Transactions of the American Fisheries Society
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/utaf20

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Matthew D. Waterhouse[a], Brian L. Sloss[b] & Daniel A. Isermann[b]

[a] Wisconsin Cooperative Fishery Research Unit, College of Natural Resources, University of Wisconsin-Stevens Point, Stevens Point, Wisconsin 54481, USA

[b] U.S. Geological Survey, Wisconsin Cooperative Fishery Research Unit, College of Natural Resources, University of Wisconsin-Stevens Point, Stevens Point, Wisconsin 54481, USA

Published online: 30 Apr 2014.

To cite this article: Matthew D. Waterhouse, Brian L. Sloss & Daniel A. Isermann (2014) Relationships among Walleye Population Characteristics and Genetic Diversity in Northern Wisconsin Lakes, Transactions of the American Fisheries Society, 143:3, 744-756

To link to this article: http://dx.doi.org/10.1080/00028487.2014.880742

Transactions of the American Fisheries Society 143:744–756, 2014

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2014.880742

ARTICLE

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Matthew D. Waterhouse
Wisconsin Cooperative Fishery Research Unit, College of Natural Resources, University of Wisconsin–Stevens Point, Stevens Point, Wisconsin 54481, USA

Brian L. Sloss* and Daniel A. Isermann
U.S. Geological Survey, Wisconsin Cooperative Fishery Research Unit, College of Natural Resources, University of Wisconsin–Stevens Point, Stevens Point, Wisconsin 54481, USA

Abstract

The maintenance of genetic integrity is an important goal of fisheries management, yet little is known regarding the effects of management actions (e.g., stocking, harvest regulations) on the genetic diversity of many important fish species. Furthermore, relationships between population characteristics and genetic diversity remain poorly understood. We examined relationships among population demographics (abundance, recruitment, sex ratio, and mean age of the breeding population), stocking intensity, and genetic characteristics (heterozygosity, effective number of alleles, allelic richness, Wright's inbreeding coefficient, effective population size [N_e], mean d^2 [a measure of inbreeding], mean relatedness, and pairwise population Φ_{ST} estimates) for 15 populations of Walleye *Sander vitreus* in northern Wisconsin. We also tested for potential demographic and genetic influences on Walleye body condition and early growth. Combinations of demographic variables explained 47.1–79.8% of the variation in genetic diversity. Skewed sex ratios contributed to a reduction in N_e and subsequent increases in genetic drift and relatedness among individuals within populations; these factors were correlated to reductions in allelic richness and early growth rate. Levels of inbreeding were negatively related to both age-0 abundance and mean age, suggesting N_e was influenced by recruitment and generational overlap. A negative relationship between the effective number of alleles and body condition suggests stocking affected underlying genetic diversity of recipient populations and the overall productivity of the population. These relationships may result from poor performance of stocked fish, outbreeding depression, or density-dependent factors. An isolation-by-distance pattern of genetic diversity was apparent in nonstocked populations, but was disrupted in stocked populations, suggesting that stocking affected genetic structure. Overall, demographic factors were related to genetic diversity and stocking appeared to alter allelic frequencies and the genetic structure of Walleye populations in Wisconsin, possibly resulting in disruption of local adaptation.

The conservation of genetic diversity and protection of genetic integrity are common goals of fisheries management (Vrijenhoek 1998; Wang et al. 2002). Genetic integrity refers to the relative temporal stability of genetic diversity within and among populations of a species. Maintaining genetic integrity helps to conserve genetic diversity by ensuring the adaptability and subsequent viability of a species in a given region (Quattro and Vrijenhoek 1989). Genetic diversity has been directly connected to fitness in a broad range of taxa (Reed and Frankham 2003), including numerous fish populations (Kartavtsev 1998; Thelen and Allendorf 2001). Cumulatively, populations of a species, each with their own unique genetic composition, exhibit genetic structure across the landscape that is largely influenced by migration routes and connectivity (Manel et al. 2003). This structure is critical to the distribution of local adaptations among populations. Identifying and understanding genetic structure is a central tenet of the stock concept (Berst and Simon 1981) and a foundation of contemporary fisheries management.

*Corresponding author: bsloss@uwsp.edu
Received September 4, 2013; accepted November 19, 2013

However, the influence of demography and anthropogenic factors on this structure is often difficult to incorporate into fisheries management.

Walleye *Sander vitreus* is an iteroparous freshwater fish with a broad distribution in the United States and Canada (Hartman 2009). Wisconsin lies in the center of the native range of Walleye and, with over 900 populations occurring in northern Wisconsin lakes, Walleye has both an ecological and a socioeconomic importance to the state (Staggs et al. 1990; Hewett and Simonson 1998). In 1998, the Wisconsin Department of Natural Resources (WDNR) developed a statewide Walleye management plan to better guide management decisions (Hewett and Simonson 1998). One of the goals of this plan was to maintain the genetic integrity of naturally reproducing Walleye populations in Wisconsin. The objectives of this goal were to determine and utilize any performance benefits of genetically distinct stocks, examine the influence of historical stocking on population genetics, and ensure stocking does not have a negative effect on naturally reproducing Walleye populations. To ensure the genetic integrity of Walleye and other fish species, it is necessary to understand the relationships among population characteristics, management practices, and genetic diversity.

Population size is perhaps the single most influential predictor of genetic diversity and integrity in natural populations (Frankham 1996; Reed 2004). Frankham (1996) documented numerous positive associations between total population size and genetic diversity that contribute to the loss of viability and fitness in small populations (Reed 2004). While this broad connection may set the upper limit of a population's genetic diversity, the actual rate of genetic change in a population is related to the effective population size (N_e). The N_e is the size of an idealized population that exhibits the same level of genetic drift as the population in question (Waples 1989) and, thus, N_e determines the rate of genetic drift, inbreeding, and the fixation of deleterious alleles in the population (Schwartz et al. 1998). In essence, this is the "genetic size" of the population and is nearly always less than the total population size (Turner et al. 2002; Shrimpton and Heath 2003). Various demographic attributes may play a role in reducing N_e including fluctuating population size (Vucetich et al. 1997), unequal sex ratios (Allendorf and Luikart 2008), total reproductive output (Felsenstein 1971), and generational overlap (Gaggiotti and Vetter 1999). All of these attributes are common in Walleye populations and in many cases have the potential to impact the genetic integrity of the population.

Stocking is another key influence on the distribution and potential disruption of natural patterns of genetic diversity and integrity in fish (Englbrecht et al. 2002; Marie et al. 2010). In fact, stocking fish from inappropriate brood sources has been proposed as an explanation for many anomalies observed in the genetic structure of Walleye over broad geographic regions (Billington et al. 1992; Stepien and Faber 1998; Stepien et al. 2009). Currently, Wisconsin's Walleye propagation program selects brood sources based on watershed management units (Fields et al. 1997) that have recently been shown to be partially inconsistent with contemporary Walleye genetic structure (Hammen 2009). The transfer of fish across natural genetic boundaries can result in the introduction of nonnative genetic material to the recipient population that could result in a measurable increase in genetic diversity. This process likely explains the positive relationship between genetic diversity and stocking intensity observed in Walleye populations across Ontario (Cena et al. 2009). However, stocking can threaten the genetic integrity of Walleye, as was demonstrated in Escanaba Lake, Wisconsin, where the influx of nonnative alleles associated with stocking events eventually led to the displacement of the original gene pool (Franckowiak et al. 2009). Higher genetic diversity should not be presumed to be better, as a majority of natural selection mechanisms result in reductions of genetic diversity; the fittest allelic variants supplant the less-fit variants. Therefore, each population of a species will have a dynamic optimum level of genetic diversity that can be difficult to predict but easy to perturb. Assuming natural genetic diversity is adapted to local conditions, the addition of exogenous genetic diversity with or without displacement of endogenous diversity can represent a distinct threat to population viability.

