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Pedigree-based decadal estimates of lake sturgeon adult spawning numbers and genetic diversity of stream-side hatchery produced offspring

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ABSTRACT

For species of conservation concern, including lake sturgeon (*Acipenser fulvescens*), maintaining genetic diversity is critical for successful restoration. Great Lakes lake sturgeon restoration increasingly relies on hatchery supplementation and stream-side rearing facilities that utilize collections of eggs and offspring of early life stages. The number of wild-caught eggs/larvae sampled is not likely an accurate predictor of spawning adult number, nor of hatchery cohort diversity. We used microsatellite loci and likelihood-based pedigree reconstruction to quantify offspring diversity, and the number and effective number of adult lake sturgeon contributing to offspring reared in the Manistee River stream-side hatchery facility in Michigan. Over 10 years (2005–2014) 1129 samples from stream collections of eggs, dispersing larvae, and juveniles were genotyped. Inter-annual variation in estimated mean offspring co-ancestry (Θ 0.013–0.030), numbers of contributing adults (N_s 23–72), and effective number of breeding adults ($\widehat{N}_b$ 17–43) were documented. Combining samples across 10 years we estimated that mean offspring co-ancestry was 0.005, the number of spawning adults (N_s) contributing to offspring released was 326, while the harmonic mean effective number of breeding adults ($\widehat{N}_b$) was estimated to be 29.5 (lower than 10 times 29.5 for semelparous species). Forty-eight percent of adults contributed one or more offspring in two or more years. Demographic (non-Poisson distribution of adult reproductive success, low annual N_s and repeated spawning) and genetic (low annual $\widehat{N}_b$, relatedness among offspring within and among year cohorts) features depressed levels of diversity. Implications to species recovery planning are discussed considering low numbers of adults recruiting offspring annually.

Introduction

Information pertaining to the number of adults contributing to offspring produced annually in natural populations is increasingly important in light of concerns associated with population levels of genetic relatedness and inbreeding (Ryman and Utter, 1987), and are essential to the design of management plans (Myers et al., 1995), especially for rare or threatened species (Meffe, 1986). In the Great Lakes, many lake sturgeon (*Acipenser fulvescens*) populations lack adequate data regarding spawning adult abundance, relationships between adult spawning numbers and larvae produced, or genetic diversity and inter-individual relatedness (or co-ancestry) among offspring produced and released each year. Because lake sturgeon are long-lived and iteroparious, repeated spawning events by the same adults across years could increase levels of co-ancestry among offspring from different year cohorts. This potential outcome is increasingly likely in populations characterized by low spawning adult abundance.

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Adult lake sturgeon occupy spawning grounds for brief periods during late April through early June (Forsythe et al., 2012a). In many Great Lakes tributaries, spawning numbers are relatively low (Holey et al., 2000) compared to historical abundance. Inferences concerning the number of adults producing offspring naturally and which provide offspring to populate restoration hatcheries is generally lacking. Estimates of spawning adult abundance are not easily made from observed spawner abundance data because of the species' complex life history including delayed sexual maturity, intermittent spawning (Forsythe et al., 2012b), low adult mortality (Pledger et al., 2013), low to moderate rates of straying among rivers (Homola et al., 2012, and extreme longevity (Peterson et al., 2007). Determining whether low levels of larval recruitment can be attributed to low adult spawner abundance or to post-ovulatory egg or larval mortality as important impediments to lake sturgeon recovery is important to direct management actions.

Until recently, little has been known about lake sturgeon adult reproductive success or survival during early life history stages (Crossman et al., 2011; Duong et al., 2011a,b; Mann et al., 2011; Forsythe et al., 2014), or about adult reproductive ecology (Forsythe et al., 2012a,b; Duong et al., 2013) in Great Lakes tributaries. In less well studied stream systems, such as the Manistee River in Michigan, USA, managers would benefit from applications of genetic methods for estimating the number of spawning adults that contribute to offspring (N_s) and to the mean ($\bar{k}$) and variance ($V_{\bar{k}}$) in reproductive success, that can influence the effective number of breeding adults (N_b) and population levels of genetic diversity and co-ancestry. Fertilized eggs deposited on substrate and dispersing larvae are present for more predictable periods of time (approximately 2–4 weeks after spawning), and can be captured by non-invasive methods, such as egg mats (Caroffino et al., 2010; Chiotti et al., 2008) and drift nets (Auer and Baker, 2002), thereby allowing larger sample sizes to be collected over diverse habitats (e.g., Hunter et al., 2020a).

Molecular techniques enable biologists to examine aspects of the physical and biotic environment that affect recruitment immediately after breeding (Pemberton, 2008), allowing estimates of the number of adults contributing to offspring produced. Molecular genetic techniques and methods of statistical inference are available (Almundevar and Field, 1999; Blouin, 2003; Fiumera et al., 2001; Kalinowski et al., 2006; Queller and Goodnight, 1989; Wang, 2004), and are amenable for estimation of pedigree relationships among offspring and the number of reproductively successful adults in large and ecologically complex riverine systems (Hunter et al., 2020a; Scribner et al., 2016; Wang and Scribner, 2013). Using a combination of statistical and genetic techniques, genetic determination of parentage (Duong et al., 2013) or pedigree analysis (Hessenauer et al., 2012; Jay et al., 2014) can be used for offspring genotypes sampled during early life stages to estimate the number of parents consistent with genealogical relationships represented in sampled eggs and larvae (e.g., Hunter et al., 2020a, Hunter et al., 2020b; Jay et al., 2014). Genetic data may also provide insight into adult breeding dynamics of a population, such as estimates of mate number, mean and variation in adult reproductive success, effective population size, and offspring relatedness or co-ancestry (Crossman et al., 2011; Duong et al., 2013). Co-ancestry is of particular importance because even in a randomly mating population, population levels of co-ancestry in the current generation will equal expected levels of inbreeding in the subsequent generation (Malécot, 1948).

The Little River Band of Ottawa Indians (LRBOI) and State of Michigan both have approved conservation plans for lake sturgeon, and both require information on population abundance, growth rate and recruitment to guide management actions (Hayes and Caroffino, 2012; LRBOI, 2008). Because sturgeon, as a group, are of conservation concern (Billard and Lecointra, 2001; Bruch et al., 2016), studies are needed to increase understanding of the barriers to population recovery, including estimates of the number of adults producing offspring and levels of genetic diversity in naturally produced and hatchery reared individuals that represent annual recruitment (e.g., Duong et al., 2013).

The LRBOI Nmé (Lake Sturgeon) Stewardship Plan guides restoration of lake sturgeon within the Manistee River and 1836 reservation. Documentation of egg deposition and larval drift, has been implemented in the Manistee River lake sturgeon population since 2002 (Chiotti et al., 2008). A stream-side rearing facility (SRF) has been operated by the LRBOI since 2003 where wild captured Lake Sturgeon eggs and larvae are reared until they may be released at a larger size. Stream-side lake sturgeon hatcheries (Holtgren et al., 2007) have been widely embraced as the preferred rearing option for U.S. populations of lake sturgeon in the Great Lakes. However, concerns have been raised regarding whether naturally deposited eggs or drifting larval sampling within large and complex riverine systems reflect contributions of the majority of spawning adults, or whether spawning also occurs in locales where sampling could not capture eggs or larvae (Crossman et al., 2011).

This study was designed to provide information to evaluate management practices associated with SRFs, to inform knowledge gaps identified in the state of Michigan and LRBOI Lake Sturgeon Stewardship Plans, and to inform management of the Manistee River lake sturgeon population and Great Lakes tributary-spawning populations generally. Given the large and increasing numbers of lake sturgeon stream-side hatcheries in operation or planned across the Great Lakes, this study provides insights into operational goals in terms of demographic contributions by adults and consequences for cohort levels of genetic diversity.

The main objective of this study was to determine egg, larval, or juvenile membership to half- and full-sibling family groups within and among years, and to estimate the number of adults contributing to offspring of different early life stages captured during each of 10 consecutive years (2005–2014) in the Manistee River, Michigan. Additionally, data regarding membership of wild-caught and streamside hatchery-reared eggs and larvae (SS) and wild (W) juveniles (captured in lower river sections later in the year) to half- and full-sib families permitted evaluation of the effectiveness of SRF's to capture the contributions of spawning adults to offspring reared and released. Finally, recommendations have been communicated (e.g., Welsh et al., 2010) regarding goals for levels of genetic diversity in juvenile lake sturgeon released, including the effective population sizes (N_e) for progeny released from SRFs (Crossman et al., 2011). As such, the final objective was to estimate the effective number of breeding adults ($\widehat{N}_b$) that produced larvae collected each year and harmonic mean N_b and N_e over all years as a means of evaluating whether the Manistee River lake sturgeon restoration program was achieving recommended genetic restoration goals. Results have relevance to lake sturgeon restoration associated with stream-side hatcheries in operation and planned across the Great Lakes basin and hatchery operations generally.

