ASSESSMENT OF HISTORICAL AND CONTEMPORARY GENETIC DIVERSITY OF STEELHEAD (*ONCORHYNCHUS MYKISS*) IN THE LAKE MICHIGAN BASIN

By

Meredith Lynn Bartron

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ABSTRACT

ASSESSMENT OF HISTORICAL AND CONTEMPORARY GENETIC DIVERSITY OF STEELHEAD (*ONCORHYNCHUS MYKISS*) IN THE LAKE MICHIGAN BASIN

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Steelhead (*Oncorhynchus mykiss*) were introduced into the Great Lakes in the late 1800's. Subsequently, natural recruitment across the Lake Michigan basin has been regularly supplemented by hatchery production of strains derived from widely dispersed locales within the species' native range along the west coast of the United States. We used microsatellite markers to assess how the populations of steelhead in Lake Michigan are genetically structured 1) spatially within and among tributaries to lake Michigan, 2) temporally based on time of entry into spawning runs (fall versus spring) as well as between historical and contemporary populations, and 3) between naturalized populations and hatchery strains used for supplementation. Hierarchical analysis indicated significant genetic differentiation between the naturalized populations and hatchery strains (Θ_s=0.060, P<0.05), among naturalized populations (Θ_p=0.003, P<0.05), and among hatchery strains (Θ_p=0.105, P<0.05). However, few significant pairwise genetic differences among naturalized populations exist, no significant differences in allele frequencies between different spawning populations within rivers were observed, and no significant differences in allele frequency between sympatric fall and spring spawning runs were observed.

Prior to 1983, hatchery supplementation of Lake Michigan steelhead populations in Michigan utilized primarily on strain and was largely unsuccessful due to low survival

estimates (0.01%) of small (<120mm) hatchery yearlings to smolt stage. Accordingly, contributions of hatchery fish to historical adult spawning runs in Michigan tributaries were low (0-30%) across six major drainages. Large (>150mm) yearlings of multiple hatchery strains have been stocked exclusively since 1983, increasing estimates of survival (90%) to smolting. Consequently, the proportion of hatchery adults in spawning runs increased to 13-79%. We examined the effects of changes in stocking practices on straying of hatchery steelhead and to temporal changes in levels of genetic diversity and relationships among populations for steelhead populations sampled for two time periods (1983-1984 and 1998-1999). Measures of inter-population divergence (mean F_{ST}) were not significant for either time period. However, spatial genetic relationships among historical and contemporary populations were significantly correlated with geographic distance. Increased numbers of alleles in spawning adults from populations can be attributed to alleles specific to recently introduced hatchery strains.

The increased contribution of hatchery origin individuals to spawning runs in Michigan rivers increases the potential for introgressive hybridization between hatchery and river origin individuals. Therefore, maintenance of genetic diversity within hatchery strains is important to management. We empirically compared six mating strategies used by hatcheries in the Great Lakes region to examine the effects of reproductive variance on measures of genetic diversity. Treatments that minimized reproductive variance by not pooling gametes from multiple individuals, and did not use individuals repeatedly for matings resulted in the lowest estimates of coancestry (inbreeding) and highest effective population size estimates.

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INTRODUCTION

Steelhead (*Oncorhynchus mykiss*) are native to the west coast of North America. Steelhead are anadromous, but differ from other ocean-migrating Pacific salmon due to their ability to spawn multiple times, whereas other *Oncorhynchus* species are semelparous. Steelhead home to their natal stream, though low levels of straying occurs (Quinn 1993). High levels of natal homing (Quinn 1993) and local adaptation (Taylor 1991) have contributed to the spatial and temporal patterns of genetic variation within and among steelhead populations in their native range (Busby et al. 1996).

Steelhead populations or stocks are often defined by drainages in which they spawn (Busby et al. 1996). For larger river systems, multiple genetically distinct populations may be present (Beacham et al. 1999; Heath et al. 2002). Typically, populations spawning in close proximity are more similar genetically than more geographically distant populations (Busby et al. 1996).

Steelhead in Lake Michigan

Steelhead were initially introduced to the Great Lakes in the late 1800's (MacCrimmon and Gots 1972). Within Lake Michigan, spawning primarily occurs in Michigan tributaries (Seelbach and Whelan 1988). Due to the lack of suitable spawning habitat, natural reproduction contributing to natural recruitment in other areas is believed to be negligible (Seelbach and Whelan 1987). Historically, the state of Michigan stocked juvenile steelhead as fall fingerlings (<110 mm; Seelbach 1987) with an estimated survival to smolt stage of 0.01% (Rand et al. 1993). Following a change in stocking practices in 1984, large yearlings (>150 mm; Seelbach 1987a) with higher estimated

survival to smolt stage (90%; Rand et al. 1993) were used for stocking. Prior to this change, average contributions of hatchery fish to spawning runs in six Michigan rivers was generally low (12%; Seelbach & Whelan 1988). Following increases in size at stocking, the average contribution of hatchery fish to spawning runs increased (40%; Bartron & Scribner, in press).

Approximately 1.8 million juvenile steelhead are stocked into the Lake Michigan basin annually. Four hatchery strains, including Skamania, Michigan, Chambers Creek, and Ganaraska are currently stocked into the basin. The Skamania strain originated from the Washougal River in southwest Washington state. The Skamania strain was introduced into the Great Lakes in 1975 by the Indiana Department of Natural Resources. The Skamania strain is maintained by annual gamete takes from the St. Joseph River to propagate the strain stocked by Indiana, and through separate broodstock rivers in Wisconsin. The Chambers Creek strain originated from the Puget Sound region of Washington State, was introduced to Lake Michigan in 1987, and is stocked by Wisconsin. The Ganaraska strain (also stocked by Wisconsin) originated from naturalized reproduction in the Ganaraska River, a tributary to Lake Ontario, and was also introduced to Lake Michigan in 1987. Both the Ganaraska and Chambers Creek hatchery strains are maintained by annual gamete takes from broodstock rivers in Wisconsin. Michigan and Indiana stock the Michigan steelhead strain. The Michigan strain is maintained from gametes taken annually from a naturalized population in the Little Manistee River, a tributary to Lake Michigan.

Ecological, morphological or genetic differences among steelhead strains have emerged over long periods of time in their native environments. Population differences

in these strains are of adaptive significance, and are maintained in nature by the balance between evolutionary forces of selection, genetic drift, and gene flow. The four strains of steelhead used for supplementation in waters of the Lake Michigan basin exploit strain-specific heritable differences in the timing of adult returns to rivers for spawning to increase recreational fishing opportunities for anglers. Additional strains of non-anadromous rainbow trout and other non-native salmonid species have and are continuing to be stocked widely throughout the basin. Stocking often is conducted without directed efforts to assess whether numbers stocked are justified relative to returns to the fishery. There has been little effort to assess the proportional contributions of hatchery fish to the spawning runs in the major tributaries of Lake Michigan or of the potential impacts hatchery fish may have on naturalized populations either competitively or via introgressive hybridization. Introgression between individuals from different hatchery strains will reduce between-strain differences in allele frequency, although measures of genetic diversity within strains (or populations) will increase. Of concern to management is the decrease in between-strain genetic differences, and the potential for the loss of the heritable differences in run-timing among strains. This thesis research focuses explicitly on these questions.

There is widespread confusion among fishery managers as to how management actions related to stocking can compromise the genetic integrity of naturalized populations. One widely held perception is that because the stock or strain composition of steelhead and most other introduced species is highly "contrived" or artificial relative to areas from the species native range, fisheries managers need not worry about levels of genetic variation or of introgression. Is the management goal to manage the resource for

sustainable yields for the public? Year to year variation in the number and strain composition of fishes stocked is widely believed to leave little opportunity for fishes to adapt (or evolve) within the environment. If there is no adaptation and survival fails to meet management prescriptions then managers can simply restock with a different strain. Using genetic markers we seek to provide empirical data that demonstrates that management actions can affect the genetic characteristics of populations within a highly manipulated lake ecosystem.

There is much confusion in fisheries management as to what levels of genetic variation within and among populations are optimal. If inbreeding is to be discouraged due to the negative effects of expression of deleterious recessive alleles in homozygous condition in offspring (and lower fitness; Figure 1), then should we not be encouraging outbreeding? Would not introgression of genetically different fishes (e.g., from different hatchery strains or between hatchery and wild fish) result in hybrid vigor in progeny? However, introgression between genetically different fishes could also potentially negatively reduce the fitness or progeny. Outbreeding depression is the decrease in fitness of offspring resulting from matings between highly genetically differentiated individuals (Figure 1).

By quantifying how genetic diversity is partitioned within and among hatchery strains and naturalized populations of steelhead in the Lake Michigan basin, we provide recommendations that minimized threats of inbreeding and outbreeding given existing constrains imposed by management.

Stocking strategies: inbreeding versus outbreeding

Two main concerns exist regarding genetic diversity within and among steelhead populations in Lake Michigan. The first concern relates to inbreeding depression, and the associated decreased fitness of offspring resulting from matings among highly related individuals (Figure 1). In general, high levels of alleleic diversity and heterozygosity within most of the hatchery strains and naturalized populations do not indicate inbreeding depression is currently of concern to steelhead in Lake Michigan.

Due to the highly significant genetic differences among hatchery strains and between hatchery strains and naturalized populations, outbreeding depression and associated potential for decrease in fitness of offspring resulting from matings between genetically distinct populations should be a concern for managers. Hatchery gamete-take practices that use large numbers of adult male and female individuals for spawning, minimize re-use of adults for matings, and do not pool gametes from more than two adult males (to minimize sperm competition) act to minimize coancestry (inbreeding) among resulting offspring.

Spatial comparison of genetic diversity

In this study, we used molecular microsatellite markers to quantify levels of genetic diversity within and the magnitude of variation among steelhead populations across the Lake Michigan basin. We found that the major component of genetic diversity among Lake Michigan steelhead populations was attributed to differences among the hatchery strains (mean F_{ST}=0.095), whereas genetic differentiation among naturalized

populations was low (mean F_{ST}=0.003). We found no evidence for significant differences in allele frequency between steelhead spawning in different locations within rivers. Additionally, we found no evidence for significant differences in allele frequencies between steelhead sampled from fall and spring-spawning runs within rivers, though previous studies (Seelbach 1993) documented differences in adult sex ratios between runs.

Temporal comparison of genetic diversity

Assessments of the magnitude of changes within and inter-relationships among populations across the Lake Michigan basin would profit from knowledge of historical benchmarks of genetic characteristics of steelhead prior to changes in hatchery management. Reisenbichler & Phelps (1989) hypothesized that introgression between native steelhead and hatchery steelhead widely stocked across large areas led to lower inter-population variance in allele frequency between steelhead populations in Washington State. Reisenbichler & Rubin (1999) suggested that although inter-breeding may occur between hatchery and wild individuals, the potential reproductive contribution of hatchery fish to wild spawning populations could be quite low due to lower reproductive success of hatchery individuals. Increased survival of juvenile steelhead stocked into the Lake Michigan watershed can in turn increase the proportion of hatchery origin adults in spawning runs, and increase the potential for introgression between steelhead of hatchery and natural origin.

Comparisons of allele frequencies assessed from samples collected during two time periods allowed assessment of changes in genetic diversity following changes in

stocking practices. Inter-population variance in allele frequency across six naturalized populations assessed before (1983-1984) and after (1998-1999) changes in stocking practices decreased (mean F_{ST}= 0.006 versus 0.002) respectively. The correlation between inter-population geographic distance and genetic distance also decreased over time (for historical populations R=0.55, P<0.01; for contemporary populations R= 0.39, P<0.02; Bartron & Scribner in press). Additionally, alleles unique to hatchery strains currently stocked in the basin were not present in naturalized populations sampled prior to the change in management, but were found in unmarked spawning adults in contemporary populations. The presence of alleles unique to hatchery steelhead currently stocked provides evidence that introgression has occurred between steelhead of natural and hatchery origin.

Straying of hatchery individuals

The four states surrounding Lake Michigan use identifying marks (e.g. fin clips, maxillae clips, or combinations thereof) to identify hatchery-origin steelhead. However, not all states clip all hatchery steelhead prior to release. Clip data are used to estimate hatchery strain-specific rates of straying, and to estimate the contribution of both hatchery- and naturally-produced steelhead to the recreational fishery in Lake Michigan and its tributaries. Accurate assessments of straying and relative contribution of hatchery fish are likely compromised due to duplication of specific clips for more than one strain, and incomplete clipping of hatchery-origin steelhead prior to stocking. Further, abundance of certain hatchery strains are likely overestimated due to confusion of strain-specific clips (i.e., maxillae clips) with hooking injuries. We observed a bias in the

estimates of contribution of hatchery individuals to spawning runs in Michigan rivers when estimates were obtained solely based on observation of hatchery clips in comparison to estimates obtained by scale pattern analysis and genetic identification to strain. Because each method identifies two separate components of the hatchery contribution (clipped and not clipped), we combined the results from methods to better estimate the strain-specific proportion of hatchery individuals in the spawning runs of Michigan rivers.

Hatchery propagation

A large proportion of realized steelhead recruitment to the creel and to spawning populations is provided by hatcheries. There is a high probability that hatchery individuals inter-breed with naturalized individuals. Therefore, it would be prudent to ensure that hatchery fish are produced with a goal to maintain genetic diversity. Genetic evaluation of alternative mating strategies used for propagation of hatchery strains is important to the long-term maintenance of genetic variation in steelhead across the basin.

Recommendations to improve hatchery management often focus on effective population size which is affected by different mating strategies (Kincaid 1995; Allendorf & Ryman 1987), sex ratios of adults (Simon et al. 1986), and total numbers of adults bred. Genetic guidelines have frequently been based on long-standing theory (Wright 1931) that unequal numbers of males and females can lead to reduced effective population size (N_e), when defined as the probability that two randomly selected genes in the current generation are copies of the same parental gene. Though Wright's formulation of N_e is an important conceptual basis for management, the use of N_e as

described by Wright (1931) does not account for variance in reproductive success among males and females.

Rigorous quantitative and comparative evaluations of the effects of alternative hatchery mating strategies on measures of genetic diversity of progeny have not been conducted. We compared six mating strategies commonly used in the Great Lakes basin for hatchery propagation. We estimated the magnitude of variation in male and female reproductive success associated with each mating strategy, and the effects reproductive variance had on the genetic diversity of resulting progeny. We found that mating strategies which pooled gametes from multiple individuals resulted in a higher mean coancestry and lower estimates of effective population size due to disproportional reproductive contributions of relatively few of the males spawned. Strategies based on matings of one or two males per female resulted in the highest percentage of unrelated offspring and highest estimates of effective population size.

Conclusion

Why should genetic diversity be considered in any comprehensive management program for steelhead in Lake Michigan? Based on the results of this dissertation research, steelhead in the Lake Michigan basin do not represent a single, panmictic population. Significant differences in allele frequencies exist between hatchery and naturalized populations. Among naturalized populations, few significant differences exist, potentially due to stocking practices and straying of hatchery individuals. Straying of hatchery individuals stocked elsewhere in the Lake Michigan basin into Michigan rivers occurs. The proportional contribution of hatchery individuals to spawning runs has

increased following changes in stocking practices that increased the survival of stocked juveniles. Due to differences in management practices used for marking hatchery origin individuals by management agencies around the Lake Michigan basin, identification of hatchery individuals can over- or underestimate the contribution of hatchery individuals in spawning runs. Additionally, mating strategies used for hatchery propagation demonstrably effect levels of coancestry, and the probability of increase in inbreeding, due to variance in male reproductive success most likely resulting from sperm competition. Due to the potential for continued introgression between hatchery and natural origin individuals, it is important to maintain genetic diversity within hatchery strains.

Chapter 1

SPATIAL GENETIC STRUCTURE AMONG LAKE MICHIGAN STEELHEAD (*ONCORHYNCHUS MYKISS*) POPULATIONS

ABSTRACT

Steelhead were originally introduced to the Great Lakes in the late 1800's, and large self-sustaining naturalized populations have existed in Michigan tributaries to Lake Michigan since introductions began. Despite high levels of natural recruitment, widespread supplemental stocking of hatchery fish occurs in almost all major rivers in Michigan, and throughout the Lake Michigan basin. We seek to learn how the populations of steelhead in Lake Michigan are genetically structured 1) spatially within and among Lake Michigan tributaries, 2) based on time of entry into spawning streams (fall vs. spring), and 3) between naturalized populations and hatchery strains used for supplementation. Sampling included adults from tributaries from fall and spring spawning runs, and multiple spawning locations within three of the 10 naturalized populations. Hierarchical analysis indicated there was significant genetic differentiation between the naturalized populations and hatchery strains (Θ_s=0.060, P<0.05), among naturalized populations (Θ_p=0.003, P<0.05), and among hatchery strains (Θ_p=0.105, P<0.05). Analyses of specific pairwise relationships between populations revealed that differences in allele frequencies are more evident between hatchery strains rather than naturalized populations. We compare levels of genetic variation for Michigan steelhead populations to populations of comparable geographic spatial scales to within their native range.

INTRODUCTION

Pacific salmonids spawn as discrete populations despite multiple year residence times across wide expanses of open-ocean. Within each species, high natal philopatry to spawning areas (Scheer 1939; Ricker 1972; Quinn 1993), adaptive differences in life history characters such as timing of spawning, utilization of different spawning habitats, and varying residence times in rivers, lakes, and ocean environments have contributed to reproductive isolation and concomitantly in genetic differentiation even over microgeographic scales (National Research Council 1996). Adaptations to specific stream environments are influenced to some degree by genetic differences among individuals and populations (Taylor 1991). Genetic diversity is evident within and among salmon populations at a variety of spatial and temporal scales (i.e., from within drainage to large regional levels and among different spawning runs, respectively) (see review in Allendorf and Waples 1996).

Management of Pacific salmonids in their native range has historically recognized regional and population differences in phenotypic, genotypic or ecological characters (Moulton 1939; Hard et al. 1996; Busby et al. 1996; Weitkamp et al. 1995), and strives to balance conservation (e.g., preservation of stock structure) with commercial and recreational exploitation (Waples 1991a). Management of naturalized populations of Pacific salmonids introduced into United States and Canadian waters of the Great Lakes differs appreciably. While the abundance and distribution of non-native salmonid species are considered in Great Lakes fisheries community plans and objectives (Eschenroder et al. 1995), management focuses on resource utilization by recreational fisheries. Other

management issues based on population structure are generally not considered, as abundance is assumed to be primarily based on hatchery production, even though many species and populations in the Great Lakes are believed to be self-sustaining (e.g. Eschenroder et al. 1995; Seelbach and Whelan 1988).

Steelhead (*Oncorhynchus mykiss*) are an anadromous salmonid native to the west coast of North America. Steelhead populations have evolved variations in life history traits such as differences in run timing and time of spawning (Leider et al. 1984; Busby et al. 1996). Within their native range, local adaptation associated with stream environments (Taylor 1991), combined with natal fidelity (Ricker 1972; Schroeder et al. 2001) have contributed to genetic differentiation among steelhead populations at both levels of macro and micro-geographic spatial scales (Parkinson 1984; Reisenbichler and Phelps 1989; Reisenbichler et al. 1992; Beacham et al. 1999; Nielsen 1999; Nielsen and Fountain 1999; Osterberg and Thorgaard 1999). However, in the presence of hatchery supplementation, stocking and subsequent introgression of hatchery fish with native populations has been shown to reduce genetic differences between steelhead populations (e.g., in the state of Washington; Reisenbichler and Phelps 1989).

Steelhead were the first non-indigenous naturalized species purposely introduced into the Great Lakes (MacCrimmon and Gots 1972), and have been stocked periodically in Lake Michigan since initial introductions began in the late 1800's (Biette et al. 1980; Fielder 1987). Steelhead used for introductions originated from locations in California and Washington, though additional sources have been used since initial introduction (MacCrimmon and Gots 1972). As a result, a large fraction of the genetic diversity that historically existed in geographically widely distributed spawning populations across the

Pacific Coast of North America was introduced into the Great Lakes. Previous genetic studies of steelhead populations in tributaries of the Great Lakes have shown that steelhead spawning in geographically widely dispersed populations around Lake Superior are genetically differentiated (Kruger and May 1987). Adjacent tributaries in Lake Ontario (Dueck and Danzmann 1996) were found to differ in mitochondrial DNA haplotypes. Genetic differentiation among steelhead populations in the Great Lakes could have accrued due to 1) retention of spatial dispersion of genetic variation reflecting different geographic sources of populations initially used for stocking or 2) local adaptation and genetic drift that occurred subsequent to initial stocking.

Steelhead abundance and distribution across the Great Lakes have been maintained by natural reproduction and extensive hatchery supplementation (Seelbach and Whelan 1988). The majority of natural reproduction of steelhead in the Lake Michigan basin occurs in Michigan drainages due to the lack of favorable spawning habitat elsewhere in the basin (Seelbach and Whelan 1988). A total of four hatchery strains are currently stocked into Lake Michigan and its tributaries by the four states surrounding the basin, representing an average of 1.8 million juvenile steelhead stocked into Lake Michigan per year since 1993 (Michigan fish stocking records, Burzynski 1999; Palla 1999; Table 1). These strains have been used for supplementation to provide a variety of angling opportunities taking advantage of strain-specific heritable differences in the timing of return of adults to rivers to spawn.

Within the state of Michigan, two hatchery strains (Michigan strain and the Skamania strain) are currently stocked (Table 2). The Michigan strain originates from the naturalized population in the Little Manistee River in Michigan. The Skamania strain

originated from the Washougal River in southwest Washington State. This strain was introduced into the Great Lakes in 1975 by the Indiana Department of Natural Resources, and in Indiana is maintained by annual gamete takes from adults of hatchery origin returning to the St. Joseph River. Wisconsin also maintains a separate Skamania strain which was obtained from Indiana. Additional strains stocked into the Lake Michigan basin include the Ganaraska strain (stocked by Wisconsin), which originated from naturalized reproduction in the Ganaraska River, a tributary to Lake Ontario, and the Chambers Creek strain (also stocked by Wisconsin), which originated from the Puget Sound region of Washington State. Both the Ganaraska and Chambers Creek hatchery strains are maintained by annual gamete takes from broodstock rivers in Wisconsin.

Recent changes (mid 1980's) in hatchery stocking practices resulted in increased survival of hatchery-raised juveniles (Seelbach 1987a) and higher return rates of hatchery steelhead adults (Seelbach and Miller 1993). Consequently, the proportion of hatchery individuals to the steelhead spawning runs in Michigan significantly increased (Bartron and Scribner in press). Increased contribution of individuals of hatchery origin to spawning runs has resulted in introgression between steelhead of natural and hatchery origin (Bartron et al. in press). Although maintaining genetic differences among naturalized steelhead populations has not been stated as a management goal, the homogenization of gene frequencies among populations may act to effectively negate any adaptive evolution that may have evolved during the species approximate 120 year tenure in the basin (Bartron and Scribner, in press).

Our objective was to determine how genetic variation is partitioned within and among Lake Michigan tributaries. To assess how genetic variance was partitioned within

and among naturalized populations and hatchery strains, we sampled ten naturalized steelhead populations in Michigan, which represent the majority of the natural reproduction contributing to the Lake Michigan steelhead populations. Within larger drainages supporting natural reproduction, multiple tributaries were sampled to determine if significant differences in allele frequency were apparent among discrete spawning areas in different tributaries within rivers. Additionally, for a subset of rivers, comparisons were made between adults sampled during the fall and spring spawning runs to determine whether time of entry into spawning streams conferred significant levels of reproductive isolation. The null hypothesis was that no spatial heterogeneity in gene frequencies exist across populations of steelhead spawning across Michigan tributaries of Lake Michigan. Results will be tied directly to extensive current and historical data on stocking intensity, distribution, and success. Findings will be compared to known levels of spatial and temporal genetic diversity within the species native range

METHODS

Stocking history

Stocking records for Michigan rivers were obtained for the years between 1993 through 1997, representing the time period that steelhead returning to rivers in 1998-1999 and the spring of 2000 would have been stocked. Specifically, information was collected regarding the hatchery strain stocked, the number of individuals stocked, size and age of the hatchery juveniles stocked, and location of stocking. Stocking records were obtained from *http://www.michigan.gov/dnr* (Michigan Department of Natural Resources).

Sampling Locations and Tissue Collection

Samples (total N=667) were collected from adult steelhead from ten rivers in Michigan, representing the majority of the naturalized steelhead populations in the Lake Michigan basin (Figure 1). Rivers sampled for adult steelhead included Thompson Creek, Black River, Platte River, Betsie River, Manistee River, Little Manistee River, Pere Marquette River, White River, Muskegon River, and St. Joseph River. Sampling occurred multiple times throughout the spawning runs.

To determine if fall and spring-run steelhead collected from the same stream represented genetically different populations, samples were obtained from the fall and spring spawning runs of the Little Manistee River (fall N=57; spring N=60), Manistee River (fall N=52), Pere Marquette River (fall N=29), Platte River (fall N=50; spring N=55), and Muskegon River (fall N=37). Individuals from the fall run were collected from the mainstem of each river through creel surveys during September 1998 to December 1998. Individuals from the spring run were collected by electroshocking and creel surveys during March 1999 to May 1999. Spring-run steelhead were sampled on spawning areas, identified by the presence of gravel substrate, spawning redds, and observations of spawning behavior.

To examine the partitioning of variance in allele frequency within drainages, adult steelhead from multiple spawning locations were sampled during the 1999 spring run in the Pere Marquette River, the Manistee River, and the Muskegon River (Figure 2). Tributaries sampled in the Pere Marquette River included the Little South Branch (N=25), the Middle Branch (N=25), and Baldwin River (N=25; Figure 2). Tributaries to

the Pere Marquette River were sampled by electroshocking. Spring-run steelhead from the Manistee River included the river mainstem (N=55) and Bear Creek (N=50; Figure 2). Sampling was completed by creel survey and electroshocking, respectively. Spring-run steelhead samples from the mainstem (N=13) of the Muskegon River were collected by creel survey. Samples from Bigelow Creek (N=25) were obtained by electroshocking.

Steelhead from additional populations naturalized populations in the Lake Michigan basin were also sampled. The St. Joseph River (N=15) was sampled in the fall of 1998 by electroshocking. The Black River (N=43), and Thompson Creek (N=12) were sampled in the spring of 1999 by electroshocking. The Betsie River (N=25) and White River (N= 17) were sampled in the spring of 2000 by electroshocking.

Adult steelhead from hatchery strains sampled included the Michigan strain (obtained from the Little Manistee River), Skamania strain, Chambers Creek, and Ganaraska strain. Wisconsin maintains and stocks steelhead from the Skamania strain (N=52), Chambers Creek strain (N=60), and Ganaraska strain (N=60), and provided fin clips from individuals of each strain from the spring spawning run of 1998 (Figure 1). Indiana maintains steelhead from the Skamania strain (N=60), which was sampled in 1998. Because the Wisconsin and Indiana Skamania strains have been managed separately (each state collects broodstock from different rivers), samples were obtained for each states collection.

Small fin clips from the upper lobe of the caudal fin were taken from each individual and stored either in urea storage buffer (4M Urea, 2M NaCl, 0.1M Tris-HCl, 0.5% Sarcosine, 10mM EDTA), 95% ethanol, or were dried and stored in scale envelopes. Buffer and ethanol preserved fin clips were stored at -20° C until DNA

extraction. Samples were taken only from steelhead that did not have hatchery strain-specific marks.

Scale pattern analysis (SPA)

Scale samples were also taken from each individual sampled for genetic analysis to determine river or hatchery origin. Origin was determined by scale growth pattern analysis (ratio 23; Seelbach and Whelan, 1988). Ratio 23 analyzes the differences in growth between the five circuli preceding the first annulus and the five circuli following the first annulus (Seelbach and Whelan 1988). Individual steelhead identified as hatchery origin by scale pattern analysis (SPA) were removed prior to analysis [see Bartron et al. (in review) for further discussion of the SPA technique].

