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Multi-year evidence of unbiased sex ratios in hatchery and wild-reared age-0 lake sturgeon (*Acipenser fulvescens*)

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ABSTRACT

Assessment of population sex ratios allows managers to forecast recruitment dynamics and loss of genetic diversity in natural populations and is important when the focal population is in low abundance and subject to demographic stochasticity. If levels of natural or artificial selection differ for males and females and levels of mortality likewise vary, lower levels of population recruitment, loss of genetic diversity, and genetic drift can occur. Lake sturgeon (*Acipenser fulvescens*) are a species of conservation concern, where restoration efforts increasingly rely on hatchery supplementation. Raising larvae to the juvenile stage can increase survival during important early life stages; however, knowledge is lacking concerning effects of artificial rearing environments on differential sex-specific survival before release. We genetically determined the sex of 1459 age-0 lake sturgeon from three cohorts (2016 through 2018) using PCR assays of the ALLWSex2 acipenserid sexing marker. Sexed individuals represented three groups: (1) wild-captured dispersing larvae that died during hatchery rearing, (2) wild-captured dispersing larvae that survived hatchery rearing to release, and (3) wild-captured, wild-raised age-0 individuals. Sex ratios of wild-captured larvae (dead + live) were nearly 50:50 in all years surveyed. We observed slight, but non-significant, directionality in sex ratios in the live and dead hatchery-reared larvae and in wild-captured age-0 individuals. Genetic sexing methods allow for analyses during prolonged pre-reproductive periods and associated variable environmental and demographic circumstances, in situations where physical determination of sex is not possible.

Introduction

Lake sturgeon have experienced declines in numerical abundance and distribution throughout the last century in the Great Lakes region (Harkness and Dymond, 1961; Holey et al., 2000), and are a species of concern to managers. Remnant populations of lake sturgeon are of small size (Hayes and Caroffino, 2012; Holey et al., 2000; Scribner et al., 2022), and are characterized by low natural annual recruitment (Caroffino et al., 2010; Schloesser and Quinlan, 2019). The species has evolved unique life history traits, such as delayed sexual maturity, which is characterized by the species' approximately 15–20 year pre-reproductive period (Scott and Crossman, 1973). Males and females differ in age at sexual maturity (Lyons and Kempinger, 1992; McLeod et al., 1999), inter-spawning interval (Forsythe et al., 2012), and resource allocation to gamete production (McGuire et al., 2019). These species and sex-specific characteristics have hindered the lake sturgeon's ability to recover from negative human-mediated effects that have reduced the species' abundance and distribution.

Hatcheries are widely embraced as a management tool to restore lake sturgeon populations through stocking (Holey et al., 2000; Welsh et al., 2010). Existing demographic stochasticity in remnant lake sturgeon populations, due to factors such as low numerical abundance, may be compounded by aspects of the species' life history, such as delayed sexual maturity, iteroparity, and prolonged inter-spawning intervals for females (Forsythe et al., 2012). Hatchery supplementation of natural populations may exacerbate future population demographic variability if skewed sex ratios exist in stocked cohorts. Identifying a skewed sex ratio in stocked cohorts would be of importance to assess future reproductive potential of females from a hatchery cohort. Additionally, sex ratios of the few wild-raised individuals that survive the first year of life are important determinants of future recruitment associated with females from each year's cohort. Accurate forecasting

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of future lake sturgeon reproductive potential requires knowledge of sex ratios of all sources contributing to each year's cohort.

Current, non-molecular sexing techniques are limited in their ability to determine sex of lake sturgeon, particularly during early, pre-reproductive life stages. Lake sturgeon lack sexually dimorphic features (Webb et al., 2019), and only adults are capable of being definitively visually sexed externally in the field during the spawning season through the expression of gametes (Crossman et al., 2011). Ultrasonography and morphological measurements have been used to assign sex in lake sturgeon during field surveys using portable ultrasound units, however this technique requires sexually mature adults, and the accuracy varies depending on maturity stage (Chiotti et al., 2016). Past laboratory studies have utilized histology techniques to determine sex of sturgeon as early as age 4 (McGuire et al., 2019; Van Eenennaam and Doroshov, 1998); however gonadal tissue must first be invasively removed from the fish, preserved, and then examined under a microscope. Standardized molecular techniques would avoid the limitations of current techniques and provide a more time-efficient methodology to sex lake sturgeon of all life stages. Several molecular studies attempted to determine sex of acipenserid species (Hale et al., 2010; Keyvanshokooh et al., 2007; McCormick et al., 2008), but were not successful. Recently, Kuhl et al. (2020) described an evolutionarily conserved region consistent with a female heterogametic ZZ/ZW system and established a polymerase chain reaction (PCR)-based assay to determine the sex of six acipenserid species. The PCR-based assay was subsequently demonstrated to accurately determine the sex of lake sturgeon (Scribner and Kanefsky, 2021), a species not evaluated by Kuhl et al. (2020). This assay requires a small non-invasively sampled fin clip that can easily be taken from wild or hatchery individuals. This newly developed PCR-based genetic method enables determination of lake sturgeon sex for individuals of all developmental ages, thereby allowing evaluation of sources of natural and human-mediated environmental stressors on sex ratios that may adversely affect natural and hatchery-mediated recruitment dynamics.