Long-term monitoring data available for Walleye populations in northern Wisconsin provided an opportunity to evaluate relationships among population characteristics, stocking intensity, and the genetic diversity of an intensively managed fish species. The objectives of this study were to: (1) determine whether relationships existed between Walleye population demographics (i.e., adult abundance, sex ratios, mean age of the breeding population, and recruitment) and genetic diversity in populations with different stocking intensities, and (2) determine whether growth and body condition were related to genetic or demographic variables in northern Wisconsin Walleye populations.

METHODS

Study site and sample collection.—During the spring and fall of 2010 and 2011, 849 Walleyes were collected by boat electrofishing using AC from the littoral habitat of eight stocked lakes and seven lakes with no record of Walleye stocking (i.e., nonstocked lakes). All lakes were located in Oneida and Vilas counties in northern Wisconsin (Table 1; Figure 1). Eight populations were sampled in the spring of 2010, all Pelican Lake samples and 15 of the 55 samples from Kawaguesaga Lake were collected in the fall of 2010, and five populations and the rest of the Kawaguesaga Lake samples were collected in the spring of 2011. All Walleyes were measured for TL (mm) and weight (nearest 5 g). When possible, sex was determined by extrusion of gametes. A small fin clip (~15 mm) was removed from the anal fin and preserved in 95% nondenatured ethanol for subsequent genetic analysis. The third spine from the anterior end of the dorsal fin was removed for age and growth estimation by cutting the spine as close to the body as possible.

TABLE 1. Genetic sample size (n), expected heterozygosity (H_e), effective number of alleles (A_E), allelic richness (A_R), F_{IS} values, mean d^2, mean pairwise relatedness (R_L), mean age-0 abundance (Age 0; $\log_e$ total abundance), mean male : female sex ratio (M:F), mean adult population estimates (PE; $\log_e$ total abundance), the average age of the breeding population in years (Age_P), stocking index (SI), the mean back-calculated lengths (mm) at ages 1 (L_1), 2 (L_2), and 3 (L_3), and mean relative condition factor (K_n) for 15 Walleye populations in northern Wisconsin lakes.

Lake population	n	H_e	A_E	A_R	F_{IS}	d^2	R_L	Age 0	M:F	PE	Age_P	SI	L_1	L_2	L_3	K_n
Big Arbor Vitae (BAV)[a]	88	0.770	4.57	9.24	–0.002	23.8	0.057	11.0	9.32	8.86	3.72	0.0	151	244	317	101.0
Eagle Chain (EC)[a]	56	0.762	4.48	8.98	–0.004	27.6	0.057	11.0	7.00	8.69	4.71	0.0	132	199	253	107.0
Kawaguesaga (KL)[a]	55	0.758	4.41	9.23	0.018	24.3	0.053	7.1	2.97	7.98	5.67	0.0	141	231	310	99.8
Little Arbor Vitae (LAV)[a]	62	0.757	4.33	9.65	0.019	22.7	0.053	9.2	3.08	8.13	6.52	0.0	131	225	290	106.0
Pelican (PEL)	49	0.756	4.43	8.44	–0.023	28.9	0.056	11.0	8.92	9.09	5.22	0.0	126	222	305	108.0
Plum (PLU)	50	0.761	4.31	9.22	–0.031	22.2	0.052	10.6	3.67	8.34	6.25	0.0	122	204	281	
Willow Flowage (WIL)[a]	49	0.766	4.27	9.32	–0.002	22.1	0.053	11.3	2.40	9.90	4.92	0.0	162	247	321	105.0
Big St. Germain (BSG)	48	0.778	4.57	9.17	–0.004	26.5	0.051	10.4	5.89	8.48	7.16	66.0	146	243	321	104.0
North Twin (NTL)	62	0.761	4.68	9.85	0.001	26.8	0.050	11.5	3.80	8.75	4.85	51.1	150	238	295	96.9
Papoose (PAP)	60	0.759	4.50	8.90	0.020	26.1	0.052	8.2	4.33	6.85	5.21	13.1	124	216	283	96.1
Thunder (THU)	50	0.783	4.63	10.00	0.051	25.9	0.048	4.2	0.18	8.09	7.20	77.2	149	233	304	98.0
Tomahawk (TOM)	49	0.775	4.55	9.92	–0.046	28.1	0.051	8.8	1.84	8.79	9.81	132.2	159	260	335	97.1
Trout (TRO)	67	0.779	4.71	9.50	0.015	26.0	0.054	9.2	1.65	9.06	6.21	98.5	136	223	292	94.6
Two Sisters (TWS)	44	0.770	4.54	9.40	0.030	20.5	0.050	6.8	1.73	7.63	5.75	33.9	152	265	354	98.7
White Sand (WSL)	60	0.766	4.38	9.35	0.003	23.1	0.055	8.2	7.17	7.45	6.54	30.5	150	235	301	97.5

[a]Nonstocked systems.

Genetic analysis.—Walleye DNA was extracted from fin samples using the Promega Wizard Genomic DNA purification kit (Promega, Madison, Wisconsin). Ten fluorescently labeled dinucleotide microsatellite loci (*Svi-17* and *Svi-33*; from Borer et al. 1999; *Svi-L5* and *Svi-L9* from Wirth et al. 1999; *Svi-2*, *Svi-4*, *Svi-6*, *Svi-7*, *Svi-20*, and *Svi-26* from Eldridge et al. 2002) were amplified using three multiplex reactions (Table A.1 in the Appendix), and amplicon length was determined using an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California) with an in-lane standard (Geneflo 625, Chimerx, Milwaukee, Wisconsin). Resulting genotypes were identified using GeneMapper 4.0 (Applied Biosystems) and compiled using Microsoft Office Excel 2010 version14.0.6 (Microsoft, Redmond, Washington). A minimum of seven successfully genotyped loci were required for a given sample to be included in subsequent analyses and to ensure consistency of the genetic data; 10% of the samples were genotyped a second time.

All loci were tested for deviations from Hardy–Weinberg expectations (HWE) in each population using a chi-square test implemented in GenAlEx version 6.4 (Peakall and Smouse 2006). To account for multiple comparisons, significant deviations from HWE were evaluated using sequential Bonferroni correction (Rice 1989). To reduce the extraneous effects of highly polymorphic loci on HWE (Pamilo and Varvio-Aho 1948), significant tests from the original analysis were reanalyzed after pooling rare genotypes (expected frequency < 1) into one observed and expected frequency value (modified from Hedrick 2000). Linkage disequilibrium was tested between all pairs of loci in each population using the exact test of Guo and Thompson (1992) implemented in GENEPOP 4.0 (Raymond and Rousset 1995; Rousset 2008) with a Markov chain method of 10,000 dememorization steps, 100 batches, and 10,000 iterations per batch. Evidence of null alleles, sequence stutter, and typographic errors were examined using MICRO-CHECKER version 2.2.3 (Oosterhout et al. 2004).

