EVALUATING COLLECTION, REARING, AND STOCKING METHODS FOR LAKE STURGEON (*ACIPENSER FULVESCENS*) RESTORATION PROGRAMS IN THE GREAT LAKES

By

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ABSTRACT

EVALUATING COLLECTION, REARING, AND STOCKING METHODS FOR LAKE STURGEON (ACIPENSER FULVESCENS) RESTORATION PROGRAMS IN THE GREAT LAKES

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Lake sturgeon (*Acipenser fulvescens*) are native fishes of the Great Lakes that have been numerically depressed throughout their range due to anthropogenic factors such as over-harvesting, degradation in water quality and spawning habitat and loss of connectivity due to impoundments. Management and conservation strategies have been implemented throughout the Great Lakes that focus on restoring remnant populations through supplementation of hatchery produced juveniles. Alternative management prescriptions have been advocated, largely in the absence of data, focusing on the best way to collect gametes/larvae, the use of different hatchery rearing environments, and the appropriate size or age of fish to stock. I determined the effects of different gamete/larval collection methods, rearing environments, and stocking strategies on juvenile lake sturgeon growth, survival, and levels of genetic diversity. I used three methods to collect lake sturgeon progeny, 1) direct gamete takes from spawning adults, 2) collection of naturally produced eggs from the stream substrate, and 3) collection of larvae dispersing downstream from adult spawning grounds. Lake sturgeon offspring were reared in two environments, half in a streamside hatchery constructed on the natal Upper Black River and half at a traditional hatchery in southern Michigan. I also examined how hatchery

rearing environment and age affect survival and movement following release. Progeny from each hatchery were marked and released at 8, 13, and 17 weeks of age. Overwinter mortality was quantified by surgically implanting 20 juvenile lake sturgeon from each hatchery with ultrasonic transmitters at 6 months of age and releasing them in late fall. Finally, I investigated the effects of four predators on survival, behavior, and habitat preference of three age classes of juvenile lake sturgeon reared in two hatchery environments. I found that collecting dispersing larvae downstream captured the highest amount of genetic diversity present in the adult breeding population. Streamside rearing and the addition of habitat complexity within rearing tanks improved survival and maintained natural variation in the size of lake sturgeon offspring. Significantly higher rates of recapture were realized for progeny reared in the streamside hatchery compared to the traditional hatchery at 8 and 13 weeks of age. Recapture rates and dispersal distances were significantly higher for 17 week old fish compared to releases of earlier ages. Large body size was negatively correlated with timing of movements across all ages. Overwinter survival of juvenile lake sturgeon was 40% irrespective of rearing location. Predation by crayfish is likely to be a significant source of mortality for juvenile lake sturgeon up to three months of age. Results demonstrate that non-lethal indirect effects potentially contribute to higher rates of mortality between alternate predator types. Work described in thesis provides a framework for evaluating alternative strategies for managers designing conservation programs for lake sturgeon and other long-lived iteroparous species. Results indicate that supplementation protocols for lake sturgeon should be developed on a site specific basis and demonstrate the importance of hatchery rearing environment.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Lake sturgeon (*Acipenser fulvescens)* are a native fish of the Great Lakes that have undergone significant reductions in population abundance over the past century resulting from over-harvest, habitat loss and degradation, and loss of habitat connectivity (Holey et al. 2000). Currently, it is estimated that lake sturgeon are present at approximately 1% of historical levels (Hay-Chmielewski and Whelan 1997). Lake sturgeon restoration and protection has become a high priority for all state, federal, and tribal management agencies throughout the Great Lakes. The lake sturgeon receives protection to varying degrees throughout their range at the state and provincial levels, listed as threatened, endangered, or of special concern (Welsh 2004). Management and conservation strategies have been implemented throughout the Great Lakes that focus on restoring remnant populations through supplementation of hatchery produced juveniles (Holey et al. 2000). Unfortunately, there are numerous impediments that complicate current lake sturgeon restoration and recovery efforts, including the presence of dams on rivers that historically supported spawning runs, habitat alterations, low spawner abundance, and the species' unique life history characteristics (late age at maturity, infrequent spawning, and low recruitment rate). Lake sturgeon exhibit delayed sexual maturation, ranging between 15 to 25 years (Houston 1987) which greatly complicate efforts to document effects of hatchery supplementation on recruitment into the adult breeding population. Once lake sturgeon mature, spawning occurs intermittently with males spawning every one to two years and females spawning less frequently, every three

to seven years depending on age (Auer 1999; Beamish et al. 1996; Forsythe, unpublished data).

Hatchery programs have been widely advocated as a viable means to enhance declining wild fish populations, but are receiving increased scrutiny because of the potential negative impacts of captive reared individuals to natural populations (Ford 2002; Araki et al. 2007a; Miller et al. 2004). Criticisms have been raised based on empirical evaluations of the relative effects of different management options including; hatchery rearing environments (Berejikian et al. 2000), age of release into the natural environment (Paragamian and Kingery 1992), and gamete, juvenile, or broodstock collection and maintenance protocols (Flagg and Nash 1999). Currently, most scientific data on hatchery fish are based on literature from a limited number of species that have very different ecologies than lake sturgeon. Data on the complexities and inter-dependencies among components of natural ecosystems have become more clearly defined for well studied fish such as salmonids (Fraser 2008). In light of recent findings, empirical and experimentally-based evaluations of hatchery rearing and stocking programs are needed to develop and incorporate new data based on the biology of lake sturgeon into scientifically-based management programs.

Research is needed directed at determining the most effective culture and stocking techniques that will repatriate lake sturgeon and also ensure the persistence of remnant populations existing around the Great Lakes. My dissertation research empirically determined the effects of different gamete/larval collection methods, hatchery rearing environments, and stocking strategies commonly used by management agencies on juvenile lake sturgeon growth, survival, movements, and levels of genetic

diversity. In my first chapter I investigate the effects of different gamete/larval collection methods on levels of genetic diversity using modern molecular approaches. In the second chapter I quantified and compared growth and survival rates of juvenile lake sturgeon acquired using three different gamete/larval collection methods and reared in two different hatchery environments. The hatchery environments I evaluated included a streamside hatchery constructed on the natal river and a traditional hatchery environment located on a non-natal water source. In chapter 3, I quantified survival and movements of juvenile lake sturgeon reared in two different hatchery environments and released at three different ages. In chapter 4, I quantified overwinter survival of age-0 lake sturgeon using ultrasonic telemetry. In addition I examined overwinter survivorship between fish reared in two different hatchery environments. In my fifth chapter, I investigated the effects of four different predators on survival, behavior, and habitat preference of three age classes of juvenile lake sturgeon reared in two different hatchery environments. I discuss the significance of my results in terms of designing and managing lake sturgeon restoration programs throughout the Great Lakes.

Chapter 1

EVALUATION OF GAMETE AND LARVAL COLLECTION METHODS FOR LAKE STURGEON; EFFECTS ON GENETIC DIVERSITY

INTRODUCTION

Hatchery supplementation has been employed as a management prescription for the last century in order to augment existing stocks (Cowx 1994; Waples 1999), mitigation for habitat destruction (Waples 1991), enhance fishing opportunities through introduction of desirable sport fish (Peck et al. 1999; Heidinger 1999), and to conserve threatened populations (Waples 1991; Ryman 1991). Traditional hatcheries have developed efficient processes to maximize progeny production. Over the past decade, conservation hatchery programs are increasingly being used to facilitate the recovery of threatened or endangered species (Ryman et al. 1995; Brown and Day 2002). The role of a conservation hatchery is to assist in the recovery of population by minimizing the probability that the genetic and ecological impacts of supplemental progeny on the wild stock will be negative (Flagg and Nash 1999).