Genetic analysis

DNA was extracted using the PurGene® extraction kit (Gentra Systems) and Qiagen DNeasy® Tissue Kit (Qiagen Inc.). DNA concentrations were determined using fluorimetery and working stocks of 20ng/μl were made for each sample. Seven microsatellite loci were used to estimate allele frequencies and population levels of genetic variability. Loci included Ogo1a and Ogo4 (Olsen et al. 1998), Oneμ10 and Oneμ11 (Scribner et al. 1996), Omy77 (Morris et al. 1996), Ots103 (Beacham et al. 1998), and Oki200 (Beacham et al. 1999).

PCR reactions for Ogo1a, Oneμ10, Oneμ11, and Omy77 were conducted in 25 μl reaction volumes using 100 ng DNA, 2 μl 10x PCR Buffer (0.1 M Tris-HCl, ph 8.3, 0.015 M $MgCl_2$, 0.5 M KCl, 0.1% gelatin, 0.1% NP-40, 0.1% Trition-X 100), 0.2 mM

dNTPs, 0.6 μM fluorescently labeled forward primer, 0.6 μM unlabeled reverse primer, and 0.3 Units Taq Polymerase. PCR reactions for Ogo4 were conducted in 25 μl volumes, with 2.5 mM $MgCl_2$, and primer concentrations were reduced to 0.5 μM. PCR reactions for Ots103 and Oki200 were conducted in 10 μl volumes using 40 ng DNA, 1 μl 10x PCR Buffer (0.1 M Tris-HCl, pH 8.3, 0.015 M $MgCl_2$, 0.5 M KCl, 0.1% gelatin, 0.1% NP-40, 0.1% Triton-X 100), 0.2 mM dNTP's, 0.4 μM fluorescent labeled forward primer, 0.4 μM unlabeled reverse primer, and 0.3 units Taq Polymerase. PCR reactions for all loci utilized an initial denaturing step at 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, annealing temperature for one minute, and extension at 72° C for 1 minute. A final extension period of 2 minutes, 30 seconds was followed by storage at 6° C until electrophoresis. Annealing temperatures for Ogo1a, Ogo4, Omy77, Oneμ10, Oneμ11, Ots103, and Oki200 were 56° C, 54° C, 54° C, 52° C, 62° C, 50° C, and 50° C respectively. Denaturing acrylamide gels (6%) were used for electrophoresis. Genotypes were visualized using a Hitachi FM-BIO II scanner and LI-COR® IR^2 Global Edition DNA Sequencer. Molecular weight standards and individuals of known genotype were run on each gel to standardize scoring.

Statistical analysis

Allele frequencies for each population at each locus and estimates of observed and expected heterozygosity were generated using the program FSTAT (Goudet 1995; ver. 2.9.3.2). Exact tests (1000 iterations) for deviations from Hardy-Weinberg equilibrium (Weir 1990, Guo and Thompson 1992) were conducted using the program GENEPOP (Raymond and Rousset 1995). Pairwise F_{ST} (Weir & Cockerham 1984)

comparisons were used to determine if differences in allele frequency existed among tributaries within drainages, between fall and spring spawning runs within drainages, and between naturalized populations and hatchery strains. Estimates of pair-wise and overall population differentiation were summarized using F-statistics, implemented in the program FSTAT (Goudet 1995) v.2.9.3.1, and nominal alpha levels were adjusted for multiple comparisons using Bonferroni corrections (Rice 1989). If pairwise comparisons of F_{ST} among tributaries within drainage were not significant, the samples were pooled. If pairwise comparisons of F_{ST} between fall and spring spawning runs within drainage were not significant, then the samples were pooled.

Hierarchical analyses of variation in allele frequency were used to determine how variance was partitioned within and among naturalized populations or hatchery strains. Variance was examined among alleles within individuals (F), among individuals within populations (f), among populations within groups (naturalized or hatchery; Θ_p), and among groups (Θ_s) were conducted using program Genetic Data Analysis (GDA; Lewis and Zaykin 2001).

The program CONTRIB (Petit et al. 1998) was used to calculate allelic diversity or "richness", and relative contributions of each population to the total genetic diversity (C_t). Allelic richness is used to estimate the numbers of alleles for a given sample size (El Mousadic and Petit 1996), and allows for comparisons between numbers of alleles between populations when sample sizes differ. Contributions of individual populations to overall diversity were also useful in understanding spatial patterns of diversity. Contributions of an individual population to total diversity were described two ways. C_t was estimated based on an individual populations contribution to the total diversity. C_{rt}

was estimated based on allelic diversity or richness. Measures of C_t and C_{rt} were composed of two parts: the contribution of an individual population to total diversity based on its own diversity, and based on its degree of divergence from the other populations.

RESULTS

Stocking history

Of the ten naturalized populations examined, only three rivers were not stocked during 1993-1997 (Table 1). The rivers not supplemented were Thompson Creek, Black River, and Platte River (Table 1). Of the seven rivers stocked, an average of 44,515 juvenile steelhead were stocked into each river each year. The smolt-equivalent adjustments described by Rand et al. (1993) were used to standardize juvenile numbers to account for differential juvenile survival based on size, age, and stocking location. An average of 28,044 smolt-equivalent juvenile steelhead were stocked per year into each of the seven stocked rivers (Table 1). The Betsie River, Little Manistee River, White River, and Muskegon River were stocked with individuals from the Michigan strain (Table 1). The Manistee River, Pere Marquette River, and St. Joseph River were stocked with individuals from both the Michigan strain and the Skamania strain (Table 1).

Genetic analysis

Estimates of degree of differentiation in allele frequency (pairwise F_{ST}) among spring-run spawning populations collected from different tributaries sampled within the Muskegon River, Manistee River, and Pere Marquette River were not significant

(P>0.05). Samples from different spawning populations within drainages were therefore combined for further analyses. Estimates of pairwise F_{ST} between adults sampled from fall and spring spawning runs from the Manistee River, Pere Marquette River, Platte River, Muskegon River, and Little Manistee River were also not significant (P>0.05). Individuals from different temporal segments of the spawning run were combined within each drainage for further analyses. Pairwise comparison of mean F_{ST} between samples from the Indiana Skamania strain and the Wisconsin Skamania strain was not significant, and samples from the two strains were combined.

Deviations from Hardy-Weinberg equilibrium were observed in the Manistee River, Pere Marquette River, White River, and Chambers Creek hatchery strain. Deviations were determined by Hardy-Weinberg exact tests (1000 iterations), most likely result from pooling populations even though no significant pairwise differences in allele frequency were observed. Mean observed heterozygosity over seven loci ranged 0.651 in the Betsie River to 0.513 in the Skamania strain (Table 2). Inbreeding values (F) were estimated to vary between -0.073 in the Betsie River to 0.083 in the St. Joseph River (Table 2). Mean allelic richness over seven loci ranged from 3.90 in the Skamania hatchery strain to 5.8 in the St. Joseph River (Table 2). The portion of returning Indiana uses adult steelhead to the St. Joseph River, which flows through Michigan and Indiana for egg takes to propagate the Skamania strain. The discrepancy between allelic richness between the naturalized population and the hatchery strain derived from that population may be due to hatchery spawning practices which utilize a small number of males and females for mating, and the mating strategy employed to fertilize gametes.

Pairwise comparisons of differences in allele frequency between naturalized populations and hatchery strains were significant ($P<0.05$; Table 3), and overall difference between the naturalized populations and hatchery strains, accounted for a significant portion of the total variance in allele frequency observed across the Lake Michigan basin ($\Theta_s=0.060$; $P<0.05$; Table 2). Pairwise comparisons of F_{ST} between the Ganaraska strain and each of the naturalized populations with the exception of the St. Joseph River were significant ($P<0.05$; Table 3). The Michigan strain (source from the Little Manistee River), which has been widely stocked in Michigan, differed significantly from steelhead sampled in the Manistee River and Black River ($P<0.05$; Table 3).

Overall, the variance in allele frequencies among populations within hatchery strains ($\Theta_p = 0.105$; $P<0.05$; Table 3) and among naturalized populations was also statistically significant ($\Theta_p = 0.003$; $P<0.05$; Table 3). Pairwise comparisons of F_{ST} between each of the hatchery strains were significant ($P<0.05$; Table 3), and the mean F_{ST} among hatchery strains over seven loci was 0.095. In contrast, pairwise comparisons of population differentiation indicate few naturalized populations significantly differ in allele frequency from other naturalized populations, and the mean F_{ST} among naturalized populations over seven loci is 0.003 (Table 3). Only the Black River was significantly different from the Manistee River, Pere Marquette River, and Muskegon River ($p<0.05$; Table 3). The Black River was not stocked between 1993-1997 (Table 1), and is geographically distant from the Manistee River, Pere Marquette River, and Muskegon River (Figure 1).

Contributions to genetic diversity

The two of the four hatchery strains and the St. Joseph River population contributed the most to the total observed diversity among the populations examined in this study (C_t; Figure 2). For example, the St. Joseph River had the highest contribution to total diversity (C_t=0.014; Figure 2), due to the diversity observed within the population (C_s=0.016; Figure 2). The Skamania hatchery strain also contributed to the total diversity (C_t=0.013; Figure 2), due to the divergence of the hatchery strain from the other populations (C_d=0.017). The Skamania strain has been derived from adult steelhead returning to the St. Joseph River, therefore similarities in their contribution to the total diversity of steelhead in the Lake Michigan basin is not unexpected. Also, similar contributions to the total diversity of steelhead in the basin is important in that it shows there is not overwhelming levels of straying of other fish into the St. Joseph River. The naturalized populations contributed proportionally less to total diversity relative to the hatchery strains. This finding reflects the lack of significance of inter-population variance in allele frequency among the naturalized populations, and significant allelic variation values between the Skamania strain and each naturalized population. The Michigan strain and the naturalized populations (with the exception of the St. Joseph River) did not contribute to total diversity (C_t), likely due to the lack of divergence of this strain, which is widely stocked (Table 1), from other populations (C_d) and similarity in presence/absence of allele and allele frequency across populations (C_s).

Comparisons of individual population (or strain) contribution to total diversity were based on the allelic richness of each of the populations. Proportional contributions of Skamania and Chambers Creek hatchery strains to the total diversity were higher than

proportional contributions for other populations (C_{rt}= 0.030 and 0.033 respectively; Figure 2), due primarily to their divergence (presence of alleles not shared with other populations) from the other populations. Most of the allelic diversity is apportioned among hatchery strains.

DISCUSSION

Steelhead have been present in the Great Lakes for approximately 100 years, or 36 generations. Using data from populations in the species (or other salmonids) native range as comparisons, this is a sufficient period of time for populations to genetically differentiate. Given that Hendry et al. (2000) found that significant genetic differences between two different sockeye (*Oncorhynchus nerka*) populations in geographically close proximity had evolved within 56 years (approximately 13 generations), steelhead populations in Lake Michigan may have had sufficient time for spatial genetic structuring to become apparent. Another component potentially contributing to differences in allele frequency among the Lake Michigan basin steelhead populations were the use of different source populations (MacCrimmon and Gots 1972). Differences in genotypic diversity among strains used for stocking reflecting different phylogeographic origin may have introduced high amounts of genotypic diversity into the Lake Michigan basin. Limited survival of hatchery-produced fish (Seelbach 1987a) may have acted to maintain genetic population structure that may have evolved since initial introduction, and the more recent concurrent stocking of four significantly genetically differentiated hatchery strains may have provided an additional influx of genotypic diversity into populations found in the basin.

However, the widespread use of hatchery supplementation within the Lake Michigan basin and subsequent introgression between hatchery and naturalized individuals may have reapportioned genetic variation, effectively decreasing variation among populations due to elevated levels of gene flow (natural straying and purposeful and directed stocking), while increasing diversity within populations. Reisenbichler and Phelps (1989) demonstrated that introgression between hatchery and native steelhead in Washington may have resulted in the homogenization of allele frequencies among populations. Recently, survival of stocked hatchery fish has greatly increased following changes in stocking practices by the Michigan Department of Natural Resources in 1984 (Seelbach 1987a). Significant increases in the proportion of hatchery individuals to the spawning runs of naturalized populations in Michigan indicated an increased potential for introgression between hatchery and naturalized individuals (Bartron and Scribner in press).

Introgression between hatchery individuals and individuals from Michigan steelhead populations has been possible through indirect straying of hatchery individuals returning as adults to spawn or directed stocking events (of juveniles) in rivers. Estimated rates of straying to rivers other than their natal river for spawning by adult steelhead in the Great Lakes range from 3-10% (Biette et al. 1981). Individuals of hatchery origin were found in each of the naturalized populations sampled for this study, including rivers that were not recently stocked (Bartron et al. in review; Bartron unpublished data). Adult steelhead originating from both Indiana and Wisconsin hatcheries identified through both strain-specific fin clips and genetic assignment to strain (Bartron et al. in review) have been observed in both the fall and spring spawning

runs in Michigan rivers. Therefore, straying of adult hatchery steelhead was not limited to straying into neighboring river drainages. Rather, straying occurred around the Lake Michigan basin.

Stocking of hatchery steelhead occurs widely throughout the Lake Michigan basin. In total, approximately 1.8 million juvenile hatchery steelhead are stocked in the Lake Michigan basin annually (Bartron et al. in review). Within Michigan, both the Skamania strain and Michigan strain are stocked. Considering the contribution of the hatchery strain to spawning runs have increased, the potential for homogenization of gene frequencies among populations stocked with Michigan strain steelhead resulting from introgression also increased. Significant pairwise differences between each naturalized population examined and the Skamania, Chambers Creek, and Ganaraska strains (Table 3) indicate relatively little if any introgression has occurred between the naturalized populations and those hatchery strains. Bartron and Scribner (in press) demonstrated that alleles unique to the Skamania, Chamber Creek, and Ganaraska strains were not present within naturalized populations in Michigan prior to the introduction of those strains to the Lake Michigan basin. However, alleles unique to the Skamania, Chambers Creek, and Ganaraska hatchery strains are currently present in most of the naturalized populations examined in this study (Bartron and Scribner in press), indicate some amount of introgression has occurred.

Most likely, introgression between naturalized populations and the Michigan strain has had a greater impact on the partitioning of genetic variation among naturalized populations. Evidence for introgression between the Michigan strain and naturalized populations in Michigan includes few significant pairwise comparisons of allele

frequency among the Michigan strain and naturalized populations (Table 2), and low contribution to total genetic diversity observed within the basin due to the divergence of these populations (Figure 3). The only populations representing significant pairwise differentiation in allele frequencies with the Michigan hatchery strain (Little Manistee River) were the Manistee River and Black River. The Manistee River is heavily stocked (Table 1), but the Black River has not been recently stocked. Additional significant pairwise differences in allele frequency between the Black River and other naturalized populations support the consideration of the Black River as a genetically distinct population, whereas no other pairwise comparisons between natural populations and the Manistee were significant. Therefore, in the absence of stocking, and relative geographic isolation, genetic differentiation of naturalized populations in the Lake Michigan basin is possible.

Levels of population differentiation for steelhead in their native range on the West Coast of North America provide insightful comparisons for Lake Michigan steelhead populations. Lack of significant differentiation between sympatric within river fall and spring-spawning runs of the naturalized populations examined in this study was consistent with comparisons of winter and summer-run steelhead in their native range (Utter and Allendorf 1977; Chilcote et al. 1980; Leider et al. 1984). Although sex ratios of fall and spring run steelhead in Michigan differ (Seelbach and Whelan 1988), significant differences in allele frequencies were not observed (Table 3). Estimates of the mean population differ in allele frequency among between naturalized populations, including the Little Manistee River population, were quite low (mean F_{ST}= 0.003; Table 3), compared to mean F_{ST} values between geographically proximate populations of

steelhead in their native range along the West Coast of North America. Between steelhead populations in northern and southern California, Nielsen (1999) found mean F_{ST}=0.064. Mean F_{ST} (over seven loci) among tributaries within the Skeena River and Nass River in British Columbia, Canada were 0.026 and 0.024 respectively (Beacham et al. 2000). However, Beacham et al. (1999) did not find significant differentiation among neighboring populations within the same drainage of the Thompson River in British Columbia, Canada.

The mean estimate of the degree of population differentiation among the hatchery strains stocked into the Lake Michigan basin is quite high (F_{ST}=0.095; Table 3), greater than differences between geographically distant populations. Pairwise comparisons of differences in allele frequencies between steelhead populations in Alaska and California ranged from 0.025-0.029 (Nielsen 1999). The observance of such high allelic variance between the hatchery strains is most likely representative of the differences in phylogeographic origin of the hatchery strains.

Most of the genetic diversity among Lake Michigan steelhead populations is partitioned among the hatchery strains. The hatchery strains used for stocking into the Lake Michigan basin were chosen to increase angling opportunities due to heritable life history traits, primarily the timing of the return of adults to rivers to spawn. Straying of adults around the basin increases the potential for introgression between strains due to different management practices by each of the various agencies (Bartron et al. in review). Introgression between strains would decrease the among-strain differences in genetic variation, and could change the heritable life history characteristics important to

management goals, and also reduce the total genetic diversity observed in the Lake Michigan basin.

CONCLUSION

Most of the genetic diversity in steelhead in the Lake Michigan basin was attributed to the differences among the hatchery strains used for supplementation. We found no evidence for significant genetic structuring among spawning populations within drainages, nor between fall and spring spawning runs within drainages. Levels of genetic differentiation among drainages in Michigan were greatly reduced in comparison to those found among drainages in the native range of steelhead. Widespread stocking of hatchery fish and introgression with the Michigan strain was likely the major factor contributing to the low degree of genetic differentiation among naturalized populations of Lake Michigan steelhead.

Chapter 2

GENETIC EVALUATION OF ALTERNATIVE HATCHERY MATING STRATEGIES

ABSTRACT

Hatchery supplementation of natural populations is increasingly used in fish conservation efforts to restore or repatriate populations. Diverse mating strategies used by hatcheries employ different numbers of males and females, varying sex ratios, and strategies to mix gametes. Due to sex ratio skew and variation in reproductive success, often the effective number of individuals contributing gametes to subsequent generations is significantly less than the total number spawned. Accordingly, mating strategies affect levels of genetic diversity, coancestry, and concomitantly long-term fitness of hatchery stocks or natural populations into which hatchery fish are placed. Using steelhead (*Oncorhynchus mykiss*) we mimicked six commonly employed hatchery mating regimes. We determined parentage using highly variable microsatellites. We calculated the mean and variance of reproductive success for each parent used in each mating regime. We determined summary measures of percentage unrelated offspring, coancestry, and effective population size for each mating regime that could be used to provide guidelines more consistent with conservation goals for maintaining gene diversity. We found that male reproductive variance in numbers of progeny produced was highest when male gametes were pooled prior to fertilization (38.7-233.3). Mating strategies using single pair matings (1:1) and those that used gametes from two unique males for each female (1:2) resulted in comparatively lower mean coancestry and the percentage of related offspring while maximizing variance effective population size relative to other gamete pooling treatments. To examine long-term effects of each mating regime to domestic

broodstocks or closed populations, we project estimates of coancestry and effective population size over time. We show that 1:1 and 1:2 mating regimes are most efficient at minimizing coancestry and maintaining genetic diversity in hatchery or other closed populations.

INTRODUCTION

Numerous complex mating strategies have evolved in natural systems to maximize fitness, maintain genetic diversity, and adaptive potential (DeWoody & Avis 2001). In wild self-sustaining populations, male and female reproductive success varies greatly due to selection for traits that increase fitness (Fleming & Gross 1994). Size, fecundity, and other phenotypic, behavioral, or life history characteristics contribute to inter-individual variance in mating opportunities and reproductive success (Fleming & Gross 1993; Fleming & Gross 1994).

In artificial environments, such as fish hatcheries, selection of mates is imposed by hatchery personnel and is often a function of availability and synchrony in maturation of male and female gametes. In addition, selection during hatchery mating and rearing of juveniles may not necessarily confer adaptive advantage once in the wild (Heggberget et al. 1993; Fleming et al. 1996; Negus 1999; Reisenbichler & Rubin 1999; Lynch & O'Hely 2001; Ford 2002).

Natural populations are increasingly augmented by releases of hatchery offspring for many reasons. Reasons for use of hatchery supplementation as a management prescription include augmentation of existing stocks (Levin et al. 2001), mitigation for dam construction (Waples 1991a), enhancement of fishing opportunities through

introduction of desirable sport fish, and conservation (Waples 1991a; Ryman 1991). Due to the large numbers of fish produced in hatcheries and stocked into natural environments, there is great concern about the potential impacts of hatchery fish on native populations (Waples 1991a; Thomas & Mathisen 1993). Increasing evidence for negative impacts of hatchery stocks to wild populations has prompted much discussion and research regarding the efficiency of hatchery use for conservation and management (Waples 1991a; Ryman 1991; Hilborn 1992; Ryman et al. 1995; Busack & Currens 1995; Philippart 1995; Waples 1999). Interactions between hatchery and wild populations have been widely studied both from biological and genetic perspectives to determine potential for introgression, biological interactions, and threats to conservation (Hindar et al. 1991; Waples 1991a; Hutchings 1991; Washington & Koziol 1993; Heggberget et al. 1993; Thomas & Mathisen 1993; Gharrett 1994; Fleming et al. 1996; Reisenbichler & Rubin 1999).

Concerns regarding the potential interactions of hatchery and wild populations have led to evaluations of hatchery practices as they relate to the maintenance of genetic diversity (Allendorf & Phelps 1980; Kincaid 1983; Allendorf 1993). Guidelines have been presented to improve hatchery management. Recommendations focus on effective population size related to assessment of mating strategies (Kincaid 1995; Allendorf & Ryman 1987), sex ratios (Simon et al. 1986), and total numbers of adults bred. Genetic guidelines have frequently been based on long-standing theory (Wright 1931) that unequal numbers of males and females can lead to reduced effective population size (N_e), when defined as the probability that two randomly selected genes in the current generation are copies of the same parental gene. Though Wright's formulation of N_e is

an important conceptual basis for management, the use of N_e as described by Wright (1931) does not account for variance in reproductive success among males and females. N_e has been used to suggest guidelines for hatchery management (Ryman 1991; Ryman et al. 1995; Allendorf & Ryman 1987). Quantitative and comparative evaluations of hatchery mating strategies currently in practice and those suggested as alternatives have not been rigorously tested. While theory dictates that factors related to N_e are important, there have been few empirical experimental studies that investigate the effects of different hatchery gamete-take practices on measures of genetic diversity that are directly linked to long-term probabilities of population persistence.

Variation in reproductive success has been known to occur in situations where the gametes of males have been pooled prior to fertilization (Gharrett & Shirley 1985; Simon et al. 1986; Danzmann & Ferguson 1988; Withler 1988; Gile & Ferguson 1990; Fleming & Gross 1993; Gile & Ferguson 1995). In hatchery matings either milt or eggs are often pooled in order to maximize possibility of fertilization. Pooling of gametes increases the possibility of competition among the gametes (i.e. sperm competition) that could increase reproductive variation. Due to variation in reproductive success, the use of pooled matings may bias estimates of N_e derived solely on the basis of total numbers of individuals bred because of high potential for unequal parental contribution (Simon et al. 1986). Inclusion of reproductive variance into estimates of N_e (Lande & Barrowclough 1987; Wood 1987; Crow & Denniston 1988) can provide a more accurate assessment of the effective number of individuals contributing to subsequent generations than is likely realized using estimates of N_e based solely on total numbers of adult males and females spawned.

Relatedness among progeny provides another measure useful in evaluations of hatchery mating regimes. Kinship has been used as a surrogate measure of relatedness in situations where actual pedigree and actual relationships among individuals are unknown (Bentzen et al. 2001; Ruzzante et al. 2001). When the pedigree of individuals is known, measures of the probability of identity by decent of alleles among individuals, or coancestry can be empirically determined. (Cockerham 1967; Cockerham 1969; Cockerham 1973; Chesser 1991a; Chesser 1991b). However, measurements of coancestry are rarely available for assessments of hatchery spawning practices because parentage resulting from hatchery matings is not often determined as a direct measure of individual parental reproductive success. In closed populations and depending on the mating system, mean population coancestry can be a direct measure of potential for future inbreeding.

It is desirable to minimize the increase in coancestry. In hatcheries, this can be accomplished in several ways. The mating strategy determines how coancestry will be accumulated with each subsequent generation. Though the importance of maintaining genetic diversity is universally recognized, the emphasis placed on specific variables to offer in management recommendations vary. Further, different mating regimes have been promoted to aquaculturists and hatchery managers on the basis of the same population genetic theory. Unfortunately, the quantitative and comparative analyses of the efficiency of alternative spawning regimes have not been rigorously pursued. To empirically determine the effects of hatchery mating strategies on measures of genetic diversity, we examined the effects of different mating strategies used by management agencies. We genetically determined parentage for offspring produced from six different

mating strategies to assess individual adult male and female reproductive success, coancestry, percentage of unrelated offspring, and several measures of effective population size. We evaluated each mating strategy and project generational changes in population levels of coancestry, and the impacts resulting from stocking juveniles propagated using each mating strategy onto existing populations (Ryman & Laikre 1991).

METHODS

Gamete take/Fertilization

We examined six mating strategies used widely by hatcheries to propagate many fish species. Gametes were obtained from 10 female and 20 male sexually mature adult steelhead (*Oncorhynchus mykiss*) captured at the Little Manistee Weir on the Little Manistee River in Michigan. To standardize the number of gametes used from each individual in all experimental matings measured volumes (50 ml of eggs from each female and 1.5 ml of milt from each male) were used for each treatment. Before fertilization, eggs from one female were placed in a bowl and mixed with eggs from additional females according to treatment. Male milt was subsequently added and thoroughly mixed (as required by each protocol; see below). Fertilization was completed by cold-water hardening the eggs and milt for 5 minutes before pooling with the other fertilized eggs for each treatment. After fertilization was completed, egg lots were kept separate by treatment. Eggs were transported to the Wolf Lake Hatchery (Michigan Department of Natural Resources) and incubated in vertical stack flowing water trays separately by treatment. Once the fry had reached swim-up stage and the yolk sac was completely absorbed, all progeny were sacrificed and stored in 95% ethanol.

Experimental treatments

Treatments 1-4 used equal volumes of milt from the same 10 males, and treatments 5 and 6 used an additional 10 males (Figure 5), also with an equal volume of milt. Treatment 1 consisted of 10 replicates of 1:1 female to male crosses. Eggs from female 1 (F_1) were placed in a container and fertilized with milt from male 1 (M_1). This was repeated for each female (N=10) and the first ten males. Treatment 1 also provided a measure of the viability of each male and female used for the experimental crosses. Treatment 2 consisted of 10 replicates of 1:2 female to male crosses, where each male was used to fertilize two females. Eggs from F_1 were fertilized by the combined milt of M_1 and M_2, as were the eggs from F_2, but in a separate container. Treatment 3 was completed in four containers: eggs from females 1 through 5 were combined, mixed, and split into two lots and eggs from females 6 through 10 were combined, mixed, and split into two lots. The first lot of eggs from each of the two egg groups was fertilized by the combined milt of males 1 through 5, and the second lot was fertilized by the combined milt of males 6 through 10. Treatment 4 combined the eggs from all females (1-10), which were fertilized by the combined milt of males 1 through 10. Crosses is treatments 5 and 6 both consisted of a 1:2 female to male ratio, using each male only once (i.e. F_1 was fertilized with the milt of M_1 and M_2, F_2 was fertilized were the milt of M_3 and M_4). In treatment 5, milt was mixed prior to fertilization; in treatment 6 the milt from each male was added sequentially.

