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Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*)

James A. Crossman [a,*], Kim T. Scribner [b], Duong Thuy Yen [a], Christin A. Davis [a], Patrick S. Forsythe [b], Edward A. Baker [c]

[a] Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, Michigan 48824-1222, USA
[b] Department of Zoology, Michigan State University, 13 Natural Resources Building, East Lansing, Michigan 48824-1222, USA
[c] Michigan Department of Natural Resources, 484 Cherry Creek Road, Marquette, Michigan 49855, USA

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ABSTRACT

Hatchery supplementation is widely advocated as an important means to achieve management goals of sustainable populations of lake sturgeon (*Acipenser fulvescens*). Increasing evidence for negative impacts of hatchery practices has prompted research regarding the efficacy of hatchery prescriptions that have been largely adopted from other well-studied species. Using a well-studied population in the Black River, MI drainage, we evaluated the effects of different gamete and larval collection methods and hatchery rearing environments (streamside and traditional) on measures of offspring genetic diversity estimated based on known or inferred pedigree and multi-locus genotypic data. Offspring produced from direct gamete takes (DGT) were more related (higher mean coancestry (θ) and significantly higher mean relatedness (r_{xy})) than were offspring produced from collections of naturally produced eggs (NPE) from stream substrate and larvae collected while dispersing from spawning areas (DL). Pedigree and genotypic data also revealed that greater numbers of adults contributed to offspring (effective number of breeders) from DL and NPE collections than from DGT collections. Comparatively higher levels of inter-family variation in egg and juvenile mortality in the traditional hatchery rearing environment relative to the streamside rearing environment resulted in more pronounced increases in mean coancestry and decreases in effective number of breeders across sequential ontogenetic stages. Methods of gamete and offspring collection and rearing have demonstrable effects on levels of offspring genetic diversity which has significant implications for restoration of numerically depressed populations.

1. Introduction

Concerns regarding the potential interactions of hatchery fish on wild populations (Ryman, 1991; Waples, 1991; Hilborn, 1992; Ryman et al., 1995; Waples, 1999; Hutchings and Fraser, 2008) have led to evaluations of the effects of hatchery practices on levels of genetic diversity (Allendorf, 1993; Utter, 1998; Mobrand et al., 2005). Genetic diversity is important for long-term population viability by providing greater potential for adaptation to environmental change (Ford, 2002). The success of hatchery restoration programs is typically measured by total numbers of progeny available for stocking or survival up to or following release (Brown and Day, 2002). As an alternate measure, successful conservation hatchery programs could focus on maximizing levels of genetic diversity (Ryman, 1991) and decreasing inter-relatedness among supplemental progeny (Ryman and Laikre, 1991). For hatchery programs focused on species that do not use captive broodstocks, factors such as the number of spawning adults, limited access to spawning adults, and unique life history characteristics (i.e., intermittent spawning, delayed maturity, skewed sex ratios) can lead to practices that reduce offspring levels of genetic diversity relative to levels represented in the adult breeding population (Allendorf and Phelps, 1980; Ryman, 1991). Current hatchery practices, combined with different methods of gamete collection, have been shown to negatively impact levels of genetic diversity (Bartron, 2003; Page et al., 2005). Different management options including how gametes, juveniles, or broodstock are collected and maintained prior to release back into the natural system have been cited as important factors to retention of levels of genetic diversity in hatchery progeny (Flagg and Nash, 1999).

Lake sturgeon (*Acipenser fulvescens*), a native fish of the Great Lakes, has been numerically depressed throughout their range (Hay-Chmielewski and Whelan, 1997). Management and conservation strategies have been implemented throughout the Great Lakes that

* Corresponding author. Tel.: +1 250 365 4560; fax: +1 250 365 4589.
E-mail address: james.crossman@bchydro.com (J.A. Crossman).

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focus on restoring remnant populations through supplementation of hatchery produced juveniles (Holey et al., 2000). Successful use of hatchery supplementation for sturgeon conservation is likely dependent upon the degree of domestication during rearing as proposed generally for other fish species (Lynch and O'Hely, 2001; Ford, 2002), adaptation to natural conditions following release, and the extent hatchery-reared juveniles retain levels of genetic diversity present in the adult population (Ireland et al., 2002; Secor et al., 2002).

Conservation hatchery programs are increasingly being used to facilitate the recovery of threatened or endangered species (Ryman et al., 1995; Brown and Day, 2002), while minimizing potentially negative genetic and ecological impacts of supplemental progeny on wild populations (Flagg and Nash, 1999). There has been recent interest in the use of streamside hatcheries for propagation as part of a comprehensive conservation hatchery program for lake sturgeon (Holtgren et al., 2007). Streamside hatcheries use water taken directly from natal rivers, providing natural daily and seasonal fluctuation in water conditions (i.e., temperature, oxygen, and turbidity) and chemistry (i.e., dissolved organics, pheromones, predator cues, etc.) that differ from more traditional hatchery environments with constant environments. Unfortunately, despite such advancements in rearing technologies, there is still a critical need for studies evaluating the efficacy of standard gamete and larval collection methods, how progeny collected from different methods perform in different hatchery environments, and relative rates of loss of genetic diversity.

Most scientific data on hatchery fish are based on literature from a limited number of species that have very different ecologies than long-lived iteroparous species like lake sturgeon. Even for widely studied groups such as salmonids (Fraser, 2008), considerable effort over many years and by many investigators has been required to accumulate information to formulate scientifically defensible hatchery-based restoration programs (Mobrand et al., 2005). As data have become available, the complexities and inter-dependencies among components of natural ecosystems have led to continual refinement of operational practices. It is necessary to develop and incorporate new science that is based on empirical data on the biology of lake sturgeon into management programs. Data from lake sturgeon collected in this study will allow the development of a scientific framework to be used to evaluate past supplementation activities and to predict outcomes of future management actions.

Collection of progeny for lake sturgeon hatchery and restoration programs throughout the Great Lakes is limited to a few standard methods, and use of a particular collection method varies by agency and population. Some methods such as captive broodstock are not used for lake sturgeon hatchery programs due to logistical constraints imposed by the species' large body size, low abundance of wild populations (Holey et al., 2000), long inter-spawning interval (Bruch and Binkowski, 2002), and genetic differentiation between populations (DeHaan et al., 2006). Without a captive broodstock program, collection of progeny for stocking has been almost entirely reliant on gametes collected from wild-caught adults.

In order to successfully design conservation programs for lake sturgeon and other species with similar life history characteristics, comparisons are needed between the standard collection methods currently available and different hatchery rearing environments. Long-term success of restoration programs that rely on supplementation will require that managers minimize relatedness between offspring released and maximize the genetic diversity represented by available spawning adults. The objectives of this study were to: 1) quantify differences in measures of genetic diversity of offspring produced based on different methods of gamete and larval collection for lake sturgeon, and 2) quantify the effect of two different hatchery rearing environments (streamside and traditional) on levels of juvenile genetic diversity. This research provides guidance for managers involved in collecting and rearing lake sturgeon progeny for restoration efforts.

2. Materials and methods

2.1. Study site and hatchery rearing

Research was conducted during each of three years (2005 to 2007) on the Upper Black River (UBR) in Michigan (Fig. 1). UBR is a fourth order stream located in the northeastern corner of Michigan's Lower Peninsula. The river's small size and hydrology allow enumeration of a large proportion of the adults reproducing each year (Smith and Baker, 2005; Forsythe, 2010), collection of gametes from the stream substrate (Forsythe, 2010), and collection of dispersing larvae (Smith and King, 2005). The adult reproductive season comprise different spawning runs. Adults migrate upstream to spawning grounds, reproduce over several days and immediately depart. Sequential groups composed of different subsets of adults arrive over a period ranging from 18 to 42 days. Timing of female lake sturgeon reproduction is highly repeatable across years (Forsythe, 2010). Reproduction occurs in wadeable sections of the river which have been delineated as distinct spawning zones based on a long-term data set collected from this population (Fig. 1).

