus mdhpi

chi	-	0.730	df	-	2			
G	-	0.726	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (13,23,33)	
observed	-	11	16	0	10	2	0	2
expected	-	9.26	18.51	0.97	9.26	0.97	0.03	1.97

pop 0 locus mpi

chi	-	0.252	df	-	1
G	-	0.255	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	14	18	4	
expected	-	14.69	16.61	4.69	

pop 0 locus pgk2

chi	-	0.400	df	-	1
G	-	0.403	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	2	6	2	
expected	-	2.50	5.00	2.50	

pop 0 locus dpep1

Expected value for smallest class was made equal to one

chi	-	1.055	df	-	1
G	-	0.637	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	35	7	0	0
expected	-	35.29	6.42	0.29	1.00

pop 0 locus tapep1

chi	-	1.097	df	-	1
G	-	1.102	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	9	26	10	
expected	-	10.76	22.49	11.76	

pop 0 locus sod1

chi	-	0.080	df	-	1
G	-	0.079	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	20	20	6	
expected	-	19.57	20.87	5.57	

pop 1 locus aat2

Expected value for smallest class was made equal to one

chi	-	1.748	df	-	1
G	-	0.831	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	48	8	2	2
expected	-	46.62	10.76	0.62	1.00

pop 1 locus maat1

Expected value for smallest class was made equal to one

chi	-	0.079	df	-	2			
G	-	0.017	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (22,23)	
observed	-	36	4	15	0	1	2	1
expected	-	35.69	3.92	15.69	0.11	0.86	1.72	1.00

pop 1 locus ada1

Expected value for smallest class was made equal to one

chi	-	1.002	df	-	1
G	-	0.081	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	54	3	0	0
expected	-	54.04	2.92	0.04	1.00

pop 1 locus ada2

Expected value for smallest class was made equal to one

chi	-	1.029	df	-	1
G	-	0.458	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	50	7	0	0
expected	-	50.21	6.57	0.21	1.00

pop 1 locus gpi3

Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1
G	-	-0.009	crVal	- 3.84	
genotype	-	11	12	22 (12,22)	
observed	-	53	1	0	1
expected	-	53.00	0.99	0.00	1.00

pop 1 locus idh3

Expected value for smallest class was made equal to one

chi	-	1.079	df	-	1							
G	-	0.910	crVal	- 3.84								
genotype	-	11	12	13	14	15	16	17	18	22	23	24
	25	26	27	28	33	34	35	36	37	38	44	45
	46	47	48	55	56	57	58	66	67	68	77	78
	88 (88)											
observed	-	50	-	-	-	-	-	-	10	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	0	0										
expected	-	50.42	-	-	-	-	-	-	9.17	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	0.42	1.00										

pop 1 locus idh4
Expected value for smallest class was made equal to one

chi	-	0.917	df	-	1
G	-	-0.755	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	57	2	1	1
expected	-	56.07	3.87	0.07	1.00

pop 1 locus mdhpi
Expected value for smallest class was made equal to one

chi	-	0.066	df	-	2			
G	-	0.056	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (13,23,33)	
observed	-	11	24	0	15	1	0	1
expected	-	10.37	24.80	0.45	14.83	0.54	0.00	1.00

pop 1 locus mpi

chi	-	3.477	df	-	1
G	-	3.312	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	30	16	7	
expected	-	27.25	21.51	4.25	

pop 1 locus pgk2

chi	-	0.157	df	-	1
G	-	0.155	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	26	20	5	
expected	-	25.41	21.18	4.41	

pop 1 locus dpep1
Expected value for smallest class was made equal to one

chi	-	0.010	df	-	1
G	-	-0.329	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	46	12	1	1
expected	-	45.83	12.34	0.83	1.00

pop 1 locus tapep1 ** Deviation **

chi	- 18.072	df	-	1	
G	- 19.439	crVal	- 3.84		
genotype	-	11	12	22	
observed	-	4	46	10	
expected	-	12.15	29.70	18.15	

pop 1 locus sod1
Expected value for smallest class was made equal to one

chi	-	2.570	df	-	3			
G	-	1.873	crVal	- 7.81				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	21	28	3	5	3	0	0
expected	-	22.20	24.94	3.65	7.00	2.05	0.15	1.00