Materials and methods

Study area

Naturally deposited eggs and larvae dispersing from spawning sites during or immediately after spawning, and juveniles in the Manistee River were collected using standardized procedures in the spring and summer, respectively for ten consecutive years (2005–2014; Fig. 1). The Manistee River is located in northwestern lower Michigan on the eastern shore of Lake Michigan, and is the

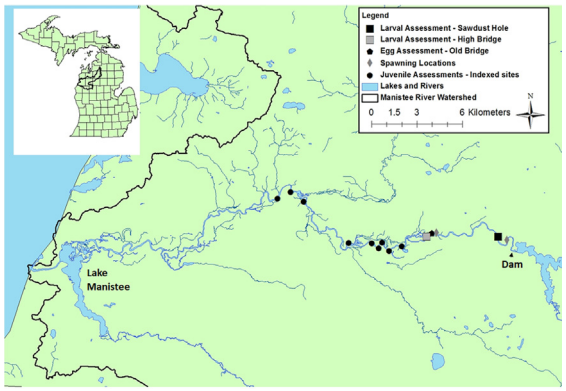

Fig. 1. Map of juvenile lake sturgeon collection sites along the Manistee River in Michigan from 2005 to 2014.

fourth longest river in Michigan. The river runs southwest for 373 km through portions of 11 counties and the Little River Band of Ottawa Indians Reservation before entering Manistee Lake and eventually Lake Michigan. The Manistee River watershed contains sandy, permeable soils throughout a primarily forested landscape draining a 4,610-km^2 area and producing an annual average discharge of 56.7 m^3/s. In 1918, Consumers Power Company built Tippy Dam, a hydroelectric facility 47 km upstream from Manistee Lake. The dam impedes migration of lake sturgeon to possible spawning areas upstream of the dam. Lake sturgeon spawning has been documented at two locations below the dam (Fig. 1). Additional larval drift surveys have been conducted below both presumed spawning sites, but at only one site have drifting lake sturgeon larvae been consistently captured (Chiotti et al., 2008; Mann et al., 2011).

Field methods

Egg assessment – Egg collection mats were used to quantify wild egg deposition, general timing, and location of lake sturgeon spawning. Eggs that were determined to be fertilized and viable were used for rearing within the SRF. Egg collection mats consisted of furnace filters (Flanders Precision-Aire, St. Petersburg, Florida) surrounding 12.3-kg (40 × 20 × 10 cm) cement cinder blocks (Chiotti et al., 2008). The coarse filter texture provided a surface conducive to egg collection and retention. Groupings of two – five mats were set 1.5 m apart with 0.64-cm nylon-coated wire, and a buoy. Egg mats were placed annually in the river by 13th April when the river temperature approached 10.0 °C and were removed by the 12th of June. Spawning was documented when eggs were present on the egg mats, which were checked every Monday, Wednesday, and Friday during the sampling period.

Larval assessment – Wild larval drift assessments were conducted at two locales, Sawdust Hole and Old Bridge (Fig. 1) that were located approximately 2 km and 0.5 km downstream from spawning locations, respectively. Sampling involved use of four index nets and additional three or four nets per site. Nets consisting of a "D-shaped" frame with a 0.75 × 0.57 m opening, 3 m length, a mesh size of 1600 μm, and a detachable cod-end were set where drift larvae congregated and were used to sample the drift (Kempinger, 1988; Auer and Baker, 2002). Sampling for drifting larvae started 15 days or after river temperatures reached 11 °C, which is the earliest projected spawning temperature (Chiotti et al., 2008). Drifting larvae were collected for three consecutive hours per night through the duration of the larval dispersal period (14th of May through as late as the 19th of June) and taken to the SRF. Nets were deployed at 2200 hr and retrieved at 0100 hr. Net cod ends were retrieved every hour.

Juvenile assessment – Visual surveys for wild age-0 lake sturgeon were conducted from July to October on the Manistee River as described by Mann et al. (2011). Two survey strategies were used throughout the program. From 2004 to 2007, 22 2-km sections were randomly sampled while from 2008 to 2014, nine index sites were surveyed at least once per field season (Fig. 1). Collection of wild juvenile lake sturgeon occurred during nighttime visual surveys conducted from a boat using long handled dip nets. Surveys started at dusk and were completed within 3 hr by slowly motoring upstream in a zig-zag fashion across the river to cover all adequate habitats. Fish were located visually by scanning the river bottom using Halogen Q-beam lights (Nite Tracker, 1.5–2 million candlepower [1 candlepower = 0.981 cd]) (Chiotti et al., 2008). Sites where juvenile lake sturgeon were visually observed and captured were marked using a GPS (Garmin: GPS V® or Montana 650 T). Fin clips were taken for genetic analysis.

Genetic analyses

Fin clips from all samples collected were cataloged in a data base at Michigan State University. DNA was extracted from tissue samples using QIAGEN DNeasy® kits (QIAGEN Inc.) according to manufacturers' protocols. DNA was quantified using a NanoDrop® ND-1000 spectrophotometer. All samples were diluted to a concentration of 20 ng/μl for use in PCR reactions.

Individuals were genotyped at 13 disomic and 4 polysomic microsatellite loci as described by Hunter et al. (2020a,b). The disomic microsatellite markers include *LS-68* (May et al., 1997), *Afu68b* (McQuown et al., 2002), *Spl120* (McQuown et al., 2000), *Aox27* (King et al., 2001), *AfuG9, AfuG56, AfuG63, AfuG74, AfuG112, AfuG160, AfuG195, AfuG204* (Welsh et al., 2003), and *Atr113* (Rodzen and May, 2002). The polysomic microsatellite markers include *Atr114, Atr117, Atr100* (Rodzen and May, 2002), and *AciG35* (Börk et al., 2008). PCR reactions were conducted in 25 μl volumes containing 100 ng DNA, 10X PCR Buffer (1 M Tris-HCl, 1 M MgCl2, 1 M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), additional $MgCl_2$ as determined by optimizations, 2 mM of each dNTP, 10 pmol each of forward and reverse primer, and 1 Unit *Taq* polymerase. PCR conditions were as follows: 94 °C for 2 min, followed by 30 cycles (35 cycles for *Atr100*, *Atr113*, *Atr114*; 33 cycles for *Atr117*; 32 cycles for *Aox27* and *AciG35*; 28 cycles for *AfuG204*) of 94 °C for 1 min, 1 min (30 s for *AfuG112*) at primer-specific annealing temperatures (48 °C for *AfuG112*, *AfuG9*, and *AfuG63*; 50 °C for *AfuG74*; 53 °C for *Aox27*; 56 °C for *LS-68*, *Afu68b*, and *Atr113*; 58 °C for *AfuG56*, *AfuG195*, and *AfuG160*; and 62 °C for *AfuG204* and *Spl120*), 72 °C for 1 min, and a final extension for 2.5 min (5 min for *Atr100* and *Atr113*) at 72 °C (excluding *Atr114*, *Atr117*, and *AciG35*). PCR products for disomic loci for all years and polysomic loci for the year 2014 were run on 6% denaturing polyacrylamide gels and genotypes were visualized using a Hitachi FMBIOII scanner. PCR products for polysomic loci for years 2005–2013 were diluted and then genotyped on an ABI 3730xl at the Genomics Core Facility within the Research Technology Support Facility at Michigan State University.

Allele sizes for all samples were determined using commercially available size standards (MapMarkerTM, BioVentures Inc.), and based on several standard samples of known genotype that were run on the same gels. Electropherograms were analyzed and scored using GeneMarker software (Softgenetics, State College, PA). A ladder was created in GeneMarker to score electropherogram peaks according to, and corresponding with, the standardized sizing established by running year 2014 disomic loci on polyacrylamide gels. All genotypes were independently scored by two experienced laboratory personnel and verified again when entered into an elec-

tronic database. Any disputed genotypes were re-gelled and/or re-amplified. As an additional measure of quality control and assurance of accurate scoring, ~10% of all individuals were randomly selected and reanalyzed for each year at all loci. We found no evidence of genotyping error based on quality control checks for years 2005–2009. The error rate for years 2010–2014 was 0.0051.

Data conversion – Increasing the number of microsatellite loci, and thus number of alleles, has been shown to improve the accuracy of full-sibling and parentage assignments in the program COLONY (Wang and Scribner, 2013). To increase the total number of alleles, four polysomic microsatellite markers were added to the original 13 disomic markers, resulting in an increase from 66 to 122 total alleles. In order to run both types of data (disomic and polysomic) in program COLONY, alleles were treated as dominant data and converted into a $1 \times n$ vector as described in Rodzen et al. (2004) and Hunter et al. (2020a) where n is the number of bands or peaks at the locus. If a band was present in an individual's genotype it was coded as a 1, whereas if a band was absent it was coded as a 0. For example, polysomic locus *AciG35* has a total of 11 possible alleles. If an individual has peaks at the 1st, 3rd, 4th, 5th, and 7th alleles, the 1×11 vector would be coded as [1,0,1,1,1,0,1,0,0,0,0]. This conversion was done for all samples with each locus across all 10 years (n = 1129).

