

ARTICLE

Performance and cost advantage of stocking locally sourced Walleye fry into southern Minnesota lakes

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Abstract

Objective: A genetically distinct strain of Walleye *Sandervitreus* (southern Minnesota strain [SMS]) persists in southern Minnesota lakes despite decades of stocking more easily obtained strains from outside of the region. Because of the regional performance advantage inferred by this persistence, we conducted in situ experiments to compare survival and stocking cost of SMS Walleye fry against those of a frequently stocked northern Minnesota strain (Mississippi headwaters strain [MIS]) to determine whether it is beneficial to expand SMS fry stocking in lieu of historic practices.

Methods: We conducted four paired stockings of oxytetracycline-marked SMS fry and MIS fry into six southern Minnesota lakes and then sampled the fish as fall fingerlings during 2018, 2019, 2021, and 2022. We then used fluorescent microscopy and parentage-based tagging techniques to discern the stocked strains for comparison of growth and survival. We also obtained production and stocking cost data from agency records to calculate relative stocking costs.

Result: By the end of their first summer, SMS Walleye from all but two stockings exhibited higher survival than MIS Walleye. Despite higher initial costs to produce SMS fry, their higher survival to fall fingerlings made the stocking of SMS fry more cost effective than MIS fry stocking. Natural reproduction was also identified in several stocked lakes, most of which consisted of either high or increasing levels of SMS ancestry.

Conclusion: A local strain can outperform nonlocal strains at a level that can overcome additional costs of developing a new egg source. In addition, natural reproduction of local-strain Walleye may reduce or eliminate the need for future stocking.

KEYWORDS

early life history, fisheries, genetics, tags and tagging, Walleye

INTRODUCTION

Stocking is a widespread and frequently conducted fisheries management activity. The latest survey of U.S. management agencies (Halverson 2008) documented that stocking was conducted nationwide, with a cumulative total of 1.7×10^9 fish stocked in 2004 alone. Because of this magnitude, improvements in stocking practices can have substantial implications for fisheries and for the operating costs of management agencies across the

continent. Fish have been widely distributed by managers even though previously isolated populations may have diverged genetically due to selective pressures and random genetic drift (Allendorf et al. 2022). This divergence often results in local adaptations that may be important in different ways for different stocking scenarios. First, supplementing wild populations with fish from a different source creates gene flow between populations that may have been isolated through a lack of physical connectivity. Gene flow can cause a reduction in fitness

if introduced genes are poorly adapted to the conditions into which they are stocked, or gene flow can result in unfavorable interactions between inherited genes from fish with different co-adapted gene complexes (Templeton et al. 1986). This type of fitness reduction is termed “outbreeding depression,” and its risk increases when stocked fish are known to differ genetically from the recipient population or when stocked fish come from waters with substantially different environmental conditions (Philipp 1991; Miller and Kapuscinski 2003). Many stocking programs now include a goal of maintaining genetic integrity of local populations to reduce the risk of outbreeding depression (Jennings et al. 2010; Porak et al. 2015; Hammen and Sloss 2019). Second, local adaptations can benefit introductory stockings when a local population exhibits prior adaptation to conditions in introduced waters (Vahsen et al. 2018). This type of adaption can occur when environmental selective forces are similar between the source and recipient waters, leading to better survival and a higher potential for establishing a self-sustaining population. In both stocking scenarios, principles of local adaption suggest that the choice of source population will have an impact on the performance of stocked fish.

Due to its popularity as a sport fish, the Walleye *Sander vitreus* is the most frequently stocked percid in North America (Halverson 2008; Kerr 2011). The Minnesota Department of Natural Resources (MNDNR) alone stocks Walleye into 1113 lakes at an annual cost of over US\$3 million (P. Phelps, MNDNR, personal communication). Minnesota fisheries managers have been stocking Walleye since at least 1910 (Cobb 1923), and the selection of a brood source in early decades was based only on availability and efficiency. Later, increased awareness of the genetic heterogeneity of native Walleye populations (McInerny et al. 1991; Billington et al. 1992; Fulton et al. 1992; Fields et al. 1997) and concerns about outbreeding depression prompted changes to stocking by MNDNR. The resulting guidelines (MNDNR 1996) recommended following the genetic management units concept expounded by Palsbøll et al. (2007) by stocking fish from local sources within the same major watershed whenever possible. Stocking fish from local sources was most practical in the northern two-thirds of the state, where most egg collections occurred. However, southern Minnesota continued to be stocked with Walleye obtained from northern sources because the demand exceeded the output of local egg sources. This stocking practice was considered harmless in southern Minnesota because a combination of (1) largely agricultural watersheds with degraded spawning habitat and (2) a long history of stocking nonlocal fish was thought to have diminished genetic

Impact statement

We show that a local Walleye strain can outperform nonlocal strains at a level that can overcome additional costs of developing a new egg source. In addition, higher natural reproduction of the local strain may reduce or eliminate the need for future stocking.

integrity in the remaining native Walleye populations. However, recent genetic analysis using both microsatellite data (L. M. Miller, unpublished data) and single-nucleotide polymorphism data (Bootsma et al. 2021) indicated that a distinct strain of Walleye associated with the Minnesota portion of the Mississippi River watershed below St. Anthony Falls (Figure 1) persists in numerous southern Minnesota waters despite decades of stocking fish from nonlocal sources. Persistence of this southern Minnesota strain (SMS) suggests that it has survival or reproductive advantages over the northern Minnesota strains that were historically stocked into these waters. Genetic studies elsewhere have also documented the persistence of native Walleye strains despite extensive stocking of fish from nonlocal sources (New River, Virginia: Palmer et al. 2006; Ontario bays of Lake Superior: Wilson et al. 2007). Further, it is likely that Eldridge et al. (2002) inadvertently observed superior performance of the SMS in southern Minnesota lakes when wild fish increased in relative abundance during a stocking assessment of two northern Minnesota strains. Because of the possible performance advantage of the SMS in southern Minnesota lakes, an annual egg collection was initiated at Lake Sarah, Minnesota, in 2015 and the fry produced from those eggs were distributed to other lakes across the area. However, the Lake Sarah egg collection is labor-intensive and inadequate to provide enough fry for the 260 lakes and seven rivers that are stocked in Minnesota's portion of the Mississippi River watershed downstream of St. Anthony Falls.

