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Table Errata

Page	Table	Correction
13	4	Delete entire Table
14	5	Rename - Table 4
15	6	Rename - Table 5

Text Errata

The following text was omitted from the References:

Leary, R.F., and H.E. Brooke. 1990. Starch gel electrophoresis and species distinctions. Page 141-170 *in* C. B. Schreck and P.B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda.

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Abstract.- Genetic characteristics of walleye *Stizostedion vitreum* collected from 11 locations (13 samples) in Minnesota were analyzed with protein electrophoresis on either starch gels or cellulose acetate. Ten polymorphic loci were found in these walleye. Allele frequencies at *ADH-1**, *IDHP-1**, *sMDH-B**, and *PROT-4** among populations differed significantly ($P \leq 0.0002$). A dendogram constructed from unbiased genetic distances calculated from these four loci identified two genetically similar groups. Walleye from Lake Pepin were the most genetically distant. The other populations were more similar to each other than to Lake Pepin walleye.

Genetic variation in Minnesota walleye appeared related to isolation caused by glaciation. Lake Pepin walleye hypothetically originated from a Mississippi River glacial refugium, and the other populations hypothetically originated from a Missourian refugium.

Genetic variation in Minnesota walleye could have been affected by indiscriminate statewide stocking. All systems except possibly Lake Pepin had been stocked with walleye fry or fingerlings from other sources. Walleye from those systems receiving fry and fingerlings from the most sources also had higher mean heterozygosity per locus.

Effects of indiscriminate stocking on these populations were unknown; however, any changes in genetic composition were probably irreparable. Effects from other factors such as overexploitation, habitat alteration, and interactions with other fish species could have masked effects caused by indiscriminate stocking.

Operation of spawning stations probably had a negligible effect on the genetic integrity of those walleye populations used for stripping of eggs and milt. Effective population sizes of brood stock at each station ensured a minimum of 99.94% retention of genetic diversity per generation, even if these were founder populations. Genetic diversity of these walleye populations was further preserved because new brood stock of varying ages and sizes were used each year, and natural reproduction occurred within each respective system.

[1] This project was funded in part by the Federal Aid in Sport Fish Restoration Program. Completion report, Studies 308 and 674, D-J Project F-26-R Minnesota.

Numbers of dorsal spines, dorsal rays, and gill rakers among populations differed significantly ($P < 0.001$). Morphometric characteristics including total length/head length, total length/head width, head length/jaw length, head length/snout length, jaw length/head width, and snout length/head width also differed significantly ($P \leq 0.004$) among populations.

Variation of walleye populations based on meristic characters was generally similar to variation of populations based on allele frequencies. Therefore, meristic characteristics could be useful for identification of genetically distinct walleye populations. Variation among populations based on morphometric characters was not similar to population variation based on allele frequencies.

Introduction

Genetic considerations have become increasingly important in fisheries management. Fisheries scientists have recognized that genetically distinct subpopulations within a population can be treated as stocks (Kapuscinski and Philipp 1988), and managed accordingly. Fisheries management now includes genetic conservation of wild stocks, maintenance of genetic characteristics of hatchery-reared stocks, stock enhancement, and selective breeding for production traits (Allendorf et al. 1987).

Several populations of walleye *Stizostedion vitreum* in Minnesota have been analyzed for genetic variation, but relationships among these populations have not been addressed. Murphy and Lee (1986) described electrophoretically detectable loci in walleye from six locations in Minnesota and suggested that genetically distinct populations probably occur. Waltner (1988) found that walleye from Navigation Pools 5 and 8 of the Mississippi River were similar to each other, but differed from populations in the Missouri River and a small lake in South Dakota. Allele frequencies in walleye from the St. Louis River were compared with similar data in other walleye populations from the eastern Great Lakes and Manitoba (Ward et al. 1989).

Mitochondrial DNA (mtDNA) was also analyzed in several walleye populations in Minnesota. Two mtDNA haplotypes were detected in walleye populations from the Lake of the Woods, Lake Winnibigoshish, Lake Vermilion, and the St. Louis River (Ward et al. 1989; Billington et al. 1990; N. Billington, University of Guelph, personal communication).

For nearly 100 years the Minnesota Department of Natural Resources (MNDNR) has been operating spawning stations where millions of eggs and milt are stripped from walleye captured during their spawning migrations. Fry and fingerlings produced from these stations have been distributed statewide. Many of those water bodies have naturally reproducing, self-sustaining walleye populations; therefore, those fry introductions could have compromised the genetic integrity of resident populations.

Operation of a spawning station could potentially reduce genetic diversity of the walleye population sampled for stripping of eggs and milt. This could occur provided that those walleye stripped for sex products were the only contributors to the gene pool of subsequent generations. Typically, 10 to 20% of fry produced at a spawning station are returned to the system where their parents were captured. Furthermore, the same streams and locations are used each year for collection of walleye, and adult walleye will spawn in the same streams each year (Crowe 1962; Olson and Scidmore 1962; Forney 1963; Olson et al. 1978). Matings utilizing a few closely related individuals (inbreeding) could result, and inbreeding reduces genetic diversity (Gall 1987).

The objectives of this study were to determine genetic variation in selected walleye populations in Minnesota, to determine genetic differences among these populations, and to determine factors affecting genetic

variation. Meristic and morphometric characteristics were also evaluated as tools for identification of genetically distinct walleye stocks in Minnesota.

Methods

Twenty to 35 walleye were collected from MNDNR spawning station traps at Little Cutfoot Sioux Lake (Lake Winnibigoshish), Pike River (Lake Vermilion), and Seagull River (Saganaga Lake) (Figure 1) in April 1987; and at Lake Sallie, Ottertail River (Ottertail Lake), Pine River (Whitefish Lake), Big Lake Creek (Lake Andrusia), and Boss Creek (Lake Osakis) (Figure 1) in April 1988. In April 1990, an additional 25 walleye were collected from spawning stations at Seagull River and Pike River. Twenty-five walleye from Lake of the Woods and Lake Winnibigoshish, and 24 walleye from Lake Pepin (Figure 1) were collected by electrofishing or trawling.

Pike River, Seagull River, and Lake of the Woods are within the Rainy River watershed, and within the larger Hudson Bay drainage (Waters 1977). Lake Sallie (Pelican River drainage) drains into the Ottertail River, also within the Hudson Bay drainage (Waters 1977). Big Lake Creek is connected to a lake that drains directly into the Mississippi River. Little Cutfoot Sioux Lake is connected to Lake Winnibigoshish, a reservoir on the Mississippi River approximately 50 km downstream of Big Lake Creek. The Pine River and Boss Creek (Sauk River drainage) systems drain into the Mississippi River further downstream (Waters 1977). Lake Pepin is a widening of the Mississippi River along the Minnesota-Wisconsin border (Figure 1).

