

## STUDY PERFORMANCE REPORT

Project No: F-81-R-4

State: Michigan

Title: Effects of exploitation and fisheries management on genetic diversity of fish stocks in inland and Great Lakes waters of Michigan.

Study No: 723

Period Covered: October 1, 2002 to September 30, 2003

**Study Objectives:** (1) To use molecular markers and population genetics theory to investigate genetic stock relationships and status of indigenous fish species of importance in Michigan, (2) to estimate the effects of past and proposed management on population and meta-population levels of genetic diversity.

**Summary:** Research is ongoing in each of several topical areas, all related to historical and contemporary factors affecting the genetic diversity of fisheries in Michigan waters. Studies involve numerous native species of economic importance that are intensively managed. This project seeks to provide information to better understand how recent management practices have impacted the genetic diversity of Michigan's fisheries. Review of available literature conclusively shows that historically, Michigan's fisheries were composed of taxonomically diverse fish assemblages. Most species were also genetically quite diverse as the result of admixture events whereby fishes that were geographically isolated into glacial refugia mixed following the retreat of glacial ice. All available literature suggests that humans have left an indelible and mostly negative mark on the genetic architecture on Michigan's fishes. Management efforts have contributed to community and species declines in genetic diversity. Each job addresses issues of importance to fish species in Michigan.

For Lake Sturgeon in Black Lake, we found evidence that previous management efforts to supplement the Black Lake population resulted in high levels of relatedness among progeny stocked. Individuals stocked during the 1980s will shortly make up approximately 50% of the adult spawning population, which possess a high risk of future inbreeding.

Walleye populations in Great Lakes waters of Michigan have recovered numerically, principally through extensive stocking efforts that continue today. We found evidence that past indiscriminant stocking without regard to basin of origin resulted in genetic homogenization of walleye stocks across formerly genetically structured populations. We found evidence for the existence of remnant stocks in impounded upstream areas.

Spawning populations of steelhead are increasingly composed of hatchery strains, including those produced by other agencies across the Lake Michigan basin. We've documented that introgression of hatchery strains has already occurred in the tributaries examined.

Coaster brook trout restoration is a primary goal in Lake Superior. We've found no evidence for introgression of hatchery brook trout into 6 Upper Peninsula tributaries to Lake Superior. Preliminary data suggests that migratory and resident brook trout are reproductively isolated.

Populations of northern muskellunge have been maintained in inland Michigan waters primarily through supplementation. Interest is increasing in the use of Great Lakes muskellunge.

Preliminary analyses suggest that northern and Great Lakes muskellunge are highly differentiated genetically. Introgression of northern pike and northern muskellunge has occurred.

**Findings:** Jobs 1 through 7 were scheduled for 2002-03, and progress is reported below.

**Job 1. Title: Review literature and unpublished studies on Great Lakes and inland fish genetics, population and community dynamics, regional hatchery practices, stocking levels and locations and rates of exploitations that relate to levels and partitioning of genetic variation for fisheries in Michigan waters. Synthesize literature for use in ongoing genetic assessment activities.**—Over the past year extensive literature searches have been conducted in each area outlined in Job 1. Below is a brief summary of findings.

Historical biogeographic events have played important roles in the development of fish species assemblages in aquatic communities and in shaping contemporary intraspecific genetic structure in waters in and surrounding Michigan. Pleistocene glaciations were arguable the most significant historical events to have occurred within the evolutionary life span of most extant species of freshwater fishes in north-central North America, including Michigan. Large areas of North America were repeatedly covered by massive amounts of glacial ice representing expansive extirpation and recolonization opportunities for fishes during up to 20 glacial episodes spanning up to 100,000 years separated by 10,000-12,000 inter-glacial periods. The last (Wisconsinan) glacial episode in North America reached areas of maximum coverage 18,000-23,000 years ago with major deglacial warming occurring between 8,000-15,000 years ago.

#### *Inferences from species distributional patterns*

Many northern (Palearctic) fish species have geographic distributions that are more wide-spread than are species from nonglaciated areas, likely due to opportunities for postglacial dispersal provided by proglacial lakes. Glacial impacts on fish communities as seen through reduced species numbers and low incidence of endemism coincide with limits of maximal glacial advance. Patterns of species diversity may be explained more effectively by a model of extinction and recolonization and admixture in glaciated or climatically stressed areas than from an equilibrium between speciation and extinction such as might be expected from areas characterized by long-term climatic stability.

