

Genetic Evaluation of a Great Lakes Lake Trout Hatchery Program

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Abstract.—Efforts over several decades to restore lake trout *Salvelinus namaycush* in U.S. waters of the upper Great Lakes have emphasized the stocking of juveniles from each of six hatchery broodstocks. Retention of genetic diversity across all offspring life history stages throughout the hatchery system has been an important component of the restoration hatchery and stocking program. Different stages of the lake trout hatchery program were examined to determine how effective hatchery practices have been in minimizing the loss of genetic diversity in broodstock adults and in progeny stocked. Microsatellite loci were used to estimate allele frequencies, measures of genetic diversity, and relatedness for wild source populations, hatchery broodstocks, and juveniles. We also estimated the effective number of breeders for each broodstock. Hatchery records were used to track destinations of fertilized eggs from all spawning dates to determine whether adult contributions to stocking programs were proportional to reproductive effort. Overall, management goals of maintaining genetic diversity were met across all stages of the hatchery program; however, we identified key areas where changes in mating regimes and in the distribution of fertilized gametes and juveniles could be improved. Estimates of effective breeding population size (N_b) were 9–41% of the total number of adults spawned. Low estimates of N_b were primarily attributed to spawning practices, including the pooling of gametes from multiple males and females and the reuse of males. Nonrandom selection and distribution of fertilized eggs before stocking accentuated declines in effective breeding population size and increased levels of relatedness of juveniles distributed to different rearing facilities and stocking locales. Adoption of guidelines that decrease adult reproductive variance and promote more equitable reproductive contributions of broodstock adults to juveniles would further enhance management goals of maintaining genetic diversity and minimize probabilities of consanguineous matings among stocked individuals when sexually mature.

Low natural recruitment or elevated levels of mortality caused by the effects of habitat loss,

overfishing, and introductions of nonnative species often decrease the long-term viability of fish populations (Lange et al. 1995; Lassuy 1995; National Research Council 1996). Hatcheries have been used widely for conservation and supplementation of declining, endangered, or extirpated species and populations (Carmichael et al. 1995; Philippart 1995; Anders 1998; Dodson et al. 1998). Preservation of genetic diversity and coadapted ecological, physiological, and phenotypic traits of individuals maintained within hatchery programs (and of juveniles stocked) are widely embraced as im-

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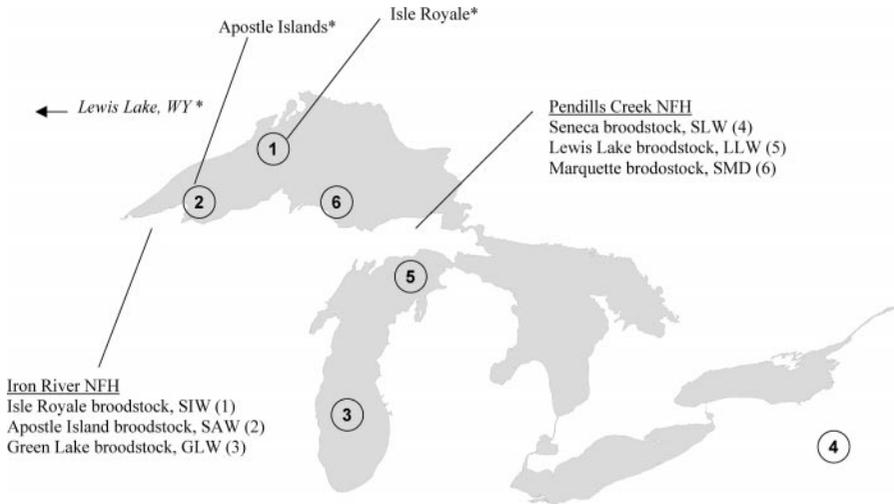


FIGURE 1.—Locations of hatcheries and wild populations of lake trout sampled for the genetic evaluation of the Great Lakes lake trout hatchery program in 1998 and 1999. Numbers refer to the original source sites of broodstocks. Names with an asterisk represent wild progenitor source populations of associated broodstocks sampled for genetic comparisons. Lewis Lake Hatchery broodstock were developed from a feral lake trout population in Lewis Lake, Wyoming.

portant goals of hatcheries associated with conservation or restoration programs (Hynes et al. 1981; Krueger et al. 1981; Ryman 1991; Utter 1991; Waples 1991). This “conservation aquaculture” approach has been advocated as a key component of the comprehensive recovery program for Great Lakes populations of lake trout *Salvelinus namaycush* (Meffe 1995).

During the 1950s and early 1960s, lake trout populations throughout the Great Lakes were decimated, principally as a result of overfishing and increased adult mortality caused by the parasitic sea lamprey *Petromyzon marinus* (Eshenroder et al. 1995; Hansen et al. 1995; Holey et al. 1995). Wild lake trout populations were completely extirpated from Lake Michigan (Eshmeyer 1957) and U.S. waters of Lake Huron. Reductions in both fishing intensity and sea lamprey abundance probably prevented extirpation of wild populations in Lake Superior (Rahrer 1965; Swanson and Swedberg 1980; Hansen et al. 1995).

Lake trout restoration efforts have emphasized the development and maintenance of hatchery broodstocks for the production of juveniles for stocking in U.S. waters of the upper Great Lakes (Fetterolf 1980). The National Fish Hatchery System of the U.S. Fish and Wildlife Service (USFWS) is responsible for nearly all lake trout stocking within U.S. waters of the upper Great Lakes, annually producing an average of 3.5 million yearling lake trout for stocking across 40 sites.

Six hatchery broodstocks have been developed and are maintained by the USFWS hatchery system, including 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; Page et al. 2004).

Selection of source populations for broodstock development was based in part on the desire to capture the remaining genetic and ecological diversity historically present in lake trout populations across the upper Great Lakes basin (Kincaid et al. 1993; Krueger et al. 1983; Krueger and Ihssen 1995); thus, hatchery broodstocks serve as “gene banks” of historical genetic diversity. Hatchery broodstocks are representative of the regional (i.e., lake basin) genetic structure historically present in lake trout populations (Guinand et al. 2003; Page et al. 2004). Hatchery broodstocks also represent the sole remnants of historically abundant Lake Michigan populations (Coberly and Horrall 1982; Krueger et al. 1983; Visscher 1983) and of remnant nearshore populations within U.S. waters of southern Lake Superior (Krueger et al. 1983).

Given the generation time of lake trout (6–8 years), stocking and evaluation of each broodstock was not deemed feasible if done sequentially, one broodstock at a time. Accordingly, individuals from multiple broodstocks were stocked simultaneously at release sites, assuming that lake envi-

ronments would subsequently select for juveniles produced from broodstocks with the highest fitness (Krueger et al. 1981; Holey et al. 1995) for conditions at each release site (e.g., depth, temperature, abundance of predators and prey; Bronte et al. 2003). Multiple hatchery strains developed from wild lake trout populations from throughout the Great Lakes basin have been used for restoration efforts in Lake Ontario (Krueger et al. 1989). Development of additional broodstocks from lake trout populations that exhibit unique behavioral, ecological, and phenotypic traits has been advocated for stocking programs in Lake Michigan (Bronte et al. 2003; Page et al. 2004).

Over 30 years of restoration efforts have failed to restore self-sustaining lake trout populations in Lakes Michigan and Huron (Krueger et al. 1995; Selgeby 1995). Numerous factors have been identified as potential impediments to lake trout restoration (Bronte et al. 2003). Factors include poor adult survival as a result of continued sea lamprey predation and overfishing; low recruitment as a result of predation on eggs and juveniles; habitat degradation; disease (i.e., early mortality syndrome); inefficient stocking practices, such as stocking in high-energy nearshore zones and in low-quality spawning habitats; and poor imprinting of juveniles to spawning sites. Because natural recruitment is not sufficient to rebuild lake trout stocks, restoration efforts will rely heavily on hatchery-produced lake trout until sufficient natural recruitment is realized.

Long-term dependence on hatchery production as the primary source of recruitment will require that ecological and genetic diversity be maintained within lake trout hatchery broodstocks and in the juveniles stocked. Hatchery programs have adopted a number of practices to minimize changes in genetic diversity (Holey 1997). Broodstocks were developed from gametes taken from a large number of adults over multiple years from populations of wild progenitors. Large numbers of adults from multiple year-classes are maintained and spawned annually for each broodstock (Holey 1997).

Hatchery management practices can alter the genetic characteristics of broodstocks and their progeny released into natural environments, even when guidelines emphasizing the importance of genetic diversity are in place (Allendorf and Ryman 1987; Waples et al. 1990; Busack and Currens 1995; Lynch and O'Hely 2001; Dushesne and Bernatchez 2002). Conservation of genetic variation of hatchery broodstocks and progeny is dependent on the adoption of fundamental population genetics prin-

ciples related to levels of relatedness (coancestry), inbreeding, genetic drift, and effective population size, all of which have been shown to be influenced by hatchery practices related to spawning methods and to methods of adult and juvenile collection and distribution (Figure 2).