Sex was determined, and morphometric and meristic characteristics were measured and counted, respectively, on walleye collected in 1987 and 1988. Little Cutfoot Sioux Lake and Boss Creek samples consisted of 20 males and 15 females, and 15 males and 5 females, respectively. Sex ratios of the other samples were 1:1. Total length (mm), total weight (g), head length (mm), intraorbital head width (mm), jaw length (mm), and snout length (mm) of each fish were measured, and all dorsal spines, dorsal rays, branchiostegal rays, and gill rakers were counted. Walleye age was determined by counting scale annuli. Annuli were distinguished from circuli by crossing over and spacing patterns (Jearld 1983).

Enzyme systems in walleye collected in 1987 and 1988 were examined with starch-gel electrophoresis, but those collected in 1990 were examined with cellulose-acetate electrophoresis. Methodologies for each technique are discussed below.

Starch-gel electrophoresis

In the field, eye retina, heart, liver, and white muscle tissue near the head were excised from each fish. Each individual tissue sample was placed into a 1.5 Ml cryovial and immediately stored in liquid nitrogen.

In the laboratory, frozen tissue, weighing 0.2 to 1.7 g, was added to an equivalent weight of distilled water (two equivalent weights of distilled water were used with white muscle tissue) and homogenized. Carbon tetrachloride (0.5 Ml) was added to liver-water mixtures before homogenization which improved partitioning of the aqueous phase of these mixtures during centrifugation. Homogenates were then placed in a refrigerated centrifuge (2° C) and centrifuged (10,000 rpm) for 15 to 30 min. Supernates were decanted or pipetted into cryovials and stored in liquid nitrogen until electrophoresis.

Sixteen enzyme systems (Table 1) were analyzed primarily with horizontal starch-gel electrophoresis. Vertical starch-gels were occasionally used to confirm results determined from horizontal starch-gels. Two buffers, TC 8.5 (tris, citric acid, lithium hydroxide, and boric acid, pH 8.5)(Ridgeway et al. 1970), and NaP 5.9 (sodium phosphate, citric acid, pH 5.9)(Harris and Hopkinson 1976), were used for specific enzyme systems (Table 1).

Starch-gels consisted of 54 g of hydro-

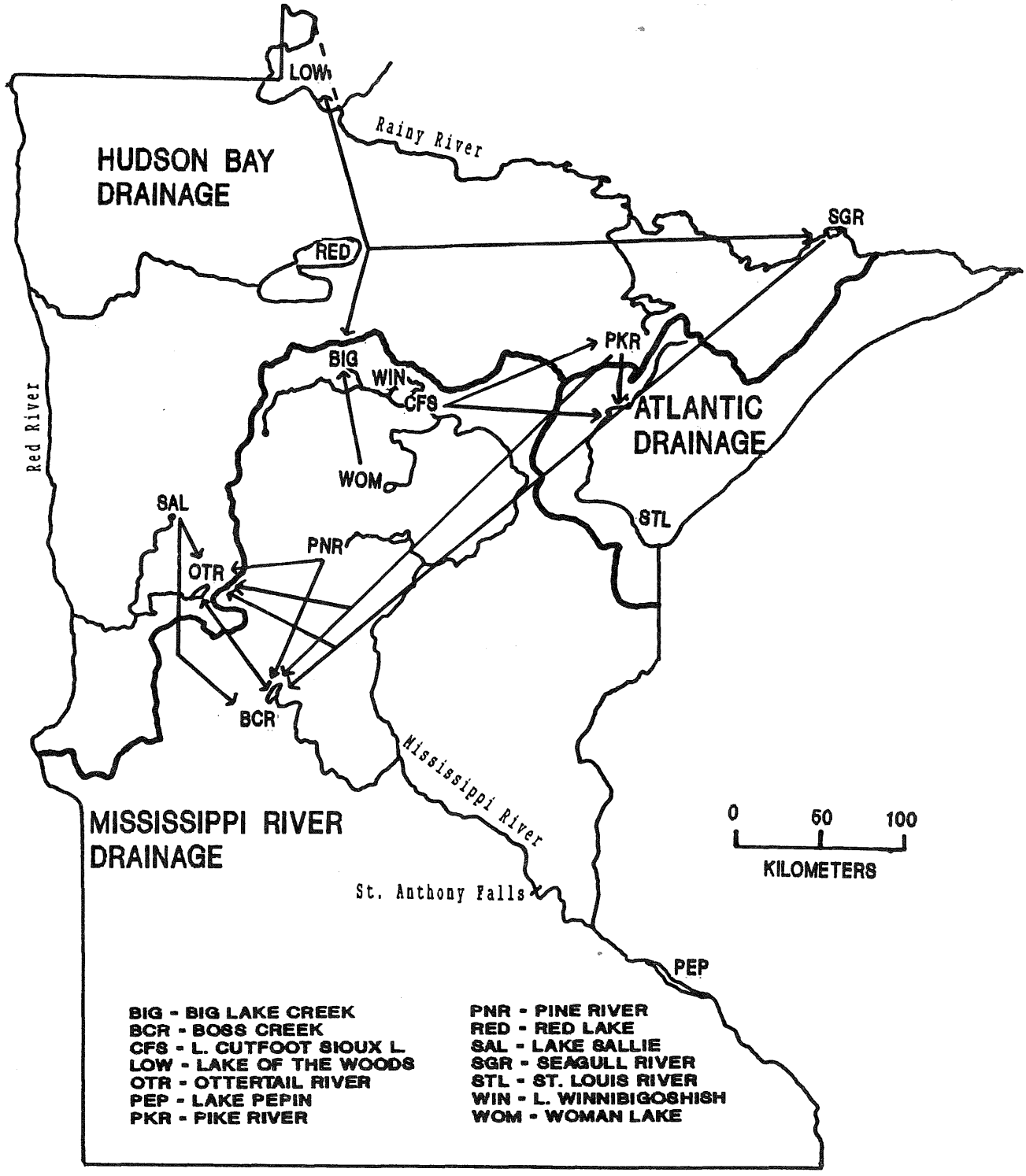

Figure 1. Map of Minnesota with locations of walleye spawning stations and walleye collection sites within three major drainage areas. Arrows (from spawning station to lakes stocked) indicate direction that walleye fry and fingerlings were transported by the Minnesota Department of Natural Resources.

Table 1. Enzyme systems, E.C. number, tissues analyzed, buffers used, and number of loci resolved with starch-gel or cellulose-acetate electrophoresis conducted on walleye collected from ten locations in Minnesota (E = eye retina, H = heart, L = liver, and M = muscle).