Newly accessible habitats created and colonized by freshwater fishes following glacial periods are also believed to have provided ecological diversity that prompted in adaptive morphological, ecological, and behavioral differentiation in colonizing species. Fishes inhabiting more southern environments have had proportionally fewer opportunities for similar levels of diversification, presumably because due to greater temporal consistency of environmental regimes.

High levels of faunal diversity in Michigan and adjoining areas of the upper Great Lakes are believed to have been due to historical post-glacial inter-connections with up to four glacial refugia. The large proglacial Lake Agassiz provided dispersal routes from proposed Beringian and Missouri refugia. Colonization of habitats in the Michigan area from the Mississippi refugia was likely facilitated via Lake Maumee in western Lake Erie basin via the Wabash and Ohio Rivers and from Lake Chicago in the Lake Michigan basin via the Illinois River. Michigan and the upper Great Lakes basin were historically connected to the proposed Atlantic refugia via the Susquehanna and Hudson Rivers.

#### *Inferences from genetic characterizations*

Comparative phylogeographic analyses can be used to assess the relative contributions of historical events and demographic process in shaping levels of genetic diversity and how it is

spatially distributed in nature. Significant latitudinal variation in phylogeographic patterns suggest that the genetic diversity of Palearctic freshwater fish species was affected by Pleistocene glaciations. Depending on a species evolutionary effective population size, some species may show genetic structuring where allopatric groups are reciprocally monophyletic (i.e., exhibit fixed genetic differences in populations from different regions of the species distribution). However, for species that were separated into multiple refugia but for comparatively short periods of time, this may not be the case. Vicariant events may not have isolated populations for sufficient periods for fixed differences to accrue.

Two major patterns emerge from examination of data from across multiple co-distributed temperate (cold-tolerant) fish species. Low levels of genetic diversity are commonly observed within populations currently inhabiting glaciated areas, presumably due to habitat loss during glacial advances. Glacial epics are believed to have isolated northern temperate populations of fish and other aquatic organisms into geographically isolated refugia. Diversity of northern populations is consistently lower than observed for congeners in more southerly locales suggesting that isolation was accompanied by low effective population size, drift and loss of genetic variation. There is a trend across 42 species for reduced mtDNA nucleotide diversity with increasing latitude, largely coincident with the median latitude of maximum Pleistocene glacial advance in North America.

Mitochondrial (mt)DNA is often used to examine patterns in intra-specific genetic variation. mtDNA diversity is commonly found to be divided into phylogeographically distinct groups suggestive of separate refugia of origin. However, fish species inhabiting northern latitudes show less intra-specific divergence and appear “younger” evolutionarily than species from non-glaciated regions. Populations in each refugium evolved over thousands of years in isolation from congeners in other refugia resulting in the development of genetically distinct intraspecific groups. When continental ice sheets melted, large proglacial rivers and lakes provided opportunities for fishes to mix and to recolonize vast areas. Degree of admixture and dispersal was likely based on the length of time aquatic connections were available between locales, the types of habitats and differential permeability of habitats to dispersal by species with different ecological requirements.

Ecological differences among taxa suggest that fish species will likely differ markedly in phylogenetic patterns though they may exhibit concordant spatial distributions. Dispersal capabilities of different species were likely sufficiently different to make proglacial connections from different refugia differentially permeable to migration. Phylogeographic signatures provide evidence of multiple species having survived in multiple glacial refugia, although the relative contributions of different refugia to contemporary populations varies considerably among taxa.

*Specific case studies of species found in Michigan and adjacent waters*

Lake whitefish *Coregonus clupeaformis* from across the species range are believed to have originated from 3 major clades with significant divergence within one of the three clades. Based in the geographic distributions of the clades across the 41 surveyed populations, each clade could be putatively placed to a single glacial refuge. Two clades were believed to be associated with the Beringian refuge in Alaska and northwest Canada. The most abundance and wide-spread clade was believed to have radiated from the Mississippian refuge. Findings of wide dispersion for this clade corroborated previous dispersal predictions based on multi-species distributions. The final group was believed to have originated in the Atlantic refugia.

Phylogeographic structuring has been investigated in lake trout *Salvelinus namaycush* from 61 locales throughout the species range in North America. The dispersion of 21 haplotypes

comprising 3 major clades was consistent with origins within 3 major glacial refugia (Beringian, Mississippian, and Atlantic). However, in contrast to data from lake whitefish, mtDNA data suggest that dispersal from the Mississippian refuge was more extensive, allowing fish to recolonize large portions of the species' contemporary range. Contact between phylogenetic groups of lake trout was implicated as being responsible for regional variation in levels of genetic diversity. Locations of areas with high levels of genetic diversity coincide with areas formerly covered by major Wisconsinan proglacial lakes.