Most empirical studies investigating the influences of hatchery practices on genetic diversity have focused on one or a few stages of a broodstock program (e.g., review in Allendorf and Ryman 1987; Secor et al. 1992; Brown et al. 2000); however, genetic diversity may be incrementally lost throughout a hatchery broodstock program (Figure 2). For this study, our objective was to chronicle events over a major portion of the Great Lakes lake trout hatchery program. We evaluated (in discrete stages) how well-established goals of retaining genetic diversity were achieved. Historical aspects of broodstock development and maintenance differ greatly among the different broodstocks and have been described in detail previously (Page et al. 2004). Because some changes in genetic characteristics within a hatchery system are inevitable, we specifically focused on the identification of large and pervasive changes in levels of genetic diversity that could be attributed to specific practices and could be readily altered. We evaluated how effectively genetic characteristics (genetic diversity and allele frequencies) of wild progenitor populations were retained during initial development of captive broodstocks (stage 1) as well as changes between broodstock adults and juveniles (stage 2). We also evaluated current collection and distribution procedures of fertilized gametes to rearing facilities and stocking programs (stage 3).

Methods

Stage 1: Broodstock Development from Wild Populations

Sample collection.—Wild populations representing progenitors of the LLW, SIW, and SAW hatchery broodstocks were sampled. The LLW source population ($N = 77$) was sampled from Lewis Lake, Wyoming, by USFWS personnel from the Yellowstone Fisheries Resource Office. At the turn of the century, Lewis Lake was stocked with wild lake trout collected from northern Lake Michigan populations (Page et al. 2004). The current LLW broodstock was developed from a broodstock derived in 1983 from wild Lewis Lake lake trout and, therefore, represents an indirect comparison between hatchery lake trout and wild progenitor

Stages in Broodstock Development and Management	Issues Pertinent to Broodstock Development and Management	Conditions Commonly Realized in Hatchery Settings
<p>Stage 1. Wild Source</p> <p>Stage 2. Hatchery Broodstock</p> <p>Stage 3. Juveniles</p> <p>Stocking</p>	<p>Subpopulations Sampled^{4,19,22}</p> <p>Number Individuals Sampled^{4,6}</p> <p>Sampling Events¹²</p> <p>Sex Ratios Sampled^{7,8}</p> <p>Spawning Ratios^{7,8}</p> <p>Fertilization Methods^{7,8}</p>	<p>Few^{12,17}</p> <p>Small^{9,11,14}</p> <p>Few^{9,12,14}</p> <p>Unequal^{9,14}</p> <p>Unequal^{9,14,17,21}</p> <p>Pooling or Sequential^{7,8,9,17}</p>
	<p>Founder Numbers^{5,6,20}</p> <p>Numbers Spawned^{6,13,19}</p> <p>Broodstock Sex Ratio^{3,13}</p> <p>Spawning Ratios^{6,7,8,13}</p> <p>Fertilization Methods^{3,7,8,13}</p>	<p>Small⁵</p> <p>Small^{11,13,18}</p> <p>Unequal¹⁰</p> <p>Unequal^{5,13,15}</p> <p>Pooling or Sequential^{7,8}</p>
	<p>Numbers Stocked^{2,19}</p> <p>Stocking Locations¹³</p> <p>Strain Combination^{13,15}</p> <p>Juvenile Distribution^{3,13}</p>	<p>Large¹</p> <p>Existing Populations^{3,13,16}</p> <p>Multiple Per Location^{13,15,16}</p> <p>Nonrandom^{3,13,15,16,17,21}</p>

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. Ilynes et al. 1981; 13. Busack and Currans 1995; 14. Mueller 1995; 15. Campton 1995; 16. Flagg et al. 1995; 17. Rees and Harrell 1990; 18. Uiter 1991; 19. Ryman 1991; 20. Cross and King 1983; 21. Secor et al. 1992; 22. Philippart 1995

FIGURE 2.—General stages in the development of hatchery broodstocks and juvenile production and factors that may contribute to changes in the levels of gene diversity within a broodstock across generations and for stocked progeny. Factors identified within this genetic evaluation of the Great Lakes lake trout hatchery broodstock program are in bold.

lake trout. Wild lake trout from Isle Royale (the source population of the SIW broodstock [$N = 119$]) and the Apostle Islands (the source population of the SAW broodstock [$N = 68$]) were sampled in the summer and fall of 1995. Isle Royale and Lewis Lake samples consisted of liver tissue preserved in ethanol. Scales collected from Apostle Islands lake trout in 1991 and 1993 were used to supplement samples of the SAW source population. Scales were sampled from archival collections located at the Wisconsin Department of Natural Resources Bayfield Station. Wild source populations were sampled from the same or adjacent locales to develop the LLW, SAW, and SIW broodstocks.

Hatchery personnel collected samples of adults from six captive hatchery broodstocks (Figure 1) during routine spawning events in the fall of 1998. The LLW, SLW, and SMD broodstocks were sampled at Pendill's Creek National Fish Hatchery (NFH) in Michigan, and the GLW, SIW, and SAW hatchery broodstocks were sampled at the Iron River NFH in Wisconsin. In all, 200 adults were sampled from the GLW, LLW, SAW, SIW, and

SLW strains, and 166 adults were sampled from the SMD strain. Samples consisted of fin clips (~ 1 cm²) removed from caudal fins and stored individually in 1.5-mL vials containing 1 mL of a high-salt buffer (4 M urea, 0.2 M NaCl, 0.1 M tris-HCl, 0.5% Sarcosine, and 10 mM EDTA). Fin clips were stored at -20°C until analysis.

Lake trout reach spawning condition asynchronously over a spawning period (males mature earlier), which typically lasts approximately 1 month. Thus, adults were spawned when a sufficient number of individuals reached spawning condition, which resulted in multiple spawning events over the entire spawning period (hereafter referred to as spawning lots). Spawning adults from broodstocks at Pendill's Creek NFH were sampled uniformly across multiple spawning events or spawning lots. Adults sampled from broodstocks at Iron River NFH were sampled from one or two spawning lots and were representative of the broodstocks spawned. Spawning lots sampled at Iron River NFH represented large proportions (45–65%) of the total number of adults spawned. Spawning

dates for these lots did not reflect an overrepresentation of early- or late-spawning individuals.

Extraction of DNA.—We extracted DNA from liver and fin tissues with the use of proteinase K digestion and a modified Puregene extraction protocol (Gentra, Inc., Minneapolis, Minnesota). The DNA was resuspended in 50 μ L of tris-EDTA (10 mM tris-HCl, pH 8.0, 1 mM EDTA). Fluorometry was used to determine DNA concentrations. Before fluorometry, RNase (2 μ L of 20 mg/ μ L stock) was added to each sample. For each polymerase chain reaction (PCR), 100 ng of DNA was used.

We used a chelating procedure to extract DNA from scale samples. Scales (3–5 per individual) were added to 250 μ L of a 5% Chelex and 10 mM tris-HCl (pH 7.5–8.0) suspension. Scales were digested overnight with 3 μ L of proteinase K. Proteinase K was subsequently inhibited by heat denaturation at 95°C for 5 min, and samples were centrifuged at 14,000 rpm for 10 min. The resulting supernatant was removed and 2.5 μ L of the supernatant was used for each PCR reaction.

Microsatellite screening.—Microsatellite markers used in this study were previously developed from brook trout *S. fontinalis* (*Sfo1*, *Sfo12*, and *Sfo18*; Angers et al. 1995), sockeye salmon *Oncorhynchus nerka* (*One μ 9* and *One μ 10*; Scribner et al. 1996), pink salmon *O. gorbuscha* (*Ogo1a* and *Ogo1c*; Olsen et al. 1998), bull trout *S. confluentus* (*Sco μ 19*; Taylor et al. 2001), and Atlantic salmon *Salmo salar* (*Ssa85*; O'Reilly et al. 1996). Primer annealing temperatures, sequences for each marker, and all PCR reaction conditions were as described in Page et al. (2004). The PCR products were screened with the use of 6% polyacrylamide vertical gels. Products were visualized with a Hitachi FMBIO II Multi-View scanner and associated software. Microsatellite fragments were sized manually with a 20 base pair internal lane standard. Several individuals of known genotype served as positive controls in each gel for standardization.

Statistical analysis.—We estimated allele frequencies and expected and observed heterozygote diversity for wild source and hatchery populations with the program BIOSYS-1 (Swofford and Selander 1981). Exact tests implemented with the program GENEPOP (version 3.1b; Raymond and Rousset 1995) were used to determine significance of differences ($P < 0.05$) in allele frequency between wild source and hatchery broodstock samples. Significance values were adjusted using sequential Bonferroni methods. Allelic richness was calculated for each population with the program

CONTRIBUTE (Petit et al. 1998). Allelic richness provides a measure of the number of alleles per locus standardized for differences in population sample size. If sampling and spawning of individuals from wild source populations were effective in capturing the genetic diversity of these populations, then estimates of allele frequency and genetic diversity of source populations and hatchery broodstocks should not differ appreciably.