In natural populations, skewed sex ratios, often caused by differential mortality rates (Quinn and Buck, 2001), competition and inbreeding (Hamilton, 1967), and anthropogenic degradation or changes in the environment (Larsson and Förlin, 2002) can have negative effects on population growth and stability. Sex ratios are important to forecast population levels of recruitment and retention of genetic diversity. Skewed sex ratios are known to lower effective population size (Wright, 1938), and in turn reduce the power of natural selection and increase rates of genetic drift, loss of genetic diversity, and inbreeding (Charlesworth, 2009). This is especially true for populations in low abundance and/or populations subject to demographic stochasticity. Skewed sex ratios may also be the result of natural environmental stressors or selection. Natural selection can act on resource allocation to reproduction and growth (Charnov, 1979), which is expected to vary with age between males and females. Physiological costs are differentially imposed on parents through the production of male or female offspring (Trivers and Willard, 1973; Williams, 1979). Environmental stress can lead to resource allocation decisions due to reproductive tradeoffs to maximize progeny numbers based on environmental quality (Myers, 2001). Shyu and Caswell (2016) developed case studies where progeny sex ratios vary depending on natural conditions, such as age and physiological condition, which is valuable for understanding population sex ratio evolution.

Effects of skewed sex ratios can be exacerbated in long-lived iteroparous species with limited recruitment, as deviance from a 1:1 sex ratio is likely to remain in populations for generations (Charnov, 1990), as relatively few individuals reach maturity and few adults die in any given year. Species with delayed sexual maturity, such as the approximately 10-year difference between male and female lake sturgeon (Bruch and Binkowski, 2002), experience delayed demographic recovery when one sex takes longer to reach maturity than the other (Charnov, 1990; Congdon et al., 1993). Knowledge of population sex ratios is critical to management planning for lake sturgeon because of the species' conservation status, regardless of whether a population is supplemented with hatchery intervention.

The goal of this study was to determine sex ratios during early life stages for lake sturgeon during three consecutive years (2016–2018). Two primary groups were evaluated in this study: (1) hatchery-reared larvae that either survived to stocking or died in the hatchery environment prior to stocking in years 2016, 2017, and 2018 and (2) wild-captured, wild-raised age-0 individuals that were surveyed in August of 2017 and 2018. Specific objectives of this study were to: (1) estimate sex ratios during early life stages for wild-caught larvae which survived rearing in a streamside hatchery for stocking purposes, (2) quantify whether more male or more female larvae died in the hatchery environment prior to stocking release, and (3) quantify sex ratios of wild-captured age-0 fish. Sex ratios in age-0 fish from a wild population may indicate whether there is selection for either sex in a population of lake sturgeon for which natural recruitment is limited.

Materials and methods

Study site

Research was conducted during each of three consecutive years (2016–2018) on the upper Black River (UBR) and the Black River Streamside Rearing Facility (BRSRF) in Cheboygan County, MI (Fig. 1) under Michigan State University Institutional Animal Care and Use approved protocols 03/14-037-99, 03/14-038-99, 04-17-056-99, and 04-17-057-99. The UBR is a fourth order stream that flows into Black Lake (Smith and King, 2005). The lake sturgeon population is isolated by dams upstream of the spawning and larval rearing area of the river above Black Lake (Smith and King, 2005). The BRSRF is a 312 m^2 flow-through system supplied with river water from the nearby Kleber Reservoir (~680 L/min) immediately upstream from the UBR (Bauman et al., 2015). Water entering the facility is filtered by a two stage, six chamber felt bag filtration system. During the first stage, water passes through a 100-μm, 81.28 × 17.78 cm felt filter bag (VB100, Pentair Aquatic Ecosystems, Apopka, FL). The second filtration stage forces water through a 50-μm, 81.28 × 17.78 cm felt filter bag (VB50, Pentair Aquatic Ecosystems, Apopka, FL), and into a gravity fed head tank where water is degassed and distributed throughout the streamside rearing facility.