Statistical Analyses. - We used program COLONY (version 2.0.5.8, Jones and Wang, 2009) that implemented a full maximum likelihood method described in Wang (2004) and Wang and Santure (2009) to assign wild egg, larval, and juvenile lake sturgeon to full- and half-sibship groups within and across years. This method can also be used to estimate the minimum number of spawning adults without knowledge of the identity of parents, as in a classic parentage study. The maximum likelihood estimator implemented in program COLONY can determine whether an unsampled adult produced one or more juveniles during the same reproductive event (yearly cohort) and in >1 year cohorts produced over several years based on inferred pedigree relationships among sampled offspring. Recent extensions in program COLONY (Wang and Santure, 2009) allow for polyandry and polygyny (females mating with multiple males and males mating with multiple females, respectively), which has been inferred for lake sturgeon based on direct observation (Bruch and Binkowski, 2002) and directly confirmed based on genetic determination of parentage (Duong et al. 2011; Duong et al., 2013).

Samples were analyzed and offspring pedigree relationships were estimated using program COLONY independently for each year as well as all years combined. Visualization of pedigree relationships among offspring and inferred parents were conducted as described in Weise et al. (n.d.) (in revision; https://github.com/weiseell/NbdLamprey/blob/master/Homebrew/pedigree.plot.R; Electronic Supplementary Material (ESM) Methods S1). The input parameters used for all runs across all years included: (a) male and female polygamy without inbreeding and clonality, (b) dioecious and diploid species parameters, (c) one long run using the full likelihood analysis method with high precision, (d) no updating of allele frequencies, and (e) sibship size scaling with no sibship size prior. Because we only have samples from surviving (and released SS) or wild (W) offspring, all parameters involving maternity and paternity were unknown. While the overall genotyping error rate was low (see above), an error rate of 2% was used. Previous simulations (Wang and Scribner, 2013) have found that a larger mistyping rate maintains larger sibships and does not split them due to possible rare alleles.

The estimated number of parents detected ($\widehat{N}_{det}$) in the reconstructed pedigree was summarized among genotyped wild-caught juveniles ($\widehat{N}_{det-Wild}$), wild drifting larvae ($\widehat{N}_{det-Drift}$), wild eggs ($\widehat{N}_{det-Eggs}$) and over all stages ($\widehat{N}_{det-Total}$). We also estimated the effective number of breeding adults ($\widehat{N}_b$) based on the frequencies of full- and half-sib progeny arrays using the method of Wang (2009) and implemented in program COLONY. The general rationale for the N_b estimator is based on the fact that the effective number of breeding adults will vary in proportion to the probability that two random individuals from a year cohort will be siblings that share the same mother, father, or both parents. Effective breeding number of adults will be small if the cohort of juveniles sampled contains a large number of siblings. Because non-disomic loci were included in pedigree analyses, effective numbers of breeding adults (N_b) and effective population size (N_e) could not be calculated using other commonly used methods from multilocus genotype data (e.g., linkage disequilibrium; Waples and Do, 2010). However, based on extensions of effective population size theory for iteroparous species using life history data (Waples et al., 2013), we were able to estimate N_e. Waples et al. (2013) established that the following relationship explained two-thirds of the variation in N_b/N_e across 63 species:

$$N_b/N_e = 0.485 + 0.758 \times \log(AL/\alpha) \quad (1)$$

where AL is adult life span and α is age at sexual maturity. Based on estimates of lake sturgeon adult life span and age at maturity (Bruch et al. 2016) we can use our data for lake sturgeon to estimate the N_b/N_e ratio and our empirical harmonic mean of N_b to estimate N_e.

Based on inferred kin-groups and assignments of offspring to individual adults within and across years, we estimated co-ancestry (Θ), mean ($\bar{k}$) and variance in number of offspring ($V_{\bar{k}}$) (ESM Methods S2) per adult as described in Bartron et al. (2018). Estimates of Θ, were based on the number of offspring dyads that shared a maternal and paternal parent (number full siblings (n_{fs}); $\Theta = 0.25$), the number of dyads that shared a single parent (number half-siblings (n_{hf}); $\Theta = 0.125$), and the number of dyads with no shared parent (number unrelated (n_u); $\Theta = 0$) using Colony output for Best Configuration pedigrees and tabulated using program R 4.0.1 (R Development Team, 2020). Mean co-ancestry, or the probability of two individuals possessing alleles that are identical by descent (Blouin, 2003), was estimated among all offspring pairs from each year and for all offspring genotyped over 10 years and were calculated as described by (Cockerham, 1967; Chesser, 1991; Eq. (1); empirical example for lake sturgeon in Crossman et al., 2011):

$$\Theta = \frac{n_{fs}(0.25) + n_{hs}(0.125) + n_u(0)}{n_t} \quad (2)$$

Estimates of pair-wise inter-individual relatedness (r_{xy}) were estimated between all pairs of individuals collected within and between life stages during each year. Specifically, we estimated pairwise relatedness using the triadic likelihood method (Wang, 2007), implemented in the R package 'related' (Pew et al., 2015). For each year, we calculated mean (±stdev), as well as minimum and maximum relatedness values for the six possible comparisons among wild-caught eggs (mats), larvae (drift), and juveniles (visual surveys).

Simulations were used to assess the power to infer familial relationships based on offspring genotypes alone using COLONY and to determine if genetic-based reconstructed pedigrees affected N_s estimation. For each simulation created by COLONY, a breeding matrix was created to represent the reproductive ecology of lake sturgeon. We used the R library *mater* (Sard et al., 2021) to create breeding matrices (details in https://github.com/nicksard/mater and in ESM Methods S3). The size of each breeding matrix was dependent on the number of successfully breeding adults and sex ratio. Given that both values are unknown in the Manistee River, we randomly drew the number of adults from a uniform distribu-

tion ranging from 10 to 100 parents, reflecting a range of biologically relevant possibilities. The sex ratio (males to females) was also randomly drawn from a uniform distribution ranging from 1.5 to 3 (Duong et al., 2013). The sex ratio was used to determine the number of males and females in the breeding matrix. The mean and variance mating success (i.e., λ) was set to 3 (assuming a Poisson distribution), and the minimum and maximum fecundity values possible were 50,000 and 700,000 (Hunter et al., 2020a). Once the full breeding matrix was created, a large number of offspring (>1,000,000) could potentially be sampled that survive to release from the SRF and genotyped; however, in this study over the period 2005–2014, the number of offspring sampled and genotyped ranged from 21 to 349 annually. Given that offspring sample sizes can affect COLONY's ability to correctly infer pedigree relationships (Hunter et al., 2020a), we evaluated 100 replicate simulations across each of five different offspring sample sizes covering the empirical range of offspring sample sizes genotyped across the 10 year period (n = 25, 50, 100, 200, or 500). To obtain the offspring sample sizes, we randomly subsampled the full breeding matrices using the 'brd.mat.sample' function in *mater* and converted the data to a 'sampled' breeding matrix. The sampled breeding matrix was used to simulate offspring genotypes and create the input file for COLONY. Importantly, the sampled offspring can be used to detect some, but not all, successfully being adults in a given breeding season (N_{det}); however, information within the reconstructed pedigree can be used to estimate the asymptotic number of adults producing offspring each breeding season (N_s; see Sard et al., 2021) which is akin to species accumulation analyses in community ecology. Given that allele frequencies cannot be reliably estimated with polysomic datasets, we simulated allele frequencies from a uniform distribution for 17 loci. However, we expected that inferences will be insensitive to assumed allele frequency distributions (see Hunter et al., 2020a). For all COLONY simulations, polygamous mating was allowed, the allele frequencies were considered unknown and were not updated, no sibship prior was used, and class 1 and 2 error rates were 2% and 1% across all loci, respectively. Finally, the full-likelihood method was used to reconstruct pedigrees during a "long" run with "high precision". Best Configuration pedigrees for each simulation were analyzed by visualizing a prediction error plot that compared the asymptotic estimated number of successfully breeding adults (N_s) to estimates (Sard et al., 2021). In addition, an assignment matrix was created to visualize the proportion of full-sibling, half-sibling, and unrelated dyads that were correctly classified and misclassified (Hunter et al., 2020a).

Results

Distribution of samples genotyped and measures of genetic diversity

Over 10 consecutive years (2005–2014), 1129 samples were obtained and genotyped from lake sturgeon sampled as naturally produced eggs (n = 291) and dispersing larvae (n = 534) immediately downstream from the suspected spawning site, and as juveniles (n = 304) later in the summer further downstream (ESM Table S1; Table 1). The first subset of samples that were genotyped included fish that were collected from naturally produced eggs and larval dispersal collections. These individuals were brought into the SRF and survived to the end of the summer and were released (streamside reared, SS, n = 839). A second subset of juvenile fish from the same year cohort were captured throughout the river later in the summer (wild caught; W; n = 291; numbers by year provided in ESM Table S1). The number of alleles observed in this population across the 17 loci ranged from 2 to 20 (ESM Table S2). The average number of alleles per locus differed slightly across years (range of means 5.5 to 6.8 (SD 0.38), ESM Table S1). The total number of alleles across all 17 loci ranged from 94 to 116 across the 10 years. The proportion of SS juveniles representing the total number genotyped varied from 0 to 0.97 over the 10 years, with year 2012 having no SS juvenile samples genotyped, while the proportion of W juveniles representing the total number genotyped ranged from 0.03 to 1.0 (ESM Table S1).