Estimation of intergenerational accrual of gene correlations within individuals (inbreeding coefficients, F) or among individuals (coancestry, θ) and the effective size of captive (hatchery) populations is critical for predicting the impact that hatchery supplementation will have on natural populations (Ryman and Laikre 1991). In the absence of pedigree data, estimates of coancestry can be estimated through the use of surrogate measures of relatedness (r_{xy} ; Queller and Goodnight 1989; Blouin et al. 1996). Pairwise interindividual estimates of r_{xy} values were derived with the program KINSHIP 2.1 (Queller and Goodnight 1989). Estimations of mean r_{xy} were then summarized for every wild source and hatchery population. We expected that, if large numbers of wild-caught adults were spawned and if spawning methods were effective in equalizing contributions by wild adults to the next generation coefficients of relatedness should not differ significantly between wild progenitor and hatchery populations. The significance of differences in distributions of pairwise coefficients of relatedness ($P < 0.05$) between wild source populations and the hatchery broodstocks derived from them were tested with nonparametric Mann-Whitney U -tests. We estimated the proportion of r_{xy} values of 0.5 or greater (consistent with the level of full siblings) for wild populations and hatchery broodstocks with the program KINSHIP 2.1 (Queller and Goodnight 1989). A high incidence of r_{xy} values 0.5 or greater between pairs of breeding adults would provide evidence of reproductive skew among wild adults used to create the broodstocks.

Stage 2: Broodstock Production

We sampled broodstock adults and juveniles to identify changes in allele frequencies and measures of genetic diversity that could be attributed to spawning regimes and other aspects of juvenile production (Figure 2). If spawning techniques effectively captured the genetic diversity within the broodstocks sampled, allele frequencies and genetic diversity of broodstocks and associated progeny should not differ appreciably.

Sample and data collection.—Juveniles were sampled in the spring of 1999 and 2000 (SMD only). The LLW broodstock was not evaluated in stage 2 because juveniles produced by this broodstock were unavailable. Juveniles of the SMD broodstock were the F1 progeny of the spawned SMD adults from 1999. All broodstock juveniles were segregated within each hatchery based on the specific adult spawning lots from which they were produced. Therefore, we were able to directly match and sample juveniles produced from sampled adult spawning lots. All juveniles were collected as swim-up fry from hatchery tanks with dip nets. Effort was made to limit sampling bias by collecting equal numbers of juveniles from within and between tanks. Juveniles were stored in 95% ethanol at room temperature. Juveniles of the SAW, SIW, and GLW broodstocks were sampled from Iron River NFH, and juveniles of the SMD and SLW broodstocks were collected from Jordan River NFH in Michigan. Approximately 200 juveniles were collected from each broodstock (except GLW juveniles; $N = 114$).

Based on hatchery records, we also documented the total number of lake trout adults from each broodstock that did not contribute to the subsequent generation because eggs were discarded either before or after fertilization. Eggs discarded before fertilization were identified by hatchery personnel as nonviable (too green or overly ripe). Eggs discarded after fertilization were identified as exhibiting low survivorship. For some spawner lots, only a portion of the total number of fertilized eggs was discarded. We defined the number of “available spawners” to be the number of adults documented before spawning events for each broodstock. We derived the actual “number spawned” by subtracting the number of adult females whose gametes were identified as of poor quality and the associated number of males that would have spawned with these females (Iron River NFH only) from the number of “available spawners.” By subtracting the number of adults whose eggs were excised after fertilization from the “number spawned,” we derived the total number of adults that could have contributed to the subsequent generation and designated these adults as “potential contributors.” Incremental reductions in the number of adults to account for discarded eggs were calculated.

Laboratory analysis.—We followed the same DNA extraction protocols for adults and juveniles described in stage 1. For juveniles, a sample of the anal fin was utilized for DNA extraction. Three

microsatellite loci with high allelic diversity (*Sfo18*, *Scoμ19*, and *Ssa85*) were utilized.

Statistical analysis.—Summary measures of genetic diversity, including observed (H_o) and expected (H_e) heterozygosity, allele frequencies, allelic richness, and tests of significance of allele frequencies were calculated as described in stage 1. Average coefficients of relatedness (r_{xy}) and distributions of pairwise estimates of coefficients of relatedness for adults and juveniles were compared as described in stage 1.

If spawning methods were effective in equalizing the contributions of spawning adults to progeny (i.e., in minimizing reproductive variance), then the effective number of breeders (N_b) should approximate the actual numbers of adults spawned. We derived estimates of temporal variance in allele frequency (see equation 8 in Waples 1989) between adult and juvenile samples (F_c) for a single locus as

$$F_c = \frac{1}{k} \sum \frac{(x_i - y_i)^2}{(x_i + y_i)(2 - x_i y_i)}, \quad (1)$$

where F_c is the average variance in allele frequency over k alleles between individuals from two generations (e.g., adults [x_i] and progeny [y_i]). Estimates of N_b were based on Waples' (1989) plan I sampling methodology, that is,

$$N_b = \frac{t}{2[F_c - 1/(2S_o) - 1/(2S_t) + 1/N]}, \quad (2)$$

where N is the total adult population size sampled before reproduction (generation 0), S_o is the sample size of adults, and S_t is the sample size of juveniles ($t = 1$ generation). We calculated a weighted mean N_b for each broodstock by applying a mean F_c across all loci for each broodstock (weighted for number of alleles) to equation (2). Confidence intervals (95%) for estimates of N_b for each broodstock were calculated by estimating the confidence intervals of F_c (Waples 1989). The model assumes random mating, that populations were closed to migration, and there was no selection or mutation to new alleles.

Stage 3: Collection and Distribution of Eggs

Evaluations of the final stage of the lake trout broodstock program involved assessments of the potential impacts of collection, distribution, and stocking on the genetic diversity and relatedness of juveniles allocated to rearing stations and stocking locations (Figure 2). Genetic diversity of juveniles stocked in each Great Lakes release site

would be maximized by equalizing, to the extent possible, representation of all broodstock adults spawned. This goal could be achieved if juveniles collected from spawning lots produced across the spawning season were mixed before distribution to rearing facilities or stocking sites (e.g., Waples et al. 1990).

Spawning and distribution records of all fertilized lake trout eggs from hatchery broodstock spawning sessions during 1998 (LLW, SLW, SIW, and SAW) and 1999 (SMD) were provided by Iron River NFH and Pendill's Creek NFH. The number of eggs distributed to different rearing facilities was calculated to infer potential loss of genetic diversity (heterozygosity and allelic richness) and increased levels of relatedness of fertilized eggs distributed to different hatchery facilities and stocking programs. Using estimates of spawning efficiencies (N_b/N ratios) derived for each broodstock in stage 2, we estimated the realized effective number of breeders that contributed the eggs that were distributed to rearing stations and stocking programs.

Results

Stage 1: Broodstock Development

Overall, after one generation (SAW and SLW) or two generations (LLW), adults in broodstocks appear to have retained the genetic characteristics of the wild progenitor populations. Estimates of genetic diversity for wild source populations and hatchery broodstocks were similar, as measured by observed (H_o) and expected (H_e) heterozygosities and allelic richness (Table 1). Estimates of inbreeding coefficients were not significantly different from zero and were similar for all broodstocks compared with wild source populations (Table 1). No significant differences were found (Mann–Whitney U -tests; LLW, $P = 0.539$; SIW, $P = 0.256$; SAW, $P = 0.829$) between distributions of coefficients of relationship (r_{xy}) values for wild and hatchery populations. Frequencies of pairwise r_{xy} estimates consistent with full-sibling levels of relatedness were similar for source populations and hatchery broodstocks (Table 1). Only allele frequencies of adults of the LLW and SAW broodstocks differed significantly from their respective source populations (LLW for loci *Ogo1a*, *Scoμ19*, and *Ogo1c*; SAW for loci *Sfo18* and *Scoμ19*; Table 1).

Stage 2: Broodstock Production

Estimates of observed heterozygosities (H_o) were generally lower in juveniles than in adults

(four of five broodstocks; Table 2). The largest difference in estimates of H_o (8.3%) was observed between adults and juveniles of the GLW broodstock. Estimates of allelic richness were lower in juveniles than adults for the SMD, GLW, and SIW broodstocks (Table 2), while slight increases in allelic richness were observed for the SLW and SAW juveniles compared with their respective adult broodstocks.

Estimates of inbreeding coefficients were higher in juveniles than in adults for four of the five broodstocks, though none differed significantly from zero ($P < 0.05$; Table 2). Significant differences ($P = 0.005$) between juvenile and adult distributions of r_{xy} were observed only in the SLW broodstock; however, we observed no consistent trend toward increases in levels of relatedness (r_{xy}) between adults and juveniles and little evidence of differences based on degrees of kurtosis or skewness in distributions of r_{xy} .

Hatchery records revealed that because of the discarding of eggs, all broodstocks experienced reductions in the number of adults contributing to offspring (Table 3). The number of adults spawned was reduced by 6–12% from the number of “available spawners” for four out of the six broodstocks (LLW, SIW, SLW, and SMD). In four broodstocks (GLW, LLW, SAW, and SIW), the number of “potential contributors” was reduced between 2% and 40% from the total number of adults spawned (Table 3). Eggs were typically eliminated at the beginning and end of the spawning period; the exception was eggs from GLW broodstock females that were discarded throughout the spawning period.