The goal of this study was to directly compare survival and stocking costs of SMS Walleye fry against those of a frequently stocked northern Minnesota strain (Mississippi headwaters strain [MIS]) in southern Minnesota lakes. First-summer survival of SMS Walleye was compared to that of MIS Walleye by simultaneously stocking equal numbers of fry of each strain into six different study lakes and then—through identification using oxytetracycline (OTC) and parentage-based tagging (PBT; Steele et al. 2019)—comparing the ratio of the strains in fall age-0 samples. We focused on the fall fingerling life stage because we were

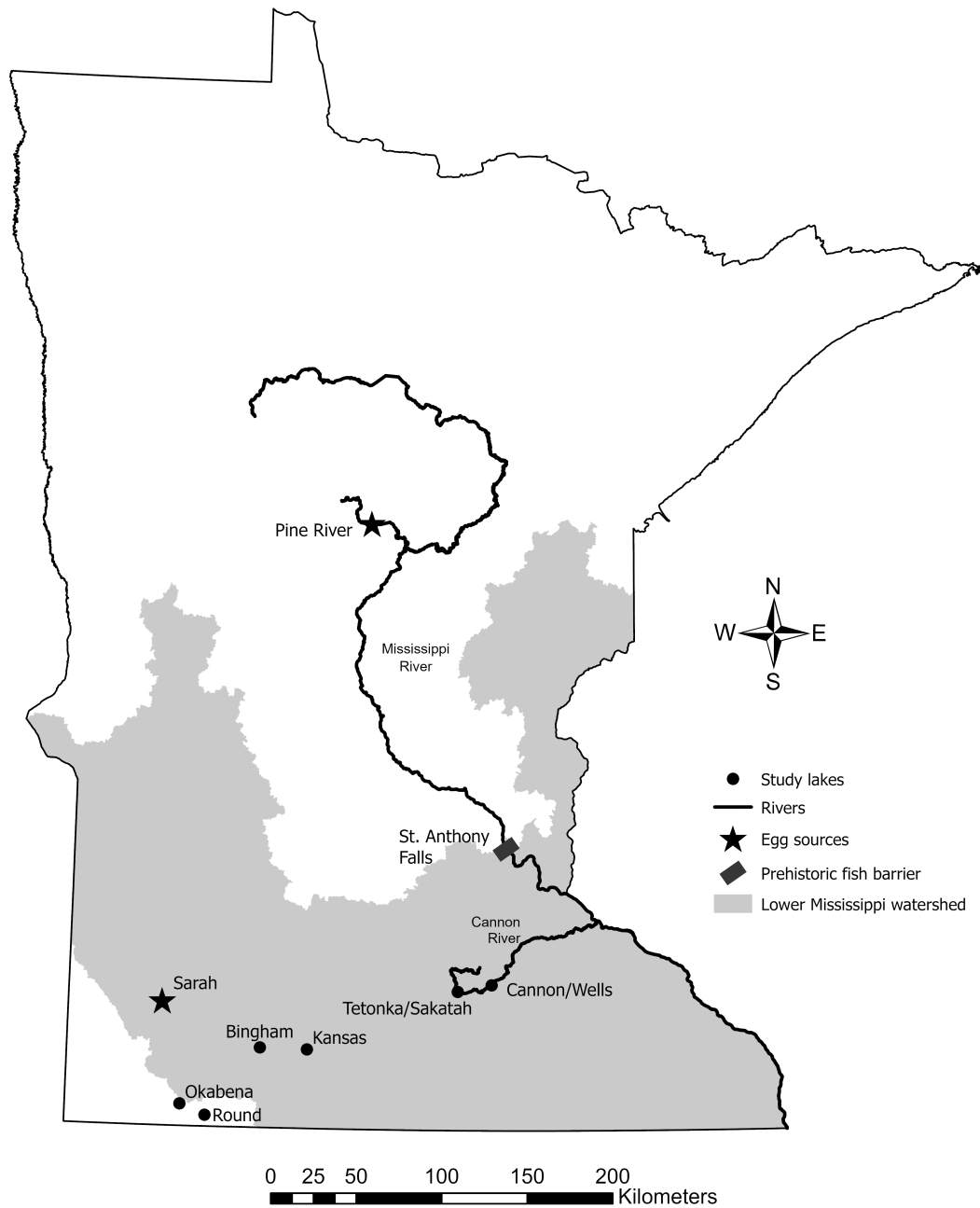


FIGURE 1 Map of Minnesota, showing the locations of the study lakes, Walleye egg sources, St. Anthony Falls (prehistoric barrier to upstream fish movement in the Mississippi River), and watersheds in Minnesota that drain to the Mississippi River downstream of St. Anthony Falls.

able to obtain adequate sample sizes through electrofishing without the bycatch mortality typical of gill nets used by MNDNR to collect Walleye at older ages. Although survival to the fall fingerling stage does not guarantee recruitment to a fishery, it is a prerequisite, and fall fingerling catch per unit effort is often used as an index of potential year-class strength (Kempinger and Carline 1977; Lucchesi 2002; Hansen et al. 2012). Differences in lengths at capture between the strains were also investigated as a measure of growth, a variable that could provide a competitive advantage through

enhanced feeding opportunities (Mathias and Li 1982; Galarowicz et al. 2006; Bozek et al. 2011) or reduced susceptibility to predation (Walters and Martell 2004; Kerns and Lombardi-Carlson 2017). Because of the large costs involved in Walleye stocking, we also calculated the costs to stock each strain and then accounted for differences in survival to obtain a relative cost per return of fingerling for each strain. Finally, we compared microsatellite DNA genotypes of unstocked fish in our samples against those of known source populations to identify the ancestry of wild fish from our study lakes.

METHODS

Study area

Two sets of lakes, representing both native and introduced Walleye populations, were selected to evaluate relative SMS survival and stocking costs in southern Minnesota (Figure 1). The first set of lakes included a series of eutrophic water bodies in the upper reaches of the Cannon River, where the SMS is likely the native strain (Miller, unpublished data), and the river is a probable source of SMS fish that became naturalized in Lake Sarah. The Cannon River is a tributary to the Mississippi River downstream of St. Anthony Falls, but the lakes are separated from the lower reaches of the Cannon River by a dam (built in 1862) that prevents upstream fish movement. Lakes of the Cannon River chain that were of interest during this study were Lake Tetonka, Upper Sakatah Lake, and Lower Sakatah Lake, which we treated collectively as one lake (Tetonka/Sakatah Lake) because of the ease of fish movement between them; and Cannon and Wells lakes, which were similarly treated as a single lake (Cannon/Wells Lake) for analysis. Over 100 million Walleye fry sourced from northern Minnesota have been stocked into the Cannon River system lakes since 1982.

The second set of lakes consisted of four isolated basins in southwest Minnesota (Lake Okabena and Kansas, Round, and Bingham lakes) where Walleye are not native but have been stocked by MNDNR for decades. Records indicate that only northern Minnesota strains were stocked there between 1992 and 2015, but it is possible that previous stockings included SMS fry from eggs collected in the Cannon River between 1982 and 1992. These lakes are also near Lake Sarah, where a naturalized Walleye population supports the recently developed SMS egg collection, and they are of similar latitude and trophic status as the Cannon River system.

