

# Stocking success of local-origin fry and impact of hatchery ancestry: monitoring a new steelhead (*Oncorhynchus mykiss*) stocking program in a Minnesota tributary to Lake Superior

David C. Caroffino, Loren M. Miller, Anne R. Kapuscinski, and Joseph J. Ostazeski

**Abstract:** The inception of a hatchery program to rebuild a naturalized steelhead (*Oncorhynchus mykiss*) population in Minnesota waters of Lake Superior gave us the rare opportunity to monitor the success of fry stocking and determine if hatchery ancestry can reduce fitness of stocked fish in the early generations of a stocking program. Through genetic monitoring of two year classes, we determined that hatchery adults produced 1.3–6.2 times as many age-2 juveniles per female than naturally spawning fish. Survival of stocked fry of parents born in a hatchery relative to those of parents born in the wild was 70% in paired-stocking comparisons. These results suggest that stocking local-origin fry can increase the short-term abundance of depleted populations and that fish with no hatchery history are a better source for supplemental stocking. Additionally, sampling small numbers of adults for broodstock created genetically distinct groups, which could potentially cause long-term genetic change in the population. Genetic monitoring of adults will be essential to determining whether differences observed persist through the life cycle of the stocked fish.

**Résumé :** La mise en marche d'un programme de pisciculture pour restaurer une population naturalisée de truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) dans les eaux du lac Supérieur au Minnesota nous a fourni une occasion unique de suivre le succès de l'alevinage et de déterminer si l'ascendance en pisciculture peut réduire la fitness des poissons ensemencés durant les premières générations d'un programme d'empoissonnement. Lors d'un suivi génétique de deux classes d'âge, nous avons déterminé que les adultes de pisciculture produisent 1,3 à 6,2 fois le nombre de jeunes d'âge 2 par femelle que les poissons qui fraient de manière naturelle. Dans des comparaisons appariées d'empoissonnement, la survie des alevins ensemencés dont les parents sont nés en pisciculture représente 70 % de celle des alevins dont les parents sont nés en nature. Ces résultats indiquent que des empoissonnements avec des alevins d'origine locale peuvent augmenter à court terme l'abondance des populations épuisées et que les poissons sans antécédent en pisciculture sont une meilleure source pour les empoissonnements complémentaires. De plus, le prélèvement de petits nombres d'adultes pour former les stocks reproducteurs crée des groupes génétiquement distincts qui peuvent potentiellement causer des changements génétiques à long terme dans la population. Il est donc nécessaire de faire un suivi génétique des adultes afin de déterminer si les différences observées persistent tout au long du cycle biologique des poissons ensemencés.

[Traduit par la Rédaction]

## Introduction

Salmonid hatcheries have been used in various roles since the mid-1800s (Schramm and Piper 1995). Hatchery fish have been stocked in many locations and at various life stages, all in the hope of increasing local populations (Steward and Bjornn 1990). Monitoring of such stockings is imperative to determining their success or failure and the effects

they have on natural populations (Winton and Hilborn 1994; Miller and Kapuscinski 2003; Brannon et al. 2004).

Many studies have evaluated the fitness of hatchery fish relative to native stocks, and inferior performance is a common result. Hatchery fish of a nonlocal origin often experience reduced survival and do not significantly contribute to natural populations (e.g., Chilcote et al. 1986; Kostow et al. 2003; McLean et al. 2003). These introduced fish lack al-

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**D.C. Caroffino,<sup>1</sup> L.M. Miller,<sup>2</sup> and A.R. Kapuscinski.** Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, 1980 Folwell Avenue, 200 Hodson Hall, St. Paul, MN 55108, USA.

**J.J. Ostazeski.** Minnesota Department of Natural Resources, 5351 North Shore Drive, Duluth, MN 55804, USA.

<sup>1</sup>Present address: School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 905 Koyukuk Drive, 245 O'Neill Building, Fairbanks, AK 99775, USA.

<sup>2</sup>Corresponding author (e-mail: [Imm@umn.edu](mailto:Imm@umn.edu)).

les adapted to the local environment, a likely major reason for their reduced survival. In addition to poor performance throughout their life cycle, hatchery fish of a different genetic origin may threaten native stocks through outbreeding depression (Miller and Kapuscinski 2003; Miller et al. 2004).

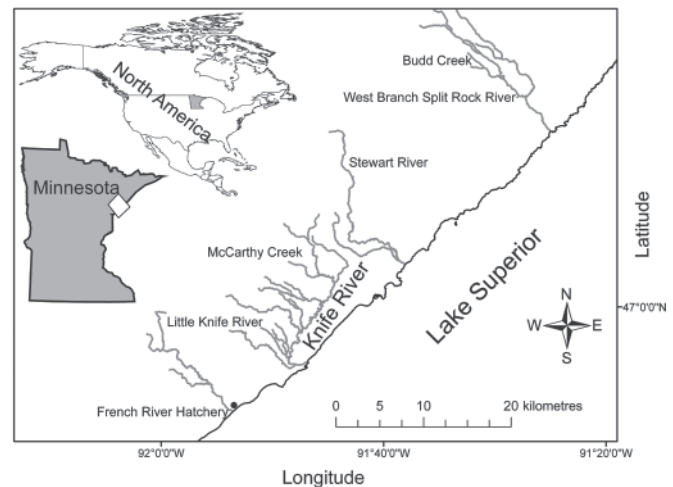
Fewer studies have evaluated supplemental stocking when a local-origin broodstock is used. Most of the research of this type has focused on a local broodstock that had been propagated in a hatchery for multiple generations (Reisenbichler and McIntyre 1977; Fleming et al. 1997; Dannewitz et al. 2003). Hatchery fish of a common genetic origin were more successful than nonlocal broodstocks, but still often experienced reduced survival compared with that of wild fish.

