STUDY PERFORMANCE REPORT

State: Michigan

Project No: F-80-R-1

Study No: 688

Title: Evaluation of alternative mechanisms underlying spatial genetic diversity of Lake Michigan steelhead: an assessment using molecular genetic markers.

Period Covered: October 1, 1999 to September 30, 2000

Study Objectives: (1) To quantify the degree of genetic variation among naturalized populations of steelhead from drainages of Lake Michigan, (2) to estimate the effective breeding population size of summer and winter run hatchery strains used in supplementation programs in the state of Michigan, (3) to estimate the effective population size of naturalized populations in Michigan drainages, (4) to correlate inter-population differences in gene frequency with population differences in life history and morphology, (5) to utilize genetic data from naturalized and hatchery populations to test and explain current spatial patterns of genetic differentiation and to make predictions of future trends in levels of genetic diversity, and (6) to assess the feasibility of using gene frequency data in Mixed Stock Analysis for open-water Lake Michigan steelhead sport fisheries.

Summary: Steelhead (*Oncorhynchus mykiss*) represent one of the first directed introductions of non-indigenous fish into the Great Lakes. Stocking began in the late 1800's, and present distribution and abundance is maintained by recruitment both from widely dispersed naturalized populations and supplemental releases of hatchery-reared offspring from several steelhead strains. Recent increases in stocking levels and greater survival of hatchery juveniles is believed to have shifted basin-wide recruitment from predominantly natural to predominantly hatchery origin. Given that hatchery strains are derived from phylogeographically distinct native West Coast steelhead stocks, and that naturalized populations may have diverged, molecular genetic markers may be useful in distinguishing strains and populations.

During fall 1998 and spring 1999 adult steelhead were obtained from all hatchery sources in the Lake Michigan basin and from spawning runs from tributaries from Michigan waters of Lake Michigan (see Table 1 for sample sizes and sources). DNA's were extracted from all adults and juveniles used in directed spawnings of Michigan strain sources of gametes from the Little Manistee River. Statistical analyses that will determine parentage of all offspring from each mating scheme are nearly completed. From estimates of male and female parentage, we will determine mean and variance of male and female reproductive success, effective population size and mean coancestries of progeny. Experimental crosses cover the range of gamete-take regimes employed by management agencies in the Great Lakes region. Analyses are ongoing and when completed will provide a basis for recommendations as to how spawning would be best conducted for all state anadromous species and strains.

Preliminary analyses of samples genetically assayed to date revealed significant differences in allele frequencies among six Lake Michigan fall-run populations and among the hatchery strains used by state agencies across the Lake Michigan Basin. Much of the interpopulation variation

could be attributed to differences between strains ('Skamania' strain vs 'Michigan' strain and naturalized populations, putatively of Michigan strain origin). The Skamania hatchery strain exhibited the lowest levels of genetic variability. Evidence of population structuring on micro-geographic scales (i.e., among tributaries within a single drainage) was observed for the Pere Marquette drainage, a non-stocked drainage whereas no evidence for within-drainage structuring was noted in other drainage systems. Management options regarding amounts and strains for supplementation and decisions on locations for hatchery releases would most prudently be based on fundamental knowledge of genetic relationships among strains and populations.

Job 1. Title: <u>Use molecular genetic markers to characterize gene frequencies of each of several naturalized spawning steelhead from tributaries across the Lake Michigan basin</u>.

Findings: Sampling of all wild and hatchery stocks has been completed as planned. During the spring of 2000, sampling was conducted in two additional streams (Betsie River and White River). These samples will be added to the steelhead, which were collected during fall 1998 and spring 1999 (Tables 1 and 2). Numbers reflect sampling from all 7 hatchery strains of either anadromous steelhead or resident rainbow trout currently stocked into Lake Michigan, as well as from representative populations from 17 sites in 12 drainages in the upper and lower peninsula. Sampling occurred throughout the fall and spring runs, and often across multiple tributaries within drainages. We also sampled from Eagle Lake and Shasta rainbow trout strains for comparison to strains of anadromous steelhead (samples sizes N>50 per strain).

Laboratory analyses were initiated using five bi-parentally inherited microsatellite loci. To date we have assayed fall-run samples from the Little Manistee River, Big Manistee River, Pere Marquette River, Platte River, and Muskegon River. Laboratory analyses of spring-run samples from the Pere Marquette and Big Manistee Rivers have been completed. Also completed were laboratory analyses for all hatchery strains including Skamania strain produced by Wisconsin and Indiana, Ganaraska and Chambers Creek strains produced by Wisconsin, and Michigan strain from the Little Manistee River (Table 3). Laboratory analysis of domestic rainbow from Eagle Lake and Shasta strains has also been completed. We also analyzed samples from the Pine River to ascertain whether genetic data could be used to determine whether these fish were a distinct resident rainbow strain or descendents of anadromous steelhead that had previously spawned (or were planted) above the dam in the lower reaches of the river

Genetic differentiation in the fall run was based on differences in allele frequencies across five microsatellite loci (F_{ST}=0.045, P< 0.001). Most of the variance in allele frequency could be attributed to differences between the hatchery strains (particularly Skamania and Ganaraska) and naturalized populations or populations putatively derived from Michigan strain hatchery steelhead. However, there was evidence for variation among the Michigan fall run populations (F_{ST}= 0.004, P<0.034). The Skamania hatchery strain had the lowest levels of genetic variability (H_O=0.52). All populations except the Pere Marquette were within Hardy-Weinberg equilibrium. Most likely, this was due to sampling of individuals originating from genetically heterogeneous spawning aggregations across the drainage (as seen in analysis of geographically separate spawning sites between tributaries of the Pere Marquette; data not shown).