Stocking

Newly hatched SMS fry were stocked in equal abundance with MIS fry into each study lake during 2018, 2019, 2021, and 2022. Lakes were stocked at total combined densities from 417 to 510 fry/littoral acre (1030 to 1260 fry/littoral hectare; <4.6 m deep), following the MNDNR Walleye Stocking Operational Plan that was in effect at the onset of this study (MNDNR, unpublished internal report). Mississippi headwaters-strain fry that were produced from eggs collected at the Pine River, Minnesota (Figure 1), were selected as a reference with which to compare SMS stocking success because the MIS was most frequently stocked into the lakes during recent years. In addition, a

spawning run up the Pine River occurs at a similar time as that of Lake Sarah, which allowed for an overlap of hatch dates with those of SMS eggs.

Caudal fin clips of broodstock adults were collected during all years at Lake Sarah and during 2021 and 2022 at the Pine River for strain identification through PBT of their offspring. Sampling included all parents that provided eggs to Waterville State Fish Hatchery for stocking the study lakes and other lakes across southern Minnesota. In total, 359–532 adults/year were used for PBT at Lake Sarah and 128–157 adults/year were used at the Pine River. Individual contributors were recorded by spawning batches, which predominantly included eggs of one or two females and two males fertilized simultaneously, but some batches involved as many as four females or males. After water hardening, eggs of both strains were transferred to the hatchery, where they were incubated simultaneously but in separate batteries of egg jars until hatch. Immediately after hatching, fry were enumerated gravimetrically with a formula relating fry weight to egg size ($\text{fry/kg} = 2.084 \times [\text{eggs/L}]$; A. Scholten, MNDNR, personal communication) and then were immersed for 6 h in a 700-mg/L solution of OTC to produce a fluorescent mark on their otoliths for later identification (Lucchesi 2002; Logsdon et al. 2004).

Equal numbers of OTC-treated fry of each strain were stocked immediately after treatment into the same offshore locations of the lakes. Both strains were transported and stocked together during 2018, 2019, and 2021, whereas the strains were transported separately and stocked 1 day apart during 2022. Samples of OTC-treated fry were also stocked into rearing ponds at Waterville State Fish Hatchery each year to evaluate mark production and retention.

Sampling

Age-0 Walleye were sampled with pulsed-DC electrofishing during evenings in September and October in each year of stocking. Target sample sizes for age-0 Walleye were determined by first calculating the sample size of stocked Walleye necessary to detect a difference of 10 percentage points from the 50%:50% (SMS : MIS) composition of stocked fry at an α of 0.05 and a power $(1 - \beta)$ of 0.80 (Lenth 2006), and the resulting value was then inflated to compensate for nonstocked Walleye from natural reproduction. Based on previously reported levels of natural reproduction, we initially sought to collect 210 age-0 Walleye from each lake. Target sample sizes were later raised to 350 Walleye in both Cannon/Wells Lake and Tetonka/Sakatah Lake for 2019, 2021, and 2022 after higher-than-anticipated levels of natural reproduction

occurred during 2018. We sampled multiple sites across different habitats to reduce the possibility of strain segregation bias (Eldridge et al. 2002), and all Walleye of lengths that could possibly be age 0 (i.e., based on previous aging data) were held on ice for processing the next day. All fish were measured for total length (TL) and weight, a caudal fin clip was collected and preserved in 95% ethanol for later strain identification, and either the head or the whole body was frozen for later mark inspection. One-hundred Walleye were also collected from rearing ponds during each October for mark efficacy evaluation.

Aging and mark detection

Immediately preceding mark inspection, samples were thawed and the sagittal otoliths were removed and secured to a microscope slide with cyanoacrylate cement. If inspection for annuli at 100× magnification confirmed that the fish were age 0, the otoliths were further prepared and inspected for an OTC mark following the methods described by Logsdon et al. (2004). Otoliths were inspected with a Nikon Eclipse 55i epifluorescent microscope with 100–200× magnification, fluorescent lighting, a 505-nm dichroic mirror, a 420–490-nm exciter filter, and a 520-nm barrier filter. Samples from rearing ponds were inspected prior to examining those from study lakes to assure that the treatment produced marks, to confirm microscope settings, and to become familiar with both the appearance and amount of polishing required for exposure of marks.

Strain determination and ancestry

Parentage or ancestry of sampled Walleye was determined via the methods described by Logsdon et al. (2009) to genotype tissue samples at 14 microsatellite DNA markers. We used markers *Svi4*, *Svi6*, *Svi17*, *Svi18*, *Svi26*, and *Svi33* from Borer et al. (1999); *SviL6* and *SviL9* from Wirth et al. (1999); *Svi2*, *Svi7*, *Svi16*, and *Svi20* from Eldridge et al. (2002); *SviL2* from Cena et al. (2006); and *Svi100* from Coykendall et al. (2014). Measures of genetic diversity, Hardy–Weinberg tests, and power to determine parentage were based on assessment of the 2018 Lake Sarah broodstock sample using the software CERVUS (Marshall et al. 1998; Kalinowski et al. 2007). CERVUS automates parentage assignment by determining whether an offspring shares alleles with each member of a candidate parental pair. Based on population allele frequencies, it calculates the likelihood that compatible parents are the true parents versus a random pair in the population. CERVUS also estimates a measure of power in parentage assignment known as nonexclusion probability, which is

the probability that a set of markers fails to exclude unrelated candidate parents from parentage of a random offspring. A low nonexclusion probability is desirable because it indicates a low chance of falsely assigning an individual to a nonparental pair.

Genetic analysis differed upon occurrence of an OTC mark. If a mark was present, then the genotype was compared to genotypes of the broodstock pairings at Lake Sarah (all years) and the Pine River (2021 and 2022) by using PBT analysis in CERVUS (Marshall et al. 1998; Kalinowski et al. 2007). Presence of an OTC mark and a match to a Lake Sarah broodstock pairing identified a fish as a stocked SMS individual, whereas the presence of a mark without a broodstock match in 2018 and 2019 or a match to a Pine River broodstock pairing in 2021 and 2022 identified a fish as a stocked MIS individual. CERVUS evaluates pairwise comparisons between all candidate mothers and fathers but not specific pairs. Therefore, CERVUS was used to first identify candidate mothers and fathers with the highest likelihood of being parents, and the results were then compared to mating records to confirm whether they were an actual pair.

Genotypes of a subset of fish without an OTC mark were compared using STRUCTURE (Pritchard et al. 2000) to reference genotypes from 11 historical stocking sources in Minnesota. STRUCTURE determines the most likely number of genetically distinct clusters (K) in a data set and then estimates, for each individual, the percentage of its ancestry contributed by each cluster. A series of 10 independent runs was evaluated for each number of distinct genetic clusters set between 1 and 12 using a burn-in period of 50,000 iterations, followed by 200,000 Markov chain–Monte Carlo steps with assumptions of admixture and correlated allele frequencies. A value of K was then chosen based on plateauing likelihood values and biological sensibility (Pritchard et al. 2010). The criterion used for biological sensibility was resolution of clusters that associated most closely with our reference populations. Ancestry from SMS Walleye was estimated as the average assignment within each sample to clusters associated with southern Minnesota reference populations. Baseline populations provided estimates of potential error in resolving ancestry between southern and northern strains.