Three mtDNA lineages have been described in Great Lake populations of walleye *Stizostedion vitreum* that could be attributed to Mississippian, Atlantic, and Missourian refuges. These authors described a decided east-west gradient in haplotype frequencies. Michigan is in the zone of overlap between the major haplotype groupings. Another Percid species, the yellow perch *Perca flavescens*, in Michigan and adjacent waters is also believed to have originated from multiple origins.

Evidence based on spatial variance in allozyme allele frequency (Seeb et al. 1987) from relatively few and westerly distributed populations of northern pike *Esox lucius* suggests that contemporary population to the north and west of the Great Lakes originated from Mississippi and Missouri refuges.

After several millennia of relative geological stasis, in the past several hundred years, human activities have changed distributional areas for species and faunal groups and established or negated natural barriers to dispersal across large areas of North America, including Michigan. Man-made diversions in the Great Lakes may have played a significant role in the distribution of some species. Alteration of waterways can disrupt spatial barriers resulting in range expansions. Development of man-made connectivity has caused formerly allopatric species to come into contact and hybridize.

Additional anthropogenic effects in the form of massive levels of hatchery production and stocking have made indelible marks on species distributions and abundance as well as naturally evolved levels and spatial dispersion of genetic diversity. The genetic architecture of all species of commercial and sport interest have been indelibly altered through whole-sale stocking of hatchery fishes without regard to location of origin without regard to natural stock structure or to genetic issues concerning inbreeding, outbreeding.

**Job 2. Title: Determine effects of hatchery supplementation on relatedness among adult lake sturgeon in Black Lake. Assess potential for future inbreeding. Estimate effective population size of remnant Black Lake lake sturgeon population.**—During 1983, 1984, and 1987 two females were spawned using milt from one or two males. Over 11,000 progeny from these matings were stocked into Black Lake in the northern Lower Peninsula of Michigan. Little natural recruitment was noted for this population and the Michigan DNR hoped that plants of fall fingerlings would enhance population numbers to levels that would continue to support sport harvest. We believed that due to the extremely small number of adults involved, that the majority of juveniles stocked were related. Thus, when these juveniles recruit to the spawning population there is a high probability that matings will occur among close relatives. Inbreeding depression could push this population to extinction, as the current adult spawners senesce or die and are replaced by highly related younger adults.

We have established that there is credible evidence for significantly negative impacts to future reproduction in Black Lake and to the long-term viability of this population. Adult spawners were sampled as were numerous individuals of a size consistent with progeny stocked during the three years of supplementation. All fish were genotyped at 8 microsatellite loci. We found that

smaller fish had significantly higher levels of inter-individual relatedness than was characterized for adult spawners.

**Job 3. Title: Estimate stock structure of walleye in Michigan waters of the Great Lakes. Determine the impacts of extensive transfers of walleye across drainages.**—Walleye (N=452) from 9 populations were collected by Michigan DNR biologists and other cooperators. Sampling locales were surveyed during the spawning season and included the St. Mary's River, Little Bay de Noc, Muskegon river, Tittabawassee River, Saginaw Bay Reefs, Holloway Reservoir, Flint River, Clinton River, and near-shore spawning areas in eastern Lake Erie. All individuals were characterized using 8 microsatellite loci that have previously been characterized in walleye.

Preliminary findings suggest that walleye populations spawning in tributaries of the Great Lakes could be partitioned into 3 genetic "clusters" based on genetic affinities established using pairwise estimates of population similarity in allele frequency. One group was composed of spawners from the St. Mary's River and Little Bay de Noc. A second group was composed of populations from the Clinton river and eastern Lake Erie. A third group included the Flint River, Tittabawassee River, Saginaw Bay Reefs, and Muskegon River. Genetic affinities between the Muskegon River (a tributary to Lake Michigan) and walleye obtained from waters in and draining to Saginaw Bay in Lake Huron reveal the overwhelming influence of previous stocking efforts in tributaries to Saginaw Bay that primarily utilized progeny from spawners from the Muskegon River. Interestingly, analyses suggest that walleye from Holloway River were not part of the Muskegon/Saginaw Bay cluster. Lack of genetic similarity to other populations surveyed suggest that this population, which has been isolated in the headwaters of a Saginaw Bay tributary, may represent historical genetic characteristics of walleye from the Saginaw drainage that were extirpated elsewhere.

