fishes

MDPI

Article

Parentage Analysis Reveals Unequal Family Sizes during Hatchery Production

Mary Akers [1], Henry Quinlan [2], Andrew Johnson [1], Edward Baker [3] and Amy Welsh [1,*]

[1] Division of Forestry and Natural Resources, West Virginia University, Morgantown, WV 26506, USA
[2] Ashland Fish and Wildlife Conservation Office, U.S. Fish and Wildlife Service, Ashland, WI 54806, USA
[3] Marquette Fisheries Research Station, Michigan Department of Natural Resources, Marquette, MI 49855, USA
* Correspondence: amy.welsh@mail.wvu.edu

Abstract: Lake sturgeon (*Acipenser fulvescens*) is a species of conservation concern that has been stocked in several Great Lakes (North America) rivers. Lake sturgeon were extirpated in the Ontonagon River in Lake Superior and stocking began in 1998. In 2017, gametes were collected from spawning lake sturgeon (9 females, 36 males) caught at the nearby Sturgeon River spawning ground, generating nine family groups using a 1:4 mating design (n = 862). In 2018, gametes were collected from 3 females and 15 males, generating three family groups, and additional collections of drifting fry from the Sturgeon River were reared in the hatchery, resulting in 84 hatchery-produced and 675 wild-caught fry for stocking in the Ontonagon River. The objective of this study was to compare paternal representation and genetic diversity between the two stocking strategies. Parentage analysis based on genetic data from 12 microsatellite loci determined none of the family groups in the hatchery had equal paternal representation ($p < 0.001$), while wild-produced offspring had equal paternal representation. Despite the larger number of breeders contributing to the wild-caught larvae, there was no significant difference in genetic diversity between the wild-caught larvae and representative hatchery-produced offspring.

Keywords: lake sturgeon; parentage testing; variance in family size

Key Contribution: We have determined that hatchery rearing of lake sturgeon results in unequal family sizes despite initial equalization of families and that genetic diversity can be maintained in the hatchery if a sufficient number of breeders is used.

check for updates

Citation: Akers, M.; Quinlan, H.; Johnson, A.; Baker, E.; Welsh, A. Parentage Analysis Reveals Unequal Family Sizes during Hatchery Production. *Fishes* **2023**, *8*, 140. https://doi.org/10.3390/fishes8030140

Academic Editor: Deshou Wang

Received: 31 January 2023
Revised: 22 February 2023
Accepted: 24 February 2023
Published: 28 February 2023

1. Introduction

Fisheries managers can utilize stocking as an important tool for population recovery with the ultimate goal of many restoration projects being the establishment of self-sustaining populations. Genetic diversity is important to maintain long term population abundance and potential for adaptation to future conditions [1,2]. For restoration projects to be successful long term, managers must focus on minimizing the relatedness of the propagated and collected offspring in order to maximize the genetic diversity of surviving adults [2]. Stocking into a vulnerable population does have associated risks that vary in effect dependent on the stocking practices. Effective population size (N_e) can influence the rate of genetic diversity loss, as a reduced N_e can lead to quicker genetic diversity loss. Many of the factors that lower N_e relative to the census population size, such as variance in family size, unequal sex ratios, and fluctuations in population size, can be managed and controlled in captive settings.

There are multiple methods to acquire or collect individuals for stocking including collections of gametes, fertilized eggs, and drifting larvae. Each method yields varying levels of genetic diversity in the surviving progeny. Additionally, the life stage stocked influences the retention of genetic diversity [2,3]. Collections of naturally produced offspring incorporate the spawning nature of the species in question. Lake sturgeon (*Acipenser fulvescens*) are

polygamous broadcast spawners, and offspring produced from these methods have low relatedness due to the high number of parental crosses [2]. While direct gamete takes can be an effective method for managers to produce a large number of progeny, the offspring can have a highly variable level of relatedness depending on the chosen mating method and survival.

Parental crosses in the hatchery can be conducted in various ways, with the primary types being monogamous or factorial designs. Factorial setups can include a partial factorial mating design where the eggs of each female are mated with a subset of available males, and full factorial mating where male and female gametes are subject to multiple parental combinations [4]. Over multiple generations, full and partial factorial mating designs can reduce inbreeding within a population, even when the initial F1 offspring is composed of many half-siblings [5–7]. When compared to a single male–female cross, a partial factorial mating scheme can increase the estimated number of breeders (N_b) and is recommended for population recovery plans focused on retaining a high value of N_b [2,4,7]. Further steps can be taken to ensure equal family sizes are stocked to reduce any decreases in the effective population size of the stocked population [8]. When no additional measures are taken, a >22% decrease in N_e was observed in coho salmon (*Oncorhynchus kisutch*) [9].