Significant differences in allele frequencies were observed between adults and juveniles for two of the five broodstocks (SLW and GLW; Table 2). The SLW adult and juvenile samples differed significantly ($P = 0.002$) for the *Scoμ19* locus, and the GLW adults and juveniles differed significantly at *Scoμ19* ($P < 0.001$), *Ssa85* ($P = 0.009$), and *Sfo18* ($P = 0.020$). Estimates of N_b based on variance in allele frequency between adults and juveniles were lower than the number of adults spawned for the spawning lots analyzed (Table 3). The number of adults in each spawning lot evaluated ranged from 112 to 436. Estimates of the average number of effective breeders ranged from 20 to 115. The ratio of the effective number of breeding adults to the total number of adults spawned (N_b/N) within spawning lots (an estimate of spawning efficiency) was 0.41, 0.27, 0.26, 0.11, and 0.09 for the SMD, SIW, SAW, SLW, and GLW

broodstocks, respectively. Extrapolating N_b/N ratios estimated from spawning lots to all potential breeding adults (Table 3), the total number of realized adult contributors was 233, 100, 181, 87, and 31 for the SAW, SMD, SIW, SLW, and GLW broodstocks, respectively.

Stage 3: Collection and Distribution of Eggs and Juveniles

Distribution of eggs from production facilities to rearing stations and stocking programs (as sac fry and eggs) were not representative of the total number of adults spawned (Table 4). Using the SAW broodstock as an example, of the 898 adults potentially contributing to juvenile production, the Bayfield State Fish Hatchery received eggs produced from 268 adults and the Jordan River NFH received eggs from a total of 240 adults, reflecting a maximum of 30% and 27% percent of all potentially contributing adults, respectively. In addition, juveniles from 96 adults representing 11% of all potentially contributing adults were utilized for one juvenile release. A more representative proportion of juveniles representing approximately 436 adults (49%) was retained at Iron River NFH.

For broodstocks SLW, SMD, and LLW, we documented numerous examples in which the eggs distributed to rearing facilities and stocking programs were not representative of the entire spawning period (Table 5). For example, eggs from SLW females distributed to Iron River NFH and retained at Pendill's Creek NFH were all collected on 14 and 15 October 1998 and represented 44% of the total potential contributors. In contrast, a more equitable cross section of the juveniles produced across the spawning season was sent to the Jordan River NFH. Similar results were observed for the SMD broodstock. Eggs from LLW adults were designated for an astroturf egg-stocking program and represented eggs from all available spawning lots.

Discussion

The ability of hatcheries to conserve levels of genetic variation characteristic of progenitor wild source populations throughout a hatchery program is contingent upon proactive management related to spawning regimes and on decisions to allocate eggs and juveniles to rearing facilities and stocking sites (Allendorf and Ryman 1987; Busack and Currens 1995). Changes in genetic characteristics of hatchery broodstocks and between adults and progeny stocked (including increased levels of re-

latedness or coancestry, inbreeding, and genetic drift) can occur. The loss of locally adapted gene pools can negatively impact the fitness of supplemented natural populations (Lynch and O'Hely 2001). Preservation of genetic diversity within lake trout broodstocks used for restoration efforts throughout the Great Lakes has been repeatedly emphasized (Krueger et al. 1981, 1989; Kincaid et al. 1993; Krueger and Ihssen 1995; Holey 1997).

Stage 1: Broodstock Development

Changes in the levels of genetic diversity and allele frequency between a source population and a newly developed broodstock, as during stage 1 (broodstock development), are important because changes in genetic diversity can be exacerbated by genetic drift over generations in captivity due to low effective population size (Allendorf and Phelps 1980). Hatchery programs do not always successfully capture the genetic variability of source populations (Dodson et al. 1998). Loss of genetic diversity, differences in allele frequencies, or both that are attributed to genetic drift have been documented between source populations (wild and hatchery) and newly developed broodstocks (Allendorf and Phelps 1980; Cross and King 1983; review in Utter 1991). Adequate sampling of source populations can be difficult and, frequently, only small numbers of fish are sampled and often from a disproportionately small period of the spawning session (Allendorf and Ryman 1987). Sampling is often 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, to maximize returns per effort expended, salmonid populations are typically sampled at the peak of the spawning run, when fish are most plentiful (Hynes et al. 1981).

The differences observed between wild-source lake trout populations and lake trout hatchery broodstocks in expected heterozygosities, allele frequencies, allelic richness, estimates of relatedness, and inbreeding were not large. The number of wild lake trout sampled (typically $N > 100$) was sufficiently large to prevent large-scale changes in allele frequencies and loss of genetic diversity. However, much larger population sizes ($N = 500, 1,000$) have been advocated to effectively capture the diversity of genetic, ecological, and behavioral traits (Allendorf and Ryman 1987; Lande and Barrowclough 1987).

Significant differences in allele frequencies were found between wild source and hatchery

TABLE 1.—Allele frequencies and measures of genetic diversity for three hatchery strains of lake trout and their wild progenitor populations. The *P*-values of exact tests for significant differences in allele frequencies between source and hatchery broodstock populations are given; *P*-values in bold italics represent significant differences after sequential Bonferroni adjustment.

Locus and statistic	Allele	Lewis Lake		Isle Royale		Apostle Islands	
		Source	Broodstock	Source	Broodstock	Source	Broodstock
<i>Sfol8</i>	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
<i>N</i>		61	112	55	155	65	193
<i>P</i>			0.106		0.032		0.000
<i>Sfo1</i>	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
<i>N</i>		47	76	55	66	61	75
<i>P</i>			1.000		0.490		0.483
<i>Oneμ9</i>	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
<i>N</i>		66	76	67	66	33	75
<i>P</i>			0.031		0.078		0.063
<i>Oneμ10</i>	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
<i>N</i>		48	74	52	65	46	75
<i>P</i>			0.174		0.033		0.063
<i>Ogo1a</i>	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
<i>N</i>		75	78	64	65	67	76
<i>P</i>			0.000		0.295		0.105
<i>Scop19</i>	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
	<i>N</i>		74	114	70	166	63
<i>P</i>			0.006		0.044		0.002
<i>Ssa85</i>	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

TABLE 1.—Continued.

Locus and statistic	Allele	Lewis Lake		Isle Royale		Apostle Islands	
		Source	Broodstock	Source	Broodstock	Source	Broodstock
<i>N</i>		68	113	68	166	67	194
<i>P</i>		0.433		0.536		0.726	
<i>Sfo12</i>	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
<i>N</i>		53	75	63	67	66	74
<i>P</i>		0.639		0.679		0.210	
<i>Ogo1c</i>	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
<i>N</i>		70	50	63	57	31	54
<i>P</i>		0.004		0.055		1.000	
<i>H_o^a</i>		0.396	0.436	0.380	0.370	0.355	0.392
<i>H_e^b</i>		0.422	0.448	0.427	0.410	0.387	0.411
<i>A^c</i>		3.0	3.1	3.2	3.3	3.1	3.2
<i>F^d</i>		0.062	0.027	0.110	0.097	0.082	0.046
<i>r_{xy}^e</i>		0.006	-0.002	0.013	-0.003	-0.009	-0.009
<i>S^f</i>		0.064	0.057	0.100	0.072	0.080	0.051
<i>U^g</i>		<i>P</i> = 0.539		<i>P</i> = 0.256		<i>P</i> = 0.829	

^a Observed heterozygosity.
^b Hardy–Weinberg expected heterozygosity (Nei 1978).
^c Allelic richness (average number of alleles standardized for sample size; Petit et al 1998).
^d Wright’s (1951) inbreeding coefficient.
^e Average coefficient of relatedness (Queller and Goodnight 1989).
^f Proportion of coefficients of relatedness at the full-sibling level (*P* < 0.05).
^g Mann–Whitney *U* test for significance of difference in distributions of coefficients of relatedness (*r_{xy}*) between wild populations and broodstocks.

broodstocks for LLW and SAW that were probably a result of nonrepresentative sampling, implementation of spawning methods that promote high reproductive variance (Page et al. 2004), or genetic drift within the wild populations. The LLW broodstock was not developed directly from wild lake trout as were the SAW and SIW broodstocks; it is a second-generation broodstock (Page et al. 2004). Differences in genetic diversity between the Lewis Lake wild progenitor population and the current LLW hatchery broodstock may be related to events that occurred during initial development of the LLW strain.

Even though cumulative evidence suggests that the genetic diversity of wild source populations has been largely maintained in the hatchery broodstocks, it should be cautioned that most broodstocks were developed relatively recently (over the period 1987–1994; Page et al. 2004). Periodic development of new broodstock year-classes from wild populations has been one of the methods adopted by the USFWS hatchery system to address concerns of genetic drift and domestication asso-

ciated with the perpetuation of captive broodstocks over multiple generations (Holey 1997).