Genetic analysis

We used microsatellite loci to genotype each parent and a large random subsample of offspring from each experimental treatment. DNA was extracted from fin clips of all male and female parents (N=30) and progeny (N=220 per treatment) following the PurGene (Gentra Inc.) protocol. Fin clips from parents were stored in 95% ethanol. Microsatellite loci Ogo1a (Olsen et al. 1998), Ots1 (Banks et al. 1999), Ots100 (Nelson et al. 1998), and Omy77 (Morris et al. 1996) were used for all treatments. Locus Ogo2 (Olsen et al. 1998) was also used for Treatments 3 and 4 to increase the statistical power of likelihood-based parental assignment when the pool of potential parents was increased. PCR reactions for Ogo1a, Ots 1, and Omy77 were conducted in 25 µl reaction volumes using 100 ng DNA, 2 µl 10x PCR Buffer (0.1 M Tris-HCl, ph 8.3, 0.015 M $MgCl_2$, 0.5 M KCl, 0.1% gelatin, 0.1% NP-40, 0.1% Trition-X 100), 0.2mM dNTP's, 0.6 µM fluorescently labeled forward primer, 0.6 µM unlabeled reverse primer, and 0.3 Units Taq Polymerase. PCR reactions for Ogo2 were conducted in 25 µl volumes, 3.5 mM $MgCl_2$, and primer concentrations were reduced to 0.5 µM. PCR reactions for Ots100 used 12.5 µM $MgCl_2$. PCR reactions for all loci utilized an initial denaturing step at 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, locus-specific annealing temperature for one minute, and extension at 72° C for 1 minute. A final extension period of 2 minutes, 30 seconds was followed by storage at 6° C until electrophoresis. PCR annealing temperatures varied by loci. Annealing temperatures for Ogo1a, Ogo2, Omy77, Ots1 and Ots100 were 56° C, 54° C, 54° C, 54° C, and 58° C respectively. 6% non-denaturing acrylamide gels were used for electrophoresis and gels were visualized on a Hitachi FM-BIO II scanner. Allele sizes were determined based on a fluorescently

labeled ladder from BioVentures and individuals of known genotype were run on each gel.

Parentage assignment

Parentage was assigned for all offspring in each treatment using the program PROBMAX (Danzmann 1997). Pedigree relationships for all offspring from each of the six treatments were empirically determined by genetic determination of parentage. Data were used to examine the effects of each mating regime on measures of genetic diversity. Parentage for all offspring used for analyses were based on total exclusions of all but one maternal and paternal parent. Parentage was assigned to 194 offspring from Treatment 1, 178 offspring from Treatment 2, 162 offspring from Treatment 3, 171 offspring from Treatment 4, 181 offspring from Treatment 5, and 189 offspring from Treatment 6. Offspring were randomly chosen within each treatment for genetic analysis.

Statistical analysis

Based on the number of offspring genetically assigned per adult, we were able to determine the mean and variance of male and female reproductive success for each treatment. Variances were transformed using the Box-Cox power transformation because they greatly varied between treatments (Rao 1998), and uniformity among variances was tested using SAS software (SAS Institute). We estimated the percentage of all offspring in each treatment that were determined to be unrelated (i.e. not full or half siblings). To calculate the number of related individual pairs for both full and half siblings, the number of offspring and their relation to each other was determined by the establishment of

family groups based on the genetics-based parentage assignment. The total number of pairs was determined by factorial analysis to sample without replacement (Lindgren 1976).

Percent contribution of male parents to offspring production was calculated for each treatment to examine inter-treatment variation in male reproductive success. Milt and eggs from the same males and females were used in all treatments. For treatment 1 (1 male: 1 females), each of ten males would be expected to contribute 10% of the total progeny sampled. Results from treatment 1 form the basis for comparisons of the treatments, representing the natural background level of variation likely encountered due to female differences in reproductive condition and egg quality and size and number.

Coancestry (Θ) values for each treatment were calculated following Cockerham (1969),

$$\Theta = \frac{n_{fs}(0.25) + n_{hs}(0.125) + n_u(0)}{n_t} \qquad (1)$$

where n_{fs} is the number of full-sib pairs in each treatment, n_{hs} is the number of half-sib pairs, n_u is the number of unrelated pairs, and n_t is the total number of pairs of offspring in the treatment. The number of individuals for each sibling relationship as determined by the genetically determined pedigree was multiplied by the appropriate coancestry value for each pairwise relationship (i.e., 0.25 for full sibling and 0.125 for half-sibling pairwise relationships). The estimates of relatedness between full siblings calculated by coancestry differ from those calculated by r_{xy} coefficients because coancestry calculates the probability of identity by descent.

To place different coancestry levels resulting from each mating strategy in an applied context, such as the maintenance of a captive broodstock, we used a recursive

model to estimate and compare the rates of accumulation of coancestry over time for a simple scenario of a captive broodstock population. We assumed the population was closed, generations did not overlap, and the only reproduction that occurred was through the hatchery (artificial) mating. Six separate initial populations were started using the progeny of unrelated individuals, and the progeny were created following each of the six mating regimes evaluated in this study.

$$\Theta_{t+1} = \Theta_t + (\Theta_t r)(1 - \frac{\Theta_t}{k}) \qquad (2)$$

Initial coancestry estimates among parents used for mating in each treatment were 0. Initial levels of coancestry for population derived and maintained using each mating regime were empirically determined from the parentage analyses. The coancestry estimates resulting from each mating strategy was defined as (r), the rate of increase in coancestry for each successive generation. Coancestry estimates for successive generations (Θ_{t+1}) were a function of the coancestry estimate for the previous generation, (Θ_t), the rate of increase in coancestry as a function of the mating strategy (r), and the asymptotic value of coancestry (k, or 1).

Effective populations size (N_e) based solely on the numbers of males (N_m) and females (N_f) used were determined following Wright (1931) as

$$N_e = \frac{4N_m N_f}{(N_m + N_f)} \qquad (3)$$

An alternative and more fully parameterized measure of effective population size was calculated by first calculating the effective numbers of males (N_{em}) and females (N_{ef}) for each treatment (Lande & Barrowclough 1987) utilizing our empirical estimates of male and female reproductive variance.

$$N_{em} = \frac{\left(N_m \overline{k_m} - 1\right)}{\overline{k_m} + \left(\sigma^2{}_{km} \big/ \overline{k_m}\right) - 1} \tag{4}$$

and

$$N_{ef} = \frac{\left(N_f \overline{k_f} - 1\right)}{\overline{k_f} + \left(\sigma^2{}_{kf} \big/ \overline{k_f}\right) - 1} \tag{5}$$

respectively, where $\bar{k}$ is the mean number of progeny produced by either the males (k_m) or female (k_f) for each treatment, and σ^2 is the variance in the number of progeny for the males or females. The variance population size (N_{ev}) for each treatment was then calculated (Lande & Barrowclough 1987)

$$N_{ev} = 4\left[\frac{1}{N_{em}} + \frac{1}{N_{ef}}\right]^{-1} \tag{6}$$

We also calculated coancestral effective population size ($N_{e\Theta}$) for each of the size mating strategies. Defined by Chesser et al. (1993), $N_{e\Theta}$ is the number of breeding individuals consistent with observed accrual of gene correlations over generations. Due to our ability to calculate coancestries of progeny arrays resulting from each mating strategy (assuming Θ=0 for adults spawned to produce the progeny in all mating strategies), we calculated the instantaneous coancestral effective size (Chesser et al. 1993)

$$N_{e\Theta} = \frac{1}{2\Delta\Theta} \tag{7}$$

To compare relative input of reproductive variance on N_e for each of the six mating regimes for situations where hatchery fish are used to supplement a wild population, we followed the model

$$\frac{1}{N_e} = \frac{x^2}{N_c} + \frac{(1-x)^2}{N_w} \quad (8)$$

used by Ryman and Laikre (1991) where x and $(1\text{-}x)$ are the relative contribution of the offspring of captive (x) and wild ($1\text{-}x$) parents, where the probability that two random individuals were derived from the same group (captive or wild) were estimated to be x^2 and $(1\text{-}x)^2$ respectively. Estimates of N_e, N_c, and N_w were the effective populations sizes of the total, captive, and wild populations respectively. Variance effective population sizes were calculated for each treatment and were used to determine total effective size given a range of relative contributions by hatchery fish to a wild population as per Ryman & Laikre (1991). Initial effective population sizes for the total population were 40 and 400 individuals.

RESULTS

Assessment of reproductive variation

The viability of the gametes of each individual male and female was determined by the single paired (1:1) matings in treatment 1 (Figure 5). Mean numbers of offspring produced by each female did not significantly differ between treatments. Tests of heterogeneity of variance revealed mean female reproductive output did not differ significantly among treatments ($P>0.05$). In contrast, comparisons of male reproductive output among all treatments revealed significant differences between treatment 1 and treatment 5 ($P<0.05$). The mean number of offspring produced per male also did not

significantly differ among treatments 1 through 4, but was reduced in treatments 5 and 6 to reflect the increased number of males used for fertilization (Table 5). Variance in number of offspring per male differed greatly among treatments. High variances reflect non-uniform contribution of males to reproduction, and were greatest in treatment 3 (Table 5).

Estimated contributions varied among males for each treatment (Figure 6). In treatment 1, the estimated contribution of progeny ranged from 14.4% for males 1 and 9, and 4.2% for male 7 (Figure 6). Male contribution for treatments 5 and 6 (n=20) would be expected to be 5%, but estimated contribution varied greatly. Males 6 and 8 contributed the majority of progeny (18.5% and 16.7% respectively) for treatment 3 (5 males: 5 females; Figure 6). In treatment 4 (10 males: 10 females), males 4, 8, and 9 contributed 10.5%, 16.4%, and 29.8% respectively of the progeny (Figure 6). In treatments 3 through 6, male 10 did not contribute any progeny (Figure 6). Female contribution was relatively consistent among individuals and among treatments (Figure 6).

Effects of reproductive variation on measures of genetic diversity

We determined the effects of each mating treatment on summary measures of genetic variation for progeny sampled. The controlled nature of all experimental treatments allowed us to estimate all measures empirically. Mating regimes resulting in the lowest mean levels of coancestry are desirable, as lower average levels of gene correlations among progeny indicate a lower rate of allele sharing and lower levels of inbreeding in progeny of the next generation. In treatment 1, a higher proportion of all

offspring surveyed were unrelated (90.2%; Table 6) compared to progeny from all other treatments. In contrast, treatment 3 had the lowest proportion of unrelated offspring (74.4%; Table 6). Progeny produced in treatments 1, 5, and 6 exhibited the lowest mean coancestry values (mean Θ=0.024, 0.023, and 0.023 respectively; Table 6). Estimates of coancestry of progeny from treatments 2, 3, and 4 were comparatively higher (mean Θ=0.029, 0.034, and 0.033 respectively; Table 6) due to greater reproductive skew of males.

Estimates of effective population size varied by treatment and method of estimation. N_e estimated based solely on numbers of males and females used in each mating regime were consistent for treatments 1 through 4 due to the use of 20 individuals. Increased estimates of N_e for treatments 5 and 6 reflect the use of 10 additional males (total N=30). A more fully parameterized estimate of variance effective size (N_{ev}) incorporated male and female reproductive variance for each treatment. Across all treatments, estimates of N_{ev} were lowest for treatments 3 and 4 (N_{ev}=14.1 and 14.7 respectively; Table 6). Estimates of N_{ev} for the treatments employing 20 parents were highest for treatment 1, being most representative of the actual number of parents mated (N_{ev}=19.2; Table 6). The highest estimate of N_{ev} were documented for treatments 5 and 6, but unlike Treatment 1, estimates were not reflective of the actual number of individuals used (Total N=30; N_{ev}=21.8, 21.7 respectively; Table 6). Data conclusively show that estimates of N_e offered solely based on numbers and sex ratio of parents mated, without inclusion of reproductive variance are decidedly upwardly biased.

Population projections of the accumulation of mean coancestry over time assuming closure for population (or domestic broodstock) demonstrated that the expected

rate of increase in coancestry over successive generations was dependent on the hatchery mating regime employed. Coancestry values accrued at higher rates in populations maintained by breeding regimes characterized by induced polygny (individual males mated with multiple females; Figure 7). Mating regimes used in treatments 2, 3, and 4 resulted in higher coancestry values than treatments 1, 5, and 6 (Table 6). Accordingly coancestry values associated with those mating regimes increased more rapidly over successive generations than was observed for other treatments. Mating regimes that resulted in progeny showing the slowest increase in coancestry were those that did not use either parent repeatedly during spawning, or those that pooled gametes prior to fertilization (Table 6; Figure 7). The use of each parent once throughout the treatments decreased the number of sibling relationships through the reduction in the number of half-sibling relationships (data not shown). Mating regimes that resulted in progeny with the highest mean coancestry also reached the level of full sibling relationships (Θ=0.25) over the shortest time interval (Figure 7).

The effects of supplementation of a wild population on the total N_e also varied by hatchery mating strategy. When total population size was small (e.g., N=40; Figure 8a), supplementation can demonstrably increase the total N_e ranged over a relatively broad range of hatchery contributions. This trend was observed with each mating strategy. The treatments that produced the highest N_{ev} (treatments 1, 5, and 6; Table 6) had the largest range of relative contribution resulting in an increase in total N_e (Figure 8a). When the total population size of is large (N_e=400), only contributions of less than 10% of hatchery-produced individuals (for all treatments) resulted in an increase in total N_e (Figure 8b).

DISCUSSION

The use of different guidelines for hatchery mating strategies has emphasized the importance of maintaining genetic diversity (Waples et al. 1990; Kincaid et al. 1993). The need for analysis of hatchery mating strategies is increasing as the role of hatcheries changes from supplementing to sustaining fish populations (Ryman 1991). By experimentally creating artificial crosses in a hatchery environment, we were able to quantify the effects of six commonly employed mating regimes on measures of genetic diversity, and offer recommendations regarding the relative success by which each mating regime can achieve goals for the production of progeny with high levels of genetic diversity.

Effective population size is commonly used predictive measure of generational changes in allele frequency, heterozygosity, and inbreeding (Ryman & Laikre 1991; Kincaid 1995; Tringali & Bert 1998). In the absence of information on male and female reproductive success, estimates of N_e offered based on evaluations of hatchery mating strategies are typically derived using the numbers and sex ratio of adults spawned. We document that estimates of N_e based on the numbers and sex ratio of parents used overestimated the effective population size when estimates of reproductive variance were not incorporated. Previous studies have documented variance in reproductive success (particularly of males) in mating scenarios occurred when gametes from multiple individuals were combined prior to fertilization (Gharrett & Shirley 1985; Withler 1988; Gile & Ferguson 1990; Gile & Ferguson 1995). With the exception of the single pair

mating treatment (1 male: 1 female), all other mating strategies we examined spawned females with multiple males.

Male reproductive variance has often been attributed to variation in male potency (Gharrett & Shirley 1985), timing of the application of the sperm to the eggs (Gharrett & Shirley 1985; Gile & Ferguson 1995), number of males used (Gile & Ferguson 1990), other characteristics such as biochemical interactions with the sperm and egg (Aas et al. 1991), or to sperm competition. The effects of reproductive variance on levels of genetic variation and relatedness of progeny, while potentially adaptive in the wild (through selection on traits associated with fitness), were found to result in decreased genetic variation and increased relatedness among hatchery-produced offspring (Table 6).

Coancestry has not been commonly used to evaluate relatedness among progeny produced in hatcheries. Many studies have used kinship (r_{xy}) as a surrogate measure of Θ to estimate genetic variation within populations (Blouin et al. 1996; Norris et al. 2000; Bentzen et al. 2001; Ruzzante et al. 2001). We used coancestry rather than estimates of r_{xy} to measure the degree of relatedness among individuals because we were able to determine parentage of each individual offspring directly, and subsequently determine the relationships among all pairwise combinations of progeny. Empirical documentation of coancestry among all progeny allowed us to predict population level increases in relatedness over time assuming the parents used for initial matings were not related. By incorporating the coancestry values resulting from each of the mating strategies examined into the models described in Chesser (1991a; 1991b) and Chesser & Baker (1996), we were able to demonstrate that mating strategies differently affect the rate at which gene correlations accrue in a closed population. Maintaining low levels of mean coancestry

over successive generations is important because higher mean coancestry of potential parents leads to higher levels of inbreeding in progeny in the next generation, and concomitant declines in fitness (Lynch & O'Hely 2001; Ford 2002). The management and conservation implication of long-term maintenance of genetic variation applies to situations where a broodstock or captive population is maintained in order to act as a source for supplementation or restoration of a wild population (see Hedrick et al. 2000).

Hatchery supplementation of wild populations has been a commonly used management strategy, and is increasingly used for purposes of conservation (Waples 1991a; Ryman et al. 1995; Hedrick et al. 2000; Hansen et al. 2001). We apply the Ryman and Laikre (1991) model to evaluate whether effective population size of wild populations can be enhanced through hatchery supplementation. We incorporate an additional comparison based on the expected effects of different hatchery mating regimes. Even if the genetic diversity of a captive population is maximized (by choosing a strategy that maximizes the effective population size), the relative contribution of the hatchery population can greatly impact the total effective population size for natural populations. Realized increases in total effective population size only occurs at very small stocking rates, particularly when there is a large discrepancy between the effective population sizes of the hatchery and wild populations as was the case across the six mating regimes.

This study is the first to empirically examine the impacts of multiple hatchery-mating strategies used by management agencies on multiple measures of genetic diversity. We expand upon earlier contributions (e.g., Ryman & Laikre 1991) by incorporating more fully parameterized models for derivation of N_e contrast different

mating strategies. Although reproductive variance is known to occur when gametes are pooled, the extent to which this variance would impact estimates of effective population size or coancestry were not known, neither have projections of long-term retention of genetic diversity resulting from each mating strategy been possible without empirical demonstrations of expected reproductive variation. Mating regimes that minimize reproductive variance, maximize the percentage of unrelated offspring, minimize coancestry, and maximize variance effective population size are recommended for hatchery use of maintenance of genetic variation is a management goal. Of the mating regimes we used, single-pair matings between one male and one female, or those that mated two unique males per female, best met these criteria. We offer these mating strategies be adopted as the preferred method of propagation. Summary measures of genetic diversity (e.g. proportions of unrelated offspring and mean coancestry) can provide valuable insight into likely trajectories of generational change in population levels of genetic diversity when different hatchery mating regimes are emphasized, and should, when possible, be incorporated into hatchery management.

Chapter 3

TEMPORAL COMPARISONS OF GENETIC DIVERSITY IN LAKE MICHIGAN STEELHEAD (*ONCORHYNCHUS MYKISS*) POPULATIONS: EFFECTS OF HATCHERY SUPPLEMENTATION

ABSTRACT

Steelhead (*Oncorhynchus mykiss*) were first introduced into the Great Lakes in the late 1800's. Subsequently, natural recruitment across the Lake Michigan basin has been regularly supplemented by primarily one hatchery strain. Recently, multiple strains derived from locations across the species native range along the west coast of the United States have also been stocked by different management agencies. Prior to 1983, hatchery supplementation of Lake Michigan steelhead populations in Michigan was largely unsuccessful due to low smolting rates of small (<120mm) hatchery yearlings (estimated survival 0.01%). Accordingly, contributions of hatchery fish to historical adult spawning runs in Michigan tributaries were low (0-30%) across six major drainages. Large yearlings of different hatchery strains (>150mm) have been stocked exclusively since 1983, increasing estimates of survival to smolting (90%). Consequently, the proportion of hatchery adults in spawning runs increased to 13-79%. We examined the effects of changes in stocking practices on straying of hatchery steelhead and to temporal changes in levels of genetic diversity and relationships among populations. We used microsatellite loci to estimate allele frequencies for six populations sampled for two time periods (1983-1984 and 1998-1999). Measures of inter-population divergence (mean F_{ST}) were not significant for either time period. However, spatial genetic relationships among historical and contemporary populations were significantly correlated with geographic distance; a result not expected if gene flow (natural straying) among

populations was mediated solely by hatchery supplementation. Increased numbers of alleles in spawning adults from populations can be attributed to alleles specific to recently introduced hatchery strains.

INTRODUCTION

Molecular markers have been used widely in studies of Pacific salmon species (*Oncorhynchus spp.*) to document interactions between natural populations and between fish from natural and hatchery origins. For steelhead (*O. mykiss*), molecular markers have identified genetic population structure at micro- and macro-geographic spatial scales (Parkinson 1984; Reisenbichler & Phelps 1989; Reisenbichler et al. 1992; Beacham et al. 1999; Nielsen 1999; Nielsen & Fountain 1999; Osterberg & Thorgaard 1999). Changes in population estimates of allele frequency and genetic diversity have been used to examine how levels of genetic variation within and among populations have changed over time (Heath et al. 2002), and have provided insight into factors (both natural and anthropogenic) contributing to temporal change (Waples 1998; Garant et al. 2000).

Hatchery supplementation has been used widely for salmon populations. Concerns regarding the potential for interactions between individuals of wild and hatchery-origin have increased the awareness of the need to re-evaluate hatchery management practices (Ryman 1991; Waples 1991a; Lynch & O'Hely 2001). Theoretical and empirical evidence (Washington & Koziol 1993; Gharrett 1994; Lynch 1997; Reisenbichler & Rubin 1999) has focused on potential consequences of introgression and breakdown of physiological or biochemical compatibilities between genes (co-adapted gene complexes) in wild and/or naturalized populations (Lynch &

O'Hely 2001). One hypothesized mechanism for outbreeding and potential fitness reduction in salmonids relates to increased levels of gene flow (straying) between hatchery and native or naturalized populations leading to the breakdown of locally adapted gene complexes.

Pacific salmon generally exhibit low levels of straying to non-natal spawning grounds (Quinn 1993). However, increased levels of gene flow between hatchery and native or naturalized populations may occur as hatchery fish exhibit higher rates of straying from rivers into which they were stocked (see Waples 1991a). While co-occurrence of hatchery and native fishes has been widely reported (largely from direct observations of tags or fin clips), evidence for introgression and consequences to population viability are not widely known. Assessments of the magnitude of genetic change within and among populations would profit from knowledge of historical benchmarks of genetic characteristics prior to outbreeding events. Reisenbichler & Phelps (1989) hypothesized that introgression between native steelhead and hatchery steelhead widely stocked across large areas led to lower inter-population levels of genetic diversity. However, Reisenbichler & Rubin (1999) suggest that although interbreeding may occur between hatchery and wild individuals, the potential reproductive contribution of hatchery fish to wild spawning populations could be quite low (due to lower reproductive success of hatchery individuals).

Steelhead were introduced into the Great Lakes in the late 1800's (MacCrimmon & Gots 1972). Natural reproduction primarily occurs in Michigan rivers due to the lack of suitable spawning habitat elsewhere in the Lake Michigan basin (MacCrimmon & Gots 1972). Widespread natural reproduction in the Great Lakes led to development of

self-sustaining populations. However, natural recruitment has been supplemented by hatchery-production (Biette et al. 1981; Seelbach 1987a). Management agencies around the Lake Michigan basin stock multiple steelhead strains to take advantage of strain-specific variation in life history characteristics (i.e., run timing) that are valued by recreational anglers. Because naturalized steelhead spawn in drainages in only a portion of the basin, stocking acts to increase the number of fish present in rivers during spawning runs, and to increase the distribution of steelhead in open-water areas throughout the basin. One hatchery strain (the Michigan strain, derived from the Little Manistee River) has historically been used across the basin, and is presently used for supplementation by the state of Michigan. Recently released hatchery strains include the Chambers Creek and Skamania strains originating from Washington, and the Ganaraska strain established from the naturalized population in the Ganaraska River in Ontario. Currently, genetic differences between the four hatchery strains represent the major component of genetic diversity in Lake Michigan steelhead (Bartron et al. in review). Introgression among hatchery strains stocked by other agencies across the Great Lakes basin may lead to increasing levels of straying, outbreeding, and loss of strain-specific hatchery traits.

Changes in hatchery management practices have increased the relative contribution of hatchery steelhead to naturalized populations in Michigan. The state of Michigan changed the size and age of stocked juvenile steelhead in 1983, from stocking fall fingerlings (<110mm) to large yearlings (>150mm; Seelbach 1987a). Larger juveniles had higher rates of smolting (mean 48.2% vs. 0.5-2.9%; Seelbach 1987a) and survival to smolt stage (90% vs. 0.01%; Rand et al. 1993) compared to juveniles stocked

at smaller sizes. Prior to changes in age and size at stocking, the average contribution of hatchery fish to spawning runs in six Michigan rivers was generally low (0-30%; Seelbach & Whelan 1988). Following the change in stocking practices, the contribution of hatchery fish in the same six rivers during the 1998-1999 spawning run substantially increased (13-79%; Bartron et al. in review).

In this study, we compared allele frequencies and levels of genetic diversity for two different time periods (1983-1984 and 1998-1999) for each of six populations before and after changes in juvenile age at stocking. Comparisons should show whether increased survival of hatchery juveniles and abundance of adult hatchery steelhead led to increased levels of introgression as evidenced by altered genetic characteristics within, and inter-relationships among naturalized populations. Our two main objectives were 1) to determine if time series data on survival and stocking histories for steelhead in the Lake Michigan basin were suggestive of increasing threats of introgression between hatchery and wild individuals, and 2) using time-series data of population allele frequencies and estimates of genetic diversity, to determine if the increased presence of hatchery individuals during the spawning period had demonstrable effects on population genetic characteristics of naturalized populations.

METHODS

Stocking history

Original introductions of steelhead into the Great Lakes in the late 1800's utilized gametes taken from rivers in California (primarily McCloud River, Klamath River, and Redwood Creek), and the Willamette and Rogue rivers in Oregon (MacCrimmon & Gots

1972). Within Lake Michigan specifically, steelhead (or non-anadromous rainbow trout) from the McCloud River were first stocked in 1896 (MacCrimmon & Gots 1972). Naturalized populations were well established and widely distributed around the Lake Michigan basin by the 1920's although primary reproduction occurred in Michigan due to the abundance of spawning habitat (MacCrimmon & Gots 1972). Supplemental stocking within Lake Michigan did not occur again until the mid 1950's following a decline in the lake-wide steelhead population (MacCrimmon & Gots 1972). Until the mid-1980's, stocking primarily utilized one hatchery strain, derived from returning adults captured at the Little Manistee weir in Michigan's Little Manistee River (Figure 9). Starting in the mid 1980's, additional hatchery strains have been used for stocking by states around the Lake Michigan basin. These additional hatchery strains include: the Chambers Creek strain from the Puget Sound region of Washington; the Skamania strain, from the Washougal River in Washington; the Ganaraska strain, from the Ganaraska River in Ontario, Canada. The Ganaraska and Michigan strains originate from naturalized populations within the Great Lakes. Each of the four hatchery strains stocked into Lake Michigan are maintained by gametes taken from adults returning to weirs located on four rivers around the Lake Michigan basin each year. No specific broodstocks are maintained. Thus, unintentional straying and introgression of fish of multiple strains could compromise the genetic integrity of each strain.

Stocking records of Michigan hatchery strain steelhead for the Lake Michigan basin (Table 7) and specifically for the Lake Michigan tributaries examined in this study (Table 8) were obtained from the Michigan Department of Natural Resources for two time periods (1979-1982 and 1993-1997). Time periods represent years preceding the

two sampling events of adult steelhead spawning runs, and reflect cohorts that may contribute to the spawning runs sampled. Smolt-equivalents were calculated from estimates of survival rates of juvenile steelhead stocked into Lake Michigan based on age, size, and stocking location (Rand et al. 1993).