Microsatellite Toolkit version 3.1 (Park 2001) was used to calculate expected heterozygosity (H_e) and GenAlEx version 6.4 was used to calculate the effective number of alleles (A_E) for each population. The program HP-RARE version 1.0 (Kalinowski 2005) was used to estimate allelic richness (A_R) using the rarefaction method described by Leberg (2002) to account for biases caused by unequal sample sizes. Inbreeding was estimated in each population using F_{IS} (Wright 1922) calculated with ARLEQUIN version 3.11 (Excoffier et al. 2005). Individual d^2, a measure proposed by Coulson et al. (1998) as a potential measure of inbreeding, was calculated within each population as the mean squared difference in repeat units of alleles at each locus and averaged across all loci; mean d^2 was calculated as an average of the individual d^2 for each population. Pairwise estimates of relatedness ($\hat{r}_{xy}$,Lynch and Ritland 1999) were calculated within each population using the maximum likelihood method implemented in ML-RELATE (Kalinowski et al. 2006) and averaged for each population (R_L).

Demographics, growth, and body condition.—Demographic data used in this study were collected by the WDNR and the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) between 1990 and 2009. Adult population estimates were based on mark–recapture surveys conducted during the spring spawning period (April–May). Adult Walleyes ($\geq$38 cm or that produced gametes upon extrusion) were captured in fyke nets and

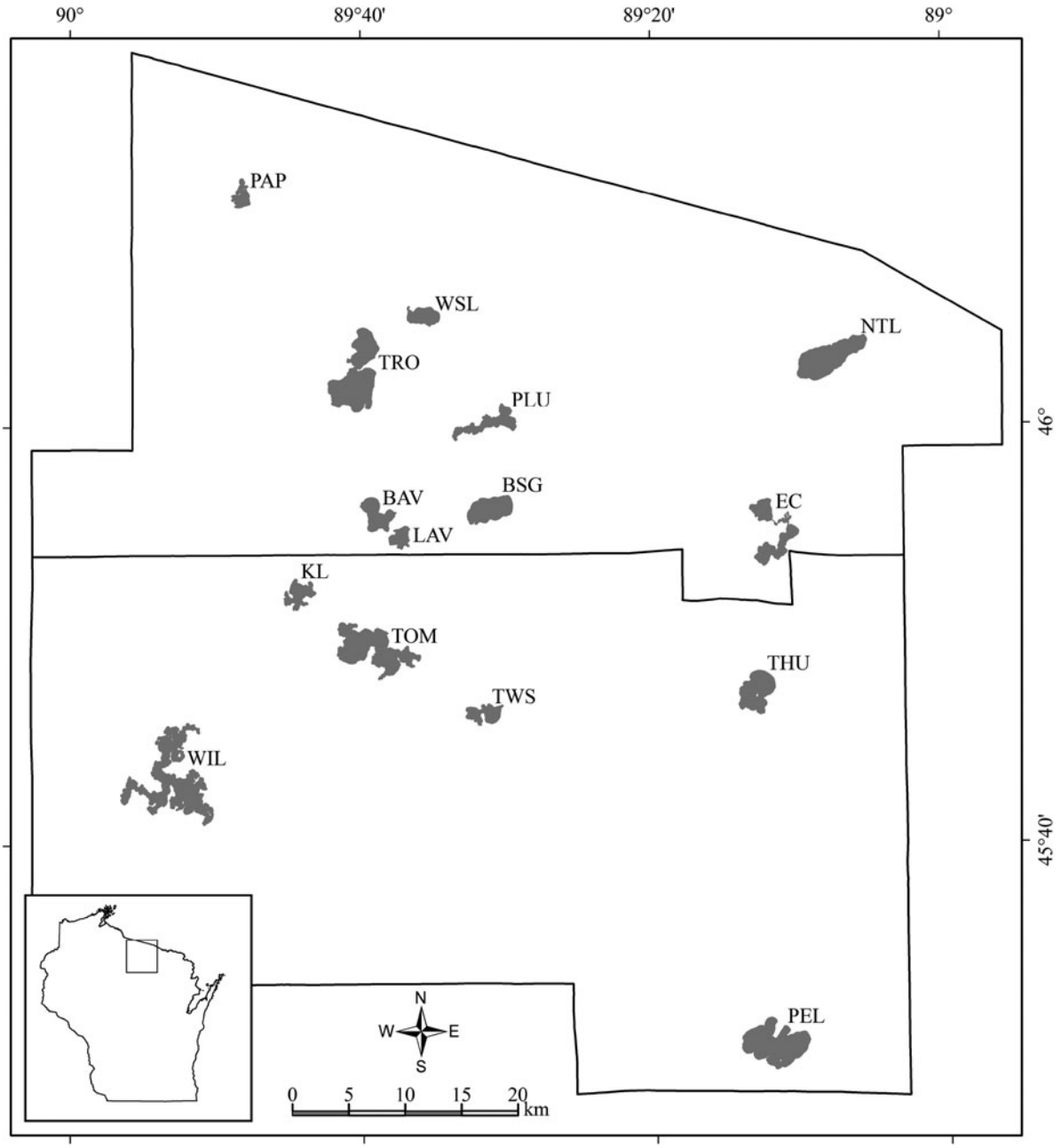

FIGURE 1. Vilas County (upper) and Oneida County (lower) in the state of Wisconsin containing the 15 study sites. Lake names corresponding to abbreviations are listed in Table 1.

marked by partial removal of one or more fins. The entire lake shoreline was electrofished using AC 1–2 d after fyke-net sampling; all Walleyes caught were examined for the presence of fin clips (Beard et al. 1997) and adult abundance was estimated using the Chapman modification of the Petersen estimator (Ricker 1975). Electrofishing data were also used to calculate male : female sex ratios (M:F) with the knowledge that these ratios would be inherently biased towards males because of differences in spawning-related behavior that contribute to sex-specific differences in catchability (Schneider et al. 2007). We averaged estimates of adult abundance and sex ratio for populations where mark–recapture surveys were conducted in multiple years and then $\log_e$-transformed population estimates (PE) to normalize the data.

Standardized fall electrofishing surveys were used to estimate age-0 Walleye abundance. Electrofishing surveys for age-0 Walleyes were conducted by WDNR and GLIFWC personnel in September and October when water temperatures were 41–74°F

(5–23°C). The majority of the shoreline (including islands) was surveyed and catch per effort (CPE) was reported as the mean number of age-0 Walleyes caught per mile of shoreline surveyed. All Walleye populations included in the study were required to have a minimum of 10 age-0 Walleye surveys, with the most recent survey occurring during or after 2004. Catch per effort of age-0 Walleyes was converted to an estimate of density using a temperature-corrected nonlinear model described by Hansen et al. (2004) as follows:

$$\text{CPE} = 31,101\left(\frac{N}{A}\right)^{0.686} \times \text{Temp}^{-2.045},$$

where N/A refers to age-0 Walleye density (number of fish/acre), and Temp is the recorded water temperature (°F) during the survey. Mean age-0 Walleye abundance for each lake was calculated by multiplying mean age-0 fish density during 1990–2008 by lake surface area and was subsequently normalized by a $\log_e$ transformation.

