

Lower fitness of hatchery and hybrid rainbow trout compared to naturalized populations in Lake Superior tributaries

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Abstract

We have documented an early life survival advantage by naturalized populations of anadromous rainbow trout *Oncorhynchus mykiss* over a more recently introduced hatchery population and outbreeding depression resulting from interbreeding between the two strains. We tested the hypothesis that offspring of naturalized and hatchery trout, and reciprocal hybrid crosses, survive equally from fry to age 1+ in isolated reaches of Lake Superior tributary streams in Minnesota. Over the first summer, offspring of naturalized females had significantly greater survival than offspring of hatchery females in three of four comparisons (two streams and 2 years of stocking). Having an entire naturalized genome, not just a naturalized mother, was important for survival over the first winter. Naturalized offspring outperformed all others in survival to age 1+ and hybrids had reduced, but intermediate, survival relative to the two pure crosses. Averaging over years and streams, survival relative to naturalized offspring was 0.59 for hybrids with naturalized females, 0.37 for the reciprocal hybrids, and 0.21 for hatchery offspring. Our results indicate that naturalized rainbow trout are better adapted to the conditions of Minnesota's tributaries to Lake Superior so that they outperform the hatchery-propagated strain in the same manner that many native populations of salmonids outperform hatchery or transplanted fish. Continued stocking of the hatchery fish may conflict with a management goal of sustaining the naturalized populations.

Keywords: anadromous rainbow trout, hatcheries, naturalized populations, outbreeding depression, survival

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Introduction

Wildlife managers commonly release captive-bred animals derived from a native or previously introduced alien species in order to maintain, rebuild or increase numbers of animals for fishing, hunting or conservation objectives. There is growing evidence, however, of failure of captive-bred releases to raise abundance when suitable habitat and other ecological factors do not appear to be limiting (NRC 1995, 2004; Fischer & Lindenmayer 2000; Waples *et al.* 2004). This has led geneticists to suggest that an evolutionary mechanism may be operating: reduction in fitness when the captive-bred released individuals hybridize

with residents of local populations. This loss of fitness, or outbreeding depression, can arise from loss of local adaptation or breakdown of coadapted genes at multiple loci (Templeton *et al.* 1986; Lynch 1991). Of the two, outbreeding depression due to loss of local adaptation is better documented and considered less controversial (Templeton 1986). Use of captive-bred animals is especially prevalent in fisheries management (Schramm & Piper 1995), where relatively few hatchery adults can produce thousands of offspring for stocking, raising hopes for enhanced fisheries or rehabilitated fish populations. As a consequence of stocking hatchery fish, outbreeding depression due to loss of local adaptation may arise from either or both the use of nonlocal fish that have evolved in different environments from the resident population or from genetic changes in

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captive populations due to adaptation to the hatchery environment, i.e. domestication (Hallerman 2003; Miller & Kapuscinski 2003). Either case could result in hybrids between hatchery and wild fish having lower fitness than individuals from the resident population.

Most studies of interactions between captive-bred and wild fish have focused on hatchery populations stocked into areas containing native populations of the same species, particularly for the commonly artificially propagated species of the family *Salmonidae* (e.g. Reisenbichler & Rubin 1999; Einum & Fleming 2001; Fleming & Petersson 2001; Waples *et al.* 2004). Evolutionary theory and empirical studies of the fate of released fish suggest that, because native salmonid populations have undergone natural selection for thousands of years to become adapted to their local environment (Ricker 1972; Taylor 1991), hatchery fish with different genetic characteristics should be less fit in the natural environment of the native population (NRC 1995; Reisenbichler 1997; Einum & Fleming 2001; NRC 2004). Past introductions also have created many naturally reproducing populations of salmonid and other fish species outside their native ranges. These 'naturalized' populations may also exhibit superior fitness relative to hatchery fish if they are from a source population that is better suited to the novel environment, if they have evolved rapidly to become better adapted to their novel environment (e.g. Stockwell *et al.* 2003) or if the hatchery fish suffer from negative fitness consequences of domestication selection (e.g. Reisenbichler & Rubin 1999). The viability of naturalized populations, therefore, could also be reduced by ongoing stocking of hatchery fish that are less adapted to conditions in the wild.

In the US State of Minnesota, there are concerns about negative interactions between hatchery and naturalized populations of rainbow trout *Oncorhynchus mykiss* in the State's waters of Lake Superior. Rainbow trout from various Pacific coast sources were translocated into Lake Superior beginning in the late 1800s and many naturalized anadromous populations now spawn in tributaries throughout the lake (MacCrimmon & Gots 1972; Kreuger and May 1987). Naturalized rainbow trout have provided a popular recreational fishery in Lake Superior for almost a century, but catch rates in Minnesota have declined since peaking in the 1960s (Negus 1999). In the late 1960s, the Minnesota Department of Natural Resources (MNDNR) introduced a hatchery strain of rainbow trout to create new fishing opportunities (Close & Hassinger 1981). The MNDNR once stocked this hatchery strain along much of the Minnesota shore of Lake Superior but now stocks only a few streams because of concerns about potential negative impacts of hatchery fish on naturalized populations (Negus 1999).