Larval collection and hatchery rearing

BRSRF staff annually survey lake sturgeon larvae as they disperse, or drift, downstream from the spawning grounds (Fig. 1) in the UBR (Auer and Baker, 2002; Crossman et al., 2014; Crossman et al., 2011; Larson et al., 2020; Smith and Baker, 2005; Smith and King, 2005). Dispersing larvae are used for hatchery propagation rather than offspring reared from direct gamete takes because a far larger number of adults contribute to dispersing larvae than can be realized based on direct gametes takes. Thus, levels of genetic diversity in fish released are much higher (Crossman et al., 2011). We deployed five D-framed nets at equal intervals across the river (~25 m width) during the entire period of larval downstream dispersal from spawning areas each year to capture larvae. The nets were set ~2 km downstream from the known spawning area (Fig. 1). Nets were deployed at night from 21:00 to 02:00. Larval lake sturgeon were counted hourly as

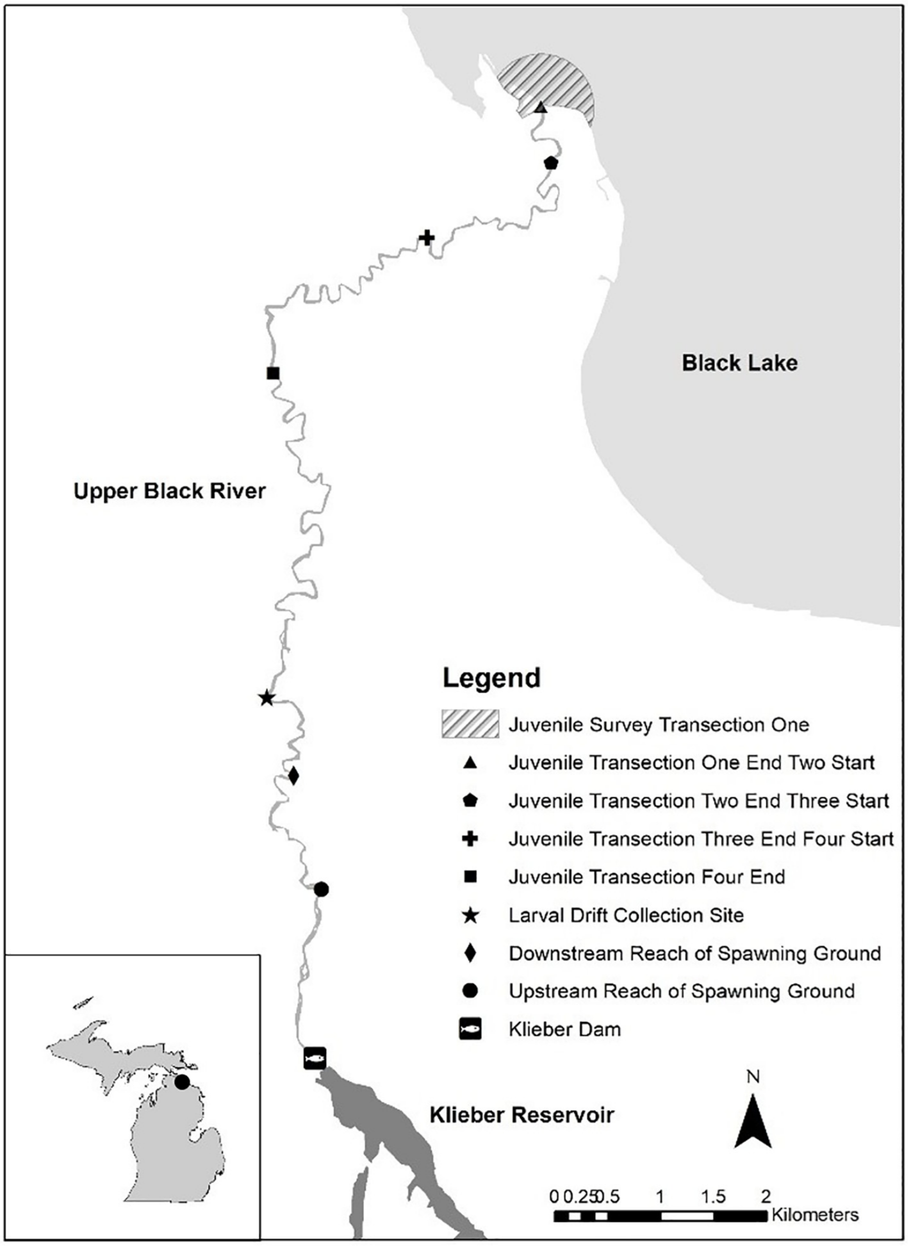

Fig. 1. Map of the Upper Black River drainage in Cheboygan Co., Michigan showing locations of collection sites of lake sturgeon of different ages and the location of the stream-side hatchery facility.