2.2. Gamete/larval collection methods

Different methods of progeny collection focused on different life history stages (eggs or larvae) that may differentially capture diversity that can be quantified based on empirically measurable variables including inter-individual relatedness, heterozygosity, and numbers of contributing adults. Collection methods evaluated in this study represent the standard methods currently available for lake sturgeon for systems where maintenance of captive broodstock is not a viable management option. Our design involved collections of lake sturgeon gametes or larvae over multiple years. Data allow for comparisons between methods within and across years as well as examining the effects of rearing environment.

2.2.1. Directed gamete takes (DGT)

Lake sturgeon supplemental progeny are typically produced through direct removal and fertilization of gametes from spawning adults. Traditionally, fertilizations have been conducted with a relatively few females mated with several males (Folz et al., 1983; Anderson, 1984; Ceskleba et al., 1985; Pyatskowit et al., 2001) due to logistical challenges of river hydrology and reduced population sizes which limit access to spawning adults (Holey et al., 2000).

In this study, adult lake sturgeon were captured on spawning grounds using large hand-held dip nets and marked individually with passive integrated transponder (PIT) tags. We attempted to collect gametes from all individuals. Eggs were removed by hand-stripping females captured while in the act of spawning. Hand stripping involved applying pressure from the anterior section of the abdomen to the posterior in order to extrude eggs from the uro-genital opening. Eggs were kept in ovarian fluid and placed in plastic bags and maintained at ambient river temperatures. Milt was removed from individual males by applying pressure anterior to the uro-genital opening and collected using a 30 ml syringe. Milt was immediately placed on ice. Fertilizations were conducted within 12 h of egg collection. We used a partial factorial mating design (Miller and Kapuscinski, 2003), where eggs from each female were divided into two equal lots (by volume) and each lot was crossed with single male creating paternal half-sib family groups of approximately equal size. Individual males were never used twice within or across years, and fertilizations occurred independently to avoid issues of sperm competition (Campton, 2004). This mating design also increased the effective number of breeders compared to single pair crosses (Busack and Knudsen, 2007) and provided a quantitative genetic framework to test for variation between males and females while accounting for interactions between females (Vandeputte et al., 2004). We chose not

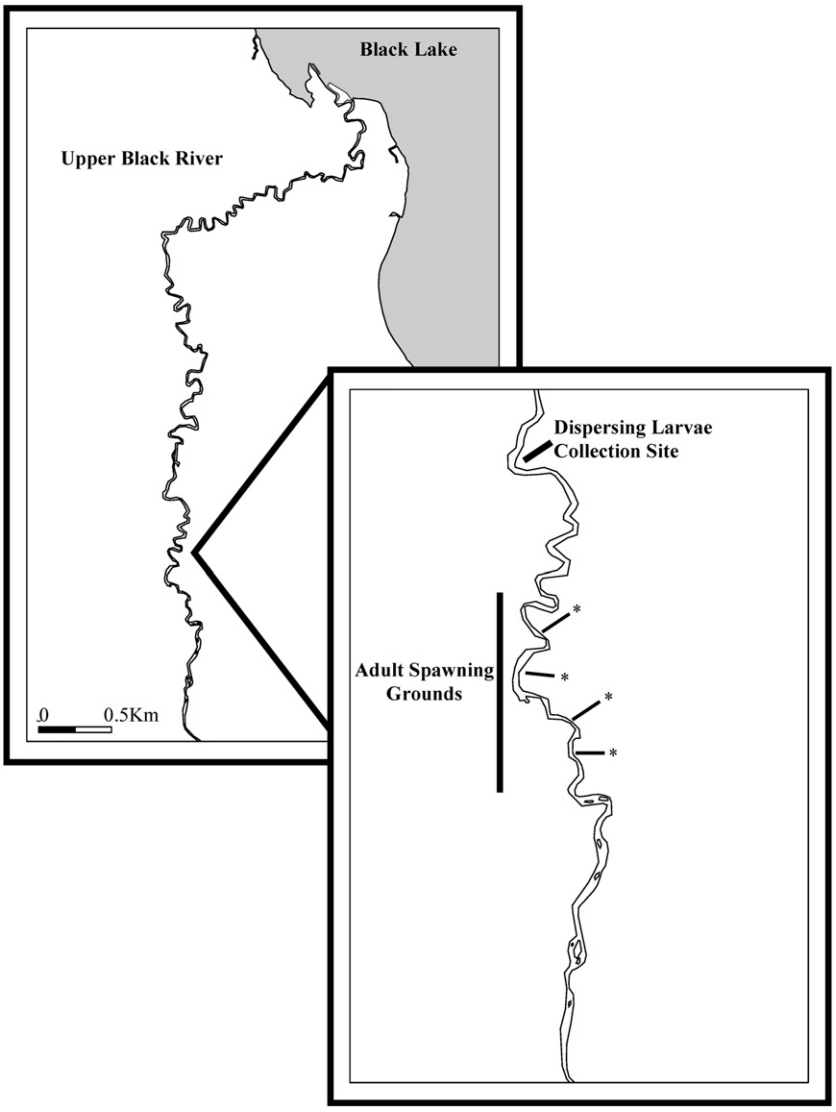

Fig. 1. The Black Lake study site in Michigan, showing the adult spawning grounds that extend over 2 km of stream, the collection sites for naturally produced eggs (*), and the collection site for dispersing larvae.

to employ a full factorial mating design, despite reported positive effects on effective population size (Fiumera et al., 2004) because of the likelihood of increased inter-individual relatedness (Miller and Kapuscinski, 2003.) which poses a threat to elevated future levels of inbreeding for numerically depressed populations of lake sturgeon. Half of the fertilized eggs from each paternal half-sib family were transported to a traditional hatchery and half were maintained at a streamside hatchery for incubation.

2.2.2. Dispersing larvae (DL)

A less intrusive collection method involved collecting larvae dispersing downstream from the spawning grounds (Auer and Baker, 2002; Smith and King, 2005). Sampling for larval lake sturgeon passively dispersing downstream has been conducted to quantify recruitment and chronology and duration of dispersal (Auer and Baker, 2002; Smith and King, 2005), and represents a viable collection strategy. Larvae obtained using this method may represent contributions from a larger and more equitable cross-section of spawning adults relative to offspring obtained using other collection methods, but genetic assays have not yet been conducted. Systematic larval sampling was conducted during a 5 h period, beginning at dusk (21:00) and ending in the early morning (03:00). The sampling location was located approximately 2 km downstream from all spawning areas on the Upper Black River (Fig. 1) allowing for collection of offspring from all spawning groups. Sampling began seven days after the first observation of reproduction. We deployed five D-framed drift nets across the stream channel in equal intervals to capture dispersing larval sturgeon. Nets were checked hourly and the larvae within each net were enumerated. This design was replicated nightly each year for the entire drifting period. Larval lake sturgeon captured each evening were reared at the streamside hatchery until the end of the drifting period. The larvae were then divided equally between the two hatchery rearing environments.

2.2.3. Naturally produced eggs (NPE)

A third method evaluated involved collecting eggs that have been naturally fertilized and deposited on the stream substrate at the spawning sites. This method takes into account the broadcast spawning nature of the species (Bruch and Binkowski, 2002) and has been used mainly to verify successful reproduction (McCabe and Beckman, 1990; Caswell et al., 2004). The effectiveness of collecting eggs in this manner depends on specific knowledge regarding adult spawning site locations, but represents a viable collection method in certain systems (Crossman, 2008). Systematic kick-net sampling was conducted below observed spawning aggregations (Fig. 1) to collect

eggs. Transects were conducted across the stream at 1 m intervals. At each interval we conducted kick-net sampling for 10 s. Transects continued downstream in intervals of 5 m until no eggs were collected in consecutive transects. The number of sampled versus non-sampled locations varied across years. Eggs were enumerated upon arrival at the streamside hatchery. Eggs from this collection method were incubated and hatched at the streamside rearing environment because of pathogen concerns of the traditional hatchery. Larvae were then divided equally between the two hatchery rearing environments.