The declines in naturalized populations coincident with the stocking of hatchery fish prompted several studies into

the interactions among naturalized and hatchery rainbow trout in Minnesota tributary streams. Close (1999) confirmed that naturalized and hatchery rainbow trout interbred in a natural stream environment following release of gravid adults into a confined section of stream, yet Krueger *et al.* (1994) found no genetic evidence for successful reproduction of hatchery fish in the wild. However, their study could not exclude the possibility of nonintrogressive hybridization, i.e. production of hybrids but poor survival to the age at which offspring were sampled. In hatchery mating experiments, Negus (1999) showed that hybrid crosses had higher egg-to-hatch mortality and their fry displayed less wariness compared to naturalized crosses. This lesser wariness was postulated to reduce survival through increased predation in the wild (Negus 1999).

Here we report on experiments involving crosses between naturalized and hatchery anadromous rainbow trout to assess outbreeding depression in natural stream environments. We tested the hypothesis that offspring of naturalized and hatchery rainbow trout, and reciprocal hybrid crosses, survive equally from fry to age 1+ in isolated reaches of Lake Superior tributary streams in Minnesota. The previously described studies (Krueger *et al.* 1994; Close 1999; Negus 1999) suggest that naturalized rainbow trout are better adapted than hatchery rainbow trout to Lake Superior and its tributary stream environments. This study examines an early in-stream component of the life history of *O. mykiss* that may contribute to the superior fitness displayed by naturalized fish and may further explain the apparent lack of introgression of hatchery genes into naturalized rainbow trout populations.

Materials and methods

Study populations

The history of the hatchery strain, locally called 'kamloops', is uncertain. Despite the name, genetic analysis indicated that they more closely resembled Pacific Coast anadromous populations than inland rainbow trout from the Kamloops region of British Columbia (Krueger *et al.* 1994). The strain was developed from eggs shipped from the Ennis National Fish Hatchery in the late 1960s. The Minnesota hatchery strain is propagated through mating of feral adults at the MNDNR French River Coldwater Hatchery. Offspring are reared in the hatchery and stocked as yearlings (age 1+) in or near mouths of tributary streams, including the French River (46°90' N, 91°89' W). Hatchery fish migrate to Lake Superior and mature before returning to the French River in sufficient numbers to sustain the hatchery programme.

Two naturalized anadromous rainbow trout populations were used in the study. In the first year we used naturalized adults from the French River spawning run, distinguishable from hatchery fish by their lack of clipped fins.

In the second year we used Knife River adults because of the timing and low abundance of naturalized fish in the French River spawning run. The Knife River (46°94' N, 91°77' W) is a relatively productive trout stream located 10 km northeast of the French River. Both of the naturalized populations have been exposed to some recent hatchery influence. In the early 1960s, the MNDNR constructed a complete barrier to upstream migration near the French River Hatchery. Since that time the MNDNR has collected all naturalized French River adults, mated them in the hatchery and stocked fry back into the river above the barrier to sustain the population. Thus, selective pressures related to spawning behaviour and egg mortality have been altered for the French River population. Knife River spawners used in the second year were stocked offspring whose parents were collected from the wild. Therefore the Knife River parents had minimal captive history consisting of one generation of rearing to yearling age in the hatchery.

In this study, we use different names and symbols than those used in earlier reports on Lake Superior rainbow trout in Minnesota. Close (1999) and Negus (1999) used the vernacular name kamloops for what we call hatchery rainbow trout (H) because this strain derives from a domesticated broodstock and has always been sustained in Minnesota by hatchery spawning (Negus 1999). These same reports used the name steelhead for their wild-caught naturalized anadromous rainbow trout, which came from French River. We use naturalized rainbow trout (N) collectively for the French River and Knife River populations described above.

