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GENETIC DIVERSITY AND INTERRELATIONSHIPS OF WILD AND HATCHERY LAKE TROUT IN THE UPPER GREAT LAKES: INFERENCES FOR BROODSTOCK MANAGEMENT AND DEVELOPMENT OF RESTORATION STRATEGIES

By

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ABSTRACT

GENETIC DIVERSITY AND INTERRELATIONSHIPS OF WILD AND HATCHERY LAKE TROUT IN THE UPPER GREAT LAKES: INFERENCES FOR BROODSTOCK MANAGEMENT AND DEVELOPMENT OF RESTORATION STRATEGIES

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Lake trout (*Salvelinus namaycush*) populations were extirpated from most of the upper Great Lakes due to overfishing and the invasion of the predatory sea lamprey (*Petromyzon marinus*). A primary component of efforts to restore lake trout has involved the stocking of juveniles from six lake trout broodstocks. Preservation of the genetic diversity of these lake trout broodstocks and remaining wild populations is considered an important component in lake trout hatchery and stocking programs. We used molecular genetic techniques to 1) evaluate how effective management practices have been in preserving genetic diversity during the development and maintenance of lake trout hatchery broodstocks and in production of offspring for release and 2) develop a fundamental understanding of the levels and partitioning of genetic diversity among hatchery broodstocks and remnant wild populations. We observed significant differences in allele frequencies among cohorts sampled at each stage of the broodstock program. Most importantly, we present evidence of extremely low effective population sizes during the production of lake trout juveniles. Analyses revealed that a significant portion of genetic diversity in wild populations was structured based on basin of origin and among lake trout morphotypes (humpers, leans and siscowets). Recommendations related to lake trout management are developed based on these findings.

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INTRODUCTION

Historically lake trout (*Salvelinus namaycush*) of the upper Great Lakes were abundant and biologically diverse. The size of the Great Lakes basin, heterogeneous nature of the lakes, and contributions from multiple isolated Pleistocene glacial refugia (Wilson and Hebert 1996) promoted geographical and eco-phenotypic variation among lake trout stocks (Brown et al. 1981; MacLean et al. 1981; Goodier 1981). Based on phylogeographic evidence, lake trout are believed to have recolonized the Great Lakes region from three discrete glacial refugia, Beringian, Mississippian, and Atlantic (Wilson and Hebert 1996). It is likely that during long periods of isolation in these refugia lake trout became diverged genetically and likely evolved locally adapted suites of traits related to resident habitats. High levels of phenotypic diversity historically present in lake trout in the upper Great Lakes may in part reflect the diversity of ancestral forms and physiological features formerly isolated in glacial refugia (Wilson and Hebert 1996).

Discrete lake trout stocks were differentiated by spawning time and location, body type and coloration, and occupancy of different water depths (Goodier 1981). Numerous anecdotal accounts describe the diversity of lake trout populations in Lake Michigan, Lake Huron, and Lake Superior and add testimony to the status of different lake trout stocks to the Great Lakes fish community (Thomson 1883; Goode 1884). Three distinct lake trout types were recognized from Lake Michigan: the shallow water "Mackinaw" or lean trout, a deep-water "fat" or siscowet form, and a "Green Bay" variety of lake trout that has been described in accounts of historical commercial fisheries (Brown et al. 1981). Variable phenotypes of lean trout, such as the "moss trout," were also described in Lake Michigan (Brown et al. 1981). As many as twelve phenotypes are believed to

have existed in Lake Huron alone (Eshenroder 1995) and as many or more were thought to have been present in Lake Superior, particularly in waters around Isle Royale (Rakestraw 1964). Goodier (1981) advocates evidence of at least four general types of lake trout in Lake Superior; leans, siscowets, humpers, and half-breeds, noting evidence of river-run lake trout in Canadian tributaries to Lake Superior.

The lean lake trout is a slender lake trout with a streamlined shape that inhabits inshore waters (<70m). Lean lake trout spawn in shallow near-shore waters (<18m) during the months of October and November (Goode 1884). The siscowet lake trout has a more robust body and higher body fat content, and inhabits deeper offshore waters (70-150m) compared to the lean lake trout (Goode 1884; Eshmeyer and Phillips 1965). Siscowet populations have been observed in spawning condition at various times of the year (Eshmeyer 1957; Bronte 1993). The humper lake trout resides near isolated offshore reefs (or "humps") commonly surrounded by water deeper than 100m (Rahrer 1965). Phenotypically, humper lake trout are intermediates of leans and siscowets and possess intermediate levels of body fat (Eshmeyer and Phillips 1965). Humper lake trout have a restricted size distribution, mature at smaller sizes than leans and siscowets, and are long-lived (Rahrer 1965; Burnham-Curtis and Bronte 1996). Humpers spawn in September, sometimes as early as August (Rahrer 1965).

Goodier (1981), described an intermediate form of lake trout ("half-breeds") that was commonly captured at depths shallower than those inhabited by the siscowet lake trout. Half-breeds possessed characteristics resembling those of both leans and siscowets (Krueger and Ihssen 1995). Half-breeds are associated with specific areas of Lake

Superior consistent with areas of transitional depth (Goodier 1981), with sympatric or spatially overlapping populations of leans and siscowets.

Phenotypic variation among lean, siscowet, and humper lake trout could reflect adaptations to local environmental regimes (i.e., water depth and temperature). Experimental evidence suggests that variance in physical traits such as gas retention (Ihssen and Tait 1974) and fat content (Eshmeyer and Phillips 1965), related to existence at different water depth is heritable and not a plastic response to variable environments. Genetic data collected from wild lake trout populations in Lake Superior reveals that significant differences in allele frequency exist among populations within and between lake trout phenotypes (review in Krueger and Ihssen 1995).

Through the 1950s and early 1960s, a combination of habitat degradation from pollution and eutrophication, overfishing, and the invasion of the sea lamprey decimated lake trout populations throughout the upper Great Lakes (Cornelius et al. 1995; Elrod et al. 1995; Eshenroder et al. 1995; Eshmeyer 1957; Holey et al. 1995; Hansen et al. 1995). Wild lake trout populations were completely extirpated from Lake Michigan (Eshmeyer 1957) and U.S. waters of Lake Huron. Isolated remnant wild lean populations survived in Georgian Bay of Lake Huron (Berst and Spangler 1973). Timely reductions in both fishing intensity and sea lamprey abundance likely prevented extirpation of wild populations in Lake Superior. Remnant wild populations exist around Isle Royale, the Apostle Islands, Caribou Reef, and Stannard Rock (Figure 1; Rahrer 1965; Swanson and Swedberg 1980; Curtis 1990; Hansen et al. 1995). Of the historical diversity that once existed, only a few recognized types (lean, siscowet, half-breed and humper) remain (review in Krueger and Ihssen 1995), and only in Lake Superior.

In 1955, the Great Lakes Fishery Commission was established to facilitate efforts to control the sea lamprey and restore lake trout populations in the Great Lakes. A major emphasis of the lake trout restoration effort has been placed on stocking of offspring from domestic lake trout hatchery strains (Fetterolf 1980). Currently six hatchery broodstocks are maintained in the U.S. Fish and Wildlife Service hatchery system, and stocked in U.S. waters of the upper Great Lakes. Broodstocks include the Isle Royale (SIW), Apostle Islands (SAW), Marquette (SMD), Green Lake (GLW), Lewis Lake (LLW), and Seneca Lake (SLW) broodstocks (Figure 1; Krueger and Ihssen 1995).

Chapter 1

INFLUENCES OF HATCHERY PRACTICES ON THE PRESERVATION OF GENETIC DIVERSITY IN LAKE TROUT HATCHERY BROODSTOCKS USED FOR RESTORATION EFFORTS IN THE UPPER GREAT LAKES

The overall goals of this chapter are: 1) To provide an overview of the history of lake trout hatchery broodstock development and management and how management practices have impacted genetic diversity of lake trout in hatcheries and of progeny released into the Great Lakes, and 2) To examine specific areas of the broodstock program (outlined in Figure 2) that can cause changes in gene frequency and levels of genetic diversity and relatedness. We used molecular genetic techniques to evaluate the effects of hatchery practices at three stages of the lake trout broodstock program. We evaluated how effectively the genetic characteristics of wild populations were retained during initial development of captive broodstocks (Stage 1). Hatchery broodstocks should represent the genetic and ecological diversity present in wild populations. We documented changes in allele frequencies and levels of genetic diversity that occurred as successive generations of broodstocks were developed and during juvenile production (Stage 2). Allele frequencies and levels of genetic diversity should not change appreciably between broodstock generations. We examined several components of the broodstock production program by evaluating broodstock spawning records, documenting changes in allele frequency and in levels of genetic diversity between adults and progeny, and estimating the effective number of breeding adults (N_b). Allelic frequencies and levels of genetic diversity should not change appreciably between adults and juveniles. Broodstock management should maximize the number of adults

contributing progeny and minimize male and female reproductive variance to maximize the effective number of breeders. Finally, we evaluated management practices related to collection and distribution of fertilized gametes (Stage 3). Ideally, eggs and juveniles should be collected, distributed, and released to maximize the number of adults spawned across the entire spawning period.

History of Hatchery Broodstocks

Hatchery broodstocks maintained by the U.S. Fish and Wildlife Service constitute a large portion of the remaining lake trout diversity in the upper Great Lakes (this study) and form the basis for lake trout restoration in U.S. waters of Lake Michigan (Holey et al. 1995) and Lake Huron (Eshenroder et al. 1995). Two of these broodstocks, LLW (Visscher 1983) and GLW (Kincaid et al. 1993), represent remnant genetic vestiges of Lake Michigan populations. The SMD broodstock represents what remains of extirpated near-shore lean populations in Michigan waters of southern Lake Superior (Krueger et al. 1983). These broodstocks also may still possess genetically determined suites of co-adapted traits (e.g., ecological and behavorial traits) characteristic of their respective source populations (Krueger et al. 1983). These co-adapted gene complexes are important because they provide a blueprint for survival within the habitat that each source population occupies. The historical background pertaining to establishment and perpetuation of each lake trout broodstock provides important predictive potential of the likelihood of changes in genetic characteristics of each broodstock and for identification of factors that are of importance for the preservation of genetic diversity.

Apostle Island (SAW).- Collections were made from a wild lake trout population from Gull Island Shoal in the Apostle Islands (eastern Lake Superior) over a five-year period to produce five broodstock year classes (1986, 1987, 1996, 1998 and 1999). Wild fish (≥100), were spawned employing a male to female ratio of 2:1 and 1:1 to develop the 1986 and 1987 broodstock year classes. The 1993 and 1994 broodstock year classes were developed from reciprocal crosses of the 1986 and 1987 broodstock year classes from the matings of 138 individuals and greater than 120 individuals respectively (D. Bast, Iron River National Fish Hatchery, personal communication; S. Schram, Wisconsin Department of Natural Resources, personnel communication).

Green Lake (GLW).- The Green Lake strain is one of two broodstocks (together with the Lewis Lake broodstock) that originated from historic lake trout populations of Lake Michigan. GLW is the oldest of the six domestic broodstocks and likely the most influenced by hatchery practices. Between 1886 and 1944, Green Lake, Wisconsin was stocked with lake trout obtained from spawning reefs in southern Lake Michigan and Green Bay. In 1958 and 1959, Green Lake lake trout were spawned to form the original Green Lake strain broodstock year classes 1959 and 1960. During 1958 and 1959, 187 (20 females and 167 males) and 309 (78 females and 231 males) individuals respectively were spawned to create these two broodstock year classes. However, the genetic integrity of these two broodstock year classes is suspect. Lake trout stocked into Green Lake after 1952 were of Lake Superior origin and although identifiable by fin clips, some individuals may have been spawned during the development of early broodstock year classes. A large number of juveniles stocked in 1944 may have originated from Lake Superior populations and a large portion of this the 1944 year class likely contributed to

the 1959 and 1960 year classes. Additionally, the 1959 and 1960 broodstocks were combined with males of Lake Superior origin to form a composite broodstock year class referred to as the 1959/1960 Green Lake broodstock. Introduced Lake Superior males were believed to count for as much as 10% of the Green Lake broodstock males (Krueger et al. 1983). The1959/1960 Green Lake broodstock was used to create a 1965 year class that was subsequently back-crossed with the 1959/1960 parental broodstock to produce the 1975 year class. The 1975 year class in turn was used to produced the 1984 year class. The 1959/1960 Green Lake broodstock and the 1965 year class were eliminated in 1975. The 1975 Green Lake year class now resides in the Genoa National Fish Hatchery (Coberly and Horall 1982; Krueger et al. 1983).

During, 1970-1975, juveniles from crosses of the 1959/1960 Green Lake broodstock were stocked in southern Lake Michigan. Individuals from the 1970-1975 production years were the primary source of the contemporary Green Lake strain (Coberly and Horall 1982; Krueger et al. 1983). Four Green Lake year classes, 1986, 1987, 1988, and 1989, were derived from the spawning of hatchery released lake trout sampled from Julian's Reef and Black Can Reef in southern Lake Michigan. The 1986 broodstock year class was composed of separate broodstock lots, 86A and 86C. Individuals spawned for each broodstock lot were 9 and 34 respectively. Eleven individuals were spawned to create the 1987 broodstock year class. All year classes except for 1987 and a small fraction of 1986 (N<100), were eventually lost due to hatchery mechanical failures. As a result, the contemporary 1992 and 1993 Green Lake year classes were derived from crosses of the remaining individuals of the 1986 and 1987 year classes, and the 1984 year class. Seven females from each year class were spawned

1:1 with males from each year class. Juveniles from each paired mating and within each year class spawning combination were segregated, which effectively created 63 different but related families. Juveniles were sampled equally across families to create the current Green Lake 1992 and 1993 broodstock year classes (Kincaid et al. 1993).

Isle Royale (SIW).- The original Isle Royale broodstock was developed from a wild population sampled from seven sites around Siscowet Bay, Isle Royale (northern Lake Superior) (D. Bathel, Minnesota Department of Natural Resources, personal communication). Four broodstock year classes, 1981, 1982, 1984 and 1986, were developed from gametes taken from 56, 76, 126 and 54 individuals respectively. A male to female spawning ratio of 2:1 or 1:1 was employed. A 1989 broodstock year class was developed from crosses between 212-306 adults of the 1981 and 1982 year classes. A 1993 year class was developed from crosses of 192 adults from the 1982, 1984 and 1986 year classes. Approximately 371 and 469 individuals are maintained for the 1989 and 1993 year classes respectively (D. Bast, IRNFH, personal communication).

Lewis Lake (LLW).- Lake trout from northern Lake Michigan were sampled in 1889 from several shallow reefs near Beaver Island. Of the nearly 5,000,000 eggs collected, 100,000 were designated for stocking in Yellowstone National Park, Wyoming. As few as 15-20 females could have contributed to this original egg take. Of greater than 42,000 fry, 12,010 were stocked into Lewis Lake. The remaining fry were stocked into Shoshone Lake. In 1941, an additional 5,890 fish of unknown origin were stocked into Lewis Lake. The initial LLW broodstock was developed at the Jackson Hole National Fish Hatchery (NFH) in 1983 (Krueger et al. 1983; Visscher 1983). The 1989 and 1991

year classes at the Pendill's Creek NFH were derived from 136 individuals of the captive broodstock at Jackson Hole NFH (Kincaid et al. 1994).

Marquette-Superior (SMD).- The Marquette broodstock represents extirpated lake trout populations from in shore reefs in Keweenaw Bay, Lake Superior. From the 1960s through the 1980s, the Marquette strain was the predominant strain stocked in Lake Michigan. The initial Marquette broodstock was developed in 1948 from wild fish captured in Marquette Harbor, Copper Harbor and an unidentified reef east of the Keweenaw Peninsula. Crosses among individuals of this year class produced five additional domestic year classes (1954, 1955, 1956 and 1957). A 1962 year class was produced from crosses between adults from the 1954 and 1955 year classes. A 1963 year class was produced from crosses among 1950's year classes (Coberly and Horall 1982; Krueger et al. 1983).

During the 1960's the Marquette strain became partially integrated with lake trout from the Green Lake (GLW) strain and hatchery fish derived from a wild remnant lake trout population near Gull Island Shoal in the Apostle Islands (western Lake Superior). Individuals from the 1959 GLW year class in addition to individuals from the 1955 and 1957 Marquette broodstock year classes were utilized to create the 1968 Marquette year class. A 1956 year class, derived from Apostle Island lake trout, created a 1965 year class that was directly integrated into the Marquette broodstock. Additionally, two broodstock year classes, created purely from the 1970 and 1969 broodstock year classes, were created during this time (Cobberly and Horall 1982; Krueger et al. 1983).

Six additional year classes were created prior to the current Marquette broodstock. These year classes include the 1974,1975,1976,1977,1978 and 1981. The 1976

broodstock was partially developed from the Apostle Island 1965 broodstock. The history of the 1976 year class is of particular interest since crosses among adults within this one year class were used to create the current 1988 year class. A 1987 year class is also maintained (Kincaid et al. 1994).

Seneca Lake (SLW).- The Seneca Lake strain is the only strain originating from outside of the upper Great Lakes basin. This broodstock was developed from deep-water lake trout populations from Seneca Lake, New York. Evidence of successful reproduction (Grewe et al. 1994) and the propensity of Seneca Lake strain fish to avoid sea lamprey predation (Eshenroder et al. 1995), has made this strain an attractive option for supplemental stocking in the upper Great Lakes.

In 1987 and 1992, respectively 137 (49 females and 88 males) and 315 (105 females and 210 males) wild lake trout from Seneca Lake were spawned to develop the 1987 and 1992 SLW year classes. Of the 107,000 eggs collected and fertilized from adults in 1987, 60,000 survived to the fingerling stage, of which approximately 900 were collected and maintained at Pendill's Creek NFH as the 1987 SLW brodstock year class. A total of 98,990 eggs were collected and fertilized from adult lake trout sampled in 1992. Of these 98,990 eggs, 70,000 survived to the fingerling stage. Approximately 500 fingerlings were collected to develop the 1992 SLW broodstock year class maintained at Pendill's Creek NFH (D. Blick, Allegheny NFH, personal communication).

Traverse Island (STW).- In 1996, 1997, and 1998, native lake lake trout from near-shore waters around Traverse Island (Lake Superior) were sampled and spawned to develop the STW broodstock. Traverse Island was one putative site sampled in the late 1940's to develop the original SMD broodstock. The 1996 broodstock year class,

analyzed in this study, was developed from 128 males and 64 females spawned at a 2:1 ratio. Eggs from each 2:1 male to female mating (54 families, some sets lost) were maintained segregated from one another. A total of 5,361 eggs were sampled from these families (100-131 from each family), of which, 4,088 were used to create the 1996 broodstock year class. This year class is maintained by Pendill's Creek National Fish Hatchery (M. Donofrio, Keweenaw Bay Indian Community Natural Resources Department, personal communication).

The 1997 broodstock year class was developed from 24 females and an unknown number of males sampled from Traverse Island waters in 1996. The spawning method used to create this broodstock year class is unknown (M. Donofrio, Keweenaw Bay Indian Community Natural Resources Department, personal communication). In 1997, 66 lake trout (33 females, 33 males) were collected and spawned 1:1 to create the 1998 STW broodstock year class. A total of 4,950 eggs were collected, 150 from each of 33 families. Of the resulting juveniles, 2,031 were designated for the 1998 STW broodstock year class. This year class is maintained at Iron River National Fish Hatchery (Michael Donofrio, Keweenaw Bay Indian Community Natural Resources Department, personal communication).

In recognition of the genetic and ecological diversity represented by the different lake trout broodstocks, the U.S. Fish and Wildlife Service recently adopted a hatchery broodstock management plan (Holey 1997). The management plan recognizes the importance of maintaining genetic diversity within and among existing broodstocks and outlines hatchery practices that should be adopted to minimize loss of genetic diversity. Included in the plan are guidelines for identification and sampling of source populations,

broodstock development and maintenance, and spawning. Recommendations outlined include: 1) a minimum of 200 individuals sampled from source populations; 2) 1:1 paired matings; 3) egg collections representative of numbers of individuals spawned and over greater than 60% of the spawning period; and 4) broodstock production numbers sufficient to minimize loss of genetic variation over three generations (i.e., ≤1% loss of genetic variation or 150 individuals).

There is increased awareness of the degree to which hatchery management practices can influence genetic characteristics of broodstocks and their progeny released into natural environments (Allendorf and Ryman 1987, Waples et al. 1990, Waples 1991). The ability of hatcheries to conserve levels of genetic variation characteristic of progenitor wild source populations hinges upon proactive hatchery management with respect to several fundamental population genetics principles related to coancestry or degree of inter-individual relatedness, inbreeding, genetic drift, and effective population size.

Differences in genetic characteristics either between year classes or between adults and progeny are a reflection of different spawning practices during production of each cohort. Large breeding populations that were evaluated in this study were expected to show minimal change in genetic characteristics across generations. Levels of inbreeding and inter-individual relatedness, gene frequencies and genetic diversity (i.e., heterozygosity), and effective population sizes between lake trout cohorts were estimated to measure the influences of the lake trout hatchery program on actual and potential changes in these genetic characteristics.

Inbreeding results from matings between two related individuals. Inter-individual relatedness or coancestry can accrue rapidly under circumstances routinely realized in hatcheries (e.g., Figure 2). Inbreeding coefficients (F) of offspring are directly related to the coancestry (θ) of their parents. In the absence of immigration, as is typical for most hatchery broodstocks, the accumulation of coancestry among individuals within groups is affected by the manner in which male and female gametes are mixed as well as by the means and variance in number of progeny produced by each mating (Chesser 1991a, b). F and θ will vary from cohort to cohort based on efficacy of spawning practices of each spawning period. In closed populations, extreme values of coancestry are realized by male polygamy (Chesser 1991a, b), which is commonly realized in hatchery spawning practices involving the pooling of gametes (Figure 3; Gharrett and Shirley 1985; Withler 1988). High coefficients of relationship among members of a domestic broodstock can have profound impacts on levels of inbreeding and population fitness of progeny to be released into the natural environment.

The inbreeding coefficient, "F", is a statistical measure of the rate of inbreeding among individuals. In a finite population that is closed to immigration (e.g., a hatchery broodstock), F will always increase. Inbreeding can be considered in the context of loss of genetic variation in which increases in F over multiple generations can be compared to expected levels of gene correlation within individuals (i.e., if all were mated at random). Inbreeding affects genetic diversity by reducing heterozygosity in a population. The number of homozygous individuals will increase with the number of individuals possessing correlated genes (Busack and Currens 1995). As F increases across generations, the fraction of heterozygotes in the population decreases (Kincaid 1983;

Tave 1993). Population fitness will decline as a result of decreased genetic variability (Allendorf and Leary 1986) and inbreeding depression.

Inbreeding depression is the reduced "performance" measured for traits in a population due to the expression of deleterious alleles (Kincaid 1983). The most common traits affected by inbreeding depression are traits related to population sustainability such as fecundity, gamete viability, juvenile size or other surrogate measures of juvenile survival (Kincaid 1983). Numerous examples of reduced population fitness related to inbreeding in aquaculture systems have been documented (Kincaid 1983; Simon 1991; Tave 1993). Mating systems that avoid unequal sex ratios, high variance in reproductive success, and consequently support large effective population sizes will decrease the probability of incestual matings and minimize inbreeding (Waldman and McKinnon 1993).