Domestication selection may explain why even local broodstocks with hatchery ancestry exhibit poor survival in the wild. Domestication refers to any change in the selection regime of a cultured population relative to that experienced by the natural population (Waples 1999). Bringing fish into an artificial environment for a part of their life exposes them to different selective pressures than would be encountered in the wild in terms of both survival and mate selection (Busack and Currens 1995). In one less-considered component of domestication selection, the hatchery environment relaxes the natural process of selection and allows some individuals to survive that otherwise would not have (Waples 1999). The offspring of hatchery fish, therefore, may experience poor survival in the wild due to inheritance of traits that are maladapted to the natural environment.

It is unclear how much hatchery ancestry is required to reduce survival of locally adapted fish. Berejikian and Ford (2004) reviewed the current literature and found only one (unpublished) study (Blouin 2003) that examined a first-generation hatchery stock. That work, later published in Araki et al. (2007a) and extended in Araki et al. (2007b), found that although supplementation was initially considered successful, locally derived hatchery steelhead (*Oncorhynchus mykiss*) experienced reductions in reproductive success relative to wild fish over the first two generations of the hatchery program. The lack of data indicates the rarity of opportunity and ability to monitor fish stocking programs from their inception. Such an opportunity to monitor a stocking program existed in the Minnesota portion of Lake Superior as the Minnesota Department of Natural Resources (MNDNR) initiated a steelhead stocking program.

In Minnesota, declining steelhead populations in tributaries to Lake Superior have led the MNDNR to begin a supplemental stocking program using local broodstock with limited hatchery experience. Steelhead were first introduced into Lake Superior in the late 1800s (MacCrimmon and Gots 1972) and first reached Minnesota waters in 1895 (Hassinger et al. 1974). They have since established genetically distinct populations spawning within the various tributaries along the shore (Krueger et al. 1994). The habitat in this area is characterized by steep slopes, and often barrier waterfalls occur a short distance upstream from Lake Superior. Nearly half of the entire spawning and nursery habitat accessible to steelhead in Minnesota waters is found in the Knife River and its tributaries (Fig. 1; Schreiner 2003). It is thought that historic spawning runs in this river included at least 1000 individuals; however, the current number of spawners is between 200 and 400 adults as factors such as harvest, stream

**Fig. 1.** Map of the southern portion of the Minnesota north shore of Lake Superior, showing only those rivers used in this study.



degradation, and fish community changes in Lake Superior have likely reduced the annual migration. Declining populations since the 1970s led to a period of stocking both local and nonlocal steelhead fry. Although the success of these stockings was never directly evaluated, the Knife River population failed to recover. To protect the remaining spawning population, the harvest fishery for steelhead in the Knife River was closed in the mid-1990s.

The 1996 construction of an adult and juvenile trap less than 1 km upstream of the mouth of the Knife River now allows monitoring of the population as both down-migrating juveniles and up-migrating adults. This makes it possible to distinguish between stream and lake sources of mortality and provides an accurate estimate of both the adult and juvenile populations (Schreiner 2003). The length of time that juvenile steelhead spend in the river depends on environmental conditions and density of fish in the stream. Age-0 emigrants experience nearly 100% mortality and have not been found to recruit to spawning age. Steelhead migrating at age 1 are smaller than age-2 migrants (1997–2004 mean difference 5.0 cm; J.J. Ostazeski, unpublished data) and have a mean survival rate of 0.25%, making their contribution to the population largely dependent on the size of the emigrating class. Most steelhead that move into Lake Superior before age 2 have not undergone smoltification and purposely migrated, but rather ended up in the lake as a result of natural movement in short-run streams (Negus 2003). Fish emigrating at age 2 are of most interest to managers as roughly 80% of adults that return to spawn migrated to Lake Superior as age-2 juveniles (Hendrickson 2004). The operation of both traps makes it possible to carefully monitor the success of a stocking program, measured by the number of age-2 juveniles produced.

A new hatchery program began after construction of the adult trap in 1996. Using only naturalized steelhead captured at the Knife River adult trap as broodstock, the MNDNR bi-annually stocked age-1 juveniles beginning in 1997. Each age-1 steelhead stocked upstream in the Knife River had a fin clipped for later identification as a hatchery-born fish. Some of these stocked age-1 fish survived and returned to the Knife River as adults in spawning condition. When cap-

tured moving upstream to spawn, none of the hatchery-born steelhead was passed above the adult trap, but all were returned to the hatchery as broodstock.

The success of this age-1 stocking program was low, with adult return rates around 1% (Ward 2004). The high cost of production and poor recruitment of the stocked age-1 juveniles shifted recovery plans to include a focus on fry stocking. In 2002, fry produced from the hatchery-born, clipped adults captured at the Knife River trap were stocked into upstream areas of the Knife River. In 2003, fry from both hatchery-born and natural-born adults were stocked into the Knife River.

Our primary objective was to evaluate the effectiveness of fry stocking in increasing the number of age-2 emigrants. Secondarily, we compared the survival of fry of parents born in the hatchery with fry of parents born in the wild. This allowed us to examine the rate at which domestication selection could affect this population. In addition, data collected for these objectives allowed us to evaluate another potential consequence of hatchery propagation: a shift in the genetic composition of hatchery subpopulations due to the use of a limited number of adults for hatchery spawning.

## Materials and methods

We addressed our primary objective by collecting samples from steelhead at the Knife River fish traps during 2002–2005. Scale samples were taken from adult steelhead when they were captured during their upstream spawning migration in 2002 and 2003. Some adults were passed upstream to spawn naturally, and the rest were taken to the hatchery for spawning. Fry were then stocked into upstream areas of the Knife River. Scale samples were collected from age-1 juveniles during their down-migration in 2003 and 2004 and from age-2 juveniles during 2004 and 2005. Because the stocked fry were not marked in any way, we used DNA-based parentage analysis to determine if the sampled juveniles belonged to a hatchery group or were naturally reproduced in the stream.

