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Muskellunge stock assessment and population estimation in two north-central Minnesota lakes
using microsatellite markers and angler participation

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ABSTRACT

Past Muskellunge *Esox masquinongy* management activities in Minnesota included stocking non-local strains to supplement native populations. These stocking events occurred at times when the genetic implications of these activities were not considered or able to be evaluated. Advances in technology and reductions in cost have made genetic analysis a viable stock assessment tool. We used DNA microsatellite analysis of samples collected by the Minnesota Department of Natural Resources (MNDNR) and anglers from two connected lakes in north central Minnesota to assess changes in genetic ancestry in response to past stocking activities, estimate population size, determine effects of genetic ancestry on length-at-age, growth (K), and ultimate length (L_{∞}), and describe seasonal movements within and between lakes. Although a strain of Muskellunge of non-local origin had not been stocked in the study lakes since 1979, the percentage of non-local ancestry an average individual had was 13% in 1995 and 9% in 2012. Respective mark-recapture methodologies using MNDNR to mark and recapture and MNDNR to mark and anglers to recapture were compared and the MNDNR-angler approach produced the narrowest confidence interval around the population estimate. Higher proportions of non-local ancestry only significantly influenced length-at-age for males. Although higher proportions of non-local ancestry were associated with increasing K, the regression fits were not significant for either sex. Similarly, higher proportions of non-local ancestry were associated with decreasing estimates of L_{∞} , but the relationship was only significant for males. Throughout the spring season 54% of males and 42% of females were recaptured at least once with multiple males captured as many as five times. Post-spawn movements between lakes were observed, with more individuals migrating from the lake characterized as having optimal spawning and nursery habitat to the lake characterized as having preferred summer habitat and prey. This study validates the utility of microsatellite techniques as a stock assessment tool, and highlights the contribution anglers can make to Muskellunge stock assessments.

INTRODUCTION

Minnesota is located in the northwestern portion of the native range of Muskellunge *Esox masquinongy* (Crossman 1978) and has over 100 Muskellunge lakes covering nearly 500,000 acres (Wingate and Younk 2007). High exploitation led to declines in abundance and size of Muskellunge populations during the first half of the 20th century (Olson and Cunningham 1989). In response, the MNDNR initiated a propagation and stocking program to address these declines in native populations and to establish new populations to expand angler opportunities (Wingate and Younk 2007). The four strains stocked from the 1950s to present include Shoepack and Leech Lake strains from Minnesota, a Wisconsin River drainage strain, and a Spirit Lake, Iowa strain of uncertain origin (Miller et al. 2012). The Shoepack Lake strain was used exclusively from the 1950s through the early 1980s (MNDNR 2011). Subsequent observations by anglers and biologists indicated a reduction in size structure in lakes where Shoepack strain fish were stocked compared to non-stocked lakes with naturally occurring populations (Wingate and Younk 2007). This resulted in requests to evaluate the genetics (Hanson et al. 1983) and growth rates (Younk and Strand 1992) of source fish. While these evaluations were occurring, a Wisconsin River drainage strain and a Spirit Lake Iowa strain were stocked in the 1980s (Miller et al. 2012). Younk and Strand (1992) concluded Leech Lake strain fish attained greater lengths, weights, and had the greatest growth potential (L_{∞}). Since these evaluations, the MNDNR has exclusively used Leech Lake strain fish for its brood source and has observed an improvement in size structure of fish caught by anglers in stocked lakes (Younk and Pereira 2007; Muskies Incorporated database, unpublished data).

While prior Muskellunge stocking evaluations have focused primarily on survival and growth (Johnson and Margenau 1993; Szendrey and Wahl 1996; Larscheid et al. 1999), advances in genetic technology now allow biologists to include genetic considerations when making stocking decisions (Miller et al. 2009; Jennings et al. 2010). Various genetic markers and

techniques have been developed to analyze population structure (Sloss et al. 2008), assess survival and growth (Hanson et al. 1983), and quantify relationships within and among genetic stocks (Koppelman and Philipp 1986; Kapuscinski et al. 2013). Although physical tags are sufficient to evaluate survival of stocked fish, only genetic markers can verify their reproductive contributions where they supplement existing populations. Miller et al. (2009; 2012) showed that ancestry from the four stocked Muskellunge strains could be traced in Minnesota populations, even years after stocking concluded.

These advances in genetic technology also provide an additional stock assessment tool. Advances in molecular approaches for Muskellunge have led to the ability to identify individual fish (Sloss et al. 2008). A genetic fingerprint can be recovered whenever a DNA-containing tissue (e.g., fin clip, scale) is collected. Fingerprinting individual fish at the molecular level provides an approach to identify individuals for mark-recapture studies. Traditional methods for marking fish range from physical mutilation (e.g., fin clips) to visible (e.g., T-bar anchor tags), subcutaneous (e.g., coded wire tags), and internal acoustic or radio tags (Guy et al. 1996). However, all of these techniques require the ability to estimate mark retention. The rate at which traditional numeric anchor tags are lost in Muskellunge can be 6% after one year and 10% thereafter (Rude et al. 2011), and tag losses can result in information on recaptured fish being unknown (Crossman 1990). As a result, alternate tagging methods with higher retention rates, such as PIT (Passive Integrated Transponder) tags, are becoming more commonly used and evaluated for long-lived species (Younk et al. 2010; Rude et al. 2011). However, no tagging method has been shown to have 100% retention in Muskellunge for long durations. Although Rude et al. (2011) estimated PIT tag loss for Muskellunge would be less than 1% for up to 10 years post tagging, Jennings et al. (2011) reported that 6.5% of fish given PIT tags had lost them within one year. Thus, genetic fingerprinting not only provides a tool to evaluate other tagging methods, but also to monitor a

long-lived species without concerns for mark retention (Deroba et al. 2005), tag reporting rate (Hoenig et al. 1998), and misreading tags (Schwartz and Stobo 1999).

Another application of microsatellite markers for individual identification is assessment of seasonal movements. Traditional Muskellunge seasonal movement studies in Canada (Minor and Crossman 1978; Stronks 1995) and the United States (Dombeck 1979; Miller and Menzel 1986; Strand 1986) have used telemetry and required extensive time, personnel, financial resources, and have relatively low sample sizes. Recapturing tagged fish can provide information on movements with increased sample sizes at reduced cost, although with loss of detailed information on movement patterns. Some Muskellunge movement studies have relied on physical tags (Crossman 1956; Haas 1978; Crossman 1990), but tag loss was indicated as a concern (Crossman 1990). Microsatellite markers provide a less expensive and labor intensive option than telemetry while avoiding the concern of physical tag loss, as noted previously for population estimation.

Past studies have demonstrated that anglers and private user groups have assisted biologists and provided information including genetic material. Maintaining good working relationships among biologists, anglers, and private user groups continues to be an important component of Muskellunge management (Oehmcke 1986). Muskellunge anglers have assisted biologists with various studies involving movement (Haas 1978; Weeks and Hansen 2009), catch rates (Younk and Cook 1992; Kerr 2007), exploitation rates (Muir and Sweet 1964; Miles 1978), population characteristics (Mosindy and Duffy 2007), post-release survival (Richards and Ramsell 1986), and angler education (Dent 1986). These studies demonstrate the dedicated nature and willingness of Muskellunge anglers to participate in management activities (Graff 1986).

The goal of this study was to assess numerous Muskellunge population metrics using novel approaches involving genetic markers and angler assistance with sampling. Our specific objectives were to: 1) quantify the percentage of non-local ancestry an average individual had compared to the previous assessment; 2) estimate abundance by mark recapture using

microsatellite analysis to identify individuals; 3) compare population estimates and precision between two recapture methods, one relying on MNDNR and the other on anglers; 4) quantify genetic differences in length-at-age, growth, and L_{∞} , 33 years after the last stocking; and, 5) assess spawning and seasonal movements within and between lakes.

STUDY AREA

The Leech Lake watershed is located in north-central Minnesota and contains a native Muskellunge population (Figure 1). Baby and Man Lakes are two of fifteen lakes within a chain connected by the Boy River and its tributaries within the Leech Lake watershed upstream of Leech Lake. The two study lakes are separated by a channel that is approximately 1 m deep, 5 m wide, and 30 m long. Baby Lake is 295 ha, has maximum and mean depths of 22 and 7 m, and is 44% littoral (≤ 5 m). It has 19 km of shoreline and a Shoreline Development Index (SDI; Jennings 2011) of 3.10. Man Lake is 198 ha, has maximum and mean depths of 29 and 9 m, and is 25% littoral. It has 7 km of shoreline and a SDI of 1.45. Baby Lake is mesotrophic and has a very diverse aquatic plant community with sedges (*Cyperaceae*), white waterlily (*Nymphaea tuberosa*), yellow waterlily (*Nuphar variegata*), floating-leaf pondweed (*Potamogeton natans*), hardstem bulrush (*Scirpus acutus*), muskgrass (*Chara sp.*) and bushy pondweed (*Najas flexilis*) all being common. Man Lake is oligotrophic and has a simple plant community, with hardstem bulrush and muskgrass being the only two common species. Yellow Perch (*Perca flavescens*) gill net catch rates were 8.6 and 1.6 in Baby and Man Lakes, respectively; while Cisco (*Coregonus artedii*) gill net catch rates were 0.2 and 7.0 in Baby and Man Lakes, respectively (MNDNR unpublished data). Northern Pike (*Esox lucius*) gill net catch rates were 2.8 and 5.4 in Baby and Man Lakes, respectively.