Simulation analyses

Simulated data can be used draw conclusions from actual reconstructed pedigrees. Simulation analyses indicate that a non-zero proportion of full-siblings may be misclassified as half-siblings (Fig. 2), leading to an upwards bias in the number of spawning adults inferred. Splitting full-sibling samplings into half-siblings occurred less frequently at higher offspring sample sizes. As offspring sample size increases, the proportion of half-siblings correctly inferred plateaus; the known half-siblings were often inferred as being unrelated. Results highlight that the limitations in the genetic information observed in the system can be partially overcome by using larger sample sizes, which likely enables COLONY to capitalize on the genetic information among related larger groups of siblings when trying to maximize the likelihood of the pedigree as a whole. Finally, the vast majority of known unrelated dyads were correctly inferred.

Due to the splitting of some known full-sibling families into half-siblings and some known half-sibling dyads being inferred as unrelated, COLONY can over-estimate the number of adults that produced the offspring sampled (N_{det}), especially when few adults successfully bred (ESM Fig. S1). As offspring family sizes decreased, due to more parents successfully breeding, COLONY can still inflate $\widehat{N}_{det}$ but to a lesser extent. That is, given uncertainties about the number of breeding adults in the system and the sex ratio during the spawning season, estimates are likely 0.75 to 1.81 times that of $\widehat{N}_{det}$.

When no errors exist in the pedigrees, the asymptotic estimates of the number of adults contributing to offspring in the pedigree ($\widehat{N}_s$) are unbiased, and precision increases with offspring sample sizes (Fig. 3). When errors are present in the pedigree relationships, estimated N_s can still be inferred based on simulated data. For instance, in 2012 a total of 26 offspring were genotyped and used a reconstructed pedigree. Based on simulated data (Fig. 3), $\widehat{N}_s$ likely ranged 20–50 given the estimate ($\widehat{N}_s$ = 23). Similarly, $\widehat{N}_s$ = 42 in 2005, which was likely relatively unbiased given the simulation (Fig. 3). Finally, while all simulations resulted in biased low N_s estimates, $\widehat{N}_s$ was consistently positively correlated with N_s. Thus, even when consistent bias is present, estimates provide useful information about N_s.

Analyses of empirical data

Based on pedigree assignment of offspring to half- and full-sib groups, we estimated that the total number of adults that contributed to the juveniles assayed ($\widehat{N}_{det}$) varied over the 10 years from 21 to 79 annually (Table 1). The number of half-sib families (kin groups in Table 2, annually in Fig. 4, and over all years in Fig. 5) varied from 10 to 29 and the number of full-sib families varied from 22 to 250 (Fig. 4). The number of adults and families varied among years in accordance with the number of juveniles sampled, as did the effective number of breeding adults which ranged from 17 to 43 ($\widehat{N}_s$, Table 1). Though the number of juveniles collected varied, the number of inferred adults ($\widehat{N}_{det}$) and asymptotic estimates of inferred reproductively contributing adults ($\widehat{N}_s$)

Table 1
Summary lake sturgeon sampled and genotyped during each life stage (eggs and drift larvae reared in the stream-side hatchery (SRF) and wild juveniles (W)) and year. Estimates are provided of the total number of adults that were inferred to have produced offspring that year (2005–2014) (N_{det}) and all years combined. Asymptotic estimates of the number of adults contributing to all offspring collected by year and over all years (N_s) (Sard et al. in press) are also provided.

	Number of Juveniles Genotyped				$\widehat{N}_s$	$\widehat{N}_{det\text{-}SRFegg}$	$\widehat{N}_{det\text{-}SRFdrift}$	$\widehat{N}_{det\text{-}Wild}$	$\widehat{N}_{det\text{-}Total}$	$\widehat{N}_S$ Est	Mean $\widehat{k}$[a]	$\widehat{V}_k$[a]	Θ[a]
	SRF_{egg}	SRF_{drift}	Wild	Total									
2005	0	51	47	98	29 (19–51)	0	35	36	41	41.7 (41–48)	4.78	14.68	0.017
2006	0	94	15	109	37 (24–59)	0	45	22	47	47.4 (47–52)	4.64	10.63	0.014
2007	0	29	39	68	30 (18–53)	0	29	37	40	44 (41–59)	3.4	7.43	0.017
2008	15	31	31	77	33 (21–55)	10	28	29	39	40 (39–47)	3.95	6.52	0.015
2009	6	27	1	34	24 (14–44)	8	22	2a	24	25.8 (24–36)	2.83	2.84	0.021
2010	0	75	71	146	34 (22–57)	0	46	44	52	53.8 (52–64)	5.62	22.24	0.015
2011	0	4	17	21	25 (14–50)	0	6	18	19	19.8 (19–26)	2.21	0.95	0.02
2012	0	0	26	26	17 (9–35)	0	0	21	21	23.2 (21–34)	2.48	3.86	0.03
2013	179	181	34	394	43 (30–66)	41	53	32	70	72 (70–92)	11.26	90.16	0.012
2014	91	42	23	156	29 (18–48)	27	27	25	47	53.1 (48–78)	6.64	34.71	0.017
All Years	291	534	304	1129	29[b]	88	225	206	293	326.8 (310–360)	7.71	117.32	0.005

[a] Mean k, V_k, and Θ are the mean and variance in reproductive success of adults inferred from pedigree data each year and over all years and Θ is mean offspring co-ancestry.
[b] Harmonic mean effective number of breeding adults over 10 years.

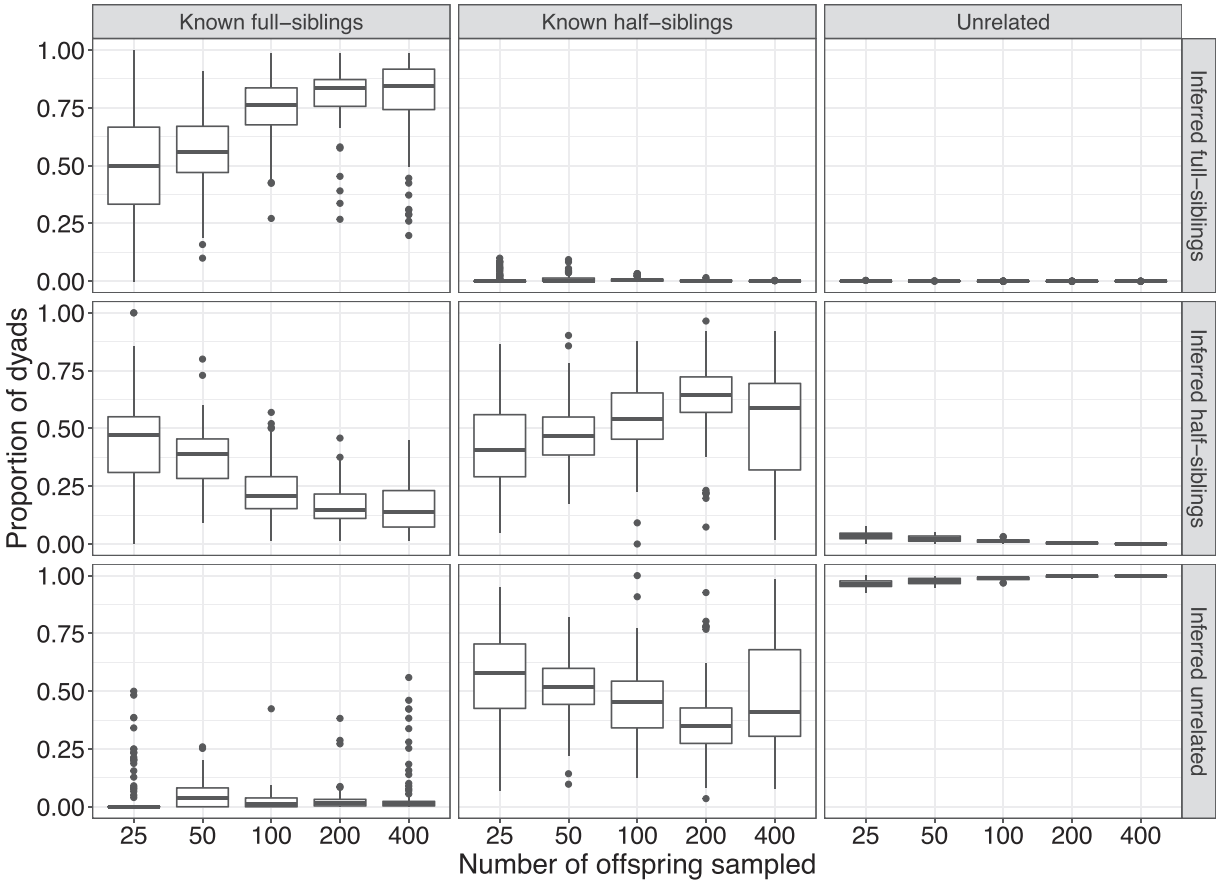

Fig. 2. Assignment matrix showing boxplots depicting the proportion of known full-siblings, half-siblings, and unrelated dyads that were inferred as full-siblings, half-siblings, and unrelated dyads across five different offspring sample sizes. Column totals sum to one. Each boxplot represents 100 simulations when 25, 50, 100, 200, or 400 offspring were sampled when the number of adults that produced the offspring were randomly drawn from a uniform distribution ranging from 10 to 100. In addition, the sex ratio among the adults was randomly drawn from a uniform distribution ranging from 1.5 to 3. Comparisons along the diagonal from the top left to the bottom right panel identify correct inferences made by COLONY, whereas off-diagonal comparisons identify incorrect inferences.

varied similarly across years (Table 1), the estimates of the effective number of breeding adults ($\widehat{N}_s$) varied less across years (Table 1).