Enzyme system	E.C. no.	Tissue	Buffer	No. of loci
Starch-gel electrophoresis				
Alcohol dehydrogenase (*ADH**)	1.1.1.1	L	NaP 5.9	1
L-Iditol dehydrogenase (*IDDH**)	1.1.1.14	L	TC 8.5	1
L-Lactate dehydrogenase (*LDH**)	1.1.1.27	E,H,L,M	TC 8.5	3
Malate dehydrogenase (*MDH**)	1.1.1.37	E,H,L,M	NaP 5.9	3
Malic enzyme (*MEP**)	1.1.1.40	H,L	TC 8.5	1
Isocitrate dehydrogenase (*IDHP**)	1.1.1.42	E,H,L,M	NaP 5.9	2
Phosphogluconate dehydrogenase (*PGDH**)	1.1.1.44	L	NaP 5.9	1
Xanthine dehydrogenase (*XDH**)	1.2.3.2	L	TC 8.5	1
Superoxide dismutase (*SOD**)	1.15.1.1	L	TC 8.5	1
Aspartate aminotransferase (*AAT**)	2.6.1.1	E,H,L,M	NaP 5.9	3
Creatine kinase (*CK**)	2.7.3.2	E,H,L,M	TC 8.5	5
Adenylate kinase (*AK**)	2.7.4.3	E,H,L,M	NaP 5.9	2
Fumarate hydratase (*FH**)	4.2.1.2	L	TC 8.5	1
Glucose-6-phosphate isomerase (*GPI**)	5.3.1.9	E,H,L,M	TC 8.5	2
Phosphoglucomutase (*PGM**)	5.4.2.2	E,H,L,M	TC 8.5	2
General protein (*PROT**)	n.a.	M	TC 8.5	3
Cellulose-acetate electrophoresis				
Alcohol dehydrogenase (*ADH**)	1.1.1.1	L	TG 9.0/9.6	1
L-Iditol dehydrogenase (*IDDH**)	1.1.1.14	L	TM 7.8	1
L-Lactate dehydrogenase (*LDH**)	1.1.1.27	H	TEMB 7.8	2
Malate dehydrogenase (*MDH**)	1.1.1.37	M	TEMB 7.8	2
Malic enzyme (*MEP**)	1.1.1.40	M	P 7.0	2
Isocitrate dehydrogenase (*IDHP**)	1.1.1.42	L	TG 9.0/9.6	2
		H	TM 7.8	2
Phosphogluconate dehydrogenase (*PGDH**)	1.1.1.44	L	P 7.0	1
Glyceraldehyde-3-phosphate dehyd.(*GAPDH**)	1.2.1.12	H,L,M	CA 6.0	1
Aspartate aminotransferase (*AAT**)	2.6.1.1	L,M	P 7.0	3
Adenylate kinase (*AK**)	2.7.4.3	M	PC 6.4	1
Adenosine deaminase (*ADA**)	3.5.4.4	L	PC 6.4	1
Fumarate hydratase (*FH**)	4.2.1.2	M	P 7.0	1
Mannose-6-phosphate isomerase (*MPI**)	5.3.1.8	L	P 7.0	1
Glucose-6-phosphate isomerase (*GPI**)	5.3.1.9	H	PC 6.4	2
Phosphoglucomutase (*PGM**)	5.4.2.2	M	PC 6.4	1
General proteins (*PROT**)	n.a.	M	TG 8.5	4

n.a. = not applicable.

lyzed potato starch (Sigma or Starch-Art) and 400 Ml of buffer. Electrophoresis was accomplished in 4-5 h at 20 W. Voltages floated around 150 and 230 V for starch-gels made with the NaP 5.9 and TC 8.5 buffers, respectively.

Starch-gels were sliced into 1.5 mm thick slices and stained utilizing stain recipes of Shaw and Prasad (1970). Staining was accomplished by either immersing gel-slices into 50 Ml of stain contained in a glass cake dish or overlaying on gel-slices a mixture of 16 Ml of stain and 1% bacto-agar. Stained gel-slices were incubated in the dark at 37°

C until stains completed development. Each gel-slice was either photographed or photocopied.

Cellulose-acetate electrophoresis

Liver, heart, and white muscle tissue were removed from freshly killed fish and snap-frozen in dry ice/methanol, transported on dry ice, and subsequently stored at -70° C. Approximately 0.5 g of tissue was ground with an equal weight/volume of 0.1% solution of 2-mercaptoethanol in water. Samples were centrifuged at 20,000 rpm for 10 min at 4° C. Supernatants were removed and stored at -20° C for up to 2 months before electrophoresis.

Protein separation on cellulose acetate (Helena Laboratories, Beaumont, Texas) was conducted at 200 V for 25 min. Forty-five min was required for separation of *PROT-2**.

A total of 7 buffers was used to resolve the 16 analyzed enzyme systems (Table 1). Buffer recipes (Richardson et al. 1986; Aebersold et al. 1987; Hebert and Beaton 1989; M. Murdock, University of Guelph, personal communication) were as follows: *TG 9.0/9.6* - 0.025 M Tris and 0.192 M glycine adjusted with HCl to a pH of 9.0, and an identical solution adjusted with HCl to a pH of 9.6; *TM 7.8* - 0.05 M Tris and 0.02 M maleic acid. Maleic acid was used to adjust pH to 7.8; *CA 6.0* - 0.04 M citric acid adjusted with N-3 aminopropyl morpholine to a pH of 6.0; *P 7.0* - 0.0116 M sodium phosphate dibasic and 0.0084 M sodium phosphate monobasic adjusted with NaOH to a pH of 7.0; *PC 6.4* - 0.01 M sodium phosphate dibasic and 0.0025 M citric acid adjusted with citric acid to a pH of 6.4; *TEMB 7.8* - 0.015 M Tris, 0.005 M Na EDTA, 0.010 M MgCl , and 0.0055 M boric acid adjusted with boric acid to a pH of 7.8; and *TG 8.5* - 0.025 M Tris and 0.0192 M glycine adjusted with NaOH to a pH of 8.5. Each buffer except TG 9.0/9.6, was used for both rehydration of gels and electrophoresis. With TG 9.0/9.6, a 50/50 mixture of both buffers was used to rehydrate gels. For electrophoresis, the 9.6 pH buffer was at the cathode and the 9.0 pH buffer was at the anode (discontinuous buffer system).

Stain recipes were from Richardson et al. 1986, Aebersold et al. 1987, Hebert and Beaton 1989, and M. Murdock (University of Guelph, personal communication). Two Ml of 1.6% agar was added to each stain immediately before use which minimized dispersion of the stained bands.

Alleles of polymorphic loci *ADH-1**, *IDHP-1**, *sMDH-B**, and *PROT-4** were identified equally well with either electrophoresis technique conducted on the same tissue samples. Polymorphism at *PROT-2** was detectable only on cellulose-acetate gels.

Allele frequencies were determined from banding patterns (phenotype) displayed on gel-slices (Utter et al. 1987). Mean heterozygosity per locus (Nei 1978), tests for random mixing of alleles (Hardy-Weinberg), and clustering of unbiased genetic distances (Nei 1978) were determined with the aid of BIOSYS-1 (Swofford and Selander 1989). Only polymorphic loci with frequencies exceeding 0.05 in at least one population and equally resolved with either technique of electrophoresis were used in these calculations. Allele frequencies of these same loci determined from 12 walleye collected from the St. Louis River (Ward et al. 1989) were also included in the cluster analysis. Significant differences in allele frequencies were determined with Chi square contingency tests. No discrimination by sex or age was made. Nomenclature of enzyme systems and loci follow Shaklee et al. (1990).