Stocking records from 1998 to 2009 were used to describe stocking intensity for each population (WDNR 2012). The index of hatchery effort used by Cena et al. (2006) was modified to account for differential survival to age 3 of the various size-classes (fry, small, large, or extended growth fingerling) of Walleyes stocked into Wisconsin lakes. The index of stocking intensity (SI) used in this study was calculated as

$$\text{SI} = \frac{\sum \text{surv}_i \times \text{YOY}_i}{\log_e(\text{SA}) \times t}.$$

where surv_i refers to the expected survival to age 3 of the ith size-class as reported by WDNR (1999), YOY_i refers to the number of Walleyes of the ith size-class that were stocked, t indicates the number of years from the first recorded stocking event to the most recent event, and SA refers to lake surface area (ha).

Growth rates and body condition were estimated from all fish sampled for genetic analysis. Age and growth methods were adapted from Borkholder and Edwards (2001). Digital images of thin-sectioned dorsal spines were interpreted by two independent readers to estimate the age of each fish. If readers did not agree on an age for an individual fish, both readers viewed the spine simultaneously to reach a consensus age. In four cases, a consensus age was not reached; these fish were not included in subsequent growth analyses. Since our sampling targeted adult Walleyes, all fish with consensus ages of <3 were excluded from subsequent analyses. Mean age estimates were calculated for each population (Age_P).

Growth was described using back-calculated lengths at age i (L_i) estimated from a linear regression incorporating TL of fish at time of capture (L_c), total spine radius along the anterior elongated axis (S_c), and the distance from spine focus to the outside edge of each ith annuli as follows:

$$L_i = K + (L_c - K) \times (S_i/S_c).$$

The length at which Walleyes develop the third dorsal spine (K) was estimated from the x-intercept of the linear regression between L_c and S_c (Borkholder and Edwards 2001). Sexually dimorphic growth was evaluated using t-tests ($\alpha = 0.05$) comparing mean back-calculated lengths at age of all known males and females. Mean lengths at age (both sexes combined) for all ages before significant sexually dimorphic growth was apparent were used as response variables in subsequent analyses.

Relative condition factor (K_n; Blackwell et al. 2000) was used to describe the body condition of Walleyes:

$$K_n = {}^{W_i}/_{W_i'} \times 100,$$

where W_i is the weight of an individual Walleye at length i and W_i' is the expected weight of a Walleye at length i. To estimate W_i', we used the weight–length relationship for all Walleyes collected from the study lakes determined as follows:

$$W_i' = (2.051 \times 10^{-6})L_i^{3.236}.$$

Mean K_n was calculated for each population and used as a response variable in subsequent analyses. All fish from Pelican Lake and fish collected during the fall from Kawaguesaga Lake were excluded from condition analysis to avoid problems with seasonal variability in body condition.

Statistical analyses.—Genetic variables were tested for normality using Shapiro–Wilk tests in SYSTAT version 11.0 (SYSTAT Software, Chicago, Illinois) and nonnormally distributed variables were $\log_e$ transformed. A two-tailed t-test in PASW Statistics (version 18.0.0) was used to test for differences in each genetic characteristic between nonstocked ($N = 7$) and stocked ($N = 8$) Walleye populations. A correlation matrix was used to assess covariance. Simple linear regressions were used to relate each genetic characteristic to each demographic variable. All models were tested for significance ($\alpha = 0.05$) and were visually inspected for nonlinear trends. Residuals from each regression model were tested for normality using Shapiro–Wilk tests. Forward stepwise regression was used to consider more complex demographic models for each genetic characteristic ($\alpha = 0.05$ to enter a characteristic, $\alpha = 0.10$ to remove a characteristic). The same series of simple and multiple linear regressions were used to test for relationships between mean lengths at age or mean K_n and all genetic and demographic variables.

To test whether Walleye populations naturally exhibited isolation by distance (IBD), genetic distances were measured between all nonstocked populations using Φ_{ST} (Excoffier et al. 1992) in GenAlEx version 6.4 (Peakall and Smouse 2006) with 10,000 permutations to determine significance. Geographic distance (km) was measured between the approximated centers of each lake using ArcGIS 9.3.1 (ESRI, Redland, California), and

the resulting matrices were correlated using a Mantel test implemented in GenAlEx version 6.4 (Peakall and Smouse 2006) with 1,000 permutations to determine significance ($\alpha = 0.05$). To test whether stocking disrupted IBD, this process was repeated for all pairwise combinations of stocked populations. A disruption of IBD by stocking would be indicated whether this model was significant in nonstocked populations but not significant in stocked populations. Additionally, to assess whether the relationship between genetic distance and geographic distance differed between nonstocked and stocked populations we used ANCOVA.

TABLE 2. Mean (± SD) back-calculated length (mm) at age (years) for all known male and female Walleyes in this study. Degrees of freedom (df), *t*-value, and *P*-value are shown for each one-way *t*-test comparing mean back-calculated length at age between male and female Walleyes. Significance was assessed following sequential Bonferroni corrections ($\alpha_o = 0.0125$).

Age	Male length	Female length	df	*t*	*P*
1	146 ± 28	151 ± 34	105	1.467	0.073
2	236 ± 37	240 ± 38	112	0.987	0.163
3	304 ± 41	316 ± 45	108	2.223	0.014
4	345 ± 38	382 ± 49	103	6.532	<0.001

RESULTS

Genetic Characteristics

The sample size for each population varied from 44 to 88 Walleyes (mean = 57, SD = 10.9). Initial chi-square analysis showed 10% (15 of 150) of all comparisons deviated from HWE expectations ($\alpha = 0.05$). Following the pooling of rare genotypes and sequential Bonferroni correction ($\alpha_1 = 0.0003$), no comparisons significantly deviated from HWE expectations. No evidence of linkage disequilibrium was observed. Three populations and two loci showed evidence of null alleles (Two Sisters Lake for *Svi-L9* and Thunder and Pelican lakes for *Svi-20*). However, expected frequencies of null alleles were low (<0.1%) and no evidence of null alleles at a given locus was observed in more than two populations; therefore, no correction was performed. Genetic diversity was high with mean H_e of 0.767 (SD = 0.009), mean A_E of 4.49 (SD = 0.133), and mean A_R of 9.34 (SD = 0.410; Table 1). Values for F_{IS} averaged 0.003 (SD = 0.024) across all populations; Thunder Lake had the highest F_{IS} (0.051). The mean d^2 across all sampled populations was 24.94 (SD = 2.53) and the mean R_L was 0.053 (SD = 0.003).

Demographics, Growth, and Body Condition

Mean adult population estimates were based on an average of 3.9 population surveys (SD = 2.0) for each lake. Population sex ratios were generally skewed towards males (mean = 4.3, SD = 2.8). Stocked lakes had a total of 101 recorded stocking events since 1998 (mean = 12.6, SD = 8.0). The intercept for the linear regression between Walleye spine radius and TL indicated that Walleyes first develop dorsal spines at 29.75 mm TL ($F = 2{,}450.7$, df = 824, $P < 0.001$). Sexually dimorphic growth was not apparent for age-1, age-2, or age-3 Walleyes but mean lengths did differ between sexes for age-4 fish (Table 2). Therefore, mean lengths at ages 1 (L_1), 2 (L_2), and 3 (L_3) were calculated with both sexes combined and were used in subsequent analyses.

