STUDY PERFORMANCE REPORT

State: <u>Michigan</u>   **Project No.:** <u>F-35-R-24</u>

Study No.: <u>688</u>   **Title:** <u>Evaluation of alternative mechanisms underlying spatial genetic diversity of Lake Michigan steelhead: an assessment using molecular genetic markers</u>

Period Covered: <u>April 1, 1998 to September 30, 1999</u>

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, (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). Directed spawnings were made using Michigan strain sources of gametes from the Little Manistee weir during the spring of 1999 in order to establish experimental crosses covering the range of gamete-take regimes employed by management agencies in the Great Lakes region.

Preliminary analyses of samples genetically assayed to date revealed significant differences in allele frequencies among six Lake Michigan fall run populations. which comprise a portion of the fall run (mean over 5 loci $F_{ST}=0.045$; P<0.001). 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) were observed within 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: **Use molecular genetic markers to characterize gene frequencies of each of several naturalized spawning steelhead from tributaries across the Lake Michigan basin.**

Findings: 1857 adult steelhead were collected during fall 1998 and spring 1999 (Table 1). Numbers reflect sampling from all 7 hatchery strains of either anadromous steelhead or resident rainbow trout currently stocked into Lake Michigan as well as representative populations from 11 drainages in the upper and lower peninsula. Sampling occurred throughout the fall and spring runs, and often across multiple tributaries within drainages.

Laboratory analyses was 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. Also sampled was the Skamania strain which were produced by Wisconsin. Preliminary results for a single microsatellite locus is presented in Table 2.

Genetic differentiation was observed in the fall run, based on differences in allele frequencies across five microsatellite loci (F_{ST}=0.045, P< 0.001; Table 3). Most of the variance in allele frequency could be attributed to differences between the hatchery Skamania strain and naturalized populations or populations putatively derived from Michigan strain hatchery steelhead (Table 3). However, there was also evidence for variation among the Michigan fall run populations (F_{ST}= 0.004, P<0.034; Table 4). The Skamania hatchery strain had the lowest levels of genetic variability (H_O=0.52; Table 3). All populations except the Pere Marquette were within Hardy-Weinberg equilibrium (Table 3). 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), suggested 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 appeared 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 occur within a single stream system.

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

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: The statistical methodology underlying estimation of effective population size calls for sampling of adults and juveniles or of adults from each of several generations. We completed the sampling of adults for each of several tributaries this past year. Allele frequencies will be determined in the context of analyses for Job 1. Subsequent analyses will be carried out on subsequent year's samples to facilitate these analyses.

Job 4. Title: <u>Estimate the effective breeding populations size of Summer and Winter Hatchery stocks and estimate short- and long-term trends of genetic diversity.</u>

Findings: Crosses were made at the Little Manistee weir during gamete takes this past spring. We conducted experimental crosses (2 replicate experiments) for 6 distinct "treatments" which represent each of several spawning strategies employed across the Great Lakes region for salmonids. Treatments included 1) 1:1 sex ratio of males to females, 2) 2 males per female (using sequential mating) , 3) mixing gametes from 5 males and 5 females, 4) mixing gametes from 10 males and 10 females, 5) 2 males per female where the milt is pooled prior to fertilization, and 6) 2 males per female where gametes from both males are used to fertilize the eggs of 2 females. We will genotype offspring and assign parentage for each treatment. From these estimates we will determine variance in male and female reproductive success and hence effective breeding population size. Based on these numbers and the gamete-take protocols used by Michigan and Indiana for Michigan strain and Skamania strain, respectively, we will estimate the effective population size of each cohort produced through hatchery activities.

Prepared by: <u>Kim T. Scribner and Meredith Bartron</u>
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Table 1.–Inventory of river and hatchery steelhead samples collected from fall and spring run (1998-1999). The hatchery samples also included the two resident rainbow trout strains stocked by Michigan.