Due to the large numbers of fish produced in hatcheries and subsequently stocked into natural environments, there is great concern about the potential impacts of hatchery fish on native populations (Waples 1991; Utter 1998; Waples 1999; Lynch and O'Hely 2001). Increasing evidence for negative impacts of hatchery stocks on wild populations has motivated research regarding the efficacy of hatchery use for conservation and management (Waples 1991; Ryman 1991; Hilborn 1992; Ryman et al. 1995; Waples 1999; Hutchings and Fraser 2008). Recent data indicates that hatchery produced fish have

lower fitness than wild fish (Araki et al. 2007a; Miller et al. 2004) and have demonstrated lower fitness following release into the wild (Ford 2002; Araki et al. 2007b; Araki et al. 2008). Concerns regarding the potential interactions of hatchery and wild populations have led to evaluations of hatchery practices of maintenance of levels of genetic diversity (Allendorf 1993). Genetic diversity is important for the long-term population viability by preserving 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 should focus on maximizing the genetic diversity present in the adult breeding population (Ryman 1991), and when possible, attempting to recreate natural reproductive features of the species reproductive ecology by incorporating information that reflects how variation in reproduction is apportioned naturally (e.g. locations or timing of reproduction). There is increasing evidence that different methods of gamete take and current hatchery practices can negatively impact conservation and management programs (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 (Flagg and Nash 1999) have been cited as important factors to retention of levels of genetic diversity in hatchery progeny. Mating strategies have evolved in natural systems that maximize fitness and maintain genetic diversity. Reduced levels of genetic diversity in captive environments have been attributed to the use of a small number of breeders (Allendorf and Phelps 1980; Ryman 1991), as well as the differential selection pressures of the captive environment (Hutchings and Fraser 2008). For

hatchery programs focused on species that do not use captive broodstocks there are many factors such as population size, limited access to spawning adults, unique life history characteristics (i.e. intermittent spawning, delayed maturity, skewed sex ratios), or knowledge of species reproductive ecology that increase the probability that offspring reared in hatcheries will represent a disproportionally small subset of the genetic diversity represented in the adult breeding population.

Lake sturgeon (*Acipenser fulvescens*), a native fish of the Great Lakes, has been numerically depressed throughout their range due to anthropogenic factors (Hay-Chmielewski and Whelan 1997). Management and conservation strategies have been implemented throughout the Great Lakes that focus on restoring remnant populations through supplementation of hatchery produced juveniles (Holey et al. 2000). Successful use of hatchery supplementation for sturgeon conservation is dependent upon the degree of domestication during rearing, 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).

There has been recent interest in the use of streamside hatcheries as a means of minimizing domestication and facilitate imprinting (Holtgren et al. 2007). Streamside hatcheries use water taken directly from natal rivers providing natural daily and seasonal fluctuation in water quality (i.e., temperature, oxygen, turbidity) and chemistry (i.e., dissolved organics, pheromones, predator cues etc). Unfortunately, despite advancements in rearing technologies, there is still a critical need for studies evaluating the efficacy of available gamete/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, including 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. As data have become available, the complexities and inter-dependencies among components of natural ecosystems have become more clearly defined. Agency logistical and financial constrains must also be considered. 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 development of a scientific framework to be used to evaluate past supplementation activities and 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 methods. Captive broodstock are not used for lake sturgeon conservation due to logistical constraints imposed by the species large body size, low abundance of wild populations (Holey et al. 2000), and genetic differentiation between populations (DeHann et al. 2007). Without a captive broodstock program collection of progeny for stocking is entirely reliant on gametes or larvae from the wild. In order to successfully design conservation programs for this and other species, comparisons are needed between available collection methods and different hatchery rearing environments. Long-term success of these 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) use demographic and genetic variables to provide a means of comparisons for three different methods of gamete/larval collection for lake sturgeon, 2) determine the effect of two different hatchery environments (streamside and traditional) on levels of genetic diversity within and among the different collection methods, and 3) add to current knowledge of lake sturgeon reproductive ecology that can be used to critically evaluate restoration programs that rely on hatcheries. This research provides much needed guidance for managers involved in collecting and rearing lake sturgeon progeny for restoration efforts. Results can also be used to improve the effectiveness of lake sturgeon collection and culture techniques.

METHODS

Study Site and Hatchery Rearing

Research was conducted during each of three years (2005 to 2007) on the Upper Black River in Michigan (Fig 1). The Upper Black River (see details in Smith and Baker 2005; Smith and King 2005) is a fourth order stream located in the northeastern corner Michigan's lower peninsula. The hydrology of the Upper Black River provides a unique opportunity to enumerate a large proportion of the adults reproducing each year (Forsythe, unpublished data), collect gametes from the stream substrate (Forsythe, unpublished data), and to collect dispersing larvae (Smith and King 2005). The adult reproductive season is comprised of different spawning runs. Adults migrate upstream to spawning grounds, reproduce over several days and depart. Sequential groups comprised of different subsets of adults arrive over a period ranging from 18-42 days. Timing of

female lake sturgeon reproduction is highly repeatable across multiple years (Forsythe, unpublished data). 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; Forsythe, unpublished data). Adults reproducing earlier in the season spawn primarily in upstream spawning zones (Zones 1-3, Fig. 1) while fish reproducing later frequent more downstream spawning zones (Zones 4-6, Fig. 1). Detailed knowledge of the reproductive behavior of lake sturgeon in this system allows for examination of genetic differences among different subsets of adults on both temporal and spatial scales. Importantly, long-term ecological data provide a measure of the extent of reproductive isolation between different spawning groups. We used capture-mark-recapture and observational data to delineate different spawning aggregations in each of three years. Spawning groups were assigned based on several consecutive days of zero captures or a combination of capture data and observational data of spawning activity. This included documenting whether females were actively releasing eggs or documenting eggs deposited on the stream substrate following reproductive.

Gamete/Larval Collection Methods

Restoration of lake sturgeon or populations of any long-lived iteroparous fish species requires a long and sustained program focused on preserving genetic diversity and local phenotypic and life history adaptations. Different methods of collection focus on different life history stages (eggs or larvae) capture diversity that can be quantified based on empirically measurable variables including inter-individual relatedness, heterozygosity, and numbers of contributing adults. Typically, lake sturgeon

supplemental progeny are collected 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). A second, and less intrusive, collection method involves collecting larvae dispersing downstream from the spawning grounds (Auer and Baker 2002; Smith and King 2005). This method may prove to be closer to an unbiased representation of the relative reproductive success of all spawning adults but has yet to be genetically assessed. A third potential method is collecting eggs that have been naturally fertilized and deposited on the stream substrate immediately downstream of spawning sites. This method has been used mainly to verify successful reproduction (McCabe et al. 1990; Caswell et al. 2004). Recent work has focused on the importance of environmental covariates including features of the stream substrate on egg deposition and survival and has documented success collecting large numbers of eggs (Forsythe, unpublished data). 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. Our design involved collections of lake sturgeon gametes or larvae over a three-year period. Data allow for comparisons between methods within and across years as well as examining the effects of rearing environment. We quantified effort for each collection method by the number of personnel hours needed by day over the entire sampling period each year.

1) Directed Gamete Takes (DGT): Adult lake sturgeon were captured on the spawning grounds using large hand-held dip nets. 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, allowing the mil to pool, and using a 30ml syringe to collect it. Milt was immediately placed on ice. Fertilizations were conducted within 12 hours of egg collection. We used a partial factorial mating design for lake sturgeon where eggs from each female were divided into equal lots and crossed with a maximum of two males (depending on the available amount of eggs for each female) creating paternal half-sib family groups of approximate equal size. Individual males were never used twice 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 to employ a full factorial mating design, all males with all females, despite reported positive effects on effective population size (Fiumera et al. 2004). Full factorial designs increase inter-individual relatedness (Miller and Kapuscinski 2003.) which may pose a threat to populations of lake sturgeon that are numerically depressed. 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) Dispersing Larvae (DL): Sampling for larval lake sturgeon larvae passively dispersing downstream has been conducted in several studies to quantify recruitment and chronology and duration of dispersal (Auer and Baker 2002; Smith and King 2005) and represents a viable collection strategy. Systematic larval drift sampling was conducted during a 5-hour period, beginning at dusk (2100) and ending in the early morning (0300). The sampling location was located approximately 2km 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. Sampling was terminated after adult spawning ceased and zero larval captures were observed for consecutive nights. 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.