Relative survival and growth

Exact binomial tests in the stats package of R version 4.2.2 (R Core Team 2022) were used to compare, by lake and year, the percentage of age-0 SMS Walleye in fall samples to the 50% that would be expected given equal survival of the two strains stocked in equal numbers. Therefore, values greater than 50% would indicate higher survival rates

of SMS Walleye relative to MIS Walleye, whereas values lower than 50% would indicate lower survival rates of SMS Walleye relative to MIS Walleye.

Relative first-summer growth of the two strains across all samples was then investigated with a linear model of TL at fall capture, which contained a two-way interaction effect between sample year and lake to account for lake-year-specific differences in growth, with a strain effect parameter added to estimate the difference between strains within each lake-year:

$$TL_{ijkm} = Y_i \times L_j + S_k + \varepsilon_{ijkm},$$

where TL_{ijkm} is the total length of the m th age-0 Walleye in year i from lake j , Y_i is year i , L_j is lake j , S_k is strain k , and ε_{ijkm} is a random error term. This analysis was also conducted in R (R Core Team 2022). Differences were deemed significant at p -values less than 0.05 for all statistical tests.

Relative stocking costs

Two different aspects of stocking cost were compared between strains. The first analysis compared the costs to produce and stock the two strains as fry. Salary and employee expenses were obtained through the Minnesota payroll system (SEMA4 [Statewide Employee Management]), supplies and utility expenses were obtained through the Minnesota purchasing system (SWIFT [Statewide Integrated Financial Tools]), and vehicle expenses were obtained through the Minnesota fleet management system. Fry stocking costs were estimated as the sum of costs to acquire and incubate the eggs required to produce stocked fry, by strain, plus fry transport costs:

$$\begin{aligned} \text{Fry stocking cost (by strain)} = & \\ [& (\text{Egg acquisition cost} + \text{Egg incubation cost}) \times \text{Eggs needed}] \\ & + \text{Fry transport cost.} \end{aligned}$$

Costs were calculated for each strain, year, and lake because each cost component varied for at least one of these factors. Costs for egg acquisition and incubation were calculated by dividing the total cost of each operation by the total number of eggs involved to determine a per-egg price. Egg acquisition costs included vehicle, labor, supplies, and utilities to collect and fertilize the eggs plus vehicle and labor costs to transport them to Waterville State Fish Hatchery. Incubation costs included the costs of labor, supplies, and utilities to incubate the eggs until hatch. The number of eggs needed to produce each strain of fry was calculated by dividing the number of fry stocked by the hatch rate of the strain. The cost of fry

production was then calculated by multiplying the sum of per-egg costs by the number of eggs needed to produce the fry stocked. Finally, the total cost of fry stocking was calculated as the cost of fry production plus the cost of fry transportation. Relative fry stocking costs for the SMS were then calculated as a percentage of MIS costs because northern Minnesota strains such as the MIS have typically been stocked into these lakes and are thus considered the baseline from which to judge the nonstandard practice of stocking SMS fry.

The second cost analysis accounted for differential first-year survival of the strains to determine the relative cost to produce surviving fall fingerlings through SMS fry stocking. This analysis was restricted to relative cost comparison between strains because the absolute number of surviving fall fingerlings was unknown, but the proportion of each strain in fall age-0 samples could be considered representative of the strain proportion in the population at the time of capture. Absolute cost per return of fingerling was first calculated by strain for each year and each study lake by dividing the total cost to stock the strain by the number of the corresponding strain captured in each fall age-0 sample:

$$\begin{aligned} \text{Cost per return of fingerling (by strain)} = & \\ (\text{Fry stocking cost}) / (\text{Fall fingerling catch}). & \end{aligned}$$

Relative costs per return of SMS fingerlings, by year and lake, were then calculated as a percentage of MIS costs.

RESULTS

Mark efficacy and genetic discrimination

Mark efficacy tests and genetic power analyses indicated a strong ability to identify stocked fish and their strain as well as the ancestry of wild fish. Otoliths from all but one Walleye inspected from efficacy ponds ($n=600$) expressed fluorescent marks when polished and viewed under an epifluorescent microscope. The otolith without a visible mark appeared vateritic (Campana 1999; Austad et al. 2020), with a very opaque appearance that prevented adequate light penetration to incite fluorescence. Otoliths of one Walleye from the study lakes also appeared vateritic, and the data from that fish were omitted from further analysis.

The 14 genetic markers had moderate to high genetic diversity in the 2018 Lake Sarah brood sample of 359 adults (heterozygosity range=0.54–0.89; number of alleles=5–18), providing power to identify the parent-age—and, thus, the strain—of stocked offspring. A single

locus had indications of possible deviation from Hardy–Weinberg equilibrium ($p=0.003$), but its expected and observed heterozygosity values were within 0.01. This locus was retained because the test was likely sensitive to small deviations from expected genotypes due to the large sample. Genetic diversity of the markers resulted in nonexclusion probabilities of 0.08–0.61 per marker, for a combined probability of 4.6×10^{-9} . Not all samples had complete genotypes, but even the eight markers with the lowest power had nonexclusion probabilities of 8.1×10^{-4} . These probabilities were based on population-wise error rates where any two fish were candidate parents. However, we had OTC marks to provide secondary confirmation of stocked fish and mating records to vastly reduce the number of possible parental pairs, thus further reducing the probabilities of false parentage assignment.

Ancestry estimates for wild fish were derived from STRUCTURE runs at a K -value of eight distinct genetic clusters. STRUCTURE likelihoods began plateauing at a K -value of 7, but an additional reference population was resolved as distinct at a K -value of 8. Four of the clusters had their highest ancestry in reference samples from the Mississippi River and its tributaries below St. Anthony

Falls, whereas the other four clusters had their highest ancestry in reference populations of historical northern Minnesota stocking sources. Estimates from the four clusters associated with southern Minnesota were then combined to obtain a single estimate of overall SMS ancestry. Southern Minnesota reference samples had average estimates of 88% SMS ancestry, while northern Minnesota samples averaged 15% SMS ancestry.

Relative survival and growth

For Walleye sampled in the fall at age 0, the catch of SMS fish in higher proportions than they were stocked indicated higher survival of the stocked SMS fry in 20 of the 22 stocking events (Figure 2). Proportions of SMS Walleye in all samples from Tetonka/Sakatah Lake, Cannon/Wells Lake, and Lake Okabena exceeded 80% ($p < 0.001$), whereas proportions of SMS fish in Kansas, Round, and Bingham lakes varied more among lakes and years. Proportions of SMS Walleye in samples from Kansas Lake exceeded 70% during all years, but those in Round and Bingham lakes were below 20% in 2018.