Based on inferred full- and half-sibling kin group size we estimated that mean co-ancestry of offspring produced per year ($\boldsymbol{\Theta}$) ranged from 0.013 to 0.030. Mean ($\bar{k}$) and variance ($V_{\bar{k}}$) in adult reproductive success ranged from 2.1 to 10.0 and 1.70 to 75.4, respectively across years (Table 1). We note that there are two adults that contribute to each offspring, and thus the mean and variance in offspring produced was not estimated simply as that total number of progeny genotyped divided by $\widehat{N}_{det-Total}$. Similar to N_s and N_{det}, co-ancestry increased, and the mean and variance in reproductive success decreased as a function of the number of offspring sampled per year (Table 1). Over all 10 years mean co-

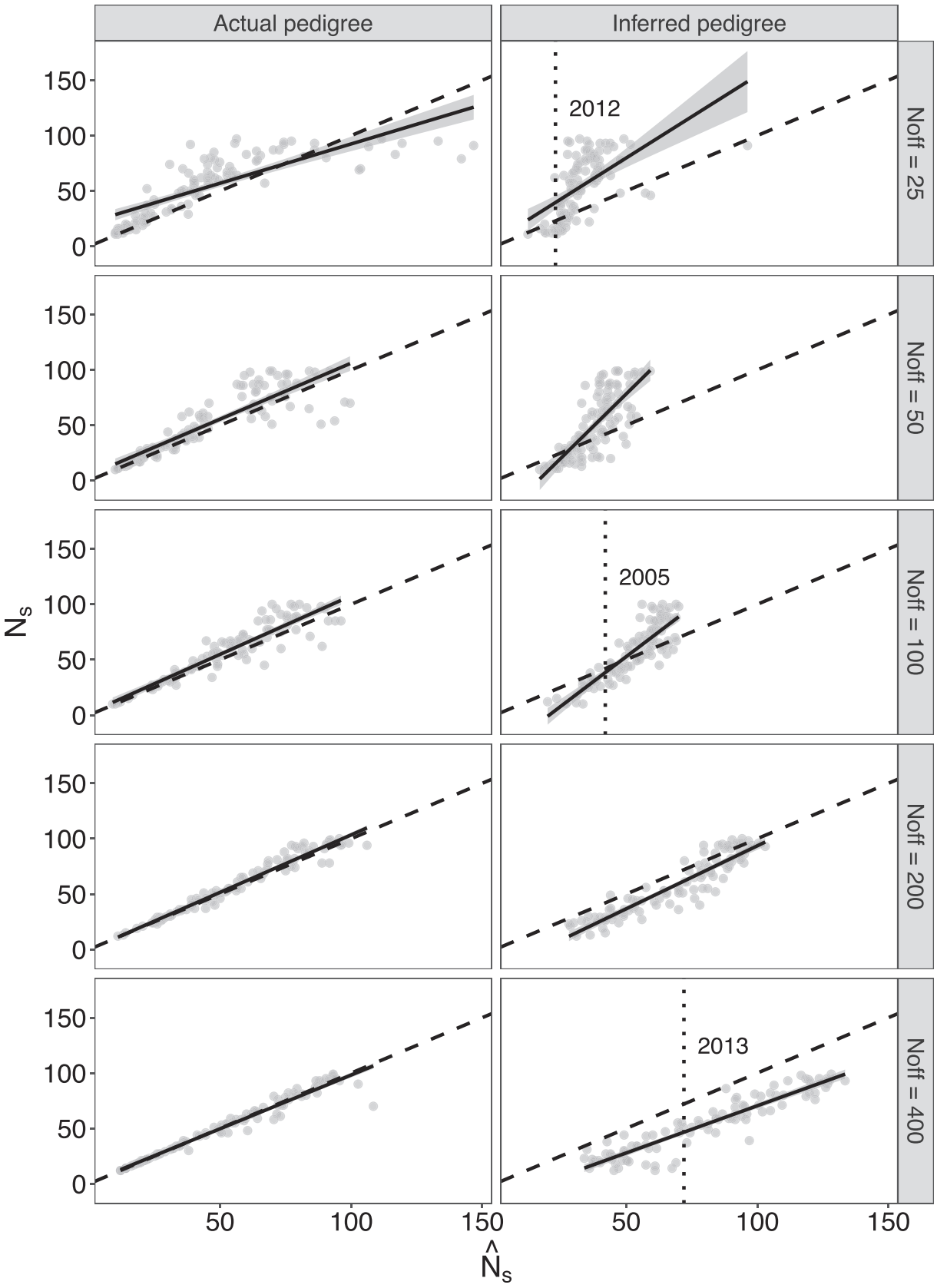

Fig. 3. Prediction error plots from simulated data depicting the relationship between estimated ($\widehat{N}_s$) and true (N_s) number of adults that successfully bred in a given spawning season when the actual (i.e., error-free) or inferred (i.e., reconstructed based on genotypes) pedigrees were used to estimate N_s. Subplots differentiate among offspring sample sizes (Noff) simulated. The relationships are visualized with linear regression, with 95% grey confidence intervals. The dashed line depicts a 1:1 relationship between $\widehat{N}_s$ and N_s. Vertical dotted lines depict $\widehat{N}_s$ for 2005, 2012, and 2013 and are placed in subplots that are similar to the number of offspring sampled in each year.

Table 2
Numbers of full- and half-sibling families with representation from lake sturgeon offspring collected from either or both wild-caught (W) or stream-side reared (SS) collections.

Year Juveniles Collected	Stream-side (SS) or wild (W) origin for members of half-sibling families			Stream-side (SS) or wild (W) origin for members of full-sibling families		
	SS & W	SS	W	SS & W	SS	W
2005	15	3	4	8	35	27
2006	8	20	1	6	65	9
2007	9	1	7	6	19	30
2008	7	7	4	5	28	15
2009	0	15	1	0	28	1
2010	14	7	3	14	53	44
2011	2	0	8	1	2	14
2012	0	0	11	0	0	22
2013	9	12	0	8	228	14
2014	5	16	2	5	76	14
All years	84	72	16	79	437	148