described by Crossman et al. (2014), Crossman et al. (2011), and Crossman et al. (2010). Sampling was conducted each night from the date larvae were first captured until three consecutive nights of no captures (Crossman et al., 2014). Larvae were placed in the hatchery nightly. The number of larvae retained per night of larval drift was set proportional to the number of larvae captured each night. In 2016, we captured 4053 larval lake sturgeon from 22 May to 24 June. In 2017, we captured 19,135 larvae from 15 May to 18 June. In 2018, we captured 48,048 larvae from 23 May to 22 June (Fig. 2).

A randomly selected subset of the total number of larvae captured each night were retained and returned to the hatchery. Larvae were reared in the stream-side hatchery in 3.0-L flow through polycarbonate tanks (Pentair Aquatic-Ecosystems, PCT3-D) at densities of 50 individuals per tank (Bauman et al., 2015), and were reared in separate tanks containing fish from a single drift collection night. Drift nights remained separated during the duration of the rearing period. Larvae were fed a diet of live *Artemia nauplii* (Brine Shrimp Direct, Ogden, UT) at feeding rates consistent with those described by Deng et al. (2003) and modified for lake sturgeon as described by Bauman et al. (2016). Larvae in all tanks were fed 26 % of body weight (BW) per day during the first and second weeks post-exogenous feeding, 13 % of BW per day during the third week, and 11 % of BW per day during the fourth and fifth weeks (Bauman et al., 2016; Deng et al., 2003). When larvae reached 0.4 g, they were moved into 0.5 m diameter round, center drain tanks, generally around four weeks. Additionally, transition from brine shrimp to bloodworms (Diptera: Chironomidae; Brine Shrimp Direct, Ogden, UT) began four weeks post-exogenous feeding (Klassen and Peake, 2008; Volkman et al., 2004). Fish were fed 5 % dry BW per day (Bauman et al., 2016) until individuals reached a recommended stocking size of 150 mm