Sample collection

Samples from adult steelhead spawning populations were obtained from six populations for each of two time periods, before (1983-1984) and after (1998-1999, 2000) changes in stocking practices. Adult steelhead from both time periods were sampled from the Betsie River, Manistee River, Little Manistee River, Pere Marquette River, White River, and Muskegon River (Figure 9). Historical samples were based on archived scale samples held at the Michigan Department of Natural Resources Institute for Fisheries Research (Ann Arbor, Michigan) that had been obtained by creel surveys and hook and line sampling of adult steelhead. Archived scales were sampled from adults during the fall 1983 and spring 1984 spawning runs in each river (Seelbach & Whelan 1988). Among historical populations, fall 1983 and spring 1984 samples were obtained for the Betsie, Little Manistee, and White rivers. The Manistee, Pere Marquette, and Muskegon rivers were only sampled in the fall of 1983. Contemporary populations in the Manistee, Little Manistee, Pere Marquette, and Muskegon rivers were sampled during both fall 1998 and spring 1999. The White and Betsie rivers were sampled in the spring of 2000. Among historical populations, the Betsie (fall run N=17; spring run N=33), Little Manistee (fall run N=29; spring run N=29), and White (fall run N=7; spring run N=6) rivers were sampled during both fall and spring runs. Among contemporary

populations, the Manistee (fall run N=53; spring run N=105), Little Manistee (fall run N=57; spring run N=60), Pere Marquette (fall run N=29; spring run N=73), and Muskegon (fall run N=37; spring run N=39) rivers were sampled during both fall and spring runs. Contemporary populations were sampled by electrofishing and creel surveys of adult steelhead returning to Michigan rivers throughout the fall of 1998, spring 1999, and spring of 2000, to include samples from the entire spawning period.

Scale pattern analysis (SPA)

Scale pattern analysis (SPA) was used to determine hatchery or natural origin of each adult steelhead (Seelbach & Whelan 1988). SPA for fish sampled from both historic and contemporary time periods was performed using the ratio 23 method (Seelbach & Whelan 1988). Ratio 23 quantifies differential winter and spring growth rates in juvenile steelhead through comparison of the width of the five intercirculus spaces prior to the first annulus to the width of the five intercirculus spaces that follow the first annulus (Seelbach & Whelan 1988). For contemporary populations, juvenile stream resident time (Bartron et al. in review) was also used to identify hatchery-origin individuals. Juvenile stream resident time was used when the ratio 23 value was between 0.7 and 0.8 (0.7 being the threshold for determination of hatchery or natural-origin). Hatchery yearlings migrate downstream to Lake Michigan within a year after stocking, whereas juvenile steelhead produced in the wild may reside in the stream for more than one year prior to smolting (Seelbach 1987a). The use of an additional technique to identify hatchery-origin individuals was necessary due to changes in growth patterns resulting from the increased size and age-at-stocking of hatchery fish. The likelihood of

classification errors resulting from the ratio 23 technique is discussed in Seelbach & Whelan (1988). All steelhead identified as originating in hatcheries based on SPA were removed from the genetic analysis.

Genetic analysis

After removal of hatchery individuals from each population, sample sizes for historical and contemporary populations for each river were: Betsie River (N=49 and N=25), Manistee River (N=52 and N=107), Little Manistee River (N=57 and N=116), Pere Marquette River (N=10 and N=102), White River (N=15 and N=18), and Muskegon River (N=7 and N=75). DNA was obtained from scales and fin clips. DNA from scales was extracted using Qiagen DNeasy® Tissue Kit (Qiagen Inc.). DNA from fin clips was extracted using PurGene® (Gentra Inc.) protocols. Individuals were genotyped at six microsatellite loci, including Ogo1a and Ogo4 (Olsen et al. 1998), Omy77 (Morris et al. 1996), Oneμ10 (Scribner et al. 1996), Ots103 (Beacham et al. 1998), and Oki200 (Beacham et al. 1999). PCR reactions for Ogo1a, Omy77, Oneμ10, and Ogo4 followed protocols described in Bartron et al. (in review). PCR reactions for Ots103 and Oki200 were conducted in 10 μl volumes using 40 ng DNA, 1 μl 10x PCR Buffer (0.1 M Tris-HCl, pH 8.3, 0.015 M $MgCl_2$, 0.5 M KCl, 0.1% gelatin, 0.1% NP-40, 0.1% Triton-X 100), 0.2 mM dNTP's, 0.4 μM fluorescent labeled forward primer, 0.4 μM unlabeled reverse primer, and 0.3 units Taq Polymerase. PCR reactions for all loci utilized an initial denaturing step at 94° C for 2 min., followed by 30 cycles for tissue-derived DNA and 40 cycles for scale-derived DNA of 94° C for 1 min., annealing temperature for 1 min., and extension at 72° C for 1 min., and a final extension period of 2.5 min. The annealing

temperature for Ots103 and Oki200 was 50° C. Genotypes for Ogo1a, Ogo4, Omy77, and Oneμ10 were visualized on a Hitachi FM-BIO® II scanner. Genotypes for Ots103 and Oki200 were visualized on a LI-COR® IR^2 Global Edition DNA Sequencer. Molecular weight standards and individuals of known genotype were run on each gel to standardize scoring.

Statistical analysis

Samples were taken over two portions of the spawning run (fall and spring) from most populations. We used Fisher's exact test (GENMOD procedure) in SAS software (SAS Institute 1999) to determine if proportions of hatchery and river-origin individuals differed between fall and spring runs, and between historical and contemporary time periods.

Estimates of observed and expected heterozygosity and allele frequencies for each population were calculated using BIOSYS-1 (Swofford & Selander 1981). Significance of deviations of genotypic frequencies from Hardy-Weinberg expectations were determined for each population using Markov chain methods of Guo & Thompson (1992) implemented in program GENEPOP (Raymond & Rousset 1995). Allelic richness values for each population for each locus (Petit et al. 1998) were calculated using FSTAT (Goudet 1995) v2.9.3.1 to standardize population measures of allelic diversity for differences in sample size. Mean allelic richness estimates for each population were presented as the sum of the allelic richness values assayed.

Pairwise F_{ST} (Weir & Cockerham 1984) comparisons were used to determine if genetic differences existed between fall and spring runs for those rivers that were

sampled for both runs. In the absence of differences, fall and spring samples were combined for analyses of population differences. Estimates of pair-wise and overall population differentiation were summarized using F-statistics, implemented in the program FSTAT (Goudet 1995) v.2.9.3.1. Nominal alpha values were corrected for multiple pair-wise comparisons using Bonferroni corrections (Rice 1989). Cavalli-Sforza & Edwards (1967) chord distances were estimated for all population comparisons during both time periods using BIOSYS-1 (Swofford & Selander 1981) and were used to construct neighbor-joining (Saitou & Nei 1987) dendrograms. Neighbor-joining trees and associated bootstraps (500 iterations) were generated using PHYLIP v3.5 (Felsenstein 1993). The resulting trees were displayed using TREEVIEW (Page 1996).

Generalized Mantel tests (Smouse et al. 1986) were used to test for correlations between genetic relationships (inter-population genetic distance) and population geographic proximity, providing measures of spatial autocorrelation in allele frequency. Geographic distances (km) were estimated between river mouths along the Lake Michigan shoreline.

RESULTS

Hatchery contributions to spawning runs

Stocking histories for steelhead of the Michigan hatchery strain into rivers in Michigan revealed a decline in the number of steelhead stocked over time (Table 7). On average, 1,045,737 Michigan-strain steelhead were stocked into Lake Michigan each year between 1979 and 1982, whereas an average of 524,895 were stocked each year between 1993 and 1997 (Table 7). Following the change in age and size at stocking, the estimated

number of stocked juvenile steelhead that survive to smolting increased from 4.1% to 83.2% between the two time periods (Table 7). Survival estimates were comparable to those of river-origin steelhead in Lake Michigan, which ranged from 13-90% for presmolt winter survival (Seelbach 1987b). Between 1978 and 1982, the estimated yearly mean number of Michigan strain juveniles stocked into Lake Michigan surviving to smolt stage was 33,509 (Table 7), compared to a mean of 437,061 between 1993 and 1997 (Table 7). Between 1983-1984 and 1998-1999, the mean number of Michigan strain steelhead stocked into the six rivers examined in this study that survived to smolt stage increased from 3,054 to 28,269 (Table 8). Estimates of natural recruitment are not available for the six rivers, however the ten-fold increase in hatchery contributions that survive to the smolt stage suggests an increased potential for straying of adult hatchery fish into natural spawning populations across the Lake Michigan basin.

We observed an increase in the proportion of hatchery-origin individuals found in adult steelhead spawning runs for six rivers in Michigan between the 1983-1984 and 1998-1999 spawning runs (Table 8). The contribution of hatchery steelhead in the six rivers examined averaged 12% during 1983-1984 spawning run, and 40% during the 1998-1999 spawning run. Significant differences were observed in hatchery contributions to the spawning run of each river between the historical and contemporary populations for the Little Manistee River ($X^2_{df=1}=37.86$; $P<0.0001$), Manistee River ($X^2_{df=1}=15.54$; $P<0.0001$), Muskegon River ($X^2_{df=1}=10.49$; $P<0.0012$), and White River ($X^2_{df=1}=5.38$; $P<0.02$).

Genetic analysis

The mean number of alleles per locus across all loci increased over time (comparisons of historical and contemporary populations; Table 9). The mean number of alleles per locus ranged from 4.2-4.7 for the historical populations and 5.3-7.8 for the contemporary populations (Table 9; specific allele frequencies are provided in Appendix 1). When measured by allelic richness to correct for sample size, the mean number of alleles per locus increased between time periods for three of the six rivers (Table 9). Alleles not found in historic populations were observed in contemporary populations, half of which are found in one or all of the Skamania, Chambers Creek, or Ganaraska hatchery strains (Appendix 1). Five alleles (in loci Ogo4 and Omy77) were found in the contemporary populations, and in three of the hatchery strains, but not in the historic populations (Appendix 1). One allele (in locus Ogo1a) was absent from historic populations except for the Little Manistee, but was found in three of the other five contemporary naturalized populations (Appendix 1). Given that the majority of common alleles are shared across strains and naturalized populations, estimates of introgression based solely on the appearance of rare alleles are likely under-estimates. Mean observed heterozygosity for the historical populations ranged from 0.563 to 0.632 and from 0.555 to 0.686 for the contemporary populations (Table 9). Deviations from Hardy-Weinberg equilibrium were not observed in any population sampled during either time period ($P>0.05$).

We found no evidence for significant differences in allele frequency between fall and spring portions of spawning runs within rivers. Variance in allele frequency as estimated using F_{ST} values for comparisons between fall and spring runs sampled within each river for each time period did not indicate evidence for significant genetic

differentiation ($P>0.05$ for mean F_{ST} values across all pair-wise comparisons). Consequently, samples from each run were combined for those populations that included samples from the fall and spring runs. Mean F_{ST} values when estimated across all six populations for each time period were not statistically significant (mean F_{ST} for historical and contemporary populations were 0.006 and 0.002 respectively; Table 10).

Mantel tests were conducted using genetic distances among populations from each time period and for correlations between inter-population genetic distances among populations. The Mantel tests indicated that genetic relationships among populations were consistent between the two time periods ($R=0.65$; $P<0.01$; Figure 10). Correlations between the geographic and genetic distances between each spawning population were significant for historic populations ($R=0.55$; $P<0.01$), indicating significant spatial autocorrelation in allele frequencies between population (isolation by distance) relationships. Relationships between genetic and geographic distance were also evident for contemporary populations, though the relationship was less strongly supported ($R=0.39$; $P<0.02$). Neighbor-joining tree topology representing inter-population variation in allele frequency across loci as viewed in the Cavalli-Sforza & Edwards (1967) distances among historic populations generally grouped populations with geographic neighbors, but topology may have been biased by populations with small sample sizes (i.e. Muskegon, Pere Marquette, and White river populations). Among contemporary populations grouping was more random, and again may be biased by populations with small sample sizes (i.e. Betsie and White rivers; Figure 10). Nodes for both trees were weakly supported by bootstrap values (Figure 10).

DISCUSSION

Widespread stocking of hatchery fish has raised concerns regarding the potential for interactions between hatchery and wild populations of many fish species. Particular emphasis has been placed on salmonid populations in their native range due to their socio-economic importance and to dramatic declines in population numbers and distribution. Individuals of hatchery origin commonly co-occur with wild individuals, which pose increased risks of introgression. Outbreeding can lead to the loss of genetic adaptations within populations (Lynch & O'Hely 2001). Loss of naturally evolved co-adapted gene complexes may not be a pressing issue in introduced and artificially maintained systems, such as those that currently exist in the Great Lakes. However, phenotypic differences (e.g. variation in run timing) are often exploited by managers to provide increased recreational fishing opportunities for anglers. In Lake Michigan, multiple hatchery strains with evolved tendencies to enter rivers at different times of the year are stocked to extend the duration of once seasonal fisheries. Concurrent stocking of multiple strains increase risks of introgression among strains and between individuals of hatchery and natural origin. Variation among strains in phenotype or life history, whether adaptive or not, is useful to managers and is at risk of loss through outbreeding.

Although large numbers of juvenile steelhead are stocked yearly (Table 7), natural reproduction provides a substantial component of the steelhead production to Lake Michigan, and hence to the basin-wide recreational fishery. Rand et al. (1993) estimated that steelhead smolts emigrating from the Little Manistee River contribute 13-21% of the total basin-wide smolt yield. Although the total number of hatchery steelhead stocked in Michigan has decreased since the early 1980's (Table 7), survival of hatchery fish to

smolt-stage has increased (Table 8). As a consequence, the proportion of hatchery fish present in spawning runs increased significantly between the two time periods (Table 8). Clearly, increased abundance of hatchery adults in spawning runs increases the potential for introgression between the hatchery and naturalized populations of steelhead in tributaries across the Lake Michigan basin. Previously, there was a lack of information regarding how the threat of increased introgression translated to actual outbreeding.

We did not observe strong evidence of increased levels of gene flow on the basis of changes in magnitude of inter-population variance in allele frequency as seen from F-statistics. However, our ability to detect effects of potential introgression if occurring (i.e. statistical power) was low, as historical populations were not significantly diverged genetically. We did however observe significant correlations between genetic distance and geographic distance among populations, suggesting that steelhead populations in Michigan (both historical and contemporary) do not represent a single panmictic population, but rather are structured as a function of geographic distance among populations. Decreased correlations between genetic and geographic distance in contemporary populations as compared to historical populations are suggestive of some level of changes in patterns of gene flow or homogenization of allele frequencies due to increased introgression as would be expected between hatchery and naturalized individuals.

Recent introduction of new genetically differentiated hatchery strains by states across the Lake Michigan basin has let to increasing incidence of these strains in Michigan rivers (Bartron et al. in review). Introgression most likely has contributed to increased levels of within-population diversity for most contemporary populations

surveyed in this study. Spatial variance in allele frequency represents a small component of the total genetic variation apportioned across the Lake Michigan basin. Genetic diversity within populations was higher in contemporary populations than in historical populations due to increased mean number of alleles per locus. The appearance of alleles not found in historical populations is due to the recently introduced hatchery strains (Appendix 1). Since all fish surveyed are of wild origin, the formerly unseen alleles can only be attributed to past introgression.

The absence of significant genetic structure among historical populations was not unexpected because of the proximity of natural populations to each other, and the relatively short time steelhead have been present in Lake Michigan. Differentiation over relatively short geographic distances was detected among steelhead populations within the Skeena River (Beacham et al. 2000). On a larger spatial scale, Krueger & May (1987) analyzed steelhead populations within the Lake Superior basin and found greater levels of population differentiation (F_{ST}=0.026). Observation of significant spatial structure by Krueger & May (1987) was potentially due to the sampling of juveniles, and spatial structure could reflect family groups rather than population differences (Allendorf & Phelps 1981).

Despite lack of significant spatial genetic differentiation, genetic characteristics observed within steelhead populations in Lake Michigan (such as the lack of significant genetic differences between fall and spring run steelhead) are consistent with comparisons of winter and summer-run steelhead in their native range (Chilcote et al. 1980; Leider et al. 1984). Heath et al. (2002) found temporal comparisons of genetic diversity among steelhead populations to be much greater over a shorter time period than

was found among Michigan populations. The lack of greater temporal genetic divergence among Michigan populations could be attributed in part to disparities in sample size obtained for populations in each time period. However, three of the historic populations had large sample sizes for both time periods and pair-wise comparisons between those populations and their contemporary counterpart did not indicate significant differences.

Within regional spatial contexts such as the Great Lakes, gene flow among fish from naturalized populations may be high due to natural levels of straying. However, the Great Lakes basins share a number of characteristics of other multi-jurisdictional fisheries resources that are increasingly at risk to accelerated levels of outbreeding due to increased reliance on hatchery supplementation. In the Great Lakes, different agencies stock fish of different genetic backgrounds. Due to higher rates of straying by hatchery fish (Waples 1991a), the potential to interbreed with native or naturalized fish from locally adapted gene pools increases as seen by levels of gene diversity within and among steelhead populations in Lake Michigan. Concomitantly, apportionment of genetic diversity may be altered within and among populations. Findings of significant correlations of geographic and genetic distance in contemporary populations suggest that contributions to recruitment from naturalized fish continue to be proportionally higher than is realized by fish of hatchery origin despite changes in management practices used for hatchery supplementation.

Chapter 4

METHODOLOGICAL BIAS IN ESTIMATES OF STRAIN COMPOSITION AND STRAYING OF HATCHERY-PRODUCED STEELHEAD IN LAKE MICHIGAN TRIBUTARIES

ABSTRACT

Steelhead (*Oncorhynchus mykiss*) were first introduced into the Great Lakes in the late 1800's. Subsequently, natural recruitment of steelhead from spawning runs in streams across the basin has been regularly supplemented by hatchery production of strains derived from widely dispersed locales within the species' native range. Estimates of hatchery contributions to spawning runs of naturalized populations may be under-represented by observations of clipped fish, as not all hatchery fish are marked prior to release. To assess the potential bias to estimates of hatchery contribution to steelhead spawning runs in four major rivers in Michigan, we used scale pattern analysis (SPA) to identify non-clipped hatchery fish, and multi-locus genotypes to estimate proportional contributions of each hatchery strain to spawning runs. The largest component to genetic diversity in Lake Michigan steelhead is among the five strains currently stocked (mean F_{ST}=0.077), making strain-specific identification using likelihood-based assignment tests possible. The main cause for differences between direct (clip observations) and indirect (SPA and genetic analysis) estimates of hatchery contribution were due to variations in percentage of hatchery fish clipped by states prior to release and the potential for confusion of certain marks with injuries. Combining direct and indirect assessment methodologies, we estimated that the percentage of hatchery fish returning to four rivers ranged from 13-31% of total spawning runs. The large contribution of hatchery fish to the non-stocked rivers differed significantly from expectations of strain-specific stocking

rates across the Lake Michigan basin and for individual streams, indicating a high rate of straying into Michigan streams.

INTRODUCTION

Many fisheries exist in waters that extend across multiple state, provincial, national boundaries, and are jointly managed by several management agencies. Different agencies often use different assessment methodologies, making coordination and multi-jurisdictional fisheries data comparison difficult. Such an example is found for steelhead (*Oncorhynchus mykiss*) fisheries in Lake Michigan, where the four states surrounding the lake use identifying marks (e.g. fin clips, maxillae clips, or a combination thereof) to identify hatchery-origin steelhead. All states stocking steelhead around the Lake Michigan basin clip a portion of the hatchery steelhead prior to release (Table 11). Clip data are used to estimate hatchery strain-specific rates of straying, and to estimate the contribution of both hatchery- and naturally-produced steelhead to the recreational fishery in Lake Michigan and its tributaries. Accurate assessments of straying and relative contribution of hatchery fish are likely compromised due to duplication of specific clips for more than one strain, and because not all individuals of hatchery-origin are clipped by each state prior to stocking. Additionally, overestimates of certain hatchery strains are likely, as general strain-specific clips may be confused with hooking injuries (i.e., maxillae clips) resulting in upward bias in abundance estimates.

The use of multiple assessment techniques to identify strain of population contributions to mixtures has been widely used in fisheries management. Traditional techniques (scale pattern analysis, coded wire tags, fin clips, otolith analysis) have been

useful to place individuals to geographic locations (Candy and Beacham 2000), hatchery strain (Burzynski 1999), or stocking location (Thedinga et al. 2000; Hard and Heard 1999). Genetic stock identification techniques are also commonly used to discriminate among stock contributions to fisheries (Pella and Milner 1987; Scribner et al. 1998; Beacham et al. 2000; Hansen et al. 2001; Potvin and Bernatchez 2001).

Molecular techniques have become a common tool in fisheries management. Molecular analysis of degree of population structure and assessment of strain or population contribution to fisheries (Marshal et al. 1991; Scribner et al. 1998; Beacham et al. 2000) represent just a few applications. In the absence of physical marks, the ability to assign individuals to population or strain of origin is based on degree of population differences in allele frequencies for a suite of genetic markers (Cornuet et al. 1999), and has been particularly useful in fisheries assessments. Molecular markers, when used in association with more traditional fish identification techniques can increase classification accuracy and effectively define the potential for hatchery and wild interactions.

Steelhead were first introduced into Lake Michigan in the late 1800's (Biette et al. 1981). Adult steelhead return in the fall and spring to natal rivers, and spawn from early to late spring. Spawning habitat in Wisconsin, Indiana, and Illinois is marginal or limited in distribution (Seelbach 1986). Accordingly, reproduction in rivers primarily occurs in Michigan tributaries due to the abundance of favorable spawning habitat. After hatching, juveniles spend one to three years in the river before emigrating as smolts to the Lake Michigan (Seelbach 1993). Juveniles spend one to three years in the lake or ocean environment before returning to spawn in their natal river (Seelbach 1993). Adults are believed to stray to rivers other than the natal river for spawning, but at low rates (Quinn

1993). Estimated straying rates were historically around 6% in Lake Michigan (Stauffer 1955) and 3-10% in Lake Huron (Dodge 1972).

Presently, spawning runs of steelhead in many tributaries to Lake Michigan are maintained by both natural reproduction and hatchery supplementation. Hatchery production averages 1.8 million annually (Table 11), and is used to supplement natural recruitment to enhance recreational river and lake fisheries. High levels of stocking and changes in age at release (fall fingerling to large yearling; Seelbach 1987a) in some states have likely increased juvenile survival, and led to greater returns of adult hatchery steelhead (Seelbach 1987a; Seelbach and Miller 1993; Rand et al. 1993).

Stocking location, size, and age of juvenile steelhead can produce large variations in survival to smolt-stage for juvenile steelhead and in straying rates of spawning adults. Stocking locations of steelhead have been widely distributed around the Lake Michigan basin. Estimated probability of survival of stocked juveniles in Lake Michigan can vary from 0.0001 over two years for a fingerling stocked into a marginal river (not optimal trout habitat) to 0.9 for a large yearling (>150 mm) stocked into a trout river (Rand et al. 1993). Rates of straying of anadromous salmonids have been found to be impacted by stocking location within a river (upstream versus river mouth; Thedinga et al. 2000), date of release (Pascual et al. 1995), size of juveniles at time of release (Pascual et al. 1995), and if stocking location differed from the location where juveniles were raised (Pascual et al. 1995).

Our objectives were to assess degree of bias in assessments of hatchery strain composition of spawning runs and to determine whether strain-specific rates of straying were consistent with levels of stocking and origins of release. We examined several

hypotheses pertaining to straying patterns of hatchery steelhead for Lake Michigan tributaries. The null hypothesis being tested was that steelhead of hatchery origin stocked into Lake Michigan represent a single panmictic population, and the abundance of adults of all hatchery strains was proportional to the rates at which they were stocked. Each population would be expected to be composed of adults of natural origin and hatchery fish present in proportions reflecting relative stocking proportions. Support for this general hypothesis comes from the fact that Lake Michigan represents a relatively small basin in comparison to the Pacific Ocean, and steelhead have been found to be widely distributed throughout open-water habitats in the Lake Michigan basin. We further wished to examine potential differences in hatchery strain composition in each of four rivers surveyed.

METHODS

Stocking history

Stocking records for the Lake Michigan basin were obtained for the years between 1993 and 1997; representing the time period that hatchery steelhead returning to rivers in 1998-1999 would have been stocked. Specifically, for each state around the Lake Michigan basin, information was collected regarding the hatchery strain stocked, the number of individuals stocked (marked and not marked), size and age of the hatchery juveniles stocked, location of stocking, and type of mark used. The specific stocking records were obtained from Burzynski (1999), J. Palla (Indiana Department of Natural Resources, personal communication), http://www.michigan.gov/dnr (Michigan

Department of Natural Resources), and S. Krueger (Illinois Department of Natural Resources, personal communication).

Hatchery marks observed in each of the four rivers examined were identified to strain of origin using stocking records obtained from each state agency that has stocked steelhead into the Lake Michigan basin (Table 11). Due to the use of a single fin clip to designate both the Michigan strain and the Indiana Skamania strain, all fish with clips identifying individuals to one of those two strains were classified as Michigan strain. Due to the small portion of Indiana Skamania steelhead marked prior to release (15%; Table 11), the bias introduced by combining the information would be small. Therefore, individuals identified as Skamania strain by clip observations represented only the Wisconsin Skamania strain. To determine the hatchery composition of the non-clipped fish, we collected fin clips for genetic analysis and scale samples (for SPA) from all non-clipped individuals.

We developed expectations for proportional contributions of adults of hatchery origin to spawning runs based on total numbers of juveniles stocked. However, there is likely to be increased mortality of hatchery steelhead stocked as fingerlings in comparison to those stocked at a larger size in Lake Michigan (Seelbach 1987a). To account for differential mortality to smolt stage (smolt equivalents) based on age and size at stocking (i.e., small yearlings <150 mm versus large yearlings $\geq$ 150 mm), and stocking location, the number of hatchery juveniles stocked were adjusted as described by Rand et al. (1993). These adjustments allowed estimated abundances of hatchery fish stocked into the Lake Michigan basin to be standardized and to more accurately represent the proportional abundance of each strain. Adjustments were made by multiplying the

number stocked by an estimate of percent survival to smolt stage. The resulting numbers of smolt-equivalents were used as the total expected abundance of each hatchery strain of steelhead in each river.

Sampling locations and tissue collection

Adult steelhead were sampled from four Michigan rivers during the fall 1998 and spring 1999 spawning runs. Samples were obtained by electro-shocking and creel sampling on spawning grounds in the Pere Marquette River, Bear Creek, and Platte River, and at a weir for the Little Manistee River (Figure 11). Samples were collected on multiple dates over the course of the spawning season to ensure representative samples were obtained from spawning adults. The Pere Marquette River and Little Manistee River were sampled during the fall and spring, while Bear Creek and Platte River were sampled only in the spring. Due to absence of genetic differences between the fall and spring run and no difference in the proportional contribution of individuals of hatchery origin to the fall run and spring run within the Pere Marquette and Little Manistee (Bartron and Scribner, in press), fall and spring samples for these river systems were combined.

Scale pattern analysis (SPA)

We used scale pattern analysis to estimate stream growth and residence time of juveniles to determine if non-marked individuals were of wild or hatchery origin. Specifically, we used Seelbach and Whelan's (1988) ratio 23 metric of scale growth. Ratio 23 is the ratio of the distance from the first stream annulus to the fifth circulus

measured towards the focus (band 2), and to the distance from the first stream annulus to the fifth circulus measured towards the scale margin (band 3; Seelbach and Whelan 1988). Distributions of ratio values from fish of known origin (hatchery or river) were used to determine a point at which hatchery-origin fish could be differentiated from river-origin fish (Seelbach and Whelan 1988). We also considered the number of years an individual spent in a river to improve the determination of origin for individuals whose ratio 23 value fell within the area of overlapping distributions of known origin individuals. Hatchery yearlings migrate downstream to Lake Michigan within a year after stocking, whereas juvenile steelhead produced in the wild may reside in the stream for more than one year prior to smoltification (Seelbach 1987a). Incorporation of juvenile stream age likely reduced errors in misidentification of stream origin fish in situations where estimates of ratio 23 were close to 0.70. Therefore, any steelhead with a ratio 23 value $\leq$ 0.80 that also had a stream age of 2 or greater was classified as being of wild origin. To test whether the observed and expected numbers of hatchery steelhead present in each river system differed significantly based on the proportions of steelhead stocked into Lake Michigan, the expected number of steelhead was based on smolt-equivalent estimates to account for differential survival juveniles stocked at different size, age, and location into the Lake Michigan basin.