METHODS

MNDNR Stocking.-Baby Lake was stocked with Shoepack strain fingerlings annually from 1971-1979 (range 150-968; mean, 517), while Man Lake was stocked with Shoepack strain fingerlings in 1972, 1973, and 1976 (range, 100-232, mean, 164). Shoepack Lake is located in Voyageurs National Park in northern Minnesota (Frohauer et al. 2007). The first Muskellunge spring assessment on the study lakes occurred in 1995, and was followed by a 2012 assessment.

MNDNR Field Collection.-From 3-April through 27-April 2012, fish were captured using 1.5 x 1.8 m double framed trap nets with 15.2 m leads (Younk and Pereira 2006). Thirteen nets were set and checked daily in Baby Lake while six nets were used in Man Lake. Prior to initiating the electrofishing assessment, fish were allowed to mix for 10 days. From 6-May through 20-May, fish were captured at night (2200-0400 hours) using a two-person crew in a Coffelt electrofishing boat (VVP 2E; single array anode; pulsed-DC). A spot and stalk technique was used with a spotlight, locating each fish and then positioning the boat with the fish under the anode prior to depressing the pedal. The entire perimeter of Baby and Man Lakes was sampled on five and two nights, respectively. Depths sampled were 0.5-1.5 m. Individuals sampled for the first time by either gear were measured (TL), sexed (Lebeau and Pageau 1989), photographed, scales were removed for growth estimation and genetic analysis, and the first four anal fin rays were removed for age estimation. Recaptured individuals were identified by the anal fin clip and only had scales removed for genetic analysis. Genetic analysis determined individual fish identification and ancestry analysis.

The first spring assessment occurred on Baby and Man Lakes in 1995, and adults were processed similarly to those captured in 2012. Summer standard lake survey assessments were conducted on Baby and Man Lakes in 2012 and 2013, respectively (MNDNR Special Publication 147; MNDNR unpublished data). All Muskellunge sampled were processed similarly to those

captured during the 2012 spring assessment; however, mortalities had clithera removed for age estimation.

Anglers.-MNDNR made a targeted effort to recruit angler assistance with collection of data to be used for genetic analysis and determine population estimates. Specifically, information regarding the assessment and a request for anglers to collect the length and scale samples from all captured Muskellunge was placed in the newsletter of the lake association for each study lake, emailed to local angler groups, and provided to the resort on each study lake. MNDNR scale envelopes were distributed to all respondents. Phone calls and emails were made throughout the angling season to respondents to encourage participation and scale collection.

Genetic Analysis.-Muskellunge scale samples from 1995 and 2012 were genotyped (DNA fingerprinted) using 13 microsatellite DNA markers (Miller et al. 2012). Genotype data were used in two ways. First, to identify individual fish, matching genotypes were identified using automated procedures in the Microsatellite Toolkit (<http://animalgenomics.ucd.ie/sdepark/ms-toolkit/>). Size and sex of individuals with matching genotypes were compared to be sure they were consistent with recaptures. Second, to assign ancestry to individual fish consisting of either native ancestry or some combination of native and stocked Shoepack strain ancestry, using the software STRUCTURE (Pritchard et al. 2000). Hereafter, Shoepack strain ancestry will be referred to as Shoepack ancestry. Miller et al. (2012) used STRUCTURE to show that Shoepack ancestry persisted in a small sample from Baby Lake.

Ancestry groups selected (0-1/16, 1/16-3/16, 3/16-5/16, 5/16-7/16, 7/16-1) were somewhat arbitrary but based on inheritance patterns. The first group, 0-1/16 Shoepack ancestry includes likely pure native fish with allowance for a small portion of misassignment to Shoepack ancestry.

The other categories potentially capture various descendant generations with the expected halving of ancestral contributions per generation. A first generation cross between Shoepack ancestry and native fish would have $\frac{1}{2}$ Shoepack ancestry, a backcross $\frac{1}{4}$ Shoepack ancestry, a second generation backcross $\frac{1}{8}$ Shoepack, etc. A low criterion of $<1/16$ (or 0.0625%) was used to reduce the chance of misclassifying advanced-generation descendants with non-local genetics and misclassifying native individuals as having low levels of Shoepack ancestry. Unequivocally determining if individual fish have Shoepack ancestry is complicated by two factors. First, there is some error in assignment so some fish assigned a low percentage of Shoepack strain ancestry may actually be pure native fish. Assignment error rates cannot be estimated directly from the data because we have no samples of the “pure” native population prior to stocking. Secondly, the percentage of Shoepack ancestry will halve each generation if Shoepack strain descendants continually backcross to native fish. Over time it becomes difficult to distinguish advanced-generation backcrossed Shoepack strain descendants from pure native fish.

Population estimates.-Population estimates were calculated using the Chapman modification of the Peterson method (Kohler and Hubert 1999) for Muskellunge captured ≥ 762 mm. This length interval was selected because no immature or fish of unknown sex sampled were greater in length. Hereafter these fish will be referred to as adults. Several independent estimates were calculated where: (1) trap netted fish were the mark and electrofished fish were the recapture; (2) trap netted fish were the mark and angler caught fish were the recapture; and, (3) trap netted and electrofished fish combined were the mark and angler caught fish were the recapture. The number of fish per ha and littoral ha were calculated.

Age and growth analysis.-Anal fin rays were prepared by transversely slicing the base with a Beuhler IsoMet[®] low speed saw (e.g. Brenden et al. 2006). The uncut end was placed in clay and

the cross sectioned end was illuminated with a Fiber-Lite[®] MI-150. Images were taken using a Meiji stereo-zoom microscope fitted with a Canon EOS Rebel camera, and projected using EOS Utility software. Two readers independently estimated ages by counting annual rings on fin rays, and consensus was reached on discrepancies.

Fin rays were used to estimate Muskellunge age (Johnson 1971), however, because fin rays lacked a definitive focus, scales were used to back-calculate length-at-age. Scales were cleaned and projected on a microfiche reader. Annuli locations were recorded and digitized. Fin ray and scale ages were compared and structures were reexamined when disagreements occurred. If agreement in estimated age between structures was not reached, fish were not used in subsequent age and growth analyses.

We stratified the overall population into two groups based on the inflection point at 762 mm in the Von Bertalanffy growth model; this was assumed to be the age at sexual maturity, which can have significant effect on the annual growth rate (He and Stewart 2002). For each individual, Walford plots were fitted to estimate the Brody growth coefficient (K) and von Bertalanffy growth equations were fitted to estimate ultimate length (L_{∞}) using a modification of the methods described by Isley and Grabowski (2007). Back-calculated length-at-annulus data for the population were tested for differences among sexes using a one-way ANOVA and among lakes stratified by sex using a one-way ANOVA. Subsequent models were stratified by sex but not by lake because no lake effect was observed. Estimates of growth (K) and L_{∞} were regressed against Shoepack ancestry for individual fish. Shoepack ancestry was then partitioned into groups representative of inheritance (0-1/16, 1/16-3/16, etc.), and L_{∞} was compared among these groups using an ANOVA to determine if Shoepack ancestry categorically affected L_{∞} .

To evaluate changes in the population over time as well as differences among gear types, we used Wilcoxon tests to compare the following length- and age-frequency distributions: 1) lengths of trap netted fish between 1995 and 2012 in Baby Lake; 2) lengths of MNDNR captured

fish in 2012 between lakes; 3) lengths of fish among the three capture methods in 2012; and 4) ages of MNDNR captured fish between lakes in 2012.

RESULTS

MNDNR Field collection.-Water temperatures ranged between 5-9 °C while trap netting and 12-17 °C while electrofishing. A total of 266 individuals were sampled between both lakes by all gears (Tables 1 and 2). Of first-time captures (266), 74% (190) were by trap nets, 20% (53) were by electrofishing, 8% (20) were by anglers, and 1% (3) were sampled during the standard lake survey assessment. Of all recaptures (121), 41% (50) were by trap nets, 34% (41) were by electrofishing, and 25% (30) were by anglers. Catch rates by trap nets in Baby and Man Lakes were 0.72 and 0.40 fish/trap. Catch rates include individuals captured multiple times.

Genetics.-A total of 66 and 391 samples were genotyped from 1995 and 2012, respectively. The 1995 sample only included fish from Baby Lake as no Muskellunge were captured in Man Lake that year, despite similar effort. The 2012 sample size included recaptured individuals.

Individuals recaptured by trap nets or electrofishing were recognizable by the lack of anal fin rays taken for ageing. Fish not noted as recaptures did not have matching genotypes, indicating that microsatellites had sufficient power to distinguish individuals. Furthermore, given the genetic diversity of the markers, the probability that random individuals or siblings would share genotypes was 4×10^{-8} and 5×10^{-4} , respectively (calculated in Cervus 3.0; Kalinowski et al. 2007).

A subsample of scales ($n = 21$) from the 1995 Muskellunge assessment and the 2000 standard lake survey assessment previously reported that individuals had on average 14% Shoepack ancestry (Miller et al. 2012); reanalysis with additional archived samples from 1995 ($n = 66$) indicated a similar Shoepack ancestry rate of 13% in 1995 (Table 3). Samples from both Baby and Man Lakes in 2012 ($n = 269$) indicated that average Shoepack ancestry declined to 9%

over the 17 year period. The percentage of individuals with any Shoepack ancestry also declined from 41% in 1995 to 29% in 2012. The percentage of fish with Shoepack ancestry was much higher than the average ancestry because most Shoepack strain descendants had a mix of native and Shoepack ancestry. The proportion of individuals with relatively high Shoepack ancestry also declined. For example, the proportion of individuals with greater than $\geq 7/16$ (or 0.4375) Shoepack ancestry in Baby Lake declined from 11% to 6% between 1995 and 2012.