Hatchery matings

We made crosses within and between naturalized and hatchery rainbow trout for 2 consecutive years at the MNDNR French River hatchery. These matings produced four cross types, two pure strain crosses (N × N and H × H) and two reciprocal hybrid crosses (N × H and H × N). Spawning run adults were captured in a pool at the mouth of the French River and held in the hatchery until ready for mating. One male was mated to one female and eggs from each pair were kept separate until tested for bacterial kidney disease. An effort was made to select similar-sized females to reduce possible maternal size related effects on fry survival (Einum & Fleming 2002), although one available female for a 1999 N × H cross was considerably larger than the other spawners. When disease tests returned negative, eggs from the same cross type were combined and placed in a separate hatch tray for incubation. Each year we made two sets of matings approximately 1 week apart to reduce the chance that a hatchery or natural catastrophe would destroy the entire experiment. In 1999, two pairs of each cross type were spawned on 22 April and 28 April for a total of four

families per cross type. In 2000, four families of each cross type were spawned on 28 April and 2 May for a total of eight families per cross type. One N × N and one H × H cross had an extra male because of poor milt yield from the initial male used in the matings. Fin tissue from each adult was stored in 95% ethanol for genetic analysis.

Eggs were incubated under normal hatchery conditions using water drawn from Lake Superior. Eggs hatched in approximately 1 month and shortly after swim-up, hatchery personnel estimated the size and counted the number of fry in each hatch tray before distributing them into containers for stocking. Average size for each cross type was determined as the total weight of a subset of fry divided by the number in the subset. No individual measurements were taken so standard errors for statistical testing could not be calculated.

In-stream experiment

Known numbers of fry from each cross type were stocked into control reaches of Amity Creek (48°83' N, 92°01' W) and the Sucker River (46°93' N, 91°83' W), two streams that enter Lake Superior 11 km southwest and 4 km northeast, respectively, from the French River. Like most tributaries in this region, these streams have only a few kilometres available to migrating fish before encountering an impassible barrier falls. However, they have additional suitable rearing habitat for salmonids above the barrier falls, habitat into which the MNDNR routinely stocks rainbow trout fry (Close & Anderson 1992). During the study period, only our experimental rainbow trout were stocked in these above barrier reaches. In mid-June, equal numbers of fry from each cross type were stocked on each of 2 days separated by approximately a week (2000 per cross type each day in Amity Creek and 3000 per cross type in the Sucker River). The one exception was the second stocking in 1999, when 1500 and 2000 of the H × H cross type were stocked in the two streams, respectively, because of reduced fry production in the hatchery. Fry were stocked in several locations throughout the stream to avoid locally high densities (Close & Anderson 1992).

We sampled surviving offspring by electroshocking during the two summers following stocking (i.e. at age 0+ and age 1+). Water levels determined primarily when we could sample the streams effectively. Age 0+ offspring were sampled 70–76 days and 40–46 days after stocking in 1999 and 2000, respectively. Age 1+ offspring of both year classes were sampled approximately 13.5 months after stocking. Electroshocking began far enough downstream of the stocking sites that few fish were initially caught, indicating limited dispersal below these locations. We continued upstream through the stocked area until fish were no longer caught or we reached an impassible barrier. We sampled age 0+ fish with a goal of collecting 100–150

individuals and conducted two to three passes to collect every age 1+ fish possible. In 2000 and 2001, fish were euthanized and stored on ice until they could be frozen. In the laboratory we measured total length to the nearest 1 mm and collected tissues for genetic analysis. In 1999 we intended to collect whole fish, but poor sampling success due to high water levels led us to collect fin clips in ethanol and return the fish to the streams unharmed. Therefore length measurements were not obtained for 1999 age 0+ fish.

Genetic analysis and parentage assignment

We identified the parents, and thus cross type, of sampled offspring using parentage assignment based on four to six microsatellite DNA loci [*Ogo3* (Olsen *et al.* 1998), *Omy77* (Morris *et al.* 1996), *Oneμ2* and *Oneμ3* (Scribner *et al.* 1996), *Ssa85* (O'Reilly *et al.* 1996) and *Str2* (Estoup *et al.* 1998)]. DNA was extracted from tissue samples using a 250 μL 5% chelex (Sigma Chemical, St Louis, MO) solution following the protocol of Miller & Kapuscinski (1996) and stored at -20 °C. Microsatellite amplification via the polymerase chain reaction (PCR) was performed in 15 μL reactions containing 1 × polymerase buffer (10 mM Tris-HCl, 50 mM KCl, 0.1% Triton® X-100), 1.5 mM MgCl₂, 0.2 mM each dNTP, 0.5 μM of each primer with the forward primer fluorescently labelled and 0.5 U *Taq* DNA polymerase (Promega, Madison, WI). Each set of samples included a repeated individual as a positive control for allele scoring and a water blank as a negative control to detect possible contamination of PCR solutions.

Amplification was carried out in a thermocycler (Hybaid Omn-E, Thermo-Hybaid US, Franklin, MA) using 35 cycles and a 50 °C annealing temperature. We submitted PCR products to a genetics core facility (Advanced Genetic Analysis Center, University of Minnesota, St Paul) for electrophoresis on an ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA). Allele scores were determined relative to an internal size standard in each lane using GENOTYPER software (Applied Biosystems).