Statistical differences in meristic and morphometric variables among populations were determined with Kruskal-Wallis tests. Natural groupings of populations based on mean meristic characteristics and groupings based on mean morphometric characteristics were determined with single-linkage cluster analysis (Wilkinson 1988). Data on walleye stocking and operation of spawning stations were obtained from appropriate MNDNR area offices. Effective population sizes of brood stocks collected at spawning stations and estimates of retention of genetic diversi-

ty per generation were calculated with equations from Allendorf and Ryman (1987).

Results

Genetic relationships

A total of 40 scorable loci from 20 enzyme systems was detected (Table 1). Five loci showed polymorphism with allele frequencies > 0.05 (Table 2). One walleye from the Ottertail River was polymorphic at *sMDH-A**. Rare polymorphism (frequencies < 0.05) was observed at *sAAT-4** and *LDH-A** in the Seagull River population collected in 1990, and at *FH-1** and *PGM-1** in the Lake Pepin population. An *IDHP-1*120* allele was detected in one walleye from Lake Pepin, but was not found in the other populations.

Genetic variation among walleye populations was observed. Allele frequencies at *ADH-1**, *IDHP-1**, *sMDH-B**, and *PROT-4** differed significantly among populations (*Chi square* = 94.2, df = 12, $P < 0.0001$; *Chi square* = 37.5, df = 12, $P = 0.0002$; *Chi square* = 301.4, df = 24, $P < 0.0001$; and *Chi square* = 745.5, df = 12, $P < 0.0001$, respectively). Mean heterozygosity of polymorphic loci was highest in the Lake Pepin population and lowest in the Pike River population collected in 1987 (Table 2). Two clusters of genetically similar populations appeared in the dendrogram of unbiased genetic distances based on these polymorphic loci. Lake Pepin walleye were most genetically distant (Figure 2). The remaining populations formed the other cluster (Figure 2). The St. Louis River population also appears isolated, but was more similar to non-Lake Pepin populations.

Significant deviations from Hardy-Weinberg equilibrium were detected at one to two loci in three populations; however, these deviations were inconsistent among years. Significant deviation at *IDHP-1** in the Pine River population (*Chi square* = 5.14, df = 1, $P = 0.023$) was detected (excess heterozygotes). Significant deviation at *IDHP-1** was not detected in the Seagull River population sampled in 1987, but was detected (excess heterozygotes) in 1990 samples (*Chi square* = 6.49, df = 1, $P = 0.011$). Significant deviations were also observed at *sMDH-B** from the Little Cutfoot Sioux Lake (*Chi square* = 16.76, df = 3, $P = 0.001$) and Seagull River (*Chi square* = 30.21, df = 3, $P < 0.001$) populations sampled in 1987, both with insufficient numbers of heterozygotes. The deviation at *sMDH-B** from the Seagull River population was not significant when rare alleles were pooled. A significant deviation (*Chi square* = 4.38, df = 1, $P = 0.036$) at *sMDH-B** in the Little Cutfoot Sioux Lake population was still detected when rare alleles were pooled. Deviation at *sMDH-B** was not significant in walleye collected in 1990 from adjacent Lake Winnibigoshish.

Stocking histories

All except one of the systems where walleye were collected had been directly or indirectly stocked with walleye fry or fingerlings from other lake chains, rivers, and drainages within Minnesota (Figure 1). The Seagull River system, void of walleye before 1934, was stocked (between 1934 and 1955) with fry and fingerlings primarily from Red Lake (S. Persons, MNDNR, personal communication). This effort resulted in the establishment of a self-sustaining walleye population in the Seagull River system. Lake of the Woods also received walleye fry from Red Lake during the 1930s and 1940s (M. Larson, MNDNR, personal communication). The Pike River system was stocked with walleye fry from Little Cutfoot Sioux Lake in the late 1960s (M. Heywood, MNDNR, personal communication). Lake Sallie was probably stocked with walleye from outside sources, but these sources were not documented. The Ottertail River and Boss Creek systems have received fry and fingerlings from each other, and both systems have received fry from Lake Sallie, Pike River, Seagull River, and Pine River broodstock (MNDNR, file data).

The Pine River system, stocked annually

Table 2. Allele frequencies of the polymorphic loci with frequencies > 0.05 in at least one population, and estimates of mean heterozygosity (H) in walleye populations collected in April 1987, April 1988, or April 1990 from 10 locations and within 2 drainages in Minnesota (H was determined from *ADH-1**, *IDHP-1**, *MDH-3**, and *PROT-4** data only).

		Locus											
	Sample	*ADH-1**		*IDHP-1**		*MDH-3**			*PROT-2**		*PROT-4**		
Location	size	-100	-70	100	80	70	100	120	60	100	100	160	H
Hudson Bay Drainage													
Seagull River (1987)	30	0.88	0.12	0.52	0.48	*0.04	0.33	0.63	-	-	0.98	0.02	0.311
Seagull River (1990)	25	0.86	0.14	*0.54	0.46	0.06	0.44	0.50	0.3	0.62	0.98	0.02	0.340
Pike River (1987)	30	0.87	0.13	0.70	0.30	0.00	0.20	0.80	-	-	1.00	0.00	0.247
Pike River (1990)	25	0.80	0.20	0.58	0.42	0.10	0.24	0.66	0.2	0.72	1.00	0.00	0.333
Lake of the Woods (1990)	25	0.88	0.12	0.44	0.56	0.02	0.60	0.38	0.26	0.74	1.00	0.00	0.306
Lake Sallie (1988)	30	0.70	0.30	0.53	0.47	0.00	0.77	0.23	-	-	0.98	0.02	0.333
Ottertail River (1988)	30	0.72	0.28	0.48	0.52	0.07	0.50	0.43	-	-	0.98	0.02	0.374
Mississippi River Drainage													
Big Lake Creek (1988)	30	0.65	0.35	0.45	0.55	0.13	0.30	0.57	-	-	1.00	0.00	0.387
L. Cutfoot Sioux L.(1987)	35	0.63	0.37	0.60	0.40	*0.01	0.06	0.93	-	-	1.00	0.00	0.274
L. Winnibigoshish (1990)	25	0.62	0.38	0.64	0.36	0.12	0.08	0.80	0.22	0.78	1.00	0.00	0.324
Pine River (1988)	30	0.53	0.47	*0.45	0.55	0.04	0.53	0.43	-	-	1.00	0.00	0.386
Boss Creek (1988)	20	0.55	0.45	0.45	0.55	0.10	0.48	0.42	-	-	1.00	0.00	0.400
Lake Pepin (1990)	24	0.73	0.27	0.64	0.33	0.04	0.73	0.23	0.27	0.73	0.35	0.65	0.444

(*) denotes significant ($P \leq 0.05$) deviation from Hardy-Weinberg equilibrium.
(-) denotes no data.