Population estimates.-Estimates calculated for Muskellunge captured ≥ 762 mm in Baby Lake using trap nets to mark and electrofishing as the recapture method had the narrowest 95% confidence intervals (N=294; C.I. 209-379; Tables 4 and 5). Man Lake estimates with the narrowest 95% confidence intervals were calculated using trap nets to mark and anglers to recapture (N=62; C.I. 42-82). Estimates calculated using trap nets and electrofishing to mark and anglers to recapture had the narrowest 95% confidence intervals for Baby and Man Lakes collectively (N=322; C.I. 248-395). The estimates of fish/ha and fish/littoral ha with the narrowest 95% confidence intervals were 1.00 and 2.24 on Baby Lake, 0.31 and 1.24 on Man Lake, and 0.65 and 1.78 on Baby and Man Lakes combined.

Age and Growth.-Structures were examined from 247 fish from both lakes in 2012. Agreement of ages between fin rays and scales was 93% and 96% for Baby and Man Lakes, respectively. The exclusion of individuals due to age disagreement and regenerated scales resulted in the use of 221 fish in analyses. Muskellunge captured by the MNDNR ranged in ages from 2 through 15 (Figure 2). The mean ages of fish captured by the MNDNR was 7.6 in Baby Lake, 9.4 in Man Lake, and 8.0 overall (Table 6). As suspected, Muskellunge in Baby Lake were significantly younger than those in Man Lake (Wilcoxon: $P < 0.0001$; Figure 2).

Fish captured by the MNDNR ranged from 432 to 1270 mm (Figure 3). The mean lengths of Muskellunge captured by the MNDNR was 884 mm in Baby Lake, 986 mm in Man Lake, and 902 mm overall (Table 7). Wilcoxon tests of the length-frequency distributions determined similar distributions among 1995 and 2012 sampling events ($P = 0.24$; Figure 4). Muskellunge in Man Lake were longer than those sampled in Baby Lake in 2012 ($P < 0.0001$; Figure 4). Electrofishing selected for smaller fish than trap nets (Wilcoxin: $P < 0.0001$) or angling ($P < 0.0001$). Conversely, the length-frequency distributions of fish captured by angling and trap nets were nearly identical ($P = 0.76$; Figure 4).

There was a difference in back-calculated length-at-age-increment between sexes (ANOVA: $F = 12.96$; $df = 5, 1689$; $P < 0.0003$), meaning further analyses needed to be stratified by gender (Table 8). Growth was similar between lakes for females (ANOVA: $F = 2.77$, $df = 5, 687$; $P = 0.10$) but differed between lakes for males ($F = 7.11$, $df = 5, 996$; $P = 0.01$), with Baby Lake having faster growth rates for males. When evaluating factors influencing length-at-age with lakes combined, the variation in growth was attributed to age only in females (ANOVA: $F = 401.98$; $df = 15, 516$; $P < 0.0001$; Figures 5 and 6), but age and Shoepack ancestry in males (ANOVA: 648.88 ; $df = 12, 630$; $P < 0.0001$). Although, higher proportions of Shoepack ancestry were associated with increasing K , the regression fits were not significant for females ($P = 0.91$; $df = 1, 52$; $R^2 = 0.02$; $P = 0.34$) or males ($F = 0.67$; $df = 1, 69$; $R^2 = 0.01$; $P = 0.42$; Figures 7 and 8). Similarly, higher proportions of Shoepack ancestry were associated with decreasing estimates of L_{∞} , but the weak relationship was only significant for males (males: $F = 5.02$; $df = 1, 69$; $R^2 = 0.07$; $P = 0.03$; females: $F = 2.81$; $df = 1, 52$; $R^2 = 0.05$; $P = 0.10$; Figures 7 and 8). The ANOVA comparing L_{∞} across ancestry groups did not detect a statistical difference for female ($F = 1.16$; $df = 3, 50$; $P = 0.33$) or male ($F = 1.39$; $df = 4, 66$; $P = 0.25$) despite a declining trend overall. We suspect smaller sample sizes for all ancestry groups other than 0-1/16 influenced these results, and visual inspection of the data (Figures 7 and 8) suggest Shoepack ancestry $\geq 3/16$ could have

noticeable effects on L_{∞} . Similarly, when the ancestry of sampled fish exceeded 19% Shoepack strain no female exceeded 1057 mm, no male exceeded 1008 mm, and no fish of either sex with >50% Shoepack strain ancestry exceeded 932 mm. These data support the hypothesis that increasing Shoepack ancestry has an increasingly negative effect on individual size potential. Overall L_{∞} values for Baby and Man Lakes were 1070 for males and 1176 for females, while overall K values for Baby and Man Lakes were 0.257 for males and 0.225 for females.

Spring Movements.-Some individuals displayed a high affinity for being captured multiple times during the spring sampling period, with 54% of males and 42% of females being recaptured at least once (Tables 1 and 2). Thirteen percent of males and females were captured three times, while multiple males were captured as many as five times. Of fish captured multiple times in trap nets, 30% were captured in the same net. Twelve of the thirteen individuals recaptured in the same net occurred in Baby Lake. Some examples of fish being captured multiple times by the same gear included a male in Baby Lake that was sampled four times by the same trap net on average five days apart, and three males in Baby Lake that were sampled via electrofishing on three separate nights. Two males and one female were captured in Man Lake during the later stages of the spring sampling period after initially being captured in Baby Lake, while one female was captured in Baby Lake during the later stages of the spring sampling period after initially being captured in Man Lake.

Post Spawn Movements and Angler Recaptures.-Of individuals initially captured during the spring sampling season, 24% were recaptured during the 2012 angling season. Seventy-eight percent of individuals recaptured by anglers were caught in the same lake, while 22% had moved to the other lake. Six fish were captured by anglers twice, which included three fish of unknown sex, two females, and one male. One fish of unknown sex was captured by an angler on three occasions.

Of the 48 fish ≥ 1016 mm initially sampled by the MNDNR, 6 (15%) were recaptured by anglers. The only fish ≥ 1270 mm sampled by the MNDNR was recaptured by an angler in 2012 and again in 2013.

DISCUSSION

Muskellunge management continues to be evaluated (Brenden et al. 2007; Knapp et al. 2012; Wahl et al. 2012) and evolve (Casselman 2007; Farrell et al. 2007; Wingate and Younk 2007). Populations occur at low densities (Kapuscinski et al. 2007; Hanson et al. 1986; MNDNR 2011) and are a high-profile species for anglers (Casselman et al. 1999; Schroeder et al. 2007). Spring assessments continue to provide the best opportunity to assess adult population characteristics (Crossman 1990; Miller et al. 2009; Jennings et al. 2011). However, microsatellite techniques offer a useful addition to Muskellunge stock assessment and offer an opportunity for anglers to contribute to data collection.

MNDNR Field collection.-Catch rates of fish by trap nets in this study occurred at water temperatures (5.0-9.4 °C) lower than what is typically described for this species (9.4-15.6 °C, Becker 1983); however, Muskellunge in Shoepack Lake have the tendency to spawn at lower water temperatures that are closer to ice out (Frohnauer et al. 2007). Although electrofishing catchability is greatest for Muskellunge in spring (Schoenbeck and Hansen 2005), there is a finite window where it is an effective tool prior to fish moving offshore to deeper depths post spawn (Minor and Crossman 1978; Dombeck 1979; Miller and Menzel 1986). Electrofishing was ineffective once water temperatures exceeded 15.6° C in this study, as fish were rarely observed in depths less than 1.5 m.

Genetics. -Our results identifying Shoepack strain ancestry persisting 33 years post-stocking but declining in prominence through time is consistent with genetic changes observed in another Minnesota population over a similar time period (Miller et al. 2009). Native ancestry has persisted in the study population and currently comprises greater than 90% of the genetic composition. Similar ancestry between study lakes likely resulted from substantial movement of fish between lakes because one lake had a much higher rate of Shoepack strain stocking. The relatively high recapture of migrants within a single year supports this conclusion. Both average Shoepack ancestry per individual and the percentage of individuals with any Shoepack ancestry continue to decline, which indicates that descendants with Shoepack ancestry have reduced fitness compared to native fish. However, the level of Shoepack ancestry remaining in supplemented Minnesota Muskellunge populations has been highly variable, ranging between 0 and 95% (Miller et al. 2012). While the level of Shoepack ancestry remaining was assessed on one occasion for a 20 lake study (Miller et al. 2012), changes in Shoepack ancestry over multiple generations has only been assessed in one lake, Moose Lake (Miller et al. 2009). Moose Lake was stocked with Shoepack strain fish 11 times from 1965-1983 and ancestries were determined from four samples from 1981 through 2004. The percentage of fish with pure Shoepack ancestry declined from 17% to 2% over time, while the percentage of individuals with admixed ancestry increased from 0 to 42%. By 2004, individuals had on average 24% Shoepack ancestry, 19 years after the last stocking event.

Population estimates. -The Peterson method assumes a closed population, which our data indicate is not the case for Baby and Man Lakes treated individually; however, the sums of the individual lake estimates were generally similar to the combined lake estimates, indicating little bias from violating this assumption. The estimated number of Muskellunge captured ≥ 762 mm per ha was higher in Baby Lake (1.00/ha) than in Man Lake (0.31/ha) or any of the 10 other Minnesota

populations in which population estimates were determined (range 0.15-0.96/ha; Table 9; MNDNR 2011).