**Job 4. Title: Estimate the impacts of hatchery supplementation on naturalized steelhead populations. Estimate proportional contributions of different hatchery strains to the open water creel in Lake Michigan.**—States surrounding the Great Lakes basin annually produce and release over one million steelhead *Oncorhynchus mykiss* juveniles into Lake Michigan to enhance open water and stream sport fish harvests. Despite this large effort, basin-wide assessment has not been coordinated. Thus, an evaluation of stocking practices by state and across the basin is generally lacking. Few data are available pertaining to levels of natural steelhead recruitment or of the relative contributions of hatchery and wild fish in the sport harvest. There is little information relating stocking levels, planting locations and timing to harvest levels for each strain. Few studies exist on levels of straying of hatchery fish or of their reproductive contribution to spawning populations in tributaries to Lake Michigan. Using strain-specific differences in genetic characteristics in conjunction with scale pattern analysis and mixed stock analysis, we are working to determine the relative contribution of different hatchery strains and wild fish (and thus states of origin) to the open-water steelhead sport harvest in Lake Michigan. We are able to identify hatchery fish in adult spawning populations and estimate hatchery straying rates. We are working to compare historical and contemporary stocking records of the number of individuals of each hatchery strain stocked to determine whether changes stocking rates and juvenile age and size at release have had an appreciable effect on hatchery-wild ratios of spawning adults and concomitantly on genetic diversity of steelhead in the Lake Michigan basin.

Analyses have been completed for adult spawning steelhead in 6 tributaries to Lake Michigan. We collected historical scale samples from fish collected during 1983-1984 are archived by the Michigan Department of Natural Resources. Samples were collected from adult spawners during 1998 and 1998 using a stream shocker. Individuals of hatchery origin were identified using scale pattern analysis. We were able to assign all hatchery fish to strain of origin using microsatellite

loci. Simulation studies suggest that we can assign strain to unmarked steelhead with >95% accuracy.

We obtained historical and contemporary samples from 6 Michigan tributaries to Lake Michigan. Streams included the Betsie River, Manistee River, Little Manistee River, Pere Marquette River, White River, and Muskegon River. We found that over all 6 streams the mean proportion of wild fish in spawning runs decreased from 88% during 1983-1984 to 60% during 1998-1999. No appreciable increase in abundance was noted over this time. Results suggest that Michigan's steelhead spawning runs are increasingly composed of hatchery fish, due principally to changes in size and age at stocking. The change in steelhead run composition has not been accompanied by concomitant increases in run size. We question the efficacy of current stocking intensity.

We estimate that approximately 25% of the hatchery steelhead spawning in Michigan tributaries to Lake Michigan were produced by other states across the basin. Eighteen percent of the hatchery fish were Ganaraska strain stocked by Wisconsin. Skamenia (3%) and Chambers Creek (3%) strains also were found across all Michigan streams surveyed during 1998-1999. These strains are stocked by Wisconsin, Indiana, and Illinois. Most importantly, at the Little Manistee weir, which is the source of Michigan DNR steelhead gametes, we estimate that the proportion of hatchery steelhead to have increased 2% to 33%. Because only a fraction of the hatchery fish are marked, we estimate that a large fraction of the gametes taken annually are from unmarked hatchery fish that are mistaken for wild steelhead.

**Job 5. Title: Estimate levels of stock structure in Lake Superior coaster brook trout in and immediately surrounding the Salmon Trout River. Assess the efficacy of developing a coaster broodstock from this population.**—Brook trout *Salvelinus fontinalis* are a notable feature of the native aquatic community in the Upper Great Lakes, and represent a major focus for restoration across the basin. Brook trout were historically an important and abundant species along coastal shores and in tributaries across the Great Lakes basin. One life history form of brook trout referred to as coasters, are known to undergo migrations from lacustrine habitats into tributary rivers and streams for reproduction, feeding or refuge. During upstream migrations from Lake Superior, adult coasters were highly susceptible to anglers and by the early 1900s this highly adapted and prized coaster brook trout life history type had all but disappeared. Coaster restoration is a stated federal, state, tribal and international goal. Objectives for Lake Superior and research priorities for the entire Great Lakes basin outline the need for evaluation of the current population structure, habitat conditions, genetic profiles, affects of hatchery supplementation and potential impediments to restoration of coaster brook trout.