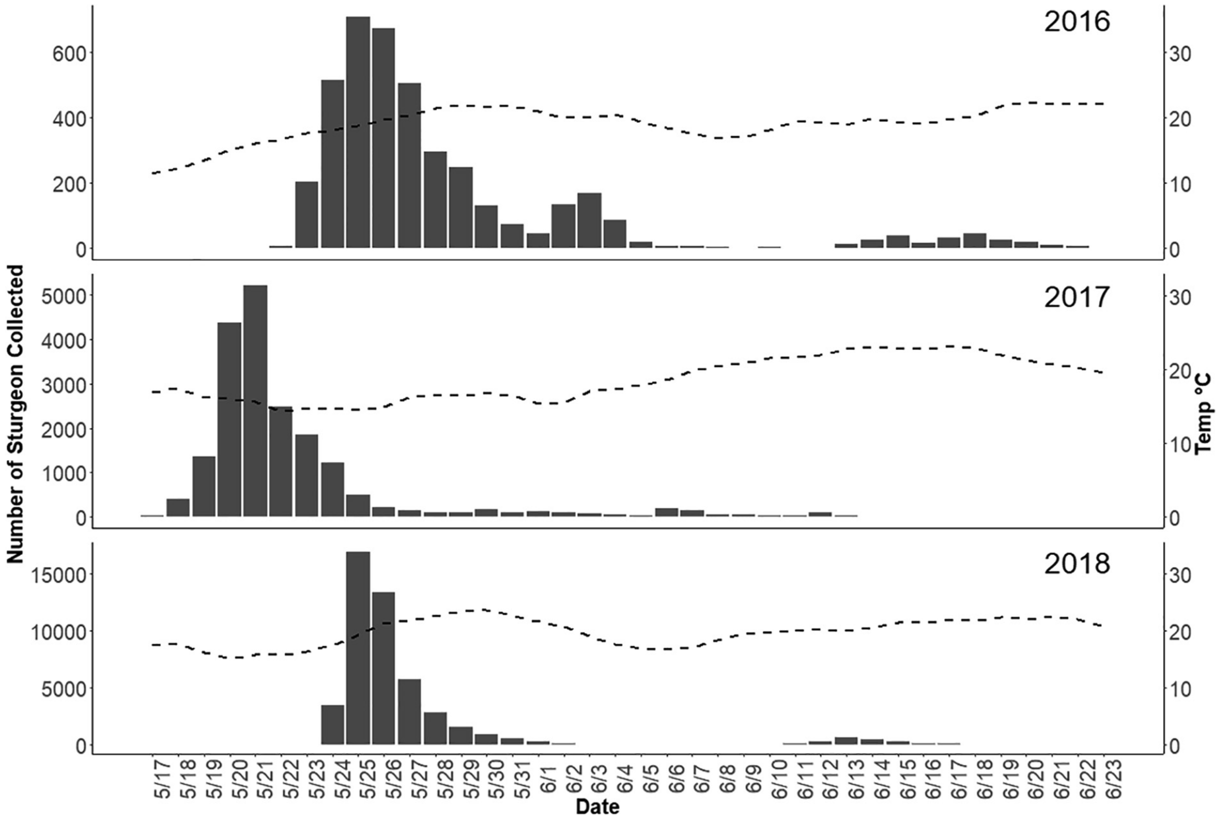

Fig. 2. Frequency histogram describing the distribution of numbers of larval lake sturgeon captured nightly during 3 consecutive years during the period of larval dispersal from adults spawning area in the upper Black River (Fig. 1). Larvae were sampled proportionally by night for rearing in the stream-side hatchery and for sex determination. Note differences in scale on the y-axes. Secondary y-axes and dashed lines denote daily average temperature during the larval collection period and during hatchery feed training.

(Baker and Scribner, 2017). The average (±SD) daily temperature and dissolved oxygen concentration of water in the hatchery in 2016 during the rearing period (23 May 2016–20 August 2016) was 22.90 °C ± 2.25 °C (range: 16.50 °C – 27.80 °C) and 8.44 mg L^{-1} ± 0.75 mg L^{-1} (range: 5.34 mg L^{-1} to 10.36 mg L^{-1}), respectively. In 2017, the average (±SD) daily water temperature was 20.90 °C ± 2.64 °C (range: 14.23 °C – 25.81 °C) during the rearing period from 18 May 2017 to 19 August 2017. A probe failure resulted in no dissolved oxygen readings in 2017. In 2018, the average (±SD) daily temperature and dissolved oxygen concentration of water in the hatchery was 23.03 °C ± 2.24 °C (range: 16.5 °C – 29.6 °C) and 7.93 mg L^{-1} ± 0.84 mg L^{-1} (range: 3.6 mg L^{-1} to 14.23 mg L^{-1}), respectively during the rearing period from 25 May 2018 to 25 August 2018. Tank densities and feeding rate were adjusted weekly to maintain the BW per day feeding regimen based on weight assessments of 20 fish measured per drift night.

A 0.5 cm^2 dorsal fin clip was collected using non-invasive methods from all hatchery-produced lake sturgeon at the time of stocking each year and organized by the night of collection to determine molecular sex. Sampling from each drift night was conducted proportionally to the number of fish captured during the entire drift season for each year. During the rearing period for each season, expired fish were removed from their respective tanks twice daily and preserved in 95 % ethanol by the drift night on which they were collected. Mortalities remained separate by drift night so future sampling could remain proportional by drift night ("dead" group; Table 1). Fish from the dead group and an equal number of fish samples from the live group were sampled proportionally across each night of the larval drift period for genetic sexing. During 2018, 472 live drift samples were available for sexing. Therefore, an equal proportion (472 samples) were sexed from both dead and live fish groups, representing only 0.09 % of the total drift collection in 2018 (Table 1).

Wild-captured age-0 collection

Age-0 lake sturgeon were captured in the Upper Black River and in Black Lake near the mouth of the Upper Black River, in August 2017 and 2018. Crews of three surveyed each of four sections of age-0 lake sturgeon river habitat indicated in Fig. 1. The survey was conducted on the nights of August 2nd, 3rd, 8th, and 9th in 2017 and August 6th-10th in 2018. Surveys began 30 min after sundown. Crews dispersed across the width of the river and waded upstream searching for age-0 lake sturgeon using headlamps and flashlights. A total of 6.7 km of the river was surveyed. Crews surveyed the mouth of the river to ~ 500 m offshore and approximately 500 m in each direction as indicated in Fig. 1. When a fish was observed, it was captured in dip nets (approximately 40 × 15 cm net and a 1.25 m pole). A 1 cm^2 fin clip was taken from each captured fish for genetic sex determination and stored in 95 % ethanol. In addition, each captured sturgeon was tagged with full duplex PIT tag; age-0 fish shorter than 150 mm were given a 9 mm tag and individuals longer than 150 mm were given a 12 mm tag (Biomark, Boise, ID). Lake sturgeon longer than 300 mm were classified as age-1 fish and were also tagged with a 12 mm PIT tag but were not included in further analyses.