Most adult estimates at similar latitudes in Wisconsin and Michigan indicate densities are commonly less than 1.2 fish/ha (Hanson et al. 1986; Hoff and Serns 1986; Jennings et al. 2011), although densities between 1.2 and 2.2 fish/ha have been estimated (Siler and Beyerle 1986; Cornelius and Margenau 1999; Weeks and Hansen 2009). Shoepack Lake is the only lake in Minnesota in which densities are known to exceed those of Baby Lake (4.79/ha; Frohnauer et al. 2007), and was the source population for fish stocked in Baby Lake (Miller et al. 2012). However, Shoepack ancestry does not explain the high density in Baby Lake, as Man Lake has similar ancestry but a lower density.

Age and Growth. -Females had significantly higher growth rates than males in both lakes, consistent with what has been observed for this species throughout its range (Hourston 1952; Hanson 1986; Casselman et al. 1999). Although fish captured in Man Lake were significantly longer and older than those sampled in Baby Lake, growth rates were similar among lakes inferring density did not play a role. The growth potential of various Muskellunge populations can be affected by prey availability (Inskip and Magnuson 1986; Casselman 1990) and density (Kapusinski et al. 2007); however, genetic ancestry may also influence growth (Younk and Strand 1992; Miller et al. 2009). Although Miller et al. (2012) concluded that stocked Shoepack strain fish have not imposed widespread limitations on L_{∞} statewide in Minnesota because of low persistence (<4% ancestry remaining) in thirteen of twenty study lakes, Baby and Man Lakes in this study and Moose Lake (Miller et al. 2009) are exceptions. From the perspective of trophy-based management, which is generally how Muskellunge are viewed by Minnesota anglers and biologists (Schroeder et al. 2007; MNDNR 2011); Shoepack strain stockings have been detrimental to statewide management goals in some lakes.

Length frequency distributions did not significantly change over the past 17 years in our study lakes despite declining levels of Shoepack ancestry, and the population still contains very few fish anglers consider trophy sized (1219 mm; Schroeder et al. 2007). These sampling events spanned a period where Muskellunge were afforded increased protection from harvest via higher minimum length limits and an increasing catch and release ethic. Similar length frequency distributions among sampling events despite increased protection suggests that harvest was not limiting the length-based quality of the population.

Younk and Strand (1992) reported higher values of K for the Shoepack strain relative to other Muskellunge strains. This may appear surprising but K is the rate of change in TL from y to $y+1$. Thus, for two fish of the same age where one is longer than the other, less growth is required to maintain a higher slope for the shorter individual. Similarly, we found that K tended to increase with increasing Shoepack ancestry overall, but the trend was variable and not significant among ancestry groups likely due to small sample sizes. Although higher proportions of Shoepack ancestry were associated with decreasing estimates of L_{∞} , this relationship was not significant for each sex. Similar to tests of K among ancestry groups, we suspect the statistical outcomes of tests evaluating Shoepack ancestry on L_{∞} were also a product of small sample sizes as a declining trend among ancestry groups was obvious, consistent, and comparable to the findings of Younk and Strand (1992). However, this declining trend was more of a generality as low estimates of L_{∞} were also observed for some individuals with relatively low (<10%) Shoepack ancestry. Poor growth could reflect environmental factors associated with home range, such as differing availability of thermal refuge habitat between lakes (Clapp and Wahl 1996; Chipps et al. 2000). Alternatively, these outliers may be a product of one or more specific genes from the Shoepack strain that have a strong influence on growth. That is, while overall ancestry may be low, the portion of the genome descending from the Shoepack strain may occasionally have genes with large contributions to the phenotype expressed as lower L_{∞} . The ultimate lengths (L_{∞}) for the male

(1070 mm) and female (1176 mm) Muskellunge in Baby and Man Lakes were intermediate between pure Shoepack strain fish (males, 683 mm; females, 749 mm) from Shoepack Lake (Frohnauer et al. 2007) and Leech Lake strain fish (males, 1092-1105 mm; females, 1201-1341 mm) from multiple Minnesota populations (Younk and Strand 1992), which is closely related to the native population in Baby and Man Lakes.

Spring Movements.-Some Muskellunge have demonstrated high rates of movement (Minor and Crossman 1978; Stronks 1995), and an affinity for being captured multiple times, throughout the spawning period (Crossman 1990). Some fish in this study were recaptured as many as five times, while other studies have had fish recaptured as many as 10 times (Crossman 1956). Thirty percent of fish recaptured by trap nets in this study were recaptured in the same net. This percentage compares to 61% in a Wisconsin study (Jennings et al. 2011; Mud-Callahan Lake) and 72% in an Ontario study (Crossman 1990). Jennings concluded that fish in lakes with a higher SDI had a higher likelihood of being recaptured in the same net, as was demonstrated in our study. Twelve of thirteen fish recaptured in the same net were in Baby Lake which has a higher SDI. Recapture rates were higher for males than females in this study with 54% of males and 42% of females being recaptured. Weeks and Hansen (2009) also demonstrated that males were more likely to be recaptured (23% of males; 16% of females).

Post Spawn Movements and Angler Recaptures.-Only two known prior studies have monitored seasonal Muskellunge movements among multiple lakes. One used telemetry and T-bar tags (Weeks and Hansen 2009), while the other used only T-bar tags (Muir and Sweet 1964). Post-spawn studies within the same lake have documented Muskellunge migrating long distances from their spawning locations prior to being recaptured by anglers (Muir and Sweet 1964; Haas 1978; Crossman 1990). Therefore, it was not surprising that 22% of fish recaptured by anglers in this

study were recaptured in the opposite lake from which they were initially tagged. On the Manitowish Chain Lakes in WI, 50% of the 36 radio-tagged fish migrated to other lakes within the 10 lake chain; while 47% of T-bar tagged fish were recaptured in different lakes from where they were tagged (Weeks and Hansen 2009). Muir and Sweet (1964) demonstrated that 55% of fish initially tagged in Pigeon Lake, were recaptured by anglers in Sturgeon Lake.

Recapture rates of individual fish by anglers were 19% and 18% on the two chain of lakes previously assessed (Weeks and Hanson 2009; Muir and Sweet 1964), compared to 12% in this study. We attribute recapture rate differences of Weeks and Hansen (2009) and this study to recent creel studies indicating the Manitowish Chain had 13 times the angling pressure compared to our study lakes (Jordan Weeks, personal communication; Shavlik 2012). In this era of catch and release angling, few studies have monitored recapture rates of individual fish (Richards and Ramsell 1986; Weeks and Hansen 2009), while no known studies have documented how many times individual fish were recaptured by anglers within the same year. Our study documented six fish that were captured by anglers two or three times during a single open water season.

We speculate that optimal spawning and nursery habitat in Baby Lake accounted for fish moving predominantly from Baby Lake to Man Lake during the later portion of the spring period or thereafter. We also observed a significantly younger population of Muskellunge in Baby Lake, which supported this hypothesis. Past research has indicated more organic sediments (Dombeck et al. 1984; Rust et al. 2002), more diverse emergent and submergent macrophytes (Craig and Black 1986; Werner et al. 1996; Murry and Farrell 2007), lower pike abundance (Dombeck et al. 1986, Farrell 2001), a higher SDI (Dombeck et al. 1986), and a greater availability of shallow water habitat (Farrell and Werner 1999) all promote Muskellunge reproduction and rearing. Past research also demonstrates a negative relationship between the abundance of *Chara sp.* and juvenile Muskellunge (Werner et al 1996; Murry and Farrell 2007), which is prevalent in Man Lake. Although, other studies have indicated Muskellunge select deeper *Chara sp.* beds to spawn

(Strand 1986; Pierce et al. 2007). However, Strand (1986) speculated this was due to a well-established northern pike population in Leech Lake that resulted in spatially segregated spawning habitats.

We also speculate more fish moved from Baby Lake to Man Lake due to Man Lake having optimal summer habitat and preferred prey. Gillnet catch rates of cisco were 35 times higher in Man Lake than Baby Lake while gillnet catch rates of yellow perch were five times higher in Baby Lake than Man Lake during the standard lake survey assessment. Although past Muskellunge diet studies have shown a high occurrence of yellow perch in diets (Hourston 1952; Bozek et al. 1999), Muskellunge have demonstrated an affinity to consume soft rayed prey when available (Gillen et al. 1981; Wahl and Stein 1988).

MANAGEMENT IMPLICATIONS

Our study demonstrates the utility of microsatellite techniques as a stock assessment tool for fisheries managers. Muskellunge are a long lived species that require high rates of tag retention for extended periods of time. As new tagging options become available, biologists continue to assess their respective retention rates. Retention rates are typically assessed by tagging individuals multiple times, in multiple locations, while often clipping fins. Genetic fingerprints give biologists a tool to evaluate the retention rates of all traditional tags or act as the sole tag. Although processing genetic samples may appear cost prohibitive, cost savings associated with not having to perform validation evaluations likely offset such expenses. One disadvantage is there is a lag time between capture and individual fish identification.

Our study also demonstrates the utility of anglers in quantifying Muskellunge population metrics. At times, some, Muskellunge anglers commonly handle more individuals than biologists capture in spring assessments (Muskie's Incorporated database, unpublished data). Therefore, cooperation is essential among biologists, anglers, and private user groups. Although 18 different

anglers contributed scale samples, scale samples were not obtained from all known angler caught fish. Reasons cited included anglers unwilling to assist the MNDNR, fishing alone and having difficulty holding a large fish while collecting scale samples, unwilling to remove the fish from water to obtain samples, or perceiving that removing scales would injure a fish despite assurances. Therefore better angler education prior to this study may have increased angler sample size.