2.3. Rearing methods and environments

Progeny from three different gamete/larval collection methods were reared in two different hatchery environments. Half of the eggs or offspring from each collection method were reared in natal water at a streamside hatchery constructed on the Upper Black River. The remaining eggs and larvae from each group were reared in a non-natal water source, a state hatchery in southern Michigan (Michigan Department of Natural Resources, Wolf Lake State Hatchery), representing a traditional rearing environment which has been a standard in fish culture for decades. The streamside hatchery represented more natural rearing conditions with temperature profiles (10–25 °C) matching the natal river. Ground water at the traditional hatchery was heated to a constant 20 °C.

Eggs were incubated and hatched using heath trays at both rearing environments. Larvae produced from direct gamete takes were enumerated by paternal half-sib family groups at hatch and then reared separately by family for the duration of the experiment within each hatchery. Larvae and juveniles were reared in round tanks (1.2 m diameter) that were divided to include two replicates of each of the three collection methods (N = 6 compartments per tank). This tank design was replicated ten times at the streamside hatchery and six times at the traditional environment. Daily husbandry activities, including cleaning and feeding, were consistent between the two hatcheries, across all collection methods, and followed protocols outlined in Crossman (2008). Feeding consisted of live brine shrimp nauplii (*Artemia* spp) starting at five days post hatch, live cultured blackworms (tubificid annelids; J.F. Enterprises, CA, USA) starting at day 21 following hatch, and frozen bloodworms (chironomid midge larvae) for the remainder of the rearing process. Tank sections were cleaned daily by purging all the water in the tank to remove excess food and waste and the tank sides were periodically wiped with providone–iodine (Betadine) for disinfection. Fish from the three collection methods were reared separately until fall fingerling size (>20 cm) and released into the Upper Black River.

2.4. Genetic sample collection

Samples were collected for use in analyses described below. Dorsal fin clips were taken from each adult captured during the enumeration of the spawning run. Adult fin clip samples were dried and stored in individual envelopes at ambient temperature. Juvenile mortalities that occurred throughout the rearing process were preserved separately by collection method, hatchery environment, and year in 95% non-denatured ethanol. Prior to release, fin clips were taken from all surviving juveniles and stored in 95% non-denatured ethanol by collection method, rearing environment, and year. A subset of juvenile lake sturgeon samples were randomly chosen from all individuals sampled within each of the NPE and DL collection methods. Juvenile samples used in analyses represented 4.6, 6.1, and 27.7% of DL samples collected in each of 2005, 2006, and 2007 respectively while 5% of NPE samples were used for analysis in each of 2006 and 2007. Samples were proportional with respect to surviving/dead juveniles and the two hatchery environments.

2.5. Genetic analyses

DNA was extracted from all adults and juvenile tissue samples using QIAGEN DNeasy® kits (QIAGEN Inc.) according to the manufacturers' protocol. DNA was quantified using a Beckman DU® 7400 spectrophotometer and diluted to a constant 20 ng/μl for use in Polymerase Chain Reactions (PCR). Individuals were genotyped at 12 disomically inherited microsatellite loci including *LS*68 (May et al., 1997), *Afu*G68b (McQuown et al., 2002), *Spl*120 (McQuown et al., 2000), *Aox*27 (King et al., 2001), *AfuG*9, *AfuG*63, *AfuG*74, *AfuG*112, *AfuG*56, *AfuG*160, *AfuG*195, and *AfuG*204 (Welsh et al., 2003). PCRs were conducted in 25 μl volumes containing 100 ng DNA, 10× PCR Buffer (1 M Tris–HCl, 1 M $MgCl_2$, 1 M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), 2 mM of each dNTP, 10 pmol of forward and reverse primer and 0.5 μl Taq polymerase (0.25 U/μl). PCR conditions for each locus followed protocols outlined in the above references. PCR products were run on 6% denaturing polyacrylamide gels and visualized on a Hitachi FMBIO II scanner. Allele sizes were scored using commercially available size standards (MapMarker™, BioVentures Inc.) and based on standard samples of known genotype. To minimize error, all genotypes were independently scored by two experienced lab personnel and verified again after data were entered into electronic databases.

2.6. Statistical analyses

2.6.1. Coancestry and relatedness

Coancestry (θ) and relatedness (r_{xy}) are two commonly used measures of kin relationship between individuals and can be used as a direct measure of potential for future inbreeding in captive or wild populations (Pemberton, 2008). In closed populations such as Black Lake or in populations restored primarily on the basis of hatchery-production, mean population coancestry and relatedness can accrue over short periods of time relative to comparatively larger populations that are open to gene flow. We calculated mean coancestry (θ) for offspring produced from fertilizations of eggs from known paternal half-sib families (offspring from DGT crosses). Data on total number males and females spawned, the manner in which eggs and milt were crossed, and the total number of eggs fertilized, and the subsequent number of larvae at hatch and later juvenile stages were used to calculate θ for each hatchery rearing environment and year.

Mean levels of θ are likely to increase over the rearing period due to different rates of mortality in different families. Accordingly, coancestry was estimated at different ontogenetic stages for both hatchery environments. Estimates were also calculated for later juvenile stages at the streamside hatchery at the time of release (56 and 90 days of age in 2005 and 140 days in 2006).

Calculations of mean θ by year and hatchery rearing environment were derived following Chesser (1991). The number of individuals in a family (b), number of families (n) and total numbers of offspring across all families (N) was used to estimate mean θ across all families for all (i) full sibs (0.25) and all (j) half sibs (0.125) as:

$$\theta = \frac{\left[0.25 \times \sum_{i=1}^{n} b_i^2 - b_i\right] + \left[0.125 \times \sum_{j=1}^{n} b_j^2 - b_j\right]}{(N^2 - N)}$$

For offspring obtained from larval drift sampling (DL) or as naturally produced eggs (NPE), we estimated θ among offspring using pedigree information derived from genetic determination of parentage (see Section 2.6.2).

In order to statistically compare coancestry estimates across gamete collection methods and hatchery rearing environments, which was not possible based on parametric measures estimated using pedigree data, we used the multi-locus genotypic data to estimate inter-individual relatedness. Marker-based estimates of

relatedness do not require prior information on pedigree relationships. Numerous studies have been conducted to develop relatedness estimators (e.g., Thompson, 1986; Ritland, 1996; Lynch and Ritland, 1999; Wang, 2002). Comparative studies that have evaluated the relative merits of different relatedness estimators under different circumstances (e.g., Van De Casteele et al., 2001; Csillery et al., 2006; Oliehoek et al., 2006) found that the performance of the Lynch and Ritland (1999) estimator surpassed other methods, particularly under circumstances expected with our upper Black River lake sturgeon population in terms of marker locus quality (moderate levels of polymorphism), the size of the study area, the closed nature of the population to immigration, and expected variance in relatedness.

Because the pedigree relationships among offspring from DGT crosses were known, individual offspring were not genotyped. In order to estimate relatedness among offspring for this collection method individual multi-locus genotypes of offspring were simulated using known genotypes of parents. To simulate offspring genotype profiles for each known parental pair, we randomly selected one allele per parent per locus to produce progeny genotypes for each locus. For each parental pair we simulated offspring numbers equal to that known to have survived at the point of sampling for coancestry estimation. Simulations were conducted using a Visual Basic macro implemented in Microsoft Excel. Samples were randomized and 2 replicates of 400 random offspring were used in subsequent analyses to estimate concordance of the mean and variance in relatedness between replicates using program COANCESTRY (Wang, in press). Estimates were highly concordant (data not shown) and results from a single replicate were used in subsequent analyses.