Genetic drift is the random change in gene frequencies over generations (Wright 1931). Drift is realized in all populations, but is most pronounced in populations of small size. Genetic drift ultimately results in loss of genetic diversity and ultimately in fixation of alleles. The probability that an allele becomes fixed or lost is dependent on its initial frequency in a population. The rate at which alleles become fixed or lost is inversely related to population size (Allendorf and Ferguson 1990).

An important principle to consider in the preservation of genetic diversity in closed populations is the effective population size (Lande and Barrowclough 1987; Nunney and Elam 1994). There are a numerous measures of effective population size including variance effective population size, inbreeding effective population size, and the coancestral effective population size. The variance effective population size (N_e) is

defined as the number of individuals in a population that would give the same variance (over time or between generations) in allele frequencies due to genetic drift as in an ideal population (i.e., equilibrium population, Wright 1931). Inbreeding and coancestral effective population sizes are population sizes that would reflect the same rate of change in heterozygosity and coancestry as an ideal population. Variance effective population size estimate in this study is usually smaller than the actual number individuals in a population (N) owing to reduced or fluctuating population size, variance in family size (reproductive success), and unequal sex ratios (Lande and Barrowclough 1987; Frankham 1995).

Large effective population sizes are expected to minimize the effects of genetic drift and inbreeding, thus conserving genetic diversity and maintaining population fitness. Lower levels of genetic variation have been shown to be positively correlated with small effective population size (review in Lande and Barrowclough 1987; Frankham 1996). Genetic diversity (heterozygosity) is lost in a population under genetic drift at a rate equal to $1/(2N_e)$ per generation. Decreased genetic diversity can lead to decreased fitness in the form of smaller individual size, lower fecundity, smaller population size and increased developmental deformities (Smith et al. 1976; Allendorf and Leary 1986; Quattro and Vrijenhoek 1989; Leberg 1990; Amos and Hoelzel 1992). Therefore, effective population size is of importance for species of conservation or management interest.

The effective population size (N_e) differs from the effective number of breeders (N_b) estimated in this study. The effective population size can be estimated directly using ecological and demographic parameters of a population (Nunney and Elam 1992), or by independent means using temporal changes in allelic frequencies across generations

(Waples 1991). Estimating N_e from ecological and demographic data for wild populations is often difficult due to the paucity of background population data needed to estimate parameters of ecological models (Nunney and Elam 1992). Even in captive populations, pedigree and juvenile survival information may be unavailable or too costly to obtain (Waples 1990). Therefore, indirect genetic methods have been advocated for these situations. Estimation of effective population sizes using temporal variance in allele frequencies for organisms with overlapping generations have been shown to be biased (Jorde and Ryman 1995). The estimate of effective number of breeders (N_b) utilized in this study is a proven method for estimating effective population sizes of discrete, closed captive populations (Waples 1990).

In recognition of the importance of these genetic principles, recommendations have been proposed to conserve genetic diversity in hatchery populations (see Appendix I; Allendorf and Ryman 1987; Allendorf and Phelps 1980; Busack and Currens 1995; Hynes et al. 1981; Kincaid 1995, Kreuger et al. 1981; Simon 1991; Kapuscinski and Jacobson 1987; Waples et al. 1990; Gharrett and Shirley 1985; Withler 1988). Figure 2 illustrates the practices and issues that are involved in a hatchery broodstock program. Genetic diversity may be progressively lost throughout a hatchery program, but diversity may also be affected at points within discrete periods of the broodstock program. We decompose the lake trout broodstock program into three major stages to better articulate how genetic diversity may be affected by specific practices within the lake trout broodstock program. These stages are: Stage 1 - the broodstock development stage in which new broodstocks are developed by sampling from source populations; Stage 2 - the maintenance and production stage in which broodstocks are perpetuated and juveniles

produced for stocking; Stage 3 - the collection and distribution of gametes and juveniles in which fertilized eggs or juveniles are collected and distributed to rearing facilities and specific stocking locations.

METHODS

Broodstocks are composed of several year classes that are defined by production year. The broodstocks analyzed in this study were composites of multiple broodstock year classes. To simplify statistical analyses and comparisons, we combined samples across year classes based on their broodstock association. We will refer to these separate year classes as simply the LLW, SIW, SAW, SLW, GLW, and SMD broodstocks. For the year classes considered in this study for each broodstock, refer to the *History of Hatchery Broodstocks* section of the introduction.

Stage 1: Broodstock Development

Sample Collection.- The LLW, SIW, and SAW hatchery broodstocks were compared to their wild populations. Samples of the three captive hatchery broodstocks were sampled by hatchery personnel during routine spawning events in the fall of 1998. The LLW broodstock was sampled from Pendill's Creek/Hiawatha National Forest Fish Hatchery in Michigan and the SIW and SAW hatchery broodstocks were sampled from the Iron River National Fish Hatchery in Wisconsin. Samples consisted of fin clips (~1cm ^{2}) removed from caudal fins and stored individually in 1.5 ml vials containing 1 ml high salt buffer (4M urea, 0.2M NaCl, 0.1M Tris-HCL, 0.5% Sarcosine, 10mM

EDTA). Two hundred adults were sampled from the SAW, LLW, and SIW broodstocks. Fin clips were stored at -20°C until analysis.

We also sampled the LLW, SIW, SAW hatchery broodstock source populations. Lake trout representing the LLW source population (N=77) were sampled from Lewis Lake, WY, by USFWS personnel at the Yellowstone Fisheries Resource Office from Lewis Lake, Wyoming. Isle Royale wild lean lake trout, representing the source population for the SIW broodstock (N=119), were sampled in the summer and fall 1995. Wild lake trout sampled from Gull Island Shoal in the Apostle Islands, WI (N=68), represent the source population for the SAW broodstock. Isle Royale and Lewis Lake samples consisted of liver tissue preserved in ethanol. The Apostle Island wild samples consisted of liver and scale tissue. Scale tissue was sampled in 1999 from archival collections located at the Wisconsin Department of Natural Resources Bayfield station. We used samples that were collected from the same or adjacent locales that were sampled to developed the LLW, SAW, and SIW broodstocks.

DNA Extraction.- DNA extraction of liver and fin tissue was performed using a proteinase K digestion and a modified Puregene extraction protocol (Gentra, Inc., Minneapolis, MN). DNA was resuspended in 50 ml of TE buffer (10 mM Tris-HCL, pH 8.0, 1 mM EDTA). Fluorometry was used to determine DNA concentrations. Prior to flourometry, RNAse (2 µl of 20mg/µl stock) was added to each sample. One hundred nanograms of DNA was used for each PCR reaction.

A Chelex procedure was utilized for DNA extraction from scale samples. Scales (3-5 per individual) were added to 250 µl of a 5% Chelex and 10 mM Tris-HCL (pH 7.5-8.0) suspension. Scales were digested overnight with 3 ul of proteinase K. Proteinase K

was subsequently inhibited by heat denaturation at 95°C for 5 minutes and samples were centrifuged at 14000 rpm for 10 minutes, the resulting supernatant was removed and 2.5 μl of the supernatant was used for each PCR reaction.

Microsatellite Screening.- Microsatellite DNA markers possess qualities well suited for population genetic analyses (Beaumont and Bruford 1999, Scribner and Pearce 2000). Microsatellite loci reside in noncoding regions of the genome and therefore are not evolving under the constraints of natural selection, nor do they code for polygenic traits (i.e., phenotypic and behavioral traits important for fitness). Microsatellite markers are useful for analysis of the influences of hatchery practices on population genetics dynamics (i.e., genetic drift and inbreeding). Allelic diversities at microsatellite loci are typically greater than levels for other genetic markers, allowing greater sensitivity in investigations of genetic drift and inbreeding. However, measures of gene diversity and loss of diversity for loci underlying polygenic traits (i.e., spawning time, fecundity) will not necessarily provide measures of diversity concordant with neutral markers (Hard 1995; Pfrender et al. 2000). Microsatellites are ideally suited to chronicle the transmission of genes across generations. In addition, microsatellite markers are readily amplified in vitro using polymerase chain reaction techniques, from small amounts of tissue, and eliminating the need for invasive and destructive sampling. Microsatellite markers used in this study were previously developed from brook trout (*Salvelinus fontanalis*) (*Sfo1*, *Sfo12* and *Sfo18*; Angers et al. 1995), sockeye salmon (*Onchorynchus nerka*) (*Oneμ9* and *Oneμ10*; Scribner et al. 1996), pink salmon (*Onchorynchus gorbuscha*) (*Ogo1a* and *Ogo1c*; Olsen et al. 1998), bull trout (*Salvelinus confluentus*)

(*Scoμl9*; Taylor et al. 2001), and Atlantic salmon (*Salmo salar*) (*Ssa85*; O'Reilly et al. 1996).

PCR reactions were performed in 25 μl volumes using conditions provided by the respective authors. The PCR profile involved a single 2 min denaturing step at 94°C, followed by 30 cycles of a 1 min denaturing step at 94°C, 1 min annealing step at the appropriate temperature and 1min extension step at 72°C. PCR profiles for scale extracted DNA required 35 cycles. Annealing temperatures were as follows: 60 °C for *Sfo1* and *Sfo12*, 56 °C for *Sfo18* and *Ssa85*, 54 °C for *Oneμ9*, 52 °C for *Ogo1a*, 46 °C for *Oneμl0* and *Scoμl9* and 48 °C for *Ogo1c*. PCR products were screened using 6% polyacrylamide vertical gels. Products were visualized by a Hitachi FMBIO II Multi-View scanner and associated software. Microsatellite fragments were sized manually using a 20 bp internal lane standard. Several individuals of known genotype served as positive controls in each gel for standardization.

Statistical Analysis.- One of the primary goals in development of domestic broodstocks is to minimize loss of genetic diversity and changes in allele frequency (Holey 1997). A variety of measures were employed to evaluate changes in genetic diversity between source and hatchery populations. Estimates of allele frequencies and expected and observed heterozygotic diversity were performed using BIOSYS I (Swofford and Selander 1981). Exact tests (Raymond and Rousset 1995a) of significance of differences in allele frequency between wild source and hatchery broodstock samples was performed using GENEPOP (V3.1b; Raymond and Rousset 1995b). The sequential Bonferroni method was used to derive nominal significance levels for multiple testing (Rice 1989). Allelic richness was calculated for each population using the program

CONTRIBUTE (Petit et al 1997). Allelic richness provides a measure of the number alleles per locus standardized for differences in population sample size.

Hatchery systems and management practices can affect gene correlations within individuals (inbreeding coefficients, F) or among individuals (coancestry between individuals, θ). An understanding of the magnitude of these effects is critical for predicting change or rates of accrual of gene correlations and effective size for successive generations of captive populations as well as of individuals introduced into natural populations (Ryman and Laike 1992). Inbreeding coefficients relate the correlation of genes within individuals to that expected if individuals had mated at random. The expected level of correlation is one minus the total variation at the same levels. We estimate expectations for fixation indices by calculation of observed and expected heterozygosities [$F=1-H_I$ and $\theta=1-H_S$] where F is the inbreeding coefficient, θ is the estimate of coancestry, and H_I and H_S are observed individual heterozyosity and mean expected heterozygosity.

Estimates of coancestry (a measure of the proportion of genes shared between individuals that are identical-by-descent) can be effectively estimated using the surrogate coefficient of relatedness in the absence of pedigree information (r_{xy}; Queller and Goodknight 1989). The average coefficient of relatedness is an appropriate surrogate measure for common pedigree measures (Blouin et al. 1995). The coefficient of relatedness we used is an unbiased measure of degree of inter-individual relationship:

$$r_{xy} = \frac{\sum_k \sum_a \left(p_y - p^*\right)}{\sum_k \sum_a \left(p_x - p^*\right)} \qquad (1)$$

where p_x refers to the frequency of a given allele possessed by an individual "x" (i.e. 0, 0.5, or 1), p_y refers to the frequency of the given allele within any individual "y" being compared to individual "x". The mean frequency of the given allele for the entire population under question is p^*. For each pair-wise estimate, the values are summed over all loci, $k = 1,\ldots,n_k$, and alleles at each locus, $a = 1,\ldots,n_a$. This statistic provides an estimate of the proportion genes between individuals that are identical-by-descent.

Pair-wise estimates of coefficients of relatedness can range from –1 to 1. A value of 1 represents complete correlation between two individuals and 0 represents no correlation. Individuals with $r_{xy} \geq 0.5$ share alleles at levels equivalent to expectations for full siblings. Individuals correlated at the level of 0.25 are correlated at the level of half siblings. Individuals with negative r_{xy} values share fewer alleles than expected based on the population average.

Pair-wise coefficients of relatedness values were calculated using KINSHIP 2.1 (Queller and Goodnight 1989). Individual pair-wise coefficients of relatedness were summarized by calculating an average coefficient of relatedness for every source and hatchery population. Calculating the average coefficients of relatedness for source populations and hatchery broodstocks summarized individual pair-wise estimates. If hatchery spawning methods are effective in equalizing contributions by adults to the next generation, coefficients of relatedness should not differ significantly. An increase in average coefficients of relatedness would suggest that disproportionately fewer adults

contributed to progeny, thus increasing coancestry among progeny. The significance of differences in distributions of pair-wise coefficients of relatedness between the wild source and complementary hatchery broodstocks were tested employing Mann-Whitney U tests (SAS, 1999). The Mann-Whitney U test is a nonparametric test of the significance of differences in location (i.e., the average) of non-normal distributions (Blaisdell 1993).

The average coancestry coefficient does not provide a complete picture of the levels of coefficients of relatedness. Another important consideration is the distribution of the coefficient of relatedness values. Populations with similar average coefficients of relatedness may possess important differences in distributions (i.e., skewness and kurtosis, or frequency of individuals related at the full sibling level, 0.50). Frequency histograms were developed to visually compare the skewness and kurtosis between source and hatchery broodstock population estimates. This is important, because distributions of coefficients of relatedness can have similar averages, but differ in the distribution of the frequencies of pair-wise coefficients of relatedness estimates. The frequency of inter-individual comparisons yielding r_{xy} values that were significant at $P \leq 0.05$ consistent with the level of full siblings, was estimated by KINSHIP 2.1 (Queller and Goodnight 1989).

Stage 2: Broodstock Maintenance and Production

Consideration of the means by which broodstocks are perpetuated and juveniles are produced for stocking is an important concern for the preservation of genetic diversity. Any genetic differences derived from Stage 1 are likely to be accentuated

during the perpetuation of a broodstock if practices to minimize changes in genetic diversity are not employed (Figure 2). This can result in changes in allele frequency and loss of genetic variance in the broodstock compared to the wild source population. These differences may subsequently be compounded in production juveniles or accentuated in juveniles due to inefficient spawning practices (Figure 2). Juveniles stocked into the wild are often the sole means of rehabilitation. Thus, it would be prudent to ensure that juveniles reflect as much of the genetic diversity of wild source populations as possible.

To examine the effectiveness of management for preservation of genetic diversity in lake trout broodstocks, we estimated allele frequencies and measures of genetic variability for the SMD broodstock, where long-term multi-generational data were available. The 1981 SMD broodstock year class, the current SMD broodstock (1987 and 1988 year classes), and the newly developed STW broodstock were the three SMD generations evaluated. Conditions that commonly influence changes in genetic variability across broodstock generations (i.e., numbers of adults spawned and fertilization conditions) are those most likely to reduce the effective population size.

Issues pertaining to the production of juveniles for restoration and enhancement purposes are also investigated under Stage 2. Lake trout broodstocks are spawned episodically throughout the spawning season. Lake trout at Pendill's Creek NFH are spawned using 5:5 male to female ratio. The gametes of five males are pooled and combined with the pooled gametes of five females. A slightly different methodology is used at the Iron River NFH where the gametes of two groups of five females are pooled separately. Gametes from two separate groups of five males are also pooled separately. Half the volume from one of the pools of male gametes is combined with one of the

female gamete pools. Half the volume of the other male gamete pool is combined with the other female gamete pool. After a period of time (several minutes) the remaining volumes of the male gamete pools are reciprocally added to the female gamete pools. This effectively results in a 10:5 male to female mating system. Due to disparities in adult male to female ratios, males are often used multiple times. In the Iron River Hatchery males were rarely reused. In the Pendill's Creek Hatchery males were reused up to two times. Within the LLW, SLW and SMD broodstocks 77%, 56% and 15% of the males were reused at least once. Eggs and juveniles developed by lake trout adults spawned episodically throughout the spawning season were retained together by spawning period through the swim-up stage until they were mixed with juveniles from other spawning periods. For our analysis, we will refer to juveniles sampled during a certain spawning period, and the adults that produced them, as "lots".

During a spawning season, hatchery personnel will spawn ripe females and a comparable number of males to fertilize the eggs. However, due to hatchery practices, portions of a spawning adult "lot" may not contribute gametes to a subsequent juvenile population. All eggs or juveniles of entire adult lots are at times discarded by hatchery personnel due to disease or nonviability (i.e., green eggs). Nonviability of eggs of entire spawning lots can be due to the lack of uniformity in egg maturation. The adults associated with such removals are eliminated from the "potential contributers" to the juvenile gene pool. Elimination of gametes or juveniles can reduce the effective population size (and the representation of early, middle, and late spawners) irrespective of spawning methodology or duration.

Sample Collection.- Information regarding spawning dates, numbers spawned, and gamete take procedures were provided by Pendill's Creek/Hiawatha and Iron River National Fish Hatchery personnel. Information was also obtained regarding numbers of juveniles produced, proportions distributed (sent to rearing stations or other programs), and identification of spawning lots from which all juveniles were culled.

Hatchery broodstock adults and offspring were genotyped to estimate the effective number of breeding adults. The LLW broodstock was not evaluated because LLW juveniles were not available. Two hundred adults were sampled from the SAW, GLW, and SLW broodstocks, and 166 from the SMD broodstock were sampled.

Juveniles from hatchery spawning periods were collected in the spring of 1999 and 2000 (SMD only). Juveniles were not random samples of the entire spawning population but a random sample from each "lot" corresponding to an identifiable number of adults spawned during a certain time period. Juveniles of the SMD broodstock were the F1 progeny of the spawned SMD adults from 1999. All juveniles were collected as swim-up fry from hatchery tanks using dip nets. Effort was made to limit sampling bias by collecting equal numbers of juveniles from within tanks (head, middle, and foot) and between tanks. Juveniles were stored whole in 95% ethanol at room temperature. Juveniles of the SAW, SIW and GLW broodstocks were sampled from Iron River NFH. Juveniles of the SMD, and SLW juveniles were collected from Jordan River National Fish Hatchery, Michigan. Several hundred juveniles were collected from each strain with the exception of the GLW juveniles of which 114 were collected.

Analysis of inter-generational changes in genetic characteristics was performed using samples from the 1981 SMD broodstock year class, the current SMD broodstock,

and the newly developed STW broodstock. Thirty-eight liver tissue samples from the 1981 SMD broodstock year class were collected randomly from fish taken out of production in 1998 and stored in 95% ethanol. The current SMD broodstock was collected as described earlier, and fin clips from 60 individuals from the STW broodstock were collected by Pendill's Creek/Hiawatha NFH personnel in 1999, preserved in scale envelopes, and stored at 0°C.

DNA Extraction.- Juvenile tails, removed posterior to the insertion of the anal fin, were utilized for DNA extraction. The DNA extraction protocol for the adults and juveniles in this analysis was performed as described under Stage 1.

Microsatellite Screening.- Three microsatellite loci, Sfo18, Scoμ19 and Ssa85 were utilized for estimation of the effective number of breeding adults (Nb). All nine loci were employed for the intergenerational analysis of the SMD broodstock.

Statistical Analysis.- The fertilized eggs from each spawning lot (egg lot) were maintained segregated from the eggs of all other spawning lots. Egg lots developed from adults spawned early or late in the spawning period may be "green" (females spawned too early) or overly "ripe" (females spawned too late). These egg lots typically exhibit low viability and are eliminated by hatchery personnel. This practice effectively removes a portion of the adults as "potential contributers". We documented the total number of lake trout spawned and numbers of hatchery adults whose eggs were eliminated. We subtracted the hatchery adults that failed to contribute to the juvenile population (due to their eggs being eliminated) from the total number of individuals spawned. Remaining individuals were designated "potential contributers". A ratio of the potential contributers to total number of lake trout adults spawned was calculated.

Genetic characteristics including observed (H_o) and expected (H_e) heterozygosities, allele frequencies, allelic richness and tests of significance were calculated as described in Stage 1. Average coefficients of relatedness (r_{xy}) and distributions of pair-wise estimates of coefficients of relatedness compared between hatchery adults and subsequent juveniles in Stage 2 were calculated using three microsatellite loci, *Sfo18*, *Scoμ19*, and *Ssa85*. Convergence of r_{xy} to actual levels of relatedness is realized when large numbers of loci are assayed (Blouin et al. 1996). We based estimates on few loci. However, as Blouin et al. (1996) point out, use of relatively few loci simply decreases statistical power (i.e., the ability to reject the null hypothesis of no relationship between individuals when non-zero coefficients of relationship exist). Further, Leberg (1992) and Spencer et al. (2000) have shown that relatively few loci can be used to document even subtle demographic changes within populations.

If spawning methods were effective in equalizing contributions of spawning adults to progeny (i.e., minimizing reproductive variance) than the effective number of breeders should be approximately the actual numbers spawned. Estimations of N_b were performed on specific lots of adults and their corresponding offspring. The same three loci, *Sfo18*, *Scoμ19* and *Ssa85* were used to estimate the N_b. Allele frequencies for the adult and juvenile populations were calculated. For each hatchery broodstock, 111 to 198 adults and 174 to 207 juveniles were screened per broodstock to minimize sampling variance. Between 99 and 113 GLW adults and juveniles were screened.

Estimates of N_b were made using temporal variance in allele frequency (Waples 1989; eqn 8). This method utilizes a standardized method for calculating allele variance (F_c) between adult and juvenile samples for a single locus:

$$Fc = \frac{1}{K}\sum_{i=1}^{K}\frac{(xi - yi)^2}{(xi - yi)/2 - xiyi} \qquad (2)$$

where F_c is the average variance in allele frequency over k alleles between two generations or samples. The allele frequency of generation 0 at allele *i* is represented by *x* and the allele frequency at generation *t* for allele *i* is represented by *y*.

The Waples model assumes that populations are closed to migration, selection and mutation are minimal, and the population is randomly mating. Estimates of N_b are based on the Plan I sampling methodology (Waples 1989):

$$Nb = \frac{t}{2[Fc - 1/(2So) - 1/(2St) + 1/N]} \qquad (3)$$

The total adult population of size *N* is sampled before reproduction (generation 0) and then juveniles are sampled at generation $t = 1$. The number sampled at generation 0 is represented by S_o (adults) and the number sampled at generation *t* is S_t (juveniles). Confidence intervals (95%) for estimates of N_b were calculated by estimating the confidence interval of the overall variance (F_c) for each broodstock (Waples 1989).