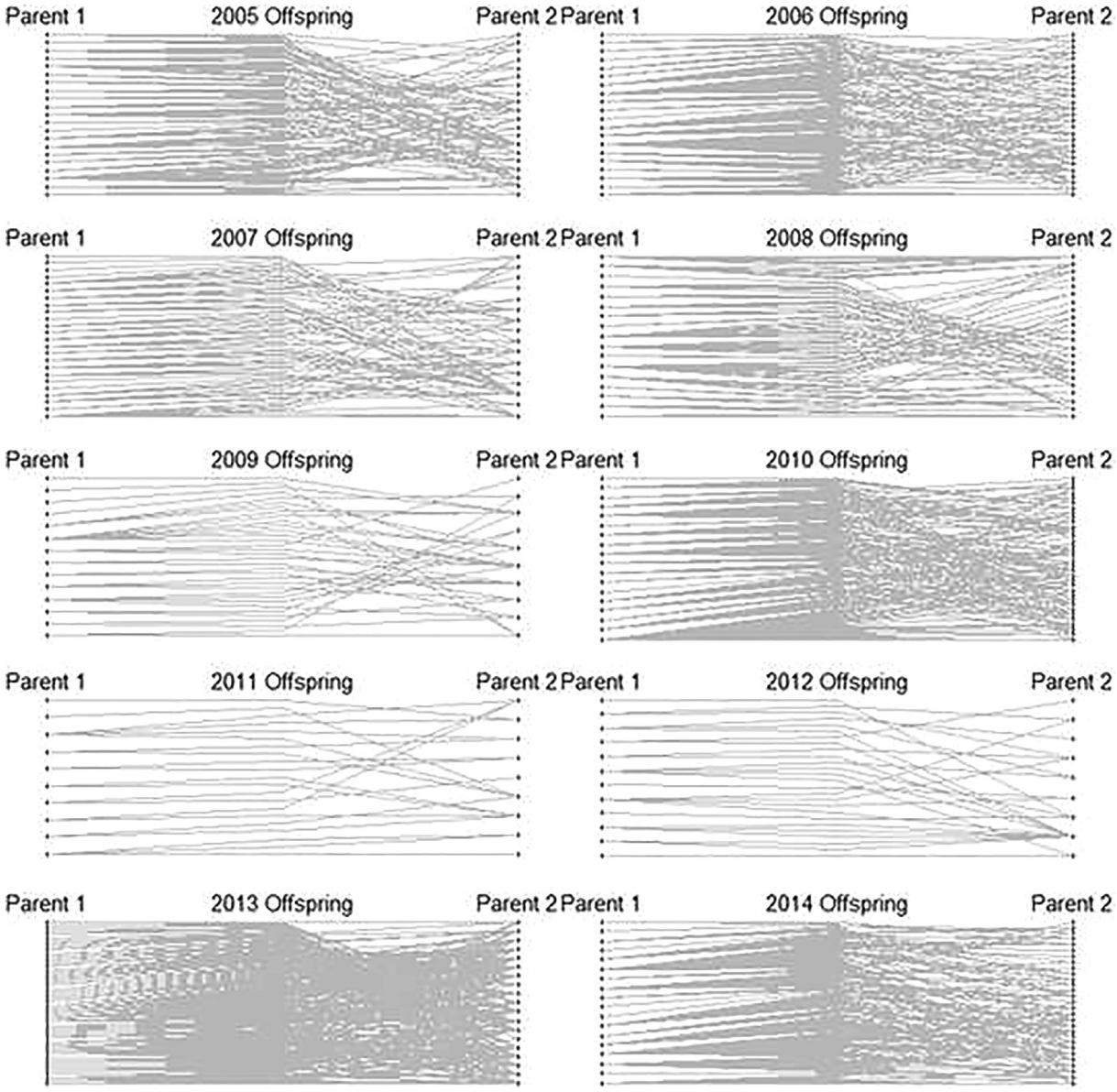

Fig. 4. Visualization of reconstructed lake sturgeon pedigrees summarizing inferred full- and half-sibling kin groups for offspring genotyped by year of collection. The center represents genotyped individuals, and dots represent inferred parents. Lines connect each reconstructed parent to each genotyped offspring in the pedigree.

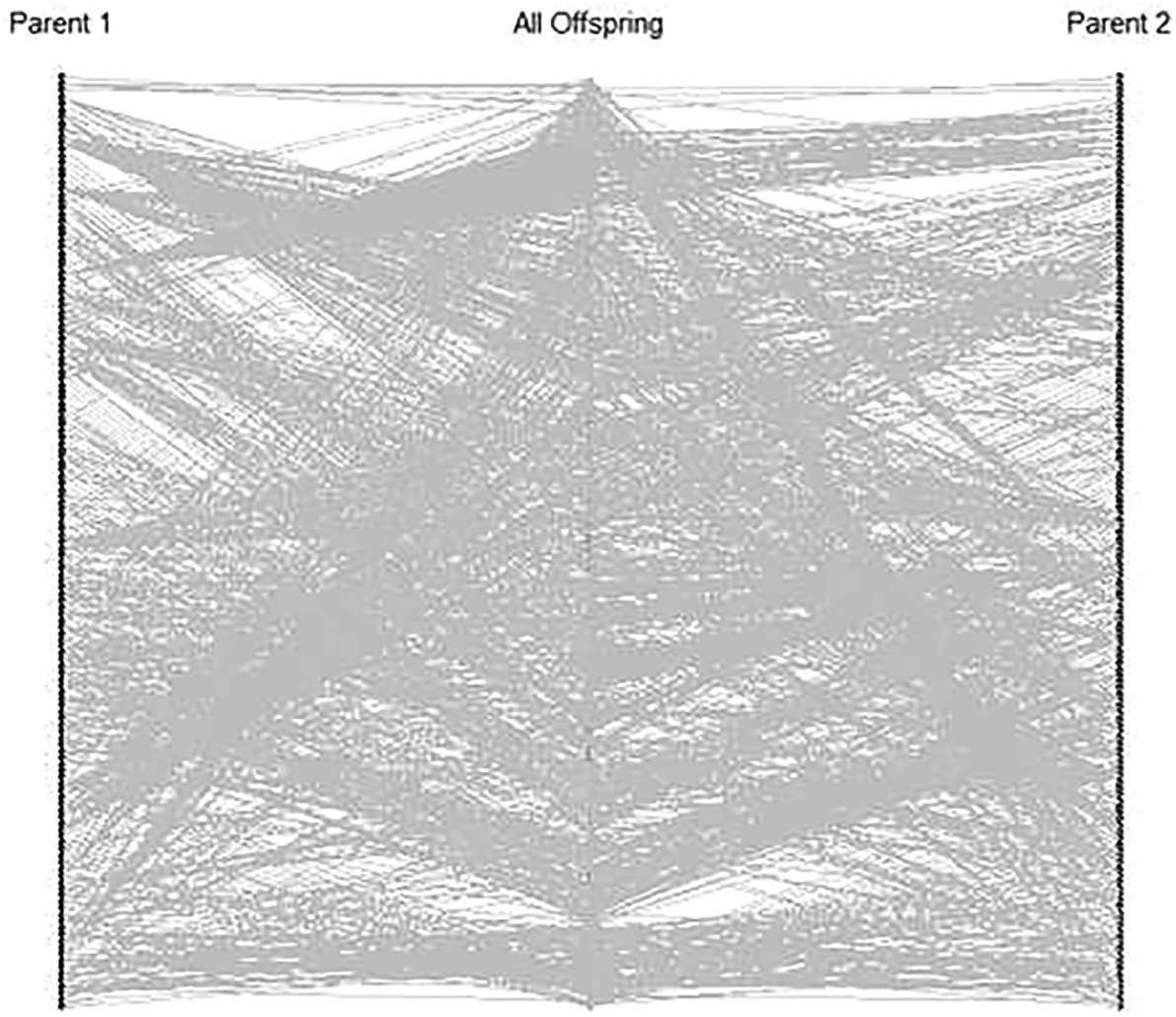

Fig. 5. Visualization of reconstructed lake sturgeon pedigrees summarizing inferred full- and half-sibling kin groups for all offspring genotyped over all ten years of collection. The center represents genotyped individuals, and dots represent inferred parents. Lines connect each reconstructed parent to each genotyped offspring in the pedigree.

ancestry was estimated to be ~0.005, while population estimates of adults' mean and variance in reproductive success were ~7.7 and ~117.3, respectively.

Comparatively low inter-annual variation in demographic parameters estimated contrast with the large inter-annual variation in total juveniles sampled and the number of half- and full-sib families represented in the juveniles sampled (Table 2). Contributions of eggs and/or dispersing larvae to SS individuals genotyped also varied considerably across years. High inter-annual variability in relative contributions of drift and eggs confound interpretation of relative numbers of adults contributing to each sample type ($N_{det\text{-}egg}$ vs $N_{det\text{-}drift}$, respectively). However, generally, greater genetic diversity indicated as numbers of adults represented in progeny ($N_{det\text{-}total}$) was contributed by individuals collected from the larval drift relative to eggs as has been described previously (Crossman et al., 2011).

Over ten years and 1129 offspring genotyped, we estimated that the total number of spawning adults in the population ($N_{det\text{-}total}$) was ~293. Asymptotic estimates of the total number of contributing adults based on pedigree accumulation analysis ($\widehat{N}_s$; Sard et al., 2021) was estimated to be slightly higher (~326). Estimated harmonic mean N_b was considerably less than expected (29.5) (i.e., observed $N_{b\text{-}total}$ is far less than the product of harmonic N_b multiplied by the number of year classes (n = 10) ($\ll gN_b$; as per Waples, 2002, Waples et al. 2013 for semelparous salmon and as advocated for iteroparous lake sturgeon Great Lakes hatchery programs by Welsh et al., 2010). The average per-year effective to total adult size (N_b/N_s) over ten years was estimated to be 0.768 (range 0.546–1.26). However, the all-years estimate of $N_b/N_{s\text{-}total}$ was considerably lower (0.316). N_e calculated using the Waples et al. (2013) estimator for iteroparous species was 29.

Data also allowed determination of distributions of the number of adults contributing offspring in one or more years (Fig. 6). Data show that 48% of inferred adults contributed offspring in collections in ≥2 years. The lower all years estimate of N_b/N_{det} or N_b/N_s relative to average per year N_b/N_{det} or N_b/N_s appears to be related to repeat spawning, which also contributed to higher V_k when some inferred adults contributed offspring in >1 year while other inferred adults contributed offspring in only one year.

A large proportion of half-sib groups contained both SS (wild eggs and larvae) and W juveniles (SS&W; Table 2) indicating that sampling of naturally produced eggs and larvae dispersing from presumed spawning areas captured the majority of spawner contributions to larvae surviving to this stage. The proportion estimated is likely low given the size of inferred family groups during 2006 and 2009 and 2011 and 2012 by the preponderance of SS fish or W fish in the combined sample, respectively. In inferred family groups of comparatively larger size (N ~ 4 or

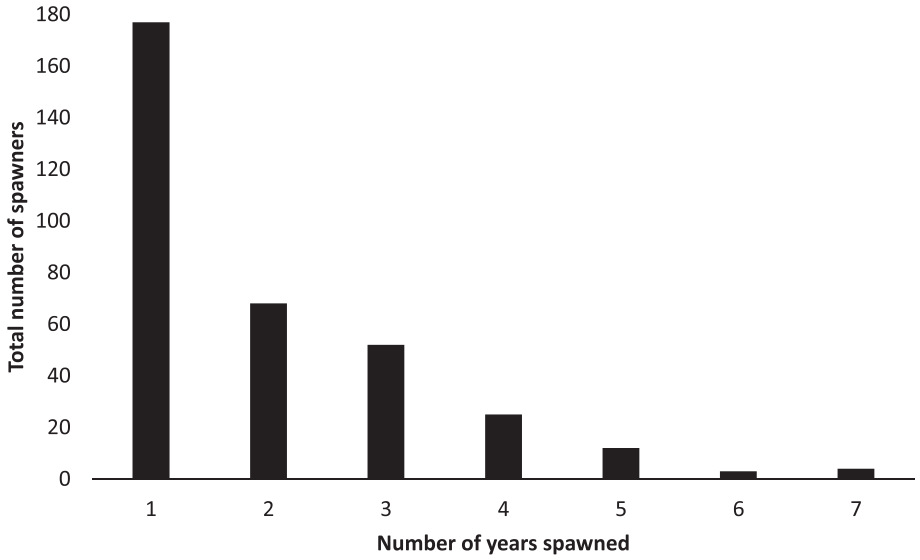

Fig. 6. Estimated number of years that adults spawned in the Manistee River based on adults contributions to kin-groups in two or more years.

greater) both SS and W juveniles were represented in the majority of the inferred kin groups.