We used the computer program PROBMAX (Danzmann 1997) to exclude systematically parent-pairs whose genotypes at one or more loci were incompatible with the offspring genotypes. When only the true parent-pair remained, the offspring was placed into one of the four cross types based on mating records for its parents. Genetic variation at the loci was sufficiently high that no offspring was assigned ambiguously to parents from more than one cross type, obviating the need for a likelihood parentage assignment approach (e.g. Marshall *et al.* 1998).

Statistical tests

We used χ^2 analysis to test the null hypothesis of no difference between the expected proportion of each cross

type (i.e. the proportion at stocking) and the known proportion in each sample. We also compared hybrid cross types (N × H and H × N combined) with each of the pure strain cross types in separate tests (i.e. N × H + H × N vs. N × N and N × H + H × N vs. H × H). Maternal or paternal effects were tested by combining cross types with common female or male strains, respectively. Finally, we tested the null hypothesis of no change from age 0+ to age 1+ in the proportion of the N × N cross type relative to all other cross types combined. A sequential Bonferroni correction (Rice 1989) was made to adjust for multiple tests.

We used analysis of variance (ANOVA) to test the null hypothesis of no difference in total length among all cross types. When differences were significant, we used the Tukey test for multiple comparisons to determine which pairs of cross types differed significantly.

Results

Parentage assignment

The six microsatellite loci had seven to 24 alleles, which provided sufficient power to assign offspring uniquely to parent pairs. Because we had samples for all parents in our study, we were able to use their genotype information to resolve some allele scoring discrepancies. Approximately 20 offspring did not assign to any parents after initial allele scoring due to a discrepancy at a single locus. Upon repeated examination or running of the sample, the genotypes were consistent with a parent-pair in all but one case. In most instances, the initial genotyping error was due to a missing allele that could be attributed to diminished signal from large allele drop-off (Taberlet *et al.* 1999) at loci with large size ranges [*Ssa85*–66 base pairs (bp); *Oneμ2*–104 bp]. One offspring's genotype was consistent with a parent pair except for one allele that differed by 2 bp. This may have been due to a mutation, but we eliminated this individual. It would have added another N × N fish to the 1999 Sucker River data.

Survival comparisons

For both year classes the proportion of cross types shifted significantly from those of the fry stocked in each stream, indicating that survival depended on the ancestry of the offspring (Fig. 1, Table 1). Maternal effects were important at age 0+. Offspring of naturalized females had significantly greater survival than offspring of hatchery females in two of four comparisons after adjustment for multiple testing. In contrast, there was no significant paternal effect at age 0+.

The N × N offspring outperformed all others in survival to age 1+ and hybrids had reduced, but intermediate, survival relative to the two pure crosses (Fig. 1, Table 1). The

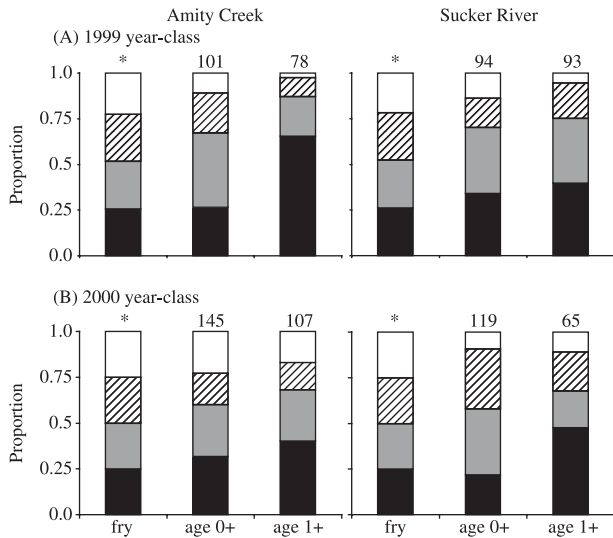


Fig. 1 Changes in the proportions of offspring from four cross types following stocking of fry produced by matings within and between hatchery (H) and naturalized (N) rainbow trout. The cross types were (female first): N × N (black bars), N × H (grey bars), H × N (hatched bars) and H × H (white bars). Fry were stocked in 1999 and 2000 into Amity Creek and Sucker River, Minnesota tributaries to Lake Superior. The numbers above each bar represents the sample size examined at each life stage (age 0+ and age 1+). The asterisks denote the initial numbers of stocked fry, which were approximately 8000 each year in Amity Creek and 12 000 in Sucker River.