Stocking can be used to build populations of various species including lake sturgeon, and prevent the adverse effects of inbreeding and genetic drift that occur in populations with a small number of individuals. One of many conservation-based stocking projects is the Ontonagon River lake sturgeon project located on the southern shore of Lake Superior in the Great Lakes of North America. The Michigan Department of Natural Resources (MIDNR; Marquette, MI, USA) and the U.S. Fish and Wildlife Service (USFWS, Ashland, WI, USA) have collected lake sturgeon from the nearby Sturgeon River in Lake Superior for stocking purposes since 1998, and continue to stock individuals in the Ontonagon River to build a reproducing population. From 1998 to 2004, lake sturgeon were reared at the MIDNR Wolf Lake state fish hatchery in Mattawan, MI, and stocked into the Ontonagon River. From 2007 to 2010, lake sturgeon were reared at a streamside rearing facility in Ontonagon, MI, but efforts were halted due to poor water quality hindering rearing efforts. A new location for rearing was secured in 2012, and from 2013 onward, stocking resumed using a streamside rearing facility on the West Branch Ontonagon River. These facilities raise the larvae in water pumped directly from the river in which they will be stocked; this technique exposes the larvae to daily changes in water conditions and chemistry in the watershed to which they will be restored, as opposed to a traditional hatchery environment with constant conditions from a distant watershed [10]. Hatchery environments can alter selection pressures and result in a different genetic makeup than wild raised fish [11–13]. Streamside rearing is still an artificial setting but by rearing the young lake sturgeon in Ontonagon River waters, hopefully these artificial selection pressures will be reduced.

The objectives of this study were to determine if the partial factorial design used in the hatchery resulted in equal family sizes, to compare this method to family size representation of naturally spawned individuals, and to assess differences in the number of breeders and genetic diversity between the two approaches.

2. Materials and Methods

2.1. Sample Collection

Adult lake sturgeon (9 females, 36 males) were caught at the Sturgeon River spawning ground in 2017, and gametes were collected for spawning at the streamside facility. Female family groups (N = 9) were created by splitting the eggs from a single female into 4 lots of approximately equal volume. Four males were then randomly selected to fertilize eggs with one male used per subset of eggs. After fertilization, the four lots of eggs were recombined for incubation and rearing. Each female family group was assigned to a single tank in the streamside rearing facility for the duration of the rearing cycle. In 2018, gametes from 3 females and 15 males were collected for the stocking program. Fin clips were collected for genetic analysis from all parents, including 21 additional adults captured in 2018 on

the spawning ground (2 females, 17 males, and 2 sex unknown) that did not contribute gametes to the stocking program but may have contributed to natural reproduction. The collected eggs (estimated egg take by female was 100 mL, 95 mL, and 120 mL) from each female were divided into five roughly equal lots, with each lot fertilized by a unique male for a 1 female:5 males mating design. Due to the low numbers of fecund females captured and substantial offspring survival from only one family group in 2018, naturally-produced larval lake sturgeon were collected from the Sturgeon River spawning ground and reared in the Ontonagon streamside rearing facility by the USFWS until time of release. At the time of release, all lake sturgeon were fitted with a PIT tag for identification and a fin clip collected from the pectoral fin of the hatchery produced larvae in 2017 (n = 1261; 862 were genetically analyzed), and both the hatchery produced (n = 84) and wild-caught hatchery raised larvae (n = 675) in 2018.