Statistical Analysis

Shapiro–Wilk tests indicated each genetic diversity measure was normally distributed. The correlation matrix showed that genetic variables were independent with the exceptions of a weak correlation between A_R and H_e ($r = 0.52$, df = 13, $P < 0.048$) and A_R and R_L ($r = 0.60$, df = 13, $P = 0.019$; Table 3). Stocked populations showed higher levels of genetic diversity as measured by H_e (mean = 0.771, SD = 0.003) when compared with nonstocked populations (mean = 0.761, SD = 0.002; $t = -2.620$, df = 13, $P = 0.021$). Also, A_E in stocked populations (mean = 4.57, SD = 0.04) was significantly higher than in nonstocked populations (mean = 4.40, SD = 0.04; $t = -3.145$, df = 13, $P = 0.008$), but mean relatedness was significantly higher in nonstocked populations (mean = 0.055, SD = 0.001) than in stocked populations (mean = 0.052, 0.001; $t = 2.715$, df = 13, $P = 0.018$). All other genetic characteristics did not significantly differ between nonstocked and stocked populations.

Genetic characteristics were significantly related to stocking intensity, age-0 Walleye abundance, and M:F ratio (Table 4). Both H_e and A_E were positively related to stocking intensity (Figure 2) and A_R was negatively related to M:F and positively related to SI. There was a positive relationship between R_L and M:F. There was a negative relationship between F_{IS} and age-0 Walleye abundance, but the residuals of this linear regression were not normally distributed (SW = 0.779, $P = 0.002$) and one lake produced a large residual value (Lake Tomahawk: residual = −2.98 SDs from the predicted value). All other models had no outlying residual values (>3 SDs from the mean), no patterns in the residuals were observed, and residuals were normally distributed. Stepwise regression analysis indicated that demographic variables explained a significant amount of variation in each genetic characteristic, with the exception of mean d^2 (Table 5). Coefficients of determination (r^2) showed more than half of the variation in each genetic parameter was accounted for by the resulting demographic model, with the exception of A_R ($r^2 = 0.471$). The demographic model for both A_E and F_{IS} had the highest r^2 values (≥0.70).

Simple linear regression showed K_n was negatively related to both A_E ($r^2 = 0.333$, $F = 6.01$, df = 12, $P = 0.030$) and SI ($r^2 = 0.328$, $F = 5.84$, df = 12, $P = 0.032$). There was also a significant positive relation between L_1 and A_R ($r^2 = 0.320$, $F = 6.11$, df = 13, $P = 0.028$); however, L_2 and L_3 had no significant relationship to any of the genetic variables. Forward stepwise regression did not result in any combination

TABLE 3. Correlation matrix showing the correlation coefficients between expected heterozygosity (H_e), effective number of alleles (A_E), allelic richness (A_R), F_{IS} values, mean d^2, mean pairwise relatedness (R_L), mean age-0 abundance (Age 0), mean male : female sex ratio (M:F), mean adult population estimate (PE), the average age of the breeding population in years (Age_P), stocking index (SI), the mean back-calculated lengths (mm) at ages 1 (L_1), 2 (L_2), and 3 (L_3), and mean relative condition factor (K_n) for 15 Walleye populations in northern Wisconsin. Asterisk (*) indicates correlation is significant at the 0.05 level (two-tailed); **indicates correlation is significant at the 0.01 level (two-tailed).

Parameter	H_e	A_E	A_R	F_{IS}	d^2	R_L	Age 0	M:F	PE	Age_P	SI	L_1	L_2	L_3
A_E	0.594*													
A_R	0.518*	0.361												
F_{IS}	0.207	0.253	0.236											
d^2	0.077	0.470	–0.148	–0.307										
R_L	–0.386	–0.260	–0.595*	–0.329	0.128									
Age 0	–0.389	–0.188	–0.396	–0.669**	0.182	0.513								
M:F	–0.374	–0.152	–0.686**	–0.377	0.249	0.758**	0.569*							
PE	0.141	0.009	0.022	–0.437	0.212	0.268	0.599*	0.07						
Age_P	0.486	0.102	0.507	–0.229	0.193	–0.486	–0.412	–0.49	–0.11					
SI	0.764**	0.675**	0.622*	–0.067	0.388	–0.477	–0.311	–0.48	0.09	0.749**				
L_1	0.508	0.212	0.563*	0.036	–0.181	–0.282	–0.123	–0.24	0.29	0.224	0.420			
L_2	0.426	0.238	0.436	0.048	–0.250	–0.383	–0.211	–0.26	0.11	0.302	0.428	0.847**		
L_3	0.390	0.130	0.249	0.009	–0.307	–0.372	–0.259	–0.24	0.08	0.309	0.337	0.689**	0.942**	
K_n	–0.409	–0.594*	–0.508	–0.265	0.059	0.424	0.464	0.45	0.40	–0.265	–0.591*	–0.266	–0.289	–0.191

of predictor variables that explained a significant amount of variation in *Kn* or L_1.

Significant genetic divergence occurred among most populations (Table A.2). Mean Φ_{ST} between populations was 0.025 (SD = 0.016). Approximately 55% (58 of 105) of the pairwise comparisons were significant after sequential Bonferroni corrections ($\alpha_1 = 0.00047$). A significant relationship existed between geographic and genetic distance in nonstocked populations ($Z = 5{,}154.7$, $P = 0.019$) but not in stocked populations ($Z = 1{,}117.9$, $P = 0.068$; Figure 3). An ANCOVA indicated that the slope ($F = 28.06$, df = 46, $P < 0.001$) and intercept ($F = 5.25$, df = 46, $P = 0.027$) of the relationship between genetic distance and geographic distance significantly differed between nonstocked and stocked populations.

DISCUSSION

Our analysis demonstrated that genetic diversity was related to demographics and stocking intensity for Walleyes in northern Wisconsin. The observed levels of genetic diversity in the sampled Wisconsin Walleye populations were comparable with values reported in other Walleye studies using similar genetic methods (Cena et al. 2006; Franckowiak et al. 2009; Hammen et al. 2009). Although our experimental design did not directly assess causative relationships among the variables, the observed relationships are important to consider for the conservation of Walleye genetic integrity. We documented apparent losses of genetic diversity associated with low levels of recruitment and skewed sex ratios. Loss of genetic diversity represents a potential threat to the genetic integrity of Walleye and has implications for the management of other fish species. Highly skewed sex ratios and low levels of recruitment likely caused reductions in N_e that, in turn, induced losses of genetic diversity via genetic drift (Felsenstein 1971; Ryman et al. 1981). Apparent effects of stocking were observed in increased intrapopulation genetic diversity and decreased interpopulation genetic divergence consistent with stocking-induced genetic introgression (Englbrecht et al. 2002; Finnegan and Stevens 2008; Marie et al. 2010). The

TABLE 4. Slope and coefficients of determination (r^2) for simple linear regressions between genetic and demographic characteristics (df = 1, 13 for all analyses). An asterisk (*) indicates a significant relationship ($\alpha_o = 0.05$). Demographic characteristics including mean age-0 abundance (Age 0), the mean male : female sex ratio (M:F), mean adult population estimate (PE), mean age of the breeding population in years (Age_P), and stocking index (SI). Genetic characteristics included expected heterozygosity (H_e), effective number of alleles (A_E), allelic richness (A_R), Wright's inbreeding coefficient (F_{IS}), d^2, and mean pairwise relatedness (R_L).