N × N cross type increased in proportion from age 0+ to age 1+ across all comparisons (significantly in two comparisons), and became the most abundant in all cases. The other three cross types had similar proportions or large decreases between the sampling periods. Crosses with

naturalized females had significantly greater survival in three comparisons and crosses with naturalized males had greater survival in two comparisons. The combined hybrid cross types had significantly lower survival than N × N in two of four comparisons at age 1+. Hybrid cross types had significantly higher survival than H × H for the 1999 years class in Sucker River. Averaging over both years and streams, survival relative to N × N offspring was 0.59 for N × H, 0.37 for H × N and 0.21 for H × H offspring (Table 2). Including the data of Negus (1999) for hatching success, we estimated egg-to-juvenile (age 1+) survival relative to N × N crosses of 0.58 for N × H hybrids, 0.30 for H × N hybrids and 0.14 for H × H crosses (Table 2).

Length comparisons

The relative size of fry of each cross type varied across stocking dates and years. In 1999, H × H and H × N fry were the second largest and smallest, respectively, at each stocking date. The N × N and N × H fry alternated as the first and third largest. In 2000, fry from the two N female cross types were consistently larger than fry from H female crosses (average fry weight approximately 15% greater). If size differences among stocked fry were real (we could not test for statistical significance; see Methods), they did not translate into corresponding size differences among juveniles. At age 0+, offspring length was equal among cross types with the exception of smaller H × H offspring in the Sucker River ($P < 0.01$) (2000 data only, 1999 data unavailable) (Fig. 2). Size differences were apparent among the survivors to age 1+. In three of four comparisons, N × N offspring were significantly smaller than all other cross types ($P < 0.01$ for each) while all other cross types

Comparison	d.f.	1999				2000			
		Amity Creek		Sucker River		Amity Creek		Sucker River	
		χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Age 0+									
All cross types	3	15.2	†	12.1	†	21.1	‡	7.0	0.07
Maternal (N♀ > H♀)	1	9.4	†	12.4	‡	5.8	*	3.0	0.08
Paternal	1	0.47	0.49	0.14	0.71	0.06	0.80	1.0	0.31
Age 1+									
All cross types	3	68.5	‡	22.6	‡	17.4	‡	19.6	‡
Maternal (N♀ > H♀)	1	37.1	‡	19.1	‡	13.5	‡	7.4	†
Paternal (N♂ > H♂)	1	16.7	‡	1.67	0.20	0.93	0.33	8.9	†
N × N > hybrid	1	37.5	‡	2.6	0.11	8.3	†	9.7	†
Hybrid > H × H	1	5.2	*	10.2	†	0.56	0.45	1.9	0.16
Age 0+ to 1+									
N × N > all others	1	25.2	‡	0.44	0.51	1.6	0.21	12.0	‡

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

Table 1 χ^2 values, degrees of freedom (d.f.) and associated probabilities (*P*) for comparisons among various crosses between hatchery (H) and naturalized (N) rainbow trout. Maternal and paternal cross types combine crosses with common female (e.g. N × N + N × H) and male strains (e.g. N × N + H × N), respectively. Hybrid cross type combines N × H + H × N. Statistics are for single tests; those tests at $P < 0.05$ that were not significant after sequential Bonferroni correction are indicated in italics

Table 2 Relative survival from fertilization to age 1+ for offspring of four cross types within and among hatchery (H) and naturalized (N) rainbow trout. Data for survival to hatch are from Negus (1999). Data for survival from fry to age 1+ are from the present study. The average and range across both year-classes and streams is given for relative survival from fry to age 1+. The streams differed in 1999 ($\chi^2 = 11.2$, 3 d.f., $P = 0.01$) but the rank order was the same: $N \times N > N \times H > H \times N > H \times H$. There was no difference between streams in 2000 and the ranking for the combined data was the same as in 1999

Cross type	Survival to hatch	Average survival from fry to age 1+ (range)	Cumulative relative survival (female first)
N × N	1.0	1.0	1.0
N × H	0.99	0.59 (0.33–0.89)	0.58
H × N	0.80	0.37 (0.16–0.49)	0.30
H × H	0.67	0.21 (0.05–0.42)	0.14

were of equal size (Fig. 2). In the one exception, the N × N offspring of the 2000 years class in Sucker River were the smallest but the only significant difference was between them and the N × H offspring ($P < 0.05$).