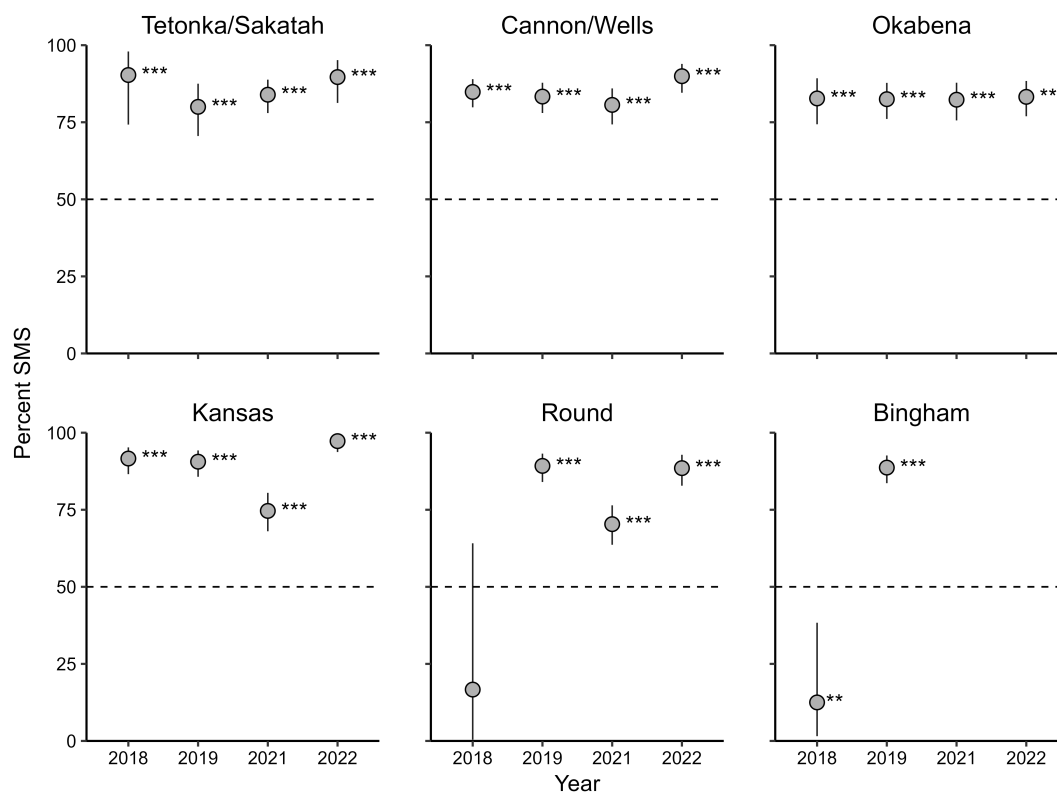


FIGURE 2 Percentages of fall samples of age-0 Walleye that were southern Minnesota strain (SMS) from experimental fry stockings in Minnesota lakes. Error bars represent 95% binomial confidence limits. Dashed lines represent the percentage of SMS fry stocked into each lake. The numbers of asterisks next to circles indicate thresholds of p -values from binomial tests and represent the probability of samples coming from a population remaining composed of equal numbers of SMS and Mississippi headwaters-strain fingerlings (*** $p < 0.001$; ** $p < 0.05$).

The 2018 sample sizes from Round and Bingham lakes were less than 10% of target values, resulting in wider confidence limits around the estimates relative to those of other samples (Figure 2). Binomial test results indicated lower survival of SMS Walleye in Bingham Lake during 2018 ($p=0.004$) but provided little support for lower survival of SMS fish in Round Lake during the same year ($p=0.220$). No age-0 Walleye were captured from Bingham Lake in 2021 or 2022. All sample sizes by source are presented in Table 1.

The TL at capture varied among lakes and among years within lakes (Figure 3). Although neither strain was consistently longer than the other in all lakes and years, the analysis of variance model contrasting the TL of the strains across all lake-year combinations identified a strain effect ($F=326.7$, $df=3457$, $p=0.0223$), with length

being 1.65 mm greater, on average, for the SMS than for the MIS.

Relative stocking costs

Costs to produce and stock SMS fry were consistently higher than those of MIS fry (Table 2; Figure 4). The greatest cost difference was in egg acquisition, with costs to obtain SMS eggs (Table 2) ranging from 20% to 130% higher than the costs of obtaining MIS eggs. Differences in incubation costs also contributed to the cost differential between strains. Incubation costs of SMS Walleye were higher than those of MIS fish in 2018 and 2019, lower than those of MIS fish in 2021, and similar to those of MIS fish in 2022 (Table 2).

Although SMS fry were initially more expensive to produce, the higher poststocking survival of SMS Walleye over that of MIS Walleye resulted in lower costs to produce fall fingerlings through fry stocking in all but two of the successful stockings (Figure 4). The cost per return of fingerling when the SMS had higher survival ranged from 43% to 96% less than those for MIS stockings. The two stockings that did result in higher costs to produce fall fingerlings occurred during 2018, when lower survival of SMS fish in Round and Bingham lakes combined with higher initial stocking costs resulted in nominal cost-per-return-of-fingerling values that were many times higher than those of MIS fish. The 2018 values from Round and Bingham lakes are of low confidence, however, because of the low sample sizes.

Natural reproduction

Percentages of wild fish in fall age-0 samples varied substantially among lakes and years (Figure 5). The highest catches of wild Walleye occurred during 2018, when the percentage of wild fish exceeded 90% in Tetonka/Sakatah Lake, 70% in Round Lake, and 40% in both Lake Okabena and Cannon/Wells Lake. The lowest catches of wild Walleye occurred during 2021, when percentages of wild fish fell below 5% in all lakes except for Lake Okabena. Wild fish in samples from the Cannon River system lakes (Tetonka/Sakatah and Cannon/Wells lakes) had consistently high SMS ancestry (72–89%), whereas wild fish from the southwest Minnesota study lakes (Lake Okabena and Kansas, Round, and Bingham lakes) had more ancestry from previously stocked strains. The southwestern lakes had 9–32% SMS ancestry in 2018 but later increased by 12–22 percentage points in each lake except Bingham Lake, where no individuals were sampled in 2021 and 2022.

TABLE 1 Sample sizes of Walleye fall fingerlings by source: stocked southern Minnesota strain (SMS), stocked Mississippi headwaters strain (MIS), and wild fish.