2.2. Laboratory Techniques

DNA was extracted from lake sturgeon fin clips following the Promega Wizard SV 96 Genomic DNA Purification System (Promega Corporation, Madison, WI, USA) per the manufacturer's instructions. Fin clip samples were eluted in 200–250 µL of nuclease free water. Samples were quantified using a NanoDrop 8000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and diluted to 10 ng/µL. Twelve microsatellite loci [14–17] were amplified in 3 multiplex groups using the Qiagen Multiplex Polymerase Chain Reaction (PCR) kit (Multiplex 1: *Afu68* (6FAM; 104–136 bp), *Afu68b* (6FAM; 172–216 bp), *Spl120* (VIC; 251–287 bp), *AfuG56* (NED; 254–270 bp); Multiplex 2: *AfuG195* (6FAM; 155–159 bp), *AfuG9* (VIC; 124–188 bp), *Aox27* (PET; 130–142 bp), *AfuG74* (NED; 214–222 bp); Multiplex 3: *AfuG160* (6FAM; 125–145 bp), *AfuG63* (VIC; 122–142 bp), *AfuG204* (PET; 133–145 bp), *AfuG112* (NED; 242–258 bp)). Each 10µL multiplex PCR reaction included 1x QIAGEN Multiplex PCR Master Mix, 0.2µM of each fluorescently labeled forward primer (0.4µM for *AfuG195*, *AfuG9*, and *AfuG112*), 0.2µM of each reverse primer (0.4 µM for *AfuG195*, *AfuG9*, and *AfuG112*), and 20 ng of extracted template DNA (40 ng for Multiplex 2). Thermal cycling protocol for Multiplex 1 has an initial denaturation of 95 °C for 15 min followed by 20 cycles of 94 °C (30 s), 70 °C (40 s) with a 0.5 °C decrease per cycle, followed by 20 cycles of 94 °C (30 s), 60 °C (40 s) with an increase of 1 s per cycle, followed by a final 10 °C hold. Multiplex 2 has an initial denaturation of 95 °C for 15 min, followed by 35 cycles of 95 °C (45 s), 54 °C (45 s), 72 °C (45 s), followed by a final extension of 72 °C for 10 min and a 10 °C hold [18]. Multiplex 3 has an initial denaturation at 95 °C for 15 min, followed by 30 cycles of 94 °C (30 s), 57 °C (90 s), 72 °C (60 s), followed by a final extension of 60 °C for 30 min and a 10 °C hold. Samples were then sent to the WVU Genomics Core Facility (CTSI Grant #U54 GM104942) for fragment analysis using a LIZ600 size standard. Allele peaks were identified and manually confirmed using GeneMarker™ Genotyping Software by SoftGenetics.

2.3. Sex Identification

In order to perform the parentage analysis of the wild-caught larvae in 2018, the sex of the two unknown adults sampled at the Sturgeon River needed to be identified. The following sex identification protocol was modified from Kuhl et al. [19] and Scribner and Kanefsky [20]. Each 25µL PCR reaction included 15.3 µL water, 2.5X GoTaq Buffer (Promega Corporation), 5.2 nmol dNTPs, 12.5 nmol of each AllWSex2 Primer F and R, 0.5 U GoTaq polymerase (Promega Corporation), and 40 ng DNA. The thermal cycling protocol had an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C (60 s), 56 °C (45 s), 72 °C (45 s) with a final extension of 72 °C for 5 min and a 12 °C hold. The PCR product was then run on a 1.5% agarose gel alongside a 100 bp ladder stained using ethidium bromide. Female lake sturgeon samples amplified a fragment around 100 basepairs long, and male samples had no amplified fragment. The microsatellite locus *AfuG56* was included in each reaction as a positive control, producing a fragment greater than 200 basepairs in length.

2.4. Parentage Analysis

Genotypes of the lake sturgeon propagated within the hatchery were compared to genotypes of the known mother and potential fathers to determine paternal representation within the family group. Paternal analysis was performed using programs CERVUS 3.0.7 [21,22] and COLONY 2.0.6.6 [23]. COLONY uses full-pedigree likelihood methods to determine sibship and parentage of sampled individuals [24]. COLONY analysis parameters were verified by simulating offspring genotypes using the same full likelihood method, male monogamy/female polygamy mating system, medium likelihood precision, genotyping error rate of 0.0001, and no sibship prior with 1 mother and 5 potential fathers at 100% sampling probability. CERVUS analysis included genotyping error rates in likelihood equations to increase the probability of successful assignment and decrease the chance of father-offspring mismatches [22]. Corroboration of paternal results were used to successfully assign parentage of the offspring by comparing relaxed (80%) and strict (95%) assignment, LOD scores, and assignment probability of the potential parent pairs. A chi-squared test was conducted on each individual tank to determine if observed paternal contributions were significantly different from the expectation of equal paternal contributions ($\alpha = 0.05$).

Parentage analysis was also conducted on the lake sturgeon larvae collected from the Sturgeon River in 2018. The 39 adults sampled at the Sturgeon River spawning grounds were all potential parents of the spawned larvae including the adults from which gametes were collected. Parentage analysis was performed to determine the most likely parent of each sampled offspring. CERVUS 3.0.7 simulation analysis was used to determine the appropriate sampling rate of parents at 0.6 based on the resulting assignment rate, and accuracy of the assignment to assign potential parents as the true parent pair sampled, one true parent sampled, or neither true parent sampled. Simulations were also run in COLONY with varying priors (simulation length, accuracy, sibship prior), polygamous mating of both sexes to reflect their mating strategy in the wild, and potential parents (5 females and 34 males), with a sampling rate of 0.6 included and excluded. Regardless of parameters used, there was low accuracy of assignment during the simulation in COLONY, and COLONY was therefore not used for parentage assignment of the wild-caught larvae. However, the true value of the number of breeders was within the estimated 95% confidence intervals, so the estimated number of breeders was included in the analysis.