Discussion

We have documented an early life survival advantage by naturalized populations of anadromous rainbow trout over a more recently introduced hatchery population and outbreeding depression resulting from interbreeding between the two strains. Krueger *et al.* (1994) found no evidence for successful natural reproduction by this hatchery rainbow trout strain in Minnesota tributaries to Lake Superior; this conclusion was based on comparing protein polymorphisms resolved in hatchery fish and fish collected in the wild. However, Close (1999) documented successful reproduction by hatchery rainbow trout, at least in hybrid matings, in a confined natural stream environment. Reduced survival following egg deposition may have been a significant factor in the lack of detectable reproduction by hatchery fish in the study by Krueger *et al.* (1994). Relative survival among cross types generally paralleled the relative hatching success found by Negus (1999). Although we found a maternal strain effect on survival to age 0+ as reported by Negus (1999), we then found a shift in favour of N × N offspring at age 1 + 0. Having an entire naturalized genome, not just a naturalized mother, was important for survival over the first winter, a time of harsh environmental conditions in northern Minnesota streams. Furthermore, most adult returns in Minnesota derive from smolts that emigrate at age 2+ (Hassinger *et al.* 1974), so survival differences may enlarge after an additional year in the streams. Our results therefore indicate that naturalized rainbow trout are better

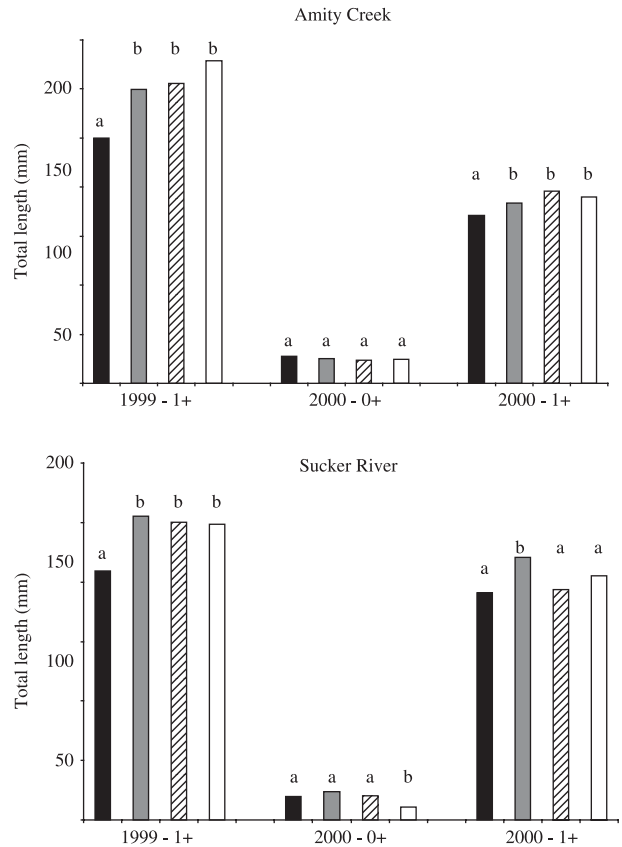


Fig. 2 Mean total length of offspring from four cross types produced by matings within and between hatchery (H) and naturalized (N) rainbow trout. Offspring from two year classes were sampled in Amity Creek and Sucker River at age 0+ and age 1+ (length measurements for the 1999 years class available only for age 1+). The cross types were (female first): N × N (black bars), N × H (grey bars), H × N (hatched bars) and H × H (white bars). Within each comparison, different letters above the bars indicate significant differences at $P < 0.05$.

adapted to the conditions of Minnesota's tributaries to Lake Superior, so that they outperform the hatchery-propagated strain in the same manner that many native populations of salmonids outperform hatchery fish (e.g. Einum & Fleming 2001; Fleming & Petersson 2001). Continued stocking of hatchery fish may disrupt this apparent local adaptation, reducing the fitness of naturalized populations (Allendorf *et al.* 2001).

Limited availability of naturalized spawners for use in our study and concerns about the power to efficiently assign offspring to families limited the scale and design of our study. Our analysis using χ^2 tests assumed that the cross type of the stocked fry, not the family, had a greater effect on survival. Although family effects can contribute to survival differences in fish (e.g. Geiger *et al.* 1997; Unwin *et al.* 2003), several lines of evidence suggest that we have detected a true strain effect on survival. First, a study of these same strains (hatchery × naturalized French River

fish) found that strain effects were significant relative to among family variation in an ANOVA analysis of hatch rates using a diallel cross design (Negus 1999). Mortality rates among cross types differed by as much as 25% while 95% confidence intervals within cross types covered ranges of only approximately 1–8%. Second, our relative survival rates among cross types were consistent across both years and in accord with relative hatch rates in the Negus study. Finally, an examination of family specific data suggests that rare atypical families have not occurred in our data. Family sizes were consistently low for crosses with hatchery females. For age 1+, they ranged from 0 to 3 in H × H crosses and 0–7 in H × N crosses of the 1999 year-class, and from 0 to 6 in both crosses of the 2000 year-class. Naturalized female crosses had higher means and greater variance, especially the N × N crosses. Family sizes for pure naturalized crosses ranged from one to 24 for the 1999 year-class and 0–14 for 2000 year-class. In addition, the N × N cross had five and six families larger than any H × H or H × K family for the 1999 and 2000 year-classes, respectively. We did not analyse explicitly family-based data using an ANOVA approach because this would incorporate both hatch rate and juvenile survival effects, as families of the same cross type were reared together in the hatchery.