Stage 3: Distribution and Stocking of Broodstock Juveniles

The final stage of the lake trout broodstock program involves the collection, distribution and stocking of juveniles. Ideally, juveniles collected from spawning lots, produced across the spawning season, should be mixed prior to distribution to rearing

facilities or stocking sites to provide equal representation of production from the entire broodstock. Egg takes, distributions, and stocking practices that do not attempt to equalize representation of the contributing adults across the entire spawning period will likely fail to capture the genetic diversity present in the hatchery broodstock or the wild source population. In addition, if progeny from different spawning lots are not mixed the levels of coancestry among juveniles will be disproportionately high, increasing the possibility of future inbreeding and potentially reduced population viability.

Data Collection and Analysis.- Information regarding egg distribution was provided by Pendill's Creek/Hiawatha National Forest and Iron River National Fish Hatcheries. Egg take and distribution records of lake trout eggs, developed from hatchery broodstock spawning sessions in 1998 and 1999 (SMD broodstock), were evaluated for representation of spawning lots and potential effects on genetic diversity and coancestry of eggs distributed. The numbers and proportions of juveniles for each spawning lot distributed to given rearing facilities were calculated. Ratios of numbers of spawning adults associated with juveniles distributed to each rearing hatchery to the total number of adults spawned at the hatchery were calculated. This provided a measure of the proportion of the total broodstock that would be represented by the juveniles distributed to each rearing station or stocking site.

RESULTS

Stage 1: Broodstock Development

Comparisons of Wild Source Populations to Captive Broodstocks.- Estimates of observed (H_o) and expected (H_e) heterozygosities were similar in wild source populations

and hatchery broodstocks (Table 1). Differences in average observed heterozygosity (H_o) between LLW, SIW, and SAW and hatchery broodstocks and their source populations were 0.040, -0.010, and 0.037 respectively. Differences in average expected heterozygosity between hatchery broodstocks and wild source populations were 0.026, -0.017, and 0.024 for the LLW, SIW, and SAW broodstocks and associated wild source populations (Table 1). Expected heterozygosities (H_e) were higher than observed heterozygosities for all populations, suggesting a modest, though non-significant heterozygote deficiency.

Estimates of average allelic richness were similar between hatchery broodstocks and wild source populations. However, all hatchery strains showed significant differences in allelic frequencies from their source populations (Table 1) suggesting genetic drift or non-random sampling of the wild populations. Across nine loci, alleles differing ≥5% in frequency were found between all hatchery broodstocks and source populations (LLW, 15 alleles; SIW, 9 alleles; SAW, 7 alleles). Differences in allele frequencies were greatest between the LLW hatchery broodstock and the Lewis Lake source population. Alleles 221 and 219 for the *Ogo1c* locus differed in frequency by 21% and 14.2% respectively between the LLW hatchery broodstock and the Lewis Lake wild source populations. The LLW hatchery broodstock and Lewis Lake wild source populations differed by 14.2% for the 171 allele at the *Sfo18* locus. Allele frequencies differed significantly between the LLW hatchery strain and the Lewis Lake wild population at the *Oneu9* ($P<0.05$) locus, *Scou19* and *Ogo1c* loci ($P<0.01$) and at the *Ogo1a* locus ($P<0.001$). After employing Bonferroni multiple test adjustments, the differences at the *Oneμ9* locus were no longer significant. Based on exact tests, the SIW

hatchery broodstock differed nominally significantly from the Isle Royale lean population at the *Sfo18*, *Oneu10*, *Scou19* ($P<0.05$) loci, but none of these differences were significant after Bonferroni adjustments. Highly significant differences were found at the *Sfo18* ($P<0.001$) and *Scou19* ($P<0.01$) loci between the SAW hatchery strain and the wild Apostle Island population and differences at both of these loci remained significant after Bonferroni adjustments. These results suggest that the individuals sampled from wild populations to develop broodstocks were sufficient in preserving allelic and genotypic diversity (Table 1). However, due to sampling over short time periods or of few numbers, appreciable drift in allele frequencies were observed (Table 1).

If spawning regimes successfully captured the diversity present in adult broodstocks and reproduction was equitably distributed across all breeding adults, average r_{xy} and distributions of r_{xy} estimates (reflecting levels of inter-individual relatedness or coancestry) would not be expected to differ between source and hatchery broodstock populations. Pair-wise estimates of relatedness (r_{xy}) for individuals within each source population and hatchery strain were calculated. Mean r_{xy} estimates and distributions of pair-wise r_{xy} estimates were compared for hatchery and wild populations (Figure 4). Frequency histograms (Figure 4) reveal that distributions of r_{xy} values were not found to differ significantly ($P<0.05$). Average r_{xy} decreased for two comparisons (Lewis Lake vs. LLW and Isle Royale vs. SIW) and remained constant for the other (Apostle Island vs. SAW). The frequency of significant pair-wise estimates at the full-sibling level also decreased slightly between source populations and hatchery broodstocks (Table 1). Qualitative inspection of distributions of r_{xy} values between broodstocks and wild source populations revealed a slight change in kurtosis (increased

peak of distribution for hatchery broodstocks), and a decrease in the frequency of extreme r_{xy} values and showing convergence on the mean r_{xy} (Figure 4). Estimates of inbreeding coefficients were similar for all broodstocks compared to wild source populations (Table 1). Estimates of inbreeding coefficients were not statistically significantly different from zero.

Overall, use of few adults spawned from wild populations mean that each broodstock was founded from a sample characterized by low variance effective population size, potentially resulting in relatively high variance in allele frequency between wild progenitor populations and hatchery broodstocks. Gene correlations do not appear to have increased as a consequence of broodstock initiation.

Stage 2: Broodstock Maintenance and Production

Intergenerational Comparisons of Marquette Broodstocks.- The SMD81, current SMD broodstock, and the preproduction STW broodstock showed little divergence in allele frequencies (Table 2). The only significant differences in allelic frequencies were found between SMD81 and STW broodstocks at the *Sfo1* and *Sfo12* loci ($P<0.05$) and for the SMD and STW comparison at the *Sfo12* locus ($P<0.01$). The difference between the STW and SMD broodstocks at the *Sfo12* locus was the only significant difference observed after Bonferroni adjustments. The average observed heterozygosity for the SMD81 broodstock (0.353) was lower than that for the SMD (0.374) and STW (0.360) broodstocks. Estimates of observed heterozygosity (H_o) were below expected heterozygosities (H_e) for both SMD81 and STW broodstocks. Allelic richness estimates were similar for the SMD and STW broodstocks (3.0), but both were higher than the

average allelic richness of the SMD81 broodstock (2.7). Inbreeding coefficients (F) of the SMD81 and STW broodstocks were high (0.089 and 0.112 respectively) relative to the inbreeding coefficient of the SMD broodstock (-0.002) but none were significant ($P<0.05$). Allelic frequencies and measures of genetic diversity appear to have remained relatively unchanged among these three broodstocks. However, we see some evidence for increased levels of inbreeding. The STW broodstock has the highest inbreeding coefficient of all three broodstocks (0.112).

Comparisons of coefficients of relationship between the three broodstocks reveal no evidence for significant changes in means or distributions (Figure 5). Average coefficients of relatedness for the SMD81, SMD, and STW broodstocks were 0.035, –0.008 and –0.047 respectively. Estimates of the proportion of inter-individual coefficients of relatedness consistent with full sibling relations ($P\leq 0.05$) were 0.079 for the SMD81 broodstock, 0.061 for the SMD broodstock (0.061), and 0.068 for the STW broodstock (Table 2). Qualitative inspection of distributions of r_{xy} values between SMD81, SMD, and STW revealed little differences in distributions based on degrees of kurtosis or skewness (Figure 5).

Comparisons Between Broodstock Adults and Juveniles.- Significant differences in allele frequencies were observed between adult and juvenile samples for a number of the lake trout broodstocks (Table 3). The SAW adult and juvenile populations differed significantly ($P<0.05$) at the *Sfo18* locus. Allele frequencies of the SLW adult and juvenile populations differ significantly ($P<0.01$) for the *Scoμ19* loci. Highly significant differences at the *Scoμ19* and *Ssa85* loci and significant differences at the *Sfo18* locus were found between the GLW adults and juveniles. Allele frequencies at the *Sfo18* locus

differed significantly for the SIW adults and juveniles. No significant differences in allele frequency were found between the SMD adults and juveniles. After Bonferroni corrections, only significant differences between adult and juveniles for the SLW and GLW broodstocks were observed. Ten instances of allele frequency differences of greater than 5% were documented. Estimates of allelic richness generally decreased between adults and juveniles for the SMD, GLW, and SIW broodstocks. The GLW broodstock showed the highest proclivity for large differences in allele frequency between adults and juveniles (for each of the three loci sampled; Table 3). Slight increases in allelic richness were observed for the SLW and SAW broodstocks.

Estimates of observed heterozygosities (H_o) were generally lower in juveniles compared to adults. Only the SIW juvenile population exhibited a slight increase in average heterozygosity. The GLW adults and juveniles showed the largest difference in H_o estimates (8.3%). With the exception of SMD juveniles and SIW adults, sampled populations showed heterozygote excess. Most adult and juvenile comparisons observed decreased average expected (H_e) heterozygosities, except for the SLW and SIW adult and juvenile comparisons. Estimates of inbreeding coefficients were higher in juveniles compared to adults for four of the five broodstocks (Table 3), however, chi-square analysis revealed that no estimates of inbreeding coefficients differed significantly from zero ($P<0.05$).

Three hatchery strains, SMD, SAW and GLW exhibited a decrease in the average coefficient of relatedness (r_{xy}) from adult to juvenile populations (Figure 6). Only the SLW and SMD strains showed an increase in average from adults to juveniles. Significant differences ($P<0.05$) between the distributions of pair wise coefficients of

relatedness were observed in the SLW broodstock. Changes in the frequencies of significant coefficients of relatedness at the full sibling level (r_{xy} = 0.50) generally decreased between adult and juvenile populations (Table 3); with the exception of the SMD broodstock in which the frequency increased. The largest deviation in the frequency of significant coefficients of relatedness at the full sibling level was observed between the SAW broodstock and juveniles, 1.8%.

Qualitative inspection of distributions of r_{xy} values between the broodstock adults and juveniles revealed little evidence of appreciable differences based on degrees of kurtosis or skewness (Figure 6). We observed no consistent trend toward the increase in levels of relatedness. Increases or decreases in r_{xy} of juveniles over adults likely reflects generational differences in total number of adults spawned or in the efficiency of the spawning regime at the time broodstock adults and production juveniles were produced.

Effective Number of Breeders (N_b).- Evaluation of hatchery records revealed that the number of adults contributing offspring for some broodstocks was substantially lower than the total number of adults actually spawned due to the elimination of entire lots of fertilized gametes (Table 4). For example, entire lots of eggs from the GLW broodstock, representing 236 adults, were discarded because eggs were of poor quality (eggs had not fully matured and females spawned prematurely). Adults that created lots of juveniles that were retained are considered "potential contributers" (Table 4). This analysis reveals how the progression of gamete maturation of lake trout selected for spawning can dramatically reduce the effective population size of hatchery broodstocks, independent of issues related to spawning regimes.

Hatchery records indicated that in four of six broodstocks, some portion of the total adult population spawned did not contribute to production (Table 4). For the SLW and SMD broodstocks, eggs from all spawning lots (spawners) were used. The SAW and SIW broodstocks experienced minor losses of egg lots, which occurred during the later half of the spawning period. Ratios of total spawners to potential contributers for the SAW and SIW broodstocks were 0.98 and 0.97 respectively. The ratios of total spawners to potential contributers for the GLW and LLW broodstocks were substantially lower, 0.58 and 0.77 respectively. Elimination of eggs in the GLW broodstock occurred episodically throughout the spawning period, while eggs were eliminated at the beginning and end of the spawning period for the LLW broodstock

Not all breeding adults whose gametes were retained contributed to the subsequent generation. The realized number of contributing adults depends on factors that affect reproductive variance (e.g., sex ratios of spawned adults and pooling or sequential mixing of gametes). Estimates of effective number of breeders (N_b) were made using adult spawning lots. A spawning lot consisted of an identifiable group of adults and associated juveniles. Estimates of N_b were much smaller than the number actually spawned (Table 4). The number of individuals in each hatchery broodstock spawning lot ranged from 436-112. All broodstocks exhibited average effective breeder numbers well below that of the number spawned (264-22). The ratio (N_b/N) of the average effective breeder numbers to the total number of individuals in the evaluated spawning lot (an estimate of spawning efficiency) were 0.61, 0.41, 0.28, 0.14, and 0.10 for the SAW, SMD, SIW, SLW, and GLW respectively. If the ratios estimated from spawning lots are applied to all potential breeding adults in Table 4, the total number of

realized contributers is 587, 35, 188, 130, and 107 for the SAW, GLW, SIW, SLW, and SMD broodstocks respectively.

Stage 3: Distribution and Stocking of Broodstock Juveniles

Distribution of eggs from production facilities to rearing stations and management projects for release are not representative of the entire number of potential spawners (Table 5). For example, the SAW broodstock produced eggs that were distributed to two hatchery facilities (Bayfield State Fish Hatchery, WI and Jordan River NFH, MI), for a management program (fry plant), and for retention at the Iron River NFH. Of the 963 potential contributers (Table 5), Bayfield SFH received eggs produced from 268 adults and Jordan River received eggs from 240 adults. These numbers represent 28% and 25% percent of the potential contributers. Juveniles from this same broodstock representing the contribution of only 96 adults (10%) were utilized for a fry planting project. Juveniles representing approximately 436 adults (45% of the potential contributers) were retained at Iron River NFH.

A similar situation exists for the SLW and SMD broodstocks at Pendill's Creek NFH. Of the 930 potential contributers for the SLW broodstock, Allegheny NFH, NY received eggs from only 16 adults (2% of the potential spawners), Iron River NFH received eggs from 426 adults (46%), and Jordan River NFH received eggs from 914 adults (98%). Eggs retained at Pendill's Creek NFH represented 418 (47%) adults. For the 260 potential contributers of the SMD broodstock, 148 (57%) adults contributed juveniles to the Allegheny NFH, 78 (30%) contributed to juveniles sent to Iron River NFH, and 112 (43%) contributed juveniles to Jordan River NFH. All juveniles for the

LLW strain were designated for management projects (astroturf). All eggs developed for the GLW and SIW broodstocks were retained at the Iron River NFH.

Eggs distributed to facilities and projects also may only represent limited portions of the spawning season. Hatchery adults are spawned at various times over a spawning period that is typically 3-5 weeks long. Table 6 illustrates the number of adults spawned per spawning period, the proportion of the total potential contributers that were spawned on each given date, and the proportion of eggs contributed by adults spawned on each date to the total egg numbers distributed to the various facilities and management programs. For progeny to reflect the diversity in life history traits represented in the adult broodstock (e.g., spawning time), eggs should be collected from throughout the spawning period and in proportion to the number of adults spawned on each date. For the SLW broodstock, Allegheny NFH received all eggs from the last spawning date which realistically represented only 2% of the potential contributers. Eggs distributed to Jordan River NFH and retained at Pendill's Creek NFH were all collected on 10/14/98 and 10/15/1998, and represented 46% of the total potential contributers. Eggs sent to the Jordan River NFH represented a more equitable cross section of the juveniles produced across the spawning season. Similar results were observed for SMD and LLW broodstocks. All eggs distributed to Iron River NFH for the SMD broodstock were from the first spawning date and represented only 30% of the total potential contributers. Eighty-two percent of the eggs distributed to Jordan River were collected from 78 adults that represented 30% of the total potential spawning from one spawning date in the middle of the spawning period. Eggs collected for Allegheny NFH were collected on two dates in the middle of the spawning period and represented three separate spawning lots.

Two lots spawned on 10/15/99 and 10/20/99 represented 12% and 15% of the total potential contributers, but contributed to 49% and 25% of the total eggs distributed to Allegheny NFH in 1999.

DISCUSSION

Hatcheries have become a widely used tool for conservation and supplementation of declining and endangered species (Anders 1998), including the mitigation of decreasing fish populations, supplementation of populations, and restoration of extirpated populations. Anders (1998) states that the role of hatcheries in conservation should be "...to conserve wild fish populations along with their locally adapted gene pools and characteristic phenotypes and behaviors." This approach, referred to by Anders as "conservation aquaculture", should be employed as part of a more comprehensive recovery program that includes habitat rehabilitation (Meffe 1995), and should not emphasize quantity of fish produced over quality. However, selection forces that occur within hatcheries and changes in genetic characteristics related to factors such as inbreeding and genetic drift can lead to the loss of "locally adapted gene pools".

Selection is a critical force to consider in the management of hatchery programs. Campton (1995) defines artificial selection as selective forces that occur as a part of normal operations in a hatchery system. Artificial selection includes advertent (purposeful; Hynes et al. 1981) selection practices such as grading of fish, selecting for larger fish, and selecting fish associated with a specific spawning time. Artificial selection can lead to deviations in life history patterns such as alterations in spawning run time and duration (Flagg et al. 1995). These selection pressures are often preventable

with minimal modification of hatchery routines. It is likely that hatcheries that employ pervasive advertent selection pressures are not as concerned about preservation of genetic diversity issues related to population genetics principles. For these activities, selection is not operating on the genotypes as we measured them in this study. Selection will operate on individuals with reduced fitness as a result of inbreeding, however we do not measure this. Other forms of selection in hatcheries are of importance as they relate to the degree of domestication and abilities of offspring to survive in natural environments.

Domestication selection acts upon fish due simply to their existence in a hatchery environment and is likely unpreventable (Campton 1995). This type of selection favors adaptations conducive to growth and survival in a hatchery environment. Hatchery fish tend to exhibit a greater propensity for capture, decreased tolerance for environmental stochasticity, and decreased growth and stamina in natural environments (Hynes et al. 1981). Selection is more pronounced in populations retained for multiple generations in a hatchery system. However, selection for individuals of higher fitness in hatchery environments can occur rapidly (Crozier 1990; Doyle et al. 1995).

Role of Hatcheries in Lake Trout Restoration

Over thirty years of effort has failed to restore self-sustaining lake trout populations to most of the upper Great Lakes (Selgeby 1995, Krueger et al. 1995). Lake Superior is the only upper Great Lake that has shown significant recovery (Hansen et al. 1995). The existence of wild populations, lower fishing pressure, reduced pollution and lower rates of sea lamprey induced mortality contributed to lake trout recovery (Hansen et al. 1995). Development of lake trout refugia and effective sea lamprey control

measures have been instrumental in increasing natural reproduction in lakes Michigan and Huron. However, levels of natural reproduction needed to establish self-sustaining populations has not been realized (Eshenroder et al 1995 and Holey et al. 1995). Continued recruitment of sea lamprey from the St. Mary's River (Eshenroder et al 1995; Holey et al. 1995) and effects of early mortality syndrome (EMS) (Fitzsimons et al. 1999, 2001) identified in stocked lake trout, continue to compromise restoration efforts.

Due to the lack of natural recruitment in Lake Michigan and Lake Huron, hatcheries will continue to play a critical role in the Great Lakes lake trout restoration program. Until conditions are favorable for self-sustaining populations, hatcheries provide a vital role as "gene banks" to preserve the genetic integrity of current and historic lake trout populations. However, continued dependence on hatchery broodstock production as the primary means of recruitment will require that ecological and genetic diversity be maintained within lake trout hatchery broodstocks. In recognition of the importance of preserving genetic diversity in hatchery broodstocks, a management plan was developed for lake trout broodstocks in the federal hatchery system (Holey 1997).

The goal of this project was to evaluate how effective the lake trout hatchery broodstock program has been in preserving genetic diversity and in producing "high quality" offspring for release into waters of the upper Great Lakes. Data were collected to genetically characterize broodstocks and juveniles to evaluate program practices at each of three stages. Generally, given that lake trout have been in the hatchery system a relatively short period of time, the genetic diversity of adult broodstocks and juveniles are high and consistent with genetic characteristics of source populations. Estimates of observed heterozygosity, allelic richness, inbreeding coefficients, and coefficient of

relatedness do not differ appreciably between lake trout broodstocks from their source populations. However, significant differences in allele frequencies were observed between wild source and broodstock samples for the SAW and LLW broodstocks. Intergenerational comparisons of the SMD broodstock revealed few differences in measures of genetic diversity (i.e., allele frequencies, observed heterozygosity, allelic richness), but showed increased levels of inbreeding (F) and relatedness (r_{xy}). Most hatchery broodstocks and juveniles produced for stocking showed a slight decrease in diversity (i.e., observed heterozygosity and allelic richness). The GLW broodstock exhibited significant differences in allele frequencies across all loci. The SLW broodstock exhibited a significant increase in the coefficient of relatedness r_{xy} between adults and juveniles. Most striking were estimates of effective numbers of breeders, which were severely depressed for all hatchery broodstocks. Nonrandom egg collection and distribution precipitated further declines in the number and diversity of adults contributing to juveniles stocked.

Stage 1: Development of Broodstocks

Measures of genetic diversity for hatchery broodstocks should not appreciably differ from their wild source populations. Differences between wild source lake trout populations and lake trout hatchery broodstocks were seen for observed and expected heterozygosties, allele frequencies, and allelic richness. These differences were, however, not extreme. The largest difference between observed and expected heterozygosities of wild source and hatchery broodstock populations was only 4%. Larger changes in allele frequencies (>10%) were observed (e.g., in the LLW broodstock

and its source population) however most differences in allele frequencies were under 5%. There were no appreciable differences between sources and broodstocks in allelic richness values, inbreeding coefficients, and coefficients of relatedness. This evidence suggests that genetic diversity of wild source populations have been largely maintained in the hatchery broodstocks.

The existence of only minor changes in measures genetic diversity does not eliminate the need for concern. Broodstocks are relatively recently developed (1987-1994). Broodstocks will likely be in production for many years and will be used to perpetuate other broodstock year classes. Even small changes (i.e., generational changes in allele frequencies) can be exacerbated over several generations due to genetic drift (Allendorf and Ryman 1987). Significant differences between allele frequencies were found between LLW and SAW wild source and hatchery broodstock populations. The most, at 3 loci, in the LLW broodstock. Differences in allele frequencies are likely the result of sampling and spawning of relatively few individuals from the wild source populations. It is likely that small numbers of adults sampled and unequal sex ratios used during spawning contributed to low founding populations that were not entirely representative of the source populations.

Hatchery programs are not always successful in capturing the genetic variability of source populations (Dodson et al. 1998). Sufficient sampling of source populations can be difficult and commonly only small numbers of fish are sampled, often from a disproportionately small period of the spawning session (Allendorf and Ryman 1987). Often, the sampling is dictated by the availability of funds, time, manpower (Kerby and Harrell 1990; Yeager et al. 1990) and source population abundance (Brown et al. 2000).

For example, white bass (*Morone saxatilis*) culture in the southeastern United States is a costly venture that limits the sampling effort employed, and results in only a few captured fish. In conjunction, white bass females are highly fecund, and only a few females ≤ 5, are required to meet production goals in most southeastern states (Kerby and Harrell 1990). Variance in reproductive success among female white bass has resulted in disproportionate contributions of parents to progeny. In 1990, as few as six females were responsible for producing 50% of the progeny in a South Carolina white bass hatchery (Secor et al. 1992). Sampling of wild populations by hatcheries may also be performed at certain times of the spawning season in order to maximize effort. Salmonid populations are typically sampled at the peak of the spawning run when fish are most plentiful (Hynes et al. 1981).