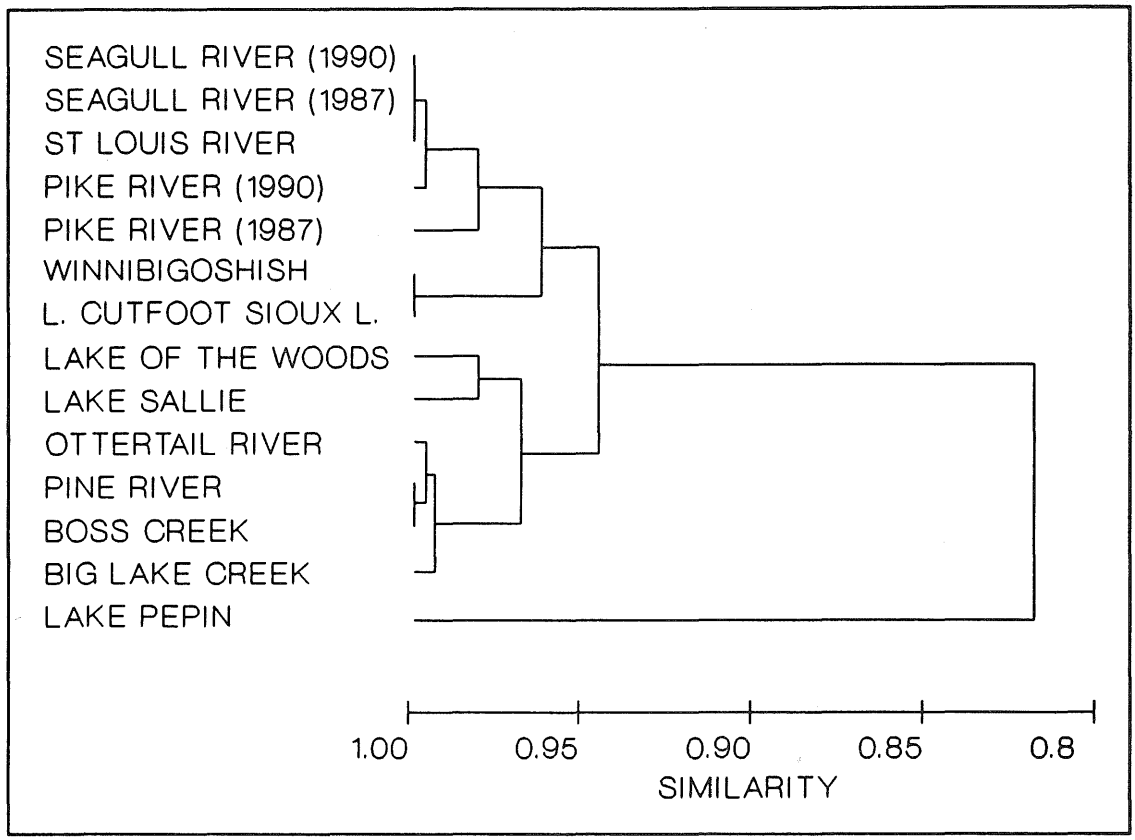

Figure 2. Genetic relationships of walleye collected from Lake Pepin, Lake of the Woods, and Lake Winnibigoshish, from spawning stations at Big Lake Creek, Boss Creek, Little Cutfoot Sioux Lake, Ottertail River, Pike River, Pine River, Lake Sallie, and Seagull River, and from the St. Louis River (data from Ward et al. 1989). Dendrogram based on allele frequencies of ADH-1*, IDHP-1*, *MDH-3**, and *PROT-4**.

since 1911, has usually been stocked with offspring from walleye collected at the Pine River spawning station; however, this station was not operating before 1923, and was not operating between 1946 and 1954 (Bonde 1963). The origin of fry during those years of inoperation was unknown.

Records indicated that the Little Cutfoot Sioux Lake system had not been stocked with fry or fingerlings from other areas, but fry from Red Lake broodstock have been introduced into the Big Lake Creek system (MNDNR, file data). Strand (1980) reported that some walleye tagged in Cass Lake (Big Lake Creek system) were found in Lake Winnibigoshish; therefore, some walleye originating from Red Lake could have immigrated into the Little Cutfoot Sioux Lake/Lake Winnibigoshish system. A dam at the outlet of Cass Lake was built around 1900. Except during periods of extreme high water, this dam probably prevents upstream movement of walleye from Little Cutfoot Sioux Lake or Lake Winnibigoshish into the Big Lake Creek system (S. Boe, MNDNR, personal communication). The Big Lake Creek system has also been stocked with fry or fingerlings from Woman

Lake (near Leech Lake) (Figure 1).

Lakes within the St. Louis River watershed have been stocked with walleye fry or fingerlings originating from Pike River and Little Cutfoot Sioux Lake (J. Spurrier, MNDNR, personal communication). Lake Pepin has not been stocked with walleye from other sources (A. Stevens, MNDNR, personal communication); however, no barriers prevent walleye stocked in lakes further upstream from migrating to Lake Pepin. St. Anthony Falls, in Minneapolis, prevents any movement of Lake Pepin walleye into lakes upstream of the falls.

Meristic and morphometric measurements

Meristic and morphometric measurements were also variable among populations (Table 3). Significant differences in numbers of dorsal spines ($H = 89.2$; df = 7; $P < 0.001$), dorsal rays ($H = 189.4$; df = 7; $P < 0.001$), and gill rakers ($H = 115.5$; df = 7; $P < 0.001$) among populations were detected. Significant differences among populations were also detected for total length/head length ($H = 42.9$; df = 7; $P < 0.001$), total length/head width ($H = 36.1$; df = 7; $P < 0.001$), head length/head width ($H = 54.4$; df = 7; $P < 0.001$), head length/jaw length ($H = 20.8$; df = 7; $P = 0.004$), head length/snout length ($H = 24.4$; df = 7; $P = 0.001$), jaw length/head width ($H = 35.6$; df = 7; $P < 0.001$), and snout length/head width ($H = 42.1$; df = 7; $P < 0.001$). No sex related differences in meristic characteristics or ratios of morphometric measurements were observed (Kruskal-Wallis tests; $P > 0.05$).

Populations based on mean meristic measurements, clustered into two groups. Ottertail Lake, Lake Sallie, Boss Creek, Big Lake Creek, and Pine River represented one cluster, while Little Cutfoot Sioux Lake, Pike River, and Seagull River represented the other (Figure 3). This clustering pattern was generally similar to clustering of unbiased genetic distances (Figure 3).