Our study also provides further evidence that supplementing naturally reproducing populations should be done cautiously, as unintentional genetic consequences may remain for generations. First, seasonal movement between our study lakes indicates that stocking has the potential to affect the genetic makeup of other lakes in the chain that were never stocked. Of the fifteen lakes in the chain in which Baby and Man are included, six were stocked with similar non-local strain fish. Second, genetic impacts from the non-local strain may be limiting the size potential of some individuals. There are at least three options that can be considered for mitigating residual non-local ancestry: 1) do nothing as the persistence of non-local ancestry may continue to decline and the proportion within individuals will decrease with further generations of backcrossing; 2) stock fish of local ancestry fish to speed up the rate of decline of non-local ancestry, as natural selection forces likely shaped local strain genetics in similar ways; or, 3) remove individuals with high rates of non-local ancestry, tagging fish and running ancestry analysis in one year and removing them in subsequent years. Considerations when selecting any of these three approaches should include, but not be limited to, the following determinations: 1) if reductions in non-local ancestry will result in significant increases in size structure; 2) if risks of potentially increasing outbreeding depression (mixing three strains instead of two) outweigh potential benefits; 3) if it is appropriate to exert the amount of effort necessary to remove individuals with high rates of non-local ancestry in subsequent years; and, 4) if removing too many individuals might induce demographic and inbreeding risks in the smaller population.

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Tables

Table 1. Capture histories for individual Muskellunge sampled in Baby Lake, 2012.

Abbreviations include: BT = Baby Lake trap net; BE = Baby Lake electrofishing; BA = Baby Lake angler; BS = Baby Lake standard lake survey assessment.

Initial capture	Number males	Number females	Number immature	Total number	1st recapture	2nd recapture	3rd recapture	4th recapture
Baby trap net	48	39	3	90				
	11	8		19	BT			
		2		2	BT	BT		
	1			1	BT	BT	BT	
		1		1	BT	BT	MA	
	2			2	BT	BA		
	4	3		7	BT	BE		
	1			1	BT	BE	MA	
	2			2	BT	BE	BE	BE
	1			1	BT	ME	ME	
	10	2		12	BE			
	1			1	BE	BA		
	1			1	BE	BG-S		
	1			1	BE	MA		
	1			1	BE	BE	BE	BA
	3	2		5	BA			
	1	1		2	MT			
		1		1	MA			
	1	1		2	MA	MA		
Total	89	60	3	152				
Baby electrofish	18	6	17	41				
		2	1	3	BA			
	1		1	2	BE			
	1	1		2	BE	BA		
Total	20	9	19	48				
Baby angler				11				
				1	BA			
				1	BA	MA		
Total	0	0	0	13				
Baby summer				3				
Total Overall	109	69	22	216				

Table 2. Capture histories for individual Muskellunge sampled in Man Lake, 2012. Abbreviations include: MT = Man Lake trap net; ME = Man Lake electrofishing; MA = Man Lake angler.

Initial capture	Number males	Number females	Number immature	Total number	1st recapture	2nd recapture	3rd recapture	4th recapture
Man trap net	13	8		21				
	5	2		7	MA			
		1		1	MA	MA		
	3	3		6	MT			
		1		1	BT	BE		
	1			1	ME			
	1			1	ME	ME		
Total	23	15	0	38				
Man electrofish	3		2	5				
Man angler				5				
				2	MA			
Total	0	0	0	7				
Total Overall	26	15	2	50				

Table 3. The proportion of captured individuals in the 1995 and 2012 assessments on Baby and Man Lakes based on estimated Shoepack ancestry. The final row indicates the sample size processed and the overall average ancestry among all captured individuals. Individuals captured multiple times were included once.

Abbreviations are as follows: N = number; % = percentage.

Shoepack criteria	1995		2012		2012	
	Baby Lake N	Baby Lake %	Baby Lake N	Baby Lake %	Man Lake N	Man Lake %
0-1/16	39	59	150	71	42	71
1/16-3/16	14	21	28	13	8	14
3/16-5/16	5	8	8	4	3	5
5/16-7/16	1	2	12	6	2	3
7/16-1	7	11	12	6	4	7
Total/Average	66	13.4	210	8.9	59	8.9

Table 4. The number of different individual Muskellunge ≥ 762 mm captured by trap nets, electrofishing, and anglers used to estimate densities in Baby Lake, Man Lake, and Baby and Man Lakes collectively. Abbreviations are as follows: M = the number of fish initially marked and released; C = the number of fish captured and examined for marks during the recapture period; R = the number of recaptures (i.e., previously marked fish) found in C.

	Mark: TN Recapture: EF	Mark: TN Recapture: AN	Mark: TN and EF Recapture: AN
Baby			
M	141	141	168
C	53	21	21
R	25	9	13
Man			
M	40	40	45
C	7	22	22
R	4	14	14
Baby and Man			
M	178	178	209
C	59	42	42
R	28	23	27

Table 5. Estimated abundance of Muskellunge ≥ 762 mm using the Chapman modification of the Peterson method. Upper and lower 95% ranges are shown using the standard error method.

Abbreviations are as follows: TN = trap net; EF = electrofishing; AN = anglers.

	Mark: TN Recapture: EF	Mark: TN Recapture: AN	Mark: TN and EF Recapture: AN
Baby	294*	311	265
Lower 95%	209	149	174
Upper 95%	379	474	356
Man	65	62*	70
Lower 95%	20	42	47
Upper 95%	110	82	92
Baby and Man	369	320	322*
Lower 95%	269	230	248
Upper 95%	470	409	395

* indicates population estimates with narrowest confidence intervals

Table 6. Mean ages and age ranges for male, female, and immature Muskellunge sampled using trap (TN) nets and electrofishing (EF) in Baby and Man Lakes, 2012.

Baby Lake	Mean age			Range of ages		
	M	F	IM	M	F	IM
Trap nets	7.6	8.8	4.3	5-11	5-15	3-5
Electrofishing	7.5	8.7	3.7	6-10	6-15	2-6
TN and EF	7.6	8.8	3.8	5-11	5-15	2-6

Man Lake	Mean age			Range of ages		
	M	F	IM	M	F	IM
Trap nets	9.5	10.7	ns	7-12	7-13	ns
Electrofishing	7.8	7.0	3.5	6-9	7-7	3-4
TN and EF	9.2	10.5	3.5	6-12	7-13	3-4

Baby and Man	Mean age			Range of ages		
	M	F	IM	M	F	IM
Trap nets	8.0	9.2	4.3	5-12	5-15	3-5
Electrofishing	7.6	8.5	3.7	6-10	6-15	2-6
TN and EF	7.9	9.1	3.8	5-12	5-15	2-6

Table 7. Mean lengths (mm) and length ranges for male (M), female (F), immature (IM), and unknown (UNK) Muskellunge sampled by trap nets (TN), electrofishing (EF), and anglers in Baby and Man Lakes, 2012.

Baby Lake	Mean length				Range of lengths			
	M	F	IM	ALL	M	F	IM	UNK
Trap nets	876	988	648	919	660-1041	737-1118	533-711	
Electrofishing	874	960	602	820	686-1016	737-1092	432-737	
TN and EF	876	980	607	884	660-1041	737-1118	432-737	
Anglers				843				406-1016

Man Lake	Mean length				Range of lengths			
	M	F	IM	ALL	M	F	IM	UNK
Trap nets	958	1090	ns	1008	864-1067	864-1270	ns	
Electrofishing	930	ns	625	843	787-1016	ns	533-711	
TN and EF	953	1090	625	986	787-1067	864-1270	533-711	
Anglers				1016				889-1270

Baby and Man	Mean length				Range of lengths			
	M	F	IM	ALL	M	F	IM	UNK
Trap nets	899	1011	648	940	660-1067	737-1270	533-711	
Electrofishing	881	960	605	823	686-1016	737-1092	432-737	
TN and EF	892	1001	610	902	660-1067	737-1270	432-737	
Anglers				927				406-1270

Table 8. Mean back-calculated length (mm) at age of Muskellunge sampled in Baby and Man Lakes, 2012.

Age	Baby Lake				Man Lake				Baby and Man Lakes			
	Males		Females		Males		Females		Males		Females	
	N	mm	N	mm	N	mm	N	mm	N	mm	N	mm
1	110	219	62	236	26	183	16	218	136	212	78	232
2	110	416	62	434	26	360	16	393	136	405	78	425
3	108	562	62	593	26	522	16	554	134	554	78	585
4	102	676	62	712	25	648	16	698	127	671	78	709
5	96	765	62	805	24	758	16	805	120	763	78	805
6	86	820	61	876	24	837	16	892	110	824	77	879
7	66	869	55	930	23	893	16	956	89	875	71	936
8	48	909	38	958	22	929	14	1005	70	915	52	971
9	27	939	22	992	18	960	13	1044	45	948	35	1011
10	12	962	16	1013	8	978	12	1082	20	968	28	1042
11	4	943	10	1032	3	1021	7	1116	7	977	17	1067
12			6	1049	2	1044	5	1140	2	1044	11	1090
13			3	1058			3	1165			6	1112
14			1	1079							1	1079
15			1	1102							1	1102

Table 9. Summary of estimated densities (fish/ha) of Muskellunge ≥ 762 mm for 10 Minnesota lakes (MNDNR 2011).