Laboratory analysis

DNA was extracted from fin clips of deceased individuals using a 96-well plate-based extraction method modified from a protocol described by Ali et al. (2016). 2X "SeraPure" beads (Rohland and

Table 1
Total number of hatchery-reared, wild-dispersing larval lake sturgeon and wild-captured age-0 lake sturgeon from the live and dead samples in 2016, 2017, and 2018. Live individuals were those which survived through hatchery rearing and were released, and dead individuals were those that died at any time throughout the hatchery rearing process.

		2016			2017			2018		
		Alive	Dead	Proportion	Alive	Dead	Proportion	Alive	Dead	Proportion
Hatchery-reared larvae	Male	25	20	54.9 %	103	101	53.4 %	252	225	50.5 %
	Female	16	21	45.1 %	88	90	46.6 %	220	247	49.5 %
	Total	41	41	–	191	191	–	472	472	–
Wild-captured age-0	Male	–	–	–	3	–	27.3 %	16	–	40.0 %
	Female	–	–	–	8	–	72.7 %	24	–	60.0 %
	Total	–	–	–	11	–	–	40	–	–
Total drift larvae collected	4,053		19,135		48,048					

Reich, 2012) were used in place of Ampure XP beads as described by Ali et al. (2016) as a more cost-effective replacement (Rohland and Reich, 2012). 70 μL of Teknova DNA Suspension Buffer (Teknova, Hollister, CA) was used to elute samples in place of low TE (Ali et al., 2016).

The ALLWSex2 marker developed by Kuhl et al. (2020) and optimized for lake sturgeon by Scribner and Kanefsky (2021) was used to determine sex. PCR reactions were carried out as described by Scribner and Kanefsky (2021) and contained 2 μL of DNA at a concentration of approximately 20 ng/μL. PCR products were visualized on a 1.5 % agarose gels alongside a 100-base pair (bp) ladder (Invitrogen, Waltham, MA) that were stained with ethidium bromide and photographed under UV light. For reference, included on each gel were the PCR products from one known adult male and female lake sturgeon whose sex was previously determined based on the expression of gametes. Samples with an amplification band slightly larger than 100 bp were scored as females and samples with no amplification or a faint band slightly less than 300 bp in size were scored as males (Scribner and Kanefsky, 2021).

Failure of the ALLWSex2 marker to amplify in an individual lake sturgeon could be caused by poor quality genomic DNA, resulting in the misclassification of a female as a male. A properly-amplified male sample, during agarose gel scoring, may appear similar to a sample which actually failed to amplify in PCR. Using a marker which amplifies for both sexes would allow for confirmation of the quality of genomic DNA and the original sex score given to the sample. To verify the quality of extracted DNAs, an empirical error check was conducted on 10 % of all samples that were scored as males. The disomic microsatellite locus AfuG160 (non-fluorescently labeled; Welsh et al., 2003) was used as a PCR-positive control to amplify putative male samples using conditions described by Homola et al. (2010). PCR products were again visualized on a 1.5 % ethidium bromide-stained agarose gel with a 100 bp DNA ladder (Invitrogen, Waltham, MA).

Statistical analysis

For hatchery produced fish, a Pearson's chi-square goodness of fit test was used to determine if numbers of males and females between live and dead fish groups within a year differed from an expected even ratio. The chi square goodness of fit test is appropriate for count data where the sample size is sufficiently large. To determine if these sample sizes had appropriate power to use the chi square goodness of fit test, we performed a power analysis using R package pwr version 1.3–0 (Champely et al. 2020). An effect size value of 0.3 consistent with a small effect size was used to evaluate power, as described in Cohen (1988). Where the calculated power exceeded the recommended value of 0.8, we used a chi square goodness of fit test. Where the statistical power was less than 0.8, indicating small sample sizes, we used a Fisher's exact test, a non-parametric analog to the chi square test which accounts for small sample sizes.