Populations based on ratios of morphometric characteristics also clustered into two groups (Figure 3). One group consisted of the Pike River population and the other group consisted of the remaining seven populations (Figure 3). These cluster patterns were considerably different than clustering of unbiased genetic distances or meristic characteristics (Figure 3).

Spawning station operation

Averages of nearly 280 to nearly 2,900 female walleye were stripped annually for eggs at MNDNR spawning stations (Table 4). Eggs were fertilized with milt from three to six males; consequently, mean effective population sizes at these stations ranged from an average of 900 to 9,843 (Table 4). Even if these populations were founder populations, nearly all genetic diversity would be retained (per generation) with these effective population sizes (Table 4).

Discussion

Genetic variation among Minnesota walleye could be related to isolation caused by glaciation. Three glacial refugia for walleye, an Atlantic, a Mississippi River, and a Missourian, have been hypothesized (Crossman and McAllister 1986; Billington and Hebert 1988; Ward et al. 1989), and we hypothesize that Minnesota walleye originated from two of these refugia.

We hypothesize that Lake Pepin walleye originated from a Mississippi River refugium. Because of high frequencies of *PROT-4*160*, Lake Pepin walleye were more similar to walleye from the eastern Great Lakes than those from Manitoba, or from Minnesota upstream and north of St. Anthony Falls (Table 5). Mitochondrial DNA analyses suggested that walleye in the Mississippi River below St. Anthony Falls were not only different than other Minnesota walleye populations, but also different than walleye from the eastern Great Lakes (Table 5). Mitochondrial DNA haplotypes 10 and 15 were found in Mississippi River walleye below St. Anthony Falls, but not found in eastern Great Lakes populations (Table 5).

Table 3. Ranges of total lengths, weights, and ages; mean meristic measurements; and mean ratios of morphometric measurements of walleye collected from spawning stations at Seagull River (SGR), Pike River (PKR), Lake Sallie (SAL), and Ottertail River (OTR) within the Hudson Bay drainage, and at Big Lake Creek (BLC), Little Cutfoot Sioux Lake (CFS), Pine River (PNR), and Boss Creek (BCR) within the Mississippi River drainage.

	Hudson Bay Drainage				Mississippi River Drainage			
Parameter	SGR	PKR	SAL	OTR	BLC	CFS	PNR	BCR
Length range (millimeters)	365-565	301-527	278-590	317-631	347-592	337-598	392-658	318-670
Weight range (grams)	430-2,050	281-1,470	148-1,670	233-2,110	293-1,720	323-1,930	456-2,690	261-2,720
Age range (years	3-6	4-9	2-9	4-12	4-8	3-10	4-8	3-11
Meristic variables								
Dorsal spines*	14.3	12.7	13.9	13.9	13.9	13.2	14.0	14.0
Dorsal rays*	19.5	19.8	22.0	21.2	21.9	19.4	22.0	22.0
Gill rakers*	10.6	11.4	11.8	11.8	11.2	11.2	12.0	12.0
Branchiostegal rays	7.0	7.1	7.2	7.0	7.1	7.1	7.0	7.0
Morphometric variables								
Total length/head length*	4.3	4.2	4.4	4.2	4.3	4.2	4.4	4.4
Total length/head width*	21.0	23.1	21.1	20.6	21.6	21.6	21.7	21.5
Total length/jaw length	9.2	9.2	9.4	9.2	9.3	9.3	9.4	9.3
Total length/snout length	14.9	14.5	14.9	15.0	14.9	15.3	14.9	14.8
Head length/head width*	4.8	5.5	4.8	5.0	5.0	5.1	4.9	4.9
Head length/jaw length*	2.1	2.2	2.1	2.2	2.2	2.2	2.1	2.1
Head length/snout length*	3.5	3.5	3.4	3.6	3.5	3.6	3.4	3.4
Jaw length/ head width*	2.3	2.5	2.2	2.2	2.3	2.3	2.3	2.3
Jaw length/snout length	1.6	1.6	1.6	1.6	1.6	1.7	1.6	1.6
Snout length/head width*	1.4	1.6	1.4	1.4	1.4	1.4	1.4	1.4

(*) denotes significant difference ($P \leq 0.05$) among populations.

Mitochondrial DNA haplotype 15 was not found in walleye from Saskatchewan, Manitoba, western Ontario, or in the other Minnesota populations (Table 5). Billington and Hebert (1988) suggested that walleye from the eastern Great Lakes originated from Mississippi River and Atlantic refugia. Because of its geographical location, the Lake Pepin walleye population probably originated from a Mississippi River refugium. This refugium was probably different from Mississippi River refugia from which the Great Lakes walleye originated. Bailey and Smith (1981) reported that several Mississippi River refugia probably existed during glaciation.

Walleye from the other Minnesota locations hypothetically originated from a Missourian refugium. These walleye were similar to walleye from Manitoba because they were either fixed at or had high (> 0.80) frequencies of *PROT-4*100* (Table 5). Walleye from Minnesota, Saskatchewan, Manitoba, and western Ontario possessed mtDNA haplotypes 4 and 10, but not mtDNA haplotypes 1 or 15 (Table 6). Ward et al. (1989) hypothesized that walleye from the St. Louis River and from Manitoba lakes