Estimates of pair-wise mean (±stdev) inter-individual relatedness (r_{xy}) were made between eggs, between larvae, between juveniles and between pairs from different life stages (e.g., eggs-larvae, eggs-juvenile, larvae-juveniles; ESM Table S3). Data indicate that generally r_{xy} for eggs is higher than larvae and juveniles, but not always so. Secondly, there is considerable variation in r_{xy} year to year within a life stage. Third, in some years (e.g., 2009), r_{xy} between individuals of different life stages can be high or higher than estimates among individuals of a single life stage, suggesting collections of individuals from all life stages (eggs, larvae, and juvenile) are sampling a higher proportion of offspring from the same adults during some years.

Discussion

Management plans to recover numerically depressed populations of lake sturgeon, and plans guiding repatriation of extirpated populations require projections of future reproductive potential and recruitment, which is in part conditional on environmental and demographic uncertainty (Boyce, 1992) and genetic diversity (Hutchings and Reynolds, 2004), and the interaction between the two (Lande, 1988). Managers need to know whether the size of extant adult spawning populations is sufficient to allow natural recruitment to achieve abundance and diversity goals annually and over time. Even in situations such as described for the Manistee River stream-side rearing program, where biologists attempt to collect individuals in the wild during all life stages to populate stream-side facilities, numbers of individuals raised to the time of release can be highly variable, which likely indicates similar inter-annual variability in the number of contributing adults. This information is particularly important for low abundance species (Kuussaari et al., 1998; Liermann and Hilborn, 2001).

Work conducted here and elsewhere (e.g., Hessenauer et al., 2012; Hunter et al., 2020a; Jay et al., 2014), has shown that fertilized eggs and larvae can be genotyped and even in the absence of information on adults (and without sampled adult genotypes), pedigree analysis can be used to ascertain genealogical relationships among offspring. Researchers often know where and when adults breed and can deploy devices that collect eggs and/or larvae in order to document evidence of reproduction (Caroffino et al., 2010; Chiotti et al., 2008; Smith and Baker, 2005). Based on pedigree information, the number of males and females contributing to offspring can be estimated, and minimum estimates of the number of adult spawners that produced offspring sampled each year can be made. By combining offspring sampled over multiple years, we have shown that multi-year pedigrees can be used to identify whether the same individuals contributed to progeny in >1 year (Fig. 6). Subsequently, estimates of total adult population size ($\widehat{N}_{det}$) and the effective number of breeding adults ($\widehat{N}_s$) can be estimated. We also extended analyses using the recently described 'pedigree accumulation' estimators ($\widehat{N}_s$, Sard et al., 2021) to quantify asymptotic numbers of adults annually and over the ten year sampling period. Estimates of the number of adults contributing to offspring sampled annually, and when collected over several years allowed plausible minimum estimates of adult spawning abundance.

Enumeration of the number of adults contributing offspring collected

Throughout most of the lake sturgeon range, the majority of remnant populations are numerically depressed and offspring survival to the point of sampling is typically low (Holey et al., 2000). Studies have consistently shown in lake sturgeon (e.g., Crossman et al. 2011) and other fish species of conservation concern generally (Osborne et al. 2020) that collections of individuals of early life stages in the wild can result in higher levels of genetic diversity than can be attained using direct gamete takes from spawning adults.

Based on egg, larval or juvenile membership to half- and full-sibling family groups we estimated the number of adults contributing to samples captured during each of 10 consecutive years. The number of spawning adults contributing samples annually were low ($\widehat{N}_{det-total}$ range 19–70) indicating low spawner abundance annually (Table 1). The range is comparable to earlier work on the Manistee River population (Lallaman et al., 2008; Peterson et al., 2002) who estimated spawner abundance at 12–36 and ~50, respectively, based on total numbers of individuals captured and body size (and inferentially aged). We acknowledge that our annual estimates ($\widehat{N}_{det}$) were correlated with the sample size of individuals genotyped; however, asymptotic estimates ($\widehat{N}_s$) based

on pedigree accumulation analyses (Sard et al., 2021) are likely more robust.

Estimates of N_s indicate that the extant population size for the Manistee River is low. However, additional considerations about sampling must be considered. First, the number of adults contributing to larvae is perhaps an over-estimate of total adult population size because a non-zero proportion of full-siblings are misclassified as half siblings and half-siblings maybe misclassified as unrelated (Fig. 2). Previous studies have found that the number of adults contributing to larvae could also be over-estimated, because the full likelihood pedigree estimate can include single offspring that are assigned to a single adult pair (e.g., Herbinger et al., 2012).

Alternatively, not all adults produce viable progeny to be sampled genetically which means that true spawning adult abundance is likely higher than estimated. In addition, eggs and drifting larvae sampled for genotyping in this study only represent the total number of samples that were brought into the hatchery and survived the season in the stream-side hatchery until the time of release. Thus, population bottlenecks could have occurred in two stages (Christie et al., 2012). First, sample sizes were reduced from the total number collected due to mortality following capture. Secondly, hatchery-based selection could have led to a bias in family-specific survival (Ford, 2002; Christie et al., 2012), therefore reducing $N_{det\text{-}total}$ and N_s annually. Downward bias introduced by sampling numbers are indicated by the association between annual $N_{det\text{-}total}$ and the total number of offspring genotyped. While single year estimates may be affected downwardly by sampling and upwardly by methodological constraints, estimates of $N_{det\text{-}Total}$ over 10 years (~293) is likely robust. Use of the Sard et al. (2021) 'pedigree accumulation' estimator of asymptotic N_s produced similar though moderately higher estimates ($\widehat{N}_s \sim 326$, Table 1).

An additional managerial concern that our sampling methodology was designed to address, was that samples of any single age or collection locale may not be sampling adults from the entire spawning run. Specifically, we were concerned that eggs and drifting larval sampled from a restricted few areas of the large and complex river system may not reflect contributions of all or the majority of spawning adults. To the contrary, we noted that a large number of adults were represented each year in samples from multiple stages and methods (i.e., eggs and larvae $N_{det\text{-}Drift}$ or $N_{det\text{-}Egg}$) and wild age-0 YOY ($N_{det\text{-}Wild}$; Table 2). Further, 48% of inferred adults were estimated to have contributed offspring in >1 year over 10 years. Sampling over 10 consecutive years should provide opportunity for a large proportion of adults to contribute at least one offspring recruited to one gear type and life stage. Further, sampling methods were standardized across years. For all LRBOI capture methods there have been standardized sampling protocols in place. Inter-annual variation in levels of mortality after samples of eggs and drift larvae were brought to the stream-side hatchery could be a factor associated with inter-annual variation.

Consistency of results for $\widehat{N}_s$ and $\widehat{N}_b$ across runs for each year (Table 1) suggests that adult estimates based on inferred pedigrees are robust. We used a full likelihood estimator as recommended by Wang (2004), rather than estimating sib membership on an individual by individual basis. Further, Wang and Scribner (2013) demonstrated that joint use of disomic and polysomic loci for polyploid species like lake sturgeon can increase the number of alleles and loci, both of which were associated with pedigree and parentage estimator accuracy (Wang and Scribner, 2013; Hunter et al., 2020a).

Complexities associated with enumeration of spawning adult population abundance are exaggerated for low abundance adfluvial species such as lake sturgeon because most individuals are present in spawning areas only briefly in large river systems and are not easily observed or handled during the brief reproductive period. Further, inter-spawning intervals vary considerably, particularly for females (mean 2.3 years for males and 3.7 years for females; Forsythe et al., 2012b, see number of reproductive events described in Fig. 6), and thus assessments necessitate sampling be conducted over many years (Pledger et al., 2013).