Lake	Year	Stocked fish (n)		Wild fish (n)
		SMS	MIS	
Tetonka/Sakatah	2018	28	3	310
	2019	76	9	234
	2021	162	31	4
	2022	78	9	301
Cannon/Wells	2018	218	39	181
	2019	200	40	56
	2021	154	37	6
	2022	161	18	39
Okabena	2018	91	19	93
	2019	146	31	35
	2021	135	29	47
	2022	149	30	16
Kansas	2018	164	15	7
	2019	183	19	8
	2021	150	51	4
	2022	178	5	16
Round	2018	1	5	18
	2019	174	21	19
	2021	147	62	16
	2022	154	20	26
Bingham	2018	2	14	6
	2019	188	24	3
	2021	0	0	0
	2022	0	0	0

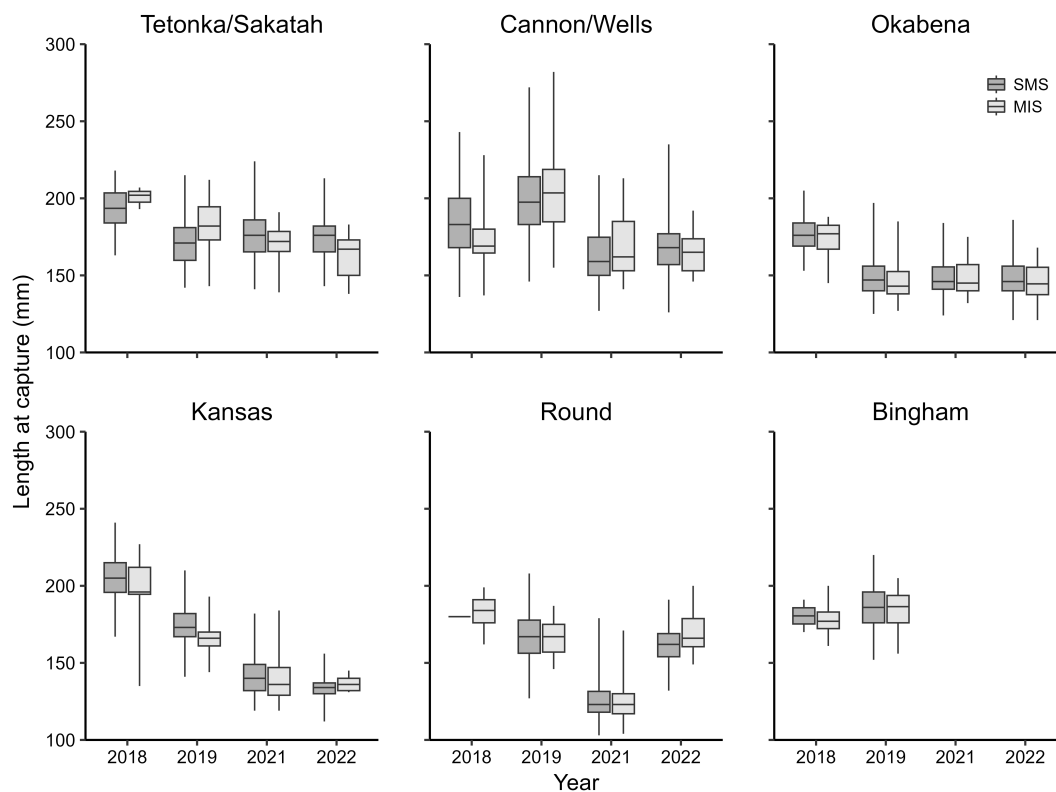


FIGURE 3 Box plots displaying ranges, medians, and interquartile ranges of total length at fall capture of southern Minnesota-strain (SMS) and Mississippi headwaters-strain (MIS) age-0 Walleye from the study lakes.

DISCUSSION

Given the extent of fish stocking (Halverson 2008), improvements in effectiveness and cost, such as those documented in this study, can provide immense benefits to fisheries management agencies. We showed that locally obtained SMS Walleye fry produced more—and less expensive—fall fingerlings than MIS fry when the two strains were stocked together in selected southern Minnesota lakes. These findings build upon those of Eldridge et al. (2002), who used PBT to evaluate survival between Walleye fry of two different northern Minnesota strains stocked into five southern Minnesota lakes. Stocked fry comparisons by Eldridge et al. (2002) were inconclusive regarding survival differences between the stocked strains, but they did document natural reproduction of a local-origin strain that increased in relative abundance across 2 years of their study. Our study compared stocking success of the same local-origin strain (SMS) identified by Eldridge et al. (2002) directly against one of the northern strains (MIS) but with the addition of OTC marking to identify and account for natural reproduction of resident SMS adults.

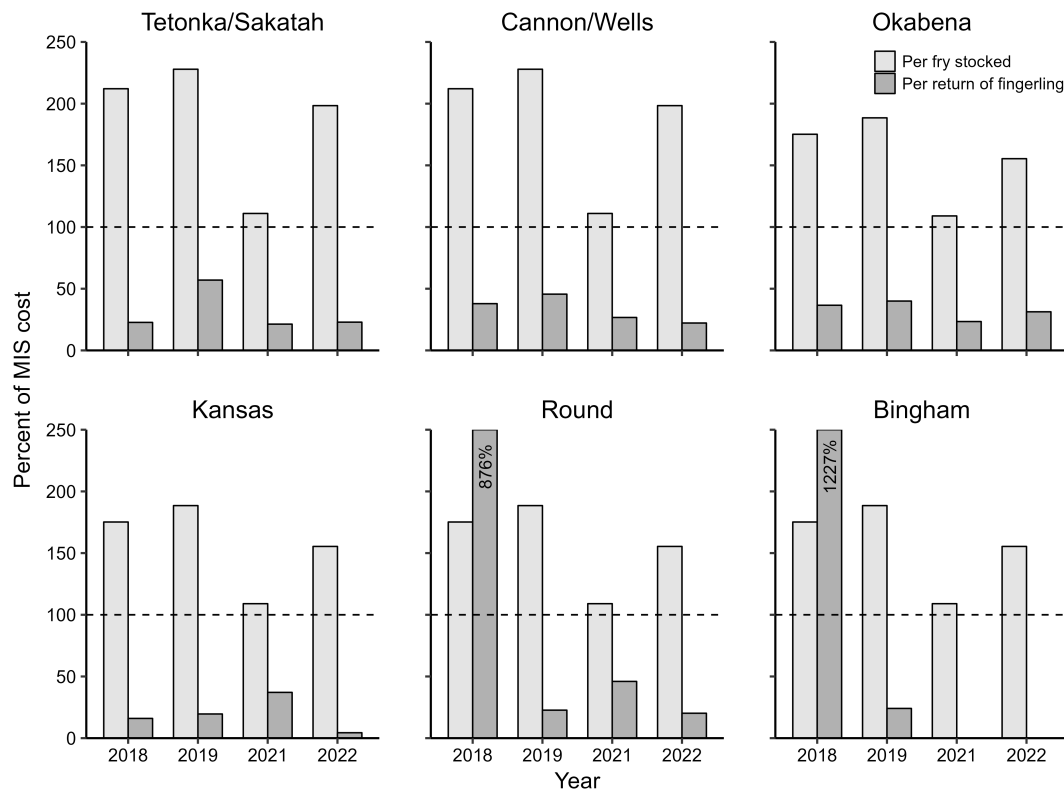
This study did not explicitly test for local adaptation, but the higher survival of stocked SMS fry and the persistence of SMS ancestry in wild fish are consistent with