We used program COANCESTRY to estimate relatedness from different estimators (i.e., five moment estimators proposed by Queller and Goodnight, 1989; Li et al., 1993; Ritland, 1996; Lynch and Ritland, 1999 and Wang, 2002; and two likelihood estimators proposed by Wang, 2007 and Milligan, 2003; see Wang, in press) and compared the results from these estimators. We found using simulations in COANCESTRY that the Lynch and Ritland (1999) estimator yielded the smallest variance in relatedness values. Therefore, the Lynch and Ritland (1999) estimator was chosen for further analysis. Statistical significance of mean differences in relatedness estimates between gamete collection methods within and between hatchery environments were tested using a bootstrapping (1000 replicates) procedure implemented in COANCESTRY.

2.6.2. Parentage analysis

There are numerous programs that estimate parentage based on multi-locus genetic data (e.g., PASOS, Duchesne et al., 2005; CERVUS, Kalinowski et al., 2007; COLONY, Jones and Wang, 2010), and the efficacy of use of different programs or combinations of programs has been widely debated (Jones et al., 2010; Christie, 2010; Walling et al., 2010). Use of offspring in analyses that were consistently assigned to the same parents based on multiple programs which are based on different statistical properties has been advocated (Lee, 2008). Accordingly, we conducted parentage analysis using complimentary aspects of two programs, the Parentage Allocation of Singles on Open Systems (PASOS) program, version 1.0 (Duchesne et al., 2005) and CERVUS version 3.0 (Kalinowski et al., 2007). Assignment of offspring to parent pairs was based on concordance of maternal and paternal assignment between the two programs.

We conducted allocation and simulation functions in PASOS to assign offspring to parental pairs, estimate the proportion of adults sampled and estimate probability that each assignment is correct (correctness). We used user-defined parameters for the program, which are recommended by the authors, including the maximum number offset tolerance, MOT, equal to 2 and the error model of "0.002, 0.008, 0.98, 0.008, and 0.002" (Duchesne et al., 2005).

Parentage analysis in CERVUS is based on a likelihood approach (Kalinowski et al., 2007). Estimates of allele frequencies, mean number of alleles per locus, and observed and expected heterozygosities were calculated by CERVUS from genotypic data collected from spawning adults each year. Chi-squared goodness of fit tests were performed for each locus using CERVUS to test for deviations from Hardy Weinberg expectations. Program CERVUS was also used to calculate the overall probability of exclusion for the 12 loci. The simulation module within CERVUS was used to generate critical values of likelihood ratios for each year so that parentage could be assigned at a given level of statistical confidence. Simulations were conducted with the rate of typing error set at 0.01%, which was empirically derived from our blind 10% genotyping error check, and the proportion of parents sampled estimated by PASOS. We chose the most likely parent pair assigned to an offspring based on a (70%) confidence level. Previous parentage studies on lake sturgeon in this population (Doung, 2010; Duong et al., in press) showed that this confidence provided both the highest assignment rate and the percentage of assignment concordance between PASOS and CERVUS (as high as 95% confidence criteria) with high numbers of offspring assigned. Genetic determination of parentage was conducted separately for offspring from each collection method during each sampling year.

2.6.3. Adult reproductive contributions

Pedigree information obtained based on genetic determination of parentage has been used with increasing frequency in natural populations (Pemberton, 2008). We calculated several measures of reproductive success based on either known or genetically determined pedigree information from offspring collected using each collection method. For directed gamete takes we calculated the mean ($\pm$1 SD) total egg number collected per female across each year and the number of larvae that hatched from all half-sib families. Mean total egg number was calculated using an egg number per volume relationship (52.5 eggs per 1 ml) that was determined empirically by counting multiple 1 ml aliquots of lake sturgeon eggs across several females. We used an analysis of variance (ANOVA) to test for differences in the number of eggs obtained across females and sampling years. We used a two-way ANOVA to test for significant differences in numbers of larvae at hatch across paternal half-sib family groups in the two different hatchery environments. For offspring collected as dispersing larvae or as naturally produced eggs, we estimated the mean and variance in male and female reproductive success (number of offspring per individual) for each collection method across each hatchery environment based on the genetically determined pedigree.

We examined whether females that we directly collected gametes from also spawned naturally. Analyses addressed concerns that direct gamete collection from spawning females and males may cause undo levels of stress and increase the probability that they will abandon spawning, thus negatively impacting natural levels of recruitment. Directed gamete takes could also potentially increase reproductive skew of wild adults, further decreasing the effective breeding population size of the wild spawning population. We used parentage information estimated for the naturally produced eggs and dispersing larvae in all years to determine the percentage of females and males that we directly removed gametes from that spawned naturally and made reproductive contributions to collected offspring.

To test for differences in the number of genetically assigned offspring contributed per male and female between naturally produced eggs and dispersing larvae within 2006 and 2007 we used a Fisher's exact test. We also estimated the number of offspring produced by each adult represented in each collection method across years. Mean differences in individual heterozygosities between collection methods within a year were assessed using a Wald Test.

2.6.4. Effective number of breeding adults

We calculated the effective number of breeding adults for each collection method, and for each hatchery rearing environment. The effective breeding number (N_b) can be estimated for adults contributing

to a single bout of reproduction but is different from effective population size for species with overlapping generations. We estimated N_b using two approaches. First, we calculated the effective number of breeders using the effective numbers of males (N_{bm}) and females (N_{bf}) for each collection method (Lande and Barrowclough, 1987) utilizing our empirical estimates of male and female reproductive variance from known (DGT) or inferred (NPE and DL) pedigree information as:

$$N_{bm} = \frac{(N_m k_m - 1)}{k_m + \left(\sigma^2_{km}/k_m\right) - 1}$$

and

$$N_{bf} = \frac{\left(N_f k_f - 1\right)}{k_f + \left(\sigma^2_{kf}/k_f\right) - 1}$$

respectively, where k is the mean number of progeny produced by either the males (k_m) or female (k_f) for each collection method, and σ^2 is the variance in the number of progeny for males or females. The variance population size (N_{bv}) for each treatment was then calculated (Lande and Barrowclough, 1987) as:

$$N_{bv} = 4\left[\frac{1}{N_{em}} + \frac{1}{N_{ef}}\right]^{-1}$$

Second, we used single sample linkage disequilibrium estimator that has been commonly used (Luikart et al., 2010), to estimate N_b using multi-locus offspring genotypes. The program LDNE (Waples and Do, 2010) was used. LDNE provides an estimate of mean N_b and 95% confidence intervals based on a jackknife procedure over loci. Because rare alleles can result in upwardly biased of N_b, alleles with frequencies less than 0.02 were excluded from the estimate (Waples and Do, 2010). We used simulated data for the DGT offspring as described above for N_b estimation. Confidence intervals were used as a means to evaluate statistical significance of differences in N_b means between gamete collection methods and hatchery rearing environments.

2.6.5. F-statistics

Estimates of F_{ST} (a measure of standardized variance in allele frequency) were calculated between collections of naturally produced eggs and dispersing larvae using FSTAT version 2.9.3 (Goudet, 2001). Per locus estimates of F_{ST} were tested for significance by jackknifing across populations and the overall estimate of F_{ST} between the collection methods was tested for significance by jackknifing across loci.

3. Results

3.1. Adult spawner abundance, spawning chronology, reproductive contributions and genetic diversity

Adults did not all spawn in the same location or at the same time and numbers of available adults varied by year (Fig. 1; Fig. 2; Table 1; Forsythe, 2010). This aspect of the adult reproductive behavior had implications to the total number of adults that contributed to offspring (N_m:N_f: Table 1) based on different sampling strategies among collection methods. Sampling focused on specific sets of adults or locations for DGT and NPE and collections of DL offspring, which were collected below the downstream most spawning locations (Fig. 1), were expected to reflect contributions of adults spawning throughout the reproductive season. For example, the timing of when gametes were collected from individual adults (DGT indicated by an asterisk; Fig. 2) and the duration of when NPE eggs were collected (indicated by arrows; Fig. 2) exemplifies issues facing managers. NPE egg collections were conducted following major spawning events. However, collections of NPE eggs [e.g., timing (Fig. 2) and location (Fig. 1)] did not represent an exhaustive attempt to collect eggs from all known spawning areas, nor did collections cover all times of the spawning season, but represented a level of effort that was logistically feasible. Gametes were obtained for DGT offspring from 26.6, 14.5, and 20.2% of the total number of adults collected during 2005, 2006, and 2007, respectively (Table 1). In the two years genetic parentage data was available for offspring from NPE collections (2006 and 2007), 23.1 and 9.6% of adults captured were estimated to have contributed to offspring (Table 1). Greater numbers of adults were estimated to have contributed to DL offspring (81.6, 68.3, 90.8% in 2005, 2006, and 2007, respectively; estimates of N_m:N_f relative to N_{AD}; Table 1).