In this paper, we refer to the success of stocking in terms of an increase in the average family size of adult female steelhead. For a stocking program to succeed, a female in the hatchery must have a larger family size than she would if she were spawning naturally. In 2002, more than one male was mated with some females, thus families are considered as all offspring with a common mother. Family size was measured at the age-1 and age-2 stages.

We conducted a separate paired-stocking experiment to determine if fry survival differed between offspring of adults born in the wild and offspring of adults born in a hatchery. Equal proportions of fry from each group were stocked into the same reaches of natural streams and recaptured 3 months after stocking. Parentage analysis allowed each age-0 steelhead to be assigned to its group of origin, and we determined if the relative abundance of each group at capture equaled the relative abundance at stocking.

## Types of adult steelhead monitored

We monitored the survival of offspring from three groups of adult steelhead, which we refer to in this paper as NS for naturally spawning and  $H_1$  and  $H_2$  for one and two genera-

tions of artificial spawning in the hatchery. Adults that were born in the wild and spawned naturally in the wild are referred to as NS adults. Their offspring, which we monitored as age-1 and age-2 emigrating juveniles, are referred to as NS offspring. Adults that were born in the wild but captured at the Knife River adult trap and taken to French River Hatchery for artificial mating are labeled as  $H_1$  adults. Their  $H_1$  offspring were stocked as fry into upstream reaches of the Knife River and then monitored as age-1 and age-2 emigrants. The final group is the  $H_2$  adults that were born in the hatchery and then stocked as age 1 with a fin clip. These fish matured in the wild but were not allowed to spawn naturally in the stream. They were captured at the Knife River adult trap, identified by their fin clip, and taken back to the hatchery for artificial mating. Their  $H_2$  offspring were also stocked as fry into the Knife River and monitored as age-1 and age-2 emigrants.

## Rearing conditions

Rearing conditions were the same for both  $H_1$  and  $H_2$  offspring. In the French River Hatchery, eggs are loaded into trays at a constant density of 1500 eggs per tray. Trays are randomly placed on egg stands, and temperatures and dissolved oxygen levels between stands are nearly identical (M. Gottwald, MNDNR, 5351 North Shore Drive, Duluth, MN 55804, USA, personal communication, 2006).

## Fry stocking

In 2002, only  $H_2$  fry were stocked into the Knife River. These fry were produced from 20 females, and matings were mostly one male crossed with one female but were not recorded. In 2003, adult steelhead were strictly mated one to one, all pairs were recorded, and both  $H_1$  and  $H_2$  offspring were stocked as fry (Table 1). Fry were stocked shortly after hatching at the time of yolk sac absorption. In the fall of 2003, 4841  $H_1$  fingerlings were stocked into the Knife River. This stocking was done independently of our study, and as a result, we could not distinguish survivors from fry versus fingerling stocking. This prevented us from making unbiased survival comparisons between  $H_1$  and  $H_2$  offspring from the 2003 year class in the Knife River.

Determining if family sizes were equal between all adult types required an accurate estimate of the number of NS females spawning in the Knife River in both 2002 and 2003. A single-census Peterson estimate was made to determine the number of females spawning upstream. All females captured in the trap on their upstream migration were marked with a floy tag, and those captured moving downstream were carefully examined for tags or evidence of a lost tag (tag stub or tagging scar). In 2002, 79 fish were marked on their way upstream, and 70 were captured on their way downstream, 53 of which were recaptures. Only one fish was found with a scar from a lost tag and was considered a recaptured fish. We thus estimated 104 NS females spawning upstream, with a 95% confidence interval (CI) estimated from the binomial distribution of 93–125 females. In 2003, 144 fish were marked on their way upstream, and 99 were captured on their way downstream, 80 of which were recaptures. Four fish were observed to have either a tag stub or tag scar, and these were considered recaptures. We thus estimated 178 NS

**Table 1.** Juvenile steelhead stocked into the Knife River during the two study years from parents with one ( $H_1$ ) or two ( $H_2$ ) generations of artificial spawning in the hatchery.

| Year class | Type  | Life stage        | Number |
|------------|-------|-------------------|--------|
| 2002       | $H_2$ | Fry               | 49 500 |
| 2003       | $H_1$ | Fry               | 24 676 |
|            | $H_2$ | Fry               | 31 918 |
|            | $H_1$ | Fingerling (fall) | 4 841  |

**Note:** Combined number of 29 517  $H_1$  offspring (fry + fingerling) used in statistical calculations of stocking success for 2003.

females spawning upstream with a 95% CI estimated from the binomial distribution of 164–203 females.

In 2003, only a portion of the offspring of  $H_1$  parents was stocked as fry for this study; the rest were kept to continue the age-1 clipped juvenile stocking program. Families were pooled as eggs, so all families could have been sampled but exact family representation was unknown. The number of offspring stocked equaled 42% of the total fry produced by the 33 females spawned at the hatchery. We thus estimated that the equivalent of 14 females would have been required to produce the amount of fry stocked and used this value in calculations of juveniles per  $H_1$  female.

### Juvenile steelhead

Fry were stocked into the Knife River in June of each study year and spent either one or two winters in the stream alongside the naturally produced fish before migrating to Lake Superior. Juvenile steelhead were captured in the Knife River juvenile trap between April and August in 2003–2005. In 2003 and 2004, scale samples for genetic analysis were taken from fish collected according to the routine MNDNR sampling protocol. Samples were taken from five unclipped fish per 10 mm size class per 2-week sampling period. This subsampling scheme was used to collect both age-1 and age-2 fish from the 2002 year class, but only age-1 fish from the 2003 year class. To obtain a larger sample size of age-2 juveniles from the 2003 year class, we collected scales from nearly all age-2 fish captured in the juvenile trap in 2005.