Even if a substantial and representative number of fish are sampled from a source population, spawning methods can reduce the realized effective number of contributing adults. Brown et al. (2000) investigated a propagation-assisted restoration program for American shad (*Alosa sapidissima*) in the James River, Virginia. Numbers of shad in the James River were insufficient to develop a broodstock, consequently requiring a broodstock to be developed from the nearby Pamunkey River. Brown et al. (2000) found that although the shad population in Pamunkey River appeared to be sampled adequately, the number of contributing adults may have been severely diminished by the spawning methodology. Greater than 1,400 American shad from the Pamunkey River were collected over the spawning season. Fish were spawned via the pooling of female gametes and sequential addition of the sperm from multiple males (female to male sex ratio averaged 1.5:1). Using an experimental family, Brown et al. (2000) discovered that

reproductive variance associated with this spawning method could have reduced the effective population size by 88%.

Mueller (1995), describes high variance in adult contributions for endangered razorback suckers (*Xyrauchen texanus*) in an enhancement project in the southwest United States. Male to female ratios of approximately 2:1 were employed following evidence that females commonly spawn with two males in the wild. Genetic analysis of a sample of the offspring, revealed that over half the juveniles in the sample were offspring of a single female. In addition, these fish were collected from the beginning of the spawning season and were likely not genetically representative of the entire population.

The broodstock development stage (Stage1) is the most influential stage of management of genetic diversity (Tave 1993). Genetic differences between the source population and new broodstock realized at this stage will potentially be perpetuated or exacerbated by genetic drift related to low N_e (Allendorf and Phelps 1980). Several studies report observed losses in genetic diversity and or differences in allele frequencies between source populations and hatchery broodstocks, and between hatchery year classes. The studies have commonly invoked founder effects and genetic drift as reasons for these differences (Cross and King 1983, Allendorf and Phelps 1980, review in Utter 1991).

Stage 2: Broodstock Maintenance and Production

Comparisons among the three SMD broodstocks revealed that genetic diversity has been maintained during successive generations. This presumably, is a result of more appropriate spawning techniques that have been employed for broodstock perpetuation

(1:1 matings and using fewer eggs per mating), compared to practices employed for broodstock production. The STW broodstock exhibited significant differences in allele frequencies at one locus with the SMD broodstock. STW also exhibited an increase in the inbreeding coefficient estimate. The wild population from which the STW broodstock was derived may be the product of the stocking of juveniles from previous SMD broodstocks. If broodstock production involves the use of unequal sex ratios and gamete pooling techniques, these conditions, and others that will be described below, will likely lead to low effective population sizes and juvenile populations that are not representative of the parent broodstock. This is an important consideration for the restoration effort if juveniles to be stocked back into the wild do not reflect their parental broodstock or ultimately the broodstock's source population.

The inbreeding coefficient (F) of the STW broodstock is the highest estimate recorded in this study. The estimate F for STW is slightly larger than that of SMD81, but far exceeds the inbreeding coefficient for SMD. One explanation for the larger estimate in STW is that the juveniles that established and perpetuated the source population of the STW broodstock were derived from small effective populations of highly related cohorts. These high numbers of related individuals were more likely to interbreed, thus increasing the inbreeding coefficient (F). An alternative explanation is that individuals that were spawned to create the STW broodstock were sampled from multiple and genetically differentiated populations. The pooling of progeny of spawning events in different populations would create a deficiency in heterozygotes (Wahlund effect) resulting in a higher estimate of the inbreeding coefficient.

Reductions in effective population size occurred at a multiple levels of the broodstock maintenance and production stage for several broodstocks in this study. Spawning records revealed that for some broodstocks, large numbers of adults were removed from the pool of potential contributers due to the excision of entire lots of juveniles. Juvenile lots were excised due to poor egg quality and were associated with adults that were spawned at the beginning or the end of the spawning period, suggesting that eggs were not ready (i.e., too green) or were no longer viable. In two cases, this reduced the potential number of contributers by 41% for the GLW broodstock and 21% for LLW broodstocks. This demonstrates that conditions unrelated to hatchery spawning or sampling methods can also decrease the numbers of adults that may contribute to subsequent generations.

Estimates of effective number of breeders revealed that several broodstocks show a considerable decrease in the number of adults that contribute to subsequent generations. The ratios of the number effective breeders to the total number spawned, or spawning efficiency (N_b/N), ranged from 0.10 to 0.61. Low spawning efficiency is likely related to particular spawning methodologies. SMD, SLW, and LLW broodstocks were spawned by pooling gametes at a 5:5 male to female ratio and GLW, SIW, and SAW broodstocks were spawned by pooling gametes at a 10:5 male to female ratio. For broodstocks at Pendill's Creek NFH, large disparities in the sex ratios require that multiple males be re-used to spawn a larger number of females. Some males were reused up to three times in the LLW broodstock. These methodologies likely increased the reproductive variance between adults. High variances in reproductive success have previously been shown to result from similar spawning techniques for salmonid species (Gharret and Shirley 1985;

Withler 1988). Genetic measures such as significant differences in allele frequencies in the SLW broodstock and increased levels in the estimates of inbreeding are consistent with these gamete pooling practices.

High reproductive variance leading to unequal contributions of adults to progeny is the most common cause of low effective population size in fishes (Allendorf and Ryman 1987; Busack and Currens 1995; Simon 1991; Hedgecock et al. 1992 and citations therein). High variance in the contribution of males to subsequent generations has been associated with spawning methods that involve the sequential addition or pooling of male gametes to fertilize the eggs (Gharrett and Shirley 1985; Withler 1988). Sequential and pooling methods are commonly employed for salmonid production and are common in hatchery systems that possess limited numbers of males (e.g., Pacific salmon hatcheries; Withler 1988). Kincaid (1995) surveyed 221 salmonid broodstocks in the U.S. and found that 48% possessed unequal sex ratios and 34% possessed excess numbers of females.

Lower effective population sizes are likely to increase the probability of the loss of alleles and increases in variance in allele frequencies. The rate of change in allele frequency and rate of loss of genetic variance is expected to be $1/2N_e$ per generation. This translates into a larger rate of loss in allelic variation that could have otherwise been avoided if more effective spawning measures had been employed. For example, applying the efficiency ratio (N_b/N) to the total potential contributers for the GLW broodstock, the effective number of breeders is 35 which translates into a 1.4% loss in variance per generation. If the effective number of breeders equaled the potential contributers, loss of allele variance would only be 0.1%. Lower effective numbers of breeders also increases

the chance that rare alleles (alleles with a frequency of ≤ 1% in a population) will be lost to genetic drift. Using the GLW broodstock as an example, the estimated N_b of 35 would translate into a less than 55% probability of retaining a rare allele over one generation of random spawning (review in Holey 1997). The chances of rare allele retention decreases to less than 17% if the estimated N_b for the GLW broodstock is applied over three generations. However, if all potential spawners (GLW=346) contribute to the subsequent generation, the probability of retention of rare alleles will increase to ~100% over three generations (review in Holey 1997).

Differences in allele frequencies were observed between hatchery adults and juveniles (Table 3). Significant differences in allele frequencies were observed between all comparisons except for the SMD broodstock. Allele frequencies differed significantly at all loci for the GLW broodstock adults and juveniles, which is consistent with the extremely low effective numbers of breeders.

No appreciable differences in genetic diversity measures were observed between hatchery adults and juveniles. All juveniles, except the SIW juveniles, exhibited decreased observed heterozygosities over that documented for adults. However, single generational differences in measures of genetic diversity (numbers of alleles and heterozygosity) were not large, and did not suggest that juveniles were no longer representative of adult broodstocks. However, as stated before, differences are important when compounded over multiple generations.

The SLW broodstock adults and juveniles differed significantly for the distributions of coefficients of relatedness. This significance is likely related to the large number of males reused (56%) during the spawning of the SLW broodstock. Males from

SMD (15%) and LLW (77%) broodstocks were also reused multiple times during a spawning period. An increased level in the average coefficient of relatedness in progeny suggest that on average, individuals within the juvenile population are more closely related to each other than adults are related to other adults in the population. High degrees of relatedness between individuals could lead to inbreeding if these juveniles survive and reproduce themselves, which is the goal for lake trout restoration.

Estimates of effective population size are sufficiently high to prevent large changes in genetic diversity over a single generation, but do represent extremely low spawning efficiencies that translates into inefficient use of these broodstocks and of resources. A substantial amount of time, money, and manpower is required to develop, maintain, and spawn individuals in these broodstocks. It is not in the best interests of the lake trout hatchery program or the lake trout restoration program to expend considerable effort and resources to maintain a large broodstock only to realize a 10% spawning efficiency. Adoption of more effective spawning practices would be prudent. The use of genetic markers would be an effective means of monitoring hatchery populations and increasing their effectiveness and efficiency.

Broodstock management employed in Stage 2, during development of new broodstock year classes and broodstock production, have also resulted in low effective population sizes and genetic drift in other hatchery programs. Allendorf and Phelps (1980) identified significant differences in allele frequencies related to genetic drift between broodstock year classes in cutthroat trout. High reproductive variance and gamete takes over a limited time period have been cited as causes for low effective population sizes of broodstocks in a red sea bream (*Pagrus major*) hatchery (Perez-

Enriquez et al. 1999). The Red Sea bream (*P. major*) hatchery program incorporates a natural spawning system using 250 individuals at a sex ratio of 1:1. A limited gamete take near the peak of egg production, in conjunction with high reproductive variance, resulted in significant differences in allele frequencies between the broodstock and the juvenile population and a low effective population size of 64. Similar patterns of genetic differences that occur between source populations, broodstocks, and juvenile year classes have been demonstrated for a number of other salmonid species (Allendorf and Ryman 1987).

Hatchery practices that increase adult reproductive variance by failing to spawn all adults across the entire spawning period, discarding gametes, pooling gametes or maintaining unequal sex ratios will lower effective population size and thereby increase the likelihood of loss of genetic diversity, increase the likelihood of large generational changes in gene frequency, and elevate levels of inbreeding over generations.

Stage 3: Distribution and Stocking

At Stage 3 of the broodstock program, great expenditures of time, human resources and funding have been expended to develop and perpetuate broodstocks and produce progeny to be stocked into U.S. waters of the upper Great Lakes. Measures should be taken to ensure that eggs and/or juveniles are distributed to rearing stations and stocking locations in a manner that accurately represents the entire broodstock from which they were derived.

Analysis of hatchery distribution records revealed that effective population sizes have been further compromised by disproportional distribution of gametes or progeny

(Table 5 and 6). Eggs produced at both Pendill's Creek NFH and Iron River NFH are distributed to several facilities and stocking programs throughout the Great Lakes. However, these collections of eggs are frequently not representative of the spawned population. For example, SAW broodstock eggs designated for a fry plant program were derived from 96 adults spawned at the end of the spawning period that represented only 10% of the potential contributers. A majority of eggs (82%) collected from the SMD broodstock were developed from adults spawned during the middle of the spawning season from 78 adults which represented only 30% of the potential contributers.

If the spawning efficiency for each broodstock is applied to the numbers of adults that were spawned on each of these dates, juveniles that were collected and distributed from limited portions of the spawning season represent extremely low effective numbers of breeders. For example, SLW eggs designated for distribution to the Allegheny NFH were all collected from 16 individuals spawned at the end of the spawning season, which represented only 2% of the potential contributers. The efficiency ratio for the SLW broodstock (0.14) applied to these 16 individuals results in an effective number of breeders of only 2 individuals! However, this is an extreme example and not all distribution sites are this biased. The distribution of eggs from the SLW, SMD, and LLW broodstocks to Jordan River, Allegheny, and a survival and imprinting enhancement study (astroturf program), are examples of more representative collections of eggs. SLW broodstock eggs distributed to Iron River NFH and retained at Pendill's Creek NFH likely represented most of the broodstock. Although these eggs were collected from a single spawning date, the adults represented almost 50% of the total potential contributers (426 individuals).

Stocking of juveniles or fertilized eggs derived from relatively few effective breeding adults poses potential problems for lake trout restoration. Juveniles collected from limited portions of the available spawners and from short intervals of the entire spawning period will fail to represent the genetic and ecological diversity of their progenitors.

Data presented in this study reveals low effective population sizes of broodstocks maintained for upper Great Lakes lake trout restoration programs. Large numbers of adults are maintained and spawned at the Pendill's Creek and Iron River National Fish Hatcheries (Table 4). However, the effective population numbers that contribute to progeny stocked at specific locales are far fewer than the number initially spawned. Managers need to re-evaluate the practices of excising entire lots of eggs due to "poor" spawning quality and implement more efficient (e.g., 1:1 male to female) spawning methods. The practice of nonrandom distribution of eggs should also be re-evaluated.

Results from Stage 2 revealed that overall levels of relatedness are not elevated in juveniles over levels described for adults. However, if progeny from comparative few adults are stocked, the average level of relatedness will be substantially higher than expected. When these year classes are sexually mature, related individuals could spawn resulting in elevated levels of inbreeding natural born progeny. This would not be consistent with restoration of self-sustaining populations.

SUMMARY

Lake trout restoration efforts across the Great Lakes region rely heavily on hatchery production. This is especially true for U.S. waters of Lake Michigan and Lake

Huron where wild lake trout populations have been extirpated. Hatchery production goals (e.g., total juvenile production) should be balanced with goals of maximizing the "quality" of progeny produced and stocked. Natural selection in the lake environment will select for phenotypes/genotypes of highest fitness. It would be prudent to stock juveniles reflecting the greatest potential diversity available. Findings of this study offer guidance to improve specific aspects of the lake trout broodstock program to produce progeny that meet these goals (see Appendix II).

There appears to be no common pattern related to the overall genetic quality of broodstocks over the entire federal lake trout hatchery program (Stage 1 through Stage 3). At each stage we've shown examples of appreciable change in particular broodstocks at some measures of genetic diversity, levels of inbreeding and coancestry, but not at others. This reflects the fact that less than optimal practices are occurring at discrete points in the broodstock program, but is not indicative of a general ineffectiveness of the program to preserve genetic diversity over the entire program. Genetic diversity and allele frequencies of lake trout of each generation reflect spawning events of a particular year. Measures of genetic diversity reflect the processes by which matings were conducted.

Based on estimates of allelic diversity, allele frequencies, observed and expected heterozygosity, inbreeding and relatedness, we found that lake trout broodstocks examined in this study are largely representative of their source populations. Although some differences were observed, we did not detect the extreme differences (i.e., in allele frequencies and average heterozygosities) that would indicate that the genetic diversity of the broodstocks fail to reflect those of their source populations. However, this does not suggest that small differences do not justify some concern.

Allele frequencies will change and levels of inter-individual relatedness will accrue over generations. Small genetic differences have the potential of being exacerbated in closed populations. Higher levels of relatedness among broodstock adults in future year classes will result in elevated levels of inbreeding in production fish to be stocked. This is especially likely in light of findings of low effective population sizes (Table 4). Most broodstocks were established relatively recently. For example, the oldest broodstock (SMD) (~ 7 generations) in production today are descendents of broodstocks initially developed in 1949. It is conceivable that these broodstocks could be perpetuated for several decades. Therefore, long-term planning is needed to preserve genetic diversity over multiple generations.

The most significant findings of this study were of the low effective breeding population size of production fish used for stocking. Although large numbers of adults are maintained and spawned, a substantial number of adults do not effectively transmit genes to the juveniles used for stocking. As an extreme example, we estimate that the effective size of the SLW broodstock of 930 adults was reduced by 98.8% through inefficient spawning methods and egg collection techniques to a realized effective population size of 2 for a group of juveniles distributed to another hatchery. Hatcheries have limited control over some activities. Reductions in effective population sizes due to uncontrollable events such as the excising of egg lots due to poor quality or disease are unavoidable. However, activities related to spawning methodologies, timing and duration of egg takes, and egg collection and distribution should be managed to ensure that juveniles stocked reflect the diversity present in broodstock adults.

Chapter 2

ASSESSING LEVELS AND PARTITIONING OF GENETIC DIVERISTY IN WILD AND HATCHERY LAKE TROUT POPULATIONS: RELEVANCE FOR LAKE TROUT MANAGEMENT AND RESTORATION IN THE GREAT LAKES

Six hatchery broodstocks, the Seneca Lake (SLW), Lewis Lake (LLW), Marquette (SMD), Green Lake (GLW), Apostle Island (SAW), and Isle Royale (SIW) and wild lake trout populations of Lake Superior, represent the remaining stocks available for restoration efforts in U.S. waters of the upper Great Lakes (Figure 1). Broodstock selection was based on political considerations, traits of source populations, source population availability, and a desire to maximize use of available genetic and ecological diversity of lake trout populations still existing within the Great Lakes basin. All broodstocks currently used for restoration efforts were developed from natural lean lake trout populations. Lean lake trout were chosen for restoration efforts due to their preference by sport and commercial fisherman (Krueger et al. 1983). Preference for lake trout phenotypes of greatest recreational value curtailed development of broodstocks from the full complement of ecologically and phenotypically differentiated forms (e.g., siscowets and humpers) in the Great Lakes basin.

The SAW, SMD and SIW broodstocks were developed from Lake Superior populations based on the availability of certain lake trout populations and a desire to utilize native lake trout diversity (Lawrie and Rahrer 1973; Peck 1975; Lawrie 1978; Swanson and Swedberg 1980, Krueger et al. 1983; G. Curtis, United States Geological Survey, Great Lakes Science Center, unpublished data). The SMD broodstock was opportunistically developed in 1948 from lake trout populations sampled near Marquette,

Michigan along the southern shore of Lake Superior. At the time the SMD broodstock was being developed, southern Lake Superior lake trout populations had collapsed and lake trout populations near Marquette were the only remaining populations available (Lawrie and Rahrer 1973; Peck 1975; Lawrie 1978). The SMD lake trout broodstock is the oldest of the hatchery broodstocks. The current broodstock year classes were developed over fifty years from the original 1948 year class (Coberly and Horrall 1982; Krueger et al. 1983; Kincaid et al. 1997).

The SAW and SIW broodstocks were derived in the middle 1990s from captive populations collected from remnant wild lake trout populations from the Apostle Islands, Wisconsin and Isle Royale, Michigan in Lake Superior, respectively. Similar to the SMD broodstock, the SAW broodstock was developed opportunistically, but also with a desire to utilize a native lake trout that had proven survivability during the collapse and extirpation of other near-shore Lake Superior lake trout populations (Swanson and Swedberg 1980; Krueger et al. 1983). Although lake trout populations inhabiting near-shore waters around the Apostle Islands collapsed concomitant to other lake trout populations of southern Lake Superior, vestigial lake trout remained and Apostle Islands lake trout populations eventually rebounded (Swanson and Swedburg 1980; Schram et al. 1995). Lake trout populations around the Apostle Islands were also economically feasible to sample given their proximity to ports and hatcheries. The SAW broodstock was developed from reciprocal crosses between two captive year classes derived from Apostle Island wild fish in 1985 and 1986 (D. Bast, USFWS, personal communication; S. Schram, Wisconsin Department of Natural Resources, personal communication).

Populations sampled from locations around Siskiwit Bay, Isle Royale between 1981 and 1986 were the progenitors of the SIW broodstock (D. Bathel, MDNR, personal communication). Multiple captive populations developed from five sampling years were reciprocally crossed to produce the two year classes (1989 and 1993) evaluated in this study (D. Bast, USFWS, personal communication). Isle Royale populations were sampled to utilize native lake trout diversity in the lake trout restoration effort (Krueger et al. 1983; Hansen et al. 1995; G. Curtis, United States Geological Survey, Great Lakes Science Center, unpublished data).

The GLW and LLW broodstocks represent the remaining vestiges of the genetic diversity that existed in Lake Michigan. All wild lake trout populations in Lake Michigan were extirpated. In an effort to develop broodstocks that reflected phenotypic and behavioral characteristics of extirpated lake trout populations, feral lake trout from Lewis Lake, WY and Green Lake, WI were sampled to develop the LLW and GLW broodstocks (Coberly and Horell 1982; Krueger et al.1983; Visscher 1982; Kincaid 1993). The history of the GLW and LLW broodstocks can be traced back to egg collections made in the late 1800's from Lake Michigan (Coberly and Hall 1982; Krueger et al. 1983; Visscher 1983; Kincaid et al. 1993). The GLW broodstock was developed from adults collected from spawning populations in southern Lake Michigan. The progenitors of the GLW broodstock were originally stocked in Green Lake, Wisconsin. The initial GLW broodstock was developed in the late 1950's from Green Lake lake trout. Juveniles from this broodstock were stocked into southern Lake Michigan during the early and middle 1970's. Due to hatchery logistical problems, the GLW broodstock was retired in 1975 (Krueger et al. 1983). The GLW broodstock was subsequently

resurrected in the middle 1980s by sampling wild domestic and feral GLW lake trout in southern Lake Michigan (Kincaid et al. 1993).

Progenitors of the LLW broodstock were developed from egg collections made in 1889 from the northern reaches of Lake Michigan that were subsequently stocked as fry into Lewis Lake, Wyoming. Gamete collections of wild Lewis Lake lake trout in the early 1980's were used to develop the current LLW broodstock (Visscher 1982).

The SLW broodstock is the only broodstock in the upper Great Lakes derived from a lake trout population outside the Great Lakes basin. The SLW broodstock was developed from deep-water wild populations within Seneca Lake, New York (Krueger et al. 1983; Eshenroder et al. 1995). Wild fish sampled and spawned in 1987 and 1992 from Seneca Lake were the basis for the current SLW broodstock (D. Blick, Allegheny NFH, personal communication). The ability of Seneca Lake lake trout to avoid sea lamprey predation during vulnerable (adult) life stages and the desire to utilize a deep water variety of lake trout made lake trout of Seneca Lake an attractive choice for a broodstock source population (Krueger et al. 1983).

Strategies for the stocking of lake trout juveniles derive from the six afore mentioned broodstocks in the upper Great Lakes have emphasized stocking of fish from multiple broodstocks and the need to correlate ecological and behavioral traits of broodstocks to habitats of specific planting sites (Krueger et al. 1981; 1983; 1995). Simultaneous use of multiple broodstocks for stocking at specific sites constitutes a "lottery" method for selecting compatible broodstocks. The theory behind this stocking strategy is to effectively offer the greatest diversity possible whereby those broodstocks most suited to the habitat will prosper and reproduce (i.e., be selected for

and survive). Perpetuation of broodstocks that exhibit greater fitness (i.e., enhanced survival and reproduction), will continue until broodstocks derived directly from self-sustaining populations can be developed. Stocking of these broodstocks would be continued until rehabilitation has been established. Due to the generation time of lake trout (6-8 years) this method was deemed preferable to stocking one lake trout broodstock at a time and assessing the success of each broodstock individually (Krueger et al. 1981; 1983; 1995). Ecological and phenotypic characteristics of progenitor wild stocks used to develop hatchery broodstocks have in part been used to design stocking strategies. However, direct genetic characterization has yet to be employed and utilized for stocking strategies in the upper Great Lakes.