Estimated measures of genetic diversity for spawning adults were relatively high for the years of study. Mean number of alleles per locus (range 5.3–5.5) mean expected multi-locus heterozygosity (range 0.591–0.587) and combined (multi-locus) probabilities of non-parental pair exclusion (5.66×10^{-5}) suggest the 12 loci surveyed provided discriminatory power to conduct analyses. Genotypic frequencies did not deviate ($P>0.05$) from Hardy–Weinberg expectations and we observed no deviance from disomic patterns of inheritance at any locus. Pooled individual heterozygosities from individuals collected using each of the collection methods were not significantly different from pooled adult heterozygosities ($t=1.96$, $df=814$, $P=0.82$; mean of 0.55).

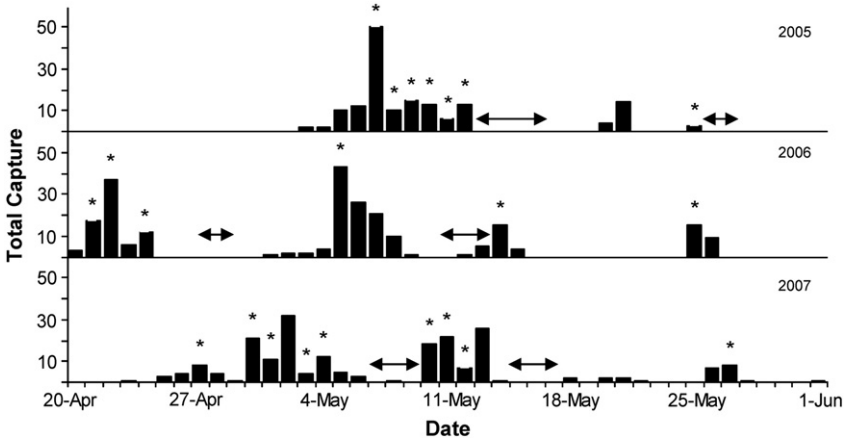

Fig. 2. Total number of adult lake sturgeon captured by day on the spawning grounds of the Upper Black River from 2005 to 2007. Asterisks represent days when female and male gametes (DGT) were collected from spawning adults and fertilized within each year. Arrows define periods when naturally produced eggs (NPE) were collected from the stream substrate. Collections of naturally produced eggs are assumed to represent a random sample of adults spawning during major spawning events immediately prior breeding in the time period prior to collection efforts.

Table 1
The number of males and females (N_m:N_f) contributing to offspring sampled and estimates (Mean ± 1 SD) of reproductive contributions by males and females. Reproductive contributions are based on known pedigree information or inferred from genetic determination of parentage for juvenile lake sturgeon. Offspring were collected using three different methods and raised in two hatchery environments (streamside and traditional) during each of 3 years.

Hatchery	Variable	Year		
		2005	2006	2007
Direct gamete takes (DGT)				
	Total N	174,839	125,433	50,170
Streamside	Mean # of eggs/female[a]	6324 ± 5056	6478 ± 4857	3345 ± 2030
	Mean # of larvae/family	669 ± 666	863 ± 820	344 ± 215
	N_m:N_f [Total N_{AD}[b]]	26:15 [154]	22:12 [234]	27:15 [208]
Traditional	Mean # of eggs/female[a]	7271 ± 5415	5963 ± 4812	–
	Mean # of larvae/family	519 ± 476	549 ± 420	–
	N_m:N_f [Total N_{AD}]	22:11 [154]	16:8 [234]	–
Naturally produced eggs (NPE)				
	Total N	1100	1130	275
Streamside	Mean # of offspring/female	–	1.3 ± 0.6	1.6 ± 0.7
	Mean # of offspring/male	–	1.3 ± 2.0	1.3 ± 0.7
	N_m:N_f [Total N_{AD}]	–	20:20 [234]	11:9 [208]
Traditional	Mean # of offspring/female	–	1.50 ± 0.9	–
	Mean # of offspring/male	–	1.33 ± 0.7	–
	N_m:N_f [Total N_{AD}]	–	9:8 [234]	–
Dispersing larvae (DL)				
	Total N	7800	5500	1400
Streamside	Mean # of offspring/female	5.6 ± 5.9	1.73 ± 1.2	2.2 ± 1.3
	Mean # of offspring/male	3.1 ± 3.5	1.22 ± 0.6	1.5 ± 0.8
	N_m:N_f [Total N_{AD}]	81:44 [154]	101:59 [234]	133:56 [208]
Traditional	Mean # of offspring/female	3.7 ± 4.2	3.0 ± 3.4	–
	Mean # of offspring/male	2.6 ± 2.0	1.67 ± 1.1	–
	N_m:N_f [Total N_{AD}]	55:37 [154]	78:43 [234]	–

[a] Mean total egg number was calculated using an egg number per volume relationship(52.5 eggs per 1 ml).
[b] Total number of adults captured on the spawning grounds in each of three years.

3.2. Comparisons of adult reproductive contributions among collection methods and hatcheries

We collected large numbers of eggs and larvae using all three collection methods, though numbers differed considerably among methods and were variable across years (Table 1). The highest numbers were realized using the DGT method (Total N = 174,839, 125,433, 50,170 in 2005, 2006, and 2007, respectively). Comparably lower egg numbers were collected from the stream substrate (NPE method; Total N = 1110, 1130, and 275 in 2005, 2006, and 2007, respectively). Large variation was also observed in DL sample numbers among years (Total N = 7800, 5500, 1400 for years 2005, 2006, 2007, respectively) despite relatively consistent adult spawning numbers (N_{AD} = 154, 234, 208 for years 2005, 2006, and 2007, respectively; Table 1; Fig. 2) and similar sampling effort each year.

The number of offspring contributed per male and female was much higher for the DGT method (Table 1) compared to samples obtained using NPE and DL methods, as anticipated given the large numbers of gametes collected from a relatively small number of females (Table 1). However, we documented higher variation in male and female contributions to offspring produced based on individuals in the DGT method (Table 1). We documented significant differences in mean egg numbers collected from females across years ($F_{2,40} = 4.44$, $P = 0.02$, Table 1) which is not surprising given that we were collecting gametes from fish in the act of spawning. We observed high inter-female variance in egg survival and high inter-male variance as seen by large SE about the mean for many females from direct gamete take collections (Fig. 3). Variance among females in egg numbers collected and egg survival to hatch contributed to estimates of the effective number of breeding adults and coancestry (Table 2).

A Fisher's exact test revealed no significant differences in the number of offspring contributed per individual adult male or female between the NPE and DL methods in either 2006 (Male: $P = 0.15$; Female: $P = 0.95$) or 2007 (Male: $P = 0.25$; Female: $P = 0.77$). There was no trend in the number of mates per male or female based on collections using NPE or DL methods based on genetic determination of parentage. Egg survival to hatch from paternal half-sib family groups reared from collections from the DGT method were significantly higher at the streamside hatchery in 2006 compared to the traditional hatchery environment in the same year and were higher than hatching rates in 2005 ($F_{3,42} = 3.39$, $P = 0.03$).

3.3. Parentage allocations

We had higher overall parentage assignment for offspring from the DL method compared to offspring from the NPE method in both years. Parentage assignment obtained from each program, PASOS and CERVUS, was high for offspring from both gamete collection methods in both years. Assignment rates from PASOS ranged 72–83%, and 86–92% from CERVUS, in which concordance in parentage assignment between the two programs was 72, 73, and 79% for DL in years 2005, 2006, and 2007 respectively while NPE was 70 and 67% in years 2006 and 2007. The final proportion of offspring assigned to both parents was 58–59% for the DL method and was similar across years while the proportion of NPE larvae assigned to parents was 58 and 48% in years 2006 and 2007, respectively.