Lake	Surface area (ha)	Number of estimates	Density (fish/ha)	
			Average	Range
Plantaganette	1,023	2	0.32	0.30-0.35
Deer	1,658	6	0.40	0.30-0.52
Alexander	1,118	2	0.47	0.44-0.52
North Star	429	4	0.54	0.15-0.82
Spider	546	6	0.59	0.17-0.89
Sugar	411	1	0.67	na
Moose	512	8	0.69	0.35-0.96
Shamineau	658	2	0.69	0.62-0.77
Little Wolf	198	1	0.84	na
Elk	110	3	0.86	0.82-0.96

Figures

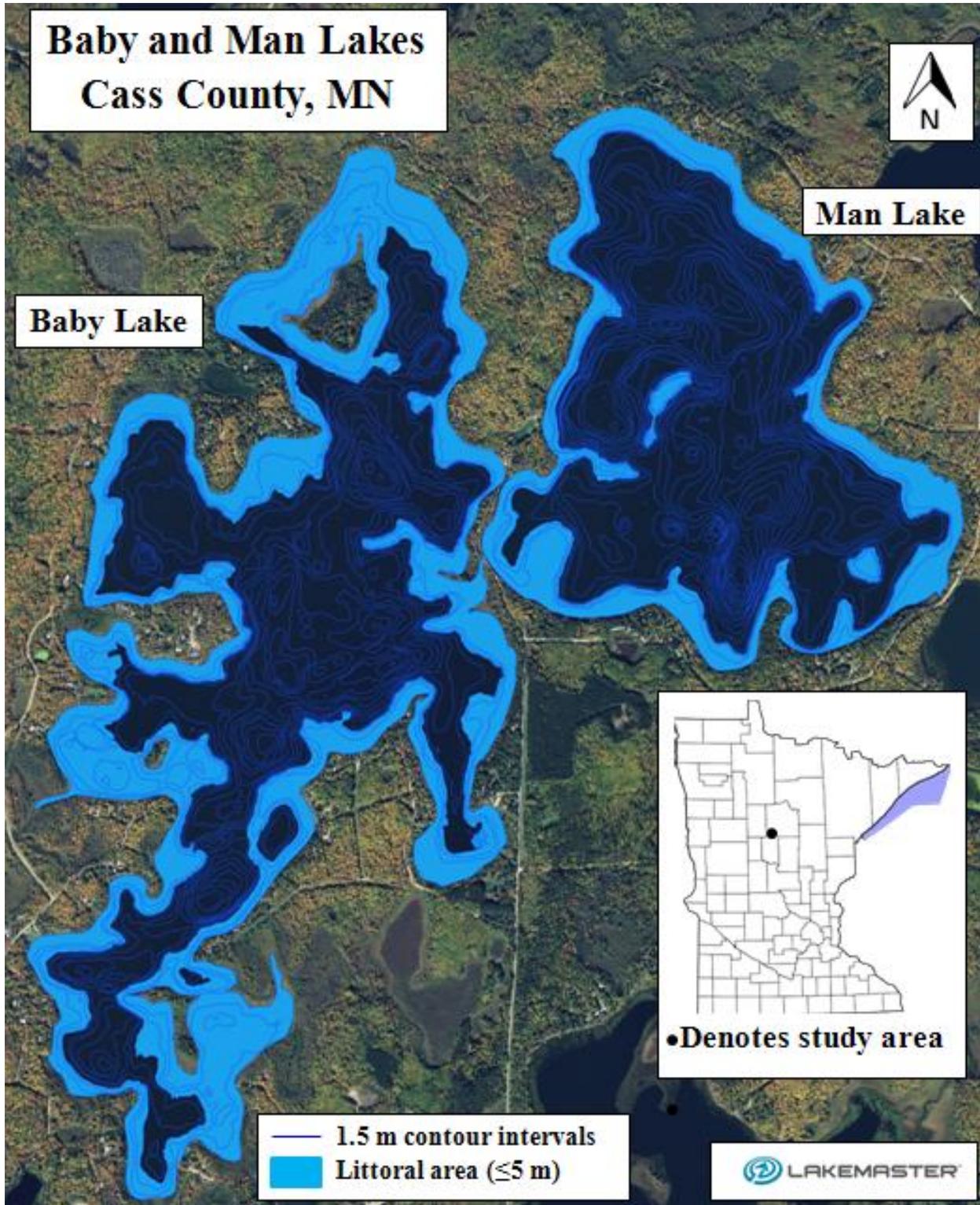


Figure 1. Map of Baby and Man Lakes with 1.5 m contour lines shaded, indicating the littoral area.

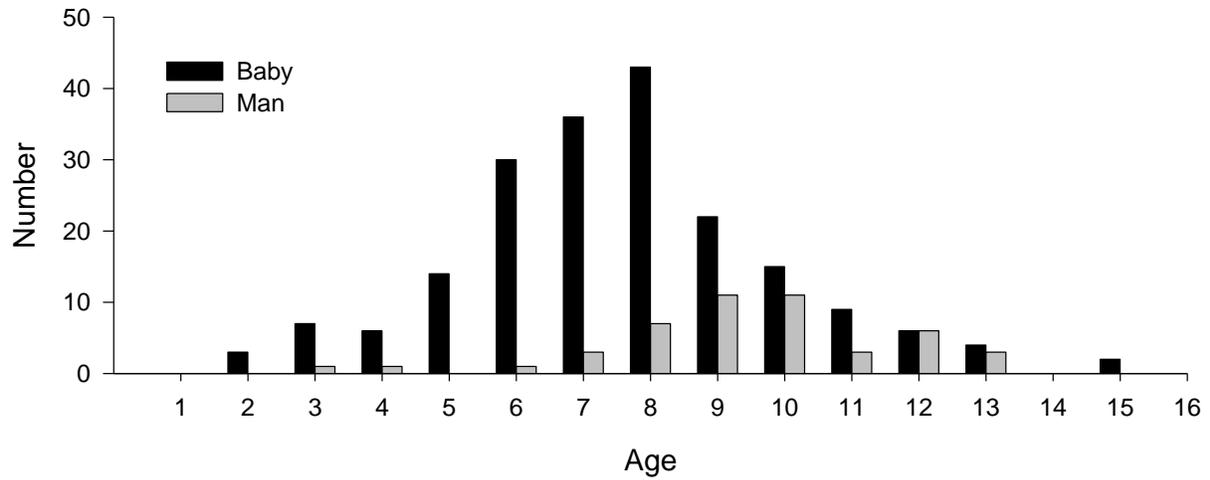


Figure 2. Age-frequency distribution of Muskellunge sampled in Baby and Man Lakes using trap nets and electrofishing combined, 2012.

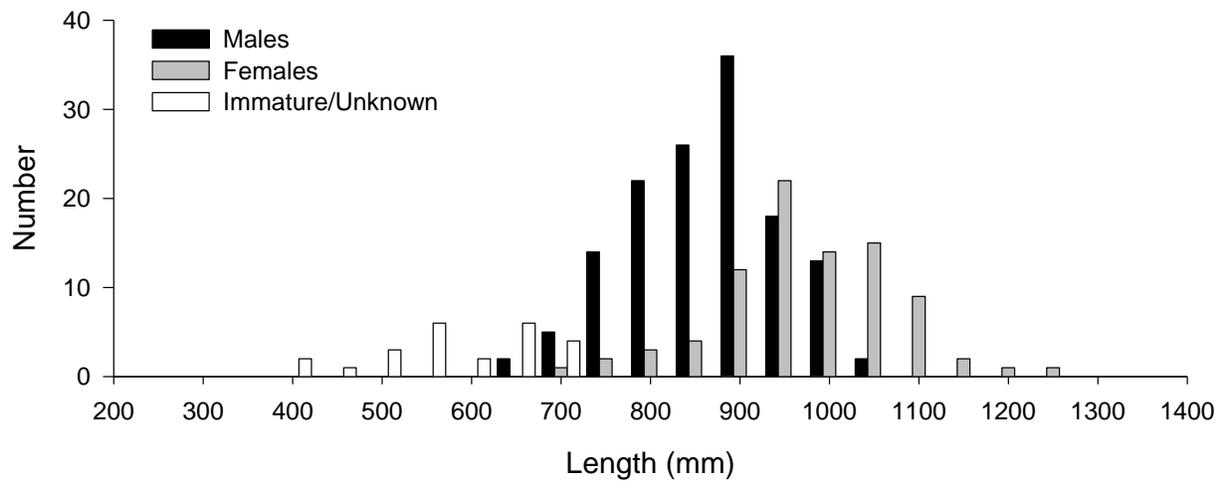


Figure 3. Length frequency distribution of male, female, and immature/unknown Muskellunge captured in Baby and Man Lakes in 2012 using trap nets and electrofishing.