In an effort to maximize the diversity of lake trout stocked into Lake Michigan, numerous broodstocks have been selected for stocking (review in Holey 1995). Broodstocks stocked into Lake Michigan included the GLW, LLW, SMD, SAW, and SLW broodstocks. The Lake Michigan derived GLW and LLW broodstocks were an intuitive selection for stocking in to Lake Michigan, whereas the SMD has historically been the dominant broodstock stocked into Lake Michigan. Developed from ancestral shallow water wild populations, in conjunction with its reputation for high rates of survival, the SMD broodstock was an attractive option (Krueger et al 1983). The SLW broodstock provided a deep-water component to the cadre of broodstocks. In addition, in an effort to develop a broodstock with both desirable hatchery and wild characteristics, wild lake trout males from the Apostle Islands of Lake Superior were crossed with females from the domesticated SMD broodstock (Krueger et al. 1983).

A similar strategy of multiple-broodstock stocking has been employed for Lake Huron. The SLW, LLW, and SMD broodstocks are currently being experimentally stocked onto specific sites of the Six Fathom Bank reef complex to evaluate relative growth, survival, and reproduction (Eshenroder et al. 1995). Concurrently, all other lake trout broodstocks are also being stocked elsewhere in the Lake Huron basin.

The marked lack of success in restoring viable and self-sustaining populations of lake trout has elicited efforts to re-evaluate recovery programs and research needs. Current stocking strategies have utilized indirect genetic considerations such as broodstock source and environmental origin. Population genetics can provide inferences into the extent of genetic differentiation between hatchery broodstocks and remnant wild populations, predict the genetic implications for the simultaneous release of progeny from multiple broodstocks, and allow development of protocols to promote preservation of the genetic variation that remains in existing wild and domestic broodstocks. Restoration efforts would be best based on biologically-sound criteria, founded on a greater fundamental understanding of the relationship between genetic diversity of lake trout broodstocks (both historical and contemporary) and extant remnant populations.

Krueger et al. (1989) employed protein-based molecular techniques to characterize the genetic diversity of broodstocks stocked into Lake Ontario. Their work has consequently led to the estimation of relative contribution of hatchery broodstocks to naturally-produced progeny in Lake Ontario. Spatial diversity of wild lake trout populations has been investigated in several other studies (Dehring et al. 1981; Ihssen et al. 1988; Wilson and Hebert 1996). However, these studies were more descriptive in nature and did not address genetic diversity of hatchery and wild populations together in

the context of management concerns. This project seeks to use molecular genetic techniques to 1) develop background data pertaining to levels and partitioning of the remaining genetic diversity of lake trout populations in the upper Great Lakes and 2) discuss the application of this information to management and restoration.

METHODS

Sample Collection.- All hatchery broodstocks were sampled in the fall of 1998 by hatchery personnel during routine spawning events. The Lewis Lake (LLW), Marquette (SMD), and Seneca Lake (SLW) broodstocks were sampled from Pendill's Creek/Hiawatha National Forest Fish Hatchery in Michigan and the Isle Royale (SIW), Apostle Island (SAW), and Green Lake (GLW) hatchery broodstocks were sampled from the Iron River National Fish Hatchery in Wisconsin (Figure 1). Samples consisted of fin clips (~1cm ^{2}) removed from caudal fins and stored individually in high salt buffer (4M urea, 0.2M NaCl, 0.1M Tris-HCL, 0.5% Sarcosine, 10mM EDTA). Two hundred adults were sampled from the SLW, LLW, GLW, SIW, and SAW broodstocks, and 166 from the SMD broodstock. Fin clips were stored at -20°C until analysis.

Liver tissue samples from the three wild lake trout morphotypes, lean, siscowet and humper, were sampled from remnant wild populations at four locations across the Lake Superior basin (Figure 1). Samples of lean, siscowet, and humper populations from Isle Royale, MI were taken in the summer and fall of 1995. Lean and siscowet lake trout were also sampled in 1995 from the Apostle Islands, WI, and Stannard Rock, MI. Apostle Island and Stannard Rock samples were supplemented with archival scale tissue collected from the Wisconsin Department Natural Resources Bayfield field station.

Siscowet lake trout were sampled from Caribou Island, MI in 1995 and humper lake trout were sampled from Caribou Island in 1995 and 1998. Caribou Island 1998 humper samples consisted of fin tissue. Due to limited samples of siscowet lake trout directly sampled from Caribou Island, siscowets from contiguous areas along the southeastern shore of Lake Superior (between Grand Marias and Whitefish Point, MI) were included with the Caribou Island samples. Lean lake trout from a remnant wild population in Parry Sound of Georgian Bay, Lake Huron were sampled in the fall of 2000. Tissue samples were preserved ethanol and scale tissue were preserved by dehydration and or stored at -20°C

DNA Extraction.- DNA extraction of liver and fin tissue were performed using a proteinase K digestion and a modified Puregene extraction protocol (Gentra, Inc.). DNA was resuspended in 50 µl of TE buffer (10 mM Tris-HCL, pH 8.0, 1 mM EDTA). Fluorometry was used to determine DNA concentrations. Prior to flourometry, RNAse (2 µl; 20mg/µl) was added to each sample. One hundred nanograms of DNA was used for each PCR reaction.

A Chelex procedure was utilized for DNA extraction from scale samples. Scales (3-5) were added to 250 µl of a 5% Chelex and 10 mM Tris-HCL (pH 7.5-8.0) suspension. Scales were digested overnight with 3 ul of proteinase K. Proteinase K was subsequently inhibited at 95°C for 5 minutes and samples were centrifuged at 14000 rpm for up to 10 minutes. The resulting supernatant was removed and 2.5 µl of supernatant was used for each PCR reaction.

Microsatellite Screening.- Nine polymorphic microsatellite markers were screened including *Sfo1*, *Sfo12* and *Sfo18* (Angers et al. 1995), *Oneµ9*, *Oneµ10* (Scribner

et al. 1996), *Ogo1a* and *Ogo1c* (Olsen et al. 1998), *Scoμ19* (Taylor et al. 2001) and *Ssa85* (O'Reilly et al. 1996). PCR reactions were performed in 25 μl volumes using concentrations recommended by the respective authors. The PCR profile involved a single 2 min denaturing step at 94°C, followed by 30 cycles of a 1 min denaturing step at 94°C, 1 min annealing step and 1min extension step at 72°C. PCR profiles for scale extracted DNA required 35 cycles. Annealing temperatures were as follows: 60 °C for *Sfo1* and *Sfo12*, 56 °C for *Sfo18* and *Ssa85*, 54 °C for *Oneμ9*, 52 °C for *Ogo1a*, 46 °C for *Oneμ10* and *Scoμ19* and 48 °C for *Ogo1c*. PCR products were screened using 6% polyacrilamide vertical gels. Products were visualized by a Hitachi FMBIO II Multi-View scanner and associated software. Microsatellite fragments were sized manually using 20 bp internal lane standards. Several individuals of known genotype were also used in each gel for standardization.

Statistical Analysis.- Allele frequencies were estimated for all populations using BIOSYS I (Swofford and Selander 1981). Levels and partitioning of genetic diversity of hatchery and wild populations was evaluated using the program CONTRIBUTE (Petit et al. 1997). F statistics, used to measure the partitioning of allelic variance within and among broodstocks and wild populations, were also calculated (Weir and Cockerham 1984).

The CONTRIBUTE program estimates the genetic diversity of a given population based on its relative contribution to the overall genetic diversity of a cohort of populations. For example, the program allows us to estimate the relative contribution of each broodstock to total diversity when all broodstocks are evaluated simultaneously. The contribution of a broodstock or wild population to the overall genetic diversity was

evaluated on two levels. First we evaluated the relative contribution each population to total diversity based on population's own diversity. Secondly we evaluated the relative contribution of each population to total diversity based on a population's divergence or uniqueness from other populations. These distinctions are useful for the identification of populations for conservation purposes. In the event that populations need to be prioritized for conservation effort, populations can be evaluated not only on their diversity, but also on their distinct genetic characteristics. The contribution of a population, k, to the total diversity (diversity of all populations combined; Nei 1973) is based on the population's intrinsic diversity that is measured by estimates of the total diversity (h_t) without the k^{th} population (n-1), the total mean diversity (h_s) without the k^{th} population, and the mean genetic differentiation of the k population (G_{st}) from all other populations. The relative contribution of a population, k^{th}, to overall diversity (C_t), is decomposed into the contribution of the population based on its own diversity (C_s) and the contribution to overall diversity based on an a population's divergence or uniqueness (C_d) from other populations.

Another means of estimating diversity is to measure allelic richness. Allelic richness provides a measure of the number alleles per locus standardized for variable sample sizes. The number of allelic states is dependent on sample size, and differences in the average number of alleles observed between populations may be influenced by differences in sample size among populations. Allelic richness is an important diversity measure in that populations that do not retain high gene diversity (heterzygosity) may however possess comparatively large numbers of alleles or unique alleles (Petit et al. 1996). Average allelic richness, *r*, was calculated for each population. Populations were

evaluated on their contributions to overall allelic richness (C_{rt}), contribution to overall allelic richness relative to intrinsic allelic richness (C_{rs}), and contributions to overall allelic richness relative to allelic divergence or uniqueness from other populations (C_{rd}). Program CONTRIBUTE was used to examine contributions to diversity using three different population groupings. Analyses were performed separately for hatchery broodstocks, wild lean populations, and all wild Lake Superior populations (all morphotypes) in order to compare levels and partitioning of genetic diversity within and between populations.

Composite estimates of F statistics that partitions variance in allele frequency into components for all loci were derived for hatchery and wild populations using the program FSTAT (vers 2.8; Goudet 1999). Allelic variance among individuals within populations (f) and over all populations (F), and variance among populations (θ_{st}) was estimated. An additional estimate of variance among morphotypes (θ_{mt}) was calculated for wild populations in Lake Superior. Hierarchical analysis of Lake Superior lake trout was performed using the program GDA (Lewis and Zaykin 2001). Variances among wild populations were calculated with and without the Parry Sound population in order to elucidate genetic structure of lake trout populations within the Lake Superior basin (Table 8).

Genetic structure of hatchery broodstocks and wild populations was visualized using Cavalli-Sforza and Edwards chord distance (1967) neighbor-joining trees. Cavalli-Sforza and Edwards chord distances were estimated using BIOSYS I (Swofford and Selander 1981). The Cavalli-Sforza and Edwards chord distance is a composite measure of differences in allele frequency between each pair of populations, summed over all

alleles and all loci. Relationships among populations as summarized by genetic measures were shown using neighbor-joining trees generated in the program MEGA (vers 2.1; Kumar et al. 1993). The neighbor-joining method for generating a tree involves the use of a phylogenetic algorithm that clusters populations based on the degree of genetic divergence, while assuming that evolutionary change is not fixed among populations (review in Avise 1994). This assumption allows branch length to vary, illustrating the number of evolutionary changes (longer branches represent a greater number of changes).

RESULTS

Three hatchery broodstocks, LLW, SLW and SMD, exhibited the largest values for genetic diversity and divergence in the CONTRIBUTE analysis (Table 7). The SLW broodstock represented the largest values for estimated expected heterozygosity (h_k= 0.449), relative differentiation or allelic variance of the k population (G_{st}= 0.110), contribution to total diversity h_t (C_t= 0.051), contribution to total diversity h_t due to intrinsic diversity (C_s= 0.014), contribution to total diversity based on genetic divergence (C_d= 0.083), and the contribution to total allelic richness based on intrinsic allelic richness (C_{rs}= 0.190). The SMD broodstock exhibited the lowest or moderate values for most measures of diversity (i.e., lowest h_k). However, the SMD broodstock was found to exhibit the highest allelic diversity. Allelic richness for SMD (3.6) represented the greatest contribution to total allelic richness based on C_{rd} (0.107), and the greatest contribution to total allelic richness due to the divergence or uniqueness of alleles. The LLW broodstock was secondary to the SLW broodstock for diversity measures, but exhibited the largest value for C_{rt} (0.059), relative contribution to total allelic richness.

The SLW and LLW broodstocks contributed the most to total broodstock diversity due to their intrinsic variability (heterozygosity), while SMD contributed considerably to broodstock total diversity due to its divergence or uniqueness (possession of discrete alleles) in allelic richness.

Evaluation of wild lean populations revealed that certain populations contributed disproportionately to allelic diversity and overall genetic variance. Lean lake trout from Stannard Rock leans contributed the most to total diversity by exhibiting the largest estimates for G_{st} (0.024), C_t (0.037), C_s (0.023), and C_{rs} (0.076), the greatest contribution related to the intrinsic diversity of Stannard Rock leans. In contrast, lean lake trout from Isle Royale contributed most to total diversity and allelic richness (r = 3.122) due to genetic divergence and allelic uniqueness, [C_d (0.151) and C_{rd} (-0.020) respectively]. Lean lake trout from Stannard Rock continue to contributed the most to total diversity relative to their own diversity, [C_t (0.007), and C_s (0.005)], and were the most divergent relative to other wild populations (G_{st} =0.031) when all wild populations are considered together. Siscowet lake trout from Stannard Rock also represent high levels of diversity, h_k (0.437) and C_s (0.005), and high contribution to total allelic richness based on this population's own allelic richness, C_{rs} (0.062). Isle Royal leans represent equal genetic divergence to the Stannard Rock leans (0.031) and the greatest contribution to total diversity due to genetic divergence (0.003). Caribou Island humpers were the most diverse with respect to allelic richness (r = 3.3), C_{rt} (0.016), and divergence C_{rd} (0.045).

Analysis of genetic variance for hatchery and wild populations revealed that variation is partitioned differently in each of the two groups (Table 8). Genetic variation of hatchery populations is partitioned among hatchery broodstocks (mean θ_{st} = 0.058,

$P<0.01$), while for the wild populations, mean $\theta_{st} = 0.024$ ($P<0.01$). This is not surprising given that the wild populations are entirely from the Lake Superior Basin while hatchery broodstocks include populations derived from lake basins other than Lake Superior (e.g., the SLW broodstock from Seneca Lake, NY). Further, hierarchical analyses for wild Lake Superior populations revealed that a significant portion of total variation in allele frequency was partitioned among morphotypes ($\theta_{mt} = 0.029$; $P<0.01$). Some basin-dependent variation was evident given the increased variance observed among the total wild populations when the Lake Huron Parry Sound population was added to the analysis ($\theta_{st} = 0.033$; $P<0.01$).

Genetic structuring that corresponds to basin of origin is further revealed by examination of genetic distances between both hatchery and wild populations (Figure 7). Broodstocks were observed to cluster together based on the their lake origin (Figure 7a). The Seneca Lake broodstock, the only broodstock developed from sources outside the Great Lakes basin, was highly divergent from all other broodstocks. Structuring across wild populations was most notable among populations from different basins, as the Parry Sound population from Lake Huron was most genetically diverged from Lake Superior populations. Within Lake Superior, among population genetic affinities were most notably based on morphotypes irrespective of location of origin (Figure 7b); a result consistent with greater overall variance attributed to differences among morphotypes (humper, leans, siscowets; $\theta_{mt} = 0.029$; $P<0.01$) relative to variance among geographic sampling locales for each morphotype.

DISCUSSION

Development of a fundamental understanding of the levels and partitioning of genetic diversity of remaining wild and hatchery lake trout populations (Burnham-Curtis 1995; Hynes et al. 1981; Meffe 1995) should be a prerequisite for establishment of restoration strategies. Although studies have characterized genetic diversity in wild populations of the upper Great Lakes and broodstocks developed from them, there has been a lack of integration of these two components. Herein we provide information on levels of genetic diversity of wild and hatchery populations and how this diversity is partitioned. These data have direct relevance for development of management strategies primarily for lake trout, but applicable to other species for which supplementation is an integral component of species recovery.

Levels and Partitioning of Genetic Diversity

The Seneca Lake (SLW) and Lewis Lake (LLW) broodstocks exhibited the highest levels of diversity among lake trout hatchery broodstocks. Nearly all measures of genetic diversity and divergence were highest in the Seneca Lake broodstock, which is likely related to its location of origin. The Seneca Lake broodstock is the only broodstock developed from fish that were completely segregated from upper Great Lakes lake trout populations. Phylogeographic evidence suggests that lake trout populations of the Great Lakes and eastern Canada originated from three separate Pleistocene glacial refugia, (Berigian, Mississipian, and Atlantic; Wilson and Hebert 1996). As lake trout reinvaded the Great Lakes region, the relative contributions of lake trout from these

various refugia could have contributed to the differences observed between Seneca Lake lake trout (predominately originating from Atlantic refugia) and those developed from within the upper Great Lakes basin (likely a mixture of all three refugia).

The Lewis Lake broodstock was developed from lake trout stocked into Lewis Lake, Wyoming, originally from various locales in the northern Lake Michigan basin plus some additional lake trout from an unknown origin (review in Grewe and Hebert 1988, and Visscher 1983). This may account for LLW's high diversity and high contribution to total allelic richness and allelic divergence or uniqueness (C_{rt} = 0.058, C_{rd} = 0.051; Table 7).

The Marquette broodstock represents the lowest genetic diversity of all broodstocks, but exhibits the highest values of allelic richness and contribution to total allelic richness based on its intrinsic allelic diversity. Low genetic diversity associated with this broodstock is expected given the long history of domestication (Krueger et al. 1983). The SMD broodstock was originally developed in 1949, prior to the collapse of the fishery in that region of the lake. Subsequent broodstocks were perpetuated from descendents of this original broodstock.

Allelic richness for the Marquette broodstock was unexpectedly high given this broodstock's degree of domestication (r = 3.6, C_{rs} = 0.024; Table 7). Previous studies have found comparatively high numbers of alleles per locus (Grewe and Hebert 1988 and Ihssen et al. 1988) and mitochondrial DNA haplotypes for the Marquette broodstock and wild lake trout populations sampled near the Marquette broodstock source population (Wilson and Hebert 1996). Our findings corroborate those of earlier studies, suggesting that the Marquette broodstock possesses a higher complement of unique alleles than other

broodstocks. In addition, lake trout from the Green Lake and Apostle Islands broodstocks were added to the Marquette broodstocks in the middle and late 1960s (Krueger et al. 1983), and potentially contributed to allelic diversity expressed in the current Marquette broodstock.

Contribution to total diversity and allelic richness, in summary, can occur on two levels: 1) contribution due to a population's intrinsic diversity and allelic richness, and 2) contribution due to a population's genetic divergence or allelic divergence. Populations therefore may contribute differently to overall diversity; both of these levels of diversity should be considered in the development of management strategies (e.g., broodstock development and stocking programs). The Seneca Lake (SLW) and Lewis Lake (LLW) broodstocks contribute the most to total broodstock diversity based on their intrinsic genetic diversity and divergence. The LLW broodstock contributes most to total allelic richness, but the Marquette (SMD) broodstock represents the largest allelic richness value due to its high C_{rd} value (uniqueness).

Analyses of wild populations revealed that lean lake trout from Stannard Rock contributed the most to total diversity based on that population's intrinsic diversity for comparisons between wild lean populations and all wild populations, while lean lake trout from Isle Royale were the most divergent population of the lean populations. Stannard Rock siscowets also exhibited high levels of diversity for comparisons among all wild populations. Lean lake trout from Isle Royale and humper lake trout from Caribou Island were most divergent of all wild populations.

Evidence suggests that hatchery broodstocks differ significantly in allele frequency (mean $\theta_{st} = 0.058$; Table 8) and that this diversity is related to lake basin of

origin (Figure 7a). The neighbor-joining tree derived with Cavalli-Sforza and Edwards chord distances shows that hatchery broodstocks most similar to other broodstocks were developed from within a given lake basin. As would be expected based on its origin, the Seneca Lake broodstock was dissimilar to all other broodstocks. Present population relationships based on basin of origin are similar to genetic relationships of historical populations in the upper Great Lakes (Guinand et al. unpublished data). Consequently, although these broodstocks have been exposed to hatchery and management perturbations, they still retain a basin-dependent genetic signature.

Wild Lake Superior lake trout are less differentiated than the hatchery broodstocks. Although wild populations differ significantly in allele frequency (θ_{st}=0.024; Table 8), more variation can be attributed to differences among wild lake trout morphotypes (θ_{mt}=0.029; Table 8). This is supported by the Cavalli-Sforza and Edwards chord distance neighbor-joining tree that reveals that wild Lake Superior lake trout morphotypes are more similar within morphotype across locales throughout the basin than to other morphotypes within the same location (Figure 7; b). Conversely, Dehring et al. (1981), in an allozyme analysis of lake trout morphotypes from similar locations as this study, found that different morphotypes within a given location were more genetically similar than similar morphotypes from other locales, suggesting a recent divergence in lake trout. Our molecular microsatellite data suggests that morphotypes had genetically diverged prior to recolonization of the upper Great Lakes or a higher rate of historical gene flow among populations within morphotypes than between morphotypes. Lake trout isolated into multiple refugia during the Pleistocene glaciation would have developed ecological and behavioral adaptations to their resident habitats.

During recolonization, lake trout may have segregated based on habitats that paralleled those in the glacial refugia.

In light of the significant evidence of genetic structuring among lake trout morphotypes, management designed to restore wild lake trout would profit from consideration of the natural fish community structure. Management policy should recognize lake trout morphotypes as "distinct" units, similar to the level of Management Units (MU; Moritz 1994a, b) utilized for a number of imperiled salmonid species (i.e., Pacific salmonids; Waples 1991). Management units are homogeneous populations where gene flow is restricted to allow for significant allele frequency divergence between populations. MUs should be managed to consider these differences.

We present evidence of allelic divergence among lake trout morphotypes and inferentially of restricted gene flow. Genetic variance of wild Lake Superior lake trout was significantly partitioned between morphotypes (Table 8). Genetic structuring (Figure 7b) of wild Lake Superior lake trout populations reveals that genetic affinities are based on morphotype irrespective of location of origin (Figure 7b). For example, Caribou Island siscowets are more similar to Isle Royale siscowets than they are to Stannard Rock leans and humpers. This suggests that gene flow is restricted among leans, siscowets, and humpers at a given location, most likely a result of spatial and temporal segregation during spawning. As a result of this segregation, each morphotype constitutes a significant independent portion of the overall genetic diversity of wild Lake Superior lake trout. Genetic data suggests that lean, siscowet and humper lake trout should not be managed inclusively as "lake trout," but should be recognized as "distinct" and managed under a fish community context.

In addition to genetic structure based on morphotype, wild populations are also geographically structured by lake basin. Addition of the Parry Sound wild population of Lake Huron to the Lake Superior populations increases the overall variance among wild populations (θ_{st}=0.033; Table 8) because it is genetically dissimilar from all Lake Superior populations (Figure 7b). This complements results obtained from the hatchery broodstock data analysis in which we found significant genetic structuring related to lake basin origin (Figure 7a).

Management Considerations

Current stocking strategies within the upper Great Lakes have focused on a "lottery" system that involves the stocking from multiple broodstocks (Krueger et al. 1981; Krueger et al. 1995; Holey et al. 1995; Eshenroder et al. 1995), relying on the lake environment to naturally-select for adapted genotypes. However, the stocking of individuals from multiple broodstocks at a single locale may result in a homogenization of genetic diversity if progeny from stocked individuals of different broodstocks interbreed. Evidence of homogenization of hatchery salmon stocks of the Pacific Northwest has resulted in the loss of the genetic distinctness between these stocks (Waples et al. 1990; Waples 1991). Although the homogenization of these salmon stocks was related to egg and juvenile transfers and the development of hatchery stocks from multiple lineages (Waples et al. 1990), it is not unlikely that a similar situation could occur from the stocking of multiple lake trout broodstocks at the same locations. Currently, the Seneca Lake broodstock, Lewis Lake broodstock, and the Marquette broodstock are all simultaneously stocked in the same location in Lake Huron. These

broodstocks are likely the three most genetically divergent broodstocks. It would be prudent to avoid stocking juveniles from such divergent broodstocks in identical locales to reduce the potential for interbreeding and homogenization of lake trout stocked.