Genetic analysis

Fin clips from each of the four hatchery strains currently stocked around the basin were obtained for genetic analysis and served as baseline. Small samples for genetic analysis were taken from the caudal fin of adult steelhead returning to each of four rivers

in Michigan. Only individuals with no hatchery identifying marks were sampled for genetic analysis and SPA. Samples were preserved in 95% ethanol, or were dried in scale envelopes. DNA was extracted using PurGene® (Gentra Inc.) protocols. Microsatellite loci used for analysis were Ogo1a and Ogo4 (Olsen et al. 1998), Oneμ10 and Oneμ11 (Scribner et al. 1996), Omy77 (Morris et al. 1996), Ots1 (Banks et al. 1999), and Ots100 (Nelson et al. 1998). PCR reactions were conducted in 25 μl reaction volumes using 100 ng DNA, 2 μl 10x PCR Buffer (0.1 M Tris-HCl, ph 8.3, 0.015 M $MgCl_2$, 0.5 M KCl, 0.1% gelatin, 0.1% NP-40, 0.1% Trition-X 100), 0.2mM dNTP's, 0.6 μM fluorescently labeled forward primer, 0.6 μM unlabeled reverse primer, and 0.3 Units Taq Polymerase. PCR reactions for Ogo4 were conducted in 25 μl volumes, following the above protocol with an additional 2.5 mM $MgCl_2$, and primer concentrations were 0.5 μM. PCR reactions for Ots100 were conducted in 25 μl volumes, following the above protocol with an additional 12.5 mM $MgCl_2$, with primer concentrations of 0.5 μM. PCR reactions for all loci utilized an initial denaturing step at 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, annealing temperature for one minute, and extension at 72° C for 1 minute, and a final extension period of 2 minutes, 30 seconds. Annealing temperatures for Ogo1a, Ogo4, Omy77, Oneμ10, Oneμ11, Ots1 and Ots100 were 56° C, 54° C, 54° C, 52° C, 62° C, 54° C, and 58° C respectively. Non-denaturing 6% acrylamide gels were used for electrophoresis. Genotypes were visualized on a Hitachi FM-BIO II scanner. Molecular weight standards and individuals of known genotype were run on each gel to standardize scoring.

Statistical analysis

Pairwise estimates of degree of inter-strain differentiation in allele frequency (F_{ST}) were determined using FSTAT (ver. 2.9.3.2), and nominal alpha levels were adjusted for multiple comparisons using Bonferroni corrections (Rice 1989). Individuals were assigned to strain of origin using likelihood-based assignment tests (Rannala and Mountain 1997). Estimates of assignment accuracy were based on resampling of the strain baseline samples using the "leave-one-out" technique (Efron 1983). Estimates of statistical confidence in individual assignment decisions were based on posterior probabilities (Pritchard et al. 2000; Blanchong et al. 2002).

Determination of hatchery contribution

We determined the percentage of adult spawners of wild origin from each river based on clip observations by summing the total number of clipped hatchery fish collected in each river and dividing by the number of fish collected. Strain totals for the genetics/SPA method were based on individuals without clips. Confidence intervals for wild contributions for each methodology were based on 95% confidence intervals (Sokal and Rohlf 1995)

$$1.96 * \sqrt{\frac{p(1-p)}{n}}$$

where p is the proportion of wild fish and n is the number of fish sampled. Direct (clip) and indirect (SPA and genetic) methods of hatchery strain identification were combined by summing the numbers of individuals assigned to each of the hatchery strains based on both methods. Estimates of hatchery strain-specific contributions derived by genetics and SPA for the Little Manistee were extrapolated when combined with clip observations as only a subsample of the fall and spring runs were analyzed with genetic methods.

Expected strain-specific counts of stocked fish were based on the yearly average of numbers stocked strain into Lake Michigan between 1993 and 1997 for both the Lake Michigan basin (Table 11*a*) and each river (Table 11*b*). The yearly averages of the numbers stocked were adjusted for age and size- specific survival to calculate numbers of smolt-equivalents (Rand et al. 1993). The Ganaraska and Chambers Creek strains are not stocked into Michigan rivers; therefore individuals belonging to these strains would not be expected to be found in any of the rivers we examined for this analysis. Comparisons were only made between the observed and expected numbers based on stream-specific and strain-specific stocking for Michigan strain individuals found in the Little Manistee River, and Michigan and Skamania strain individuals found in the Pere Marquette, Bear Creek, and Platte rivers.

Three tests were conducted to determine how observed hatchery strain composition compared to expected counts based on levels of stocking. The numbers of hatchery individuals by strain were first compared among rivers to test for differences in proportional abundance by river and by strain. Fisher's exact tests were performed to test among-stream heterogeneity using the SAS software (SAS Institute). Second, observed counts of hatchery steelhead by strain were compared (chi-square test) to expected numbers based on the proportion that each strain was stocked into the Lake Michigan basin. Finally, the observed counts of hatchery steelhead by strain were compared using chi-square tests to expected numbers based on the yearly average number of steelhead stocked into each river examined.

RESULTS

Stocking history

Approximately 1.8 million juvenile steelhead were stocked annually between 1993 and 1997 into the Lake Michigan basin (Table 11). Adjustments to the total number of juveniles stocked into the basin according to age, size, and location of stocking (smolt equivalents) reduced the annual number of juvenile steelhead stocked to 640,517 (Table 11). The proportion of juvenile steelhead marked prior to release from a hatchery varied by state for the time period between 1993 and 1997. Beginning in 1995, Michigan began clipping all hatchery origin individuals. Illinois also marks 100% of juvenile steelhead prior to release from hatcheries (Table 11). Wisconsin marks approximately 33% of the hatchery juveniles, and Indiana marks approximately 15% of hatchery juveniles.

Of the four rivers examined in this study, only the Platte River was not stocked between 1993 and 1997 (Table 11). The Little Manistee River was stocked with a small number of individuals of the Michigan strain. Portions of the Pere Marquette River and Manistee River drainages (which Bear Creek is a tributary) were stocked large numbers of both Michigan and Skamania hatchery strains (Table 11). However, Bear Creek is not directly stocked, and only one tributary of the Pere Marquette (Ruby Creek, located near the mouth) is stocked.

Genetic identification of hatchery strains

Significant differences in allele frequency were observed among the four hatchery strains of steelhead stocked in the Lake Michigan basin ($P<0.05$; Table 12). Differentiation between the Skamania strains produced by Indiana and Wisconsin was

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low and not significant (P>0.05; data not shown). Accordingly, estimates of contributions of Skamania strain steelhead based on genotypic data represent cumulative contributions from both states. Inter-strain comparisons indicated that the greatest differentiation was between the Michigan and Skamania hatchery strains (mean F_{ST}=0.127; Table 12). The smallest difference in allele frequencies was between the Chambers Creek and Skamania strains (mean F_{ST}=0.045; P<0.05; Table 12). Levels of differentiation among strains were sufficiently large to provide accurate individual classification for each strain (range 98.2-86.7%; Table 13).

Estimates of strain-specific contribution based on observation

Direct estimates of the contribution of individual hatchery strains to adult steelhead spawning runs from four Michigan rivers ranged from 1% to 15% of the samples collected from each spawning run. Total contributions across all hatchery strains if extrapolated across an entire spawning run varied between 9% of the fall and spring spawning runs in the Pere Marquette River to 27% of the spring spawning run in the Platte River (Table 14). Clips identifying each of four hatchery strains were observed in the Pere Marquette River and Bear Creek (Table 14). The Little Manistee River and Platte River both contained individuals from the Michigan, Skamania, and Chambers Creek hatchery strains.

Estimates of strain-specific contribution based on genotype and SPA

We estimated that of the non-clipped fish collected, contributions of hatchery strains ranged from 3% to 26% of the total spawning run (Table 14). Hatchery

contribution of non-clipped fish for all four strains ranged from 3% in the Pere Marquette River to 26% in the Little Manistee River (Table 14). The Michigan strain was present in all four rivers. Individuals of each of the four hatchery strains were found in the Little Manistee River (Table 14). Individuals of the Michigan and Ganaraska strains were found in the Pere Marquette River and Bear Creek (Table 14).

Combined direct and indirect estimates of strain-specific contribution

To estimate the total hatchery contribution to spawning runs for the four rivers, a combination of hatchery marks observations and SPA/genetics analysis were used to examine the composition of two separate groups of steelhead (clipped and not clipped). Individual strain contributions to spawning runs ranged from 0% to 23% (Table 14). With the exception of the Platte River, all of the four hatchery strains were present in the rivers examined. Total hatchery contribution (combined over all strains) ranged from 31% in the Little Manistee River to 13% in the Pere Marquette River (Table 14). In Bear Creek, hatchery individuals contributed 27% to the spawning run (Table 14), and in Platte River, hatchery individuals contributed 29% to the spawning run (Table 14). Estimates of Michigan strain hatchery contribution in the Pere Marquette River and the Platte River were similar using both clip observation and genetics/SPA (Table 14). Individuals of Skamania strain origin were present in all rivers as estimated by clip observations (Table 14). Genetics/SPA analysis identified Skamania strain only in the Little Manistee River, in a greater abundance to the fall and spring spawning runs than estimated by clip observations (4% versus 1% respectively; Table 14).

Observed numbers of steelhead with the Ganaraska strain clip represented 1% of the spawning runs in the Pere Marquette River and Bear Creek (Table 14). Genetic/SPA identified individuals of the Ganaraska strain in the Pere Marquette River (3% of the total run; Table 14), Little Manistee River (3% of the total run; Table 14), and Bear Creek (5% of the total run; Table 14) spawning runs. The Chambers Creek strain contributed to the spawning runs of Bear Creek and Platte River (9% and 15% respectively; Table 14) estimated by clip observations whereas this strain was not present in the genetic/SPA analysis of the non-clipped individuals. Estimated contributions by genetic/SPA analysis did not detect the Chambers Creek strain in the Pere Marquette River, compared to estimates of contribution based on clip observations (4%; Table 14). Estimates of Chambers Creek strain contribution to the Little Manistee River were comparable for the direct and indirect identification methods (1%; Table 14).

Of the rivers examined in this study, only the Platte River was not stocked with hatchery individuals, and the Little Manistee River was stocked with very low numbers of individuals. However, sizable portions of hatchery-derived adults were observed during spawning. This includes appreciable numbers of hatchery strains stocked in other states around the basin. The Little Manistee River had the highest contribution of hatchery steelhead of the two non-stocked rivers (31%; Table 14). The Platte River also has a large hatchery component (29%; Table 14).

Straying of hatchery strains into Michigan rivers

Comparisons of the observed and expected contributions of hatchery-origin individuals to the spawning runs of the four Michigan rivers provided evidence of

homing in Lake Michigan steelhead. We found significant differences among rivers in the strain composition of hatchery-origin individuals (χ^2=181.4; df=9; P<0.0001). Chi-square tests between the observed and expected contribution of each hatchery strain pooled over all rivers indicated a significant difference (χ^2= 1249.2; df =3; P<0.001) in the number of hatchery fish observed based on the proportion of smolt-equivalents stocked into the Lake Michigan basin. River-specific chi-square tests indicated significant difference in the observed and expected contribution of hatchery-origin steelhead relative to the proportions stocked into Lake Michigan for the Pere Marquette River (χ^2=5.3; df =1; P<0.05), Little Manistee River (χ^2= 1015.7; df =3; P<0.001), Bear Creek (χ^2= 5.0; df =1; P<0.05), and Platte River (χ^2= 10.5; df =1; P<0.05). Due to the low contribution of Ganaraska and Chambers Creek to the total percentage of stocked smolt-stage steelhead into Lake Michigan, and the low number of hatchery origin individuals observed in Pere Marquette River, Bear Creek, and Platte River, no individuals from these two strains were expected in these rivers. Therefore chi-square comparisons were not made for three of the four rivers between the observed and expected numbers of Chambers Creek and Ganaraska strain hatchery fish relative to what is stocked into Lake Michigan.

The observed number of Michigan and Skamania strain individuals differed from the number of Michigan and Skamania strain individuals stocked into each river. There was a significant difference in the proportions observed in the spawning run compared to the proportions stocked in of the Pere Marquette River (χ^2= 5.9; df =1; P<0.05), but not in Bear Creek. As the Michigan and Skamania strains are the only hatchery steelhead strains stocked into the Pere Marquette and Bear Creek drainages, statistical comparisons

were not made for the Chambers Creek or Ganaraska strains despite observations of individuals with clips specific to those strains.

DISCUSSION

Comparisons of estimated straying rates derived from clip observations with rates based on genetic and SPA methods indicated that potential biases exist when clip data alone are used to estimate hatchery contributions to steelhead spawning runs. We documented non-uniformity in proportions of steelhead juveniles who received clips among states around Lake Michigan (Table 11). As such, assessment information based solely on clips may misrepresent strain-specific estimates of abundance or contribution to fisheries.

The use of hatchery clips (e.g., fin clips, maxillary clips, and or a combination of marks) that are easily confused with injuries such as those resulting from hooking may bias estimates by overestimating the contribution of those strains that use those clips. The Wisconsin Skamania and Chambers Creek hatchery strains are identified by clipped left or right maxillae (Burzynski 1999). Estimates for the left maxillae clipped Chambers Creek strain were consistently higher than the Ganaraska strain, which can be identified by having both maxillae clipped (sometimes additional clips are used in combination with the maxillae clips; Burzynski 1999). Estimates of Skamania strain contribution primarily consist of steelhead from Wisconsin, although Indiana and Michigan both stock Skamania and clip a portion of the stocked fish (Table 11). Because only one-third of Wisconsin's hatchery fish are clipped prior to release, estimates of the non-clipped steelhead are expected to be approximately two times greater than estimates based on clip

observations alone. However, analysis of non-clipped individuals estimated smaller contributions of the single maxillae clipped steelhead. Assuming clipping does not affect rates of straying, a portion of single maxillae clipped individuals are not representative of the Chambers Creek or Skamania strains, but rather represent river origin individuals with hooking injuries. Support for the bias in estimates for the Chambers Creek strain due to clip and injury confusion was found in the observation of multiple individuals with the single maxillae clip in Bear Creek and Platte River (Table 14), but the lack of support for these observations using the genetics and SPA techniques.

Straying (or returning to spawn in a river other than the natal river) is an adaptive life history characteristic of salmonids, including steelhead. Therefore, the presence of individuals of hatchery origin belonging to strains not stocked into a particular system was expected. However, the extent of straying was not expected (Table 14). It has been observed that hatchery individuals may stray at higher rates than found in naturally occurring populations (Waples 1991b). The Little Manistee River has been stocked with very low numbers of individuals and Platte River is not currently stocked. However, the mouths of these drainages are in close geographic proximity to heavily stocked systems, resulting in an increased potential for straying of fish during fall and spring adult spawning migrations. The contribution of steelhead straying from Wisconsin, specifically the Chambers Creek and Ganaraska strains (the Wisconsin and Indiana Skamania strains are not genetically distinct in this analysis) differ between direct and indirect observations.

We found significant differences among rivers in the strain-specific composition of hatchery-origin steelhead. Among-river heterogeneity in strain contribution to

spawning runs indicates adult hatchery contribution was not randomly distributed between each river system. Additionally, significant differences in the observed and expected numbers of individuals of hatchery-origin to spawning runs for all rivers indicated non-uniform distribution or survival of hatchery strains. Proportions of hatchery-origin individuals found in the Little Manistee River, Bear Creek, and Platte River were significantly different from proportions of each strain stocked into Lake Michigan. However the proportion of hatchery-origin individuals did not differ in the Pere Marquette River in comparison to the proportion stocked into Lake Michigan. This result may be biased by the relatively small proportion of the Chambers Creek and Ganaraska strains to the Lake Michigan hatchery-origin steelhead population, and the relatively low observed number of hatchery-origin individuals in the Pere Marquette River.

There are positive and negative implications associated with findings of high proportions of hatchery fish in Michigan spawning runs derived from stocking by other states. On one hand, Michigan experiences an "embarrassment of riches". If strain composition of spawning adults is consistent with strain-contributions to the creel, then Michigan anglers are reaping the benefits of resources expended by other states. However, introgression between hatchery strains will lead to the breakdown of among-strain genetic differences, potentially resulting in the loss of the differences in heritable life history traits (run-timing) that currently exist between the strains and which provide managers with greater management options.

The large contribution of certain hatchery strains to the spawning runs in Michigan increased the potential for introgression among hatchery strains and between

naturalized and hatchery populations. Reisenbichler and Phelps (1989) hypothesized that reduction in among stream genetic diversity of Washington steelhead populations was due to the widespread stocking of hatchery steelhead and resulting introgression with the native populations. Introgression between the naturalized populations in Michigan and the Michigan strain has been proposed to be primary cause for lack of significant genetic differentiation among drainages (Bartron and Scribner in press). Of particular concern is the contribution of hatchery origin individuals to the fall and spring spawning runs of the Little Manistee River. The Little Manistee River serves as the source for gametes for propagation of the Michigan strain (Seelbach 1987a).

When accounting for differential survival of the various ages and stages of fish stocked into the Lake Michigan basin, we found that only approximately 39% (Table 11) of the stocked juveniles survive to the smolt stage. Due to the small size and fingerling stage at stocking, low survival rates indicate that hatchery stocking programs are producing more steelhead for stocking purposes than needed, and could better devote resources to producing few larger, older juveniles that have better survival rates (Rand et al. 1993; Seelbach 1987a). Concerted management efforts to utilize hatchery marks that are not easily misidentified with injuries, and to mark all hatchery-produced individuals may reduce bias in hatchery-mark derived information. Additionally, despite the large number of steelhead stocked into the Lake Michigan basin, the contribution of natural recruitment to the spawning runs for the four Michigan rivers examined was larger than expected. Further quantification of the amount of natural reproduction may lead to a reduction in stocking levels while the total population would be maintained at current levels.

As varying portions of hatchery-produced steelhead are marked each year prior to release, estimates of hatchery fish in spawning runs based solely on counts of clipped individuals may not adequately estimate hatchery contribution. Using additional techniques to estimate hatchery contribution, such as SPA to determine river or hatchery origin and genetic identification of hatchery strains provide a better representation of the contribution of hatchery individuals.

Appendix 1. Sample sizes (N) and allele frequencies at each of the six microsatellite loci for six Michigan steelhead populations sampled during each time period (1983-4 and 1998-9), and each hatchery strain. Hatchery individuals were excluded from river samples.

		Population										Hatchery Strains				
		Betsie		Manistee		Pere Marquette		White		Muskegon		Little Manistee				
Locus		1983-4	1998-9	1983-4	1998-9	1983-4	1998-9	1983-4	1998-9	1983-4	1998-9	1983-4	1998-9	Ska-mania	Chambers Creek	Ganaraska
Ogo1a	N	47	23	52	105	10	101	15	17	7	75	57	114	103	59	60
	115	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	125	0.000	0.000	0.000	0.029	0.000	0.040	0.000	0.000	0.000	0.020	0.009	0.018	0.000	0.000	0.000
	139	0.415	0.413	0.356	0.486	0.300	0.405	0.500	0.412	0.643	0.353	0.360	0.417	0.874	0.797	0.566
	141	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000
	143	0.234	0.239	0.327	0.247	0.250	0.223	0.367	0.239	0.143	0.293	0.325	0.254	0.107	0.186	0.267
	161	0.351	0.348	0.317	0.238	0.450	0.327	0.133	0.349	0.214	0.327	0.306	0.311	0.019	0.017	0.167
Ogo4	N	49	25	52	106	10	100	14	16	7	72	56	115	111	58	60
	118	0.328	0.360	0.490	0.473	0.550	0.375	0.393	0.438	0.429	0.375	0.348	0.370	0.148	0.387	0.558
	120	0.296	0.280	0.279	0.250	0.300	0.305	0.250	0.281	0.214	0.340	0.330	0.365	0.135	0.147	0.100
	122	0.020	0.000	0.010	0.000	0.000	0.005	0.000	0.000	0.000	0.014	0.000	0.004	0.000	0.009	0.050
	124	0.061	0.100	0.038	0.028	0.000	0.030	0.036	0.063	0.071	0.035	0.045	0.052	0.000	0.000	0.075
	126	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	128	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.000
	130	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.052	0.000
	132	0.224	0.160	0.096	0.085	0.100	0.165	0.213	0.125	0.143	0.160	0.205	0.166	0.297	0.250	0.117
	134	0.071	0.080	0.058	0.108	0.000	0.050	0.036	0.031	0.143	0.069	0.027	0.026	0.171	0.078	0.033
	136	0.000	0.020	0.029	0.028	0.050	0.030	0.036	0.031	0.000	0.000	0.027	0.013	0.162	0.034	0.017
	138	0.000	0.000	0.000	0.005	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.041	0.009	0.000
	140	0.000	0.000	0.000	0.009	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.034	0.008
	142	0.000	0.000	0.000	0.009	0.000	0.005	0.036	0.031	0.000	0.000	0.018	0.004	0.000	0.000	0.042
Omy77	N	49	25	48	104	10	100	15	16	7	75	56	116	97	59	59
	82	0.061	0.060	0.021	0.005	0.050	0.020	0.000	0.031	0.000	0.000	0.063	0.034	0.000	0.000	0.144
	98	0.000	0.020	0.021	0.019	0.100	0.005	0.000	0.000	0.071	0.000	0.009	0.009	0.000	0.000	0.017
	100	0.305	0.260	0.270	0.279	0.200	0.305	0.233	0.375	0.286	0.240	0.285	0.362	0.000	0.000	0.085
	102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	104	0.082	0.020	0.010	0.058	0.050	0.020	0.033	0.031	0.000	0.047	0.054	0.017	0.082	0.186	0.034
	106	0.082	0.040	0.094	0.091	0.000	0.055	0.200	0.063	0.143	0.053	0.089	0.056	0.000	0.000	0.169

Appendix 1 (cont'd).

	108	0.000	0.020	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.017	0.008
	110	0.041	0.000	0.115	0.043	0.100	0.110	0.100	0.031	0.000	0.033	0.045	0.056	0.000	0.017	0.153
	112	0.082	0.120	0.156	0.120	0.150	0.095	0.067	0.188	0.072	0.133	0.080	0.082	0.000	0.059	0.127
	114	0.000	0.000	0.000	0.024	0.000	0.015	0.033	0.031	0.000	0.013	0.009	0.013	0.000	0.008	0.000
	116	0.082	0.040	0.063	0.077	0.050	0.060	0.033	0.031	0.072	0.047	0.035	0.052	0.000	0.068	0.034
	120	0.000	0.040	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.160	0.220	0.000
	122	0.031	0.100	0.042	0.034	0.100	0.045	0.000	0.000	0.143	0.093	0.080	0.099	0.000	0.000	0.059
	124	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.041	0.144	0.000
	126	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
	128	0.092	0.060	0.094	0.068	0.050	0.050	0.067	0.000	0.071	0.154	0.134	0.065	0.134	0.034	0.093
	130	0.092	0.080	0.052	0.101	0.100	0.145	0.201	0.125	0.071	0.127	0.036	0.086	0.464	0.154	0.068
	132	0.020	0.080	0.021	0.029	0.000	0.020	0.000	0.063	0.000	0.027	0.027	0.039	0.119	0.017	0.000
	134	0.020	0.040	0.021	0.000	0.050	0.040	0.033	0.000	0.000	0.020	0.036	0.009	0.000	0.059	0.008
	136	0.000	0.000	0.010	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
	142	0.010	0.020	0.010	0.014	0.000	0.005	0.000	0.000	0.071	0.013	0.009	0.013	0.000	0.000	0.000
Oneμl0	N	49	24	51	107	10	95	15	16	7	75	57	115	103	60	60
	121	0.000	0.000	0.020	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	125	0.051	0.021	0.020	0.051	0.000	0.047	0.033	0.000	0.000	0.020	0.018	0.004	0.098	0.142	0.200
	127	0.102	0.104	0.196	0.178	0.167	0.237	0.133	0.250	0.143	0.200	0.149	0.170	0.184	0.142	0.192
	129	0.449	0.458	0.490	0.491	0.722	0.489	0.500	0.438	0.500	0.420	0.438	0.439	0.718	0.541	0.300
	131	0.388	0.375	0.225	0.224	0.111	0.179	0.267	0.281	0.357	0.320	0.316	0.339	0.000	0.017	0.250
	133	0.000	0.000	0.010	0.014	0.000	0.011	0.000	0.000	0.000	0.020	0.000	0.005	0.000	0.133	0.033
	135	0.010	0.042	0.039	0.033	0.000	0.037	0.067	0.031	0.000	0.020	0.035	0.039	0.000	0.025	0.025
	137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.004	0.000	0.000	0.000
Oki200	N	49	24	46	89	10	100	15	18	7	75	57	113	106	57	60
	86	0.021	0.021	0.022	0.022	0.000	0.020	0.000	0.028	0.000	0.047	0.018	0.035	0.000	0.018	0.042
	90	0.010	0.083	0.000	0.006	0.000	0.010	0.000	0.028	0.071	0.013	0.009	0.009	0.009	0.009	0.017
	92	0.041	0.083	0.054	0.096	0.000	0.050	0.000	0.000	0.000	0.073	0.053	0.058	0.317	0.298	0.042
	94	0.704	0.646	0.696	0.618	0.556	0.710	0.700	0.722	0.787	0.653	0.683	0.646	0.132	0.219	0.625
	96	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
	98	0.224	0.146	0.206	0.236	0.444	0.205	0.300	0.222	0.071	0.187	0.228	0.239	0.481	0.219	0.257
	100	0.000	0.021	0.022	0.022	0.000	0.005	0.000	0.000	0.071	0.027	0.009	0.013	0.061	0.228	0.017

Appendix 1 (cont'd).

Ots103	N	49	25	52	96	10	102	15	18	7	73	57	115	108	43	58
	55	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009
	57	0.082	0.080	0.048	0.042	0.100	0.054	0.133	0.028	0.000	0.068	0.044	0.035	0.000	0.000	0.121
	73	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.023	0.000
	77	0.051	0.080	0.087	0.068	0.000	0.044	0.033	0.056	0.143	0.041	0.061	0.083	0.125	0.117	0.078
	81	0.867	0.840	0.865	0.875	0.900	0.902	0.801	0.916	0.857	0.891	0.886	0.882	0.773	0.860	0.783
	85	0.000	0.000	0.000	0.015	0.000	0.000	0.033	0.000	0.000	0.000	0.009	0.000	0.056	0.000	0.009

APPENDIX II

TABLES AND FIGURES

Table 1. Stocking histories for each of the ten naturalized populations examined. Numbers of individuals stocked represents the average number of juvenile steelhead stocked into each river between 1993-1997. The estimates of smolt-equivalents stocked are based on survival estimates for different size, age, and location of stocking described by Rand et al. (1993). Strains used for stocking were Michigan strain (M) and Skamania strain (S).