	H_e		A_E		$A_{R.}$		F_{IS}		d^2		R_L	
Predictor	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
Age 0	–0.002	0.151	–0.012	0.036	–0.079	0.092	–0.008	0.447*	0.224	0.033	0.001	0.263
M:F	–0.001	0.140	–0.007	0.023	–0.101	0.471*	–0.003	0.142	0.226	0.062	0.001	0.575*
PE	0.002	0.020	0.002	0.000	0.012	0.000	–0.014	0.191	0.710	0.045	0.001	0.072
Age_P	0.003	0.236	0.010	0.010	0.145	0.258	–0.004	0.052	0.342	0.037	–0.001	0.236
SI	1.58×10^{-4}	0.584*	0.002	0.456*	0.006	0.387*	-3.80×10^{-5}	0.004	0.023	0.151	-2.86×10^{-5}	0.227

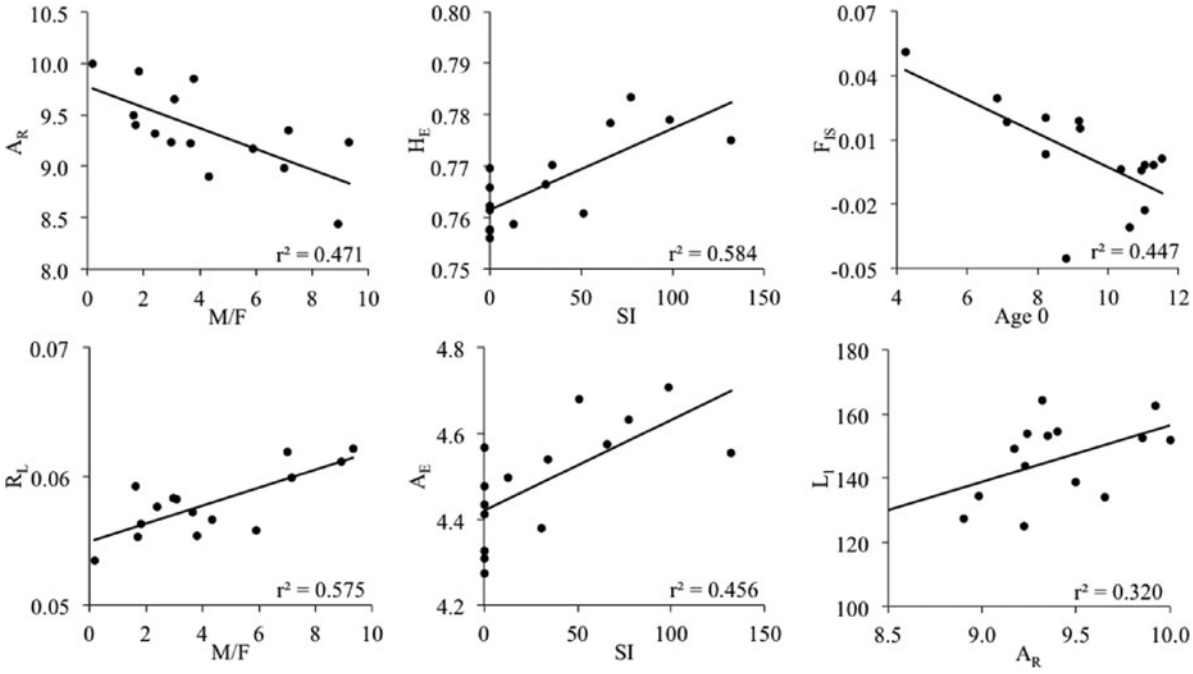

FIGURE 2. Linear regressions (black lines) for significant relationships between Walleye demographic variables, growth metrics, and genetic diversity metrics. Variables include from left to right, top to bottom: male : female sex ratio (M:F) and allelic richness (A_R), stocking index (SI) and heterozygosity (H_e), mean age-0 Walleye abundance (Age 0) and inbreeding (F_{IS}), the sex ratio (M:F) and mean relatedness (R_L), the stocking intensity and effective number of alleles (A_E), and allelic richness (A_R) and mean length at age 1 (L_1 [mm]). P-values were <0.006 for all but the regression between A_R and L_1 ($P = 0.030$).

decrease in the early growth rates of Walleyes associated with lower levels of genetic diversity was consistent with the findings of Cena et al. (2006) and suggests that genetic factors as well as demographic factors (e.g., density dependence) may be influencing Walleye growth in northern Wisconsin.

Theoretical expectations predict the total reproductive output of a population will have a strong positive influence on N_e (Felsenstein 1971); in turn, N_e is one of the main factors governing inbreeding (Newman and Pilson 1997). The strong observed relationship between age-0 Walleye abundance and F_{IS} was consistent with a reduced N_e perpetuated by low levels of reproduction. This pattern has been observed in Pacific Sardine *Sardinops sagax* and Northern Anchovy *Engraulis mordax*, where differential levels of recruitment were highly predictive of the ratio of N_e to census population size (Gaggiotti and Vetter 1999). Interestingly, Gaggiotti and Vetter (1999) also showed populations with larger generation overlaps exhibited increased temporal stability of N_e compared with populations having shorter generation overlaps. The results of our stepwise regression analysis indicated the mean age of the breeding Walleye population was an important component of the demographic model describing F_{IS}. Maturation in Walleyes is strongly correlated to average number of growing degree-days (Venturelli et al. 2010) and was unlikely to differ between populations in this

TABLE 5. Results of forward stepwise regression analyses used to determine the best-fit demographic model for each genetic characteristic. Coefficients of determination (r^2) were adjusted when more than one variable was present in the model. F-values, df, and P-values are shown for each model. Genetic characteristics included expected heterozygosity (H_e), effective number of alleles (A_E), allelic richness (A_R), Wright's inbreeding coefficient (F_{IS}), d^2, and mean pairwise relatedness (R_L). Demographic characteristics included the stocking index (SI), the mean sex ratio (M:F), and the average age of the Walleye breeding population in years (Age_P).

Response	Model	r^2	df	F	P
H_e	0.0002(SI) + 0.761	0.584	13	18.3	0.001
A_E	0.004(SI) − 0.086(Age_P) + 4.86	0.798	12	28.7	<0.001
A_R	−0.101(M:F) + 9.78	0.471	13	11.5	0.005
F_{IS}	−0.108(Age 0) − 0.010(Age_P) + 0.165	0.713	12	18.4	<0.001
d^2	No model				
R_L	0.001(M:F) + 0.050	0.575	13	17.6	0.001