Stage 2: Broodstock Production

During spawning, the effective number of breeding adults can be reduced. This increases the potential for differences in allele frequency, lowering levels of genetic diversity, and elevating levels of coancestry between broodstock adults and juveniles. There is also increased potential for inbreeding among juveniles once they mature (Kincaid 1983; Allendorf and Ryman 1987; Simon 1991; Busack and Currens 1995). For most lake trout broodstocks, spawning records revealed that adults were removed from the pool of potential contributing adults because of the stage of gamete maturation, resulting in the excision of entire lots of fertilized and unfertilized eggs (Table 3). A large proportion of adults (41%; Table 3) from the GLW broodstock did not contribute progeny because their eggs were excised. In fact, a majority of the GLW egg lots excised were from young adults of a recently developed year-class (1993)

TABLE 2.—Allele frequencies and measures of genetic variability for hatchery broodstock adults and progeny during a genetic evaluation of the Great Lakes lake trout hatchery program. The *P*-values of exact tests for significant differences in allele frequencies between adult and juvenile (juv.) populations are given; *P*-values in bold italics represent significant differences after sequential Bonferroni *t*-test adjustments. Abbreviations are as follows: NFH = National Fish Hatchery, SMD = Marquette broodstock, SLW = Seneca Lake broodstock, SAW = Apostle Islands broodstock, GLW = Green Lake broodstock, and SIW = Isle Royale broodstock.

Locus and statistic	Allele	Pendill's Creek NFH			
		SMD		SLW	
		Adult	Juv.	Adult	Juv.
<i>Sfo18</i>	169	0.000	0.000	0.000	0.000
	171	0.599	0.557	0.748	0.745
	173	0.000	0.005	0.022	0.020
	175	0.041	0.073	0.204	0.162
	179	0.000	0.000	0.000	0.000
	181	0.275	0.231	0.026	0.064
	183	0.005	0.005	0.000	0.005
	185	0.005	0.005	0.000	0.000
	187	0.068	0.120	0.000	0.005
	189	0.009	0.003	0.000	0.000
	191	0.000	0.000	0.000	0.000
<i>N</i>		111	184	115	204
<i>P</i>		0.167		0.152	
<i>Scop19</i>	157	0.005	0.000	0.000	0.000
	159	0.005	0.000	0.000	0.000
	161	0.112	0.132	0.256	0.267
	163	0.000	0.000	0.000	0.000
	165	0.009	0.000	0.000	0.010
	167	0.000	0.000	0.004	0.000
	169	0.005	0.000	0.004	0.000
	171	0.275	0.273	0.415	0.331
	173	0.014	0.005	0.047	0.100
	175	0.437	0.478	0.231	0.257
	177	0.086	0.086	0.043	0.015
	179	0.045	0.024	0.000	0.020
	181	0.000	0.000	0.000	0.000
	183	0.000	0.000	0.000	0.000
<i>N</i>		111	185	117	204
<i>P</i>		0.166		0.002	
<i>Ssa85</i>	126	0.018	0.037	0.000	0.005
	130	0.000	0.000	0.004	0.000
	134	0.694	0.724	0.470	0.478
	136	0.063	0.034	0.000	0.017
	138	0.225	0.204	0.526	0.500
<i>N</i>		111	174	117	204
<i>P</i>		0.218		0.068	
<i>H_o^a</i>		0.634	0.624	0.584	0.569
<i>H_e^b</i>		0.608	0.618	0.538	0.561
<i>A^c</i>		6.0	4.6	3.7	4.5
<i>F^d</i>		-0.031	0.062	-0.086	-0.014
<i>r_{xy}^e</i>		0.001	-0.001	-0.012	0.007
<i>S^f</i>		0.043	0.054	0.064	0.052
<i>U^g</i>		<i>P</i> = 0.835		<i>P</i> = 0.005	

^a Observed heterozygosity.

^b Hardy–Weinberg expected heterozygosity (Nei 1978).

^c Allelic richness (average number of alleles, standardized for sample size).

^d Wright's (1951) inbreeding coefficient.

^e Average coefficient of relatedness (Queller and Goodnight 1989).

^f Proportion of coefficients of relatedness at the full-sibling level (*P* < 0.05).

^g Mann–Whitney *U*-test for significance of difference in distributions of coefficients of relatedness (*r_{xy}*) between adults and offspring.

TABLE 2.—Extended.

Locus and statistic	Iron River NFH					
	SAW		GLW		SIW	
	Adult	Juv.	Adult	Juv.	Adult	Juv.
<i>Sfo18</i>	0.000	0.000	0.000	0.000	0.010	0.010
	0.562	0.527	0.465	0.454	0.510	0.515
	0.008	0.005	0.025	0.023	0.019	0.000
	0.044	0.047	0.005	0.005	0.055	0.046
	0.000	0.002	0.000	0.000	0.000	0.000
	0.228	0.252	0.449	0.394	0.271	0.270
	0.062	0.020	0.000	0.000	0.010	0.015
	0.003	0.012	0.000	0.009	0.039	0.026
	0.083	0.101	0.040	0.116	0.081	0.110
	0.008	0.020	0.015	0.000	0.006	0.008
	0.003	0.012	0.000	0.000	0.000	0.000
<i>N</i>	193	204	99	108	155	196
<i>P</i>		0.025		0.020		0.225
<i>Scop.19</i>	0.000	0.000	0.000	0.000	0.000	0.000
	0.000	0.000	0.000	0.000	0.015	0.022
	0.174	0.181	0.103	0.049	0.039	0.067
	0.000	0.000	0.000	0.000	0.003	0.010
	0.013	0.012	0.000	0.000	0.018	0.030
	0.000	0.000	0.005	0.000	0.000	0.000
	0.005	0.002	0.029	0.013	0.000	0.000
	0.265	0.234	0.279	0.398	0.352	0.413
	0.020	0.019	0.010	0.000	0.048	0.035
	0.465	0.428	0.363	0.376	0.473	0.393
	0.040	0.077	0.108	0.022	0.018	0.002
	0.020	0.043	0.103	0.142	0.027	0.027
	0.000	0.000	0.000	0.000	0.006	0.000
	0.003	0.002	0.000	0.000	0.000	0.000
<i>N</i>	198	207	102	113	166	201
<i>P</i>		0.185		0.000		0.038
<i>Ssa85</i>	0.049	0.032	0.005	0.037	0.090	0.096
	0.003	0.000	0.000	0.000	0.000	0.000
	0.657	0.687	0.505	0.606	0.500	0.470
	0.098	0.095	0.040	0.037	0.139	0.150
	0.193	0.187	0.450	0.321	0.271	0.284
<i>N</i>	194	206	100	109	166	197
<i>P</i>		0.599		0.009		0.891
H_o^a	0.598	0.539	0.697	0.614	0.640	0.685
H_e^b	0.580	0.575	0.629	0.613	0.652	0.662
A^c	6.3	6.6	5.0	4.6	6.7	5.9
F^d	-0.043	-0.011	-0.109	-0.001	0.020	-0.036
r_{xy}^e	-0.002	-0.005	0.006	-0.008	-0.001	0.000
S^f	0.046	0.028	0.050	0.042	0.063	0.051
U^g		$P = 0.624$		$P = 0.060$		$P = 0.520$

that were spawned for the first time in 1998. Egg quality was low, and all eggs produced from females of the GLW 1993 year-class were eventually discarded to prevent overrepresentation of early-maturing females within the juveniles produced for this broodstock.

For most broodstocks, the adults used in the production of excised egg lots were typically from

adults spawned at the beginning or end of the spawning period. Eggs from females spawned early in the spawning period were not uniformly mature, and eggs from females spawned late in the spawning period often exhibited low viability. Reductions in broodstock effective population sizes related to the excision of egg lots (typically for early- and late-spawning females) can be accom-

TABLE 3.—Estimates of percent reductions in the total number of hatchery adults available for spawning as a result of the excision of egg lots, and estimates of the effective numbers of breeding adults (N_b) for spawners sampled for each of six hatchery broodstocks of lake trout during a genetic evaluation of the Great Lakes lake trout hatchery program. “Number spawned” is the number of fish spawned after the removal of adults before mating that were identified as possessing nonviable gametes (i.e., green or overly ripe eggs). “Potential contributors” is the number of adults spawned for each broodstock that potentially contributed to the juvenile population after the excising of fertilized eggs. The ratio N_b/N is a measure of the spawning efficiency, or the average N_b divided by the total number of adults in the spawner lot. The numbers in parentheses are the incremental percent reductions in the number of adults that can contribute to the juvenile population. The abbreviation LLW stands for the Lewis Lake broodstock; see Table 2 for an explanation of the other abbreviations.

Broodstock	Reduction in N_b based on hatchery records			Reduction in N_b based on genetic analysis							
	Available spawners	Number spawned	Potential contributors	Spawner lot (N)	Loci ^a			Avg. N_b ^b	95% CI ^c	N_b/N	Total contributors ^d
					<i>Sfo18</i>	<i>Scop19</i>	<i>Ssa85</i>				
SAW	918	918	898 (2)	436	75	133	583	115	45, 322	0.26	233
GLW	582	582	346 (41)	224	34	17	14	20	8, 41	0.09	31
SIW	778	692 (11)	670 (3)	384	156	58	—	105	40, 307	0.27	181
SLW	848	793 (6)	793	450	79	33	77	48	18, 124	0.11	87
SMD	271	245 (10)	245	112	41	51	45	46	24, 70	0.41	100
LLW	426	375 (12)	276 (26)								

^a Effective number of breeders (N_b) was calculated by means of equations (1) and (2) for each locus.
^b Mean of N_b for each broodstock was calculated with a mean variance in allele frequency between adults and juveniles across all loci for each broodstock and weighted by number of alleles.
^c Ninety-five percent confidence intervals for estimates of N_b (Waples 1989).
^d Calculated by applying efficiency ratio (N_b/N) to numbers of potential contributors; it represents an overall estimate of the effective number of breeders for the entire year for each broodstock.

panied by a loss of additive genetic variation for an important life history trait (timing of spawning).