River	Total # stocked	Smolt-equivalent	Average smolt-equivalent per year	Strains stocked
Thompson	0	0	0	-
Black	0	0	0	-
Platte	0	0	0	-
Betsie	247,973	223,176	44,635	M
Manistee	357,739	321,965	64,393	M, S
Little Manistee	500	450	90	M
Pere Marquette	70,242	63,218	12,644	M, S
White	112,100	100,890	20,178	M
Muskegon	292,683	263,415	52,683	M
St. Joseph	476,787	429,108	85,822	M, S

Table 2. Sample size (N), number of alleles per locus (A), allelic richness (A_r), observed heterozygosity (H_o), and expected heterozygosity (H_e) for each population by locus and mean values over all loci. Additionally, mean F (inbreeding coefficient) is provided over all loci. Because the Little Manistee population is used for propagation of the Michigan Strain, it is included in the hatchery population group. Naturalized populations are defined as follows: MN (Manistee River), PM (Pere Marquette River), PL (Platte River), MU (Muskegon River), BE (Betsie River), WH (White River), SJ (St. Joseph River), BL (Black River), TH (Thompson Creek). Hatchery strains are defined as follows LM (Little Manistee River/Michigan strain), SK (Skamania strain), CC (Chambers Creek strain), and GA (Ganaraska strain).

										Hatchery populations			
Locus	Naturalized populations												
Allele	MN	PM	PL	MU	BE	WH	SJ	BL	TH	LM	SK	CC	GA
Ogo1a													
N	154	101	105	75	23	17	15	43	12	116	112	60	60
A	4	5	4	4	3	3	3	4	3	4	3	3	3
A_r	3.52	3.76	3.39	3.41	3.00	3.00	3.00	3.28	3.00	3.36	2.30	2.36	2.99
H_o	0.662	0.683	0.638	0.720	0.739	0.294	0.733	0.698	0.583	0.612	0.223	0.267	0.483
H_e	0.657	0.680	0.670	0.687	0.666	0.670	0.674	0.647	0.684	0.666	0.217	0.329	0.585
Ogo4													
N	155	100	103	72	25	16	15	42	12	117	112	60	60
A	10	11	9	7	6	7	7	7	5	8	8	9	9
A_r	5.37	5.79	5.47	4.92	5.36	6.19	6.53	6.00	5.00	4.69	6.47	6.23	6.36
H_o	0.671	0.680	0.699	0.722	0.760	0.562	0.733	0.595	0.833	0.658	0.804	0.783	0.700
H_e	0.690	0.738	0.745	0.717	0.765	0.730	0.786	0.731	0.746	0.702	0.818	0.763	0.659
Omy77													
N	151	100	103	75	25	16	15	43	12	116	109	60	60
A	18	16	17	13	15	11	12	16	11	17	6	13	13
A_r	9.92	9.48	10.15	9.14	11.4	9.39	10.9	11.3	11.0	9.54	5.44	8.65	9.36
H_o	0.815	0.770	0.825	0.747	0.920	0.938	0.933	0.837	0.917	0.784	0.706	0.700	0.900
H_e	0.866	0.855	0.860	0.872	0.898	0.820	0.901	0.910	0.917	0.832	0.725	0.864	0.890

Table 2 (cont'd).

Oneu10													
N	155	95	95	75	24	16	15	40	12	116	110	60	60
A	8	6	6	6	5	4	6	5	6	7	3	6	6
A_r	5.40	4.56	4.42	4.23	4.23	3.75	5.40	3.81	6.00	3.93	2.93	4.80	5.08
H_o	0.690	0.705	0.705	0.680	0.708	0.813	0.667	0.675	0.583	0.690	0.427	0.583	0.817
H_e	0.718	0.672	0.682	0.685	0.650	0.688	0.708	0.657	0.670	0.665	0.449	0.653	0.775
Oneu11													
N	148	97	104	71	25	16	15	39	12	116	119	60	59
A	3	5	3	3	2	3	3	3	3	3	3	3	3
A_r	2.79	3.00	2.87	2.73	2.00	2.75	3.00	2.29	3.00	2.79	2.73	2.36	2.20
H_o	0.480	0.526	0.471	0.451	0.440	0.375	0.533	0.282	0.583	0.371	0.355	0.400	0.254
H_e	0.471	0.461	0.468	0.424	0.429	0.365	0.515	0.249	0.489	0.436	0.397	0.411	0.320
Ots103													
N	144	102	79	73	25	18	15	41	12	115	104	43	58
A	4	3	4	3	3	3	3	4	1	3	4	3	5
A_r	2.89	2.44	2.77	2.51	2.87	2.56	2.97	2.83	1.00	2.48	3.40	2.45	3.27
H_o	0.257	0.196	0.190	0.164	0.280	0.167	0.267	0.195	0.000	0.209	0.413	0.209	0.362
H_e	0.272	0.183	0.201	0.202	0.287	0.160	0.432	0.225	0.000	0.214	0.381	0.248	0.367
Oki200													
N	134	100	90	75	24	18	15	41	12	113	98	57	60
A	6	6	6	6	6	4	5	5	5	6	5	7	6
A_r	4.00	3.48	4.04	4.37	4.89	3.33	4.60	4.50	5.00	3.86	4.01	4.80	4.08
H_o	0.537	0.450	0.556	0.480	0.708	0.556	0.400	0.537	0.417	0.522	0.663	0.842	0.567
H_e	0.551	0.453	0.566	0.533	0.559	0.440	0.625	0.525	0.543	0.523	0.655	0.769	0.543

Table 2 (cont'd).

Mean for all loci													
N	149	99	97	73	24	17	15	41	12	116	108	57	60
A	7.52	7.43	7.00	6.14	5.71	5.00	5.57	6.29	4.86	6.86	4.57	6.29	6.43
A_r	4.84	4.64	4.73	4.47	4.83	4.42	5.20	4.86	4.86	4.38	3.90	4.52	4.76
H_o	0.587	0.572	0.583	0.566	0.651	0.529	0.610	0.546	0.560	0.549	0.513	0.541	0.583
H_e	0.604	0.577	0.599	0.589	0.608	0.553	0.663	0.563	0.579	0.577	0.520	0.577	0.591
F	0.027	0.008	0.026	0.038	-0.073	0.045	0.083	0.032	0.035	0.048	0.014	0.063	0.014

Table 3. Hierarchical partitioning of genetic variation (Weir 1996) within and among the naturalized populations and hatchery strains (populations) is provided for each locus and mean over seven loci. Naturalized populations include the Manistee River, Pere Marquette River, Platte River, Muskegon River, Betsie River, White River, St. Joseph River, Black River, and Thompson Creek. Hatchery strains include Little Manistee River/Michigan strain, Skamania strain, Chambers Creek strain, and Ganaraska strain.

Locus	(F) Alleles within individuals	(f) Among individuals within populations	(Θ_p) Among populations within groups	(Θ_s) Among groups
Ogo1a				
Naturalized populations	0.019	0.020	-0.001	
Hatchery strains	0.236	0.099	0.152	
Combined groups				0.100
Ogo4				
Naturalized populations	0.059	0.056	0.003	
Hatchery strains	0.089	0.011	0.078	
Combined groups				0.037
Omy77				
Naturalized populations	0.067	0.062	0.005	
Hatchery strains	0.178	0.060	0.126	
Combined groups				0.062
Oneμ10				
Naturalized populations	-0.012	-0.014	0.002	
Hatchery strains	0.111	0.005	0.106	
Combined groups				0.045
Oneμ11				
Naturalized populations	-0.016	-0.018	0.002	
Hatchery strains	0.122	0.122	0.000	
Combined groups				0.003
Ots103				
Naturalized populations	0.068	0.064	0.002	
Hatchery strains	0.012	-0.008	0.021	
Combined groups	0.019			
Ots100				
Naturalized populations	0.020	0.018	0.002	
Hatchery strains	0.141	-0.032	0.168	
Combined groups				0.115

Table 3 (cont'd).

Mean for all loci				
Naturalized populations	0.030	0.028	0.003	
Hatchery strains	0.135	0.034	0.105	
Combined groups				0.060
95% Confidence				
Naturalized populations				
Upper bounds	0.053	0.050	0.004	
Lower bounds	0.003	0.001	0.001	
Hatchery strains				
Upper bounds	0.176	0.072	0.137	
Lower bounds	0.092	-0.020	0.063	
Combined				
Upper bounds				0.084
Lower bounds				0.032

Table 4. Pairwise comparisons of inter-population variance in allele frequency (mean Θ across seven loci). Significant (P<0.05) comparisons are italicized. Mean pairwise Θ among naturalized populations (including the Little Manistee River) is 0.003. Mean pairwise Θ among the hatchery strains (including the Little Manistee River) is 0.095.

River	MN	PM	PL	MU	BE	WH	SJ	BL	TH	LM	SK	CC
Pere Marq.	0.005											
Platte	0.002	0.004										
Muskegon	0.004	0.003	0.001									
Betsie	0.000	0.003	0.000	0.000								
White	0.000	0.000	0.000	0.000	0.000							
St. Joseph	0.000	0.007	0.000	0.000	0.000	0.000						
Black	*0.007*	*0.009*	*0.008*	*0.005*	0.001	0.000	0.006					
Thompson	0.008	0.004	0.008	0.000	0.000	0.005	0.000	0.012				
Lt. Man.	*0.004*	0.004	0.003	0.000	0.000	0.000	0.001	*0.009*	0.006			
Skamania	*0.134*	*0.147*	*0.140*	*0.156*	*0.152*	*0.167*	*0.134*	*0.146*	*0.141*	*0.155*		
Chambers Cr.	*0.078*	*0.097*	*0.091*	*0.101*	*0.092*	*0.107*	*0.081*	*0.085*	*0.076*	*0.105*	*0.046*	
Ganaraska	*0.021*	*0.035*	*0.033*	*0.033*	*0.028*	*0.025*	0.017	*0.020*	0.035	*0.038*	*0.146*	*0.080*

* P<0.05 adjusted using sequential Bonferroni tests (Rice 1989)

Table 5. Estimates of the mean and variance number of offspring produced by males and females for each mating treatment. The number of individual males and females (N) used for each treatment is also provided.

	Males			Females		
Treatment	N	mean	var.	N	mean	var.
1 (1:1)	10	19.4	34.0	10	19.4	34.0
2 (1:2, rep.)	10	17.8	144.0	10	17.8	25.3
3 (5:5)	10	16.2	233.3	10	16.2	16.6
4 (10:10)	10	17.1	192.5	10	17.1	45.7
5 (1:2, mixed)	20	9.1	38.7	10	18.1	65.0
6 (1:2, seq.)	20	9.5	43.0	10	18.9	71.0

Table 6. Total number of males and females used for matings (N), and summary measures of genetic diversity including the percent unrelated offspring, coancestry (Θ), and effective population size calculated using the number of males and females (N_e), reproductive variance (N_{ev}), and coancestry ($N_{e\Theta}$) for each of the six hatchery mating strategies used.

Treatment	N	% unrelated offspring	Θ[1]	N_e[2]	N_{ev}[3]	$N_{e\Theta}$[4]
1 (1:1)	20	90.2%	0.0244	20	19.2	19.3
2 (1:2, rep.)	20	83.3%	0.0291	20	16.4	17.2
3 (5:5)	20	74.4%	0.0344	20	14.1	14.5
4 (10:10)	20	75.6%	0.0329	20	14.7	15.2
5 (1:2, mix)	30	88.7%	0.0225	26.7	21.8	22.2
6 (1:2, seq.)	30	88.7%	0.0226	26.7	21.7	22.1

[1] Cockerham 1969

[2] Wright 1931

[3] Lande & Barrowclough 1987

[4] Chesser et al. 1993

Table 7. Number of juvenile Michigan strain steelhead annually stocked into Michigan rivers of the Lake Michigan basin, estimates of the number of juveniles that would be expected to survive to smolt stage, and percentage of the total number stocked based on estimated survival.

Year	Number stocked	Smolt-equivalent[1]	Estimated percent survival
1978	data not available	-	-
1979	1,092,273	21,091	1.9
1980	1,325,177	19,942	1.5
1981	674,343	77,508	11.5
1982	1,091,154	15,493	1.4
Mean	1,045,737	33,509	4.1
1993	475,139	388,765	81.8
1994	532,688	439,297	82.3
1995	544,530	448,243	82.3
1996	527,329	426,517	80.9
1997	544,791	482,485	88.6
Mean	524,895	437,061	83.2

[1] Smolt equivalents were calculated as described by Rand et al. (1993). Estimated survival to smolt stage was based on juvenile size and age at stocking, and stocking location.

Table 8. Estimates of the mean (± 95% CI) number of juvenile steelhead stocked into Michigan tributaries of Lake Michigan that were expected to survive to smolt (i.e. number of smolt-equivalents), and the proportion of wild-origin adults in the spawning runs of six rivers in Michigan estimated from two time periods prior to and following changes in juvenile stocking practices.

	1983-1984		1998-1999	
River	Mean number stocked per year	Proportion wild[1]	Mean number stocked per year	Proportion wild[2]
Betsie	2,588	0.82 (±0.10)	44,635	0.65 (±0.18)
Manistee	826	0.88 (±0.08)	42,781	0.65 (±0.06)
Little Manistee	1,400	0.98 (±0.03)	90	0.67 (±0.00)
Pere Marquette	113	1.00 (±0.00)	9,252	0.87 (±0.01)
White	4,880	0.88 (±0.16)	20,178	0.55 (±0.17)
Muskegon	8,514	0.70 (±0.28)	52,683	0.21 (±0.04)
Mean	3,054	0.88	28,269	0.60

[1]Data from Seelbach & Whelan (1988)

[2]Data from Bartron et al. (in review)

Table 9. Sample sizes (N), number of alleles per locus (A), allelic richness (A_r), and observed and expected (H_O and H_e) heterozygosity for each steelhead population during each time period (1983-4 and 1998-9 or 2000), and for each hatchery strain. Hatchery individuals were excluded from naturalized populations.

		Naturalized Populations										Hatchery Strains				
		Betsie		Manistee		Pere Marquette		White		Muskegon		Little Manistee		Ska-mania	Chambers Creek	Ganaraska
Locus		1983-4	2000	1983-4	1998-9	1983-4	1998-9	1983-4	2000	1983-4	1998-9	1983-4	1998-9			
Ogo1a	N	47	23	52	105	10	101	15	17	7	75	57	114	103	59	60
	A	3	3	3	4	3	5	3	3	3	5	4	4	3	3	3
	A_r	2.981	2.990	2.993	3.307	3.000	3.483	2.934	2.993	3.00	3.339	3.115	3.206	2.052	2.178	2.924
	H_O	0.596	0.739	0.654	0.629	0.600	0.683	0.600	0.294	0.714	0.720	0.544	0.614	0.271	0.271	0.483
	H_e	0.657	0.666	0.672	0.648	0.679	0.681	0.618	0.670	0.560	0.687	0.677	0.667	0.333	0.333	0.585
Ogo4	N	49	25	52	106	10	100	14	16	7	72	56	115	111	58	60
	A	4	6	7	10	4	11	7	7	5	7	7	8	8	9	9
	A_r	4.525	4.785	4.297	4.585	3.621	4.758	4.989	4.917	5.000	4.261	4.359	4.073	5.629	5.192	5.053
	H_O	0.673	0.760	0.654	0.670	0.500	0.680	0.857	0.563	0.714	0.722	0.679	0.652	0.802	0.776	0.700
	H_e	0.754	0.765	0.673	0.697	0.626	0.738	0.759	0.730	0.780	0.717	0.874	0.702	0.817	0.761	0.659
Omy77	N	49	25	48	104	10	100	15	16	7	75	56	116	97	59	59
	A	13	15	15	16	11	16	10	11	9	13	16	17	6	13	13
	A_r	7.549	8.516	7.479	7.762	9.164	7.315	7.144	6.902	9.000	7.292	7.817	7.247	4.807	7.005	7.630
	H_O	0.857	0.920	0.854	0.827	1.00	0.770	0.867	0.938	0.857	0.747	0.875	0.784	0.707	0.712	0.915
	H_e	0.864	0.898	0.870	0.874	0.932	0.855	0.871	0.821	0.912	0.872	0.874	0.832	0.722	0.866	0.892

Table 9 (cont'd).

Oneμ10	N	49	24	51	107	10	95	15	16	7	75	57	115	103	60	60
	A	5	5	7	7	3	6	5	4	3	6	6	7	3	6	6
	A_r	3.490	3.632	4.044	4.143	2.956	3.992	4.122	3.432	3.000	3.728	4.038	3.549	2.720	4.209	4.613
	H_O	0.673	0.708	0.804	0.692	0.556	0.705	0.733	0.813	0.857	0.680	0.649	0.696	0.417	0.583	0.817
	H_e	0.641	0.650	0.675	0.677	0.464	0.672	0.678	0.688	0.648	0.685	0.688	0.665	0.443	0.653	0.775
Oki200	N	49	24	46	89	10	100	15	18	7	75	57	113	106	57	60
	A	5	6	5	6	2	6	2	4	4	6	6	6	5	7	6
	A_r	2.854	4.034	3.107	3.390	2.000	2.948	1.999	2.767	4.000	3.635	3.010	3.257	3.595	4.389	3.366
	H_O	0.510	0.708	0.500	0.506	0.667	0.450	0.333	0.556	0.429	0.480	0.439	0.522	0.670	0.847	0.567
	H_e	0.456	0.559	0.475	0.555	0.523	0.453	0.434	0.440	0.396	0.533	0.481	0.523	0.650	0.770	0.543
Ots103	N	49	25	52	96	10	102	15	18	7	73	57	115	108	43	58
	A	3	3	3	4	2	3	4	3	2	3	4	3	4	3	5
	A_r	2.268	2.488	2.265	2.303	1.921	2.032	2.867	2.022	2.000	2.107	2.221	2.112	2.913	2.155	2.794
	H_O	0.184	0.280	0.231	0.208	0.200	0.196	0.400	0.167	0.000	0.164	0.193	0.209	0.417	0.217	0.362
	H_e	0.241	0.287	0.244	0.229	0.189	0.183	0.352	0.160	0.264	0.202	0.211	0.214	0.383	0.251	0.367
Mean	A	5.8	6.3	6.7	7.8	4.2	7.8	5.2	5.3	4.3	6.7	7.2	7.5	4.8	6.8	7.0
	A_r	3.945	4.408	4.031	4.248	3.777	4.088	4.009	3.836	4.333	4.060	4.093	3.907	3.619	4.188	4.397
	H_O	0.582	0.686	0.616	0.588	0.587	0.581	0.632	0.555	0.595	0.586	0.563	0.580	0.540	0.568	0.641
	H_e	0.602	0.637	0.601	0.613	0.569	0.597	0.619	0.585	0.593	0.616	0.610	0.601	0.540	0.606	0.637

Table 10. Pairwise estimates of F_{ST} between historic (above diagonal) and contemporary (below diagonal) populations. Pairwise F_{ST} estimates along the diagonal (italicized) represent comparisons between time periods for the same population. Comparisons were not statistically significant.

	Population					
Population	Betsie	Manistee	Little Manistee	Pere Marq.	White	Muskegon
Betsie	*0.000*	0.007	0.000	0.021	0.001	0.000
Manistee	0.002	*0.001*	0.000	0.002	0.002	0.000
Little Manistee	0.000	0.007	*0.000*	0.013	0.000	0.000
Pere Marquette	0.003	0.005	0.004	*0.007*	0.016	0.032
White	0.000	0.000	0.000	0.000	*0.000*	0.000
Muskegon	0.000	0.007	0.001	0.004	0.000	*0.000*

Table 11. *a.* Estimates of the yearly mean number (± SE) of juvenile steelhead stocked and proportion clipped by each of the four states bordering Lake Michigan between 1993 and 1997. Juvenile year classes are representative of expected returns to Lake Michigan tributaries during fall 1998 and spring 1999. Numbers represent averages of each strain stocked into Lake Michigan, based on the actual numbers stocked (total), and numbers adjusted for differential size- and age-specific survival (Rand et al. 1993). *b.* Average number of steelhead of each hatchery strain stocked into each of four Michigan rivers between 1993 and 1997.

a.

Hatchery	Average number of steelhead stocked by each state per year											
	Michigan		Wisconsin		Illinois[a]		Indiana			% of	smolt-	% of
strain	total	smolt-equiv.	total	smolt-equiv.[b]	total	smolt-equiv.	total	smolt-equiv.	Total	total	equivalent	total
Michigan	524,895 (28,826)	437,061 (34,047)	x	x	x	x	151,518 (26,208)	1,110 (2,446)	676,413	37.2	438,171	68.4
Skamania	76,580 (19,769)	41,439 (16,979)	159,374 (59,571)	4,782 (1,786)	39,502 (22,185)	395 (222)	396,729 (57,860)	143,884 (1,281)	672,185	36.9	190,500	29.7
Ganaraska	x	x	265,041 (116,366)	6,382 (1,601)	x	x	x	x	265,041	14.6	6,382	1.1
Chambers Cr.	x	x	206,285 (49,268)	5,464 (670)	x	x	x	x	206,285	11.3	5,464	0.9
Average number of steelhead stocked each year									1,819,924		640,517	
% hatchery steelhead marked prior to release	<100%[c]		33%		100%		15%					

b.

River	Year(s) Stocked	Strain(s) stocked	Number stocked	Smolt-equiv. stocked	Average number stocked per year	Average smolt-equiv. stocked per year
Pere Marquette	1993-1997	Michigan	51,400	46,260	14,048	9,252
	1993-1994	Skamania	14,100			
Little Manistee	1997	Michigan	500	450	100	90
Bear Cr. (Manistee)	1993-1997	Michigan	237,673	213,906	72,348	42,781
		Skamania	120,066			
Platte	not stocked					

Table 11 (cont'd).

[a] Illinois stocked Skamania in 1996 and 1997.

[b] Large disparity between total and adjusted stocking numbers for Wisconsin, Indiana, and Illinois reflect higher expected rates of mortality of smaller fingerling juveniles relative to pre-smolt size classes stocked by Michigan.

[c] This value is less that 100% because intensive marking programs began in 1995 and a percentage of older fish were not clipped.

Table 12. Pairwise estimates of inter-strain variance in allele frequency (mean F_{ST} over seven microsatellite loci) for four hatchery strains of steelhead stocked into Lake Michigan.

	Hatchery Strain		
Hatchery Strain	Skamania	Chambers Creek	Ganaraska
Skamania	-		
Chambers Creek	0.045*	-	
Ganaraska	0.117*	0.060*	-
Michigan	0.127*	0.080*	0.037*

* $P<0.05$

Table 13. Estimates of classification accuracy for likelihood-based assignment tests (Blanchong et al. 2002). Percentages on the diagonal are the proportion of individuals of that strain correctly re-classified to strain of origin based on seven microsatellite loci.

	Strain re-classified as			
Strain of origin	Michigan	Skamania	Chambers Creek	Ganaraska
Michigan	86.7 %	-	-	13.3 %
Skamania	-	98.2 %	1.8 %	-
Chambers Creek	-	5.0 %	95.0 %	-
Ganaraska	6.7 %	-	1.7 %	91.7%

Table 14. Numbers and percentage of steelhead from each hatchery strain identified based on fin clip observations and using a combination of genetic analysis and scale pattern analysis (SPA) to identify unclipped hatchery-produced steelhead. Strains include M (Michigan), Sk (Skamania), G (Ganaraska), and C (Chambers Creek).

	Fin clip observations only						Genetics/SPA						Total hatchery					
		Number % hatchery				% wild		Number % hatchery				% wild		Number % hatchery				% wild
River	n	M	Wi-Sk	G	C	(CI)	n	M	Sk	G	C	(CI)	n	M	Sk	G	C	(CI)
Fall/Spring-run combined																		
Pere	117	2	4	1	5	91%	105	1	0	2	0	97%	117	3	4	3	5	87%
Marquette		2%	3%	1%	4%	(0.00)[a]		1%		3%		(0.03)		3%	3%	3%	4%	(0.06)
Little	5294	283	35	0	35	93%	158	30	6	4	1	74%	5294[b]	1220	225	125	66	69%
Manistee		5%	1%		1%	(0.00)		19%	4%	3%	1%	(0.01)		23%	4%	2%	1%	(0.01)
Spring-run only																		
Bear Cr.	70	3	3	1	6	80%	56	3	0	3	0	89%	70	6	3	4	6	73%
		4%	4%	1%	9%	(0.01)		5%		5%		(0.08)		9%	4%	6%	9%	(0.11)
Platte R.	79	1	8	0	12	73%	58	2	0	0	0	97%	79	3	8	0	12	71%
		1%	10%		15%	(0.01)		3%				(0.05)		4%	10%		15%	(0.10)
Total		289	50	2	58			36	6	9	1			1232	240	132	89	
% of strain contribution to hatchery total		73%	12%	1%	14%			69%	12%	17%	2%			73%	14%	8%	5%	

[a] Confidence intervals are based on Rao (1998).

[b] We observed all clipped individuals, but only performed genetic analysis on a subsample of the individuals. In order to combine fin clip observations and genetics/SPA methods, strain-specific contributions estimated using genetics/SPA were extrapolated proportionally to entire sample.

Figure 1. Depiction of inbreeding and outbreeding on offspring fitness depending on relatedness of parents.

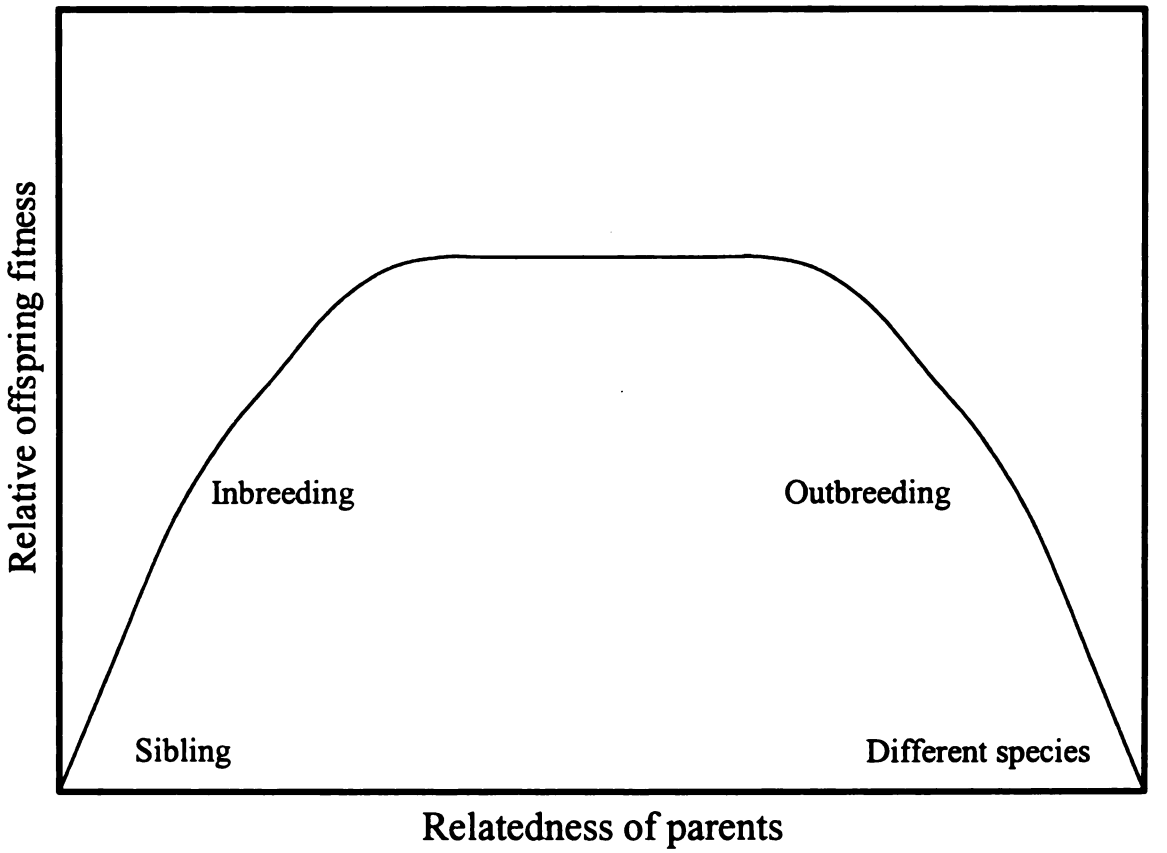


Figure 2. A map representing all naturalized populations and hatchery strains sampled in this study. Naturalized populations are numbered as follows: 1) Thompson Creek, 2) Black River, 3) Platte River, 4) Betsie River, 5) Manistee River, 6) Little Manistee River, 7) Pere Marquette River, 8) White River, 9) Muskegon River, and 10) St. Joseph River. Hatchery strains sampled are denoted as follows: C) Chambers Creek strain, G) Ganaraska strain, S) Skamania strain, M) Michigan strain.