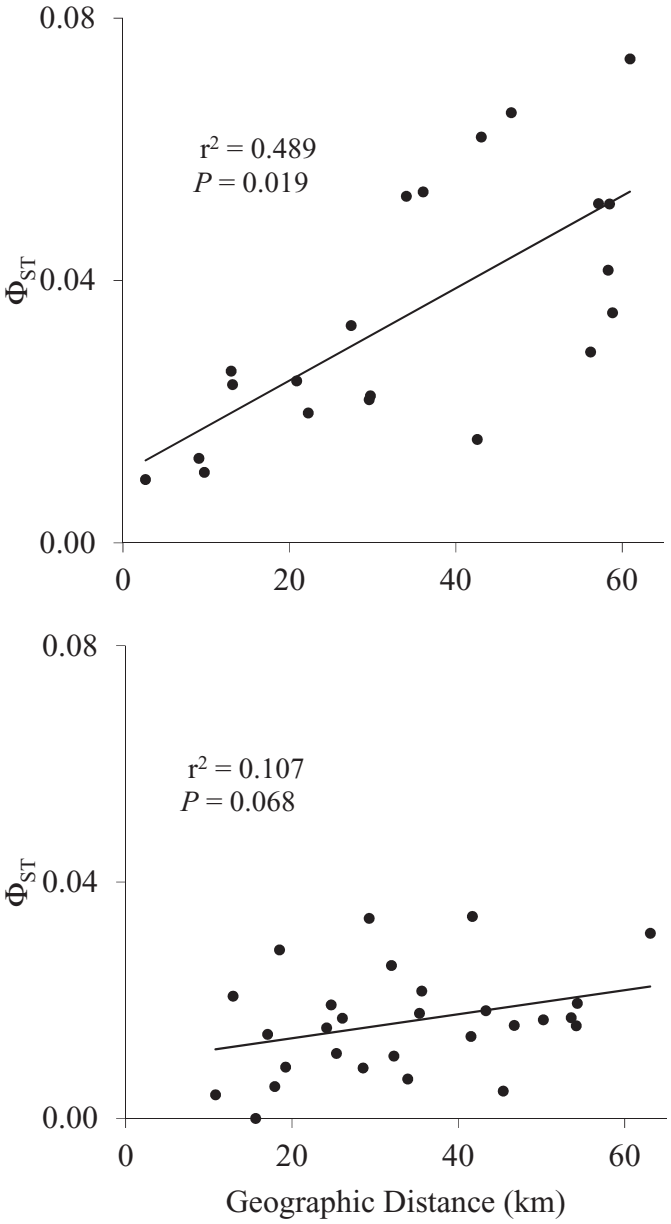

FIGURE 3. Relationships between genetic distance (Φ_{ST}) and geographic distance between all pairs of nonstocked (top panel) and stocked (bottom panel) populations. Coefficients of determination (r^2) were from a simple linear regression and significance (P) between geographic and genetic distance was calculated using a Mantel test with 1,000 permutations. An ANCOVA indicated that the slope ($F = 28.06$, df $= 46$, $P < 0.001$) and intercept ($F = 5.25$, df $= 46$, $P = 0.027$) of the relationship between genetic distance and geographic distance significantly differed between nonstocked and stocked populations.

study due to the relatively narrow latitudinal range. Therefore, the average age of the breeding population would likely indicate the degree of generational overlap, where populations with older Walleyes would have a greater generational overlap. These results indicate, as expected, that the average age of Walleyes and recruitment levels (i.e., age-0 abundance) affect the N_e of the population and have functional impacts on genetic diversity. Low levels of recruitment have been recorded in Walleye populations throughout large portions of their range (Hansen et al. 1998; Beard et al. 2003a), and our results demonstrate this may have detrimental implications to maintaining Walleye genetic integrity on a local and, ultimately, regional scale.

The differential level of allelic diversity observed among Walleye populations was a strong indicator that genetic drift was occurring (Luikart et al. 1998). Populations with highly skewed sex ratios have a lower N_e than populations with more balanced sex ratios. In turn, these populations are predicted to have greater relatedness among individuals due to inbreeding. A reduced N_e will also increase genetic drift leading to the loss of genetic diversity (Waples 1989; Schwartz el al. 1998), especially allelic richness (Luikart et al. 1998). Wisconsin Walleye populations with highly skewed sex ratios had increased relatedness and decreased allelic richness, which was consistent with the hypothesis that genetic drift was operating to decrease the genetic diversity in some populations. Skewed sex ratios have been reported in other exploited fish species (Buxton 1993; McGovern et al. 1998) and have been linked with size-selective harvest (Fenberg and Roy 2007). The average Walleye exploitation rate in Wisconsin lakes is ~12% (Beard et al. 2003b). Sexually dimorphic growth of Walleyes, where females are generally larger at age than males (Henderson et al. 2003), may disproportionately expose females to exploitation. However, a study of Walleye exploitation in Escanaba Lake from 1955 to 1979 showed no differences in sex-specific exploitation rates despite high fishing pressure (Serns and Kempinger 1981). The skewed sex ratios we observed could merely be the result of sampling during Walleye spawning, a well-known observation attributed to males being more likely to be captured than females during this time (Schneider et al. 2007). Nevertheless, if the skewed sex ratios were solely a result of sampling bias, no subsequent relationships with genetic parameters would be expected. On the contrary, significant relationships with two key genetic parameters (A_R and R_L) suggest that sex ratios are indicative of population-specific conditions and, thus, may be ecologically relevant.

Our results suggest stocking is altering allelic frequencies, but not total genetic content of the Walleye populations we sampled. The lack of a connection between SI and either d^2 or A_R indicated stocked fish did not come from sufficiently isolated brood sources to be adding novel genetic material to the population as was reported in a similar prior study over a larger geographic area (Cena et al. 2006). The restricted geographic area of this study likely afforded sufficient gene flow or recent common ancestry such that all populations maintained some degree of genetic connectivity (Manel et al. 2003). However, genetic drift and mutation operating independently in each population would have altered allele frequency distributions randomly across the landscape (Nei 1975). In fact, our data suggest nonstocked populations of Walleye independently develop divergent allelic frequencies in relation to the distance apart and inferred degree of landscape resistance to migration (i.e., IBD). Mixing disparate sources of genetic material via stocking likely altered the allelic

frequencies as measured by A_E and H_e and thereby eliminated the IBD pattern of genetic divergence among stocked Walleye populations in this study. Further evidence of this effect was observed in the F_{IS} of Lake Tomahawk Walleyes; this unusually high F_{IS} could have been the result of an inflated H_e as Lake Tomahawk was the most intensely stocked population.

The disruption of allelic frequencies by stocking and previous evidence for the interbreeding of stocked and native Walleyes (Cena et al. 2006) make genetic introgression very likely in stocked Walleye populations. Genetic introgression is common in fish populations where significant stocking occurs (Finnegan and Stevens 2008; Marie et al. 2010), but ecologically intact populations may be more resilient to genetic introgression (Englbrecht et al. 2002). There was evidence for this in our study where SI was associated with altered allelic frequencies but populations with greater generational overlaps were more resilient to change. The overall genetic effects of stocking in this study were consistent with previous studies suggesting that stocking altered the genetic structure of Walleye populations over a broader geographic area (Billington et al. 1992; Stepien and Faber 1998; Stepien et al. 2009) and indicated that stocking increases the risks of losing naturally occurring local adaptations (Hansen et al. 2002; Ayllon et al. 2006).