Age-1 steelhead, although often the largest emigrating group based on numbers alone, experience high mortality in Lake Superior, which limits their contribution to future adult returns. In our study, age-1 fish were examined to determine if the proportion of hatchery fish among age-1 emigrants equaled their proportion among age-2 migrants. A difference might indicate that hatchery ancestry affected age of emigration, which could contribute to observed differences in the number of age-2 juveniles produced.

### Paired stocking

Equal numbers of  $H_1$  and  $H_2$  fry were stocked into two streams in each of three years to determine if one type had a survival advantage. Adult steelhead of both types were captured at the Knife River trap during 2003–2005 and spawned at the hatchery. In 2003, 7485 fry of each type were stocked into Little Knife River and 6655 fry of each type were stocked into McCarthy Creek. In 2004, 7500 fry of each type were stocked into Stewart River and Budd Creek. In

2005, 7510 fry of each type were stocked into Stewart River and the West Branch Split Rock River (Fig. 1). Fry were stocked in June of each study year and were captured at age 0 by backpack electrofishing three months after stocking. Each captured juvenile fish had a portion of its caudal fin removed, which was stored in 95% ethanol for genetic analysis.

### Genetic and parentage analysis

We extracted DNA from all collected scale and fin samples with 250  $\mu\text{L}$  of 5% Chelex<sup>®</sup> (Sigma Chemical, St. Louis, Missouri) solution and stored the samples at  $-20^\circ\text{C}$  following the protocol of Miller and Kapuscinski (1996). Polymerase chain reactions (PCR) were performed to amplify 11 microsatellite loci (Table 2). Reaction volumes were 15  $\mu\text{L}$  and contained 6  $\mu\text{L}$  DNA template, 10 $\times$  polymerase buffer (10  $\text{mmol}\cdot\text{L}^{-1}$  Tris-HCl, 50  $\text{mmol}\cdot\text{L}^{-1}$  KCl, 0.1% Triton<sup>®</sup> X-100), 1.5  $\text{mmol}\cdot\text{L}^{-1}$   $\text{MgCl}_2$ , 0.2  $\text{mmol}\cdot\text{L}^{-1}$  each dNTP, 0.5  $\mu\text{mol}\cdot\text{L}^{-1}$  of each primer, 0.5 U Taq DNA polymerase (Promega, Madison, Wisconsin), and water up to the final reaction volume. Each plate included a water control to monitor for PCR contamination.

Thermal cycling conditions included 35 cycles of denaturation at  $95^\circ\text{C}$ , annealing at  $53^\circ\text{C}$ , and extension at  $72^\circ\text{C}$ . We submitted PCR products to the Advanced Genetic Analysis Center (University of Minnesota, St. Paul, Minnesota) for electrophoresis on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, California). Alleles were scored using the Applied Biosystems software program GENOTYPER.

We used the computer program WhichParents (W. Eichert, Bodega Marine Laboratory, University of California Davis, www.bml.ucdavis.edu), which uses an exclusion approach to parentage, to assign juvenile steelhead sampled at the Knife River trap to either a  $H_1$  or  $H_2$  parent pair. If a juvenile genotype was not compatible with any parent pair, it was considered to be produced by NS parents. Some juveniles from the paired-stocking experiment did not match any parents. In each of these cases, the stocking site was accessible by other fish and these mismatching individuals were removed from the analysis.

To ensure that our microsatellite set would not falsely assign a naturally produced juvenile to our set of hatchery parents, we created simulated populations of offspring to mimic what could have been produced in the wild. We used the software Whichloci (W. Eichert, Bodega Marine Laboratory, University of California Davis, www.bml.ucdavis.edu) to generate populations of 10 000 individuals based on the allele frequencies of each of the parent data sets. We then used the program WhichParents to determine if the simulated offspring genotypes matched any of our known parent pairs.

### Statistical analysis

We used  $\chi^2$  analysis to test the null hypothesis of equal family size between the NS and  $H_1$  or  $H_2$  adults, using Yates correction for tests with one degree of freedom (Zar 1985). Specifically, we tested if the proportion of types among the emigrating juveniles equaled the proportion of types among the adult females that produced them. We considered age-1 and age-2 offspring separately. In 2002 we compared NS females with  $H_2$  females, and in 2003 we compared NS with  $H_1$  and NS with  $H_2$ . Family size ratios of one type to another

**Table 2.** Diversity information of microsatellite loci used in parentage analysis, including name of each locus, number of alleles present, observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities, and frequency of null alleles in the parent generations.

| Locus name     | Source               | No. of alleles | $H_o$ | $H_e$ | Null allele frequency |
|----------------|----------------------|----------------|-------|-------|-----------------------|
| <i>OGO3</i>    | Olsen et al. 1998    | 10             | 0.77  | 0.66  | 0.00                  |
| <i>OGO4</i>    | Olsen et al. 1998    | 7              | 0.76  | 0.73  | 0.00                  |
| <i>OKE4</i>    | GenBank AF330221     | 17             | 0.91  | 0.91  | 0.00                  |
| <i>OKI23*</i>  | GenBank AF272822     | 20             | 0.76  | 0.93  | 0.08                  |
| <i>OMY1001</i> | Spies et al. 2005    | 19             | 0.84  | 0.89  | 0.00                  |
| <i>OMY1011</i> | Spies et al. 2005    | 15             | 0.96  | 0.89  | 0.00                  |
| <i>ONE108*</i> | Olsen et al. 2000    | 26             | 0.81  | 0.90  | 0.05                  |
| <i>ONE2</i>    | Scribner et al. 1996 | 27             | 0.91  | 0.92  | 0.00                  |
| <i>OTS4*</i>   | Banks et al. 1999    | 7              | 0.56  | 0.62  | 0.00                  |
| <i>OTSB5*</i>  | Naish and Park 2002  | 15             | 0.77  | 0.86  | 0.03                  |
| <i>RT191</i>   | Spies et al. 2005    | 24             | 0.88  | 0.93  | 0.00                  |

\*Significantly different from Hardy–Weinberg expectations after sequential Bonferroni correction ( $\alpha = 0.05$ ,  $k = 11$ ).

were calculated by dividing the number of juveniles produced per female of the larger group by the number of juveniles per female of the smaller group.