3) Naturally Produced Eggs (NPE): Systematic kick-net sampling was conducted below observed spawning aggregations (Fig. 1) to collect eggs that were naturally fertilized and deposited in the stream substrate. Transects were conducted across the stream at 1 meter intervals. At each interval we conducted kick-net sampling for 10 seconds. Transects continued downstream in intervals of 5 meters 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.

Rearing Methods and Environments

Lake sturgeon 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 (Fig. 1). 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. The streamside hatchery represented more natural rearing conditions with temperature profiles mimicking that of the natal river. Ground water at the traditional hatchery was heated to a constant 20^{o}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 within each hatchery. Larvae and juveniles were reared in round tanks (diameter = 1.2m) that were divided to include two replicates of each of the three collection methods. 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. Fish from the three collection methods were reared until fall fingerling size (>20cm) and released into the Upper Black River.

Genetic Sample Collection

Genetic samples were collected for use in genetic 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 were preserved separate 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.

Genetic Analyses

A subset (5%) of juvenile lake sturgeon tissue samples were randomly chosen from the naturally produced and larval drift collection methods. Samples were proportional with respect to surviving/dead juveniles, the two hatchery environments, and year of collection.

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 20ng/ul for use in Polymerase Chain Reactions (PCR). Individuals were genotyped at 12 disomically inherited microsatellite loci including *LS*68 (May et al. 1997), *AfuG*68b (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 25µl volumes containing 100ng DNA, 10X PCR Buffer (1M Tris-HCl, 1M $MgCl_2$, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X),

2mM of each dNTP, 10pmol of forward and reverse primer and 0.5µl Taq polymerase. PCR conditions for each loci followed protocols outlined in the primary literature. 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^{TM}, 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. Furthermore, we re-ran a random subset (10%) of samples to determine an empirical per-allele error rate for use in statistical analyses.

Statistical Analyses

Coancestry and Parentage: In closed populations such as Black Lake or in populations restored primarily on the basis of hatchery-production, mean population coancestry can be a direct measure of potential for future inbreeding. We calculated mean coancestries (θ) for offspring produced from fertilizations of eggs from known paternal half sib families. 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 were used to calculate θ. Mean θ were estimated at both the egg and larval stage for both hatchery environments. Estimates were also calculated for later juvenile stages at the streamside hatchery. Calculations of θ were derived as described by Chesser (1991). The number of individuals in a family (b), number of families (n) and total numbers of adults spawned (N) was used to estimate mean θ across all half sib families as:

$$\theta = \frac{\sum_{i=1}^{n} b_i^2 - b_i}{4\left(N^2 - N\right)}$$

For offspring obtained from larval drift sampling or as naturally produced eggs we estimated θ among offspring using pedigree information derived from genetic determination of parentage. Genetic determination of parentage was conducted separately for each collection method across two sampling years (2006 and 2007). Parentage was assigned using the likelihood-based approach in CERVUS Version 3.0 (Kalinowski et al. 2007). Estimates of allele frequencies, mean number of alleles per locus, and observed and expected heterozygosities were calculated by CERVUS. Chi-squared goodness of fit tests were performed for each locus using CERVUS to test for deviations from Hardy Weinberg expectations. The 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 so that parentage could be assigned at a given level of statistical confidence. Simulations were conducted with the rate of typing error set at 0.02% which was empirically derived from our blind 10% genotyping error check described above. Because we did not sample all possible parents during the annual spawning runs we used the program PASOS 1.0 (Duchesne et al. 2005) to estimate the proportion of parents sampled during the adult spawning run. Offspring were allocated to either one or both parents of known sex in PASOS with the maximum number of offsets between a parental and an offspring allele set conservatively to 0. The resulting proportions for male and females were entered into the simulation settings in CERVUS. Further analysis parameters for each simulation were as follows: 10,000 replication

cycles, the number of both female and male candidates (unique by year), and 99.54% of loci typed which was empirically determined. Output from the simulation run was then used in the parentage assignment module of CERVUS to assign offspring to a candidate mother and father using the default stringent and relaxed confidence intervals of 95% and 80% respectively. Paternity was assigned from the CERVUS output following two criteria, a) the assignment of both parents was statistically significant at either 80 or 95% confidence, or b) total exclusion between the offspring genotype and the genotype of both parents. Offspring parent combinations meeting these criteria were then used in calculation of mean θ and in further analyses described below.

Reproductive Success: We calculated several measures of reproductive success based on either known or genetically determined pedigree information from each collection method. For directed gamete takes we calculated the mean (± 1 SD) egg volume collected per female across each year and the number of larvae that hatched across half-sib families. For all collection methods we calculated the number of mates for both males and 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) and mean numbers of mates for both males and females for each collection method across each hatchery environment based on the genetically determined pedigree. Finally, we examined whether females that we directly collected gametes from spawned naturally.

This was to address concerns that direct gamete collection from spawning females may cause undo levels of stress and increase the probability that they will abandon spawning. We used parentage information estimated for the naturally produced eggs and dispersing larvae in 2006 and 2007 to examine the percentage of females that we directly removed gametes from that were represented in these samples. To test for differences in the number of genetically assigned offspring per male and female between naturally produced eggs and dispersing larvae within 2006 and 2007 we used a Fisher's exact test. We compared the number of offspring for all individual adults that spawned in the particular year of question. We were interested if offspring from one adult of a given sex were represented more by one collection method than the other. We calculated individual heterozygosities from genotypes for naturally produced and larval drift offspring. Mean differences in individual heterozygosities between collection methods within a year were assessed using a Wald Test.

Effective Population Size: We calculated three measures of effective population size for each collection method, and for each hatchery rearing environment within each collection method. Effective population sizes (N_e) based solely on the numbers of males (N_m) and females (N_f) used were determined following Wright (1931) as:

$$N_e = \frac{4N_m N_f}{(N_m + N_f)}$$

Secondly, we calculated a more fully parameterized measure of effective population size using the effective numbers of males (N_{em}) and females (N_{ef}) for each collection method

(Lande and Barrowclough 1987) utilizing our empirical estimates of male and female reproductive variance as:

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

and

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

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_{ev}) for each treatment was then calculated (Lande and Barrowclough 1987) as:

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

Finally, we calculated coancestral effective population size ($N_{e\theta}$, Chesser et al. 1993) for each collection method. $N_{e\theta}$ is the number of breeding individuals consistent with observed accrual of gene correlations over generations and was calculated as:

$$N_{e\theta} = \frac{1}{2\Delta\theta}$$

where the change in mean θ was calculated for any estimated made for the same groupings over multiple time periods.