A Fisher's exact test was used to determine if counts of male and female lake sturgeon captured in the wild age-0 fall assessment produced an odds ratio different from 1. Fish in the wild captured survey were collected from direct sampling and were not sampled proportionally from a larger population in both 2017 and 2018 making direct comparison between years possible. A binomial regression was used to model the proportion of sturgeon surviving in the hatchery as a function of sex and year produced. This regression included all genotyped fish across 2016, 2017, and 2018, and was used to determine whether survival in the hatchery was different between sexes and among years. Differences in total length and weight for wild-captured age-0 fish were analyzed using Wilcoxon–Mann–Whitney tests (WMW; Siegel and Castellan 1988). Differences between all groups in all tests were evaluated using $\alpha = 0.05$.

Results

We genetically sexed 82, 382, and 944 hatchery-reared larvae collected during 2016, 2017, and 2018, respectively (Table 1). There was no statistically significant difference in sex ratios of fish surviving hatchery rearing to stocking across years (Table 1); however, in all years, numerically more males survived to stocking age. All males subjected to an additional round of PCR as an empirical error check were confirmed to be males. All males also had PCR amplification bands (alleles) in the expected allele size range for locus AfuG160 verifying PCR amplification success.

The 2016 group of hatchery-reared larvae that survived to stocking was composed of 25 (61 %) males and 16 (39 %) females, and the mortality group was composed of 20 (48.7 %) males and 21 (51.2 %) females (Table 1). For hatchery-reared larvae collected during the 2016 larval drift period, there was no statistical difference in the proportion of males and females in both the live and dead sampled groups (p = 0.375, Fisher's exact test). In the 2017 groups, 103 (53.9 %) males and 88 (46.1 %) females were members of the live group that survived to stocking, and 101 (53.2 %) males and 90 (46.8 %) females were members of the group that died during the hatchery rearing period (Table 1). In 2017, no difference in the proportion of males and females was observed between larvae from the live and dead groups ($\chi^2 = 0.011$, df = 1, p = 0.918). The 2018 hatchery-reared larvae included members of the fish group that survived to stocking, composed of 252 (53.4 %) males and 220 (46.6 %) females, and the group of mortality fish that was composed of 225 (47.7 %) males and 247 (52.3 %) females (Table 1). In 2018, analyses also found no difference in the proportion of males to females between larvae from live and dead groups ($\chi 2 = 2.864$, df = 1, p = 0.091). Collectively, across the three years collections were made, there was a tendency for a greater proportion of hatchery mortalities to be composed of females; however, trends were not statistically significant.

We captured 11 and 40 wild-captured age-0 lake sturgeon in 2017 and 2018, respectively, during August visual river surveys. Fish captured in 2017 had an average length of 141.9 mm with a range of 115–182 mm. In 2018, the average length of wild-captured age-0 lake sturgeon was 158.6 mm with a range of 108–195 mm. Of the 11 wild-captured age-0 sturgeon genetically sexed from 2017, 3 (27.3 %) were male and 8 (72.7 %) were female. Of the 40 wild-captured age-0 fish from 2018, 16 (40 %) were male and 24 (60 %) were female. There was no statistically significant difference in number of males and females sexed between 2017 and 2018 ($p = 0.505$, Fisher's exact test). The consistently higher proportions of females in the groups of wild-captured age-0 lake sturgeon captured was notable; however, sample sizes were small and the results, while seen in both collection years, were not statistically significant. A Wilcoxon–Mann–Whitney test of size indicated that there was no statistically significant difference in the weight or total length of age-0 males or females from 2017 (total length: $W = 9$, $p = 0.609$; weight: $W = 10$, $p = 0.776$), 2018 (total length: $W = 130.5$, $p = 0.1833$; weight: $W = 139$, $p = 0.3819$), or when both years (2017 and 2018) were pooled (total length: $W = 204.5$, $p = 0.1006$; weight: $W = 207$, $p = 0.1516$).

A binomial regression, including individuals across all three years, indicated that there was no statistically significant difference in the proportion of males and the proportion of females surviving in the streamside hatchery (z-value = 1.813, $p = 0.07$). Additionally, the proportion of sturgeon surviving was not different between years (z-value < 0.05, $p > 0.95$). There was no statistically significant difference in the proportion of wild-captured age-0 males and females between 2017 and 2018 based on a chi-square test of homogeneity ($\chi^2 = 0.177$, df = 1, $p = 0.674$).