higher fitness of SMS Walleye due to adaptation to environmental conditions in southern Minnesota waters. The robust SMS population in Lake Sarah and the higher survival of SMS fry in the other introduced populations of Lake Okabena and Kansas, Round, and Bingham lakes could be due to greater environmental similarity of the lakes in southwest Minnesota to that of original source populations of the SMS. The high SMS ancestry of wild fish from Tetonka/Sakatah and Cannon/Wells lakes indicates resistance of the native SMS population in the Cannon River system to introgression of northern alleles despite decades of intensive stocking with several northern Minnesota strains. Similar resistance to introgression has been identified in other remnant populations of Walleye (Stepien et al. 2004; Palmer et al. 2006; Wilson et al. 2007; Garner et al. 2013; Haponski et al. 2014) and other fish species (Piller et al. 2005; Turnquist et al. 2017; Kazyak et al. 2022).

A broad range of environmental conditions combined with long periods of isolation likely contributed to the adaptive divergence of Walleye populations across Minnesota. Minnesota spans over four degrees of latitude, four major watersheds, and multiple ecoregions, where a diversity of factors (e.g., temperature, precipitation, and aquatic productivity) varies along a southwest–northeast gradient (Heiskary et al. 1987). Walleye in northern

TABLE 2 Costs (US\$), by activity and year, to stock equal numbers of southern Minnesota-strain (SMS) and Mississippi headwaters-strain (MIS) Walleye fry into the study lakes.

Activity	2018		2019		2021		2022	
	SMS	MIS	SMS	MIS	SMS	MIS	SMS	MIS
Egg acquisition	2716	1089	1918	577	2083	1689	2598	923
Incubation	691	406	381	312	370	496	584	564
Stocking	566	566	421	421	472	472	804	804
Total	3972	2060	2720	1310	2924	2657	3985	2291

**FIGURE 4** Relative costs of stocking southern Minnesota-strain (SMS) Walleye fry expressed as a percentage of the cost to stock Mississippi headwaters-strain (MIS) fry into the study lakes. Light-gray bars represent fry stocking costs, whereas dark-gray bars represent stocking cost per return of fingerling. The dashed line represents the level at which the stocking costs of the strains are equal. Values above the line indicate that costs for stocking SMS fry are higher than MIS costs, whereas values below the line indicate that costs for stocking SMS fry are lower than MIS costs. Values that exceed the y-axis scale are presented on the bars.

Minnesota watersheds have been isolated from those of southern Minnesota for at least 10,000 years since the formation of St. Anthony Falls by the Glacial River Warren (Pielou 1991), but many populations of Walleye in Minnesota could have been isolated for much longer. Current Minnesota Walleye populations likely originated from a mix of Missouri and Mississippi refugial populations (Billington et al. 1992; Stepien et al. 2015) that were separated by the Laurentide Ice Sheet that was present during the Wisconsin Glaciation from approximately 100,000 to 11,000 years before present (Pielou 1991) but had temporary postglacial access to northern Minnesota

through connectivity via Glacial Lake Agassiz and the Mississippi River. Bootsma et al. (2021) identified multiple genetic loci that were highly diverged among populations across Minnesota and Wisconsin. This divergence suggests local adaptation. However, no studies have evaluated whether the divergence is related to adaptive selection pressures in the environment (e.g., Andrews et al. 2023).

Although Walleye stocking is a popular management activity across North America (Goeman 2002; Kerr 2011), its outcome is often unpredictable (Ellison and Franzin 1992). Supplemental stocking of lakes that have natural reproduction is least effective (Laarman 1978;

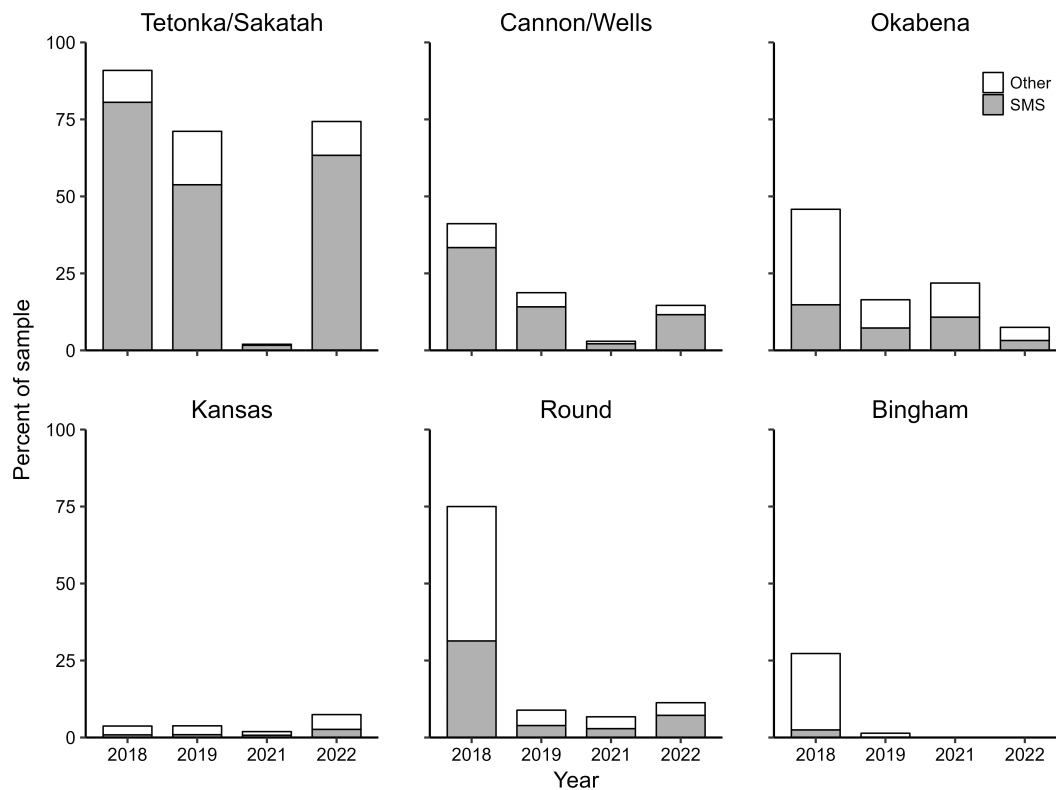


FIGURE 5 Percentages of wild fish present in fall samples of age-0 Walleye and their ancestral composition. Gray bars represent average southern Minnesota-strain (SMS) ancestry, whereas white bars represent ancestry from all other strains.