Chi-squared tests were also used to analyze the results of our paired-stocking experiments. We tested if the relative abundance of juveniles of each group at capture equaled their relative abundance at the time of stocking. A  $6 \times 2$  contingency table was used to determine if a significant difference existed between any of the years and streams used in the study.

Because female size may affect egg quality and, thus, early-life survival, we tested for a significant difference in the mean length and weight of adult females taken to the hatchery using two-sample  $t$  tests. We compared the size of  $H_1$  and  $H_2$  females during 2003, 2004, and 2005.

### Genetic composition of hatchery fish

To determine if the offspring produced in the hatchery accurately represented the genetic variation of the offspring produced naturally, we tested for population differentiation and differences in genetic diversity between the groups of hatchery and natural age-2 offspring in both the 2002 and 2003 year classes. We also tested the effect of differentiation on the genetic variation of the entire cohort following stocking. For each year class, we combined all age-2 offspring to estimate the allele frequencies of the entire cohort and compared them with the frequencies of only the NS fish. Difference in this comparison would represent a shift in allele frequencies of the entire cohort due to hatchery supplementation. Pairwise population differentiation tests were performed using FSTAT (version 2.9.3; Goudet 2001) to estimate  $F_{st}$  and conduct permutations to test its significance and Genepop (Raymond and Rousset 1995) to perform exact tests for population differentiation. Expected heterozygosities and allelic richness by locus were also estimated using FSTAT. Because increasing sample size is expected to increase the number of alleles found, we used the FSTAT application that performs a resampling routine to estimate the number of alleles expected to be found with common sample sizes. Wilcoxon signed-rank tests (Zar 1985) were then used to test for differences in heterozygosity and allelic richness between sample groups.

## Results

### Parentage analysis

Simulation results demonstrated that we were unlikely to falsely assign naturally produced juveniles to either of our hatchery groups. In the simulated population based on allele frequencies of the 2002 parents, none of the 10 000 offspring uniquely assigned at all loci to a hatchery parent pair, neither did any match a parent pair at 10 of the 11 loci examined. Less than 1% matched both a male and female parent at nine of the 11 loci. The second simulated population based on the 2003 parents yielded similar results. No individual matched hatchery parents at nine or more loci. Approximately 1% of the 10 000 simulated offspring matched the parents at eight loci. The difference in the number of loci required to uniquely assign offspring between year classes is due to documentation of the hatchery mating scheme. In 2002, we did not have records of mated pairs, making it necessary to consider all possible crosses of males and females for parentage assignment. In all other years, the mated pairs were recorded in the hatchery, thus limiting the number of possible crosses in parentage analysis. In addition to the simulation results, no offspring from the paired-stocking experiments were assigned to more than one possible parent pair.

We were able to confirm null alleles present in three of the loci used in this study. While assigning parents to juveniles captured in Budd Creek, a 2004 paired-stocking site, we found some juveniles that matched known parents at only 10 of 11 loci. Given the isolated location of this stream and the results of our power analysis, it seemed improbable that these fish were from another source yet only mismatched our parents at one locus. Upon further inspection we found that each mismatch could be explained by the presence of a null allele in a parent that was passed on to the offspring. Of all juveniles sampled in these experiments, we did not observe an offspring with a null allele at all three of these loci. Only five individuals (of 730 uniquely assigned to a parent pair) with nulls at two loci were found, and they were included in the analysis because of the results of our power tests. The frequency of null alleles at each locus was estimated for the hatchery parent samples based on the con-

**Table 3.** Number of juvenile emigrants from different parent types of Knife River steelhead, naturally spawning females (NS) and hatchery females with one (H<sub>1</sub>) or two (H<sub>2</sub>) generations of artificial spawning.

| Type                   | No. of females | Age 1            |                      | Age 2            |                      |
|------------------------|----------------|------------------|----------------------|------------------|----------------------|
|                        |                | No. of offspring | Offspring per female | No. of offspring | Offspring per female |
| <b>2002 year class</b> |                |                  |                      |                  |                      |
| NS*                    | 104            | 47               | 0.45                 | 63               | 0.61                 |
| H <sub>2</sub>         | 20             | 37               | 1.85                 | 75               | 3.75                 |
| <b>2003 year class</b> |                |                  |                      |                  |                      |
| NS*                    | 178            | 167              | 0.94                 | 806              | 4.53                 |
| H <sub>1</sub> *       | 14             | 32               | 2.29                 | 132              | 9.42                 |
| H <sub>2</sub>         | 19             | 26               | 1.37                 | 110              | 5.79                 |

**Note:** Types for which the numbers of females were estimates rather than direct counts are indicated with asterisks (\*); see Methods for description. The number of offspring per female is low because of juvenile subsampling for all groups except the age-2 juveniles from the 2003 year class.

**Table 4.** Comparisons of steelhead family size, measured as age-1 and age-2 juveniles, between naturally spawning females (NS) and hatchery females with one (H<sub>1</sub>) or two (H<sub>2</sub>) generations of artificial spawning.

| Year class | Type comparison    | Age 1          |                   | Age 2          |                   |
|------------|--------------------|----------------|-------------------|----------------|-------------------|
|            |                    | $\chi^2$ value | Family size ratio | $\chi^2$ value | Family size ratio |
| 2002       | H <sub>2</sub> :NS | 46.36**        | 4.2               | 146.20**       | 6.2               |
| 2003       | H <sub>1</sub> :NS | 21.01**        | 2.4               | 63.68**        | 2.1               |
|            | H <sub>2</sub> :NS | 3.97*          | 1.5               | 5.81*          | 1.3               |

**Note:**  $\chi^2$  tests (1 df) indicate if the proportion of types among the emigrating juveniles equaled the proportion of types among the adult females that produced them, with significant differences denoted by asterisks: \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ . Family size ratio is the number of offspring per female of the first type listed relative to the number per female of the second type, based on estimated female numbers.

firmed null alleles from parentage testing and ranged from 0.03 to 0.08 (Table 2). The presence of null alleles likely explains the Hardy–Weinberg disequilibrium at these three loci; of the remaining eight loci, only one was out of Hardy–Weinberg equilibrium after correction for multiple comparisons (Rice 1989).