Parentage data from NPE and DL offspring identified that 100, 92, and 93% of adult females that we directly removed gametes from reproduced naturally in years 2005, 2006 and 2007, respectively. Based on parentage analysis, females contributing gametes for direct gamete takes also contributed to natural recruitment and mated with different males than we used for hatchery (DGT) crosses. We also identified 84, 61, and 89% of adult males that we directly removed gametes from reproduced naturally in years 2005, 2006, and 2007, respectively. Direct egg takes did not appear to detract from female or male abilities to spawn naturally, though we were unable to determine whether spawning occurred prior to or following collection of gametes.

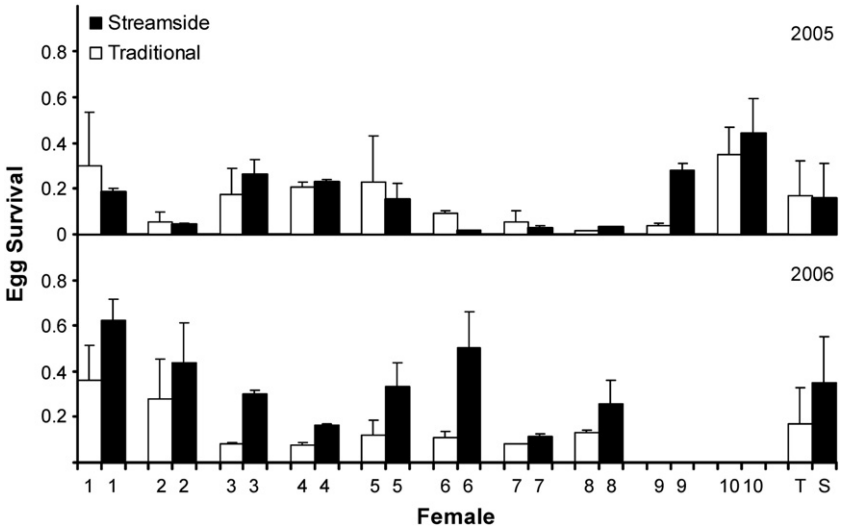

Fig. 3. Lake sturgeon egg survival (mean ± 1SE) to hatch when incubated in two different hatchery environments (streamside and traditional) in each of two years by female. Different females were used each year. Standard errors were estimated across half-sib groups from eggs fertilized using sperm from each of two males per female. Total egg survival within each hatchery environment incorporating all females is provided in each year for traditional (T) and streamside (S) hatchery environments.

3.4. Comparisons of measures of offspring genetic diversity among collection methods and hatcheries

The microsatellite loci used showed moderate levels of polymorphism in offspring, with 2 to 9 alleles observed per locus. Genotypic frequencies at all 12 loci did not deviate from Hardy–Weinberg expectations in offspring from any collection method or year. Mean number of alleles per locus and observed heterozygosity were consistently high (5.5, 0.55 and 5.42, 0.57, respectively for DL samples and 5.5, 0.56 and 5.25, 0.57 for NPE in years 2006 and 2007, respectively). There were no significant differences in individual observed heterozygosities between larvae from NPE or DL collection methods in either 2006 ($t = 1.97$, $df = 248$, $P = 0.12$) or 2007 ($t = 1.98$, $df = 122$, $P = 0.85$).

Estimating the change in θ and N_b across sequential life stages and identifying factors contributing to increasing θ and decreasing N_{bv} is important in supplemental progeny reared in hatcheries, but is seldom quantified because eggs of multiple females are typically combined during incubation. We determined levels of coancestry (θ) for lake sturgeon progeny obtained using each of three collection methods based on known (DGT) or assigned (NPE and DL) parentage. For each year, estimates of θ were consistently highest for offspring obtained from the DGT method, and comparatively lower in offspring from NPE and DL methods (Table 2). For example, coancestry estimates in 2006 were >0.03 for individuals collected using DGT compared to individuals collected using NPE (<0.02) and DL (≤0.005) in the same year. For offspring collected using the DGT method we also found that offspring θ was consistently higher at the traditional hatchery compared to the streamside hatchery during both 2005 and 2006.

We documented an increase in θ between the egg stage and larval stage for offspring collected using the DGT method across all years and in both hatchery environments (Table 3). We also documented the same increasing trend in θ across different ages at the streamside hatchery environment over two years (Table 2).

The direction and magnitude of differences in mean relatedness of offspring among collection treatments estimated based on genotypic data were highly concordant with estimated mean coancestries (mean $r_{xy} = 2 *$ mean θ, Malécot, 1948; Table 2). Relatedness estimates of DGT offspring reared in the streamside hatchery differed significantly from offspring from DL offspring in 2 of 3 years and from the traditional hatchery in both years (2005 and 2006) rearing occurred there (Table 2). Relatedness of offspring from the NPE treatment were

Table 2
Summary measures of genetic diversity for offspring obtained using direct gamete takes (DGT), collection of naturally produced eggs from the substrate (NPE), and larvae dispersing downstream from spawning grounds (DL). Estimates are presented for offspring raised in each of two hatchery environments (streamside or traditional). Letters (a, b, c) associated with mean relatedness (r_{xy}) correspond to statistical differences ($P<0.05$) between collection methods and hatcheries. Estimates of coancestry were calculated empirically using known or inferred pedigree data. Effective population size estimates include calculations incorporating both multi-locus genotypic data (N_b and 95% CI) and male and female reproductive variance (N_{bv}) based on known or inferred pedigree data.

		Streamside hatchery			Traditional hatchery		
		Collection method			Collection method		
Year	Genetic variable	DGT	NPE	DL	DGT	NPE	DL
2005	Mean r_{xy} (variance)	0.067^{a} (0.051)	–	0.068^{a} (0.034)	0.071^{b} (0.060)	–	0.039^{a} (0.037)
2006	Mean r_{xy} (variance)	0.082^{c} (0.051)	0.065^{b} (0.066)	0.008^{a} (0.056)	0.079^{b} (0.057)	0.052^{b} (0.027)	0.011^{a} (0.028)
2007	Mean r_{xy} (variance)	0.032^{b} (0.043)	0.027^{ab} (0.030)	0.015^{a} (0.027)	–	–	–
2005	Coancestry	0.029	–	0.008	0.033	–	0.009
2006	Coancestry	0.03	0.003	0.005	0.043	0.019	0.004
2007	Coancestry	0.019	0.012	0.005	–	–	–
2005	N_b (95% CI)	11.0 (8.6–13.5)	–	28.2 (22.9–34.5)	11.8 (9.6–14.2)	–	41.3 (33.3–51.5)
2006	N_b (95% CI)	12.9 (10.7–15.3)	88.0 (39–1985)	146.8 (103–224)	7.2 (5.6–8.8)	43.0 (17.9–inf)	103.3 (67.4–186.7)
2007	N_b (95% CI)	16.3 (13.4–19.4)	31.2 (17.8–73.4)	126 (105–150)	–	–	–
2005	N_{bv}	29.4	–	101.5	26.7	–	120.3
2006	N_{bv}	26.2	95.9	176.7	15.0	35.8	247.9
2007	N_{bv}	38.6	45.7	181.3	–	–	–

Table 3
Comparisons of sample size (N) and parametric estimates of genetic diversity including coancestry (θ) and effective breeding population size (N_{bv}) for individuals sampled at different developmental stages produced from direct gamete takes (DGT) over each of three years. Estimates are presented for individuals raised in streamside or traditional hatchery environments.