Li et al. 1996; Jacobson 2004), with only 5% of reported attempts considered successful (Laarman 1978). Introductory and maintenance stockings in lakes without natural reproduction were more successful—but only in 48% and 32% of attempts. Because of all the variables affecting Walleye survival (Chevalier 1973; Hansen et al. 1998; Hoxmeier et al. 2006), identifying genetic effects can be difficult and probably overlooked in many stocked populations. Our use of a paired-stocking protocol with stocked fish identification through OTC and PBT allowed us to account for annual variability in both environmental conditions and natural reproduction to isolate differences in survival that were attributable to genetic differences between strains. We also identified that survival differences among strains can occur during the first 5 months of life, but we could not identify a causal mechanism. We hypothesized that faster growth of a local strain would provide competitive advantages due to enhanced feeding opportunities (Mathias and Li 1982; Galarowicz et al. 2006; Bozek et al. 2011) or reduced susceptibility to predation (Walters and Martell 2004; Kerns and Lombardi-Carlson 2017). However, longer MIS fish occurred as frequently among individual lake-year samples as longer SMS fish even though SMS Walleye had higher survival in 90% of those same samples. The analysis of variance did estimate a 1.65-mm greater length of SMS fingerlings across all lake-year

combinations, but this size difference is less than 2% of the mean TL of fish in our samples and is unlikely to be biologically significant given the small magnitude of difference and given the variation across lakes and within lakes across years. Galarowicz and Wahl (2003) also observed similar growth among age-0 Walleye from different latitudes (different strains) during laboratory experiments even though the strains exhibited differences in metabolism and food consumption.

Although the mechanism that caused higher survival of stocked SMS fry remains unknown, we did document that higher survival occurred across a broad range of concurrent natural reproduction. The highest percentage of wild fish typically occurred in our samples from Tetonka/Sakatah Lake, which is directly downstream of the Cannon River location where Walleye eggs were collected by MNDNR during the 1980s and where spring electrofishing has confirmed the continued presence of a spawning run (D. E. Logsdon, unpublished data). The lowest percentage of wild fish in Tetonka/Sakatah Lake samples occurred during 2021, when spring water levels in the Cannon River were low. These low water levels could have reduced access to the river by adults (Colby et al. 1979), diminished incubation conditions in spawning substrate (Colby et al. 1979), or disrupted the distribution of fry after hatch (Priegel 1970; Jones et al. 2003). Samples from

Cannon/Wells Lake showed the same annual pattern in natural reproduction as that of Tetonka/Sakatah Lake, but wild fish composed a lower percentage of the samples. Natural reproduction in Lake Okabena and Kansas, Round, and Bingham lakes, which presumably occurs in-lake due to a lack of suitable tributaries, was less synchronous and had a lower percentage of SMS ancestry than that of lakes in the Cannon River system. Ancestry of wild age-0 Walleye during the beginning of this study reflected that spawning adults were already present in the lakes. High SMS ancestry in Tetonka/Sakatah and Cannon/Wells lakes is consistent with either higher reproductive success or higher survival of remnant native stocks (Palmer et al. 2006; Wilson et al. 2007). The SMS ancestry in Lake Okabena and Kansas, Round, and Bingham lakes was likely due to descendants of SMS Walleye that were stocked in the 1980s. This study was not designed to evaluate the spawning success of SMS fish, but we did observe increases in SMS ancestry for age-0 Walleye from Lake Okabena, Kansas Lake, and Round Lake toward the end of the study. Male Walleye in the study lakes typically mature at age 3, whereas females mature 1 year later (Logsdon, unpublished data); thus, the reproductive contribution from males of the 2018 stocking could have begun in 2021 and the contribution from females of the 2018 stocking could have begun in 2022. Southern Minnesota-strain fry from stockings into Round Lake during 2017 could have matured and started contributing to natural reproduction there in 2020.

Further monitoring is needed to determine whether natural reproduction continues to increase and whether higher survival of SMS Walleye will result in greater relative strength of stocked SMS year-classes. If not, there is still a benefit in maintaining current fisheries at lower cost. Further cost savings could also be achieved if some of the fisheries that are currently maintained through fingerling stocking are switched to fry stocking. Given the poor relative survival of the MIS, it is likely that some of the fisheries where fry stocking has failed in the past would succeed if SMS fish were used. Although additional monitoring of the SMS is warranted, stocking of Walleye from local sources is currently practiced in the northern two-thirds of Minnesota and is recommended elsewhere for both Walleye (Stepien et al. 2015; Hammen and Sloss 2019) and other species (Jennings et al. 2010).

We suggest that enough evidence now exists to warrant that the MNDNR policy of stocking Walleye from local egg sources be extended to the entire state. Priority should be given to fry stocking because that life stage demonstrated a substantial survival advantage of the SMS over the competing northern strain. Initially targeting lakes with the greatest reproductive potential and connectivity to the

broader watershed would also facilitate development of additional SMS egg sources.

Developing new egg sources will incur additional costs. A recently purchased instream trap and stripping platform for the Cannon River cost \$40,000 (C. Soupir, MNDNR, personal communication), and additional staff time will have to be allocated to identify and collect fish from other spawning sites. However, the higher survival demonstrated in this study can offset the higher initial costs of SMS production, and it is likely that SMS production costs can be reduced over time. The primary difference in production cost between the strains in our study was due to lower efficiency of in-lake brood collection (Satterfield and Flickinger 1996) at Lake Sarah compared to river-run brood collection (Thompson 1996) at the Pine River. The first few years of the Lake Sarah egg collection also resulted in lower hatch rates of SMS eggs, thus requiring more eggs collected per fry produced. Furthermore, SMS production in 2018 and 2019 also included additional labor to collect tissue samples for PBT, whereas MIS production costs did not. The disparity in fry production costs between the strains will likely decrease in the future when staff at Lake Sarah become more proficient at brood collection and fertilization, when they are no longer required to take tissue samples, or when MNDNR develops more efficient river-run SMS egg sources.

We have shown that a local strain can outperform nonlocal strains at such a level that can overcome the additional costs of developing a new egg source. When stocking is used to supplement native populations, use of a local strain has the additional benefit of conserving genetic integrity and reducing the risk of outbreeding depression (Jennings et al. 2010; Porak et al. 2015; Hammen and Sloss 2019). Although genetic conservation may not be a concern for introductory stockings, use of a local strain can also be advantageous when stocking nonnative waters because prior adaption to local conditions can increase the survival of stocked fish and improve the potential for natural reproduction, thus allowing for lower stocking rates and possibly eliminating the need for future stocking.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest in this article.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Handling of fish was conducted in accordance with the *Guidelines for the Use of Fishes in Research* (Use of Fishes in Research Committee 2014).

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