### Fry stocking success

Fry stocking successfully increased the average family size of female steelhead. From the 2002 year class, H<sub>2</sub> females produced significantly more age-1 and age-2 offspring than did NS females. These hatchery adults produced 4.2 times more age-1 juveniles per female than did naturally spawning females and 6.2 times more age-2 juveniles (Tables 3 and 4). These results remained highly significant, even if the lower bound of the 95% CI for the number of NS females was substituted for the population estimate (age 1  $\chi^2 = 38.25$ , 1 df,  $P < 0.001$ ; age 2  $\chi^2 = 124.74$ , 1 df,  $P < 0.001$ ). A similar trend was observed from the 2003 year class. Both H<sub>1</sub> and H<sub>2</sub> females had significantly higher family sizes of both age-1 and age-2 offspring than did naturally spawning fish, although the family size ratio for each of the hatchery groups compared with the naturally spawning females was lower in the 2003 year class than it was in the 2002 year class (Table 4). The results for age-1 and age-2 H<sub>1</sub> steelhead remained highly significant if the lower bound of the CI was substituted for the population estimate (age 1  $\chi^2 = 25.00$ , 1 df,  $P < 0.001$ ; age 2  $\chi^2 = 101.80$ , 1 df,  $P < 0.001$ ); however, the difference between H<sub>2</sub> and NS steelhead was no longer significant (age 1  $\chi^2 = 2.64$ , 1 df,  $P = 0.104$ ; age 2  $\chi^2 = 2.59$ , 1 df,  $P = 0.108$ ).

### Paired-stocking survival comparisons

Paired-stocking comparisons showed an early life survival advantage for H<sub>1</sub> offspring over H<sub>2</sub> offspring when stocked at equal numbers into the same environment. In five of the six individual paired-stocking experiments, the number of H<sub>1</sub> juveniles captured exceeded the number of H<sub>2</sub> juveniles (Table 5); however, the difference in each was not significant owing to the low abundance of both groups at capture. A contingency table showed that there was no year or stream effect ( $\chi^2 = 2.78$ , 5 df,  $P = 0.734$ ). All streams and years were then combined to achieve a larger sample size. In the resulting comparison, we found that significantly more H<sub>1</sub> juveniles were captured ( $\chi^2 = 9.51$ , 1 df,  $P = 0.002$ ). The relative survival of H<sub>2</sub> to H<sub>1</sub> fish 3 months after stocking was 70%.

### Adult female size

We found a significant difference between the body size of H<sub>1</sub> and H<sub>2</sub> females in 2003. From the 2003 spawning class, 28 of the 33 H<sub>1</sub> females taken to the hatchery successfully produced at least one age-1 or age-2 juvenile. Fifteen out of 19 H<sub>2</sub> females produced offspring during the same spawning season. The successful H<sub>1</sub> females were significantly longer and heavier than the H<sub>2</sub> females (Table 6). In 2004 and 2005, there were no significant differences in length or weight between the groups.

### Genetic composition of hatchery fish

The offspring produced at the hatchery were genetically differentiated from those of naturally produced fish and had lower allelic richness. Pairwise  $F_{st}$  was significantly greater

**Table 5.** Numbers of age-0 steelhead with parents that had one (H<sub>1</sub>) or two (H<sub>2</sub>) generations of artificial spawning captured during three years of paired-stocking experiments in which equal numbers of each type were stocked as fry.

| Location                       | Year      | Type           | Number captured | $\chi^2$ (p value) |
|--------------------------------|-----------|----------------|-----------------|--------------------|
| Little Knife River             | 2003      | H <sub>1</sub> | 39              | 1.83 (0.175)       |
|                                |           | H <sub>2</sub> | 27              |                    |
| McCarthy Creek                 | 2003      | H <sub>1</sub> | 22              | 0.00 (1.000)       |
|                                |           | H <sub>2</sub> | 23              |                    |
| Stewart River                  | 2004      | H <sub>1</sub> | 45              | 1.55 (0.213)       |
|                                |           | H <sub>2</sub> | 33              |                    |
| Budd Creek                     | 2004      | H <sub>1</sub> | 24              | 2.13 (0.144)       |
|                                |           | H <sub>2</sub> | 14              |                    |
| Stewart River                  | 2005      | H <sub>1</sub> | 13              | 1.25 (0.260)       |
|                                |           | H <sub>2</sub> | 7               |                    |
| West Branch Split Rock River   | 2005      | H <sub>1</sub> | 44              | 3.61 (0.057)       |
|                                |           | H <sub>2</sub> | 27              |                    |
| All streams and years combined | 2003–2005 | H <sub>1</sub> | 187             | 9.51 (0.002)       |
|                                |           | H <sub>2</sub> | 131             |                    |

**Note:**  $\chi^2$  tests showed whether relative abundance of each type at capture equaled relative abundance at stocking, for each year and site separately and for all data combined.