The ecological processes controlling neutral genetic diversity (e.g., inbreeding and genetic drift) have biological impacts on fitness-related traits since these processes may act to erode adaptive genetic diversity (Hansson and Westerberg 2002; Reed and Frankham 2003; Johansson et al. 2007). Anthropogenic influences on these processes may exacerbate the potential negative impacts. The positive relationship between A_R and L_1 showed Walleyes grew faster during their first year of development in populations that had higher A_R and, thus, lower inferred rates of genetic drift. Interestingly, Cena et al. (2006) also found that only early growth in Walleyes was sensitive to genetic diversity. The first year is a critical developmental period for Walleyes since it includes several ontogenetic diet shifts (Galarowicz et al. 2006). Genetic drift could have resulted in losses of adaptive genetic diversity advantageous during this complex transitional period. The observed negative relationship between condition and A_E could indicate a possible decrease in fitness in response to outbreeding depression (Allendorf and Luikart 2008) or other factors associated with stocked fish not thriving in receiving waters. However, this conclusion should be cautiously considered as small sample size ($N = 14$) and a strong connection between A_E and SI could have confounded our analysis. Further research could investigate a potential loss of fitness associated with early life history growth and survival following experimental crossings of genetically distinct strains of Walleye consistent with the aforementioned genetically designated management units in Wisconsin (Hammen 2009; Granier et al. 2011).

We suggest that relationships among demographic variables and the genetic integrity of Walleye populations warrant management consideration. Walleye life history characteristics (low levels of recruitment, highly skewed sex ratios) and common management practices (high stocking intensities) pose a continual threat to the genetic integrity of Walleye via genetic drift and outbreeding depression. These factors have likely had a measureable influence on Walleye growth characteristics. Microsatellite analysis has the potential to provide cost-effective genetic information to inform management tactics when the combination of demographic and population dynamic characteristics are presumed to be threatening genetic integrity. However, a major limitation of this study was that it only encompassed neutral genetic diversity so it remains unknown what role natural selection may play in the dynamic between demographic and genetic characteristics. Recent analyses have suggested that adaptive genetic markers have the potential to provide valuable insight into the management of genetic resources (Bonin et al. 2007). Future research could couple neutral and adaptive genetic markers (see Oosterhout et al. 2006) to determine whether important genetic adaptations occur in Wisconsin Walleye and to assess the role of natural selection in maintaining the genetic integrity of Walleye.

ACKNOWLEDGMENTS

Funding was provided by the Wisconsin Department of Natural Resources and Sport Fish Restoration. The use of trade names or products does not constitute endorsement by the U.S. Government. Jonathan Hansen and Thomas Cichosz (WDNR) and Mark Luehring (Great Lakes Indian Fish and Wildlife Commission) provided demographic and population data. Steve Gilbert and John Kubisiak (WDNR) provided invaluable guidance in sampling design and Walleye samples on select lakes. Michael Bozek and Keith Rice provided experimental design and analytical advice throughout the study. Ryan Franckowiak and Andrea Musch provided valuable laboratory, field, and administrative assistance throughout the study.

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Appendix: Supplemental Genetic Information on Wisconsin Walleye Populations

TABLE A.1. Polymerase chain reaction conditions, fluorescent labels, and thermocycler temperature profiles for all multiplexes. The column RXN refers to loci that were co-amplified in multiplex PCR with the respective temperature profiles provided in the table footnotes, dNTP and $MgCl_2$ are the final PCR concentrations of each reagent (mM), Primer refers to the final concentration (μM) of each primer, and Label refers to the fluorescent label on the forward primer of each locus. All reactions contained 1 × PCR Buffer B (ThermoFisher Scientific, Waltham, Massachusetts) and 0.5 U of *Taq* DNA polymerase (New England Biolabs, Ipswich, Massachusetts).

Locus	RXN	dNTP	$MgCl_2$	Primer	Label
Svi-2	A[a]	0.60	1.50	0.08	6FAM
Svi-4				0.06	6FAM
Svi-6				0.17	NED
Svi-7				0.20	HEX
Svi-L5	B[b]	1.00	1.90	0.30	HEX
Svi-L9				0.25	6FAM
Svi-20				0.08	HEX
Svi-17	C[c]	1.00	1.50	0.30	NED
Svi-26				0.30	6FAM
Svi-33				0.30	HEX

[a]RXN A: 94°C for 2.0 min; 31 cycles each at 94°C for 30 s; 60°C annealing for 1.0 min; then 72°C for 2.0 min. Final elongation of 72°C for 15.0 min.
[b]RXN B: 94°C for 2.0 min; 35 cycles each at 94°C for 45 s; 53°C annealing for 45 s; then 72°C for 45 s. Final elongation of 72°C for 45.0 min.
[c]RXN C: 94°C for 5.0 min; 35 cycles each at 94°C for 1.0 min; 52°C annealing for 1.0 min; then 72°C for 1.0 min. Final elongation of 72°C for 15.0 min.

TABLE A.2. Genetic distance matrix showing Φ_{ST} values (below diagonal) between all pairs of Walleye populations and corresponding *P*-values (above diagonal; $\alpha_o = 0.00048$) based on 10,000 permutations. Note *P*-values < 0.0001 are truncated to 0.0001 for presentation. Abbreviations for lake populations are defined in Table 1.

	Population														
Population	BAV	BSG	EC	KL	LAV	NTL	PAP	PEL	PLU	THU	TOM	TRO	TWS	WSL	WIL
BAV		0.0002	0.0001	0.0017	0.0041	0.0018	0.0040	0.0001	0.0001	0.0001	0.0079	0.0001	0.1649	0.0005	0.0001
BSG	0.0168		0.0001	0.0090	0.0028	0.0106	0.0005	0.0001	0.0114	0.0397	0.4632	0.0020	0.1241	0.0012	0.0313
EC	0.0535	0.0419		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
KL	0.0129	0.0117	0.0619		0.0107	0.0076	0.0695	0.0001	0.0003	0.0001	0.0092	0.0001	0.0022	0.0013	0.0002
LAV	0.0096	0.0135	0.0528	0.0108		0.0056	0.0035	0.0001	0.0001	0.0001	0.0043	0.0001	0.0547	0.0015	0.0002
NTL	0.0110	0.0106	0.0492	0.0105	0.0103		0.0001	0.0004	0.0001	0.0001	0.0006	0.0001	0.0005	0.0003	0.0001
PAP	0.0101	0.0178	0.0423	0.0061	0.0119	0.0171		0.0001	0.0002	0.0001	0.0026	0.0001	0.0009	0.0199	0.0001
PEL	0.0351	0.0404	0.0656	0.0517	0.0291	0.0185	0.0443		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
PLU	0.0241	0.0119	0.0331	0.0198	0.0262	0.0279	0.0201	0.0738		0.0001	0.0005	0.0003	0.0001	0.0006	0.0022
THU	0.0227	0.0085	0.0513	0.0284	0.0319	0.0339	0.0314	0.0603	0.0280		0.0739	0.1182	0.0009	0.0013	0.0012
TOM	0.0100	0.0000	0.0445	0.0117	0.0123	0.0158	0.0139	0.0346	0.0191	0.0067		0.0010	0.1780	0.0115	0.0350
TRO	0.0296	0.0142	0.0478	0.0281	0.0381	0.0342	0.0285	0.0636	0.0185	0.0046	0.0153		0.0001	0.0001	0.0001
TWS	0.0036	0.0054	0.0504	0.0165	0.0073	0.0182	0.0167	0.0324	0.0360	0.0192	0.0040	0.0259		0.0002	0.0012
WSL	0.0146	0.0170	0.0382	0.0160	0.0140	0.0195	0.0087	0.0395	0.0179	0.0157	0.0110	0.0207	0.0216		0.0001
WIL	0.0224	0.0093	0.0416	0.0247	0.0218	0.0286	0.0268	0.0517	0.0158	0.0164	0.0089	0.0275	0.0191	0.0230	