Relatedness: Relatedness coefficients (r_{xy}; Queller and Goodnight 1989) were calculated using the program Kinship 1.3.1 (Goodnight 2000). The estimates of relatedness between individuals calculated as r_{xy} differ from those calculated by coancestry because coancestry calculates the probability of alleles being identical by descent while r_{xy} compares the degree of relatedness between individuals as compared to the average intra-population relatedness. Allele frequencies were estimated using adult genotypes from the Black Lake population. We calculated the r_{xy} value for all parental pairs for each collection methods and calculated the distribution of r_{xy} values for the each spawning population for each year. Forsythe (unpublished data) found repeatability for spawning time to be high in female lake sturgeon so we calculated the distribution of r_{xy} for adult female lake sturgeon spawning in distinct spawning runs (temporal variation) and within distinct spawning zones (spatial variation; Fig. 1). To test for mean differences in relatedness made among assigned parents we used a permutation test appropriate for similarity data. This analysis has been used previously for genetic relatedness data (Lehmann et al. 1992; Ratnayeke et al. 2002; Wayne et al. 1991). Using a SAS-based program (A. Saxton, University of Tennessee, unpublished software), the observed difference between the means of the two groups was first computed and then data from the two groups were pooled. Pooled data was randomly subsampled 1000 times. Each subsampling calculated the difference between the observed and theoretical

mean calculated from the pooled data and the one-tailed probability of the two means being different was tested. We used a Mann-Whitney *U* test to test for differences in the distribution of r_{xy} values between females within different spawning zones and spawning runs. Tests were conducted between spawning zones and runs within a year and between the same spawning zones and runs across years with the null hypothesis being that the samples were drawn from a single population.

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. Finally, to further address the issue of genetic differentiation between spawning runs we calculated per locus estimates of F_{ST} for different spawning runs of adults within each year.

RESULTS

Collection Methods

We collected large numbers of eggs and larvae using all three collection methods (Tables 1, 2, 3) with the highest totals realized using the DGT method. Numbers were fairly consistent for both DGT and NPE across each year. However, larger variation was observed in the DL samples among years despite relatively consistent adult spawning numbers (n = 154, 234, 208 for 2005, 2006, and 2007, respectively; Fig. 2).

Microsatellite loci used showed moderate levels of polymorphism with 2 to 9 alleles observed per locus. Genotypic frequencies at all 12 loci did not deviate from

Hardy-Weinberg expectations. Mean number of alleles per locus and observed heterozygosity were 5.5/0.55 and 5.42/0.57 for 2006 and 2007 DL samples and 5.5/0.56 and 5.25/0.57 for NPE in 2006 and 2007. There were no significant differences in individual observed heterozygosities between naturally produced or larval drift samples for either 2006 (df = 248, t = 1.97, P = 0.12) or 2007 (df = 122, t = 1.98, P = 0.85). We had higher overall parentage assignment for the DL method compared to the NPE method in both years. Assignment of offspring to both parents was 68% and 74% for the DL method in 2006 and 2007 respectively while NPE had lower assignment rates of 57% and 21% in the same years.

We determined levels of coancestry (θ) for lake sturgeon progeny obtained using each of three collection methods as an indication of gene correlations among progeny with known (DGT) or assigned (NPE and DL) parentage. For each year, estimates of θ were consistently highest for DGT (Table 1) and decreased sequentially in NPE (Table 2) and DL (Table 3) methods. For example, estimates in 2005 were >0.02 for individuals collected using DGT compared to individuals collected using NPE in the same year (0.014). Estimates for individuals collected using DL in 2005 were lowest (0.004).

Three estimates of effective population size (N_e, N_{ev}, and $N_{e\theta}$) were highest for the DL (Table 3) collection method compared to both the DGT (Table 1) and NPE (Table 2) methods. This trend was consistent across both years. For example, in 2007 the estimates of N_{ev} were 38.6 for individuals collected using DGT, 59 for NPE individuals, and a much higher estimate for individuals collected using the DL method (181.3). The number of contributing adults was higher for offspring from the DL samples (Tables 5) compared offspring collected using DGT (Table 4) and NPE (Table 5) and therefore

directly influenced the estimate of N_e, which is based entirely on numbers of males and females. We documented higher variation in male and female reproductive success based on individuals in the DGT method (Table 4) which lead to estimates of N_{ev} being more similar to the simplified estimate of N_e within years. Estimates of $N_{e\theta}$ were lower than N_e or N_{ev} estimates for DGT offspring (Table 1) but were higher for both NPE (Table 2) and DL (Table 3) samples than N_e estimates and varied with respect to N_{ev} estimates.

Reproductive success is often measured by the number of offspring produced per male and female. The number of offspring per male and female was much higher for DGT method (Table 4) compared to samples obtained using NPE and DL (Table 5), as anticipated given the large numbers of gametes collected from a relatively small number of females. We documented significant differences in the mean egg volumes collected from females across years (ANOVA, $F_{2,40} = 4.44$, $P = 0.02$). A fisher's exact test revealed no significant differences in the number of offspring 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. The highest number of mates for either sex was for several females in the 2006 and 2007 DL samples that were estimated to have successfully reproduced with 6 males. The mean number of mates per male and female was highest in the 2007 DL samples (Table 5) with an estimated 2.2 and 1.5 mates per individual females and males respectively.

Permutation tests quantifying mean differences in distributions of relatedness (r_{xy}) values between collection methods within each sampling year revealed that the offspring collected from the DGT method in 2006 had a lower mean r_{xy} value than both offspring collected using the DL method (P=0.038; Table. 6; Fig. 3) and the spawning population (P=0.042; Table. 6; Fig. 3). There were no other significant differences in the distribution of r_{xy} values between offspring collected using different collection methods in each of the three years (P>0.05; Table. 6; Fig 3). Though not statistically significant, the estimates of r_{xy} for DGT offspring in 2005 and 2007 were higher than all other collection methods. This is comparable to the results found with the pedigree estimates of θ between the three groups.

Multilocus estimates of F_{ST} between individuals collected using the NPE and DL methods within a year were low significant indicating that offspring represented different subsets of adults or different adult contributions. The overall F_{ST} value between individuals collected using the NPE and DL methods in 2006 was 0.019 (95% CI 0.009-0.029). In 2007, the estimate of F_{ST} was 0.016 (95% CI 0.008- 0.024) between offspring collected using the NPE and DL collection methods suggesting that different subsets of the pool of available offspring were sampled using the different methods.

Effort expended to collect samples using each collection method was consistent across years. However, effort differed substantially among collection methods. Directed gamete takes from spawning adults required approximately 24 personnel hours per day for the duration of spawning. In addition, fertilizations of paternal half sib family groups required approximately 1 hour per female. Collecting eggs using the NPE method from

the stream substrate took approximately 30 personnel hours total for each year. NPE samples were collected from two different spawning areas in each of the three years (Fig. 1). DL sampling consisted of 10 personnel hours per day for the entire duration of drift. Drift duration was 26, 31, and 36 days for 2005 to 2007 respectively. These estimates of effort for the three collection methods reflect collection only and do not include personnel required to perform daily husbandry duties (egg incubation, feeding, mortalities, water quality etc) in the two hatchery environments (See details in Crossman 2008, chapter 2).

Rearing Environment

Proportions of larval hatching from paternal half-sib family groups in the DGT method was significantly higher at the streamside hatchery in 2006 compared to the traditional hatchery environment in the same year as well being higher than hatch in 2005 (ANOVA, $F_{3,42} = 3.39$, $P = 0.03$). For offspring collected using the DGT method we estimated consistently higher θ at the traditional hatchery compared to the streamside hatchery during both 2005 and 2006. Estimating the change in θ at different life stages is an important variable in supplemental progeny reared in hatcheries. We documented an increase in θ between the egg stage and larval stage for offspring collected using the DGT method across all years and both hatchery environments (Table 1). Furthermore, we documented the same increasing trend in θ across four different stages at the streamside hatchery environment over 2 years (Table 1).

The number of adults contributing to offspring within the different hatchery environments was similar in the controlled DGT crosses (Table 4) but varied

considerably in the NPE and DL collection methods (Table 5). This is further reflected in the estimates of N_e between the rearing environments. In both 2005 and 2006 all three estimates of effective population size for DGT were higher at the streamside hatchery compared to the traditional hatchery (Table 1). In 2006 all three measures of effective population sizes were higher at the streamside hatchery for NPE samples. In the DL samples during 2006 estimates of N_e and $N_{e\theta}$ were higher at the traditional hatchery compared to the streamside hatchery. However, taking measures of reproductive variance into account revealed a higher estimate of N_{ev} for DL samples at the streamside hatchery compared to the traditional hatchery in the same year.