Discussion

Sex ratios during larval and age-0 life stages were the focus of this study because lake sturgeon experience high mortality rates during the first ~ 3 months of life (Caroffino et al., 2010) and comparatively higher survival at the end of their first summer following spawning season (Baker and Scribner, 2017). Sex ratios observed in fish stocked from the hatchery or captured in Black River age-0 surveys at the end of the summer are likely to remain unchanged for their respective year class as they develop and recruit to the adult stage, as trends in survival are maintained throughout a cohort's growth to sexually mature stages (Baker and Scribner, 2017). Hatchery-produced lake sturgeon released into the wild with the intent to augment natural populations may be affected by differential survival which may be best documented at early life stages while high mortality rates occur between egg and larval stages (Caroffino et al., 2010) and selection occurs. Maintenance of balanced sex ratios in hatchery-produced offspring is important to future reproduction in augmented natural populations. In turn, demographic information that includes sex ratios of captive-raised cohorts may help diagnose future problems or describe how hatcheries are affecting natural populations.

This study found no statistically significant differences in sex ratios among hatchery-reared larval lake sturgeon which survived to stocking and those which died in the hatchery. Similarly, no statistically significant difference was found between the number, weight, or total length of wild-captured male and female age-0 fish from 2017 and 2018. These results indicate a lack of significant sex-related differential mortality, survival, or growth (growth measured in wild-captured age-0 fish only) in both the Black River streamside rearing facility and the wild age-0 fish captured in Upper Black River. It is, therefore, unlikely that stocked age-0 individuals throughout the three study years significantly influenced the existing sex ratio of the natural population.

While no statistically significant skews were observed, there are trends in directionality of sex ratios in some groups that may deserve further attention as cohorts age. More females than males were observed across all three years in the hatchery-raised larvae that suffered mortalities prior to a scheduled stocking event in this study (Table 1). A female bias in mortality could indicate the possibility of some differential mortality rates related to the hatchery environment. Bias towards males in hatchery-reared stocked age-0 individuals may decrease future reproductive contributions from females of the cohort. Further investigation of older cohorts would be beneficial.

Hatchery supplementation is an important management action necessary for conservation of many lake sturgeon populations. The availability of sexing methods could be profitably used to assess questions such as whether resource allocation between somatic and gonadal tissues varied between males and females as suggested by McGuire et al. (2019). In a traditional hatchery setting, abiotic parameters, such as rearing temperature, are controlled to maximize growth, survival, and hatchery output. In contrast, streamside facilities use surface waters to promote natal stream fidelity for small rearing batches in a manner that is more cost-effective than traditional rearing but exposes lake sturgeon to variable ambient temperatures consistent with the river (Bauman et al., 2015; Holtgren et al., 2007). Stressful hatchery conditions which affect physiology, behavior, and other aspects of survival, such as predation risk (Wassink et al., personal communication, Wassink et al., 2019) may also intensify the divergence between male and female development, reproductive and otherwise (McGuire et al., 2019). A study by Thompson et al. (2015), obtained similar results to this study using different methodology, and concluded there was no evidence for sex ratio skew in a population of wild steelhead (*Oncorhynchus mykiss*) due to biased survival in a hatchery. While the manipulation of natural populations by hatchery supplementation may cause concern for skewing sex ratios, this study and that by Thompson et al. (2015) did not find a significant effect on sex ratios due to hatchery influence.

Sex can now be non-invasively determined for lake sturgeon of any age using the ALLWSex2 marker described by Kuhl et al. (2020) and Scribner and Kanefsky (2021) for early, cost-effective sex determination in lake sturgeon. This methodology allows for accurate assessments of sex ratios and may be particularly useful in assessing ratios of hatchery-produced stock prior to their release into the wild. Little is known about how standard hatchery practices quantitatively impact sex ratios in stocked age-0 lake sturgeon due to the historical lack of appropriate sexing methodology. Elucidating the relationship between sturgeon hatchery production and relative abundance of males and female prior to the onset of sexual maturity is important for effective conservation. Molecular determination of sex allows managers to assess differential survival between males and females through ontogeny, which may be particularly important when considering the possible implications of hatchery influence on imperiled populations.

This study has demonstrated that hatchery mortalities, hatchery-reared stock, and wild age-0 lake sturgeon groups lack sex ratio bias in three consecutive years. Additional studies would be useful to draw more generalized conclusions about the magnitude and prevalence of skewed sex ratios and their relationship to varying rearing conditions and the pre-reproductive period, generally. The utility of molecular sexing in other settings would be useful to explore, such as for larvae reared in traditional groundwater and smaller streamside rearing facilities, such as trailers, to provide a more holistic understanding of how different hatchery rearing settings may affect sex ratios.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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