Hatchery spawning practices that increase adult reproductive variance, such as reusing males, pooling gametes, and allowing unequal sex ratios,

will lower effective population size. 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 eggs for a number of hatchery-cultured species (Gharrett and Shirley

TABLE 4.—Estimates of the number of adult lake trout that contributed eggs or juveniles to various hatchery facilities and stocking programs for each hatchery broodstock spawned in 1998 (SMD, 1999) during a genetic evaluation of the Great Lakes lake trout hatchery program. Estimates were derived from hatchery records of the numbers of adults spawned and eggs or progeny distributed. “Potential contributions” are adults that potentially contributed eggs to hatchery facilities and programs. Adults whose eggs were eliminated before (poor quality eggs) or after (low viability and disease) spawning were not considered. The numbers in parentheses are the proportions of the total number of potential contributors that contributed progeny to various hatchery facilities or stocking programs. Because facilities and programs may receive eggs from the same spawning events, the same adults may contribute eggs to multiple facilities and programs. Therefore, the number of adults contributing eggs across all facilities and programs may not equal the total number of adults spawned for each broodstock and, consequently, proportions may not sum to 1. See Tables 2 and 3 for an explanation of the abbreviations.

Broodstock	Potential contributors	Hatchery facilities receiving eggs					Programs	
		Allegheny	Bayfield	Iron River	Jordan River	Retained ^a	Fry plant ^b	Astroturf ^c
SAW	898		268 (0.30)		240 (0.27)	436 (0.49)	96 (0.11)	
GLW	346					346 (1.00)		
SIW	670					670 (1.00)		
SLW	793	16 (0.02)		403 (0.50)	785 (0.98)	403 (0.50)		
SMD	245	148 (0.60)		78 (0.32)	104 (0.42)			
LLW	276							276 (1.00)

^a Number of potential contributors whose eggs were not distributed but retained within the production hatchery.
^b Hatching of eggs delayed in order to stock fry at various times of the year.
^c Eggs planted on spawning reefs in astroturf bundles.

1985; Withler 1988; Mueller 1995; Perez-Enriquez et al. 1999). Brown et al. (2000) found that reproductive variance associated with hatchery spawning methods (i.e., pooling of gametes) of American shad *Alosa sapidissima* could have reduced effective population sizes by 88%, even though large numbers of adult shad ($N = 1,400$) were spawned. Further, high reproductive variance has been documented in salmonid production involving sequential addition or pooling of male gametes during spawning (Gharrett and Shirley 1985; Withler 1988).

Hatchery spawning methods for Great Lakes lake trout in 1998 and 1999 were similar to techniques previously reported to result in high variances of reproductive success for other salmonid species (e.g., Pacific salmon *Oncorhynchus* spp.; Withler 1988). Low mean estimates of effective numbers of breeders (N_b) and spawning efficiencies ($N_b/N = 9\text{--}41\%$) for lake trout hatchery broodstocks (Table 3) are probably related to spawning methods. At both Pendill's Creek NFH (LLW, SLW, and SMD broodstocks) and Iron River NFH (GLW, SAW, and SIW broodstocks), gametes of both males and females are pooled during spawning. Lake trout at Pendill's Creek NFH were spawned with the combined milt and eggs of 5 males and 5 females. At Iron River NFH, two sets of pooled gametes that each consisted of milt from 5 different males were used to fertilize two sets of pooled gametes that each consisted of eggs from 5 different females. One-half the volume of each male gamete pool (added sequentially several minutes apart) was used to fertilize each female gamete pool. Therefore, the milt of 10 males was combined with the eggs from 5 females, resulting in a 10:5 male to female mixture of gametes. Reciprocal crossing was performed to avoid the loss of production as a result of nonviable milt. Gharrett and Shirley (1985) showed that, during sequential spawning males spawned later in a spawning sequence will disproportionately contribute to offspring, even when other males are viable. Therefore, it is conceivable that the use of gametes from multiple males could result in only a few males dominating the fertilization of eggs within a given spawning event.

Because of unequal adult male: female ratios at Pendill's Creek NFH, males were often used multiple times. Within the LLW, SLW, and SMD broodstocks, 77%, 56%, and 15% of males were reused, respectively, increasing the potential for greater reproductive variance among broodstock adults. In addition, unequal survival of eggs and

juveniles among spawning lots within a broodstock may also contribute to high reproductive variance. Survival rates of fertilized eggs produced from adults sampled from two SLW spawning lots analyzed in this study differed appreciably (32% and 79%). However, unequal contributions of adults to progeny and, consequently, low effective population sizes for hatchery populations are more often attributed to spawning methods (e.g., Allendorf and Ryman 1987; Simon 1991; Hedgecock et al. 1992; Busack and Currens 1995).

High reproductive variance not only contributes to low effective population sizes but also promotes greater levels of relatedness among progeny. Significant differences in distributions of coefficients of relatedness between adults and juveniles for the SLW broodstock were documented and may be related to the fact that the unequal sex ratio within this broodstock required a large proportion of males to be spawned multiple times during the spawning season. Broodstocks with unequal male: female ratios are common within salmonid hatchery systems. Kincaid (1995) surveyed 221 salmonid broodstocks in the United States and found that 48% were characterized by unequal sex ratios.

With the exception of the GLW broodstock ($N_b = 31$), estimates of effective breeding population sizes were sufficiently high to prevent large-scale changes in genetic diversity over a single generation. However, spawning practices that promote high N_b/N ratios will minimize the likelihood for changes in genetic characteristics between adults and juveniles. Genetic drift can exacerbate small differences in allele frequencies over time. Low estimates of effective numbers of breeding adults indicate low spawning efficiencies. Adoption of spawning practices that increase the number of contributing adults (e.g., 1:1 matings) and minimize the reuse of males is warranted.

For stages 1 and 2 of the lake trout broodstock hatchery program, estimates of heterozygosity and allelic richness were higher (though not significantly) for a number of broodstocks and broodstock progeny than was estimated for populations of wild progenitors. This should not be construed as an "improvement" in the genetic character of broodstocks or broodstock progeny over populations of wild progenitors. Differences in estimates of allelic richness are probably a function of differences in sampling, both in terms of the number of samples assayed genetically and sampling variance (genetic drift). For example, the sample size for populations of wild progenitors was comparatively smaller (33–75) than sample sizes for

TABLE 5.—Estimates of the relative contribution of adult lake trout spawned on each date to total egg production during a genetic evaluation of the Great Lakes lake trout hatchery program. Data are presented for the Seneca Lake (SLW), Marquette (SMD), and Lewis Lake (LLW) broodstocks from the 1998 (SMD, 1999) spawning period. Total eggs received is the total number of eggs distributed to each hatchery facility or stocking program.

Strain	Spawning date	Adults spawned ^a	Proportion of total spawned ^b	Eggs distributed ^c
SLW	30 Sep	52 (7)	0.06	64,960
		272 (38)	0.30	207,120
	6–7 Oct	76 (11)	0.08	212,480
		88 (12)	0.10	201,789
	14–15 Oct	403 (56)	0.44	1,503,360
27 Oct	16 (2)	0.02	20,650	
Total eggs received				
SMD	7 Oct	78 (32)	0.30	151,250
	15 Oct	32 (13)	0.12	105,105
		78 (32)	0.30	162,615
	20 Oct	38 (16)	0.15	52,800
		34 (14)	0.13	24,371
Total eggs received				
LLW	22 Sep	64	0.17	145,464
		132	0.34	368,550
	30 Sep	60	0.16	68,277
	7 Oct	42	0.11	55,630
		84	0.22	67,398
Total eggs received				

^a Number of adults spawned is greater than the number of potential contributors (Table 3) because males within a given spawning group are treated as unique even if they have been utilized in previous spawning groups. Males reused within the same spawning group were only counted once. This allows for a measure of the number of unique adults, representing specific spawning dates and groups, that contributed eggs to a given hatchery facility. Numbers in parentheses represent the effective number of breeding adults (N_b) contributing to eggs distributed from each spawning group on each spawning date. Estimates of effective numbers of breeding adults for each spawning group on each date were calculated by applying the broodstock-specific ratio of the total number of breeders to the effective number of contributing adults (N_b/N ; Table 3).

^b Proportion of all broodstock adults spawned on each spawning date. Gametes from one or two adult spawning groups on each date (e.g., two groups for SLW on 30 September) were maintained separately.

^c Number of eggs produced from each spawning lot that were distributed to hatchery facilities or stocking programs.

hatchery broodstocks (50–198). Variance in estimates of genetic diversity between cohorts reflects conditions unique to each spawning event.