Adult Characteristics

Duration of spawning varied among years. Spawning adults were captured over periods of 23, 37, and 40 days during 2005, 2006, and 2007, respectively (Fig. 2). Sex ratios (number of males per female) of the adult spawning population were 2.38, 2.55, and 2.28 respectively for 2005, 2006, and 2007. The number of distinct spawning groups differed among years with 2, 4, and 3 distinct reproductive events in 2005-2007, respectively (Fig. 2). We sampled a high proportion of the adult spawning population in 2006 and 2007. PASOS estimates of the proportion of spawning adults sampled during the adult spawning run for both NPE and DL samples within each year were higher for DL samples (2006 Males (M):0.91, Females (F):0.93; 2007 M:0.93, F:0.96) compared to NPE samples (2006 M:0.86, F:0.89; 2007 M:0.97, F:0.76).

Individual heterozygosities of adults did not differ significantly between 2006 and 2007 (df = 440, t = 1.97, P=0.2) reflecting the large numbers of spawning adults. Pooled individual heterozygosities from individuals collected using each of the collection methods were not significantly different from pooled adult heterozygosities (df = 814, t = 1.96, P = 0.82; mean of 0.55). However, adults do not all spawn at the same time and in the same location. This aspect of the adult reproductive behavior has implications when sampling based on DGT and NPE is conducted. We found significant differences in mean r_{xy} values among adults from different spawning zones in 2007 (Fig. 4), with adults spawning in zone 2 having a significantly higher mean r_{xy} value when compared adults spawning in zones 4 and 5 (Mann-Whitney *U* test, P < 0.001 respectively). There were no other significant differences in mean r_{xy} values between zones within the two other sampling years. Tests for differences in mean r_{xy} values between adults spawning in different spawning zones across different sampling years resulted in significant difference between zone 2 in 2006 and 2007 (Mann-Whitney *U* test, P = 0.0.039). For spawning runs within a year, we found significant differences in mean r_{xy} values only within 2007 (Fig. 5). Adults spawning in the second run had significantly higher mean r_{xy} estimates than adults spawning in both the first and third run during 2007 (Mann-Whitney *U* test, P < 0.001 and 0.004 respectively). Between years, adults spawning in the second run in 2007 also had a significantly higher mean r_{xy} than both 2005 and 2006 (Fig. 5, Mann-Whitney U test, P = 0.026 and <0.001 respectively).

We estimated F_{ST} to determine if there was a difference in the variance in allele frequency between adults spawning at different times within a year. In 2005 there was no significant difference between the two spawning runs observed during that year (F_{ST} = 0.001, CI: -0.024, 0.026; Fig. 3). In 2006 the first run of spawning adults was not significantly different from the second group (F_{ST} = -0.008, CI: -0.010, -0.006). In 2007 the first group of spawning adults was significantly different from the last group of spawning adults (F_{ST} = 0.012, CI: 0.002, 0.022) which implies that managers should use caution when sampling from a limited portion of the total spawning season.

Parentage data from NPE and DL offspring identified that 67% and 60% of adult females that we directly removed gametes from reproduced naturally in 2006 and 2007 respectively. Direct egg takes do not detract from a female's ability to spawn naturally. Thus, females contributing gametes based of direct gamete takes do contribute to natural recruitment and mate with different males then used for hatchery crosses.

DISCUSSION

Hatchery programs require empirical evaluations of the efficacy 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, including the Great Lakes, have been initiated (Holey et al. 2000) despite considerable uncertainty regarding the effects gamete/larval collection methods have on levels of genetic diversity observed in supplemental 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. 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.

Collection Methods Affect Measures of Genetic Diversity in Progeny

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 broodstock 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 progeny obtained using three collection methods allows for quantification of levels of genetic diversity captured conditional on numbers and the reproductive ecology of available adults, and provides a framework to assess which method might be most effective in retaining genetic diversity present in the wild population. Each method evaluated provided large numbers of progeny for use in hatchery programs and supplemental stocking.

Collections of gametes directly from spawning adults provided the highest initial abundance of progeny (Table 1). However, offspring did not reflect the genetic diversity of the wild population as did collections of naturally produced eggs or dispersing larvae. We estimated coancestry, or levels of gene correlation, for individuals acquired using

each collection method to identify the method(s) that minimized levels of coancestry and therefore future levels of inbreeding. Estimates of coancestry were higher for offspring from directed gamete takes compared to both naturally produced eggs and dispersing larvae (Table 1). The mechanism driving differences can be attributed to several factors. First, the total number of adults contributing to offspring increased from directed gamete takes to naturally produced eggs to dispersing larvae samples (Table 2). Reproductive variance was high between females in the hatchery environment. Furthermore, 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). Though the number of adult females that we successfully spawned was quite 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. Naturally produced eggs were taken over relatively small spatial scales from substrates associated with locations of two spawning groups each year. Therefore, offspring had fewer contributing adults when compared to the larval drift samples. Greater effort could result in reproductive contributions from more adults. Importantly, naturally produced eggs and dispersing larvae samples were analyzed from tissue samples collected during the juvenile stage. Therefore, these estimates of θ refer to juveniles at older ages than those estimates produced for DGT offspring at both the egg and larval stages. These estimates could have increased during the rearing process if different families had different competitive abilities (and survival) in the hatchery environment. Even though we observed no difference in offspring heterozygosities

between the three collection methods, both the NPE and DL methods resulted in lower mean coancestry and should considered for populations where a limited number of adults are available for gamete collection.

Parentage assignment based on molecular markers is an effective approach to explore mating systems of species that are otherwise hard to study (Avise 2004). We used a combination of programs to assign parentage to sampled offspring from the naturally produced eggs and dispersing larvae methods. Allocation of offspring to candidate parents with statistical confidence can be difficult in open systems where the proportion of sampled adults is unknown. Lower assignment rates for NPE offspring was a product of the proportion of adults sampled estimated using PASOS. The estimate of the proportion of adults sampled was more sensitive for the NPE offspring because we were sampling eggs from a subset of the spawning females each year or spawning run. Unsampled females associated with spawning groups depositing NPE's for our collections could result in over estimation of the proportion of unsampled females (e.g. only 76% of females sampled in 2007). This in turn limited our ability to assign NPE offspring, particularly in 2007. The proportion of sampled adults was likely closer to the true value for the dispersing larvae (>90% of both sexes each year) as we sampled offspring from the entire reproductive period. Estimates of coancestry are contingent on parentage assignments. However, despite inconsistency in assignment rates for offspring from the NPE and DL collection methods, the rank order of our results remained consistent across years

Effective population size is a predictive measure of generational changes in allele frequency, heterozygosity, and inbreeding that has been widely used in fisheries

management when supplementing wild populations with large numbers of hatchery produced progeny (Ryman and Laikre 1991). We documented differences in three measures of effective population size of lake sturgeon progeny collected using three collection methods. Estimates were considerably higher for the DL samples compared to both the NPE and DGT samples. Estimates of effective population size are influenced by aspects of a species biology. Greater numbers of adults contributed to individuals collected using the DL method in each year. The most fully parameterized estimate of variance effective size which incorporates male and female reproductive variance is likely the most reliable indicator for hatchery programs. 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 will be high and projections of effective population size based just on numbers of males and females will lead to overestimation.

Estimates of F_{ST} are 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 three different methods. Multilocus 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 the proportional contribution of adults varied. Genetic differentiation between the two groups of offspring indicates methods of gamete 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.