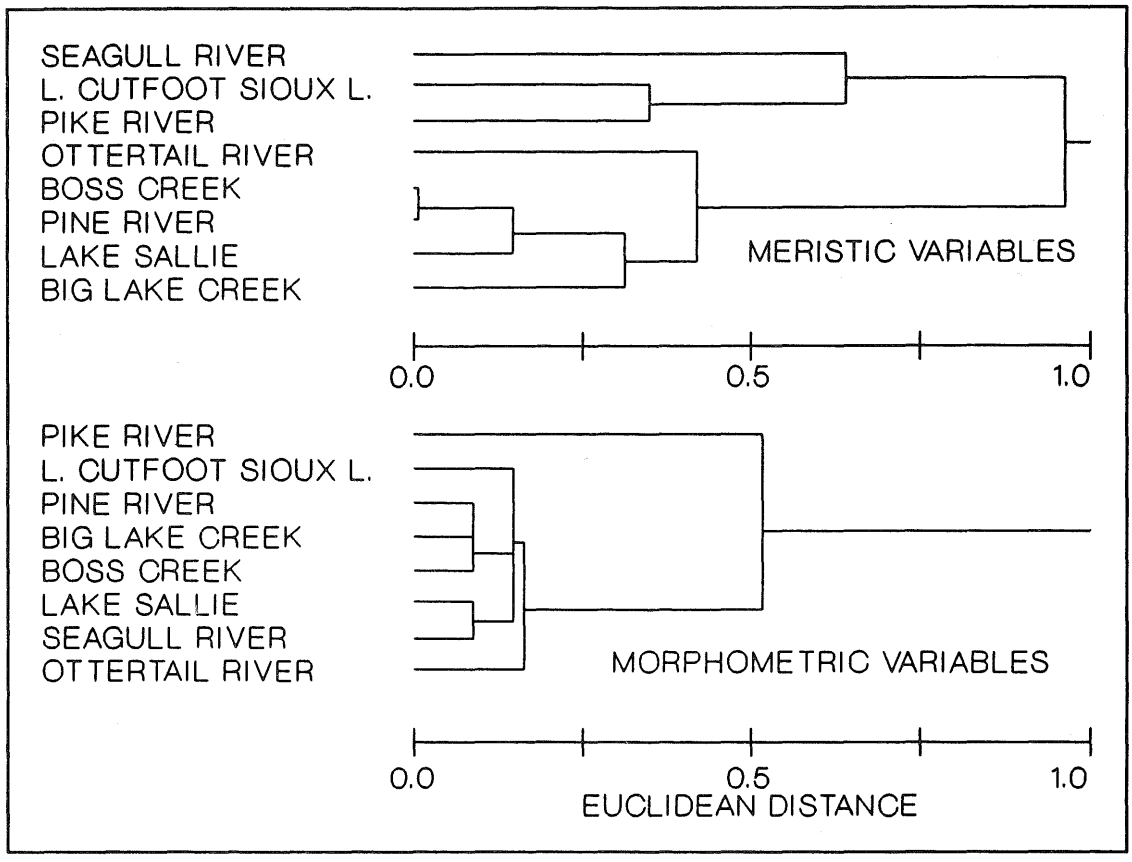

Figure 3. Dendrograms based on meristic characteristics (mean numbers of dorsal spines, dorsal rays, branchiostegal rays, and gill rakers) and morphometric characteristics (mean ratios of total length/head length, total length/head width, total length/jaw length, total length/snout length, head length/head width, head length/jaw length, head length/snout length, jaw length/head width, jaw length/snout length, and snout length/head width) showing relationships of walleye collected from spawning stations at Big Lake Creek, Boss Creek, Little Cutfoot Sioux Lake, Ottertail River, Pike River, Pine River, Lake Sallie, and Seagull River, Minnesota.

probably arose from a Missourian refugium.

Genetic divergence among Minnesota walleye, excluding Lake Pepin, was lower than divergence between Lake Pepin and the other Minnesota walleye populations possibly because of shorter periods of isolation. Walleye populations within the Hudson Bay and Mississippi River drainages have been isolated approximately 10,000 years, when the last glacier receded from Minnesota (Ojakangas and Matsch 1982). Billington and Hebert (1988) estimated that populations from the Atlantic and Mississippi River refugia shared a common ancestor 230,000 $\pm$ 100,000 years ago. Ward et al. (1989) reported that walleye from the Mississippi River refugium were genetically closer to walleye from the Missourian refugium than walleye from the Atlantic refugium. Therefore, walleye originating from Mississippi

Table 4. Mean number of meristic variables and mean ratios of morphometric variables associated with phenotypes of *ADH-1**, *IDHP-1**, and *MDH-3** in walleye collected from eight spawning stations in Minnesota during April 1987 or April 1988. Means[1] sharing underlines were not significantly different ($P \leq 0.05$).

	ADH-1*			IDHP-1*			MDH-3*					
Meristic variables	-100/-100	-100/-80	-80/-80	100/100	100/80	80/80	100/100	100/120	120/120	70/70	100/70	120/70
Dorsal spines	13.7	13.7	14.0	13.5	13.7	13.9	13.8	13.9	13.4	14.0	13.8	14.0
Dorsal rays	20.6	21.1	21.2	20.5	21.1	21.0	21.6	21.1	20.2	21.0	21.6	21.8
Gill rakers	11.2	11.5	11.7	11.1	11.6	11.2	11.8	11.5	11.0	11.0	11.9	12.0
Branchiostegal rays	7.1	7.0	7.0	7.1	7.1	7.1	7.1	7.0	7.1	7.3	7.0	7.0
Morphometric variables												
Total length/head length	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.4	4.2
Total length/head width	21.6	21.6	21.1	21.8	21.4	21.4	21.1	21.5	21.8	21.0	21.0	21.5
Total length/jaw length	9.3	9.3	9.3	9.3	9.3	9.2	9.2	9.3	9.3	9.0	9.3	9.4
Total length/snout length	15.1	14.7	14.7	15.0	14.9	14.8	14.8	14.8	15.0	14.4	15.2	15.6
Head length/head width	5.0	5.0	4.9	5.1	5.0	5.0	4.9	5.0	5.1	4.9	4.8	5.1
Head length/jaw length	2.2	2.2	2.1	2.2	2.2	2.1	2.1	2.2	2.2	2.1	2.1	2.2
Head length/snout length	3.5	3.4	3.4	3.5	3.5	3.4	3.4	3.4	3.5	3.4	3.5	3.7
Jaw length/head width	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.2	2.3
Jaw length/snout length	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7
Snout length/head width	1.4	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.5	1.3	1.4
Sample size	112	103	20	69	114	52	46	80	93	3	8	5

[1] Means associated with any combination of MDH-3*70 were not statistically compared because of zero variance or small (n < 10) sample size.

Table 5. Mean numbers[1] and ranges of females stripped for eggs per spawning season, average effective population size based on three and six males per female[2], and minimum percent retention of genetic diversity per generation based on average effective population size at eight Minnesota Department of Natural Resources spawning stations.

Location	Time frame	Number of females stripped Mean	Number of females stripped Range	Effective population size 3 males	Effective population size 6 males	Percent retention genetic diversity
Seagull River	1980-1989	300	-	900	1,030	99.94
Pike River	1981-1990	2,871	1,787-5,903	8,613	9,843	99.99
Lake Sallie	1972-1990	284	21-640	852	973	99.94
Ottertail River	1972-1989	643	0-1,583	1,929	2,205	99.97
Big Lake Creek	1971-1989	322	0-694	966	1,104	99.95
Ltl. Cutfoot Sioux L.	1980-1989	2,183	656-4,128	6,549	7,485	99.99
Pine River	1982-1989	678	344-885	2,034	2,325	99.98
Boss Creek	1974-1989	357	0-816	1,071	1,224	99.95

[1] Means do not include years when no females were trapped or spawning stations were not operating.
[2] Eggs are fertilized with milt from 3 to 6 males collected at each spawning station.

River and Missourian refugia probably shared a common ancestor more recently than 230,000 years ago.

Genetic divergence of Minnesota walleye (except for Lake Pepin and possibly St. Louis River populations) was unexpectedly unrelated to drainage areas. Those populations within the same drainage and close proximity should have been most genetically similar (Gall 1987). Although 10,000 years of isolation could have been insufficient for detectable genetic divergence to occur, indiscriminate statewide stocking of walleye could have masked any divergence related to isolation.