Successful restoration of coaster brook trout in Lake Superior will hinge upon their current status. The research initiated in this section of the project will build upon ongoing research being conducted by the cooperators Dr. Ed Baker and Dr. Casey Huckins involving characterization of abundance, distribution, and composition of brook trout in coastal tributaries. Research has been hampered by the paucity of direct observational data on movements between streams by resident brook trout and of the movements by coaster brook trout among drainages. We presently lack a means of distinguishing hatchery from wild individuals, the degree of reproductive isolation between stream-resident and migratory forms, and of the potential influence of stocking on the recruitment of populations targeted for restoration. Use of molecular genetic markers has proven successful at circumventing these problems.

The Salmon Trout River in the Upper Peninsula of Michigan represents the only known viable population of coaster brook trout along the south shore of the Lake Superior basin. Brook trout of both life history forms have been widely stocked across the basin. There is intense interest to ascertain whether stocking of different strains of resident and coaster brook trout has been

successful, and what effects stocking has had on population relationships across the southern shores of the basin.

Fish communities of the Great Lakes have been heavily managed through stocking. Lake Superior has been stocked with millions of brook trout. Concerns over impacts of releasing cultured fishes into the environment have accelerated in recent years and impacts of such releases occur both at the community level, at the population level by changing demographic parameters, or by affecting population genetic characteristics. Changes in genetic characteristics of populations following often repeated introduction scenarios can lead to reductions in effective population size, hybridization, and disruption of adapted genotypes. In light of these concerns, the objectives of our proposed research were to document whether there is evidence of hybridization between wild and planted fish, to determine whether stocking programs are contributing to the recruitment of brook trout, and to determine genetic relationships among stream resident populations in Michigan tributaries to Lake Superior and relationships between resident and migratory life-history forms

The primary questions of this portion of the project focused on the extent of dispersal and establishment of stocked brook trout. As part of our current efforts we have been conducting preliminary surveys of the fish community and habitat conditions in tributaries around the Keweenaw Peninsula (KP) and the eastern shore of northern Marquette County. Brook trout were sampled from 4 streams in each of 2 regions of Michigan's upper peninsula. Identification of stream resident and coaster phenotypes was based on diagnostic morphological traits. A fin clip biopsy was taken from the caudal fin for DNA analysis. Samples from each of the hatchery strains used for supplementation were provided by management agencies.

Genetic data in the form of multi-locus genotypes are being obtained for each individual based on microsatellite loci that are currently being used for brook trout in our lab in other projects. Summary measures of genetic similarity among populations are being used to quantitatively determine degree of reproductive isolation. Preliminary data suggest that all natural populations in Michigan tributaries to Lake Superior have not been impacted by hatchery supplementation. Data also suggest that coaster and resident brook trout from the Salmon Trout River are reproductively isolated.

**Job 6. Title: Estimate rates of loss of genetic diversity in northern muskellunge. Estimate degree of introgression between stocked muskellunge and northern pike. Determine the relationships between northern muskellunge and Great Lakes muskellunge to assist in developing a comprehensive Esocid hatchery management plan.**—Samples of northern muskellunge were obtained from the 2 Michigan DNR broodstock lakes (Thornapple Lake and Hudson Lake). We also obtained Great Lakes muskellunge from Lake St. Clair. Northern Pike were collected from Gun Lake and Gull Lake. We were also provided putative northern pike/muskellunge hybrids from the ponds behind the Wolf Lake Hatchery.

All individuals were characterized using microsatellite loci. The loci show fixed species-specific differences between northern pike and muskellunge. Thus, all hybrids (F1, F2, and backcross) can be unambiguously identified. We found that several fish samples from Gun and Gull Lakes were hybrids. We found that the majority of fish sampled from the Wolf Lake ponds were hybrids. These fish were putatively carry-over from a year when the Michigan DNR obtained muskellunge fingerlings from an outside source. The presence of large numbers of hybrids suggests that the majority of supposed muskellunge stocked across the state of Michigan during this year were hybrids and not northern muskellunge.

We found that northern muskellunge and Great Lakes muskellunge are genetically distinct and can be easily identified using microsatellite loci.

**Job 7. Title: Prepare annual report and as appropriate communicate program results in the form of peer-reviewed publications, reports, popular articles, and presentations.** Findings from the first years activities are communicated in this document and to Michigan DNR and regional fisheries biologists via oral communications at meetings, professional meetings, and written reports.

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**Date: September 30, 2003**