The differences in survival of the naturalized populations relative to the hatchery strain may have several genetic causes that are interacting and cannot be distinguished by this study. First, the naturalized strains may have become better adapted to the Lake Superior environment since their introduction. Rapid evolution over contemporary time scales is well documented for numerous species, including fish (see review by Stockwell *et al.* 2003). Adaptation can sometimes be rapid: guppies (*Poecilia reticulata*) translocated to a new environment exhibited adaptive evolution over seven generations (Reznick *et al.* 1997). Several studies have indicated contemporary evolution in chinook salmon introduced to New Zealand (Hendry *et al.* 2000; Unwin *et al.* 2000; Kinnison *et al.* 2001; Quinn *et al.* 2001). Unwin *et al.* (2003) showed that distinct populations have become adapted to local streams so that resident naturalized populations had higher survival rates than did transplanted individuals from other regional naturalized populations. The potential for rapid adaptation implies that we should be cautious of using a population's non-native status to justify indiscriminant stocking over naturalized populations. If the goal is to maintain the naturalized population, then ongoing stocking of hatchery fish or transplants may reduce the fitness and viability of the established population.

A second explanation for the lower survival of hatchery fish is that they derive from a stock that is unsuited for Lake Superior's environment and therefore is less fit relative to other stocks that founded the current naturalized populations. If this is true, they are apparently less fit than

most of the possibly multiple founding stocks. We found lower survival by the hatchery strain compared to two different naturalized populations and there is an apparent lack of introgression of hatchery genes across Lake Superior tributary populations in Minnesota (Kreuger *et al.* 1994). It will be difficult to determine the potential role of source stock differences because of the uncertain history of the hatchery strain and the stocking of fish from California, Oregon and Washington, all of which could have contributed to the naturalized populations in Lake Superior (Kreuger *et al.* 1994). Strain differences have affected stocking success for other salmonids in this region, at least as measured by survival and angler harvest (Siesennop 1992). If reintroduction or naturalization is a goal, choice of founding sources is likely to be an important factor affecting its achievement.

A third explanation for survival differences is hatchery-induced decreases in fitness caused by domestication selection and inbreeding. This cause would be expected to reduce the performance of hatchery fish relative to naturalized fish whether or not the latter have become better adapted to their novel environment. Waples (1999) argues that some degree of domestication selection is inevitable in any hatchery programme. Reisenbichler & Rubin (1999) presented strong evidence that domestication reduces fitness in the wild for Pacific salmonids in studies unconfounded by nonlocal sources of hatchery broodstocks. Domestication selection could even be affecting our naturalized populations, particularly French River fish, which have been spawned in the hatchery and stocked as fry for the past 40 years following 50–70 years of reproduction in the stream. In contrast, the hatchery strain has always been maintained in Lake Superior as a hatchery strain stocked as yearlings. In addition, it derived from an existing hatchery strain maintained for an uncertain number of years in the federal hatchery system. If domestication has reduced the fitness of the naturalized populations, it should have lessened the survival differences among strains in our study; comparisons with other Lake Superior populations without hatchery influence may reveal even greater survival differences.

Inbreeding may also cause reduced fitness in the hatchery fish. The hatchery strain began with egg shipments from an unknown number of founders and has been maintained since by mating approximately 90 adults per year. In contrast the French River run averages less than 100 spawners per year and the Knife River has recently had adult run sizes of about 200–400 (MNDNR, unpublished data). For the microsatellite data used for parentage analysis, average allele sharing among the experimental spawners was 0.34 and 0.33 for the hatchery adults in 1999 and 2000, respectively, 0.17 for French River adults and 0.23 for Knife River adults. Although these data indicate that the hatchery population is more inbred, we did not see an alleviation of inbreeding depression, i.e. heterosis, in our

hybrid crosses. This suggests that different, or additional, genetic factors are contributing to reduced survival by the hatchery and hybrid crosses. Regardless of which genetic effects are responsible, negative interactions would affect the popular naturalized rainbow trout, compromising the MNDNR goal of restoring naturalized populations (Schreiner 2003).