We believe that introduced fry and fingerlings survived to reproduce in those affected systems. Introduced walleye fingerlings comprised 10 to 95% of selected year-classes in 3 lakes in western Minnesota (B. Parsons, MNDNR, personal communication). Genetically distinct stocks of juvenile walleye introduced into Claytor Lake, Virginia, comprised an average of 67% of the 1976 to 1979 year-class strengths (Murphy et al. 1983). Fry introductions accounted for 36 and 96%, respectively, of the 1971 and 1972 year-class strengths at West Blue Lake, Manitoba (Schweigert et al. 1977).

Mean heterozygosity per locus was higher in walleye from lakes stocked from more sources. Mean heterozygosity per locus in the Boss Creek and Ottertail River populations was higher than that observed in all other populations except Lake Pepin (Table 2). These two systems were also stocked with walleye from more outside sources than the other systems (Figure 1). Increases in mean heterozygosity per locus from mixing stocks of northern largemouth bass *Micropterus salmoides*, Florida largemouth bass *M. s. floridanus*, and their intergrades were demonstrated in pond experiments (D.P. Philipp, Illinois Natural History Survey, personal communication). The high mean heterozygosity per locus observed in the Lake Pepin population was caused by the high frequency of *PROT-4*160* which was

Table 6. Mean allele frequencies (ranges in parentheses) at *MDH-3** and *PROT-4** and mitochondrial DNA haplotypes present in walleye from three Canadian provinces, Minnesota and the eastern Great Lakes (X = present in population; (-) = no data).

Location	*MDH-3**			*PROT-4**		mtDNA haplotype			
	70	*100*	*120*	*100*	*160*	1	4	0	15
Saskatchewan[1,2,3]	0.34 (0.09-0.62)	0.38 (0.01-0.67)	0.28 (0.17-0.38)	-	-		X	X	
Manitoba[1,2,3]	0.09 (0.00-0.23)	0.54 (0.31-0.80)	0.37 (0.10-0.54)	1.00 -	0.00 -		X	X	
Western Ontario[1,2,3]	0.04 (0.03-0.05)	0.66 (0.62-0.70)	0.30 (0.24-0.35)	-	-		X	X	
Minnesota/Hudson Bay Drainage[2,3,4,5,6]	0.03 (0.00-0.10)	0.41 (0.20-0.77)	0.56 (0.23-0.80)	0.99 (0.98-1.00)	0.01 (0.00-0.02)		X	X	
Minnesota/Mississippi R. above St. Anthony Falls[3,4,5,6]	0.09 (0.04-0.13)	0.29 (0.06-0.65)	0.62 (0.31-0.93)	1.00 -	0.00 -		X	X	
Minnesota/Mississippi R. below St. Anthony Falls[3,4,7]	0.01 (0.00-0.04)	0.77 (0.73-0.83)	0.22 (0.18-0.24)	0.39 (0.35-0.41)	0.61 (0.59-0.64)		X	X	X
Minnesota/Atlantic Drainage[2,3,5]	0.00 -	0.46 (0.42-0.50)	0.54 (0.50-0.58)	0.84 (0.83-0.85)	0.16 (0.15-0.17)		X	X	
Eastern Great Lakes[2,3,8]	0.01 (0.00-0.03)	0.69 (0.50-0.92)	0.30 (0.08-0.50)	0.60 (0.29-0.79)	0.40 (0.21-0.71)	X	X		

[1] Clayton et al. (1974).
[2] Ward et al. (1989).
[3] Billington et al. (1990).
[4] Present study.
[5] Murphy and Lee (1986).
[6] N. Billington, University of Guelph, personal communication.
[7] Waltner (1988).
[8] Todd (1990).

rare or absent in the other populations.

Non-random mixing of alleles, caused by mutation, migration, selection, or genetic drift (Utter et al. 1987) could also affect genetic variation. However, it was unclear whether any significant observed deviations in Hardy-Weinberg equilibrium were of concern because of inconsistencies among years. Data in Murphy and Lee (1986) show additional inconsistencies in Hardy-Weinberg equilibriums. They reported no significant deviation at *IDHP-1** in Pine River walleye, and we found deviation in that population. They did report significant deviation at *sMDH-B** in Little Cutfoot Sioux Lake walleye. Significant deviation at *sMDH-B** was also observed in the 1987 samples from Little Cutfoot Sioux Lake, but not in the Lake Winnibigoshish samples collected in 1990.

Clustering of meristic characteristics generally reflected clustering of unbiased genetic distances; therefore, meristic characteristics could be useful for identification of genetically different walleye populations in Minnesota. Meristic variables in fish have high heritabilities (Allendorf et al. 1987); however, environmental influences can alter meristic variation in fish (MacGregor and MacCrimmon 1977).

Clustering of morphometric characteristics did not reflect clustering of unbiased genetic distances. Therefore, morphometric characteristics would not be useful for identification of genetic variation in Minnesota walleye populations. Variation in morphometric measurements in fish in general were seldom linked with genetic variation (Allendorf et al. 1987). Uthe and Ryder (1970) reported no relationship in body ratios (orbit length/interorbital width, standard length/head length, interpelvic width/width of base of the pelvic fin, and upper jaw length/width of gape) of walleye and polymorphism in general muscle protein isozymes. Colby and Nepszy (1981) suggested that genetic components involved with morphometric characteristics in walleye stocks were probably masked by environmental influences.

Operation of spawning stations probably had little affect on genetics of walleye populations adjacent to these stations. Effective population sizes were large enough so that little genetic diversity would be lost, even if these same brood stocks were used as founder populations. Natural reproduction has also been documented within the stream where spawning stations are located, at other streams within the watershed, and within the adjacent lake.

Genetic diversity was further preserved because new brood stock of varying age and size (Table 3) were collected each year. New brood stocks were comprised of few individuals from the previous years brood stock. At Boss Creek, 6% of the 1988 brood stock consisted of individuals from the 1987 brood stock (MNDNR, file data). Olson et al. (1978) hypothesized that homing of walleyes was a learned behavior; consequently, walleye would not necessarily spawn in the same streams where they were hatched and may mix within drainages.

Management Implications

Effects of indiscriminate stocking on walleye populations were unknown. Indiscriminate walleye stocking had been conducted at least 60 years before any growth, survival, genetics, or other life history data were obtained. Other factors such as overexploitation, habitat alteration, biotic interactions, among others, have affected walleye populations in Minnesota (Osborn et al. 1981; Osborn and Schupp 1985). These factors could have masked effects related to indiscriminate stocking.

New genetic material from introduced walleye has probably been incorporated into the genome of the affected populations. Leary and Booke (1990), and Philipp and Whitt (1991) reported that genetic changes in fish populations resulting from mixing stocks were irreparable.

Work by Philipp and Whitt (1991) suggests that native stocks are best fit for their local environment, and that genetic changes resulting from mixing stocks reduces this

fitness. Indiscriminate stocking into systems with native self-sustaining walleye populations should not be done because this practice could cause poorer survival and growth in native stocks. Stockings of walleye between watersheds should not be done for similar reasons.

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