Wild populations enhanced with broodstocks developed from different lake basins may lose their genetic distinctness. The detrimental effects (i.e., outbreeding depression; Lynch 1997) of stocking hatchery fish within wild populations have been well documented (Waples et al. 1991; Waples 1990). As evidenced by this study, wild populations and hatchery broodstocks are genetically structured by basin. Genetic differentiation between the wild and hatchery populations may be exacerbated if the hatchery lake trout are derived from a small effective population size. Ryman and Laikre (1996) suggest that wild populations enhanced by large numbers of hatchery fish developed from a low effective population size will decrease the effective population size of the overall wild and hatchery populations. Small effective population sizes have been associated with the contemporary production of lake trout juveniles (this study). Fundamental knowledge of the levels and partitioning of genetic diversity of lake trout populations available for restoration of the upper Great Lakes is crucial for effective development of future management strategies.

Broodstock Retirement.- The number of broodstocks that can be maintained in a hatchery system is dependent upon space, time, and resources. In light of the data presented in this study, broodstocks that would be less desirable candidates for retirement would be the Seneca Lake, Lewis Lake, and Marquette broodstocks. These three broodstocks represent disproportionately high levels of genetic diversity and uniqueness present across the six hatchery broodstocks. Additionally, given that there is evidence of

genetic relationships, based on phylogenetic trees (Figure 7a) consistent with the lake basins from which the broodstocks were developed, it would be prudent to maintain representatives of each basin among the broodstock choices. Phylogenetic trees have been widely proposed as a measure for assessing distinctness among populations and identifying populations as candidates for conservation (Van-Wright et al. 1991; Crozier 1992; Faith 1992).

It is not unlikely that a broodstock would have to be retired due to logistical constraints such as money, hatchery space, or resources. Similar situations have occurred historically in the lake trout hatchery system. In the mid 1960s, the Green Lake broodstock was housed at the Marquette Hatchery in Michigan, in addition to broodstocks of Lake Superior origin. The Green Lake broodstock was found to spawn later than the Lake Superior broodstocks, causing difficulties with spawning and stocking schedules. As a result, the Green Lake broodstock was discontinued in 1975 (Krueger et al. 1983), but later resurrected.

The Marquette broodstock is currently being phased out in favor of the newly developed Traverse Island broodstock (STW). The Traverse Island broodstock was developed from wild populations of lake trout sampled from locales that were putatively used to develop the SMD broodstock. The Traverse Island broodstock exhibits lower observed heterozygosity than the Marquette broodstock and high inbreeding coefficient, suggesting an increase in homozygosity in this broodstock (this study).

Broodstock Management Strategies.- Historically, broodstocks from numerous locales representing a variety of ecological and behavioral traits have been selected to be stocked in the upper Great Lakes. By maximizing the variability of lake trout stocked, it

is hypothesized that lake trout most suited to current lake conditions will be selected for. No direct genetic measure has been employed to match lake trout broodstocks to complementary historical populations. Recent genetic characterization of historical populations (Guinand et al. unpublished data) in conjunction with the information presented in this project can be utilized to match broodstocks with proposed release sites, providing an alternative proactive stocking strategy.

Interest has grown in the development of new broodstocks from other lake trout morphotypes in addition to the lean morphotype that has been exclusively utilized (Krueger et al 1995). This project provides direct measure of genetic diversity of wild populations that may be used as a measure for selecting wild populations as potential candidates for new broodstock sources. Lean populations within Lake Superior represent a small proportion of the overall available diversity (Figure 8). If it is desirable to maximize diversity as a restoration goal, then the largest component of variation in Lake Superior is partitioned among the lake trout morphotypes (Figure 7b). Plants of these fish into comparable habitats in other Great Lakes would be desirable. Among the siscowet and humper populations, the Stannard Rock siscowet population exhibits the highest diversity ($h_k = 0.437$, $G_{st} = 0.024$, $C_t = 0.005$, $C_s = 0.005$; Table 7). Isle Royale and Caribou Island humper populations exhibit the highest measures in divergence of allelic richness ($C_{rd} = 0.006$; Table 7) and the Caribou Island humpers exhibit the greatest allelic richness ($r = 3.280$, $C_{rt} = 0.016$, $C_{rs} = 0.010$; Table 7). Selection of these wild populations as sources for future siscowet and humper broodstocks would offer the greatest diversity.

Recent investigation of the lake trout hatchery broodstock program has revealed that methodologies related to the spawning of adults and egg collection and distribution result in extreme reductions in broodstock effective population size (this study). Hundreds of lake trout adults are spawned for each lake trout broodstock during the spawning period, but subsequent juveniles in many cases only represent a small fraction of the number adults spawned (in an extreme case as low as 2%). Spawning practices utilizing the pooling of gametes and the reuse of multiple males was cited as a major contributor to low effective population size. Lake trout broodstocks are spawned utilizing a 5:5 or 10:5 male to female ratio and broodstock sex ratios are unequal, requiring the reuse of large fractions of males (as high as 77%) to spawn a greater number of females (this study).

Given the small effective population sizes realized during broodstock production, maintaining larger numbers of adults for each broodstock may be one means to increase effective population sizes. However, increasing the number of adults in the hatchery system will likely constitute the reduction in the number of broodstocks that can be maintained due to limited space. We present evidence that suggests most differentiation among lake trout broodstocks exist between broodstocks derived from different lake basins (Lake Michigan, Lake Superior, and Seneca Lake) (Figure 7; a). Thus, if space allocation is a limiting factor it may be justifiable to combine broodstocks developed from the same basin to create two composite broodstocks, a Lake Michigan (GLW and LLW), and Lake Superior (SAW, SMD, and SIW) broodstock. Development of a Lake Michigan and a Lake Superior broodstock, while continuing to maintain the Seneca Lake (SLW) broodstock, will allow the hatchery system to maintain a greater number of adults

for each broodstock while continuing to preserve most of the lake trout broodstock genetic diversity (Figure 9).

Conservation.- Populations that represent the greatest diversity and possess unique genetic characteristics should be given priority status for purposes of conservation. The Isle Royale lean, Isle Royale humper, Stannard Rock lean, Stannard Rock siscowet, and Caribou Island humper populations would be the preferred populations to capture a large portion of the diversity represented in Lake Superior. The Parry Sound population should also be given priority status given its degree of genetic differentiation from Lake Superior populations (Figure 7b). As reviewed by Petit et al. (1997) prioritization should be made using both allelic richness and heterozygotic diversity measures, along with relative contributions to total allelic richness and diversity of a population based on intrinsic diversity and divergence from other populations. As was observed in this study, populations can contribute little to overall diversity based on heterozygosity, but may contribute significantly based on allelic richness (e.g., Marquette broodstock). These populations are of importance because they possess a higher diversity of alleles despite lower heterozyogitic diversity. In addition, populations may possess low diversity overall, but still contribute substantially to total diversity based on genetic divergence. The Isle Royale humper population, for example, exhibited high divergent values for allelic richness (C_{rd} = 0.006; Table 7) despite a low allelic richness (r = 2.9; Table 7) and low contributions to total allelic richness (C_{rt} = 0.000; Table 7). Petit et al. (1997) describes populations with these characteristics as likely isolated populations of limited gene flow and subjected to significant genetic drift, resulting in low allelic diversity but high divergence. This data is consistent with mark-recapture

data of humper lake trout (review in Dehring et al. 1981), corroborating evidence of restricted range (low migration) of this lake trout morphotype (Burnham-Curtis and Bronte 1996). Thus, humper lake trout exhibit a high affinity for associated reefs, resulting in limited gene flow between populations.

SUMMARY

Fundamental understanding of levels and partitioning of genetic diversity of lake trout populations in the upper Great Lakes can help form the basis for restoration management. It is prudent to gain a basic understanding of the genetic structure of what existed previously and what is currently available if restoration goals to reconstitute diversity to historical locales. This study revealed that genetic diversity of hatchery and remnant wild populations is structured on multiple levels on the basis of morphotype and among geographically structured populations. Management efforts should recognize this diversity. Genetic data can be utilized to evaluate current management considerations, and as a means of inference and evaluation of future management endeavors.

APPENDIX I

GUIDELINES EXPRESSED IN THE LITERATURE REGARDING HATCHERY PRACTICES RELATED TO THE THREE STAGES OF HATCHERY BROODSTOCK MANAGEMENT

Preservation of genetic diversity throughout all stages of a hatchery program is important. Adaptability and the rate of evolutionary response are directly dependent on population genetic diversity (Meffe 1995). Figure 2 presents critical issues for preservation of genetic diversity during the three stages of broodstock development and maintenance. Practices commonly employed in hatchery systems during all stages of broodstock development, perpetuation and production are often contrary to what is recommended for preserving genetic diversity. Some issues (i.e., population sampling) are dependent on available resources (e.g., time, money, manpower), and accessibility to wild sources. However, logistical considerations involving gamete takes (i.e., broodstock sex ratios, spawning ratios, fertilization methods), and rearing and stocking methods can be tailored to achieve management goals of maximizing retention of levels of genetic diversity in the face of logistical constraints (Allendorf and Waples 1996). Below are guidelines expressed in the literature regarding hatchery practices related to the three stages of lake trout hatchery broodstock management investigated in this study.

Stage 1: Sampling of source populations for the development of broodstocks must be designed to capture the genetic diversity characteristic of the source population. A wild population should be sampled across the entire spawning period and include sampling across the entire population (i.e., include subpopulations) (Hynes et al. 1981; Krueger et al. 1981). Spawning methods that decrease variance in male and female reproductive contributions will also serve to reduce relatedness among progeny and increased effective population sizes are preferred (Gharret and Shirley 1985; Simon 1991; Kincaid 1995). An optimal spawning methodology would involve matings at a male to female ratio of 1:1, and avoidance of pooling and sequential fertilization of gametes (Gharrett and Shirley 1985; Withler 1988 Simon et al 1991).

Stage 2: The creation of new broodstock year classes and production of progeny for release should utilize the largest number of breeding adults possible. We recognize that the numbers of individuals that contribute to subsequent broodstock year classes and juveniles to be stocked can be compromised by a number of factors (Figure 2). It is generally accepted that a broodstock of several hundred individuals is sufficient to prevent significant deterioration of genetic diversity (Allendorf and Ryman 1987; review in Kincaid 1995). Spawning methods equivalent to those outlined in Stage 1 should be employed. Equal numbers of male and females for each broodstock should be maintained and individuals should be spawned across the entire spawning period (Simon 1991).

Stage 3: Strategies employed to collect and disperse fertilized gametes and stocking strategies should emphasize the preservation of genetic diversity (Figure 2) (Kapuscinski and Jacobson 1987). Juveniles produced from each paired mating should be combined immediately after fertilization and fertilized gametes should be dispersed randomly to rearing and release locations. Consideration should be made with regard to existing wild populations in the targeted stocking area as well as to the number of hatchery strains being concurrently stocked (Waples 1991). Mixing of hatchery juveniles with wild populations or mixing from multiple broodstocks may adversely effect the population genetic constituency of wild populations (Ryman and Laikre 1991; Waples 1991; Tringali and Bert 1998).

APPENDIX II

OVERALL RESULTS AND RECOMMENDATIONS FOR THE LAKE TROUT HATCHERY PROGRAM

Stage 1 -Overall, genetic characteristics of broodstocks reflect their wild source populations. Few appreciable differences in measures of genetic diversity or allele frequencies were observed. Broodstocks should be developed from multiple year classes or lines using large numbers of adults, or from individuals derived from multiple source populations of large N. These populations are less likely to be bottlenecked and exhibit reduced variability and increased relatedness. If a broodstock is to be developed from multiple year classes or lines, the size of each founding population should be large enough to reflect the goal of the broodstock management plan. Spawn all fish using a 1:1 male to female ratio as recommended by the broodstock management plan. The greatest source of low effective population size is high reproductive variance, typically due to differentiated sperm penetrance and competition when male gametes are pooled.

Stage 2-Intergenerational comparisons revealed few differences in measures genetic diversity. Evaluation of broodstock production revealed low estimates of the effective number of breeders (N_b) related to elimination of egg lots and inefficient spawning methods. Multiple year classes should be maintained and spawning should be carried out by mating individuals between year classes. Spawning methods should involve spawning all fish using a 1:1 male to female ratio as recommended by the broodstock management plan. Adult sex ratios should be equalized to avoid reusing males for spawning. This will serve to minimize the degree of relatedness among progeny. Spawning numbers that include entire lots of spawners whose progeny were culled or include reused spawners, are not representative of the true potential effective population size and may not meet the requirements of the broodstock management plan. If desired, practices that may improve the synchronicity of egg maturation include, grading females based on ripeness to coordinate the spawning of females possessing the same levels of egg maturation, and spawning over a larger spawning period but increasing the number of spawning events to spawn more females at peak ripeness.

Stage3-Egg take and distribution methods frequently do not attempt to maximize the total numbers of adults spawned and/or represent the entire spawning period. Gametes from larger proportions of fish from throughout the spawning cycle should be distributed and stocked when possible.

In general: The broodstock management plan (Holey 1997) provides a strong foundation for the development and management of hatchery broodstocks. However, the over reliance on genetic theory (i.e., $1/2N_e$), assuming N_e relates simply to the total numbers of adults spawned as an absolute measure of potential loss of genetic diversity (i.e., heterozygosity), is not adequate. The composition of the entire broodstock of N

individuals (i.e., female to male ratios and year classes), the history of the individuals in N and how gametes of individuals in N are combined can all effect genetic diversity.

APPENDIX III

TABLES AND FIGURES

Table 1. Comparisons of allele frequencies and measures of genetic diversities for three wild populations and respective hatchery strains.

		Population					
		Lewis		Isle		Apostle	
Locus	allele	Lake	LLW	Royale	SIW	Island	SAW
		Source	Broodstock	Source	Broodstock	Source	Broodstock
Sfo18	167	0.000	0.000	0.009	0.000	0.000	0.000
	169	0.000	0.000	0.000	0.010	0.008	0.000
	171	0.508	0.366	0.536	0.510	0.562	0.562
	173	0.000	0.000	0.018	0.019	0.015	0.008
	175	0.016	0.004	0.009	0.055	0.008	0.044
	177	0.000	0.000	0.000	0.000	0.008	0.000
	179	0.008	0.009	0.018	0.000	0.000	0.000
	181	0.361	0.451	0.345	0.271	0.308	0.228
	183	0.057	0.112	0.000	0.010	0.000	0.062
	185	0.033	0.045	0.009	0.039	0.008	0.003
	187	0.016	0.013	0.055	0.081	0.085	0.083
	189	0.000	0.000	0.000	0.006	0.000	0.008
	191	0.000	0.000	0.000	0.000	0.000	0.003
N		*61*	*112*	*55*	*155*	*65*	*193*
		P=0.106[1]		*P=0.032*		*P=0.000*	
Sfo1	108	0.000	0.000	0.036	0.015	0.057	0.027
	110	0.979	0.974	0.882	0.924	0.877	0.900
	116	0.021	0.026	0.082	0.061	0.066	0.073
N		*47*	*76*	*55*	*66*	*61*	*75*
		P=1.000		*P=0.490*		*P=0.483*	
Oneu9	224	0.000	0.000	0.007	0.000	0.000	0.000
	228	0.992	0.934	0.963	0.932	0.955	0.927
	230	0.008	0.046	0.000	0.038	0.000	0.053
	232	0.000	0.020	0.030	0.030	0.045	0.020
N		*66*	*76*	*67*	*66*	*33*	*75*
		P=0.031		*P=0.078*		*P=0.063*	
Oneu10	170	0.000	0.007	0.000	0.000	0.000	0.000
	174	0.708	0.601	0.731	0.846	0.902	0.807
	178	0.292	0.392	0.269	0.154	0.098	0.193
N		*48*	*74*	*52*	*65*	*46*	*75*
		P=0.174		*P=0.033*		*P=0.063*	
Ogo1a	142	0.000	0.013	0.000	0.000	0.000	0.000
	144	0.193	0.256	0.078	0.062	0.090	0.039
	146	0.000	0.019	0.000	0.000	0.000	0.000
	148	0.000	0.058	0.000	0.000	0.000	0.000
	150	0.493	0.481	0.719	0.800	0.701	0.671
	152	0.313	0.173	0.203	0.138	0.209	0.283
	154	0.000	0.000	0.000	0.000	0.000	0.007
N		*75*	*78*	*64*	*65*	*67*	*76*
		P=0.000		*P=0.295*		*P=0.105*	

Table 1 (cont'd)

		Population					
Locus	allele	Lewis Lake	LLW	Isle Royale	SIW	Apostle Islands	SAW
		Source	Broodstock	Source	Broodstock	Source	Broodstock
Scou19	159	0.000	0.000	0.007	0.015	0.000	0.000
	161	0.128	0.057	0.100	0.039	0.111	0.174
	163	0.000	0.000	0.000	0.003	0.016	0.000
	165	0.020	0.039	0.029	0.018	0.016	0.013
	167	0.027	0.022	0.000	0.000	0.016	0.000
	169	0.000	0.000	0.000	0.000	0.016	0.000
	171	0.176	0.250	0.300	0.352	0.278	0.265
	173	0.020	0.000	0.021	0.048	0.000	0.020
	175	0.527	0.478	0.429	0.473	0.468	0.465
	177	0.068	0.061	0.029	0.018	0.024	0.040
	179	0.034	0.092	0.079	0.027	0.056	0.020
	181	0.000	0.000	0.007	0.006	0.000	0.000
N		*74*	*114*	*70*	*166*	*63*	*198*
		P=0.006		*P=0.044*		*P=0.002*	
Ssa85	126	0.000	0.000	0.125	0.090	0.045	0.049
	130	0.000	0.000	0.000	0.000	0.000	0.003
	134	0.419	0.403	0.456	0.500	0.604	0.657
	136	0.118	0.146	0.118	0.139	0.112	0.098
	138	0.441	0.447	0.301	0.271	0.239	0.193
	140	0.022	0.004	0.000	0.000	0.000	0.000
N		*68*	*113*	*68*	*166*	*67*	*194*
		P=0.433		*P=0.536*		*P=0.726*	
Sfo12	254	0.047	0.027	0.127	0.142	0.061	0.041
	256	0.057	0.040	0.032	0.052	0.045	0.081
	258	0.877	0.920	0.841	0.799	0.894	0.858
	260	0.009	0.000	0.000	0.007	0.000	0.020
	262	0.009	0.013	0.000	0.000	0.000	0.000
N		*53*	*75*	*63*	*67*	*66*	*74*
		P=0.639		*P=0.679*		*P=0.210*	
Ogo1c	213	0.079	0.140	0.024	0.096	0.032	0.046
	219	0.421	0.570	0.683	0.640	0.645	0.620
	221	0.500	0.290	0.294	0.263	0.323	0.324
	223	0.000	0.000	0.000	0.000	0.000	0.009
N		*70*	*50*	*63*	*57*	*31*	*54*
		P=0.004		*P=0.055*		*P=1.000*	
	H_o[2]	0.396	0.436	0.380	0.370	0.355	0.392
	H_e[3]	0.422	0.448	0.427	0.410	0.387	0.411
	A[4]	3.0	3.1	3.2	3.3	3.1	3.2
	F[5]	0.062	0.027	0.110	0.097	0.082	0.046
	r_{xy}[6]	0.006	-0.002	0.013	-0.003	-0.009	-0.009
	S[7]	0.064	0.057	0.100	0.072	0.080	0.051
	U[8]	*P=0.539*		*P=0.256*		*P=0.829*	

Table 1 (cont'd)

1. *P* values of exact test for significant differences in allele frequencies between source and hatchery broodstock populations.
2. Observed heterozygosity.
3. Hardy-Weinberg expected heterozygosity (Nei 1978).
4. Allelic richness (average number of alleles standardized for sample size).
5. Wright's (1951) inbreeding coefficient.
6. Average Coefficient of Relatedness (Queller and Goodnight 1989).
7. Proportion of coefficients of relatedness at the full sibling level ($P<0.05$).
8. Mann-Whitney U test for significance of difference in location (i.e., average) of the distributions of coefficients of relatedness.

Table 2. Intrastrain comparisons of allele frequencies and measures of genetic diversity of the Marquette broodstock (SMD).

		Broodstocks							
Locus[1]	allele	SMD81[2]	SMD[3]	STW[4]	Locus	allele	SMD81	SMD	STW
Sfo18	171	0.583	0.599	0.616	*Scou19*	157	0.000	0.005	0.000
	173	0.014	0.000	0.000		159	0.000	0.005	0.000
	175	0.014	0.041	0.014		161	0.090	0.122	0.199
	181	0.319	0.275	0.261		165	0.000	0.009	0.000
	183	0.000	0.005	0.007		169	0.000	0.005	0.015
	185	0.000	0.005	0.000		171	0.231	0.275	0.221
	187	0.042	0.068	0.065		173	0.038	0.014	0.029
	189	0.028	0.009	0.036		175	0.526	0.437	0.426
N		*36*	*111*	*69*		177	0.077	0.086	0.088
						179	0.038	0.045	0.022
Sfo1	108	0.000	0.040	0.058	*N*		*39*	*111*	*68*
	110	0.936	0.905	0.819					
	116	0.064	0.056	0.123	*Ssa85*	126	0.013	0.018	0.036
N		*39*	*63*	*69*		134	0.613	0.694	0.667
						136	0.050	0.063	0.043
Oneu9	224	0.000	0.008	0.000		138	0.325	0.225	0.254
	228	0.943	0.903	0.956	*N*		*40*	*111*	*69*
	230	0.043	0.065	0.022					
	232	0.014	0.016	0.000	*Sfo12*	252	0.014	0.000	0.000
	234	0.000	0.008	0.022		254	0.111	0.048	0.181
N		*35*	*62*	*68*		256	0.083	0.040	0.022
						258	0.792	0.889	0.768
Oneu10	166	0.000	0.000	0.008		260	0.000	0.016	0.029
	174	0.865	0.893	0.818		262	0.000	0.008	0.000
	178	0.135	0.107	0.174	*N*		*36*	*63*	*69*
N		*37*	*56*	*66*					
Ogo1a	144	0.150	0.087	0.101	*Ogo1c*	213	0.076	0.059	0.059
	150	0.738	0.762	0.775		219	0.682	0.686	0.743
	152	0.112	0.151	0.109		221	0.242	0.255	0.169
	154	0.000	0.000	0.014		223	0.000	0.000	0.029
N		40	63	69	*N*		*33*	*51*	*68*
	H_o[5]	0.353	0.374	0.360					
	H_e[6]	0.387	0.373	0.405					
	A[7]	2.7	3.0	3.0					
	F[8]	0.089	-0.002	0.112					
	r_{xy}[9]	-0.008	-0.017	0.003					
	S[10]	0.079	0.061	0.068					
	U[11]	*P=0.484*	*P=0.341*[11]						

Table 2 (cont'd)

1. Differences in allele frequencies observed between SMD81 and STW broodstocks for *Sfo1* (P=0.027) and *Sfo12* (P=0.039), and between SMD and STW broodstocks for *Sfo12* (P=0.002).
2. SMD81 represents 1981 broodstock yearclass.
3. SMD, represents current broodstock year classes, 1987 and 1988.
4. STW, represents new broodstock developed from SMD broodstock feral fish.
5. Observed heterozygosity.
6. Hardy-Weinberg expected heterozygosity (Nei 1978).
7. Allelic richness (average number of alleles, standardized for sample size).
8. Wright's (1951) inbreeding coefficient.
9. Average Coefficient of Relatedness (Queller and Goodnight 1989).
10. Proportion of coefficients of relatedness at the full sibling level (P<0.05).
11. Mann-Whitney U test for significance of difference in location (i.e. average) of the distributions of coefficients of relatedness.
12. Difference between distributions of the SMD81 and STW broodstocks was insignificant (P=0.892).