**Table 6.** Results of two-sample *t* tests for difference in size between hatchery females with one (H<sub>1</sub>) or two (H<sub>2</sub>) generations of artificial spawning.

| Year | Type           | Number sampled | Mean weight (kg) | <i>t</i> (p value) | Mean length (mm) | <i>t</i> (p value) |
|------|----------------|----------------|------------------|--------------------|------------------|--------------------|
| 2003 | H <sub>1</sub> | 28             | 2.4              | 4.43 (<0.001)      | 627              | 3.69 (<0.001)      |
|      | H <sub>2</sub> | 15             | 1.7              |                    | 554              |                    |
| 2004 | H <sub>1</sub> | 7              | 2.8              | 0.26 (0.800)       | 655              | 0.62 (0.541)       |
|      | H <sub>2</sub> | 12             | 2.7              |                    | 643              |                    |
| 2005 | H <sub>1</sub> | 7              | 2.5              | -1.09 (0.298)      | 660              | -0.27 (0.789)      |
|      | H <sub>2</sub> | 6              | 2.8              |                    | 668              |                    |

**Table 7.** Comparisons of expected heterozygosity (H<sub>e</sub>), allelic richness (A), and population differentiation (F<sub>st</sub>) for samples of age-2 steelhead with parents that had one (H<sub>1</sub>) or two (H<sub>2</sub>) generations of artificial spawning or were spawned naturally (NS).

| Year class and group | Sample size | H <sub>e</sub> | A       | Pairwise F <sub>st</sub> |       |
|----------------------|-------------|----------------|---------|--------------------------|-------|
|                      |             |                |         | H <sub>2</sub>           | NS    |
| 2003                 |             |                |         |                          |       |
| H <sub>1</sub>       | 132         | 0.84           | 13.8    | 0.03*                    | 0.01* |
| H <sub>2</sub>       | 110         | 0.83           | 12.0*** | —                        | 0.02* |
| NS                   | 806         | 0.83           | 14.8    | 0.01*                    | —     |
| Cohort               | —           | —              | —       | —                        | 0.00  |
| 2002                 |             |                |         |                          |       |
| H <sub>2</sub>       | 75          | 0.78           | 9.6**   | —                        | 0.05* |
| NS                   | 63          | 0.83           | 13.9    | 0.05*                    | —     |
| Cohort               | —           | —              | —       | —                        | 0.01* |

**Note:** Means are shown for H<sub>e</sub> and A, but testing was based on the Wilcoxon signed-rank test. Significance for pairwise F<sub>st</sub> tests (i.e., F<sub>st</sub> > 0) was based on permutations tests. Significant results are indicated with asterisks: \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001. The cohort category grouped all H and NS together and compared them with NS alone.

than zero in all comparisons between H and NS offspring for both year classes (probability was <0.05 that F<sub>st</sub> = 0 following permutation tests in FSTAT; Table 7). The entire 2002 emigrating cohort (NS and H<sub>2</sub>) was also significantly differ-

entiated from the 2002 NS fish alone; however, the 2003 emigrating cohort (NS, H<sub>1</sub>, and H<sub>2</sub>) did not differ significantly from the 2003 NS fish. Exact tests for population differentiation performed in Genepop produced the same significant comparisons (data not shown). Expected heterozygosities did not differ between H and NS offspring in any comparisons (all P ≥ 0.10, Wilcoxon signed-rank tests, n = 11). In contrast, allelic richness was significantly lower for H<sub>2</sub> offspring compared with NS offspring for both year classes and lower, but not significantly, for H<sub>1</sub> offspring in 2003 (P = 0.18, Wilcoxon signed-rank test, n = 11).

### Discussion

We have shown that a steelhead fry stocking program was successful as measured by an increased number of offspring produced by hatchery females relative to naturally spawning females. By circumventing the perils of spawning and incubation in the wild (egg-to-fry survival was approximately 70% in the hatchery), hatchery rearing produced large numbers of fry per female, ultimately leading to increased numbers of age-2 offspring. This measure of stocking success is necessary, but not sufficient, to achieve a goal of population rehabilitation. To achieve a self-sustaining increase in population abundance, the stocked fish must add to the population rather than displace naturally produced offspring, and

short-term increases in abundance must not be negated by fitness impacts of hatchery fish on wild populations. We found reduced survival of offspring of parents with only one generation of hatchery ancestry. This indicates that the fitness impacts of domestication selection (Reisenbichler and McIntyre 1977; Fleming et al. 1997; Dannewitz et al. 2003) can occur in the early generations of a hatchery program.

In both years of our study, we observed that fry stocking successfully increased the number of emigrating juveniles produced per spawning female. Steelhead that emigrate to Lake Superior as age-2 juveniles are more likely than age-1 emigrants to return as spawning adults (Schreiner 2003) and are thus of most importance to managers. We found that a female steelhead spawned in the hatchery was able to produce more age-2 offspring than a steelhead spawning naturally. The success of stocking  $H_1$  fry in 2003 may have been inflated by the late fall stocking of fingerlings, which we grouped with fry in our analyses; however, the  $H_2$  females, which had only fry stocked, also produced more offspring than natural spawners, and the greater success of  $H_1$  over  $H_2$  was consistent with the results of our paired-stocking experiments. We also observed no significant difference in the proportion of emigrants, derived from stocked fry, that left the river as age 1 or age 2. This indicates that stocked fry did not differ from natural fry in their age at emigration, a critical factor in determining likelihood of survival to adulthood.

We could not directly measure whether the fry stocked in this study merely displaced some naturally reproduced juveniles or actually added to them; however, a negative correlation between adult abundance and success of fry stocking between study years was consistent with additions to the population. In 2002 we estimated that there were 104 female steelhead spawning in the Knife River and the  $H_2$ -NS family size ratio for that year class at age 2 was 6.2:1. In 2003 the number of females increased to 178, whereas the ratio of the groups spawned in the hatchery to naturally produced fish dropped to 2.1:1 and 1.3:1 for  $H_1$  and  $H_2$  females, respectively. The offspring of these extra females in 2003 would have used more habitat in the river, leaving less available for the stocked fry. Our results suggest that the fry stocking did add to the population, at least in one year, but it will cease to be productive when the carrying capacity of the river is met. Careful monitoring of stocking success and correlating the number of age-2 juveniles produced with the number of adults spawning and the environmental conditions will allow for determination of when stocking is no longer productive.