Stage 3: Collection and Distribution of Eggs and Juveniles

Eggs and juveniles are commonly distributed from production to rearing facilities. This practice has been implicated in the loss of genetic diversity in other salmonid broodstocks (Waples 1991). Analysis of lake trout hatchery distribution records revealed that disproportional allocations of gametes or progeny may lower effective breeding population sizes of adults contributing to juveniles stocked (Tables 4 and 5). Eggs distributed to several rearing facilities and to stocking programs throughout the Great Lakes were frequently not representative of the entire broodstock spawned. Facilities often received eggs representing limited

portions (2–32%) of the total number of adults spawned (Tables 4 and 5).

When estimates of spawning efficiency (N_b/N) for each broodstock were applied to the numbers of adults spawned on each spawning date, the effective numbers of breeders associated with juveniles was often extremely low. In the most extreme case, juveniles from the SLW broodstock designated for distribution to the Allegheny NFH were all collected from 16 individuals (Table 4) spawned at the end of the spawning season (Table 5), which represented 2% of the potential number of contributing adults. When the estimate of spawning efficiency was applied for the SLW broodstock ($N_b/N = 0.11$; Table 3), the effective number of breeders was estimated to be only 2. The majority of egg or juvenile distributions were not as extreme (Tables 4 and 5). Eggs collected from the SLW, SMD, and LLW broodstocks and

TABLE 5.—Extended.

Strain	Proportion of all eggs received by a given hatchery representing specific spawning dates for each broodstock				
	Allegheny	Iron River	Jordan River	Pendill's Creek	Astroturf program
SLW			0.06 0.20 0.20 0.19 0.35	1.00	
	1.00				
Total eggs received	20,650	241,920	1,053,709	366,000	0
SMD		1.00			
	0.49 0.26 0.25		0.82 0.18		
Total eggs received	212,625	151,250	132,266	0	0
LLW					0.21 0.52 0.10 0.08 0.10
Total eggs received	0	0	0	0	705,319

then distributed to Jordan River NFH, Allegheny NFH, and to survival and imprinting enhancement programs (e.g., astroturf program), respectively, were examples of more equitable apportionments of eggs. It should also be noted that eggs developed from specific hatchery broodstocks and sent to separate rearing facilities are often recombined as juveniles before stocking or may be integrated into eggs developed from another broodstock of the same hatchery strain. Eggs from the SLW broodstock that were distributed to the Allegheny NFH were subsequently combined with a large number of SLW broodstock eggs developed within the Allegheny NFH. The recombining or integration of progeny before stocking may help diminish the effects of nonrepresentative egg collection and distribution; however, the recombination or integration of progeny before stocking is typically arbitrary and dependent on stocking demands. Therefore, measures that would increase the likelihood of a proportional representation of all adults to eggs and juveniles distributed to rearing stations and stocking locations should be considered.

The examples highlighted emphasize the need to reconcile constraints relative to juvenile production, housing, distribution, and release with the desire to maximize the diversity of progeny

stocked. The cases are not reflective of the entire program; rather, the specific cases cited represent important examples of serious events that can be easily changed. Other events are beyond the control of hatchery managers. Requests for progeny derived from a specific broodstock may exceed production. In other instances, egg lots distributed to separate facilities may eventually be recombined during stocking or during additional distribution phases.

Summary

Lake trout restoration across the Great Lakes region relies heavily on hatchery production, especially for U.S. waters of Lakes Michigan and Huron, where wild lake trout populations have been extirpated. Low survival of stocked juveniles (Bronte et al. 2003) dictates that emphasis should be placed on maximizing juvenile production. However, if small changes are adopted in key areas, production goals can be met while simultaneously maximizing the likelihood of retaining high levels of genetic diversity in offspring. Through the use of molecular genetic markers and measures of genetic diversity, we found no pervasive directional changes of genetic diversity across all stages of the lake trout hatchery pro-

gram; however, at each stage we documented examples where changes have occurred in some but not all broodstocks.

Changes in the genetic diversity and allele frequencies of lake trout of each generation probably reflect spawning events each year. Given the species' potential longevity and the fact that stocking in many Great Lakes localities occurs annually, low diversity or high levels of relatedness of offspring from a single year's production can be countered if stocking occurs over multiple years. However, several aspects of lake trout ecology in current lake environments suggest that levels of diversity in each year-class should be maximized. First, lake trout typically show high fidelity to local areas (Hansen et al. 1995). This is true even for juvenile lake trout of hatchery origin (Bronte et al. 2002). High levels of site fidelity suggest that juveniles stocked on specific spawning reefs have a higher probability of returning at sexual maturity than spawning elsewhere. Second, high levels of predation by sea lampreys and humans greatly limit the number of adults that return to spawn each year. Natural recruitment is further constrained by the young age structure of spawning adults (Madenjian et al. 2004). The age distribution of spawning adults is quite homogeneous as a result of high levels of mortality. Third, Page et al. (2003) found that levels of relatedness in naturally produced progeny were negatively correlated with the abundance of spawning adults. Low survivorship, young and homogeneous age structure of spawning adults, and high fidelity suggest that low diversity and high levels of relatedness among juveniles stocked may lead to consanguineous matings and inbreeding in progeny during natural spawning.

The most significant findings of this study were reductions in the effective breeding population sizes of adults contributing to juveniles used for stocking. Excision of eggs because of poor quality or disease is unavoidable during hatchery operations. Temporary or infrequent reductions in effective breeding population sizes will probably occur throughout the history of a broodstock in any hatchery system and often result from unforeseen or uncontrollable events. However, spawning methods and activities related to egg collection and distribution can be readily changed.

Numerous factors interact to limit the success of lake trout restoration programs. This thorough review of the Great Lakes lake trout hatchery program shows that levels of genetic diversity of juveniles used in restoration need not be a confound-

ing factor that impacts the success of restoration efforts.

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References

- Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. *Transactions of the American Fisheries Society* 109: 537-543.
- Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery stocks. Pages 141-159 *in* N. Ryman and F. Utter, editors. *Population genetics and fishery management*. University of Washington Press, Seattle.
- Anders, P. J. 1998. Conservation aquaculture and endangered species: can objective science prevail over risk anxiety? *Fisheries* 23(11):28-31.
- Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. *Journal of Fish Biology* 47(Supplement A):177-185.
- Blouin, M. S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology* 5:393-401.
- Bronte, C. R., J. Jonas, M. E. Holey, R. L. Eshenroder, M. L. Toney, P. McKee, B. Breidert, R. M. Claramunt, M. P. Ebener, C. C. Krueger, G. Wright, and R. Hess. 2003. Possible impediments to lake trout restoration in Lake Michigan. *Great Lakes Fishery*