pop 2 locus aat2
Expected value for smallest class was made equal to one

chi	-	1.583	df	-	1
G	-	2.039	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	6	6	0	0
expected	-	6.75	4.50	0.75	1.00

pop 2 locus maat1
Expected value for smallest class was made equal to one

chi	-	0.179	df	-	2			
G	-	0.139	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (12,22,23)	
observed	-	6	0	4	0	1	1	1
expected	-	5.33	0.67	4.67	0.02	0.29	1.02	1.00

pop 2 locus idh3

chi	-	0.667	df	-	1							
G	-	0.652	crVal	- 3.84								
genotype	-	11	12	13	14	15	16	17	18	22	23	24
	25	26	27	28	33	34	35	36	37	38	44	45
	46	47	48	55	56	57	58	66	67	68	77	78
	88 (16,66,68,88)											
observed	-	6	-	-	-	-	1	-	5	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	0	-	0	-	-
	0	1										
expected	-	6.75	-	-	-	-	0.75	-	3.75	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	0.02	-	0.21	-	-
	0.52	1.50										

pop 2 locus mdh4

Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1			
G	-	-0.038	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (13,33)	
observed	-	12	-	1	-	-	0	1
expected	-	12.02	-	0.96	-	-	0.02	1.00

pop 2 locus mdhpi

chi	-	2.000	df	-	1			
G	-	3.244	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (11,13,33)	
observed	-	0	4	0	4	0	1	1
expected	-	0.44	2.67	0.44	4.00	1.33	0.11	1.00

pop 2 locus mpi

chi	-	1.160	df	-	1
G	-	1.146	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	5	3	2	
expected	-	4.23	4.55	1.23	

pop 2 locus pgk2

Expected value for smallest class was made equal to one

chi	-	0.003	df	-	1
G	-	-0.163	crVal	- 3.84	
genotype	-	11	12	22 (12,22)	
observed	-	2	1	0	1
expected	-	2.08	0.83	0.08	1.00

pop 2 locus dpep1

Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1
G	-	-0.045	crVal	- 3.84	
genotype	-	11	12	22 (12,22)	
observed	-	10	1	0	1
expected	-	10.02	0.95	0.02	1.00

pop 2 locus tapep1

chi	-	0.737	df	-	1
G	-	0.746	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	2	8	3	
expected	-	2.77	6.46	3.77	

pop 2 locus sod1

chi	-	0.770	df	-	1			
G	-	0.768	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (22,23,33)	
observed	-	5	6	1	0	1	0	1
expected	-	5.56	4.58	1.31	0.94	0.54	0.08	1.56

pop 3 locus aat2
Expected value for smallest class was made equal to one

chi	-	1.155	df	-	1
G	-	1.005	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	15	6	0	0
expected	-	15.43	5.14	0.43	1.00

pop 3 locus ada1
Expected value for smallest class was made equal to one

chi	-	1.005	df	-	1
G	-	0.105	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	18	2	0	0
expected	-	18.05	1.90	0.05	1.00

pop 3 locus ada2
Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1
G	-	-0.022	crVal	- 3.84	
genotype	-	11	12	22 (12,22)	
observed	-	22	1	0	1
expected	-	22.01	0.98	0.01	1.00

pop 3 locus ah
Expected value for smallest class was made equal to one

chi	-	1.042	df	-	1
G	-	0.422	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	17	4	0	0
expected	-	17.19	3.62	0.19	1.00

pop 3 locus gr
Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1			
G	-	-0.019	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (13,33)	
observed	-	26	-	1	-	-	0	1
expected	-	26.01	-	0.98	-	-	0.01	1.00

pop 3 locus idh3
Expected value for smallest class was made equal to one

chi	-	1.115	df	-	1							
G	-	0.860	crVal	- 3.84								
genotype	-	11	12	13	14	15	16	17	18	22	23	24
	25	26	27	28	33	34	35	36	37	38	44	45
	46	47	48	55	56	57	58	66	67	68	77	78
	88 (33,38,88)											
observed	-	18	-	2	-	-	-	-	4	-	-	-
	-	-	-	-	0	-	-	-	-	0	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	0	0										
expected	-	18.38	-	1.75	-	-	-	-	3.50	-	-	-
	-	-	-	-	0.04	-	-	-	-	0.17	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	0.17	1.00										