Developmental stage	Year	N	θ	N_{bv}
Streamside hatchery				
Egg stage[a]	2005	94,856	0.020	38.1
Larval stage[a]		15,383	0.029	29.4
56 days		1500	0.034	29.4
90 days		1500	0.040	29.4
Traditional hatchery				
Egg stage[a]	2005	79,983	0.026	29.3
Larval stage[a]		10,371	0.033	26.7
Streamside hatchery				
Egg stage[a]	2006	77,731	0.024	31.7
Larval stage[a]		19,129	0.030	26.2
40 days		1000	0.032	26.2
140 days		1000	0.041	26.3
Traditional hatchery				
Egg stage[a]	2006	47,702	0.037	21.3
Larval stage[a]		9894	0.043	15.0
Streamside hatchery				
Egg stage[a]	2007	50,170	0.017	38.6
Larval stage[a]		13,243	0.019	38.6

[a] Egg and larval stage refer to times of fertilization and hatch, respectively.

consistently lower than DGT offspring and generally significantly higher that DL offspring.

Estimates of the effective number of breeders (N_{bv}) were highest for offspring from the DL collection method compared to both DGT and NPE methods (Table 1). This trend was consistent across both years where comparative data were available. For example, in 2007 the estimates of N_{bv} were 38 for offspring collected using DGT, 45 for NPE, and 181 for offspring collected using the DL method. The estimated number of contributing adults was higher for offspring from the DL samples compared with offspring collected using DGT and NPE (Table 1) and therefore directly influenced the estimate of N_b (Table 2). The effective number of adults (N_{bv}) contributing to offspring also decreased across life stages (Table 3). Estimates were similar for offspring reared in different hatchery environments, but varied greatly based on method of collection (Table 2). The direction and magnitude of difference in estimated effective number of breeders (N_b) among collection treatments were highly concordant with N_{bv} estimates (Table 1). Estimates of N_b for offspring collected using the DGT method were significantly lower that estimates based for offspring collected using the NPE and DL methods in all years data were available (Table 1).

Multi-locus estimates of F_{ST} between collections of individuals obtained using the NPE and DL methods within a year were significant [0.019 (95% CI 0.009–0.029) in 2006 and 0.016 (95% CI 0.008–0.024) in 2007], indicating that offspring represented different subsets of adults or different proportional adult contributions.

4. Discussion

Hatchery programs require empirical evaluations of the relative merits of different collection methods for progeny that are to be reared and released into the natural environment. Lake sturgeon restoration programs throughout the species range have been initiated (Holey et al., 2000) despite considerable uncertainty regarding the effects gamete and larval collection methods have on levels of genetic diversity in hatchery-reared juveniles. If conservation hatchery programs are to be emphasized in lake sturgeon recovery planning, then alternative progeny collection methods should be evaluated empirically so the benefits of alternative management actions can be quantitatively assessed.

Over the past few decades there has been considerable concern expressed over artificially increasing wild population abundance and effects on long-term genetic variation (Tringali and Bert, 1998). Molecular methods have been used in captive programs to determine pedigrees in an effort to minimize levels of coancestry between adults used in artificial crosses (Ballou and Lacy, 1995; Wang, 2004). However, in programs that use limited wild broodstock of unknown pedigree, there is a need to determine the degree of genetic diversity that can be retained using collection measures that focus on different life history stages. Comparing measures of genetic diversity of lake sturgeon progeny obtained using three collection methods allowed for quantification of levels of genetic diversity conditional on numbers and the relative reproductive contributions of adults. Data from this study provide a framework to assess which method might be most effective in retaining genetic diversity present in the wild population. The relative benefits and risks associated with each collection method provide general guidelines that have direct bearing on future restoration efforts for lake sturgeon and other long-lived iteroparous fish species.

4.1. Collection methods affect measures of genetic diversity in progeny

The large numbers of progeny used in hatchery programs (Table 1) were not reflective of offspring levels of genetic diversity (Table 2). Collections of gametes directly from spawning adults (DGT) provided the highest initial number of progeny. However, despite the greater abundance of offspring from DGT collections, measures of genetic diversity were lower than for offspring from NPE and DGT collections (Table 2). The biological significance of differences in measures of diversity documented among collection methods are based on several factors. First, there was a high degree of concordance between measures of diversity estimated using two fundamentally different analytical approaches. Both coancestry and the effective number of breeding adults were estimated from known or inferred pedigree information and from multi-locus genotypic data directly. Concordance in magnitude and direction of difference across these independent methods speaks strongly to the significance of the results. Lastly, the direction and magnitude of differences between collection methods were concordant across years despite the fact that different sets of adults contributed to offspring in each year.

4.1.1. Collection methods influence mean levels of relatedness in offspring

Differences in mean coancestry and relatedness were estimated for individuals acquired using each collection method. Estimates of coancestry and relatedness were comparatively higher for offspring from DGT compared to both NPE and DL methods (Table 2). Differences can be attributed to several factors including the lower total number of adults contributing to DGT offspring relative to adult numbers contributing to samples from collections of NPE and DL samples (Table 1) and the high among family variance in family size (Fig. 3).

Hatchery environments typically increase both fertilization success and survival at critical life history stages (eggs and larvae) when compared to survival in the wild (Secor and Houde, 1998). However, for lake sturgeon, the number of eggs that can be collected typically varies among females not due to size-specific differences in fecundity but due to heterogeneity in the ability to collect eggs. After fertilization, reproductive variance was comparatively higher among females in the traditional hatchery environment relative to the streamside environment due to higher and family-specific rates of mortality during the egg and early larval periods (Fig. 3). This could be attributed to increased adaptation to natural temperature fluctuations compared to fixed temperature regimes reflective of a traditional hatchery. Though the number of adult females that we successfully spawned was high relative to other lake sturgeon studies throughout the Great Lakes (Folz et al., 1983; Anderson, 1984; Ceskleba et al.,

1985; Pyatskowit et al., 2001), adult numbers were comparably lower relative to the numbers of adults that provided offspring captured during the period of larval dispersal (N_m:N_f in Table 1) and estimates of the effective number of breeding adults (N_b and N_{bv} in Table 2) were even lower.

Naturally produced eggs were collected over relatively small areas from substrates associated with locations of two spawning groups each year (Fig. 2). Greater sampling effort for naturally produced eggs temporally across the spawning period (Fig. 2) or spatially across all 6 known spawning areas in the upper Black River (Fig. 1; Forsythe, 2010) could have resulted in reproductive contributions from a greater number of adults. Importantly, progeny from NPE and DL samples were analyzed from tissue samples collected during the juvenile stage. Therefore, these estimates of θ refer to juveniles at older ages compared to those estimates produced for DGT offspring at both the egg and the early (post hatch) larval stage (Table 2). The increase in estimates of θ during the rearing process could have occurred if different families had different competitive abilities (and survival) in the hatchery environment. Both the NPE and DL methods resulted in lower mean larval coancestry relative to offspring collected using the DGT method (Table 1) and should be considered for populations where a limited number of adults are available for gamete collection.

4.1.2. Collection methods influence effective number of breeding adults

Estimates of the effective number of breeding adults is a predictive measure of generational changes in allele frequency, heterozygosity, and inbreeding that has been widely used in fisheries management when evaluating supplementation prescriptions for wild populations (e.g., Ryman and Laikre, 1991). Estimates of effective breeding number were considerably higher for the DL samples compared to both the NPE and DGT samples (Table 2). Greater numbers of adults contributed to individuals collected using the DL method in each year. The importance of keeping family groupings separate through the rearing process so that individual contributions can be determined from each pairing of adults has been emphasized in previous work (Allendorf, 1993; Fraser, 2008). Logistically, this may be challenging due to high numbers of breeders, and limited tank space. However, managers should recognize that variance among family groups will be high (e.g., Fig. 3), and projections of effective breeding number based just on numbers of males and females spawned will lead to over-estimation of N_b (Page et al., 2005).