Predator avoidance behaviour is a possible mechanism for instream survival differences among fry from the four cross types. Negus (1999) showed that $N \times N$ fry showed greater fright response than $H \times H$ fry, and hybrid fry showed intermediate wariness. Relative survival in our study was consistent with this trend in predator avoidance. Einum & Fleming (2001) reviewed several studies that examined hatchery effects on response to predators in salmonids, all of which showed lower response by hatchery fish compared to wild fish. Berejikian (1995) found that hatchery fish with lower ability than wild fish to avoid predators in laboratory trials had lower survival in natural stream enclosures.

Migration also may have contributed to observed survival differences. A portion of the naturalized rainbow trout in Minnesota streams migrate to Lake Superior prior to our age 1+ sampling date, but few of these early emigrants survive. Hassinger *et al.* (1974) found that 31% of juvenile rainbow trout emigrated at age 1+ in two Minnesota streams but that only 12% of the surviving adults returning to the streams had emigrated at less than age 2+. MNDNR studies at the French River weir found adult return rates of approximately 10% for age 2+ emigrants but only 0.5% for age 1+ emigrants (T. Close unpublished data). Thus, while different migration rates among cross types, if they occurred, would have affected our results, they would not alter our conclusions about survival differences because early migration nearly guarantees mortality before reaching sexual maturity.

Breeding competition probably interacts with survival differences to limit the introgression of hatchery genes into naturalized populations. Studies involving native salmonid populations have documented inferior mating success by hatchery fish. In a meta-analysis of experimental studies in seminatural conditions, Fleming & Petersson (2001) found sex differences in reproductive inferiority, with hatchery males less successful than hatchery females in breeding competition with wild fish. Fleming *et al.* (2000) showed that this male bias extended to breeding competition in the wild. We found that $N \times H$ hybrids had higher survival than $H \times N$ hybrids, but $N \times H$ hybrids would be uncommon if hatchery males are reproductively inferior. $H \times N$ hybrids would be more common but we found them less likely to survive. As a result, there would be fewer hybrid adults and less chance to initiate introgressive backcross matings with naturalized fish, than would be expected if the sexes mated randomly in hybrid crosses.

The smaller size of $N \times N$ offspring at age 1+ was unexpected, considering the generally equal size of all cross types at age 0+. The shift in relative size may have resulted from equal growth rates but differential size-dependent mortality among cross types. Close & Anderson (1992) showed that young rainbow trout in Minnesota tributaries needed to reach a minimum threshold size to survive over winter. It is possible that offspring with a hatchery parent ($H \times H$, $H \times N$ and $N \times H$) needed to obtain a larger threshold size to survive the winter than did the $N \times N$ offspring. In this scenario, offspring with a hatchery parent had lower survival but increased average size because only larger offspring survived. Alternatively, hatchery offspring may have had superior growth that was not expressed until after age 0+ and achieving this extra growth resulted in greater mortality, perhaps through increased exposure to predators when foraging for food (e.g. Johnsson 1993; Abrahams & Sutterlin 1999).

In Minnesota, spawning runs of hatchery adults support sport fisheries at the mouths of several streams, so the potential for hybridization with naturalized populations exists. The introgression of hatchery genes may reduce the fitness of naturalized populations through outbreeding depression. The lower survival of first-generation hybrids may limit the extent of long-term introgression as natural selection acts against fish with hatchery ancestry. However, in this ongoing hatchery programme, the potential production of first-generation hybrids year after year would continually waste a fraction of naturalized rainbow trout gametes due to the lower fitness of these hybrid offspring. Although rainbow trout are not native to Lake Superior, there is considerable interest in conserving their naturalized populations in Minnesota (Schreiner 2003). In contrast, hatchery rainbow trout support a popular sport fishery and are currently the only *O. mykiss* that anglers can keep. Our results suggest that these are competing management goals, making it difficult to achieve both simultaneously.

Fisheries managers are increasingly concerned about the impact of hatchery-propagated fish on wild fish populations (Schramm & Piper 1995). There is ample documentation of negative effects resulting from ecological and genetic interactions between such fish to warrant this concern (for reviews, see Hindar *et al.* 1991; Waples 1991; NRC 1995; Einum & Fleming 2001; Fleming & Petersson 2001; NRC 2004). Genetic effects probably explain, in part, why hatchery fish often fail to increase, and ultimately may decrease, the abundance and viability of wild populations (Reisenbichler & Rubin 1999; Fleming & Petersson 2001; Waples *et al.* 2004). Naturalized fish populations occur throughout the world, often supporting important sport and commercial fisheries (Lever 1996). The viability of these naturalized populations could also be compromised by continued stocking of translocated or hatchery-propagated fish. Fisheries management agencies may

therefore find it easier to sustain desired fishing levels on naturalized populations by monitoring fitness effects of hatchery stocking or translocation via appropriate field studies, as well as by addressing concern in formal risk-benefit assessment of any proposed new stocking or translocation programme.

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