River	Run	Number	Collection Method
Au Sable			
Mainstem	Spring	18	Creel
Big Manistee			
Mainstem[1]	Fall	74	Creel
Mainstem[2]	Spring	77	Creel
Bear Creek	Spring	87	Electroshocking
Black River (UP)			
Mainstem	Spring	55	Electroshocking
Little Garlic (UP)			
Mainstem	Spring	44	Electroshocking/Creel
Little Manistee			
Weir	Fall	200	Weir
Weir	Spring	200	Weir
Menominee (UP)	Spring	11	Creel
Muskegon			
Mainstem	Fall	35	Creel
Mainstem	Spring	23	Creel
Bigelow Cr.	Spring	33	Electroshocking
Pere Marquette			
Mainstem	Fall	35	Creel
Lt. South Br.	Spring	29	Electroshocking
Middle Br.	Spring	47	Electroshocking
Baldwin R.	Spring	37	Electroshocking
Platte			
Mainstem[3]	Fall	129	Weir
Mainstem	Spring	100	Electroshocking
St. Joseph			
Mainstem	Fall	41	Weir
Tribs.	Spring	16	Electroshocking
Thompson Creek			
Mainstem	Spring	44	Electroshocking
Hatchery Strain			
Wisconsin			
Skamania		62	
Chambers Creek		100	
Ganaraska		100	
Indiana			
Skamania		60	
Michigan			
Little Manistee		see Little Manistee above	
Eagle Lake (RBT)		100	
Shasta (RBT)		100	

[1] Plus 26 clipped fish
[2] Plus 2 clipped fish
[3] Plus 21 clipped fish

Table 2.–Allele frequencies at locus Oneμ10 for five naturalized populations and one hatchery strain which contribute to fall run steelhead in Lake Michigan.

Allele (bp)	LMNF (n=60)	BMNF (n=59)	PMRF (n=32)	PLTF (n=60)	MUSF (n=37)	WISK (n=49)
			Population[1]			
125	.000	.042	.016	.000	.014	.122
127	.175	.203	.219	.150	.135	.143
129	.425	.492	.516	.425	.446	.735
131	.350	.203	.203	.350	.338	.000
133	.033	.025	.016	.050	.027	.000
137	.008	.000	.000	.000	.000	.000

[1] Populations are as follows: Little Manistee (LMNF), Big Manistee (BMNF), Pere Marquette (PMRF), Platte (PLTF), Muskegon (MUSF), and Wisconsin Skamania (WISK).

Table 3.–Measures of genetic diversity across five microsatellite loci for five naturalized populations and one hatchery strain which contribute to fall run steelhead in Lake Michigan.

Variable	LMNF	BMNF	PMRF	PLTF	MUSF	WISK
			Population[1]			
H_E[2]	0.679	0.673	0.668	0.678	0.682	0.530
H_{DC}[3]	0.670	0.657	0.600	0.672	0.636	0.522
Mean no. alleles/locus	7.400	8.000	8.000	7.200	6.800	4.800
Hardy-Weinberg[4] (Prob<)	0.205	0.148	0.001	0.819	0.431	0.401

[1] Populations are as follows: Little Manistee (LMNF), Big Manistee (BMNF), Pere Marquette (PMRF), Platte (PLTF), Muskegon (MUSF), and Wisconsin Skamania (WISK).
[2] Expected heterozygosity under Hardy-Weinberg.
[3] Direct count heterozygosity.
[4] Results from Fisher Exact Test over all loci.

Table 4.–Analysis of genetic diversity for Lake Michigan fall-run steelhead.

locus	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}
	All populations			Michigan populations only		
Ogo1a	.081	.066*	.016	.030	.000	.031
Ogo4	.092	.044*	.051	.064	.044*	.054
Omy77	.087	.051*	.038	.050	.006*	.045*
Oneμ10	.056	.047*	.010	.026	.004	.023
Oneμ11	.041	.045	.043	.000	.000	.000
mean	.075	.045*	.032*	.038	.004*	.034*

* p<0.05