In populations of seasonally-breeding species, individuals breeding at similar times will be more likely to mate with one another than with individuals breeding at different times. Temporal assortative mating can be further reinforced if habitats used for reproduction also vary throughout the reproductive season. If timing of reproduction is heritable, and offspring share similar tendencies, multi-modal distributions of spawning times can develop as observed in Black River lake sturgeon. Multiple spawning episodes associated with different habitats tied to seasonal variation in thermal regimes have been documented in lake sturgeon (Auer 1996). If selective regimes imposed by temporally and spatially variable environmental conditions affect offspring traits and fitness, covariation between adult spawning time and juvenile traits can develop (Hendry and Day 2005). Differences in allele frequency between adults spawning at the same time and in different locations should be considered and gametes collected from adults from multiple locations and times to maximize retention of levels of genetic diversity represented in neutral genetic markers and adaptive phenotypic traits.

Collecting gametes directly from spawning adults for use in sturgeon hatchery conservation programs may provide the numerical abundance required. However, there are several important aspects 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 dependant 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) allowing a relatively few number of adults to contribute a high proportion of the supplemental progeny if absolute gamete number is the sole criteria. In our experience, collection of milt form a large proportion of males is possible and tagging ensures that the same male is not reused twice. We recommend that programs collecting gametes form spawning adults sample in a spatial and temporally comprehensive manner. 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).

Rearing Environment Affects Measures of Genetic Diversity

Hatchery rearing environments have been shown to result in supplemental progeny that are both genetically and biologically inferior to their wild counterparts (Waples 1991; Ford 2002; Araki et al. 2007a; Miller et al. 2004). We compared two rearing environments, a traditional hatchery and a more novel streamside hatchery. We found that estimates of θ for DGT offspring were higher at the traditional hatchery compared to the streamside. Equal numbers of eggs from each paternal half-sib family group were represented at both hatcheries. However, higher proportions of eggs hatched at the streamside hatchery among family groups compared to the traditional hatchery. We documented an increase in θ for DGT offspring at the time of hatch relative to estimates based on the relative number of eggs fertilized. Consistently higher θ was documented for the traditional hatchery environment across years, resulting from differential survival

among family groups during the embryonic stage (Crossman chapter 2). Coancestry increased over time at the streamside hatchery resulting over 4 sampling periods in each of two years. Again, differential survival of larvae and juveniles among family groups throughout the rearing process lead to increases in θ (Crossman chapter 2). 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. Differences between hatchery rearing environments could influence survival as different genotypes could be favored in different environments. We documented high variation in reproductive success in adults used to produce offspring in the DGT collection method within both hatcheries across both years. Finally, assignment rates within hatchery rearing environments varied by collection method and location but our overall results remained consistent across years.

Adult Reproductive Ecology

Examining the reproductive ecology of species such as lake sturgeon, which broadcast gametes into the open water with no post-ovulatory parental care, is challenging because of difficulties in designing sampling protocols for relevant groups of offspring. Molecular-genetic markers have for the examination of reproductive patterns in fishes that utilized different reproductive tactics including broadcast spawners (Avise et al. 1992; DeWoody and Avise 2001; Avise et al. 2002). The ability to combine molecular markers with field data allows for the examination of aspects of a species reproductive ecology that are otherwise uncertain (DeWoody and Avise 2001). Information of the relative number of contributing adults, the number of mates, and the

number of offspring produced are variables that have eluded sturgeon ecologists. We were able to use molecular methods to examine these variables using two different methods of collection that represent offspring produced through natural reproductive behaviors. Though the number of mates was controlled for DGT in the hatchery environment, estimates of the number of mates per male and female among all three collection methods were not widely different (Table 2). Adult males and females contributing to the 2007 DL samples had the highest number of mates. Total natural recruitment to the larval stage was much lower in this year compared to the other two years. Offspring that were part of families that had very low initial abundance may have been missed during sampling due to low representation or in the selection of genetic samples to analyze. In the DL samples we documented individual females mating with as many as six different males. The largest number of mates for one individual male was 3. DeHaan (2003) found through genetic determination of parentage with a larger sample size that females and males mated successfully with as many as 11 and 5 different individuals, respectfully. Female lake sturgeon spawning in the Upper Black River have been observed to be surrounded by several males (Forsythe, unpublished data), a behavior that has been noted in other systems (Bruch and Binkowski 2002; Kempinger 1988). At the time females release their eggs, several males compete to position themselves close to females to increase the likelihood of fertilizing the eggs (Bruch and Binkowski 2002). However, the relative reproductive success of individual males has been difficult to assess. We used molecular markers to document fundamental aspects of this species reproductive ecology and our findings complement behavioral observations

of reproduction recorded in the wild. Furthermore, this represents one of the first attempts to examine lake sturgeon reproductive behavior using a molecular approach.

Sampling methods that represent spatial and temporal aspects of a species reproductive ecology will help to preserve adaptations that may have evolved to different spawning environments (Hendry and Day 2005). Heritability for either spawning location or spawning time is a component of diversity that has been relatively unconsidered as of yet in sturgeon. Forsythe (unpublished data) documented the timing of spawning to be highly repeatable for individual female lake sturgeon. Other species have been shown to have an increased heritability for both spawning time and location which has lead to adaptive divergence, even within a population (Hendry and Day 2005). If spawning time is a heritable trait in lake sturgeon then collecting gametes from only one subsection of the entire spawning season may compromise future population adaptive potential. We documented differences in estimates of relatedness among adult female lake sturgeon within years across spawning locations and spawning runs. Furthermore, we documented these differences in relatedness across years. These results indicate that adequate sampling of offspring from adults from the entire spawning season will increase diversity and decrease mean relatedness between progeny collected. Future studies that seek to preserve the genetic diversity of lake sturgeon populations should design sampling protocols accordingly.

Information regarding sex biased dispersal of adult lake sturgeon between spawning locations and times is difficult to estimate given the logistics of following individuals. It has been documented that approximately 15% of adult males and 0% of adult females spawn in multiple runs within a year in the Black Lake system (Forsythe,

unpublished data). Estimates of F_{ST} were different for adults spawning at different locations and at different times further reflecting low rates of dispersal between groups. For lake sturgeon, dispersal on the population scale is very low due to the species philopatric nature and the spatial genetic structuring of populations throughout the Great Lakes (DeHaan et al. 2006). Further knowledge on dispersal rates between spawning groups would be important to improve our understanding of the reproductive ecology of lake sturgeon and to help define sampling strategies for progeny.

Handling Does Not Affect Natural Reproduction

Handling-induced stress can have wide ranging effects on fish including nest abandonment (i.e., 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 (i.e., sturgeon species including stellate (*Acipenser stellatus*, Kynard et al. 2002), shortnose (*Acipenser brevirostrum*, Moser and Ross 1995), white (*Acipenser transmontanus*, Schaffter 1997), and green sturgeon (*Acipenser medirostris*, Benson et al. 2007). Observations of interrupted upstream migration of sturgeons postulated abandoned of spawning due to stress incurred during capture and tagging. The majority of these observations were been made 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 either before or after our interruption. Our estimate should be viewed as an underestimate given

that we only genotyped 5% of offspring samples. Results are encouraging for gamete removal from actively spawning females. High fecundity in this species allows for a relatively small amount of eggs to be taken for hatchery rearing.

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. Sturgeon typically spawn in large, deep, fast flowing rivers that may limit the choice of progeny collection methods. The Black Lake system is a unique system because of access to a large number of spawning adults during the entire reproductive season. Other systems may not be as conducive for collecting gametes and might expect to expend even greater effort to do so. The number of eggs we obtained through direct gamete takes represents a maximum as we attempted to collect gametes from all spawning females, some on multiple occasions. Hand stripping was only effective for females caught while releasing gametes, which might not be an efficient technique in larger systems where adults are caught by methods other than dip nets. In this situation, adults would need to be held and protocols for collecting gametes may have to follow several available methods required to induce ovulation (Doroshov et al. 1983; Parauka et al. 1991; Doroshov et al. 1997; Webb et al. 2002). All three of these collection methods 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 transferable and effective 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.