Table 3. Comparisons of allele frequencies and measures of genetic variability for hatchery broodstocks and progeny.

		Broodstocks									
		Pendill's Creek NFH				Iron River NFH					
		SMD		SLW		SAW		GLW		SIW	
Locus	allele	Adults	Juven.	Adults	Juven.	Adults	Juven.	Adults	Juven.	Adults	Juven.
Sfo18	169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010
	171	0.599	0.557	0.748	0.745	0.562	0.527	0.465	0.454	0.510	0.515
	173	0.000	0.005	0.022	0.020	0.008	0.005	0.025	0.023	0.019	0.000
	175	0.041	0.073	0.204	0.162	0.044	0.047	0.005	0.005	0.055	0.046
	179	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
	181	0.275	0.231	0.026	0.064	0.228	0.252	0.449	0.394	0.271	0.270
	183	0.005	0.005	0.000	0.005	0.062	0.020	0.000	0.000	0.010	0.015
	185	0.005	0.005	0.000	0.000	0.003	0.012	0.000	0.009	0.039	0.026
	187	0.068	0.120	0.000	0.005	0.083	0.101	0.040	0.116	0.081	0.110
	189	0.009	0.003	0.000	0.000	0.008	0.020	0.015	0.000	0.006	0.008
	191	0.000	0.000	0.000	0.000	0.003	0.012	0.000	0.000	0.000	0.000
N		*111*	*184*	*115*	*204*	*193*	*204*	*99*	*108*	*155*	*196*
		P=0.167[1]		*P=0.152*		*P=0.025*		*P=0.020*		*P=0.225*	
Scou19	157	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	159	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.022
	161	0.112	0.132	0.256	0.267	0.174	0.181	0.103	0.049	0.039	0.067
	163	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.010
	165	0.009	0.000	0.000	0.010	0.013	0.012	0.000	0.000	0.018	0.030
	167	0.000	0.000	0.004	0.000	0.000	0.000	0.005	0.000	0.000	0.000
	169	0.005	0.000	0.004	0.000	0.005	0.002	0.029	0.013	0.000	0.000
	171	0.275	0.273	0.415	0.331	0.265	0.234	0.279	0.398	0.352	0.413
	173	0.014	0.005	0.047	0.100	0.020	0.019	0.010	0.000	0.048	0.035
	175	0.437	0.478	0.231	0.257	0.465	0.428	0.363	0.376	0.473	0.393
	177	0.086	0.086	0.043	0.015	0.040	0.077	0.108	0.022	0.018	0.002
	179	0.045	0.024	0.000	0.020	0.020	0.043	0.103	0.142	0.027	0.027

Table 3 (cont'd)

	181	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
	183	0.000	0.000	0.000	0.000	0.003	0.002	0.000	0.000	0.000	0.000
N		*111*	*185*	*117*	*204*	*198*	*207*	*102*	*113*	*166*	*201*
		P=0.166		*P=0.002*		*P=0.185*		*P=0.000*		*P=0.038*	
Ssa85	126	0.018	0.037	0.000	0.005	0.049	0.032	0.005	0.037	0.090	0.096
	130	0.000	0.000	0.004	0.000	0.003	0.000	0.000	0.000	0.000	0.000
	134	0.694	0.724	0.470	0.478	0.657	0.687	0.505	0.606	0.500	0.470
	136	0.063	0.034	0.000	0.017	0.098	0.095	0.040	0.037	0.139	0.150
	138	0.225	0.204	0.526	0.500	0.193	0.187	0.450	0.321	0.271	0.284
N		*111*	*174*	*117*	*204*	*194*	*206*	*100*	*109*	*166*	*197*
		P=0.218		*P=0.068*		*P=0.599*		*P=0.009*		*P=0.891*	
	H_o[2]	0.634	0.624	0.584	0.569	0.598	0.539	0.697	0.614	0.640	0.685
	H_e[3]	0.608	0.618	0.538	0.561	0.580	0.575	0.629	0.613	0.652	0.662
	A[4]	6.0	4.6	3.7	4.5	6.3	6.6	5.0	4.6	6.7	5.9
	F[5]	-0.031	0.062	-0.086	-0.014	-0.043	-0.011	-0.109	-0.001	0.020	-0.036
	r_{xy}[6]	0.001	-0.001	-0.012	0.007	-0.002	-0.005	0.006	-0.008	-0.001	0.000
	S[7]	0.043	0.054	0.064	0.052	0.046	0.028	0.050	0.042	0.063	0.051
	U[8]	*P=0.835*		*P=0.005*		*P=0.624*		*P=0.060*		*P=0.520*	

1. *P* values of exact test for significant differences in allele frequencies between source and hatchery broodstock populations
2. Observed heterozygosity.
3. Hardy-Weinberg expected heterozygosity (Nei 1978).
4. Allelic richness (average number of alleles, standardized for sample size).
5. Wright's (1951) inbreeding coefficient.
6. Average Coefficient of Relatedness (Queller and Goodnight 1989).
7. Proportion of coefficients of relatedness at the full sibling level ($P<0.05$).
8. Mann-Whitney U test for significance of difference in location (i.e., average) of the distributions of coefficients of relatedness.

Table 4. Hatchery strain comparisons of 1) total numbers of individuals spawned, 2) the potential numbers of individuals contributing genes to the subsequent generation after excising of eggs by hatcheries, 3) estimated effective number of breeders for spawner lots evaluated (N), and 4) the realized contributers (N_b/N applied to potential contributers).

	Post Excising of Egg Lots			Effective Number of Breeders							
Strain	Total Spawners	Potential Contributers	ratio	Spawner Lot (N)	Sfo18	Scou19	Ssa85	Avg. N_b	N_b/N	95% CI	Total Contributers[1]
SAW	990	963	0.97	436	75	133	583	264	0.61	43,341	587
GLW	582	346	0.59	224	34	17	14	22	0.10	5,41	35
SIW	692	670	0.97	384	156	58	-	107	0.28	29,239	188
SLW	930	930	1.00	448	79	33	77	63	0.14	17,128	130
SMD	260	260	1.00	112	41	51	45	46	0.41	23,72	107
LLW	486	382	0.79	-	-	-	-	-	-	-	-

1. Calculated by applying spawning efficiency (N_b/N) to the potential contributers for each broodstock.

Table 5. Numbers and proportions of potential contributers representing the distribution of lake trout (*Salvelinus namaycush*) broodstock egg production to various rearing facilities and management programs.

		Hatchery Facilities					Programs	
Broodstocks	Potential Contributers	Allegheny NFH	Bayfield SFH	Iron River NFH	Jordan River NFH	Retained[1]	Fry Plant[2]	Astroturf[3]
SAW	963	-	268(0.28)	-	240(0.25)	436(0.45)	6(0.10)	-
GLW	346	-	-	-	-	346(1.00)	-	-
SIW	670	-	-	-	-	670(1.00)	-	-
SLW	930	16(0.02)	-	426(0.46)	914(0.98)	418(0.47)	-	-
SMD	260	148(0.57)	-	78(0.30)	112(0.43)	-	-	-
LLW	382	-	-	-	-	-	-	382(1.00)

Note: Total numbers of potential contributers across facilities and programs for each broodstock, may not equal the potential number contributers number for each broodstock due to overlap in the distribution of eggs from spawning lots. Consequently, proportions may not sum to 1. All egg production is the result of adults spawned in 1998 except for the SMD broodstock eggs which were produced in 1999.

1. Numbers of potential contributers who's eggs were not distributed prior to this analysis.
2. Hatching of eggs delayed in order to stock fry at various times of the year.
3. Eggs planted on spawning reefs in astroturf boundles.

Table 6. Total egg numbers distributed to hatchery facilities and programs for three hatchery broodstocks, and the proportion of total egg numbers attributed to specific spawning dates and adult numbers spawned.

		9/30/98[1]		10/6-7/98		10/14-15/98	10/27/98
		52[2]	272	76	88	426	16
SLW	Total Eggs	(0.06)[3]	(0.29)	(0.08)	(0.09)	(0.46)	(0.02)
Allengheny	20,650	-	-	-	-	-	1.00(2)
Iron River	241,920	-	-	-	-	1.00(60)	-
Jordan River	1,053,709	0.06(7)[4]	0.20(38)	0.15(11)	0.19(12)	0.35(60)	-
Pendill's Creek	366,000	-	-	-	-	1.00(60)	-

		10/7/99	10/15/99		10/20/99	
		78	32	78	38	34
SMD	Total Eggs	(0.30)	(0.12)	(0.30)	(0.15)	(0.13)
Allegheny	212,625	-	0.49(13)	0.26(32)	0.25(16)	-
Iron River	151,250	1.00(32)	-	-	-	-
Jordan River	132,266	-	-	0.82(13)	-	0.18(32)

		9/15/98		9/22/98		10/07/98
		64	132	60	42	84
LLW	Total Eggs	(0.17)	(0.35)	(0.16)	(0.11)	(0.22)
Astroturf Program	705,319	0.21	0.52	0.10	0.08	0.10

1. Spawning date.
2. Numbers spawned per spawning date (more than one "lot" may have been spawned on a given date)
3. Proportion of the total numbers spawned for the spawning season.
4. Proportion of total eggs attributed to spawner "lot". Values in parentheses represent realized effective numbers of breeders when Nb/N (Table 3) is applied to each spawner lot.

Table 7. Levels and partitioning of genetic diversity for populations of lake trout hatchery broodstocks, wild lean lake trout, and wild lake trout populations and morphotypes of Lake Superior.

Hatchery Broodstocks	$r(82)^1$	h_k^2	h_s^3	h_t^4	G_{st}^5	C_t^6	C_s^7	C_d^8	C_{rt}^9	C_{rs}^{10}	C_{rd}^{11}
Lewis Lake (LLW)	3.270	0.448	0.430	0.460	0.063	0.014	0.013	0.001	0.058	0.007	0.051
Seneca Lake (SLW)	2.375	0.449	0.430	0.493	0.110	0.051	0.014	0.038	-0.021	-0.042	0.020
Apostle Island (SAW)	3.374	0.411	0.416	0.434	0.038	-0.015	-0.003	-0.012	0.001	0.013	-0.012
Marquette (SMD)	3.591	0.373	0.400	0.423	0.050	-0.028	-0.020	-0.008	0.000	0.024	-0.025
Green Lake (GLW)	2.884	0.419	0.419	0.438	0.041	-0.011	0.000	-0.011	0.001	-0.020	0.021
Isle Royale (SIW)	3.463	0.410	0.415	0.436	0.046	-0.012	-0.004	-0.009	0.013	0.018	-0.004
Wild Leans	$r(82)$	h_k	h_s	h_t	G_{st}	C_t	C_s	C_d	C_{rt}	C_{rs}	C_{rd}
Isle Royale	3.122	0.291	0.419	0.426	0.014	0.004	-0.147	0.151	0.010	0.021	-0.011
Stanard Rock	2.816	0.436	0.421	0.433	0.024	0.037	0.023	0.013	-0.007	-0.028	0.021
Apostle Islands	3.040	0.387	0.409	0.416	0.019	-0.040	-0.035	-0.005	0.018	0.008	0.011

Table 7 (cont'd)

Wild Lake Trout	*r(82)*	h_k	h_s	h_t	G_{st}	C_t	C_s	C_d	C_{rt}	C_{rs}	C_{rd}
Isle Royale Lean	3.035	0.426	0.422	0.438	0.031	0.006	0.002	0.003	-0.009	0.001	-0.010
Isle Royale Siscowet	3.153	0.425	0.421	0.431	0.020	0.001	0.002	-0.001	-0.002	0.005	-0.003
Isle Royale Humper	2.864	0.417	0.418	0.428	0.029	-0.001	0.000	-0.001	0.000	-0.006	0.006
Apostle Island Lean	2.965	0.387	0.404	0.418	0.030	-0.008	-0.009	0.001	-0.003	-0.002	0.002
Apostle Island Siscowet	2.985	0.407	0.413	0.423	0.020	-0.004	-0.003	-0.001	-0.003	-0.001	-0.002
Stanard Rock Lean	2.755	0.436	0.426	0.440	0.031	0.007	0.005	0.002	-0.003	-0.010	-0.007
Stanard Rock Siscowet	2.944	0.437	0.426	0.437	0.024	0.005	0.005	-0.001	0.002	-0.027	0.005
Caribou Island Siscowet	3.133	0.412	0.415	0.423	0.018	-0.004	-0.002	-0.002	-0.001	0.005	-0.006
Caribou Island Humper	3.280	0.416	0.417	0.428	0.025	-0.001	-0.001	0.000	0.016	0.010	0.006

1. Allelic richness (Petit et al. 1997) rarefaction number, 2N, where N is the smallest sample size.
2. Estimate of expected heterozygosity.
3. Mean diversity without the *k* population.
4. Total diversity without the *k* population.
5. Average relative divergence of the *k* population from other populations.
6. Contribution of the *k* population to total diversity.
7. Contribution of the *k* population to total diversity based on *k*'s own diversity.
8. Contribution of the *k* population to total diversity due to *k*'s divergenge or uniqueness.
9. Contribution of the *k* population to total allelic richness.
10. Contribution of the *k* population to total allelic richness due to *k*'s own allelic richness.
11. Contribution of the *k* population to total allelic richness due to *k*'s allelic divergence or uniqueness.

Table 8. Summary of F-statistics partitioned genetic variation within and among hatchery and wild populations of lake trout (*Salvelinus namaycush*) from the upper Great Lakes.

Populations	Loci	F[1]	f[2]	θ_{st}[3]	θ_{mt}[4]
Hatchery	*Sfo18*	-0.012	-0.086	0.068**	-
	Sfo1	0.073*	-0.226	0.094**	-
	Oneu9	0.055	0.051	0.004**	-
	Oneu10	-0.054	-0.116	0.055**	-
	Ogo1a	0.086*	-0.029	0.111**	-
	Scou19	0.023	-0.005	0.028**	-
	Ssa85	0.006	-0.056	0.059**	-
	Sfo12	0.014	-0.020	0.033**	-
	Ogo1c	0.538**	0.501*	0.074**	-
	Mean	0.081**	0.002	0.058**	
	95% CI	(0.000 , 0.259)	(-0.062 , 0.205)	(0.043 , 0.082)	
Wild (Lake Superior)	*Sfo18*	0.091**	0.028	0.065**	0.086**
	Sfo1	0.115**	0.080*	0.039**	0.050**
	Oneu9	0.015	-0.002	0.017**	0.019**
	Oneu10	-0.038	-0.051	0.013**	0.011
	Ogo1a	0.043	0.030	0.014**	0.015**
	Scou19	0.046*	0.034	0.013**	0.018**
	Ssa85	-0.026	-0.045	0.019**	0.024**
	Sfo12	0.022	0.016	0.007	0.007
	Ogo1c	0.657**	0.647**	0.029**	0.031**
	Mean	0.103**	0.082**	0.024**	0.029**
	95% CI	(0.011 , 0.312)	(-0.010 , 0.296)	(0.014 , 0.040)	(0.017 , 0.051)
	All Wild[5]	0.078**	0.103**	0.033**	
	95% CI	(0.012 , 0.312)	(-0.009 , 0.291)	(0.017 , 0.042)	

Note: *$P<0.05$; **$P<0.01$

1. Allelic variance among all individuals F_{it}.
2. Allelic variance among individuals within populations F_{is}.
3. Allelic variance among populations within each morphotype (leans, humpers, siscowets).
4. Allelic variance among lake trout morphotypes.
5. All wild includes Parry Sound population from Lake Huron.

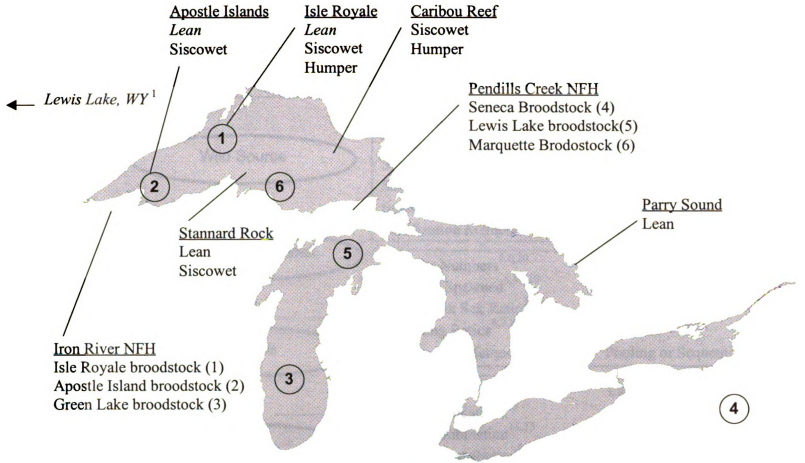
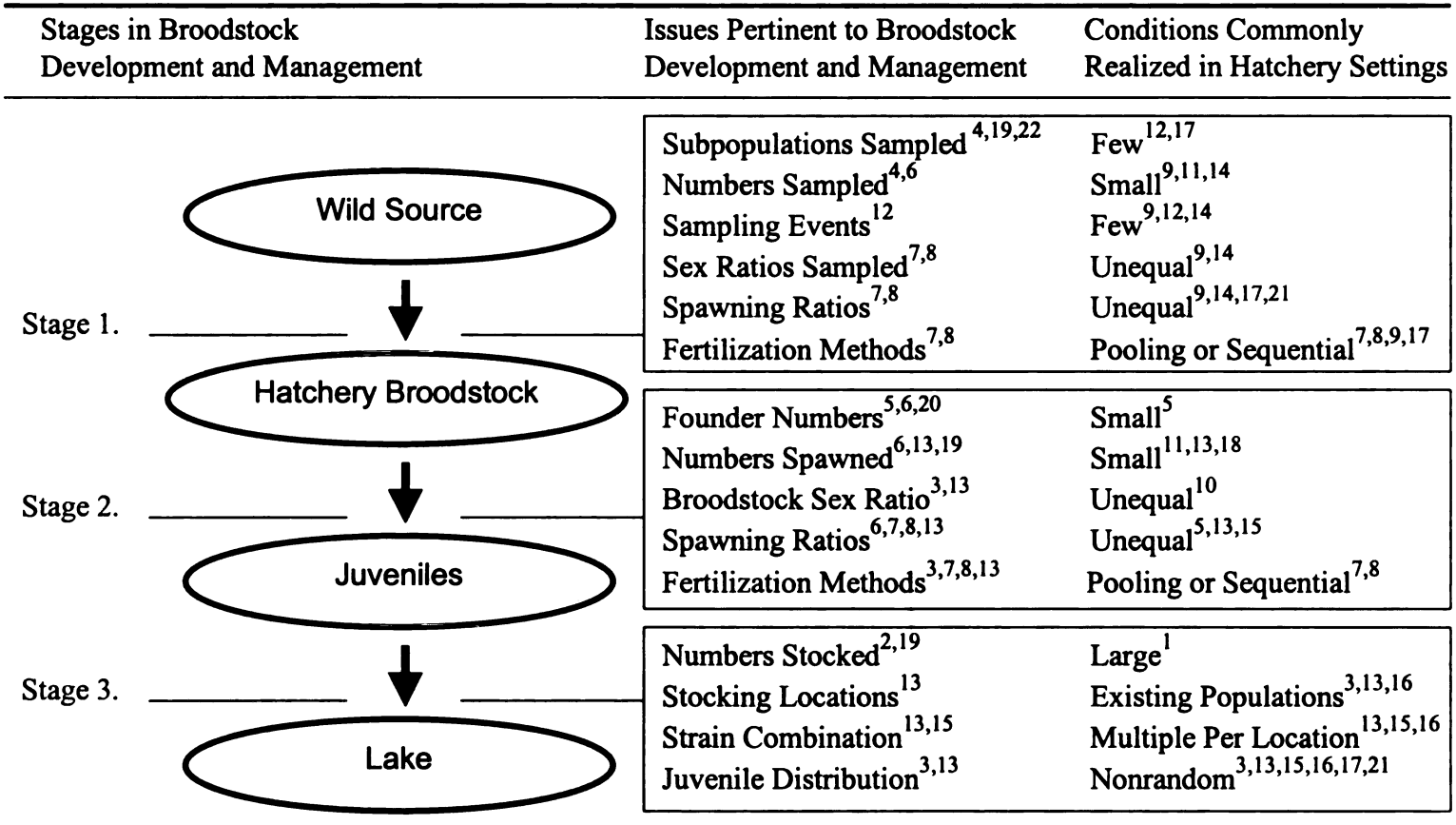


Figure 1. Locations of hatchery and wild populations of lake trout (*Salvelinus namaycush*) sampled. Numbers refer to the original source sites of broodstocks. Italicized name represent wild source populations of associated broodstocks sampled for genetic comparisons. 1. Lake trout stocked into Lewis Lake, WY were collected from northern Lake Michigan. The Lewis Lake broodstock was subsequently developed from these feral fish.

Figure 2. Stages during development of lake trout (*Salvelinus namaycush*) hatchery broodstocks and juvenile production contributing to changes in levels of gene diversity and relatedness.



1. National Research Council 1996; 2. Tringali and Bert 1999; 3. Waples et al. 1990; 4. Kreuger et al. 1981; 5. Allendorf and Phelps 1980; 6. Allendorf and Ryman 1987; 7. Gharret and Shirley 1985; 8. Withler 1988; 9. Kerby and Harrell 1990; 10. Kincaid 1993; 11. Fiumera et al. 1999; 12. Hynes et al. 1981;13. Busack and Currens 1995; 14. Mueller 1995; 15. Campton 1995; 16. Flagg et al. 1995; 17. Rees and Harrell 1990; 18. Utter 1991;19. Ryman 1991; 20. Cross and King 1983; 21. Secor et al. 1992; 22. Philippart 1995

Spawning Scenario: 1:1 male to female matings.