The paired-stocking study allowed us to directly compare the survival of fry from  $H_1$  and  $H_2$  parents. The relative survival of  $H_2$  to  $H_1$  was 70% after 3 months of in-stream life. We found no significant difference between streams or years; however, if we removed 2003 from the analysis, owing to the possible influence of female size, there was still a significant advantage for  $H_1$  offspring. Using only 2004 and 2005 paired-stocking data, the relative survival of  $H_2$  to  $H_1$  actually dropped to 64%. In addition, the relative survival of  $H_2$  to  $H_1$  was 75% among the age-2 emigrants from the 2003 year class stocked in the Knife River (data not shown), although this comparison was confounded by the additional stocking of  $H_1$  fingerlings. Araki et al. (2007a) found similar effects of hatchery ancestry on reproductive success of local-origin first-generation hatchery steelhead. Hatchery female  $\times$

hatchery male crosses had 66% reproductive success relative to wild female  $\times$  hatchery male crosses (Araki et al. 2007a; Table 5). When keeping the female constant, hatchery male  $\times$  hatchery female crosses had 90% reproductive success relative to wild male  $\times$  hatchery female crosses, although this result was not significant. Araki et al. (2007b) extended this study to additional run years and a second generation and found significant reductions in reproductive success of hatchery fish relative to wild fish over the first two generations. Their meta-analysis of relative reproductive success of local captive-reared versus wild fish suggested a decline of 37.5% per generation.

The observed difference in survival between the two groups of fry stocked in our study was likely due to lesser exposure of  $H_1$  to domestication selection than  $H_2$ . The fry groups experienced the same rearing environment, suggesting a genetic component to the survival differences. The only life history difference between parents of  $H_1$  and  $H_2$  offspring was the place of birth and rearing during their first year of life and the additional generation of artificial mating. The year of relaxed natural selection, experienced by the  $H_2$  adults in the hatchery, undoubtedly enabled some of them to survive that otherwise may not have. When offspring of those parents had to compete in the wild with fish that had no hatchery history, they were less able to do so and experienced reduced survival. Our finding is consistent with the conclusion by Reisenbichler and Rubin (1999) that rearing fish for one year or longer in a hatchery causes genetic change and decreases both their fitness and that of their offspring.

We could not explicitly test for maternal effects, but we did examine one potential contributing factor, female size. In 2003,  $H_1$  females were significantly larger than  $H_2$  females, which in other studies has been shown to increase the survival chances of offspring (e.g., Pitman 1979; Kinnison et al. 1998; Einum and Fleming 2000). Of all six paired-stocking comparisons over 2003–2005, the only one that did not favor  $H_1$  was McCarthy Creek in 2003. At all sites in 2004 and 2005,  $H_1$  was favored over  $H_2$  in spite of no size difference for the females in these years. Other maternal effects could have been present but were likely negligible because the females shared a common stream and lake environment for the majority of their lives (i.e., after the stocking of  $H_2$  at age 1).

The hatchery practices used during this fry stocking program shifted allele frequencies enough to segment the Knife River steelhead population into distinct groups; however, the long-term effect of this segmentation depends on the success of the stocking (Ryman and Laikre 1991; Waples and Do 1994). The use of a relatively small number of adults at the hatchery led to genetic differentiation between stocked fry and the naturally produced fish. This was caused by a change in allele frequencies and the loss of some alleles. In 2002, the high success of  $H_2$  fry overwhelmed the naturally produced fish, and the cohort as a whole experienced a shift in allele frequencies from the natural population. In 2003, the success of the stocked fry was not as great, and a larger number of naturally produced fish prevented the stocked fry from significantly changing the allele frequencies of the entire cohort. Even if some cohorts experienced shifts, returning different adults to the hatchery in consecutive years may eventually capture more of the genetic variation within the population. For example, Heggenes et al. (2006) documented

no significant shift in genetic diversity from 20 years of supplemental steelhead stocking in British Columbia; however, they did show that some rare alleles were lost in the population. Careful monitoring of Knife River steelhead will be required to determine what effects hatchery practices may have on the genetic variation of the population in the long term.

Reproductive success should be measured throughout an entire life cycle, but the fry that were stocked have not recruited to spawning age in sufficient numbers for evaluation. Those data would represent the most complete measure of fry stocking success and would provide the most conclusive results about the effects of one generation of hatchery experience upon fitness of offspring. Our preliminary conclusion of stocking success could be tempered if hatchery juveniles have reduced survival as they mature into adults. However, even without the rate of adult returns, our study demonstrates the reduced survival of offspring of parents that spent their first year of life in a hatchery.

The goal of steelhead management in the Knife River is to reestablish a population large enough to allow limited angler harvest, supported mainly by natural reproduction (Schreiner 2003). The MNDNR has eliminated harvest of natural steelhead and promotes appropriate land use to maintain or increase available habitat. We have shown that fry stocking increased the reproductive success of hatchery females and may also contribute to achieving the management goal; however, cautions do exist. Our data suggest that domestication selection may be reducing survival of fry after only one generation of hatchery experience. Domestication selection is not understood well enough to predict its effects on future generations or how quickly it can be counteracted by natural selection, but modeling has shown that its long-term impacts can be significant (Ford 2002). Additionally, genetic change as a result of enhancing only a small portion of the population is occurring with current practices, at least within some cohorts. Steps must be taken to ensure that sufficient genetic variability remains in the population. These risks must be weighed against the benefits of fry stocking, and a recovery method that provides an immediate boost in population size yet reduces genetic risks should be developed (Miller and Kapuscinski 2003). Any management strategy must be met with a commitment to future monitoring and evaluation of this population and the hatchery practices associated with it.

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