2.5. Genetic Diversity Comparison between Hatchery- and Wild-Produced Offspring

The number of breeders (N_b) was estimated for the hatchery-propagated and wild-collected larvae using COLONY 3.0.7 full likelihood method using the same analysis parameters for hatchery produced and wild-caught offspring outlined in the parentage analysis section. The accuracy of N_b estimation was confirmed using COLONY 3.0.7 simulations of the mating matrix. Genetic diversity (i.e., allelic richness (A_r), observed heterozygosity (H_o)) between the wild-produced larvae in 2018 and the hatchery-produced offspring in 2017 and 2018 were compared using a paired t-test to determine whether differences in genetic diversity were significant. Allelic richness was calculated using the software FSTAT [25], and heterozygosity values were calculated using the software ARLEQUIN [26].

3. Results

3.1. Sex Identification

The two lake sturgeon sampled at the Sturgeon River in 2018 during the spawning run were genotyped as males after an inconclusive field identification.

3.2. Hatchery-Produced Offspring

For the 2017 samples, a corroborating genetic analysis of 862 of 1261 hatchery-produced stocked offspring indicated that multiple tanks were successful, with all males contributing offspring. However, paternal contributions were significantly unequal for all tanks

($p < 0.001$; Table 1). The estimated number of effective breeders was calculated to be $N_b = 38$ (95% CI: 34–42), representing the minimum number of effective breeders given that ~1/3 of the released offspring were not analyzed.

Table 1. Number of offspring produced by each male in 2017 and 2018 for the Ontonagon River stocking project. Four males were used for each family group in 2017 and five males were used in 2018. One female was used for each family group, with different males and females used for each family group.

Year	Family Group	Male					X^2	*p*-Value
		1	2	3	4	5		
2017	2	33	5	31	31	N/A	21.44	8.53×10^{-5}
2017	3	21	36	2	19	N/A	29.79	1.52×10^{-6}
2017	4	35	33	2	19	N/A	31.40	6.99×10^{-7}
2017	5	35	38	18	8	N/A	24.52	1.95×10^{-5}
2017	6	23	24	5	10	N/A	13.63	0.001
2017	7	12	8	51	26	N/A	46.71	4.00×10^{-10}
2017	8	6	30	36	27	N/A	20.64	0.0001
2017	9	44	10	24	22	N/A	23.84	2.70×10^{-5}
2017	10	22	19	7	50	N/A	40.53	8.22×10^{-9}
2018	1	34	7	3	28	11	50.05	4.70×10^{-9}

For the 2018 samples, corroborating genetic analysis of the hatchery-produced stocked offspring ($n = 84$) identified offspring of the two surviving female family groups; 83 offspring were assigned to a family group with high relative survival and 1 offspring assigned to a family group with low survival rates. Additional paternity analysis of offspring assigned to the successful family group determined that male reproductive success was significantly different ($\chi^2 = 50.05$; $p = 4.7 \times 10^{-9}$; df = 4), and over half of the propagated lake sturgeon were sired by two of the five males (Table 1). However, all fathers were represented in the stocked offspring. The estimated number of effective breeders was calculated to be $N_b = 3$ (95% CI: 2–12).

3.3. Wild-Produced Larvae

A total of 675 naturally produced wild-caught larvae were stocked in 2018. Maternity was successfully assigned to 147 of the offspring with a strict assignment of 95% and relaxed assignment of 80%, with all 5 adult females sampled at the spawning ground represented in the collected offspring. Paternity was assigned to 105 of the collected larvae and included parentage from 33 of the 34 sampled males. The number of offspring produced by each male sampled at the Sturgeon River spawning grounds was not significantly different ($\chi^2 = 30.51$; $p = 0.59$; df = 33). Of the three females used for hatchery propagation, the female with multiple surviving offspring in the hatchery had an additional 27 offspring in the wild; the female with one surviving offspring from the hatchery had 26 naturally produced offspring; and the female with no successful hatchery production had 39 offspring in the wild. The two other females caught at the Sturgeon River had 49 and 6 assigned offspring, respectively. Of the paternal assignments, the average number of assigned offspring was three offspring/male with the maximum number of eight offspring to one father. Of the 15 males from which gametes were collected for hatchery production, all except 1 male had offspring identified in the naturally produced wild-caught larvae. The estimated N_b of the naturally produced larvae was 127 (95% CI: 110–147).