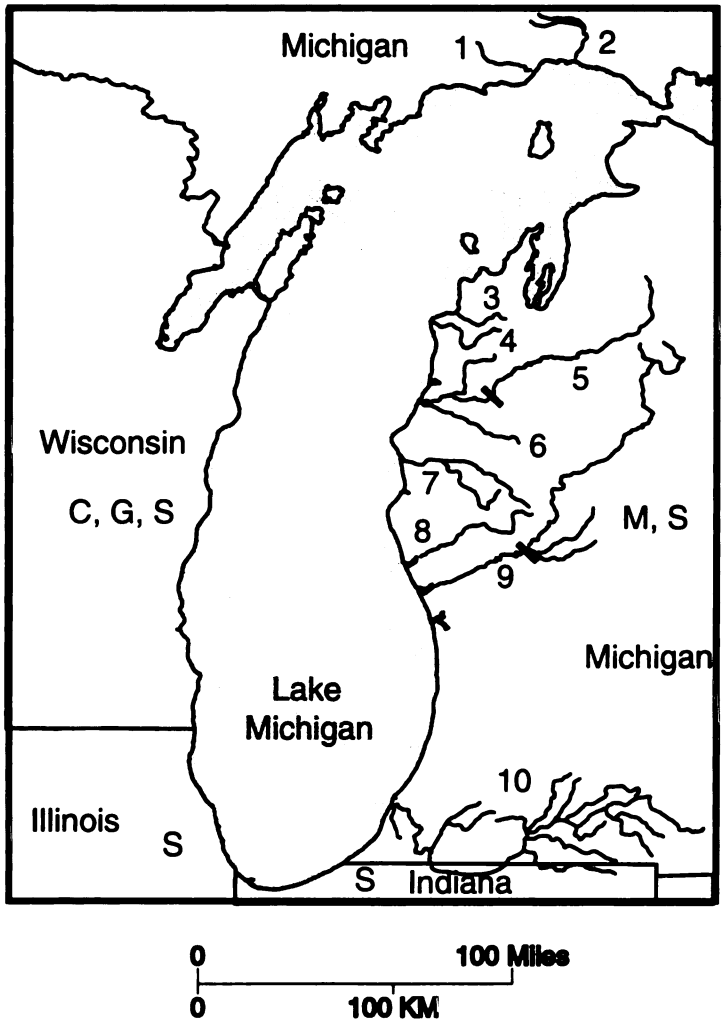


Figure 3. This map depicts locations sampled within the Manistee River, Pere Marquette River, and Muskegon River to determine whether genetically unique populations existed within river drainages. Populations sampled within the Manistee River were 1) Bear Creek, and 2) the mainstem of the Manistee River. Within the Pere Marquette River, populations sampled were 3) Baldwin River, 4) Middle Branch, 5) Little South Branch, and 6) the Pere Marquette River mainstem. Populations sampled within the Muskegon River were 7) Bigelow Creek and 8) the Muskegon River mainstem.

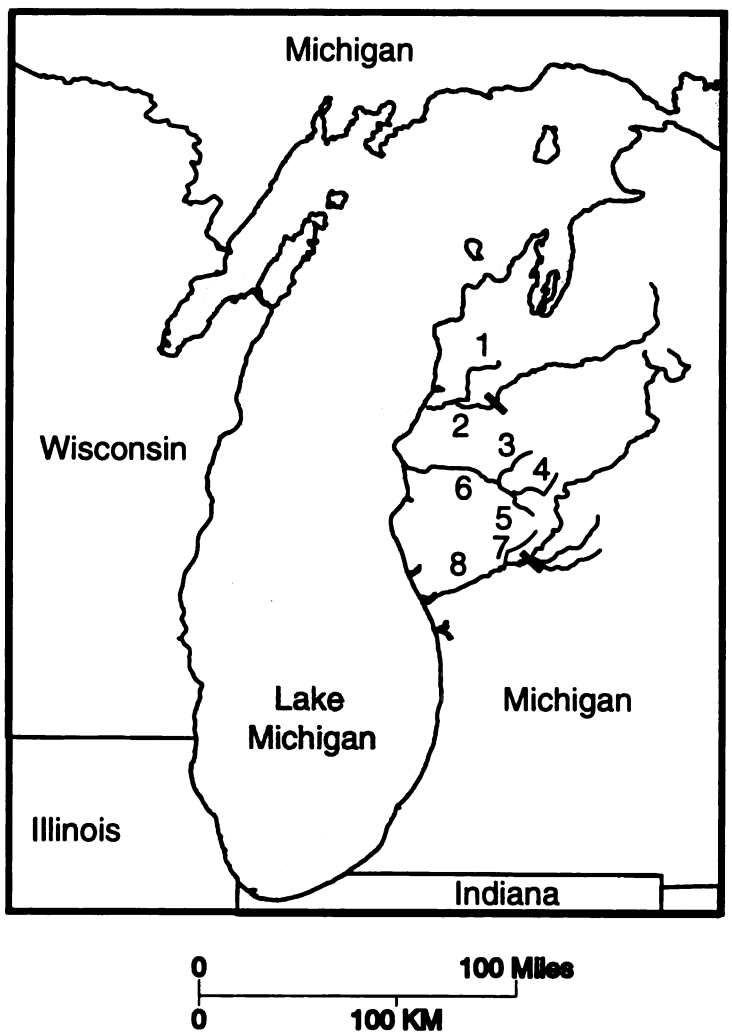


Figure 4. Estimated contributions of each population to total diversity observed based on the diversity and divergence contributed by each population. The upper graph represents the unbiased contribution to total diversity of each population, and the lower graph represents the contribution to total diversity by each population incorporating allelic richness.

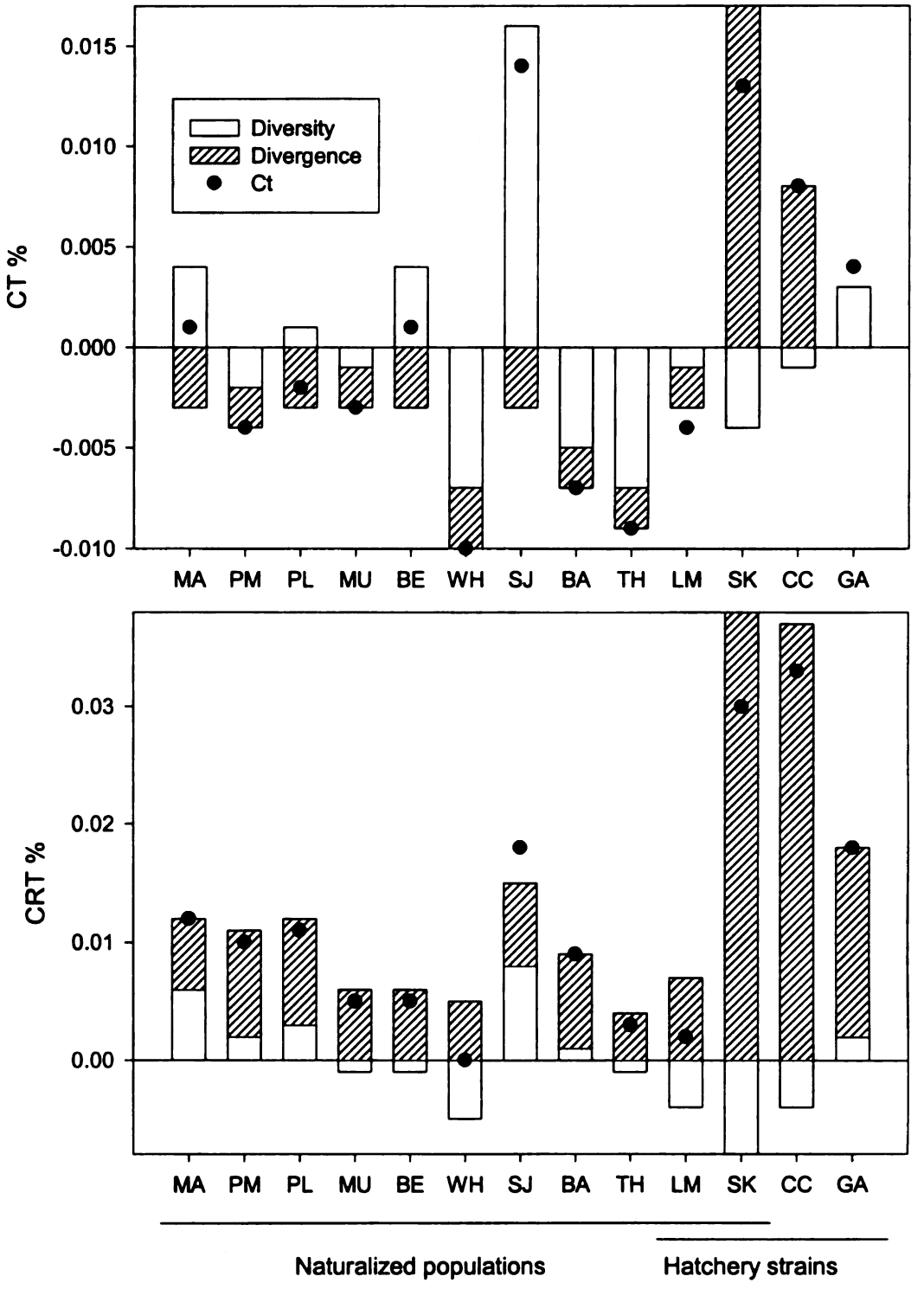
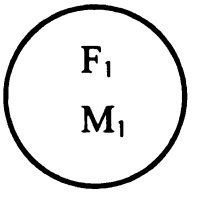
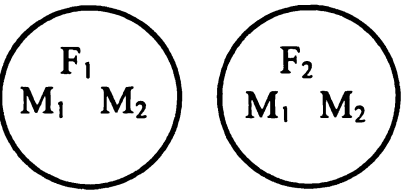


Figure 5. Diagrams of the six mating treatments used. Each circle represents the combination of gametes from representative males and females. Sex ratios are listed following the number of female to the number of males. Each treatment was repeated following the prescribed mating strategy until all males and females allotted for that treatment were used. Equal volumes of milt and eggs for each male and female were used for each individual for each treatment.

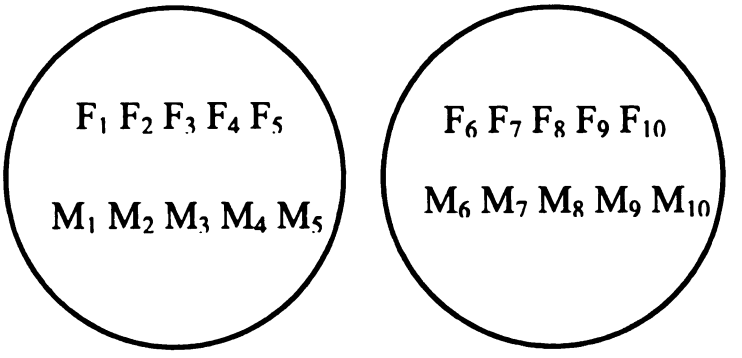
Treatment 1
Sex ratio: 1:1
No. females: 10
No. males: 10
No. matings: 10



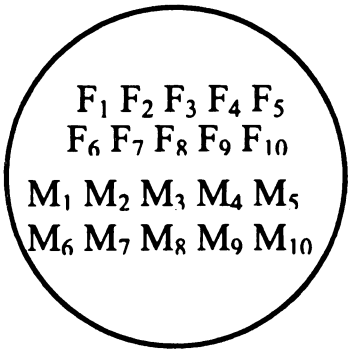
Treatment 2
Sex ratio: 1:2, repeated
No. females: 10
No. males: 10
No. matings: 10



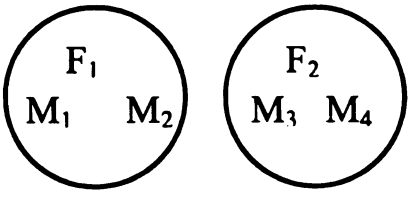
Treatment 3
Sex ratio: 5:5
No. females: 10
No. males: 10
No. matings: 4



Treatment 4
Sex ratio: 10:10
No. females: 10
No. males: 10
No. matings: 1



Treatment 5
Sex ratio: 1:2, mixed
No. females:10
No. males:20
No. matings: 10



Treatment 6
Sex ratio: 1:2, sequential
No. females: 10
No. males: 20
No. matings: 10

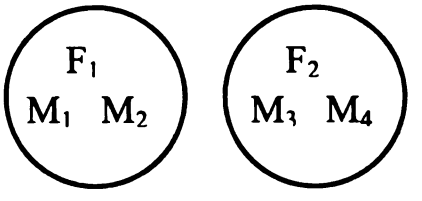
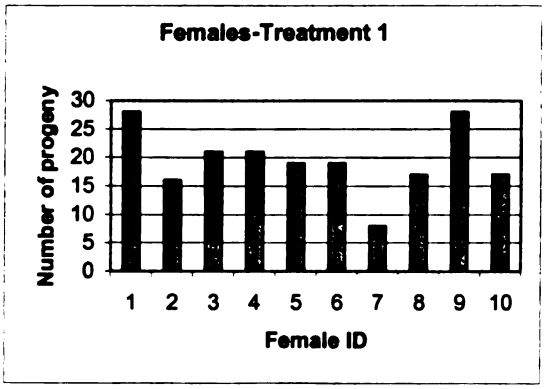
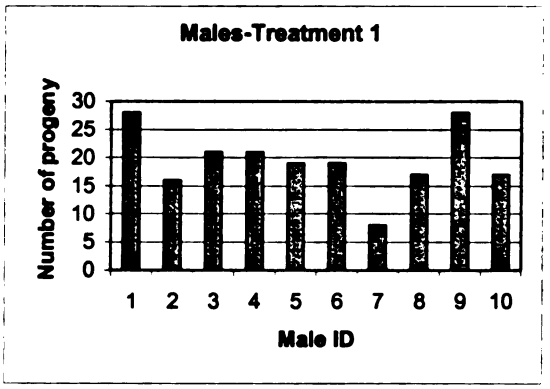
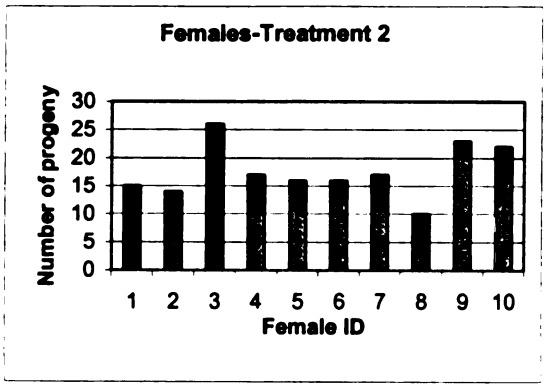
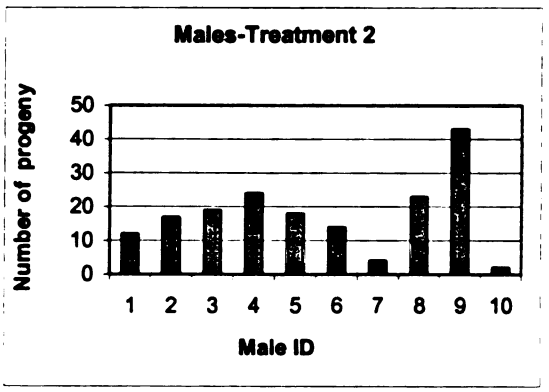


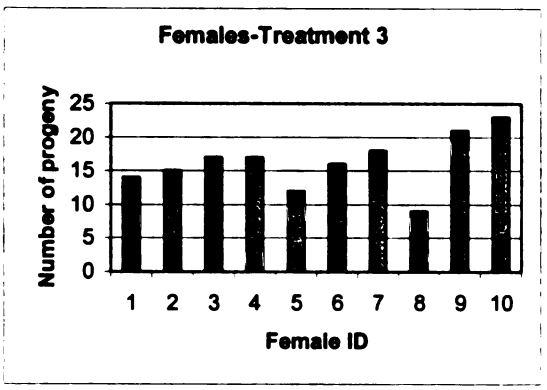
Figure 6. Number of offspring produced by each male and female in each treatment.











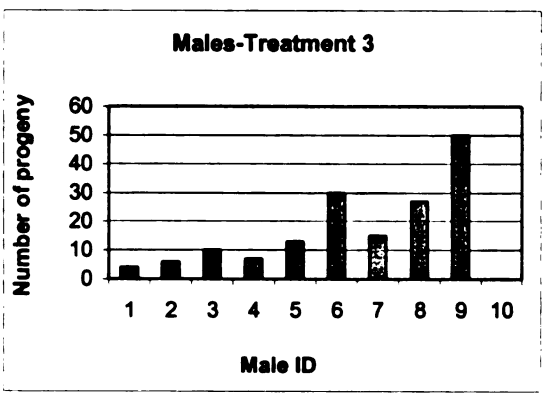
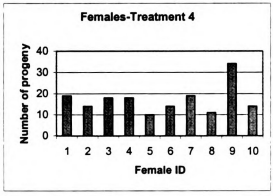
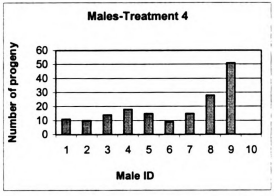
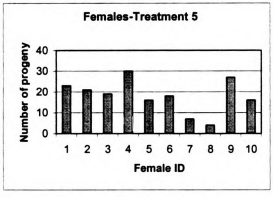
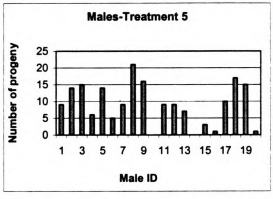


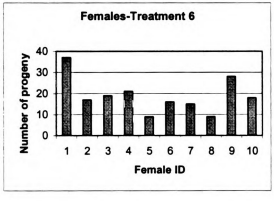
Figure 6 (cont'd).











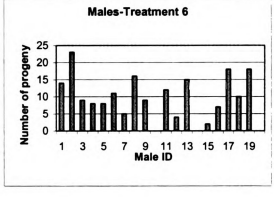


Figure 7. Predicted generational changes in population of coancestry (Θ) for populations maintained using one of the six mating strategies over successive generations. Coancestry values of 0.25 represent the relationship between full siblings.

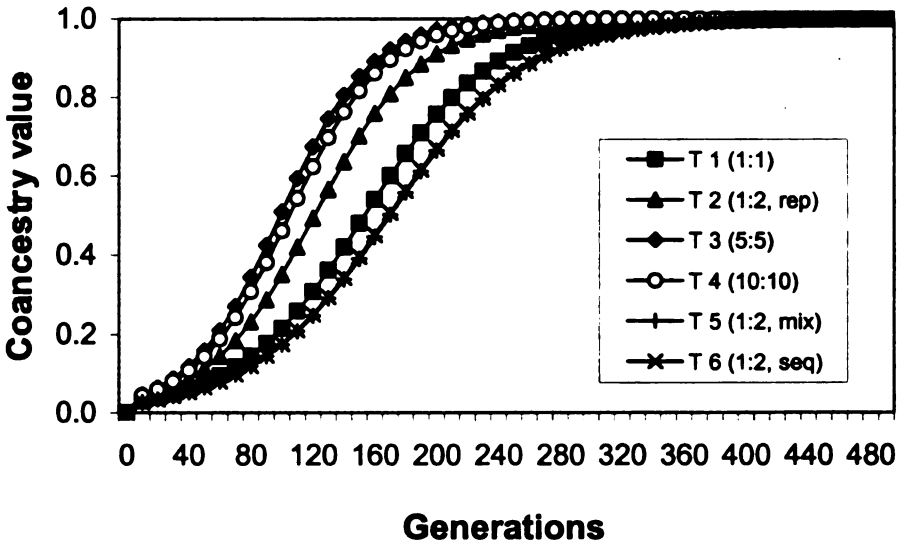
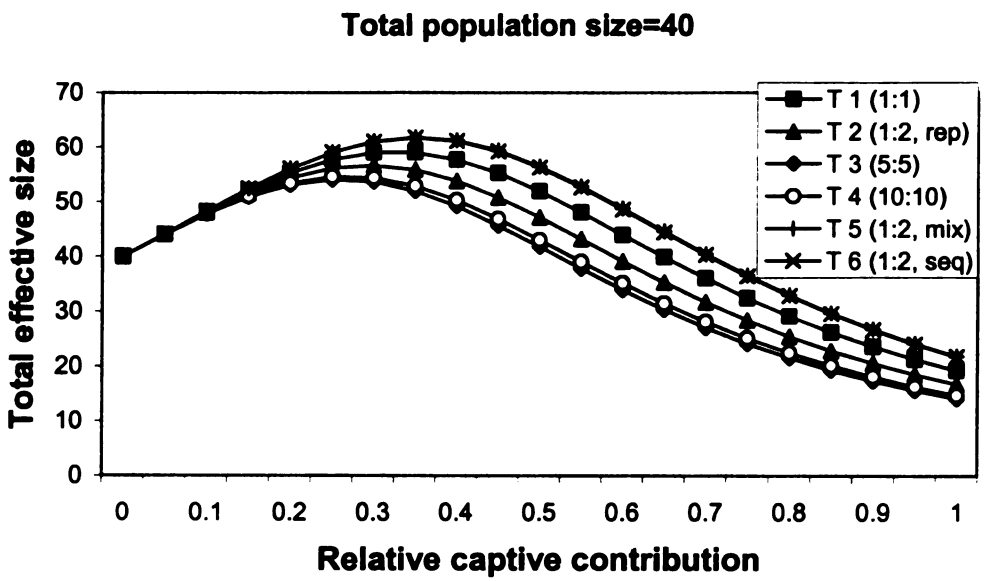


Figure 8. Demonstration of how the effective population size for captive populations maintained using six different mating can impact the total effective size of two wild populations of different size and varying contributions of the captive population, following Ryman & Laikre (1991).

a.



b.

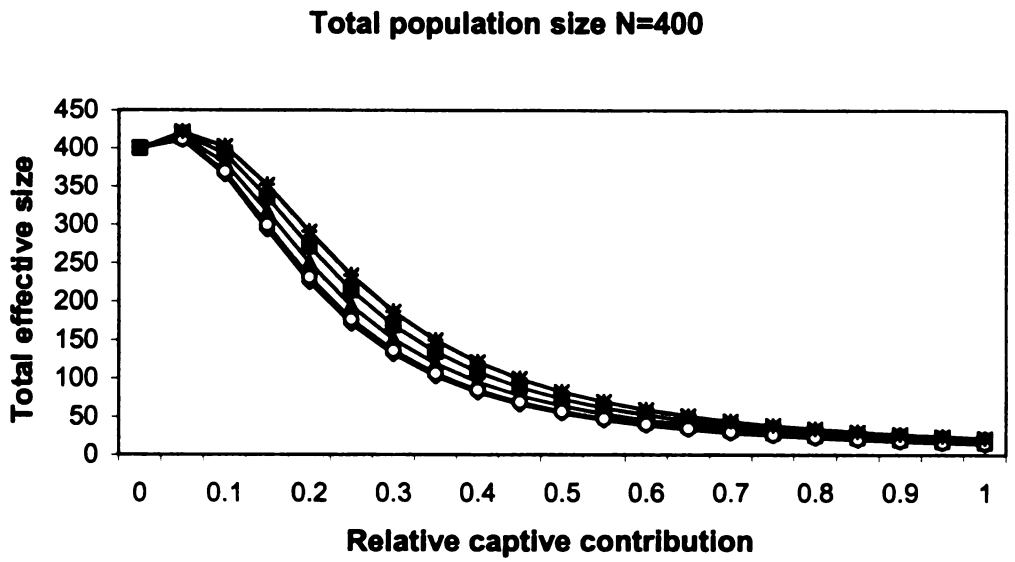


Figure 9. Geographic locations where historical and contemporary populations of steelhead were sampled from Michigan tributaries of Lake Michigan. Samples were obtained from throughout the river drainages. Numbers on maps corresponding to river names are as follows: 1) Betsie River, 2) Manistee River, 3) Little Manistee River, 4) Pere Marquette River, 5) White River, 6) Muskegon River. The bars that intersect both the Manistee River and Muskegon River correspond to dams that prevent upstream migration of adult steelhead. Sampling locations for these rivers were distributed throughout the portion of the rivers downstream from the dams.

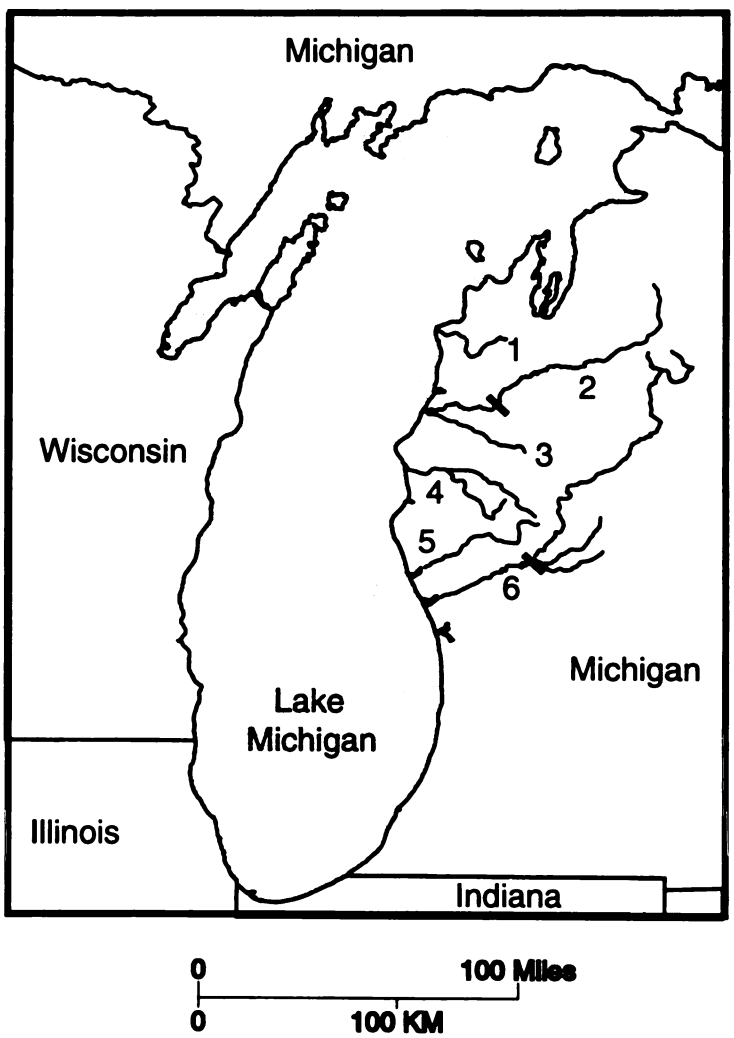


Figure 10. Unrooted neighbor-joining trees based on Cavalli-Sforza & Edwards (1967) chord distance, demonstrating the genetic relationships among each of the six populations for two time periods a) 1983-1984 and b) 1998-2000. Bootstrap values at nodes represent the percentage of 500 trees where the populations past the nodes were grouped together.