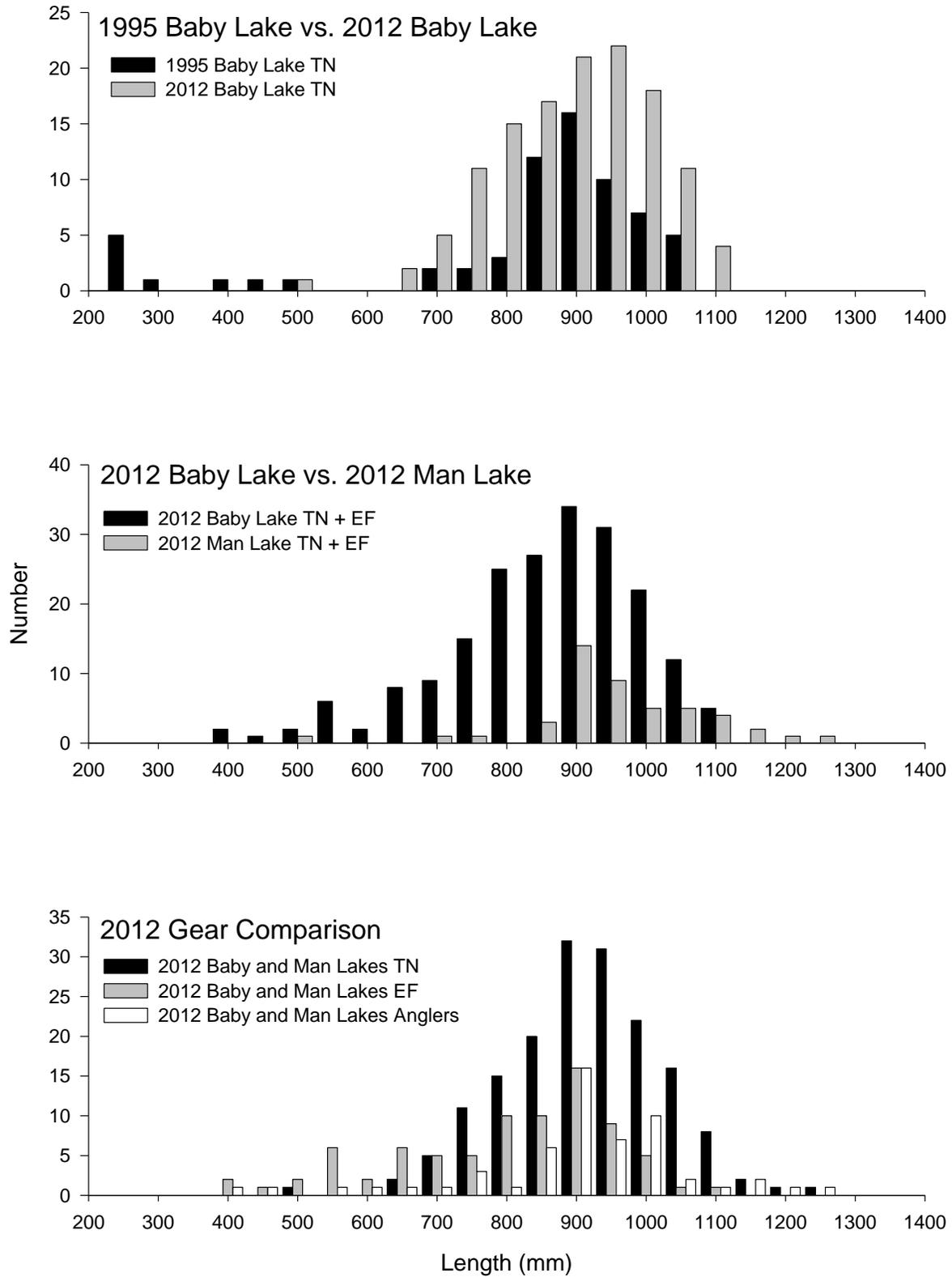


Figure 4. Length frequency distribution of Muskellunge captured in Baby and Man Lakes in 1995 and 2012 using trap nets (LTN), electrofishing (EF) and anglers.

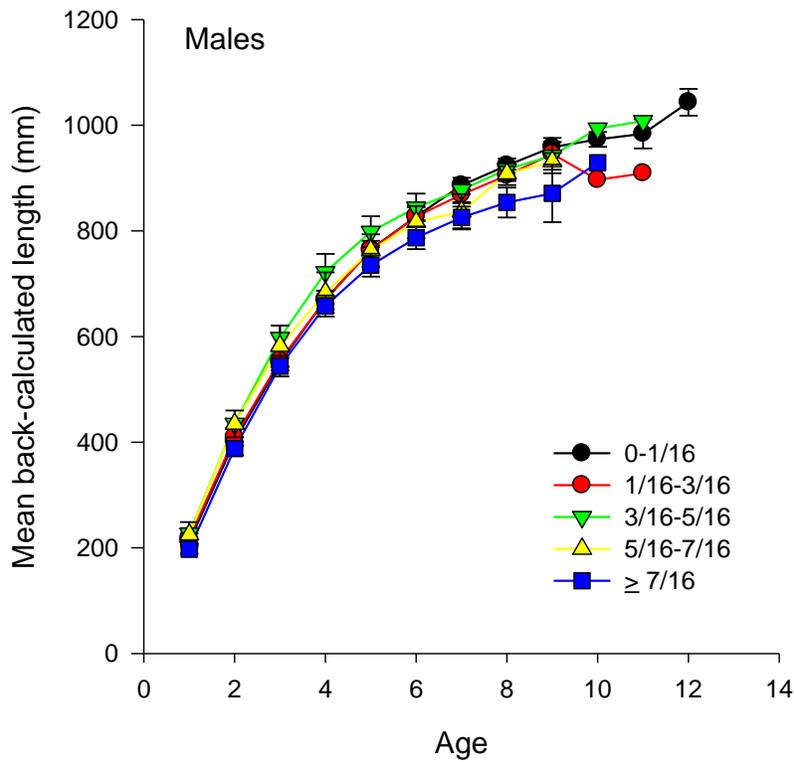
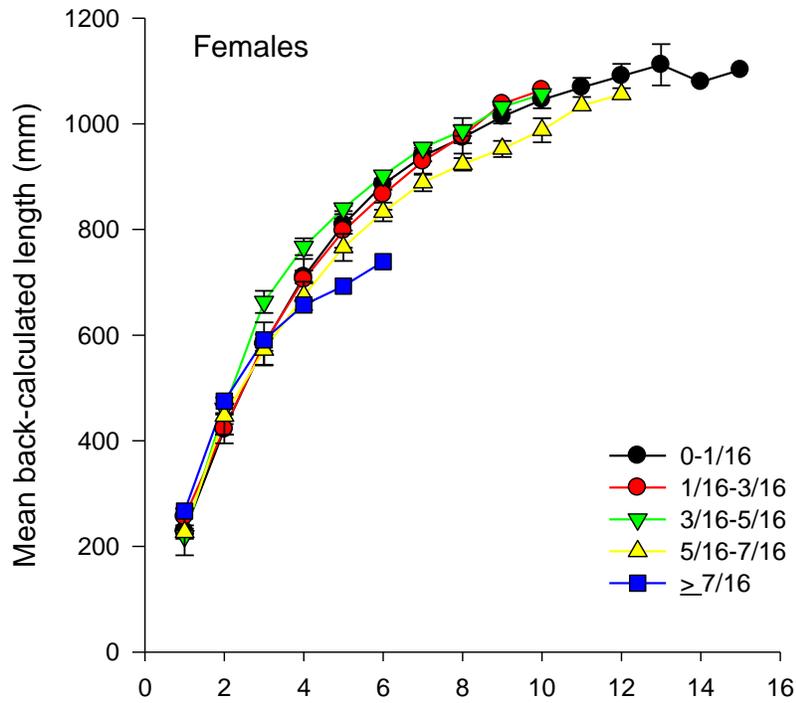


Figure 5. Mean back-calculated length (mm; \pm SE) at age for male and female Muskellunge sampled in Baby and Man Lakes combined, 2012. Individuals were grouped by estimated proportion of Shoepack ancestry.

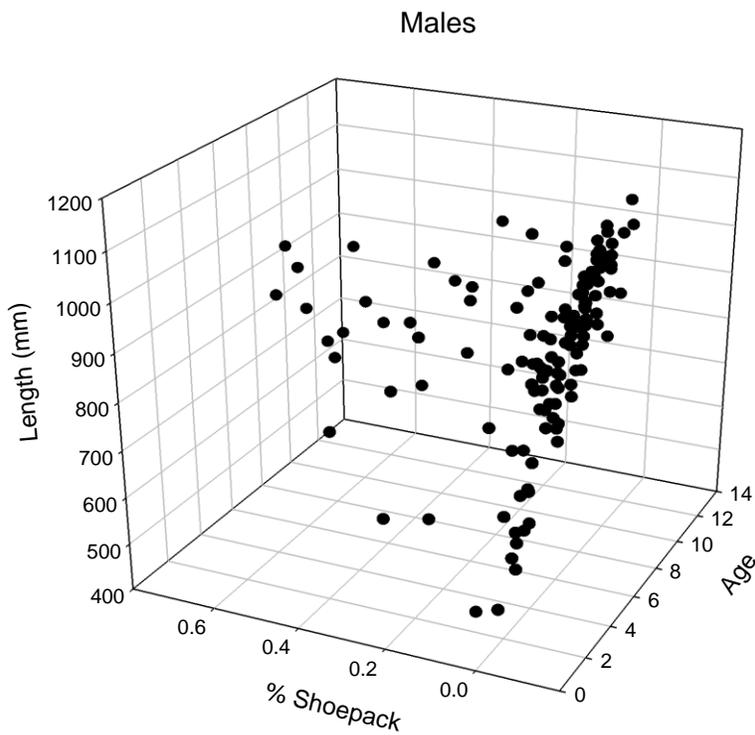
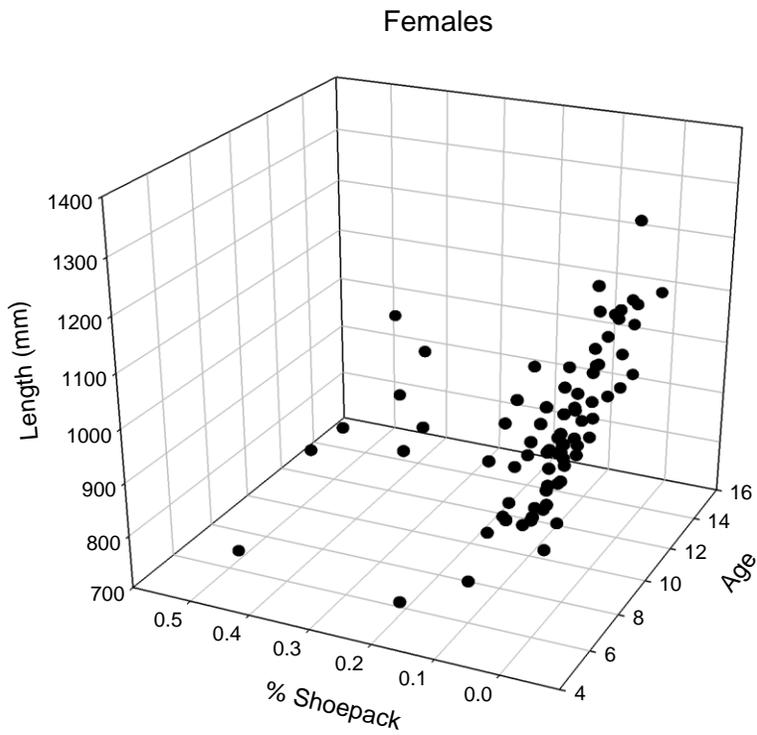