Despite the smaller number of breeders represented in the hatchery setting, there were no significant differences in genetic diversity between production from 2017 (with a larger number of breeders) and wild-produced offspring (Table 2). Allelic richness was slightly higher in the wild-produced larvae, but the difference was not significant. No significant difference in observed heterozygosity was detected. However, there was a significant difference in allelic richness between hatchery production in 2018 (with a smaller number

of breeders) and 2017 hatchery production ($p = 0.0006$) and 2018 wild production ($p = 0.004$). No significant differences in heterozygosity were observed.

Table 2. Genetic diversity comparison between hatchery-produced offspring in 2017 and 2018 and wild-produced offspring in 2018. No significant differences were observed at any of the genetic diversity measures. Sample sizes (N) and estimated number of breeders (N_b) with 95% confidence intervals are included.

	N	N_b	Allelic Richness	Observed Heterozygosity
Hatchery–2017	862	38 (34–42)	4.33	0.548
Hatchery–2018	84	3 (2–12)	3.25	0.543
Wild-produced	675	127 (110–147)	4.75	0.538

4. Discussion

Our study identified a clear difference in paternal reproductive success between naturally-produced lake sturgeon and lake sturgeon produced in a hatchery from a partial factorial mating design. The difference in the number of offspring per male between the two hatchery collection methods has multiple consequences for the effective population size of the entire cohort and resulting Ontonagon River population. The low calculated N_b of the hatchery-produced offspring relative to the total number of parents used reflects this overrepresentation by some males in the cohort. This genetic swamping and deviation from the expected level of genetic diversity can lead to reduced adaptive potential and harm the stocked population in the long term, especially if this trend is repeated over multiple stocked cohorts. Additional measures may be necessary to prevent an overabundance of full- and half-siblings present, which the partial factorial method was designed to prevent. It may be necessary to raise the offspring of each parent pair separately to reduce the variation in family sizes at the time of stocking. Further consideration needs to be given to the threshold for family size reduction for equalizing family sizes when the number of offspring produced by one or more families is low.

The variation in family sizes in the hatchery setting is likely due to selection against particular genotypes, which may be more pronounced in the absence of mate choice. The resulting reproductive skew in surviving offspring per parent can be caused by a combination of competition and differential reproductive success [27]. In typical sturgeon hatcheries, offspring from male–female pairs are initially equalized, but then offspring from a single female and multiple males are pooled together for rearing. During this time, competition among the offspring may occur, resulting in selection against particular paternal alleles and genotypes. Family differences in size (i.e., fork length) have been documented in salmonids, resulting in differences in post-release survival [28,29]. These differences based on size can be exacerbated when fish are raised in high densities [30]. In addition, the absence of mate choice in the hatchery has been correlated to observed fitness differences between hatchery-produced and wild-produced offspring [31,32]. In brown trout (*Salmo trutta*), male reproductive success was highly dependent on female mate choice as a function of body size during aggregate spawning events, a phenomenon well documented in salmonid species [27,33]. Direct gamete takes remove this effect of mate choice, and reproductive success is solely dependent on gamete quality and eventual larval development. In hatcheries where direct gamete takes are the primary method of propagation, artificial selection during the hatchery rearing process will largely affect the fitness of the stocked offspring by cultivating individuals well adapted to the hatchery environment. Hatchery-produced steelhead (*Oncorhynchus mykiss*) had lower reproductive success compared to natural-origin spawners, and this effect was observed two generations after stocking occurred [34], indicating that selective pressures in the hatchery can have potentially long-lasting effects.

Additional familial relationships were identified between larvae produced in the hatchery and the collected naturally-produced larvae. Some of the females that did not

reproduce successfully in the hatchery had successful reproduction in the wild, indicating that streamside rearing may not fully be capturing an adaptive environment for lake sturgeon, and genotype–environment interactions may be occurring. However, as knowledge about optimal field conditions continues to improve, streamside rearing conditions will likely better reflect natural conditions. The wild-caught offspring sampled did share parents with the hatchery offspring, but all offspring were produced by different male–female pairs than the hatchery produced offspring. This phenomenon has been observed in past gamete collections; over 3 years, 92–100% of female lake sturgeon and 61–89% of male lake sturgeon from which gametes were collected also reproduced naturally at the spawning grounds [2]. The presence of these familial relationships between the wild-caught and hatchery-produced larvae highlights the importance of only collecting what is needed to ensure adequate numbers of naturally produced larvae [13].