- Commission, Ann Arbor, Michigan. Available; www.glf.org-lakecom-lmc-ltrestore. (October 2004).
- Bronte, C. R., S. T. Schram, J. H. Selgeby, and B. L. Swanson. 2002. Reestablishing a spawning population of lake trout in Lake Superior with fertilized eggs in artificial turf incubators. *North American Journal of Fisheries Management* 22:796–805.
- Brown, B. L., T. P. Gunter, J. M. Waters, and J. M. Epifanio. 2000. Evaluating genetic diversity associated with propagation-assisted restoration of American shad. *Conservation Biology* 14(1):294–303.
- Busack, C. A., and K. P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. Pages 71–80 in H. L. Schramm, Jr., and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- Carmichael, G. J., J. N. Hanson, J. R. Novy, K. J. Meyer, and D. C. Morizot. 1995. Apache trout management: cultured fish, genetics, habitat improvements, and regulations. Pages 112–126 in H. L. Schramm, Jr., and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- Coberly, C. E., and R. M. Horrall. 1982. A strategy for reestablishing self-sustaining lake trout stocks in Illinois waters of Lake Michigan. Institute for Environmental Studies, Marine Studies Center, University of Wisconsin–Madison, Report 42, Madison.
- Cross, T. F., and J. King. 1983. Genetic effects of hatchery rearing in Atlantic salmon. *Aquaculture* 33:33–40.
- Dodson, J. J., R. J. Gibson, R. A. Cunjak, K. D. Friedland, C. G. De Leaniz, M. R. Gross, R. Newbury, J. L. Nielsen, M. E. Power, and S. Roy. 1998. Elements in the development of conservation plans for Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 55(Supplement 1):312–323.
- Duchesne, P., and L. Bernatchez. 2002. Investigating the dynamics of inbreeding in multigeneration supportive breeding using a recurrence equations system generator. *Conservation Genetics* 3:47–60.
- Eshenroder, R. L., N. R. Payne, J. E. Johnson, C. Bowen II, and M. P. Ebener. 1995. Lake trout rehabilitation in Lake Huron. *Journal of Great Lakes Research* 21(Supplement 1):108–127.
- Eshmeyer, P. H. 1957. Contributions to the study of subpopulations of fishes. U.S. Fish and Wildlife Service Special Scientific Report on Fisheries 208.
- Fetterolf, C. M. 1980. Why a Great Lakes Fishery Commission and why a Sea Lamprey International Symposium? *Canadian Journal of Fisheries and Aquatic Sciences* 37:1588–1593.
- Gharrett, A. J., and S. M. Shirley. 1985. A genetic examination of spawning methodology in a salmon hatchery. *Aquaculture* 47:245–256.
- Guinand, B., K. T. Scribner, K. S. Page, and M. K. Burnham-Curtis. 2003. Genetic variation over space and time: analysis of extinct and remnant lake trout populations in the upper Great Lakes. *Proceedings of the Royal Society of London* 270:425–433.
- Hansen, M. J., J. W. Peck, K. G. Schorfhaar, J. H. Selgeby, D. R. Schreiner, S. T. Schram, B. L. Swanson, W. R. MacCallum, M. K. Burnham-Curtis, G. L. Curtis, J. W. Heinrich, and R. J. Young. 1995. Lake trout (*Salvelinus namaycush*) populations in Lake Superior and their restoration. *Journal of Great Lakes Research* 21(Supplement 1):152–175.
- Hedgecock, D., V. Chow, and R. S. Waples. 1992. Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. *Aquaculture* 108:215–232.
- Holey, M. E. 1997. Broodstock management plan for wild lake trout and brook trout in the Great Lakes. U.S. Fish and Wildlife Service, Region 3, Fort Snelling, Minnesota.
- Holey, M. E., R. W. Rybicki, G. W. Eck, E. H. Brown, Jr., J. E. Marsden, D. S. Lavis, M. L. Toney, T. N. Trudeau, and R. M. Horrall. 1995. Progress toward lake trout restoration in Lake Michigan. *Journal of Great Lakes Research* 21(Supplement 1):128–151.
- Hynes, J. D., E. H. Brown Jr., J. H. Helle, N. Ryman, and D. A. Webster. 1981. Guidelines for the culture of fish stocks for resource management. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1867–1876.
- Kerby, J. H., and R. M. Harrell. 1990. Hybridization, genetic manipulation, and gene pool conservation of striped bass. Pages 159–190 in R. M. Harrell, J. H. Kerby, and R. V. Minton, editors. *Culture of striped bass and its hybrids*. American Fisheries Society, Southern Division, Striped Bass Committee, Bethesda, Maryland.
- Kincaid, H. L. 1983. Inbreeding in fish populations used for aquaculture. *Aquaculture* 33:215–227.
- Kincaid, H. L. 1995. An evaluation of inbreeding and effective population size in salmonid broodstocks in federal and state hatcheries. Pages 193–204 in H. L. Schramm, Jr., and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- Kincaid, H. L., C. C. Krueger, and B. May. 1993. Preservation of genetic variation in the Green Lake strain lake trout derived from remnant domestic and feral populations. *North American Journal of Fisheries Management* 13:318–325.
- Krueger, C. C., A. J. Gharrett, T. R. Dehring, and F. W. Allendorf. 1981. Genetic aspects of fisheries rehabilitation programs. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1877–1881.
- Krueger, C. C., R. M. Horrall, and H. Gruenthal. 1983. Strategy for the use of lake trout strains in Lake Michigan. Report of the genetics subcommittee to the Lake Trout Technical Committee for Lake Michigan of the Great Lakes Fishery Commission, Ann Arbor, Michigan.
- Krueger, C. C., and P. E. Ihssen. 1995. Review of genetics of lake trout in the Great Lakes: history, mo-

- lecular genetics, physiology, strain comparisons, and restoration management. *Journal of Great Lakes Research* 21(Supplement 1):348–363.
- Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic differentiation among lake trout strains stocked into Lake Ontario. *Transactions of the American Fisheries Society* 118:317–330.
- Krueger, C. C., M. J. Jones, and W. W. Taylor. 1995. Restoration of lake trout in the Great Lakes: challenges and strategies for future management. *Journal of Great Lakes Research* 21(Supplement 1):547–558.
- Lande, R., and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pages 87–123 in M. E. Soulé, editor. *Viable populations for conservation*. Cambridge University Press, New York.
- Lange, R. E., G. C. LeTendre, T. H. Echert, and C. P. Schneider. 1995. Enhancement of sportfishing in New York waters of Lake Ontario with hatchery-reared salmonines. Pages 7–11 in H. L. Schramm, Jr., and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- Lassuy, D. R. 1995. Introduced species as a factor in extinction and endangerment of native fish species. Pages 391–396 in H. L. Schramm, Jr., and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- Lynch, M., and M. O’Hely. 2001. Captive breeding and the genetic fitness of natural populations. *Conservation Genetics* 2:363–378.
- Madenjian, C. P., T. J. Desorcie, J. R. McClain, and A. P. Woltdt. 2004. Status of lake trout rehabilitation on Six Fathom Bank and Yankee Reef in Lake Huron. *North American Journal of Fisheries Management* 24:1003–1016.
- Meffe, G. K. 1995. Genetic and ecological guidelines for species reintroduction programs: application to Great Lakes fishes. *Journal of Great Lakes Research* 21(Supplement 1):3–9.
- Mueller, G. 1995. A program for maintaining the razorback sucker in Lake Mohave. Pages 127–135 in H. L. Schramm, Jr., and R. G. Piper, editors. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society, Symposium 15, Bethesda, Maryland.
- National Research Council. 1996. *Upstream: salmon and society in the Pacific Northwest*. National Academy Press, Washington, D.C.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 23:341–369.
- Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. *Molecular Ecology* 7:1087–1089.
- O’Reilly, P. T., L. C. Hamilton, S. K. McConnell, and J. W. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* 53:2292–2298.
- Page, K. S., K. T. Scribner, K. R. Bennett, L. M. Garzel, and M. K. Burnham-Curtis. 2003. Genetic assessment of strain-specific sources of lake trout recruitment in the Great Lakes. *Transactions of the American Fisheries Society* 132:877–894.
- Page, K. S., K. T. Scribner, and M. K. Burnham-Curtis. 2004. Genetic diversity of wild and hatchery lake trout populations: relevance for management and restoration in the Great Lakes. *Transactions of the American Fisheries Society* 133:674–691.
- Perez-Enriquez, R., M. Takagi, and N. Taniguchi. 1999. Genetic variability and pedigree tracing of hatchery-reared stock of red sea bream (*Paagrus major*) used for stock enhancement, based on microsatellite DNA markers. *Aquaculture* 173:413–423.
- Petit, R. J., A. E. Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* 12(4):844–855.
- Philippart, J. C. 1995. Is captive breeding an effective solution for the preservation of endemic species? *Biological Conservation* 72:281–295.
- Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- Rahrer, J. F. 1965. Age, growth, maturity, and fecundity of “humper” lake trout, Isle Royale, Lake Superior. *Transactions of the American Fisheries Society* 94:75–83.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Ryman, N. 1991. Conservation genetics considerations in fishery management. *Journal of Fish Biology* 39(Supplement A):211–224.
- Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. *Conservation Biology* 5(3):325–329.
- Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. *Canadian Journal of Fisheries and Aquatic Sciences* 53:685–693.
- Secor, D. H., J. M. Dean, T. A. Curtis, and F. W. Sessions. 1992. Effect of female size and propagation methods on larval production at a South Carolina striped bass (*Morone saxatilis*) hatchery. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1778–1787.
- Selgeby, J. H. 1995. Introduction to the proceedings of the 1994 international conference on restoration of lake trout in the Laurentian Great Lakes. *Journal of Great Lakes Research* 21(Supplement 1):1–2.
- Simon, R. C. 1991. Management techniques to minimize the loss of genetic variability in hatchery fish populations. Pages 487–494 in J. Colt and R. J. White, editors. *Fisheries bioengineering symposium*. American Fisheries Society, Symposium 10, Bethesda, Maryland.

- Swanson, B. L., and D. V. Swedberg. 1980. Decline and recovery of the Lake Superior Gull Island Reef lake trout (*Salvelinus namaycush*) population and the role of sea lamprey (*Petromyzon marinus*) predation. Canadian Journal of Fisheries and Aquatic Sciences 37:2074–2080.
- Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281–283.
- Taylor, E. B., Z. Redenbach, A. B. Costello, S. M. Pollard, and C. J. Pacas. 2001. Nested analysis of genetic diversity in northwestern North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*). Canadian Journal of Fisheries and Aquatic Sciences 58(2):406–420.
- Utter, F. 1991. Biochemical genetics and fishery management: an historical perspective. Journal of Fish Biology 39(Supplement A):1–20.
- Visscher, L. 1983. Lewis Lake lake trout. U.S. Fish and Wildlife Service, Denver.
- Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121:379–391.
- Waples, R. S. 1991. Genetic interactions between hatchery and wild populations: lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences 48(Supplement 1):124–133.
- Waples, R. S., G. A. Winans, F. M. Utter, and C. Mahnken. 1990. Genetic approaches to the management of Pacific salmon. Fisheries 15(5):19–25.
- Withler, R. E. 1988. Genetic consequences of fertilizing Chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture 68:15–25.
- Wright, S. 1951. The genetical structure of populations. Annals of Eugenics 15:323–354.
- Yeager, D. M., J. E. Van Tassel, and C. M. Wooley. 1990. Collection, transportation, and handling of striped bass brood stock. Pages 29–42 in R. M. Harrel, J. H. Kerby, and R. V. Minton, editors. Culture and propagation of striped bass and its hybrids. American Fisheries Society, Southern Division, Striped Bass Committee, Bethesda, Maryland.