Figure 6. Lengths (mm) of Muskellunge from Baby and Man Lakes in 2012 plotted against the estimated Shoepack ancestry by age. Immature and angler caught fish were not included because sex was not known.

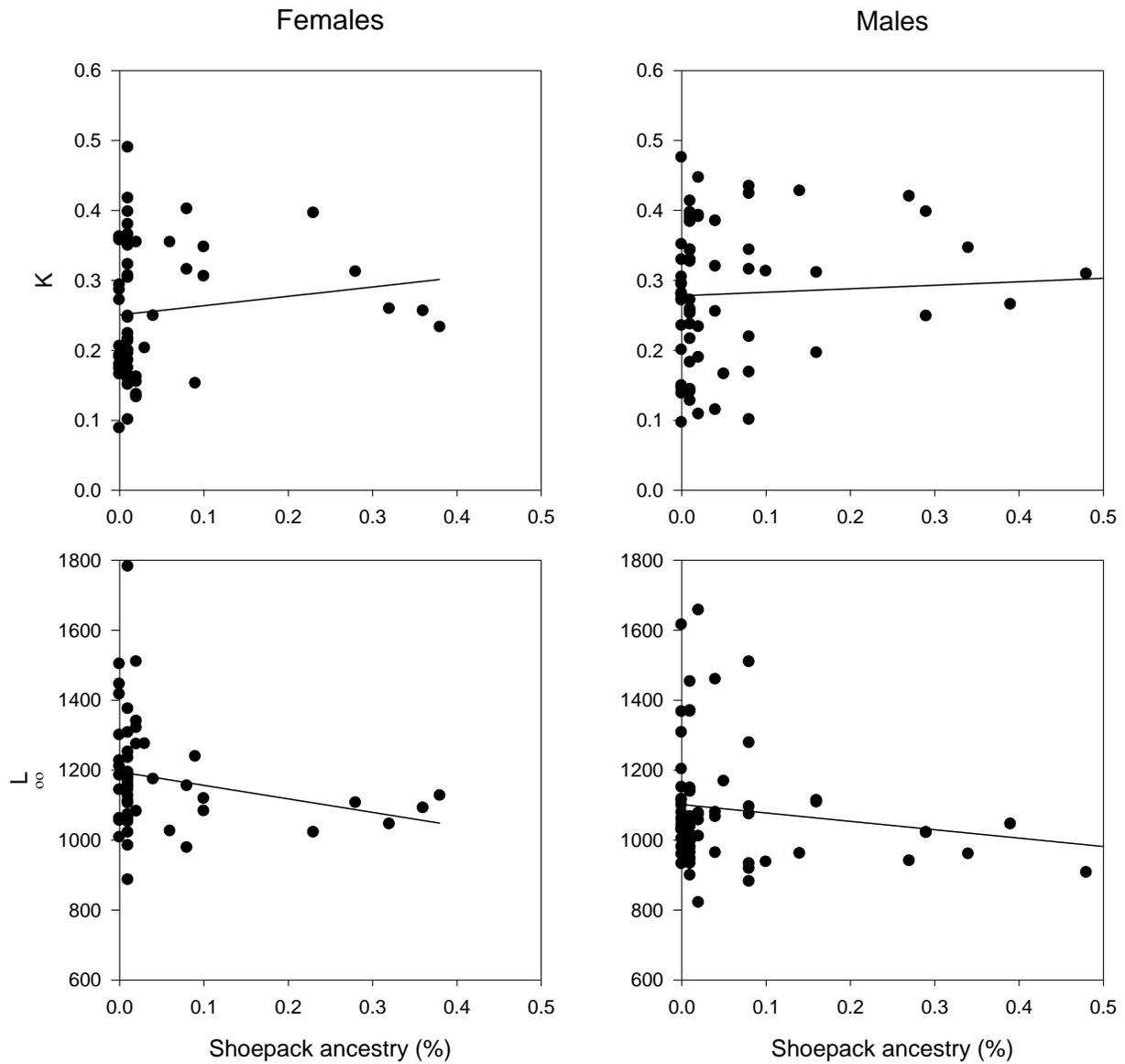


Figure 7. Comparisons of L_{∞} (ultimate length) and K (growth coefficient) to the proportion of Shoepack ancestry, by sex, for Muskellunge captured in Baby and Man Lakes, 2012.

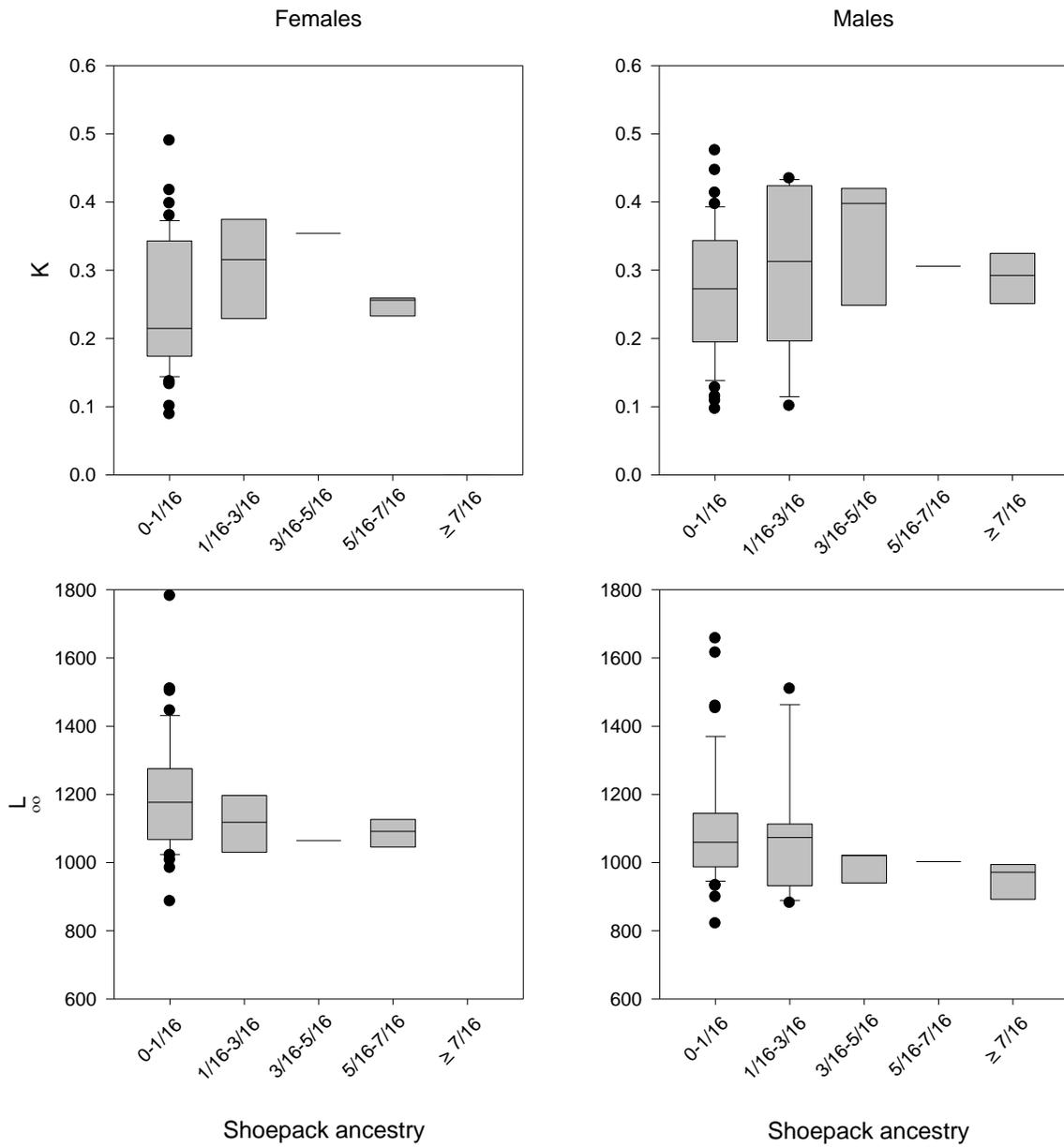


Figure 8. Comparisons of L_∞ (ultimate length) and K (growth coefficient) to the proportion of Shoepack ancestry, by sex, for Muskellunge captured in Baby and Man Lakes, 2012.