pop 3 locus idh4
Expected value for smallest class was made equal to one

chi	-	1.004	df	-	1
G	-	0.087	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	22	2	0	0
expected	-	22.04	1.92	0.04	1.00

pop 3 locus mdh4
Expected value for smallest class was made equal to one

chi	-	1.003	df	-	1			
G	-	0.074	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	26	-	2	-	-	0	0
expected	-	26.04	-	1.93	-	-	0.04	1.00

pop 3 locus mdhpi
Expected value for smallest class was made equal to one

chi	-	4.125	df	-	2			
G	-	4.661	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (13,33)	
observed	-	3	5	2	9	0	0	2
expected	-	2.22	7.87	0.68	6.96	1.21	0.05	1.00

pop 3 locus mpi

chi	-	0.277	df	-	1
G	-	0.272	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	11	9	3	
expected	-	10.45	10.11	2.45	

pop 3 locus pgk2

chi	-	0.117	df	-	1
G	-	0.119	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	13	12	2	
expected	-	13.37	11.26	2.37	

pop 3 locus dpep1
Expected value for smallest class was made equal to one

chi	-	1.038	df	-	1	
G	-	0.401	crVal	- 3.84		
genotype	-	11	12	22 (22)		
observed	-	18	4	0	0	
expected	-	18.18	3.64	0.18	1.00	

pop 3 locus tapep1

chi	-	0.631	df	-	1
G	-	0.640	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	7	13	3	
expected	-	7.92	11.15	3.92	

pop 3 locus sod1 ** Deviation **
Expected value for smallest class was made equal to one

chi	-	6.597	df	-	2			
G	-	8.992	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (23,33)	
observed	-	7	13	3	0	0	0	0
expected	-	9.78	8.48	1.96	1.84	0.85	0.10	1.00

pop 4 locus aat2

chi	-	0.402	df	-	1
G	-	0.376	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	23	10	2	
expected	-	22.40	11.20	1.40	

pop 4 locus maat1
Expected value for smallest class was made equal to one

chi	-	1.536	df	-	2			
G	-	0.986	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (22,23)	
observed	-	22	3	7	0	1	2	1
expected	-	20.83	3.09	9.26	0.11	0.69	1.03	1.00

pop 4 locus ada2
Expected value for smallest class was made equal to one

chi	-	1.016	df	-	1
G	-	0.258	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	29	4	0	0
expected	-	29.12	3.76	0.12	1.00

pop 4 locus gpi3
Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1
G	-	-0.015	crVal	- 3.84	
genotype	-	11	12	22 (12,22)	
observed	-	32	1	0	1
expected	-	32.01	0.98	0.01	1.00

pop 4 locus gr
Expected value for smallest class was made equal to one

chi	-	1.001	df	-	1			
G	-	0.050	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	39	-	2	-	-	0	0
expected	-	39.02	-	1.95	-	-	0.02	1.00

pop 4 locus idh3
Expected value for smallest class was made equal to one

chi	-	1.242	df	-	1							
G	-	1.620	crVal	- 3.84								
genotype	-	11	12	13	14	15	16	17	18	22	23	24
	25	26	27	28	33	34	35	36	37	38	44	45
	46	47	48	55	56	57	58	66	67	68	77	78
	88 (88)											
observed	-	26	-	-	-	-	-	-	10	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	0	0										
expected	-	26.69	-	-	-	-	-	-	8.61	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	0.69	1.00										

pop 4 locus idh4
Expected value for smallest class was made equal to one

chi	-	1.004	df	-	1
G	-	0.117	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	37	3	0	0
expected	-	37.06	2.89	0.06	1.00

pop 4 locus mdh4
Expected value for smallest class was made equal to one

chi	-	1.004	df	-	1			
G	-	0.108	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	40	-	3	-	-	0	0
expected	-	40.05	-	2.90	-	-	0.05	1.00

pop 4 locus mdhpi
Expected value for smallest class was made equal to one

chi	-	0.089	df	-	1
G	-	-0.308	crVal	- 3.84	
genotype	-	11	12	22 (11)	
observed	-	1	2	2	1
expected	-	0.80	2.40	1.80	1.00