Male	1		2		3		4		5	
Female	1		2		3		4		5	
	O_{11}	O_{12}	O_{21}	O_{22}	O_{31}	O_{32}	O_{41}	O_{42}	O_{51}	O_{52}
O_{11}[a]	-	θ	0	0	0	0	0	0	0	0
O_{12}		-	0	0	0	0	0	0	0	0
O_{21}			-	θ	0	0	0	0	0	0
O_{22}				-	0	0	0	0	0	0
O_{31}					-	θ	0	0	0	0
O_{32}						-	0	0	0	0
O_{41}							-	θ	0	0
O_{42}								-	0	0
O_{51}									-	θ
O_{52}										-

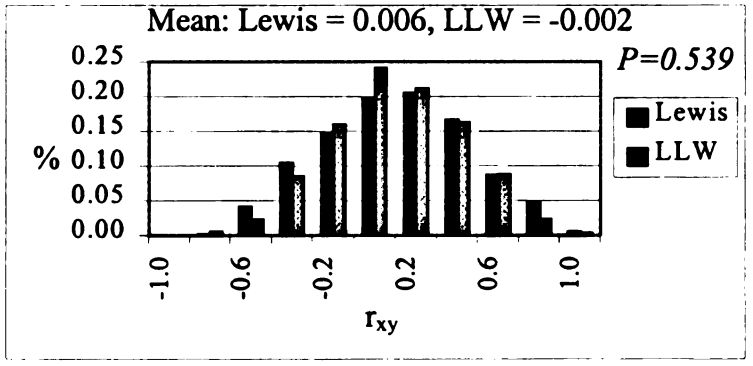
Mean θ=0.028
Offspring Unrelated=89%

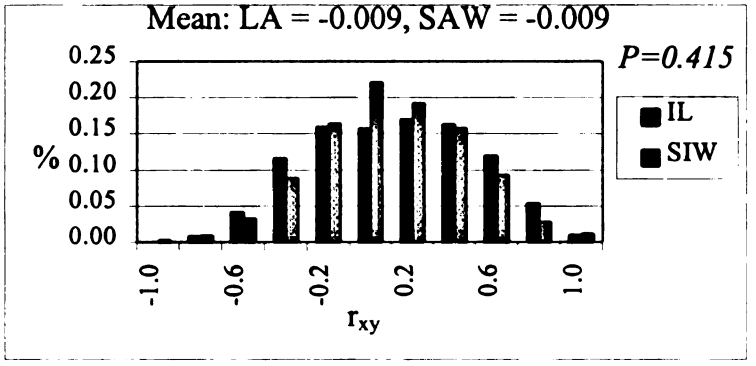
Spawning Scenario: 5:5 male to female matings, gametes pooled, unequal reproductive variance.

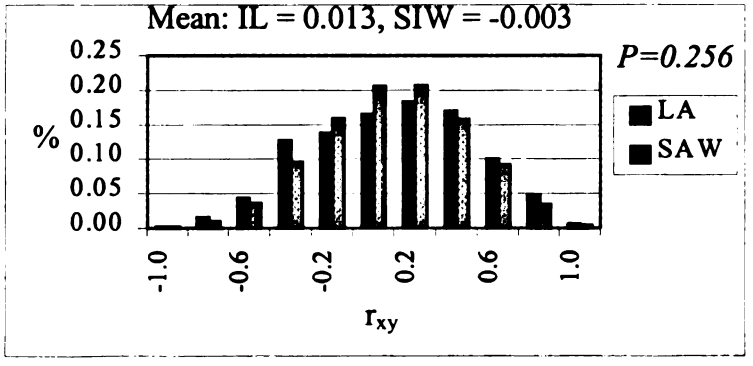
Male	*1*[b]		**2**[c]		3[d]		4[b]		5[d]	
Female	1		2		3		4		5	
	O_{11}	*O_{12}*	**O_{21}**	O_{22}	**O_{31}**	**O_{32}**	*O_{41}*	O_{42}	**O_{51}**	**O_{52}**
O_{11}	-	θ/2	θ/2	0	θ/2	θ/2	0	0	θ/2	θ/2
O_{12}		-	0	0	0	0	θ/2	0	0	0
O_{21}			-	θ/2	θ/2	θ/2	0	0	θ/2	θ/2
O_{22}				-	0	0	0	θ/2	0	0
O_{31}					-	θ	0	0	θ/2	θ/2
O_{32}						-	0	0	θ/2	θ/2
O_{41}							-	θ/2	0	0
O_{42}								-	0	0
O_{51}									-	θ
O_{52}										-

Mean θ=0.061
Offspring Unrelated=55%

Figure 3. Hypothetical spawning scenarios as advocated in the broodstock management plan (1:1 matings; Holey 1997) and actual conditions realized at the hatcheries (i.e., 5:5). In this simple example, we assume adult males and females are unrelated and each female contributes two offspring (e.g., no variance in reproductive success). θ is the probability that any two alleles shared between two individuals are inherited from a common ancestor (identical-by-descent). θ=0.25, offspring related at the level of full -siblings. θ=0.125, offspring are related at the level of half-siblings. (a) "O_{11}" represents offspring. Subscripts designate the female that produced the offspring (i.e., female 1) and the offspring number (i.e. offspring 1 of 2). (b.) Male 1 and 4 each contribute 20% of the offspring (Male 1 offspring italicized). (c.) Male 2 contributes 60% of the progeny (Male 2 offspring in bold). (d.) Male 3 and 5 do not contribute offspring. Mating strategies should seek to minimize mean θ among progeny and maximize the proportion of unrelated offspring.

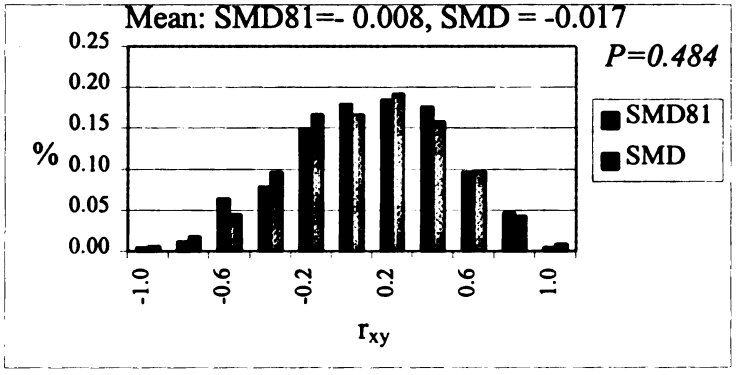


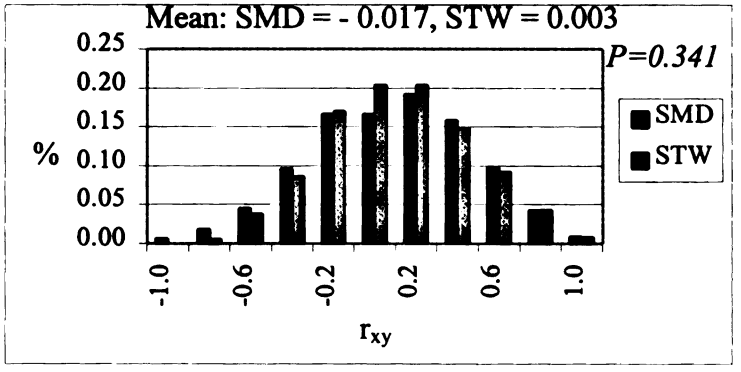


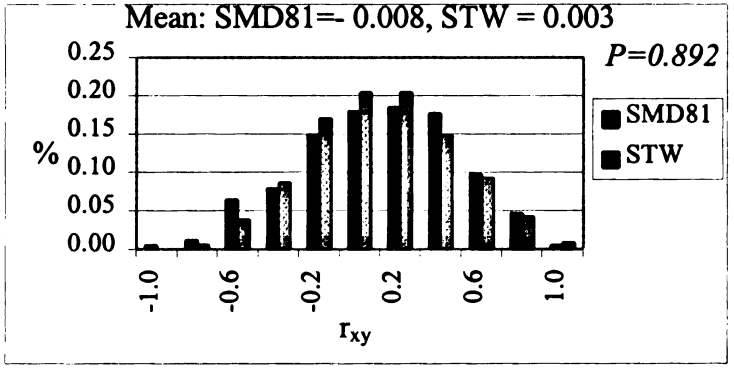


Wild Source Populations	Hatchery Broodstocks
Lewis =Lewis Lake, WY	LLW = Lewis Lake
LA = Apostle Islands	SAW = Apostle Island
IL = Isle Royale	SIW = Isle Royale

Figure 4. Frequency (Y-axis) distributions of pair-wise coefficients of relatedness (X-axis) for source populations and hatchery broodstocks.

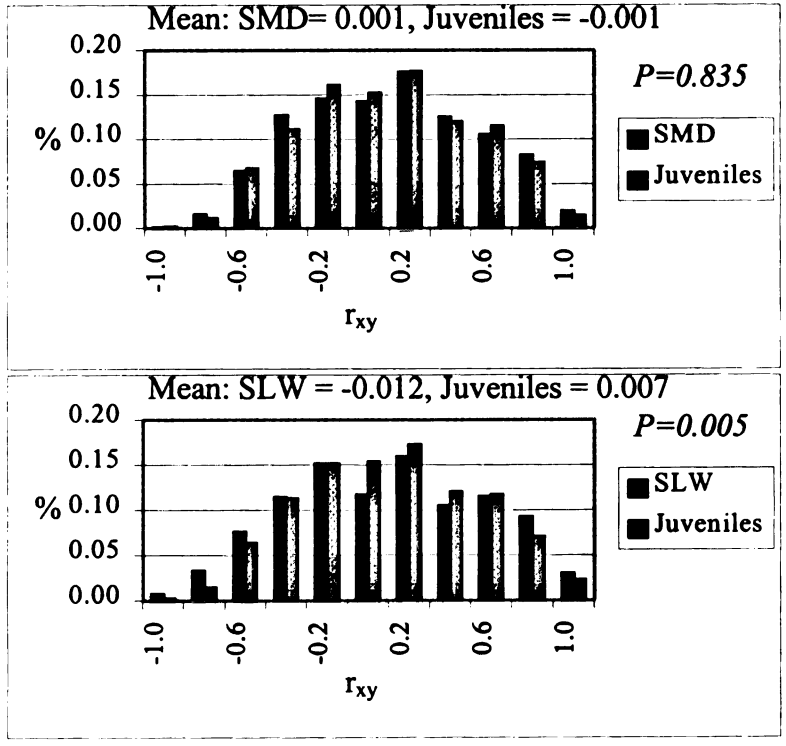


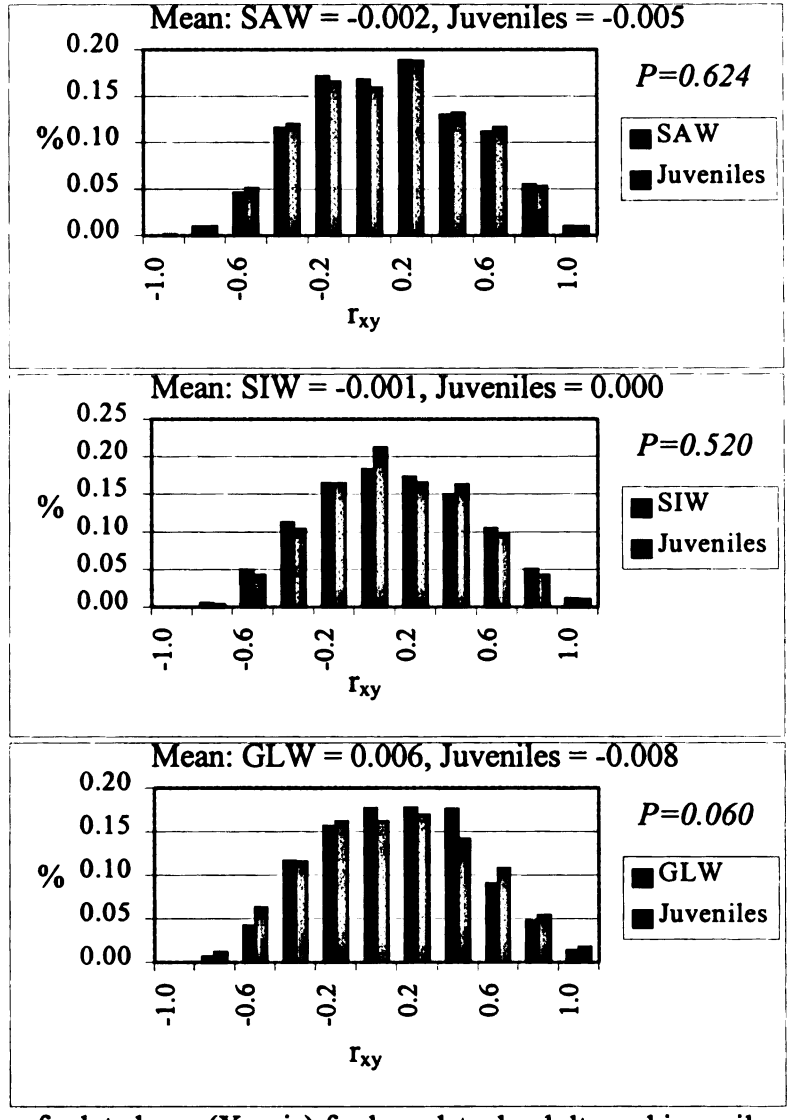




Intergenerational Hatchery Broodstocks
SMD81 = 1981 broodstock year class
SMD = 1988 and 1987 broodstock year classes
STW = Traverse Island broodstock

Figure 5. Frequency (Y-axis) distributions of pair-wise coefficients of relatedness (X-axis) for intergenerational Marquette (SMD) hatchery broodstocks.





Hatchery Broodstocks
SMD = Marquette
SLW = Seneca Lake
SAW = Apostle Islands
SIW = Isle Royale
GLW = Green Lake

Figure 6. Frequency (Y-axis) distributions of pair-wise coefficients of relatedness (X-axis) for broodstock adults and juveniles.

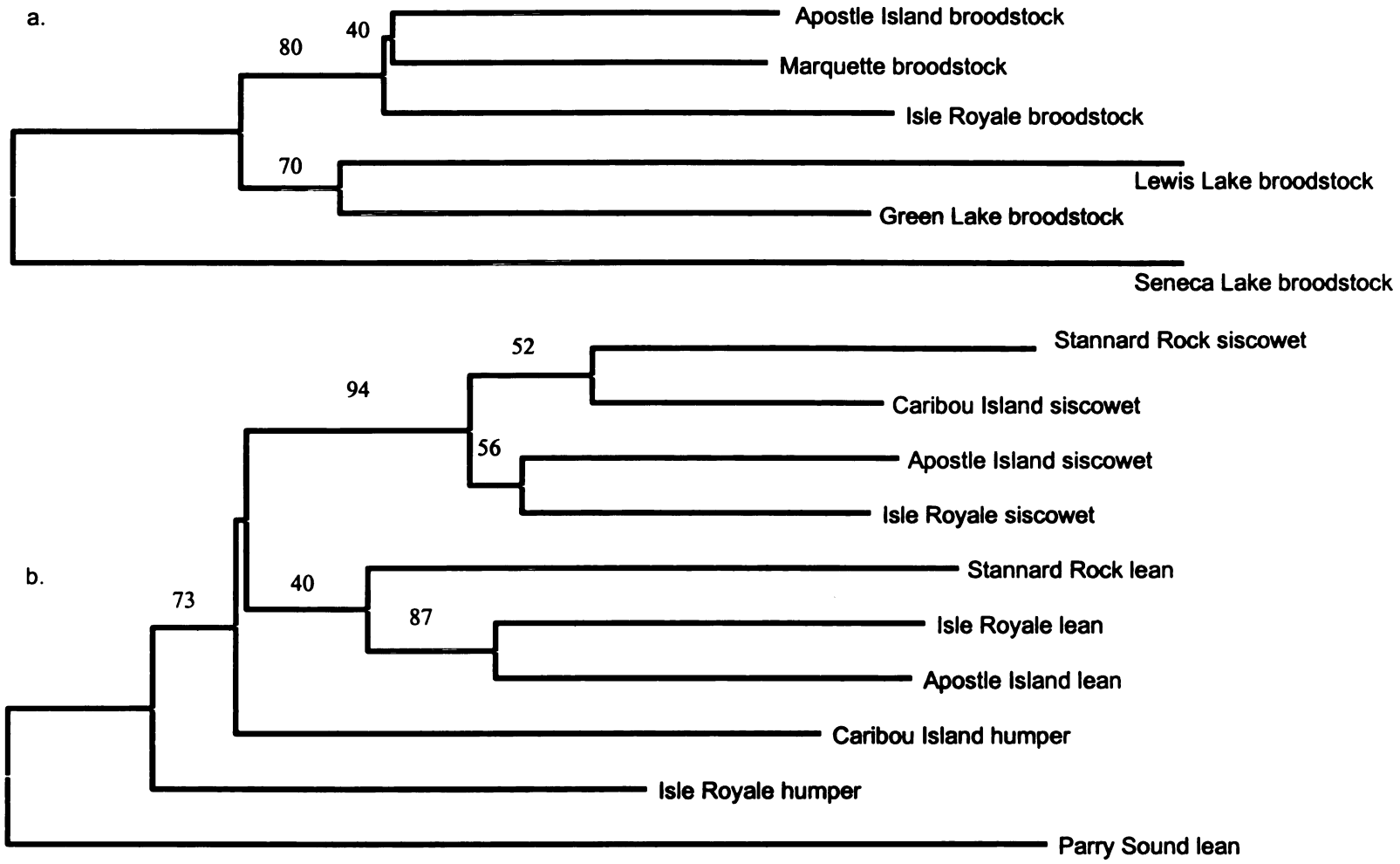


Figure 7. Neighbor-joining trees representing genetic divergences in hatchery (a.) and wild (b.) lake trout (*Salvelinus namaycush*) populations based on Cavalli-Sforza and Edwards chord distances (1967). Numbers represent bootstrap values.

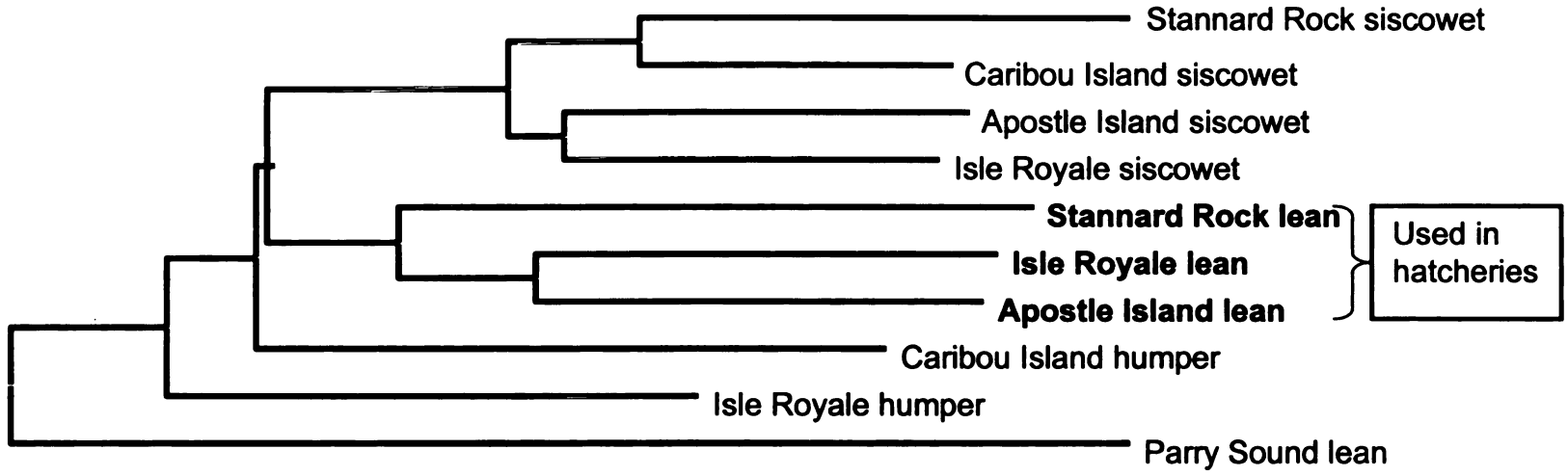


Figure 8. Neighbor-joining tree representing genetic divergence of remnant wild lake trout (*Salvelinus namaycush*) populations based on Cavalli-Sforza and Edwards chord distances (1967). The tree reveals that a comparatively small segment of lake trout diversity (leans) is currently being utilized in the lake trout broodstock program. All populations are from Lake Superior except for lean lake trout from Parry Sound that are from Lake Huron waters of Georgian Bay, Ontario.

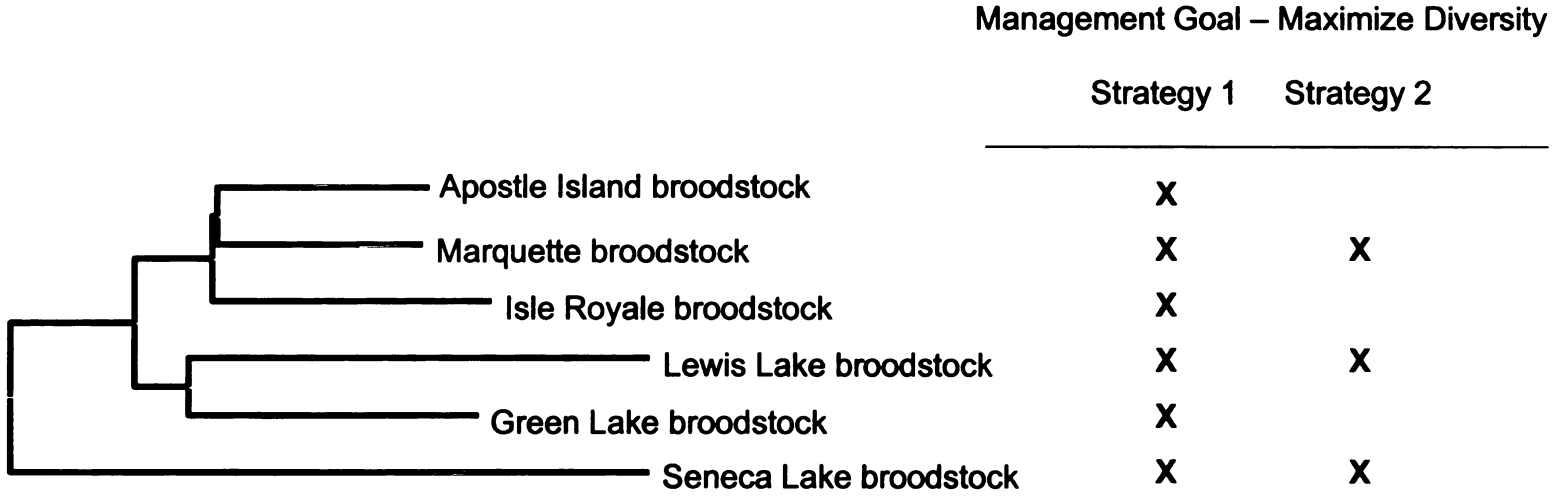


Figure 9. Neighbor-joining tree representing genetic divergence of lake trout (*Salvelinus namaycush*) hatchery broodstocks based on Cavalli-Sforza and Edwards chord distances (1967). This tree illustrates two possible strategies for maximizing genetic diversity in hatchery broodstocks. Strategy 1 advocates the use of all existing hatchery broodstocks. Strategy 2 offers an alternative strategy, advocating development of a composite Lake Michigan and Lake Superior broodstock and maintaining the Seneca broodstock. Strategy 2 allows for increased numbers of adults to be retained per broodstock within existing hatchery facilities to increase effective population sizes while simultaneously maintaining the single greatest source of genetic diversity (among lake basins).

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