4.1.3. Variance in offspring allele frequency indicates different subsets of adults contribute to offspring represented in different collection methods

Estimates of F_{ST} represent a measure of genetic differentiation among groups (Whitlock and McCauley, 1999), and were used as a measure of deviance in parental contributions of offspring collected using the different methods. Multi-locus estimates of F_{ST} between offspring obtained from collection methods NPE and DL were significantly different from zero indicating that different subsets of adults were contributing to offspring collected using each method and/or that the proportional contribution of adults varied. Genetic differentiation between the two groups of offspring indicates methods of gamete and larval collection should sample from many spawning areas (adequate spatial coverage) or spawning runs (adequate temporal coverage) to increase probabilities of retention of levels of genetic diversity present in the breeding adult population. Differences could also reflect variable (among family) rates of mortality in the stream through the incubation and post-emergence period as observed in the hatchery (Fig. 2).

Collecting gametes directly from spawning adults for use in sturgeon hatchery conservation programs may provide the numerical abundance of offspring required to meet stocking objectives. However, there are several important aspects of direct gamete takes to consider. Current and past practices have been to remove gametes from a female, fertilize with two or more males, and transfer and incubate eggs in large mixed groups to limit the number of incubation vessels and therefore reduce maintenance. Duration of egg takes around the Great Lakes has traditionally been dependent upon a number of factors including the number of spawners present, and the total amount of eggs required. Hatchery limitations (including space and personnel) may dictate the number of gametes collected. Sturgeon are highly fecund (Beamesderfer and Farr, 1997) and often produce over 150,000 eggs in the hatchery environment (R. Ek, Freshwater Fisheries Society of British Columbia, Unpublished Data). This allows a relatively few number of adults to contribute a high proportion of the supplemental progeny if absolute gamete number is the sole criteria. Collection of milt from a large proportion of males is possible and tagging ensures that the same male is not reused. If logistical constraints limit the ability to monitor families through the entire hatchery rearing process then equalizing family contributions at the egg stage and if possible at hatch should be an important consideration for conservation programs (Allendorf, 1993).

4.2. Rearing environment affects measures of genetic diversity

Hatchery rearing environments have been shown to produce progeny that are less variable genetically and biologically less fit than their wild counterparts (Waples, 1991; Ford, 2002; Miller et al., 2004; Araki et al., 2007). We compared two rearing environments, a traditional hatchery and a novel streamside hatchery. Estimates of θ for DGT offspring were higher at the traditional hatchery compared to the streamside hatchery despite equal numbers of eggs from each paternal half-sib family group represented at both hatcheries. However, variation in survival among family groups through the egg stage to hatch at both hatchery environments (Fig. 3) resulted in increases in θ for DGT offspring as a function of age from the time of hatch through the period of rearing (Table 3). Therefore, estimates of the total number of eggs sampled per family cross (Table 1) are not predictive of mean levels of coancestry or effective population size of offspring at release (Fig. 3). Levels of coancestry will increase with inter-family variation in egg and/or larval survival.

Differential survival was more pronounced at early stages (eggs, larvae, and juveniles) among family groups and persistence of this pattern throughout the rearing process led to increases in θ (Crossman, 2008). We documented consistently higher levels of θ at the traditional hatchery relative to the streamside hatchery (Table 3). This result is attributed to differential survival among family groups during the rearing process with certain half-sib family groups contributing at significantly higher proportions to the supplemental progeny (Crossman 2008). Studies on other species (Herbinger et al., 1995; Unwin et al., 2003) have indicated family group accounts for a large proportion of the variation in survival during early life stages. Increases in the inter-relatedness between supplemental progeny over time in the hatchery, combined with differences attributed to hatchery rearing environment, are important based on results demonstrating higher survival of streamside reared lake sturgeon released at earlier ages (Crossman et al., 2010). This provides further evidence for the benefits of rearing supplemental lake sturgeon progeny at streamside hatcheries when releases are to occur at earlier ages. Differences between hatchery rearing environments could influence survival as different genotypes could be favored in different environments.

4.3. Handling effects on natural reproduction

Handling-induced stress can have wide ranging effects on fish including nest abandonment (e.g., several species of bass (Suski et al., 2003; Hanson et al., 2007; Siepker et al., 2007) and interruption of spawning migration due to incidental net capture or tagging (e.g., several sturgeon species (Moser and Ross, 1995; Schaffter, 1997; Kynard et al., 2002; Benson et al., 2007). Observations of interrupted upstream

migration of sturgeons are hypothesized to indicate that adults abandon spawning however the majority of these observations are based on tagging and telemetry data. It is still unknown whether females that are subjected to partial gamete removal, either through hand stripping or induced ovulation, continue on to spawn naturally after incurring this stress. We documented through genetic determination of parentage that a large number of the females used for direct removal of gametes successfully spawned in the wild, though it is unknown whether reproduction occurred either prior to or following handling and direct gamete removal. Results are not only encouraging for gamete removal from actively spawning females but also suggest that handling for purposes of marking and collection of biological data may not affect an individual's ability to contribute to natural recruitment. High fecundity in this species allows for a relatively small amount of eggs to be taken for hatchery rearing while allowing females to contribute to natural recruitment.

4.4. Applicability of results to other systems

Application of methods evaluated in our study in other systems will require knowledge regarding the timing, duration, and locations of spawning activity, which will vary among systems. The number of eggs we obtained through direct gamete takes represents a maximum given resources available as we attempted to collect gametes from all spawning females, some on multiple occasions. For the DGT treatment we used a partial factorial mating system designed to minimize probabilities of future inbreeding. Levels of offspring diversity based on the DGT treatment relative to DL and NPE treatments may have resulted from factors associated with the collection method as well as the specific mating design. Polyandry (females mating with multiple males) has been shown to decrease mean coancestry in offspring (Chesser, 1991) but not in a linear manner (i.e., when many males are involved). Higher levels of background coancestry will result if large numbers of males are used which will lead to greater probabilities of inbreeding in future generations. Use of higher numbers of males per female will increase adult representation in progeny (the effect of mating strategy). However, variance in offspring survival across families (the effects of collection method) was high. Thus, the results based on the factorial design employed are likely to be robust if different numbers of males were used. In systems where DGT is the most applicable collection method and the number of available breeders is limited, tradeoffs between the effective number of breeders used based on the number and sex ratio of adults available and expectations of coancestry in supplemental progeny should be evaluated when designing or revising conservation programs.

All three of the collection methods evaluated are labor intensive requiring both field and hatchery components. Greater numbers of naturally produced eggs could have been obtained if additional effort was made to increase the number of spawning sites sampled. Collection numbers of dispersing larvae could have been higher if additional nets and personnel were employed. Furthermore, collecting dispersing larvae has been shown to be an effective method in a number of rivers (Kynard et al., 1999; Auer and Baker, 2002; Smith and King, 2005). Collecting eggs, whether from spawning adults or from the stream substrate, requires effort during the incubation stage to reduce mortalities and improve hatching success. Capturing dispersing larvae bypasses the incubation stage by collecting offspring that are developed to the extent where they can begin feeding immediately upon entry into the hatchery environment. One positive aspect of rearing progeny at a streamside hatchery is that the facility can be designed based on the restoration needs of a specific system or population.

Methods and results described in this study provide a framework for evaluating alternative strategies for managers designing and implementing conservation programs for lake sturgeon and other long-lived iteroparous species. To ensure high levels of genetic diversity in offspring supplemented into natural populations, programs should focus on developing collection methods that incorporate aspects of the species reproductive ecology to ensure they adequately capture the genetic diversity of the adult breeding population. Populations of lake sturgeon are composed of a mixture of individuals that reproduce at different times and in different locations (Forsythe, 2010), and collection of gametes from adults captured from a finite number of locations and times will limit the diversity represented in offspring, including traits adapted to environmental conditions where and when adults reproduce (Hendry and Day, 2005). For lake sturgeon, a species that has undergone significant reductions in abundance, conservation programs should be designed based on rigorous evaluations of the most effective methods required to collect, rear, and stock progeny back into the natural environment. In this study, we found that collecting dispersing larvae downstream of the spawning grounds minimized estimates of coancestry while at the same time increased the effective number of breeders. Differences between hatchery rearing environments have the ability to influence the genetic variation observed in the progeny.

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