Results reported here provide managers with information on annual diversity of stocked fish not previously available to assess management goals. Annual collections of eggs and drifting larvae and wild juveniles captured in lower sections of the Manistee River have been low over the ten year assessment period (2005–2014), annually 21–394 individuals (Table 1). Without estimates of the number of contributing adults ($\widehat{N}_s$ and $\widehat{N}_b$), interpretation of low annual numbers and inter-annual variation would be problematic. Our estimates of the number of adults contributing to SS and W juveniles is relatively low and varied by a factor of 3 over 10 consecutive years of sample collection (Table 2). Importantly, the effective number of adult breeders contributing to larvae remained relatively constant across years, indicating that larval numbers captured are not necessarily predictive of the genetic diversity represented in SS juveniles released or wild juveniles capture well after the reproductive season.

Given that lake sturgeon are broadcast spawners (Bruch and Benkowski, 2002) and efficiency of fertilization can be strongly dependent on depensatory dynamics including probability of fertility (Liermann and Hilborn, 2001; Dammerman et al., 2019 for lake sturgeon), low spawner abundance likely will negatively affect recovery potential (Myers et al., 1995). Estimated N_s is below Michigan population abundance goals (Hayes and Caroffino, 2012).

What do N_s and N_b estimates mean for future population demography and diversity?

We estimated the number of adults consistent with the inferred pedigree ($\widehat{N}_{det}$) was 293 and $\widehat{N}_s$ over 10 years was ~326. The harmonic mean effective number of breeding adults ($\widehat{N}_b$) was estimated to be 29 over the 10 yr sampling period. This number is considerably lower than estimated for the Black River spawning population of lake sturgeon (Duong et al., 2013), likely due to the high reproductive skew over 10 years (V_k = 117.3; Table 1). Lake sturgeon population recovery will be dictated by relative contributions to annual recruitment by wild and hatchery reared offspring, both of which appear in the kin groups identified (Table 2). Evolutionary consequences of population size (e.g., loss of alleles and heterozygosity, changes in allele frequency and accrual of population levels of co-ancestry and inbreeding) are attributed to the effective number of breeding adults (N_b). Effective population size is difficult to estimate for long-lived iteroparous species like lake sturgeon (Waples et al., 2011; Waples et al. 2013; Waples et al., 2014). Generally, $N_e \sim 500$ in a closed population is believed to be sufficient to balance loss of diversity due to genetic drift with gains due to mutation (Frankham, 1995). Estimates of N_e are typically larger than N_b for a single year but less than gN_b, where g is the number of years per generation for semelparous species (Waples, 2002).

A number of factors may affect estimates of N_b and N_e. For example, our estimated N_e (~29) may be higher and mean N_b may be lower than estimated due to 'skipped breeding' (Waples and Antao, 2014). All females and most males do not spawn each year (Forsythe et al., 2012b), which will depress spawning numbers within a year but decrease variance in reproductive success across years. Components affecting population vital rates including age-specific fecundity (b_x) and age-specific variance in reproductive success (V_x) are also important to estimation of N_e and N_b,

but are unknown for this or most populations (see Waples et al., 2018 for worked examples). Separate age-specific estimators for males and females are important and likely vary. Ratios of the variance to the mean reproductive success in one time period of individuals of age $\times$ ($\phi_x = V_x/b_x$) is often assumed to be 1.0 (Poisson distributed) in absence of other information, which is not the case in this population (Table 1).

Other factors contributing to N_s and N_b

Data collected independently by Homola et al. (2012) revealed that Lake Michigan tributary populations of lake sturgeon are not closed to immigration and emigration. Using multi-locus genotypes and individual assignment testing, these authors estimated that ~8.5% of adults captured during the spawning period in the Manistee River were strays from other natal rivers. Therefore, continual (though low) levels of infusion of adults suggests that Lake Michigan tributary populations of lake sturgeon may behave as a metapopulation (Hanski and Gilpin, 1997). Gilpin (1991) has shown that the metapopulation levels of diversity are governed by effective metapopulation size, which is related to the average number of local populations extant in the metapopulation and the rate of gene flow between local populations. Gene flow from other streams can help to reduce levels of accrual of coancestry and inbreeding relative to a completely 'closed' population. However, implications of previously documented gene flow are that the adult breeding ($N_{det\text{-}Total}$) and effective adult breeding size (N_b) of the Manistee River population is based in part on influx from other Lake Michigan basin tributary populations. The number and effective number of Manistee River adults is likely to be smaller than estimated herein based on reproductive contributions from non-resident adults (Palstra and Ruzzante, 2008).

Future applications – are genetic estimates of N_s and N_b robust surrogate measures of adult abundance?

Estimated numbers of adults that recruited offspring to sampling gear in the Manistee River varied 3-fold over 10 years (N_s point estimates 19–70; Table 1). Estimates of adults contributing to genotyped offspring that survived to release were correlated with the number of offspring sampled. Data on total number of eggs and larvae observed were not available. However, data on lake sturgeon recruitment from other Michigan drainages indicate that mortality during early life stages is high (Duong et al., 2011b). While it is doubtful that managers would be able to use juvenile numbers as a surrogate to infer adult spawner abundance, the range in inter-annual variation in $\widehat{N}_s$ is likely attributed to physical and biotic factors associated with mortality during egg and larval stages. Duong et al. (2013) studying lake sturgeon recruitment on the Black River in Michigan found there was no relationship between spawner abundance and larvae captured in D-frame drift nets during the larval dispersal period. Adult spawner abundance varied by a factor of 2 over 9 years while larvae captured varied by 40 times over the same period. Duong et al. (2013) has shown that estimates of the effective number of breeding adults (N_b) in the Black River, MI varied much less among years (range of inbreeding effective breeding number 47–167 based on measures of linkage disequilibrium; Waples and Do, 2010) even though estimates of total larval recruitment to the period of capture in drift nets varied by 40 times (475–17,500) over years (2001–2010).

Producing plausible statistical models to inform managers of the number of 'successful' adult spawning numbers as estimated herein to measures of total adult abundance that match the biological processes can be difficult (Jackson et al., 2001). Clearly, estimates of the probability of viable offspring each year per adult can be useful for population demographic analyses, and to characterize aspects of mating behavior or estimation of adult reproductive success. However, there are sources of variability (Duong et al., 2011, Duong et al., 2013) which are difficult to quantify for wild populations inhabiting expansive habitats that are difficult to survey exhaustively. In this regards, genetic data provide a powerful tool to inform managers of important population parameters.

Conclusions

Estimates of the effective number of lake sturgeon breeding adults in the Manistee River over a ten year period ranged from 48% to >100% (mean 0.768) of estimated successful adult spawner abundance ($\widehat{N}_s$) during each year ($\widehat{N}_b$ range 17–43; Table 1). Harmonic mean adult effective breeding number over all 10 years was ~29, as was our estimated N_e based on Waples et al. (2013) life history estimator developed for iteroparious species.

An effective population size per year of 20 (N_e = 20) has been recommended for donor lake sturgeon populations contributing to stream-side hatcheries (Welsh et al., 2010). This yearly target was suggested to be sufficient to ensure that the overall minimum number (N_e = 500) would be met within a 25-year period (Welsh et al. 2010). Welsh et al. (2010) assumed a relationship $N_e \sim gN_b$ established for semelparous species (Waples 2002; see discussion above). The logic is flawed, as the semelparous formulation for N_e assumes completely new sets of adults each of 'g' generations (not years). Further, we provide evidence here of discrepancies between N_e estimated for iteroparous species (Waples et al. 2013) based on species life history parameters and our estimated harmonic mean N_b (~29) and what the expectation would be for cumulative N_b over the comparable period (i.e., harmonic annual mean N_b of 29 times 10 yrs (g in Welsh et al.. 2010 formulation based on Waples 2002, or $N_e \sim 290$). Further, Welsh et al. (2010) formulations only considered possible deviations in spawning sex ratio as the sole evolutionary factor decreasing N_b and N_b/N_e. Here we documented non-Poisson estimates of reproductive success (V_k 10 times mean k over the ten year period), which substantially reduced N_b, as has been documented in other lake sturgeon programs where long-term monitoring data has been available (e.g., Duong et al., 2013). Additionally, a substantial number of adults were found to contribute to offspring during multiple years (Fig. 6), which contributes to lower decadal estimates of effective breeding number by accentuating multi-annual differences in adult reproductive success, and thus increasing V_k.

Several opportunities may be explored to increase representation of larger numbers of adults with each sampling year. For example, sampling efforts could be increased to increase the numbers of wild-caught eggs and larvae prior to major mortality events (typically predation; Forsythe et al., 2014; Crossman et al., 2018; Waraniak et al., 2019) to increase the number of spawning adults contributing to individuals captured during early life stages. Importantly, if the goal of restoration stocking programs includes production of progeny with high levels of genetic diversity and low levels of co-ancestry, direct gamete takes do not generally produce offspring with comparable levels of diversity and co-ancestry as wild-caught individuals (Crossman et al., 2011) and should not be used unless large numbers of males and females are spawned and proper mating strategies are employed (Bartron et al., 2018). Preference should be placed on wild-caught individuals to increase the likelihood of inclusion of offspring from larger numbers of adults.

While data from the Manistee River population may not be generalizable across all remnant lake sturgeon populations, or sturgeon populations generally, results described here indicate comparable data should be collected to guide management deci-

sions where hatcheries are in use or contemplated using wild caught offspring collected during early life stages from resident remnant spawning populations that are believed to be in low abundance.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2021.12.005.

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