Multi-year studies have demonstrated that offspring produced from gamete collections have a lower estimated N_b than naturally produced offspring due to the decrease in number of parental crosses [2,35]. While there is a skewed sex ratio of adult lake sturgeon at the spawning grounds, the naturally-produced larvae account for a larger pool of breeders due to the polygamous broadcast spawning nature of lake sturgeon. This strategy increases the number of male–female pairings beyond the partial factorial hatchery setup [2,36]. By increasing the number of the breeders, the genetic diversity within the offspring can increase dramatically with each allele combination. However, despite the smaller number of breeders in the hatchery setting and variance in family size, genetic diversity did not decrease relative to the wild-produced larvae when 45 breeding adults were used in 2017. This may be due to genetic erosion being hidden by the use of randomly selected broodstock adults representing various ages. However, given the long generation time of lake time (~25 years), this is unlikely. Additionally, when fewer breeders were used (seven successful adults in 2018), allelic richness was significantly lower. This was also observed in white sturgeon (*A. transmontanus)*, when only six breeding adults were used [35]. In summer chum salmon (*O. keta*), the mating design also incorporated only a small number of parents, which produced highly variable family sizes and directly resulted in a cohort with decreased genetic diversity and limited adaptive potential [34]. For captive breeding, it is estimated that 30 founders will capture 98% of the population's heterozygosity and there will be a 95% probability that all the alleles with a frequency greater than 0.05 will be represented [37]. In 2017, there were 45 founders, which exceeds the recommended 30 founders and likely resulted in higher genetic diversity, despite the high variance in family size.

Ultimately, a population should have a minimum final effective population size of 500 to ensure long term adaptive potential and adequate genetic diversity [13,38]. This objective for stocking projects, including the Ontonagon River project, is to ensure the population will continue to thrive to the point where the population is self-sustaining [39]. Over time, sampling of the Ontonagon River population should observe the population increasing in abundance to more than 750 individuals to decrease the risk of extinction and maximize long-term success of the stocking project [13,40]. The 2017 and 2018 cohorts are only a small part of the long-term stocking project for the Ontonagon River lake sturgeon restoration project, and with additional cohorts, the genetic diversity and the effective population size of the Ontonagon lake sturgeon will likely continue to improve.

5. Conclusions

We have documented unequal family sizes in the hatchery despite families being equalized prior to pooling paternal contributions. Hatchery practices should be reevaluated to prevent this occurrence because unequal family sizes can contribute to a reduced effective population size, decreasing the adaptive potential of the stocked population. Despite the potential for a low effective population size, genetic diversity was comparable to levels observed in wild-produced offspring when a sufficient number of breeders was used. This highlights the importance of breeder number and the use of unique spawners each year

to increase the number of breeding adults across the generation, which will result in the maintenance of genetic diversity in the stocked population.

Author Contributions: Conceptualization, H.Q., E.B. and A.W.; methodology, M.A., H.Q. and A.W.; software, M.A., A.J.; validation, M.A.; formal analysis, M.A. and A.J.; investigation, M.A.; resources, H.Q., E.B. and A.W.; data curation, A.W.; writing—original draft preparation, M.A.; writing—review and editing, H.Q., A.J., E.B. and A.W.; visualization, M.A.; supervision, A.W.; project administration, H.Q. and A.W.; funding acquisition, H.Q. and A.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the U.S. Fish and Wildlife Service Great Lakes Restoration Initiative–Cooperative Agreement F19AC00632 and by the USDA National Institute of Food and Agriculture, Hatch project WVA00747, and the West Virginia Agricultural and Forestry Experiment Station (Article No. 3454).

Institutional Review Board Statement: Ethical review and approval were waived for this study due to state and federal government exemption.

Data Availability Statement: Data will be made available by the corresponding author upon request.