CONCLUSIONS

To maximize genetic diversity in offspring used in supplementation, 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. Managers should acknowledge that populations are composed of a mixture of individuals that reproduce at different times and in different locations. 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. We found that collecting dispersing larvae downstream of the spawning grounds minimized estimates of coancestry while at the same time maximizing effective population size. Difference between the hatchery rearing environment and the wild have

the ability to influence the genetic variation observed in the supplemental progeny. 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.

Chapter 2

COMPLEXITY OF REARING ENVIRONMENT AFFECTS SURVIVAL AND GROWTH OF LAKE STURGEON EMBRYOS, LARVAE, AND JUVENILES

INTRODUCTION

Declines in numerical abundance have been described for the majority of fish populations' worldwide (Myers and Worm 2003). Natural populations are increasingly augmented by releases of hatchery offspring. Hatchery supplementation has been used as a management prescription to augment existing stocks (Levin et al. 2001), mitigation for habitat destruction (Waples 1991a), enhance fishing opportunities through introduction of desirable sport fish, and conservation (Waples 1991a; Ryman 1991). The ability to raise large numbers of fish at all life history stages in hatchery environments bypassing bottlenecks imposed by egg of size-specific mortality (Secor and Houde 1998).has fostered widely held beliefs by the public and managers of hatchery abilities to compensate for losses due to both fishing and natural mortality.

Due to the large numbers of fish produced in hatcheries and stocked into natural environments, there is great concern about the potential impacts of hatchery fish on native populations (Waples 1991a; Thomas and Mathisen 1993). Increasing evidence for negative impacts of hatchery stocks on wild populations has prompted much discussion and research regarding the efficiency of hatchery use for conservation and management (Waples 1991; Ryman 1991; Hilborn 1992; Ryman et al. 1995; Waples 1999). Recent evaluations of hatchery practices as they relate to management goals to maintain genetic diversity have raised concerns regarding the negative effects on individual fitness and

population sustainability if hatchery fish interact with wild counterparts (Araki et al. 2007a; Miller et al. 2004; Ford 2002; Araki et al. 2007b; Araki et al. 2008). Concerns have prompted calls for research directed at evaluating the efficacy of different culture techniques to produce fish that are ecologically and genetically compatible with intended release environments.

Conservation hatchery programs have been developed for a few species that have become listed as endangered, threatened, or have the potential to become threatened due to habitat loss or alteration (Flagg and Nash 1999; Maynard et al. 2004). The role of conservation hatcheries is to restore populations through supplemental stocking by lessening the genetic and ecological impacts of hatchery released fish on wild populations. Many of these programs have developed fish culture techniques that enable hatcheries to produce fish with more wild-like characteristics that result in increased post-release survival (Flagg and Nash 1999; Maynard et al. 2004). Techniques include rearing supplemental fish in semi-natural habitats. Semi-natural rearing incorporates components of the natural environment which may include adding structure and cover into fish culture tanks or exposing fish to a more natural fluctuation in water temperature. Many different semi-natural culture techniques have been suggested based on the assumption that rearing fish hatchery environments that emulate natural environmental conditions will promote expression of traits that improve probabilities of survival following release into the wild (Wiley et al. 1993; Olla et al. 1994; Maynard et al. 1995; Olla et al. 1998; Brown and Laland 2001). However, alternative culture techniques should be developed and evaluated on a species by species specific basis. Research aimed at empirically evaluating

the effectiveness of natural culture techniques will be important for the restoration of species of conservation concern.

The lake sturgeon (*Acipenser fulvescens*) is a native fish in the Great Lakes and has undergone significant declines in numerical abundance over the past century due to anthropogenic factors including overfishing, habitat destruction, and the presence of dams on many rivers that historically supported spawning runs impacting access to historical spawning grounds (Holey et al. 2000). Current lake sturgeon abundance in the Great Lakes is believed to be less than 1% of historic levels (Hay-Chmielewski and Whelan 1997). The lake sturgeons unique life history characteristics (late age at maturity, infrequent spawning, low recruitment rate) combined with low abundance of reproductive adults complicates recovery efforts. Management and conservation strategies have been implemented throughout the Great Lakes that focus on restoring remnant populations through supplementation of hatchery production and release of juveniles (Holey et al. 2000).

Considerable uncertainty exists among lake sturgeon managers and researchers about the impacts of current lake sturgeon culture techniques and stocking efforts on remnant populations. A variety of techniques are currently implemented throughout the region that differ in progeny collection methods, (larval collection, direct gamete takes) and rearing environments (streamside versus traditional hatcheries). The variety of methods currently used by managers and the lack of data collected on the success of each strategy reflects of the lack of consensus of the most effective methods. Currently, management prescriptions for lake sturgeon hatchery production are largely adopted from other well-studied species (e.g., salmonids). However, the reproductive ecology

and early life history of lake sturgeon are very different from salmonids. Therefore, rehabilitation strategies should be designed, experimentally tested, and developed for lake sturgeon rather than adopted from a species with dissimilar life histories.

Increasing the effectiveness of culture techniques for lake sturgeon will help guide current and future restoration programs. There is a critical need to determine if rearing lake sturgeon supplemental progeny in different hatchery environments has an effect on stage specific survival. We raised lake sturgeon in two different hatchery environments to quantify differences in stage based survival and growth between the two hatchery environments and three methods of gamete/larval collection. Our objectives were to determine the effects of rearing environment and complexity and methods of gamete/larval acquisition on survival and growth at different life stages. Specific objectives were to, 1) quantify and compare survival of eggs, larvae, and juveniles between hatchery rearing environments and methods of gamete/larval collection, 2) quantify and compare growth across the three collection methods and hatchery environments, 3) determine the effects of refuge (habitat complexity) and resource availability on growth and yolk-sac utilization in larval lake sturgeon, and 4) determine the effects of refuge availability on juvenile lake sturgeon growth.

METHODS

Study Population

Work was conducted during each of two years (2005 and 2006) using gametes and larvae from the lake sturgeon population residing in the Black Lake Michigan. This system offers a unique ability to sample large numbers gametes and individuals across all

lake sturgeon life history stages, and is founded by previous research on egg (Forsythe, unpublished data), larval (Smith and King 2005a), juvenile (Smith and King 2005b, Crossman 2008 chapters 3 and 4), and adult stages (Baker and Borgeson 1999; Smith and Baker 2005; Forsythe, unpublished data).

Hatchery Rearing Environments

Lake sturgeon eggs, larvae, and juveniles were reared in two different environments, a streamside hatchery on the natal Upper Black River and at a traditional hatchery. Streamside hatcheries have been shown to provide an environment for successful growth and survival for lake sturgeon and offer adaptability in facility design that isn't available in more traditional hatchery facilities (Holtgren et al. 2007). More traditionally, gametes have been incubated, hatched, and larvae reared at hatcheries geographically removed from natal streams and returned when stocked. Lake sturgeon are highly philopatric and rearing that occurs at non-natal environments has led to concerns regarding disruption of natal imprinting and straying among populations following release into the natal environment. Straying may artificially elevate levels of gene flow and affect the genetic conservation programs for lake sturgeon, as remnant populations of lake sturgeon within the Great Lakes basin exhibit genetic structuring (DeHaan et al. 2006).

The streamside hatchery used in this study was constructed on the Upper Black River in the spring of 2005 using a steel building (US Buildings; Dimensions: 7.3/12.2/4.3m). Water for the streamside hatchery was pumped directly from the Upper Black River. Water passed through a primary settling tank, sand filtration, a second

settling tank, and a degassing column before entering egg incubation and juvenile rearing tanks via gravity feed. Flow rate through the system measured approximately 350L/min allowing for two total water turnovers per hour. Aeration was provided to the entire facility from a single unit (Sweetwater Regenerative Blower, Aquatic Eco-Systems) to maintain levels of dissolved oxygen during periods of warm summer temperatures and elevated fish respiration. System specifics for rearing different lake sturgeon life stages at the streamside hatchery are described below.