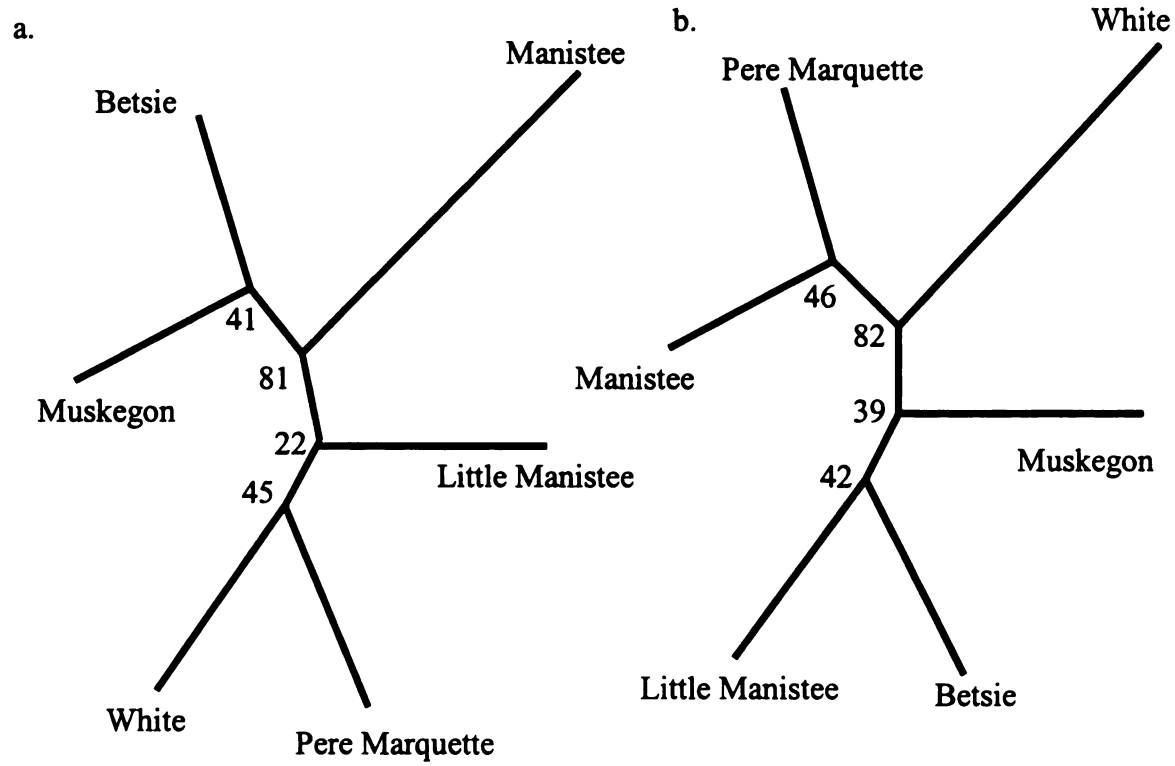
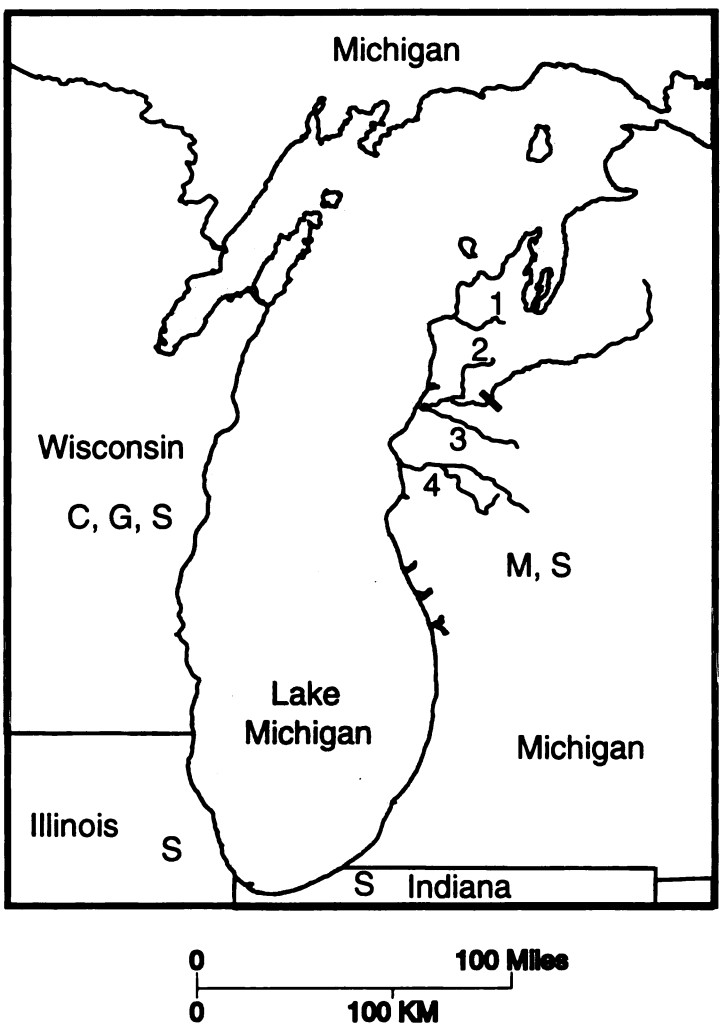


Figure 11. Geographic locations where populations of steelhead were sampled from Michigan tributaries of Lake Michigan. Numbers on maps corresponding to river names are as follows: 1) Platte River, 2) Bear Creek (tributary to the Manistee River), 3) Little Manistee River, and 4) Pere Marquette River. The bar that intersects the Manistee River corresponds to Tippy Dam, which prevents upstream migration of adult steelhead. Sampling locations for these rivers were distributed throughout the portion of the rivers downstream from the dams. Hatchery strains sampled are denoted as follows: C) Chambers Creek strain, G) Ganaraska strain, S) Skamania strain, M) Michigan strain.



LITERATURE CITED

Aas, G. H., T. Refstie, B. Gjerde. 1991. Evaluation of milt quality of Atlantic salmon. Aquaculture 95: 125-132.

Allendorf, F.W. 1993 Delay of adaptation to captive breeding by equalizing family size. Conservation Biology 7: 416-419.

Allendorf, F.W. and N. Ryman. 1987. Genetic management of hatchery stocks. in N. Ryman and F. Utter, editors. Population Genetics and Fishery Management. University of Washington Press, Seattle.

Allendorf, F.W. and R. S. Waples. 1996. Conservation and genetics of salmonid fishes. in J. C. Avise and J. L. Hamrick, editors. Conservation Genetics: Case Histories From Nature. Chapman and Hall, New York, New York.

Allendorf, F. W. and S. R. Phelps. 1980 Loss of genetic variation in a hatchery stock of cutthroat trout. Transactions of the American Fisheries Society 109: 537-543.

Allendorf, F.W. and S.R Phelps. 1981. Use of allelic frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Science. 38: 1507-1514.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tschawytscha*). Journal of Heredity 90: 281-288.

Bartron, M. L. and K. T. Scribner. in press. Temporal comparisons of genetic diversity in Lake Michigan steelhead (*Oncorhynchus mykiss*) populations: effects of hatchery supplementation. Environmental Biology of Fishes.

Bartron, M. L., D. R. Swank, E. S. Rutherford, and K. T. Scribner. in review. Methodological bias in estimates of strain composition and straying of hatchery-produced steelhead in Lake Michigan tributaries. North American Journal of Fisheries Management.

Beacham, T. D., S. Pollard, and K. D. Le. 2000. Microsatellite DNA population structure and stock identification of steelhead trout (*Oncorhynchus mykiss*) in the Nass and Skeena Rivers in northern British Columbia. Marine Biotechnology 2:587-600.

Beacham, T. D., L. Margolis, and R. J. Nelson. 1998. A comparison of methods of stock identification for sockeye salmon (*Oncorhynchus nerka*) in Barkley Sound, British Columbia. North Pacific Anadromous Fish Commission Bulletin 1: 227-239.

Beacham, T. D., S. Pollard, and K. D. Le. 1999. Population structure and stock identification of steelhead in southern British Columbia, Washington, and the Columbia River based on microsatellite DNA variation. Transactions of the American Fisheries Society 128: 1068-1084.

Bentzen P., J. B. Olsen, J. E. McLean, T. R. Seamons, and T. P. Quinn. 2001. Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. Journal of Heredity 92: 127-136.

Biette, R. M., D. P. Dodge, R. L. Hassinger, and T. M. Stauffer. 1981. Life history and timing of migrations and spawning behavior of rainbow trout (*Salmo gairdneri*) populations of the Great Lakes. Canadian Journal of Fisheries and Aquatic Science 38: 1759-1771.

Blanchong, J. A., K. T. Scribner, and S. R. Winterstein. 2002. Assignment of individuals to populations: Bayesian methods and multi-locus genotypes. Journal of Wildlife Management 66:321-329.

Blouin, M. S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology 5:393-401.

Burzynski, T. 1999. Wisconsin's Lake Michigan salmonid stocking program. Wisconsin Department of Natural Resources, Great Lakes Research Facility, Milwaukee, Wisconsin.

Busack, C. A. and K. P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium 15:71-80.

Busby, P. J., T. C. Wainwright, G. J. Bryant, L. Lierheimer, R. S. Waples, F. W. Waknitz, and I. V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. NOAA Tech. Memo. NMFW-NWFSC-27. U. S. Dep. Commerce.

Candy, J. R. and T. D. Beacham. 2000. Patterns of homing and straying in southern British Columbia coded-wire tagged chinook salmon (*Oncorhynchus tshawytscha*) populations. Fisheries Research 47:41-56.

Cavalli-Sforza, L.L. and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Chesser, R. K. 1991a Gene diversity and female philopatry. Genetics 127: 437-477.

Chesser, R. K. 1991b Influence of gene flow and breeding tactics on gene diversity within populations. Genetics 129:573-583.

Chesser, R. K., O. E. Rhodes, D. E. Sugg, and A. Schnabel. 1993 Effective sizes for subdivided populations. Genetics 135:1221-1232.

Chilcote, M.W., B.A. Crawford, and S.A. Leider. 1980. A genetic comparison of sympatric populations of summer and winter steelheads. Transactions of the American Fisheries Society 109:203-206.

Cockerham, C. C. 1967. Group inbreeding and coancestry. Genetics 56:89-104.

Cockerham, C. C. 1969. Variance of gene frequencies. Evolution 23:72-84.

Cockerham, C. C. 1973. Analysis of gene frequencies. Genetics 74:679-700.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. Comparison of methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000.

Crow, J. F. and C. Denniston. 1988. Inbreeding and variance effective numbers. Evolution 42:482-495.

Danzmann, R. G. 1997. PROBMAX: A computer program for assigning unknown parentage in pedigree analysis from known genotypic pools of parents and progeny. Journal of Heredity 88:333.

Danzmann, R. G. and M. M. Ferguson. 1988. Temperature-dependent genotypic selection and embryonic survival of rainbow trout. Biochemical Genetics 26:69-81.

DeWoody, J. A. and J. C. Avise.2001. Genetic perspectives on the natural history of fish mating systems. Journal of Heredity 92:167-172.

Dodge, D. P. 1972. Comparative bio-ecology of rainbow trout (*Salmo gairdneri*, Richardson) of three tributaries to the Owen Sound, Lake Huron. Doctoral dissertation. University of Guelph, Ontario.

Dueck, L. A., and R. G. Danzmann. 1996. Matriarchal population structure of introduced rainbow trout (*Oncorhynchus mykiss*) in the Lake Ontario watershed. Canadian Journal of Fisheries and Aquatic Sciences 53:2100-2114.

El Mousadik, A. and R. J. Petit. 1996. High levels of genetic differentiation for allelic richness among populations of the argan tree [*Argan sinosa* (L.) Skeels] endemic of Morocco. Theoretical and Applied Genetics 92:832-839.

Efron, B. 1983. Estimating the error rate of a prediction rule: Improvement on cross-validation. Journal of the American Statistical Association 78:316-320.

Eshenroder, R. L., M. E. Holey, T. K. Gorenflo, and R. D. Clark Jr. 1995. Fish-

community objectives for Lake Michigan. Great Lakes Fisheries Commission Special Publication 95-3. Ann Arbor, Michigan.

Felsenstein, J. 1993. Phylip: phylogeny inference package. Ver 3.5c. Department of Genetics, University of Washington, Seattle.

Fielder, D. G. 1987. An assessment of the introduction of summer steelhead into Michigan. Michigan Department of Natural Resources, Fisheries Research Report 1948. Lansing, Michigan.

Fleming, I. A. and M. R. Gross. 1993. Breeding success of hatchery and wild coho salmon (*Oncorhynchus kisutch*) in competition. Ecological Applications 3:230-245.

Fleming, I. A. and M. R. Gross. 1994. Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. Evolution 48:637-657.

Fleming, I. A., B. Jonsson, M. R. Gross, and A. Lamberg. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). Journal of Applied Ecology 33:893-905.

Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology 16:815-825.

Garant, D., J. J. Dodson, and L. Bernatchez. 2000. Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). Molecular Ecology 9: 615-628.

Gharrett, A.J. 1994. Genetic dynamics of a small population system: a model applicable to interactions between hatchery and wild fish. Aquaculture and Fisheries Management 25 (Suppl. 2):79-92.

Gharrett, A. J. and S. M. Shirley. 1985. A genetic examination of spawning methodology in a salmon hatchery. Aquaculture 47:245-256.

Gile, S. R. and M. M. Ferguson. 1990. Crossing methodology and genotypic diversity in a hatchery strain of rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 47:719-724.

Gile, S. R. and M. M. Ferguson. 1995. Factors affecting male potency in pooled gamete crosses of rainbow trout, *Oncorhynchus mykiss*. Environmental Biology of Fishes 42:267-275.

Goudet, J. 1995. F-STAT v. 1.2: A computer program to calculate F-Statistics. Journal of Heredity 86:485-486.

Guo, S. W. and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48: 361-372.

Hansen, M. M., E. Kenchington, and E. E. Nielsen. 2001. Assigning individual fish to populations using microsatellite DNA markers. Fish and Fisheries 2:93-112.

Hansen, M. M., D. E. Ruzzante, E. E. Nielsen, and K-L. D. Mensberg. 2001. Brown trout (*Salmo trutta*) stocking impact assessment using microsatellite DNA markers. Ecological Applications 11:148-160.

Hard, J. J. and W. R. Heard. 1999. Analysis of straying variation in Alaskan hatchery chinook salmon (*Oncorhynchus tshawytscha*) following transplantation. Canadian Journal of Fisheries and Aquatic Sciences 56:578-589.

Hard, J. J., R. G. Kope, W. S. Grant, F. W. Waknitz, L. T. Parker, and R. S. Waples. 1996. Status review of pink salmon from Washington, Oregon, and California. NTIS No. PB96-162607.

Heath, D. D, C. Busch, J. Kelly, and D. Y. Atagi. 2002. Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). Molecular Ecology 11:197-214.

Hedrick, P. W., D. Hedgecock, S. Hamelberg, and S. J. Croci. 2000 The impact of supplementation in winter-run chinook salmon on effective population size. Journal of Heredity 91:112-116.

Heggberget, T. G., B. O. Johnsen, K. Hindar, B. Jonsson, L. P. Hansen, M. A. Hvidsten, and A. J. Jensen. 1993. Interactions between wild and cultured Atlantic salmon: a review of the Norwegian experience. Fisheries Research 18:123-146.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290:516-518.

Hilborn, R. 1992. Hatcheries and the future of salmon in the northwest. Fisheries 17:5-8.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48:945-957.

Hutchings, J. A. 1991. The threat of extinction to native populations experiencing spawning intrusions by cultured Atlantic salmon. Aquaculture 98:119-132.

Kincaid, H. L. 1983. Inbreeding in fish populations used for aquaculture. Aquaculture 33:215-227.

Kincaid, H. L. 1995. An evaluation of inbreeding and effective population size in salmonid broodstocks in federal and state hatcheries. American Fisheries Society Symposium 15:193-204.

Kincaid, H. L., C. C. Krueger, and B. May. 1993. Preservation of genetic variation in the Green Lake strain lake trout derived from remnant domestic and feral populations. North American Journal of Fisheries Management 13:318-325.

Krueger, C.C. and B. May. 1987. Genetic comparison of naturalized rainbow trout populations among Lake Superior tributaries: differentiation based on allozyme data. Transactions of the American Fisheries Society 116:795-806

Lande, R. and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. in M. E. Soule, editor. Viable populations for conservation. Cambridge University Press, Cambridge.

Leider, S. A., M. W. Chilcote, and J. J. Loch. 1984. Spawning characteristics of sympatric populations of steelhead trout (*Salmo gairdneri*): evidence for partial reproductive isolation. Canadian Journal of Fisheries and Aquatic Sciences 41:1454-1462.

Levin, P. S., R. W. Zabel, J. G. Williams. 2001. The road to extinction is paved with good intentions: negative association of fish hatcheries with threatened salmon. Proceedings of the Royal Society B 268:1153-1158.

Lewis, P. O. and D. Zaykin. 2001. Genetic Data Analysis: computer program for the analysis of alleleic data. Version 1.0 (d16c). http://lewis.eeb.uconn.edu/lewishome/software.html

Lindgren, B. W. 1976. Statistical theory, 3rd edn. Macmillan Publishing Co., New York.

Lynch, M. 1997. Inbreeding depression and outbreeding depression. pp. 59-67. in W.S. Grant editor. Genetic effects of straying of non-native hatchery fish into natural populations: Proceedings of the Workshop. U.S. Dept. Comm., NOAA Tech Memo. NMFS-NWFSC-30.

Lynch, M. and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. Conservation Genetics. 2: 363-378.

Marshall, A. R., M. Miller, C. Busack, and S. R. Phelps. 1991. Genetic stock identification analysis of three 1990 Washington ocean and Strait of Juan de Fuca chinook salmon fisheries. Washington Department of Fisheries, GSI Summary Report 91-1, Olympia, Washington.

MacCrimmon, H. R. and B. L. Gots. 1972. Rainbow trout in the Great Lakes. Ontario Ministry of Natural Resources. Toronto, Ont.

Michigan fish stocking record. Fisheries Division, Michigan Department of Natural Resources, Lansing, Michigan. www.dnr.state.mi.us/fishstock.asp

Morris, D. B., K. R. Richard, and J. M. Wright. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 53: 120-126.

Moulton, F. R. 1939. The migration and conservation of salmon. Publication of the American Association for the Advancement of Science. Number 8.

National Research Council. 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington D.C.

Nelson, R. J. and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics 30: 228-229.

Nelson, R. J., T. D. Beacham, and M. P. Small. 1998. Microsatellite analysis of the population structure of a Vancouver Island sockeye salon (*Oncorhynchus nerka*) stock complex using nondenaturing gel electrophoresis. Molecular Marine Biology and Biotechnology 7:312-319.

Negus, M. T. 1999. Survival traits of naturalized, hatchery, and hybrid strains of anadromous rainbow trout during egg and fry stages. North American Journal of Fisheries Management 19:930-941.

Nielsen, J. L. 1999. The evolutionary history of steelhead (*Oncorhynchus mykiss*) along the US Pacific Coast: developing a conservation strategy using genetic diversity. ICES Journal of Marine Science 56:449-458.

Nielsen, J. L. and M. C. Fountain. 1999. Microsatelite diversity in sympatric reproductive ecotypes of Pacific steelhead (*Oncorhynchus mykiss*) from the Middle Fork Eel River, California. Ecology of Freshwater Fishes 8:159-168.

Norris, A. T., Bradley, D. G. and E. P. Cunningham. 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. Aquaculture 182:73-83.

Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1087-1089.

Osterberg, C. O. and G.H. Thorgaard. 1999. Geographic distribution of chromosome and microsatellite DNA polymorphisms in *Oncorhynchus mykiss* native to Western Washington. Copeia 1999:287-298.

Palla, J. S. 1999. 1999 Lake Michigan creel survey. Indiana Department of Natural Resources, Division of Fish and Wildlife, Michigan City, Indiana.

Page, K. S. 1996. TreeView: an application to display phylogenetic trees on personal computers. Computer Applications in Biological Science 12:357-358.

Parkinson, E. A. 1984. Genetic variation in populations of steelhead trout (*Salmo gairdneri*) in British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 41:1412-1420.

Pascual, M. A., T. P. Quinn, and H. Fuss. 1995. Factors affecting the homing of fall chinook salmon from Columbia River hatcheries. Transactions of the American Fisheries Society 124:308-320.

Pella, J. J. and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. Pages 247-276 in N. Ryman and F. Utter, editors. Population Genetics and Fishery Management. Washington Sea Grant, Seattle, Washington.

Petit, R. J., A. El Mousadik and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12:844-855.

Philippart, J. C. 1995. Is captive breeding an effective solution for the preservation of endemic species? Biological Conservation 72:281-295.

Potvin, C. and L. Bernatchez. 2001. Lacustrine spatial distribution of landlocked Atlantic salmon populations assessed across generations by multilocus individual assignment and mixed-stock analyses. Molecular Ecology 10:2375-2388.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Quinn, T. P. 1993. A review of homing and straying of wild and hatchery-produced salmon. Fisheries Research 18:29-44.

Rand, P. S., D. J. Stewart, P. W. Seelbach, M. L. Jones, and L. R. Wedge. 1993. Modeling steelhead population energetics in Lakes Michigan and Ontario. Transactions of the American Fisheries Society 122:977-1001.

Rannala, B. and J. L. Mountain. 1997. Detecting immigration using multilocus genotypes. Proceedings of the National Academy of Sciences 94: 9197-9201.

Rao, P.V. 1998. Statistical research methods in the life sciences, Brooks/Cole Publishing Company, Pacific Grove, CA.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Reisenbichler, R. R. and Phelps, S. R. 1989. Genetic variation in steelhead (*Salmo gairdneri*) from the north coast of Washington. Canadian Journal of Fisheries and Aquatic Sciences 46:66-73.

Reisenbichler, R. R., J. D. McIntyre, M. F. Solazzi, and S. W. Landino. 1992. Genetic variation of steelhead of Oregon and northern California. Transactions of the American Fisheries Society 131:158-169.

Reisenbichler, R. R. and S. P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56:459-466.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Ricker, W.E. 1972. Hereditary and environmental factors affecting certain salmonid populations. Pages 27-160 *in* R. C. Simon and P. A. Larkin, editors. The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries, University of British Columbia Press. Vancouver, B.C.

Ruzzante, D. E., M. M. Hansen, and D. Meldrup. 2001. Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. Molecular Ecology 10:2107-2128.

Ryman, N. 1991. Conservation genetics considerations in fishery management. Journal of Fisheries Biology 39:211-224.

Ryman, N., F. Utter, and K. Hindar. 1995. Introgression, supportive breeding, and genetic conservation. Pages 341-365 *in* J. D. Ballou, M. Gilpin, and T. J. Foose, editors. Population management for survival and recovery: analytical methods and strategies in small population conservation. Columbia University Press, New York.

SAS Institute. 1999. SAS/STAT Version 8.0. SAS Institute, Cary, North Carolina.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Scheer, B. T. 1939. Homing instinct in salmon. Quarterly Reviews in Biology 14:408-430.

Schroeder, R. K., R. B. Lindsay, and K. R. Kenaston. 2001. Origin and straying of hatchery winter steelhead in Oregon coastal rivers. Transactions of the American Fisheries Society 130:431-441.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53:833-841.

Scribner, K. T., P. A. Crane, W. J. Spearman, and L. W. Seeb. 1998. DNA and allozyme markers provide concordant estimates of population differentiation: analyses of U.S. and Canadian populations of Yukon River fall-run chum salmon (*Oncorhynchus keta*). Canadian Journal of Fisheries and Aquatic Sciences 55:1748-1758.

Seelbach, P. W. 1986. Population characteristics and dynamics of wild steelhead in a Michigan tributary of Lake Michigan. Doctoral dissertation. University of Michigan, Ann Arbor, Michigan.

Seelbach, P. W. 1987a. Smolting success of hatchery-raised steelhead planted in a Michigan tributary of Lake Michigan. North American Journal of Fisheries Management 7:223-231.

Seelbach, P.W. 1987b. Effect of winter severity on steelhead smolt yield in Michigan: an example of the importance of environmental factors in determining smolt yield. American Fisheries Society Symposium 1:411-450.

Seelbach, P. W. and G. E. Whelan. 1988. Identification and contribution of wild and hatchery steelhead stocks in Lake Michigan tributaries. Transactions of the American Fisheries Society 117:444-451.

Seelbach, P. W. and B. R. Miller. 1993. Dynamics in Lake Superior of hatchery and wild steelhead emigrating from the Huron River, Michigan. Michigan Department of Natural Resources, Fisheries Division, Research Report 1993, Ann Arbor, Michigan.

Simon, R. C., J. D. McIntyre, A. R. Hemmingsen. 1986. Family size and effective population size in a hatchery stock of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 43:2434-2442.

Smouse, P. E., J. Neel, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology 35:627-632.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry. 3rd ed. Freeman, San Francisco, CA.

Stauffer, T. M. 1955. Migration and growth of tagged, immature rainbow trout in the Black River, Mackinac County, and in Lake Michigan, 1951-1954. Michigan Department of Conservation, Institute of Fisheries Research Report 1441, Ann Arbor, Michigan.

Swofford, D. L. and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Taylor, E. B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98:185-207.

Thedinga, J. F., A. C. Wetherimer, R. A. Heintz, J. M. Maselko, and S. D. Rice. 2000. Effects of stock, coded-wire tagging, and transplant on straying of pink salmon (*Oncorhynchus gorbuscha*) in southeastern Alaska. Canadian Journal of Fisheries and Aquatic Sciences 57:2076-2085.

Thomas, G. L. and O. A. Mathisen. 1993. Biological interactions of natural and enhanced stocks of salmon in Alaska. Fisheries Research 18:1-17.

Tringali, M. D. and T. M. Bert. 1998. Risk to genetic effective population size should be an important consideration in fish stock-enhancement programs. Bulletin of Marine Science 62:641-659.

Utter, F. M. and F. W. Allendorf. 1977. Determination of the breeding structure of steelhead populations through gene frequency analysis. Pages 44-54 in T. J. Hassler and R. R. Van Kirk, editors. Proceedings of the genetic implications of steelhead management symposium, May 20-21, 1977, Arcata, CA. Special Report 77-1. California Cooperative Fisheries Research Unit.

Waples, R. S. 1999. Dispelling some myths about hatcheries. Fisheries 24:12-21.

Waples, R. S. 1991a. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences 48 (Suppl. 1):124-133.

Waples, R. S. 1991b. Definition of "Species" under the Endangered Species Act: Application to Pacific Salmon. NOAA Technical Memorandum NMFS F/NWC-194. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity 89:438-450.

Waples, R.S., G. A. Winans, F. M. Utter, and C. Mahnken. 1990. Genetic approaches to the management of Pacific salmon. Fisheries 15:19-25.

Washington, P. M. and A.M. Koziol. 1993. Overview of the interactions and environmental impacts of hatchery practices on natural and artificial stocks of salmonids. Fisheries Research 18:105-122.

Weir, B. S. 1990. Genetic Data Analysis. Sinauer Publishing Company, Sunderland, MA.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-Statistics for the analysis of population structure. Evolution 38:1358-1370.

Weitkamp, L. A., T. C. Wainwright, G. J. Bryant, G. B. Miller, D. J. Teel, R. G. Kope, and R. S. Waples. 1995. Status review of coho salmon from Washington, Oregon, and California, NTIS No. PB96-106554.

Withler, R. E. 1988. Genetic consequences of fertilizing chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture 68:15-25.

Wood, J. W. 1987. The genetic demography of the Gainj of Papua New Guinea, 2. Determinants of effective population size. American Naturalist 129:165-187.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.