Acknowledgments: U.S. Fish and Wildlife Service Ashland Fish and Wildlife Conservation Office (Glenn Miller, Kaitlyn Windschitl, Evan Boone, Joshua Schloesser, Angelena Sikora, and Sharon Rayford), U.S. Forest Service Ottawa National Forest (John Pagel and Anthony Saxman), Northland College (Nathan Kline), Fond du Lac Band of Lake Superior Chippewa (Thomas Howes, Terry Perrault, Brian Borkholder, John Goodreau, Matt Weske, and Eli Goodreau) assisted with field and hatchery efforts. Lauren Schumacher assisted with the laboratory work for this project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ford, M.J. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conserv. Biol.* **2002**, *16*, 815–825. [CrossRef]
2. Crossman, J.A.; Scribner, K.T.; Yen, D.T.; Davis, C.A.; Forsythe, P.S.; Baker, E.A. Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). *Aquaculture* **2011**, *310*, 312–324. [CrossRef]
3. Flagg, T.A.; Nash, C.F. *A Conceptual Framework for Conservation Hatchery Strategies for Pacific Salmonids*; NOAA Technical Memorandum NMFS-NWFSC-38; U.S. Department of Commerce, National Oceanic and Atmospheric Administration: Washington, DC, USA, 1999; 54p.
4. Fiumera, A.C.; Porter, B.A.; Looney, G.; Asmussen, M.A.; Avise, J.C. Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. *Conserv. Biol.* **2004**, *18*, 94–101. [CrossRef]
5. Engström, G.; McMillan, I.; McKay, L.; Quinton, M. Comparison of mating systems in a fish population: A simulation study. *J. Anim. Breed. Genet.* **1996**, *113*, 559–566. [CrossRef]
6. Dupont-Nivet, M.; Vandeputte, M.; Haffray, P.; Chevassus, B. Effect of different mating designs on inbreeding, genetic variance and response to selection when applying individual selection in fish breeding programs. *Aquaculture* **2006**, *252*, 161–170. [CrossRef]
7. Busack, C.; Knudsen, C.M. Using factorial mating designs to increase the effective number of breeders in fish hatcheries. *Aquaculture* **2007**, *273*, 24–32. [CrossRef]
8. Ryman, N.; Laikre, L. Effects of supportive breeding on the genetically effective population size. *Conserv. Biol.* **1991**, *5*, 325–329. [CrossRef]
9. Moyer, G.R.; Blouin, M.S.; Banks, M.A. The influence of family-correlated survival on Nb/N for progeny from integrated multi-and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* **2007**, *64*, 1258–1265. [CrossRef]
10. Holtgren, J.M.; Ogren, S.A.; Paquet, A.J.; Fajfer, S. Design of a portable streamside rearing facility for lake sturgeon. *N. Am. J. Aquacult.* **2007**, *69*, 317–323. [CrossRef]
11. Campton, D.E. Genetic effects of hatchery fish on wild populations of Pacific salmon and steelhead: What do we really know? *Am. Fish. Soc. Symp.* **1995**, *15*, 337–353.
12. Currens, K.P.; Busack, C.A. A framework for assessing genetic vulnerability. *Fisheries* **1995**, *20*, 24–31. [CrossRef]
13. Welsh, A.B.; Elliott, R.F.; Scribner, K.T.; Quinlan, H.R.; Baker, E.A.; Eggold, B.T.; Holtgren, J.M.; Krueger, C.C.; May, B. Genetic guidelines for the stocking of lake sturgeon (*Acipenser fulvescens*) in the Great Lakes basin. In *Great Lakes Fishery Commission Miscellaneous Publication 2010-01*; Great Lakes Fishery Commission: Ann Arbor, MI, USA, 2010; 62p.
14. May, B.; Krueger, C.C.; Kincaid, H.L. Genetic variation at microsatellite loci in sturgeon: Primer sequence homology in Acipenser and Scaphirhynchus. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 1542–1547. [CrossRef]