pop 4 locus mpi

chi	-	2.139	df	-	1
G	-	2.142	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	13	11	7	
expected	-	11.04	14.92	5.04	

pop 4 locus pgk2

chi	-	0.214	df	-	1
G	-	0.229	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	21	12	1	
expected	-	21.44	11.12	1.44	

pop 4 locus dpep1

Expected value for smallest class was made equal to one

chi	-	1.030	df	-	1
G	-	0.397	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	29	5	0	0
expected	-	29.18	4.63	0.18	1.00

pop 4 locus tapep1 ** Deviation **

chi	-	8.882	df	-	1
G	- 12.330	crVal	- 3.84		
genotype	-	11	12	22	
observed	-	0	23	11	
expected	-	3.89	15.22	14.89	

pop 4 locus sod1

Expected value for smallest class was made equal to one

chi	-	2.647	df	-	3			
G	-	2.167	crVal	- 7.81				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	12	15	3	2	3	0	0
expected	-	12.60	13.20	3.60	3.46	1.89	0.26	1.00

pop 5 locus aat2

Expected value for smallest class was made equal to one

chi	-	1.077	df	-	1
G	-	0.573	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	12	4	0	0
expected	-	12.25	3.50	0.25	1.00

pop 5 locus maat1

Expected value for smallest class was made equal to one

chi	-	1.158	df	-	1			
G	-	0.931	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (22,23,33)	
observed	-	11	2	3	0	0	0	0
expected	-	11.39	1.69	2.53	0.06	0.19	0.14	1.00

pop 5 locus ada1

Expected value for smallest class was made equal to one

chi	-	0.000	df	-	1
G	-	-0.029	crVal	- 3.84	
genotype	-	11	12	22 (12,22)	
observed	-	16	1	0	1
expected	-	16.01	0.97	0.01	1.00

pop 5 locus idh3

chi	-	0.373	df	-	1							
G	-	0.363	crVal	- 3.84								
genotype	-	11	12	13	14	15	16	17	18	22	23	24
	25	26	27	28	33	34	35	36	37	38	44	45
	46	47	48	55	56	57	58	66	67	68	77	78
	88 (16,66,68,88)											
observed	-	13	-	-	-	-	1	-	4	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	0	-	0	-	-
	1	2										
expected	-	12.64	-	-	-	-	0.82	-	4.89	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	0.01	-	0.16	-	-
	0.47	1.46										

pop 5 locus mdh4

Expected value for smallest class was made equal to one

chi	-	1.005	df	-	1			
G	-	0.100	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	19	-	2	-	-	0	0
expected	-	19.05	-	1.90	-	-	0.05	1.00

pop 5 locus mdhpi

Expected value for smallest class was made equal to one

chi	-	0.123	df	-	1
G	-	-0.482	crVal	- 3.84	
genotype	-	11	12	22 (11)	
observed	-	1	3	5	1
expected	-	0.69	3.61	4.69	1.00

pop 5 locus mpi

chi	-	0.062	df	-	1
G	-	0.062	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	6	6	2	
expected	-	5.79	6.43	1.79	

pop 5 locus pgk2

Expected value for smallest class was made equal to one

chi	-	0.003	df	-	1
G	-	-0.112	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	7	5	1	1
expected	-	6.94	5.12	0.94	1.00

pop 5 locus dpep1

Expected value for smallest class was made equal to one

chi	-	1.941	df	-	1
G	-	1.940	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	16	0	1	1
expected	-	15.06	1.88	0.06	1.00

pop 5 locus tapep1

chi	-	0.014	df	-	1
G	-	0.014	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	3	8	6	
expected	-	2.88	8.24	5.88	

pop 5 locus sod1
Expected value for smallest class was made equal to one

chi	-	0.070	df	-	2			
G	-	0.040	crVal	- 5.99				
genotype	-	11	12	13	22	23	33 (13,23,33)	
observed	-	7	7	0	2	1	0	1
expected	-	6.49	7.41	0.62	2.12	0.35	0.01	1.00

pop 6 locus aat2

chi	-	0.018	df	-	1
G	-	0.018	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	137	45	4	
expected	-	136.78	45.45	3.78	