Comparisons of gene frequencies across populations from stocked rivers to those of the common hatchery source (Little Manistee), suggest that hatchery supplementation can result in the homogenization of populations, even in the presence of substantial natural recruitment. If the goal of management is to maintain genetic diversity between populations, Skamania should not be introduced into Michigan streams, as variance between strains appears to account for a sizable portion of the genetic variation within the Lake Michigan basin.

The deviation of genotypic frequencies from Hardy-Weinberg for the Pere Marquette population is of interest because this population represents the only unstocked drainage with a large naturalized population, which spawns in geographically widely dispersed areas. Evidence for spatial population structuring at such micro-geographic scales suggests that spatial genetic structure can accrue within a single stream system.

Eagle Lake and Shasta strains of rainbow trout were genetically quite distinct from any anadromous strain of steelhead due to the presence of numerous alleles, which were found exclusively in either steelhead or rainbow trout. For alleles found in rainbow trout and steelhead, we observed large and significant differences in allele frequency. Genetic differences among hatchery strain, and between steelhead and rainbow trout can be used for several management-related purposes. Firstly, we were able to determine that fish from the Pine River were steelhead and not rainbow trout. This has implications for decisions as to whether the strain is used for hatchery production. Secondly, differences among strains would allow analyses of individual fish or groups of fish to determine strain of origin. In absence of coded wire tags or fin clips we were able to assign strain of origin with >95 accuracy to many of the strains.

Job 2. Title: <u>Determine whether the degree of population differentiation in genetic characteristics can be correlated to population differences in juvenile life history and to morphology</u>.

Findings: Sampling of juvenile steelhead and of spawning adults for morphometric analysis was carried out independently (for juveniles) and concurrent (for adults) with collections for genetic samples. Analyses of drainage- and site-specific differences in morphology will be carried out independently by researchers at the University of Michigan. Data on genetics and morphology will be combined to facilitate comparisons dictated by this job later in the project.

Job 3. Title: <u>Determine the effective population size of steelhead spawning in each of several drainages of Lake Michigan.</u>

Findings: All adult and juvenile samples have been analyzed for microsatellite loci for six treatments representing a range of gamete-taken strategies.

Treatment 1 – 1 female mated to 1 male
Treatment 2 – 1 female mated to 2 males where both males were used for each of 2 females
Treatment 3 – eggs from 5 females mixed with mixed milt from 5 males
Treatment 4 – eggs from 10 females mixed with mixed milt from 10 males
Treatment 5 – eggs from 1 female mixed with mixed milt from 2 males
Treatment 6 – eggs from 1 female mixed with milt from 2 males added sequentially

Ten males and ten females were used for the first four treatments. Ten females and 20 males were used for treatments 5 and 6. We standardized the amount of eggs used per female (50mls) and the volume of milt used (1 ml) per male. Milt and eggs were mixed and eggs were allowed to water harden. Eggs were incubated until emergence at the Wolf Lake Hatchery. We genotyped all adults and 160-194 progeny from each cross (Table 4). We assigned male and female parentage to all offspring. We determined mean and variance in male and female reproductive success. Based on parentage information we were able to assign a mean level of genetic relatedness across all individuals (mean coancestry). Further, we were able to estimate effective population size under each mating strategy. Male and female reproductive variance was much higher in Treatments 2, 3, and 4 presumably due to differences in male gamete quality or sperm penetrance. Effective population sizes for Treatments 2, 3, and 4 also were lower and mean coancestries were higher.

Job 4. Title: Estimate the effective breeding population sizes for Summer and Winter Hatchery stocks, and estimate short- and long-term trends of genetic diversity.

Findings: see results under Job 3. Based on past and present state mating strategies we will formulate projections for overall levels of diversity and potential for changes given estimates of total numbers of males and females spawned and stocked.

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Table 1.–Sample sizes of adult steelhead collected during fall and spring portions of spawning runs from Lake Michigan drainages.

Location	Fall 1998 (N)	Spring 1999 (N)
Muskegon		
Mainstem	37*	24
Bigelow Creek		33
Pere Marquette		
Mainstem	30*	
Baldwin River		25*
Middle Branch		25*
Little South Branch		25*
Little Manistee	60*	60
Big Manistee		
Mainstem	60*	27*
Bear Creek		60*
Platte	62*	60

Table 2.–Sample sizes of adult steelhead collected from drainages that extend geographic coverage to streams with naturalized populations.

	Season Sampled	Number
Black River (UP)	Spring 1999	55
Thompson Creek (UP)	Spring 1999	44
Menominee (UP)	Spring 1999	10
Little Garlic (Lk. Superior)	Spring 1999	44
St. Joseph	Spring 1999	16
Betsie River	Spring 2000	34
White River	Spring 2000	30

Table 3.–Sample sizes of adult steelhead from each of 5 hatchery sources. Strains constitute the breadth of strain diversity for anadromous steelhead stocked into Lake Michigan by state agencies.

	Season Sampled	**Number**
IN Skamania	Fall 1998	60*
WI Skamania	Spring 1999	60*
WI Chambers Creek	Spring 1999	60*
WI Ganaraska	Spring 1999	60*
MI Little Manistee	Fall ‘98/Spring ‘99	60*/60

Table 4.–Sample sizes of steelhead adult and progeny used in experimental treatments to examine the effects of spawning practices on variance in male and female reproductive success.

Parents	Number
Females	10*
Males	20*
Progeny	
Treatment 1	194*
Treatment 2	178*
Treatment 3	162*
Treatment 4	171*
Treatment 5	181*
Treatment 6	189*

* Indicates population/group genotyped.