15. King, T.L.; Lubinski, B.A.; Spidle, A.P. Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Conserv. Genet.* **2001**, *2*, 103–119. [CrossRef]
16. McQuown, E.C.; Gall, G.A.E.; May, B. Characterization and inheritance of six microsatellite loci in lake sturgeon. *T. Am. Fish. Soc.* **2002**, *131*, 299–307. [CrossRef]
17. Welsh, A.; Blumberg, M.; May, B. Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon. *A. medirostris. Mol. Ecol. Notes* **2003**, *3*, 47–55. [CrossRef]
18. Estep, K.; VanDeHey, J.; Raabe, J.; Schmalz, P.; Wilfond, D.; Piszczeck, P.; Borkholder, B. Genetic origins and diversity of lake sturgeon in the St. Louis River estuary. *J. Great Lakes Res.* **2020**, *46*, 1028–1035. [CrossRef]
19. Kuhl, H.; Guiguen, Y.; Höhne, C.; Kreuz, E.; Du, K.; Klopp, C.; Lopez-Roques, C.; Yebra-Pimentel, E.S.; Ciorpac, M.; Gessner, J.; et al. A 180 Myr-old female-specific genome region in sturgeon reveals the oldest known vertebrate sex determining system with undifferentiated sex chromosomes. *Philos. T. R. Soc. B* **2021**, *376*, 20200089. [CrossRef]
20. Scribner, K.T.; Kanefsky, J. Molecular sexing of lake sturgeon. *J. Great Lakes Res.* **2021**, *47*, 934–936. [CrossRef]
21. Marshall, T.C.; Slate, J.; Kruuk, L.E.B.; Pemberton, J.M. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **1998**, *7*, 639–655. [CrossRef]
22. Kalinowski, S.T.; Taper, M.L.; Marshall, T.C. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **2007**, *16*, 1099–1106. [CrossRef] [PubMed]
23. Wang, J.; Santure, A. Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics* **2009**, *181*, 1579–1594. [CrossRef] [PubMed]
24. Jones, O.R.; Wang, J. COLONY: A program for parentage and sibship inference from multilocus genotype data. *Mol. Ecol. Resour.* **2010**, *10*, 551–555. [CrossRef] [PubMed]
25. Goudet, J. FSTAT (ver. 2.9.4), a Program to Estimate and Test Population Genetics Parameters. 2003. Available online: http://https://www2.unil.ch/popgen/softwares/fstat.htm (accessed on 21 February 2023).
26. Excoffier, L.; Laval, G.; Schneider, S. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinform.* **2005**, *1*, 47–50. [CrossRef]
27. Serbezov, D.; Bernatchez, L.; Olsen, E.M.; Vøllestad, L.A. Mating patterns and determinants of individual reproductive success in brown trout (*Salmo trutta*) revealed by parentage analysis of an entire stream living population. *Mol. Ecol.* **2010**, *19*, 3193–3205. [CrossRef]
28. Thompson, N.F.; Clemens, B.J.; Ketchum, L.L.; Simpson, P.C.; Reagan, R.E.; Blouin, M.S. Family influence on length at release and size-biased survival post release in hatchery-reared steelhead: A mechanism to explain how genetic adaptation to captivity occurs. *Aquaculture* **2018**, *491*, 135–146. [CrossRef]
29. Blouin, M.S.; Wrey, M.C.; Bollmann, S.R.; Skaar, J.C.; Twibell, R.G.; Fuentes, C. X Offspring of first-generation hatchery steelhead trout (*Oncorhynchus mykiss*) grow faster in the hatchery than offspring of wild fish, but survive worse in the wild: Possible mechanisms for inadvertent domestication and fitness loss in hatchery salmon. *PLoS ONE* **2018**, *16*, e0257407. [CrossRef]
30. Thompson, N.F.; Blouin, M.S. The effects of high rearing density on the potential for domestication selection in hatchery culture of steelhead (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **2015**, *72*, 1829–1834. [CrossRef]
31. Evans, M.L.; Neff, B.D.; Heath, D.D. Behavioural and genetic analyses of mate choice and reproductive success in two Chinook salmon populations. *Can. J. Fish. Aquat. Sci.* **2013**, *70*, 263–270. [CrossRef]
32. Whitcomb, A.C.; Banks, M.A.; O'Malley, K.G. Influence of immune-relevant genes on mate choice and reproductive success in wild-spawning hatchery-reared and wild-born coho salmon (*Oncorhynchus kisutch*). *Can. J. Fish. Aquat. Sci.* **2014**, *71*, 1000–1009. [CrossRef]
33. Fleming, I.A. Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Can. J. Fish. Aquat. Sci.* **1998**, *55*, 59–76. [CrossRef]
34. Ford, M.J.; Murdoch, A.R.; Hughes, M.S.; Seamons, T.R.; LaHood, E.S. Broodstock history strongly influences natural spawning success in hatchery steelhead (*Oncorhynchus mykiss*). *PLoS ONE* **2016**, *11*, e0164801. [CrossRef] [PubMed]
35. Thorstensen, M.; Bates, P.; Lepla, K.; Schreier, A. To breed or not to breed? Maintaining genetic diversity in white sturgeon supplementation programs. *Conserv. Genet.* **2019**, *20*, 997–1007. [CrossRef]
36. Bruch, R.M.; Binkowski, F.P. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). *J. Appl. Ichthyol.* **2002**, *18*, 570–579. [CrossRef]
37. Allendorf, F.W.; Funk, W.C.; Aitken, S.N.; Byrne, M.; Luikart, G. *Conservation and the Genomics of Populations*, 3rd ed.; Oxford University Press: Oxford, UK, 2022.
38. Lande, R. Genetics and demography in biological conservation. *Science* **1988**, *241*, 1455–1460. [CrossRef] [PubMed]
39. Hay-Chmielewski, E.M.; Whelan, G.E. Lake sturgeon rehabilitation strategy. *Mich. Dep. Nat. Resour. Fish. Div. Spec. Rep.* **1997**, *18*, 1–51.
40. Hayes, D.B.; Caroffino, D.C. Michigan's lake sturgeon rehabilitation strategy. *Mich. Dep. Nat. Resour. Fish. Div. Spec. Rep.* **2012**, *62*, 1–26.