pop 6 locus maat1
Expected value for smallest class was made equal to one

chi	-	1.460	df	-	3			
G	-	1.106	crVal	- 7.81				
genotype	-	11	12	13	22	23	33 (22)	
observed	-	106	12	43	0	3	6	0
expected	-	104.84	11.78	45.55	0.33	2.56	4.95	1.00

pop 6 locus ada1
Expected value for smallest class was made equal to one

chi	-	1.008	df	-	1
G	-	0.278	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	175	10	0	0
expected	-	175.14	9.73	0.14	1.00

pop 6 locus ada2
Expected value for smallest class was made equal to one

chi	-	1.038	df	-	1
G	-	0.814	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	169	17	0	0
expected	-	169.39	16.22	0.39	1.00

pop 6 locus ah
Expected value for smallest class was made equal to one

chi	-	1.042	df	-	1
G	-	0.422	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	17	4	0	0
expected	-	17.19	3.62	0.19	1.00

pop 6 locus gpi3
Expected value for smallest class was made equal to one

chi	-	1.000	df	-	1
G	-	0.011	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	177	2	0	0
expected	-	177.01	1.99	0.01	1.00

pop 6 locus gr
Expected value for smallest class was made equal to one

chi	-	1.000	df	-	1			
G	-	0.039	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	205	-	4	-	-	0	0
expected	-	205.02	-	3.96	-	-	0.02	1.00

pop 6 locus idh3

chi	-	0.800	df	-	2							
G	-	0.905	crVal	- 5.99								
genotype	-	11	12	13	14	15	16	17	18	19	22	23
	24	25	26	27	28	29	33	34	35	36	37	38
	39	44	45	46	47	48	49	55	56	57	58	59
	66	67	68	69	77	78	79	88	89	99		
(19,33,36,38,39,66,68,69,89,99)												
observed	-	154	-	2	-	-	2	-	37	1	-	-
	-	-	-	-	-	-	0	-	-	0	-	0
	0	-	-	-	-	-	-	-	-	-	-	-
	0	-	0	0	-	-	-	1	0	0	1	
expected	-	155.46	-	1.78	-	-	1.78	-	34.64	0.89	-	-
	-	-	-	-	-	-	0.01	-	-	0.01	-	0.20
	0.01	-	-	-	-	-	-	-	-	-	-	-
	0.01	-	0.20	0.01	-	-	-	1.93	0.10	0.00	1.41	

pop 6 locus idh4

Expected value for smallest class was made equal to one

chi	-	0.236	df	-	1
G	-	-1.396	crVal	- 3.84	
genotype	-	11	12	22 (22)	
observed	-	191	10	1	1
expected	-	190.18	11.64	0.18	1.00

pop 6 locus mdh4

Expected value for smallest class was made equal to one

chi	-	1.008	df	-	1			
G	-	0.294	crVal	- 3.84				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	200	-	11	-	-	0	0
expected	-	200.14	-	10.71	-	-	0.14	1.00

pop 6 locus mdhpi

Expected value for smallest class was made equal to one

chi	-	2.486	df	-	3			
G	-	0.709	crVal	- 7.81				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	27	54	2	45	3	1	1
expected	-	22.92	61.25	2.92	40.93	3.90	0.09	1.00

pop 6 locus mpi ** Deviation **

chi	-	4.138	df	-	1
G	-	4.070	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	79	63	25	
expected	-	73.12	74.77	19.12	

pop 6 locus pgk2

chi	-	0.000	df	-	1
G	-	0.000	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	71	56	11	
expected	-	71.02	55.96	11.02	

pop 6 locus dpep1

chi	-	0.229	df	-	1
G	-	0.210	crVal	- 3.84	
genotype	-	11	12	22	
observed	-	154	29	2	
expected	-	153.47	30.06	1.47	

pop 6 locus tapep1 ** Deviation **

chi	- 17.641	df	-	1
G	- 18.015	crVal	- 3.84	
genotype	-	11	12	22
observed	-	25	124	43
expected	-	39.42	95.16	57.42

pop 6 locus sod1

Expected value for smallest class was made equal to one

chi	-	4.854	df	-	3			
G	-	4.754	crVal	- 7.81				
genotype	-	11	12	13	22	23	33 (33)	
observed	-	72	89	10	15	8	0	0
expected	-	76.09	79.54	11.27	20.78	5.89	0.42	1.00

APPENDIX B

Clustering levels of different populations plotted in Figures 2 and 3, respectively, based on Nei's (1978) unbiased genetic distance.

Figure 2:

Population or cluster numbers joined		Clustering level	Cycle
2	4	.00000	1
2	6	.00000	2
3	5	.00000	2
2	3	.00023	3
1	2	.00361	4

where 1 = Betsie River, 2 = Little Manistee River, 3 = Manistee River, 4 = Muskegon River, 5 = Platte River and 6 = Pere Marquette River.

Figure 3:

Population or cluster numbers joined		Clustering level	Cycle
2	3	.00072	1
1	2	.03046	2

where 1 = Cowlitz River, 2 = Green River, and 3 = Lake Michigan.

APPENDIX C

Allelic frequencies and deviation from Hardy-Weinberg equilibrium for individual watersheds and Lake Michigan pooled population.

Locus, allele and statistic	Swan River (*N*=44)	Michigan population (*N*=257)
*sAAT-1** -		
**100*	1.000	1.000
sAAT-2+ -		
**100*	0.917	0.868
**85*	0.083	0.132
mAAT-1+		
**-100*	0.849	0.798
**-77*	0.058	0.047
**-104*	0.093	0.155
ADA-1+		
**100*	0.966	0.972
**83*	0.034	0.028
ADA-2+		
**100*	0.989	0.961
**105*	0.011	0.039
AH-1+		
**100*	0.000	0.905
**86*	0.000	0.095
**112*	0.000	0.000
GPI-1		
**100*	1.000	1.000
GPI-2+		
**100*	1.000	1.000
**60*	0.000	0.000
GPI-3+		
**100*	1.000	0.995
**105*	0.000	0.005
**93*	0.000	0.000
GPI-H		
**100*	1.000	1.000
GR+		
**100*	0.977	0.988
**85*	0.000	0.000
**110*	0.023	0.012

IDH-3+		
**100*	0.864	0.884
**74*	0.011	0.006
**142*	0.000	0.000
**94*	0.000	0.004
**129*	0.114	0.102
**136*	0.011	0.004
IDH-4+		
**100*	0.932	0.963
**127*	0.057	0.035
**50*	0.011	0.002
LDH-3		
**100*	1.000	1.000
LDH-4		
**100*	1.000	1.000
LDH-5+		
**100*	1.000	1.000
**90*	0.000	0.000
MDH-1 -*		
**100*	1.000	1.000
MDH-2 -*		
**100*	1.000	1.000
MDH-3 -*		
**100*	1.000	1.000
MDH-4+ -		
**100*	0.943	0.969
**121*	0.000	0.000
**70*	0.057	0.031
MDHP-1+		
**100*	0.417	0.417
**92*	0.556	0.557
**105*	0.028	0.027
MDHP-2		
*100	1.000	1.000
MPI+		
**100*	0.629	0.656
**109*	0.371	0.344
**95*	0.000	0.000
		($p<0.05$)
PGK-2+		
**100*	0.697	0.713
**90*	0.303	0.287
PGM-1+		
**100*	1.000	1.000
**210*	0.000	0.000
PDPEP-2+		
**100*	0.849	0.899
**107*	0.151	0.101

TAPEP-1+		
**100*	0.295	0.424
**130*	0.705	0.576
**-350*	0.000	0.000
	(p<0.001)	(p<0.001)
PEP-LT+		
**100*	1.000	1.000
**110*	0.000	0.000
sSOD-1+		
**-100*	0.443	0.592
**-260*	0.500	0.359
**580*	0.057	0.048
mSOD		
**100*	1.000	1.000
Mean sample size per locus	40.5	220.8*
S.E.	1.7	8.1
Mean No. of alleles per locus	1.7	1.8
S.E.	0.2	0.2
*Percent of loci that were polymorphic***	46.7	53.3
Heterozygosity direct count	0.102	0.129
(standard error)	0.031	0.035
Heterozygosity expected	0.114	0.124
(standard error)	0.033	0.034

*** a locus was considered polymorphic i f more than one allele was detected.*
'-' isoloci presented as two loci with allelic frequencies estimated using a maximum-likelihood approach reported by Waples (1989).

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