Lake sturgeon reared at the Michigan Department of Natural Resources, Wolf Lake State Hatchery, representing a traditional hatchery environment. Lake sturgeon have been reared in this multi-species facility since the late 1970's following consistent rearing protocols. Water for the hatchery is provided from both well and natural spring sources in a flow through design. Water temperature in the egg incubation trays remains relatively constant year round (11.1^{o}C) and the hatchery has the capacity to warm a small percentage of the water to rear cool-water species such as lake sturgeon. Heated water for juvenile lake sturgeon rearing ranges from 11.1 to 22.0^{o}C during the summer months with dissolved oxygen levels maintained at greater than 9mg/L.

Progeny Collection

We collected gametes and larval lake sturgeon using three different methods. Methods were 1) direct gamete takes (DGT) from adults spawning in the Upper Black River, Michigan, 2) collection of naturally produced eggs (NPE) from the stream substrate, and 3) collection of larval lake sturgeon passively dispersing downstream (DL) from the spawning grounds. For the DGT collection method, adults were captured while

spawning using large hand held dip nets. Eggs were collected by hand stripping females that were caught in the act of spawning and kept in sealed plastic bags at ambient river temperatures. Milt was collected from as many males as possible using a syringe and kept on ice. Fertilizations were conducted at the streamside hatchery the same day as the eggs were collected. Eggs from an individual female were divided into two lots equally based on volume and fertilized separately with milt from two randomly chosen males. Milt was diluted at a ratio of 1:200mls and immediately poured over the eggs. The milt and eggs were mixed for 1 minute at which time a mixture of water and diatomaceous earth was added and mixed with the eggs by hand to remove the adhesiveness of the eggs. This de-adhesion process was conducted for a period of one hour at which time the eggs were rinsed with clean water. Immediately following fertilization and de-adhesion, half of the eggs from each paternal half-sib family were transported to the traditional hatchery environment. Fertilized embryos were transported from the streamside hatchery to the traditional hatchery in plastic bags kept at ambient river temperatures. Eggs were placed in incubating trays immediately following fertilization at the streamside hatchery and immediately following arrival at the traditional hatchery (approximately 5hr post fertilization).

Systematic kick-net sampling was conducted below observed spawning aggregations to collect eggs that were naturally fertilized and deposited on stream substrate. Transects were conducted across the stream at 1 meter intervals. At each interval we conducted kick-net sampling for 10 seconds. Transects continued downstream in intervals of 5 meters until no eggs were collected in consecutive transects. The number of sampled versus non-sampled locations varied across years. Eggs were

counted at the streamside hatchery. Eggs from this collection method were incubated and hatched at the streamside rearing environment because managers at the traditional hatchery environment had concern regarding potential pathogens that eggs taken from stream could contain. Larvae were divided equally between the two hatchery rearing environments within one week post hatch.

Sampling for larval lake sturgeon larvae passively dispersing downstream has been conducted in several studies to quantify recruitment and chronology and duration of dispersal (Auer and Baker 2002; Smith and King 2005) and represents a viable collection strategy. Systematic larval drift sampling was conducted during a 5-hour 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 adults. 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. We sampled the entire distribution of dispersing larvae due non-randomness described in other work (DeHaan 2003). Sampling was terminated after adult spawning ceased and zero larval captures were observed for consecutive nights. 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.

Experimental Design

Mortality and growth rates of fish during egg, larval, and juvenile stages are both high and variable (Houde 1989; Pepin 1991). It is difficult to obtain precise estimates of mortality for many teleost fishes and such estimates are critical for evaluating the extent and causes of stage-specific mortality (Houde 1997). For species with complicated life histories, such as lake sturgeon, events occurring during one stage can have important implications for the performance and survival of individuals in subsequent stages. Experimental and analytical protocols conducted in this study are described on a life stage by life stage basis.

Egg Stage: The effects of hatchery rearing environment on egg survival

Rationale: The environmental conditions (i.e. temperature) in which a sturgeon embryo is reared, have been shown to affect development and survival rates (Wang et al. 1985; Gershanovich and Taufik 1992). Lake sturgeon reproduce over a broad range of time that can last for up to 6 weeks (Forsythe, unpublished data). Eggs incubating in natural river conditions may experience environmental fluctuation in water temperature and chemistry. Furthermore, eggs that are deposited early in the spawning season likely experience environment conditions (temperature and flow) that are different from eggs deposited late in the season. Hatchery incubation of lake sturgeon eggs has traditionally been conducted at facilities that use a water source that is different from that of the natal river. Typically, these hatcheries use a well water source which remains at a constant temperature during the incubation period allowing for accurate predictions on the time to hatch which improves hatchery management efficiency. The effects of fluctuating and

constant environments on egg survival during incubation have not been compared for lake sturgeon. Streamside hatcheries have been recently developed for this species in an attempt to minimize straying following release of juveniles back into the wild and offer a unique approach to examine differences in egg survival relative to more traditional hatchery environments. Streamside hatcheries mimic the natural temperature fluctuations of the natal river. We examined overall and daily egg survival in two different hatchery environments.

Methods: Fertilizations of embryos followed protocols discussed for the DGT collection method above. Eggs were incubated separately by family group using heath tray stacks at both hatchery rearing environments. Family groups were randomly assigned to one of 8 trays within a heath tray stack. Egg volumes larger than 200mls were divided between multiple trays. Eggs were treated with a formalin drip (17ppm) for 15 minutes daily to minimize pathogen and fungal infection (Bouchard and Aloisi 2002). Daily measurements recorded included the number of dead eggs and the number of dead eggs with microbial infection. We also recorded Temperature (^{o}C, Data loggers every hour) and dissolved oxygen (mg/L, YSI) within the heath trays.

Analysis: Analyses were conducted using program R (Version 2.1.1). We used a two-way analysis of variance (ANOVA) to test for differences in overall egg survival after all eggs had hatched as a function of hatchery environment and year. Separate analyses were performed for each year because we used different subsets of females in each year. We used a two-way ANOVA to test for overall differences in egg survival between hatchery environments and years. We were not able to collect daily survival information at the traditional hatchery in 2005. We calculated daily survival at both

hatchery environments in 2006. We used a general linear model (GLM) to test for differences in daily survival based on both fixed (hatchery, day, heath tray location) and random (female) effects. Daily measurements of temperature and dissolved oxygen were used as covariates. We also examined the proportion of dead eggs that were infected by microbes between the two hatcheries over time using a GLM. Factors included hatchery and day. Temperature and dissolved oxygen were included as covariates. Finally, since we had daily survival at the streamside hatchery for two years we tested for the effect of year and day on daily survival. Environmental covariates were incorporated. We also examined the proportion of dead eggs infected by microbes as a function of year and day for the streamside hatchery. Data were tested for normality and homogeneity of variances by comparing residuals versus fitted values and using a Shapiro-Wilk test in program R. Analysis were conducted using arcsine-square-root transformed data in cases of non-normality or heterogeneity of variance.

Larval Stage: The effects of hatchery rearing environment on larval survival

Rationale: Under artificial conditions, once larval sturgeon hatch from the egg and deplete their yolk sac reserves, their survival depends on multiple factors including the tank design (i.e. water flow, access to cover) and hatchery management (i.e. feed, feeding rates, cleaning; (Conte et al. 1988). Streamside hatcheries have been implemented as restoration tool lake sturgeon and represent more natural fluctuation in incubation and rearing environments. However, there is no empirical evidence to suggest that streamside hatcheries result in elevated numbers at hatch or higher survival during the critical larval stage. We were interested in examining the effects of hatchery

environments on larval hatch and subsequent survival by comparing a streamside hatchery to a traditional hatchery environment. Results from this work will be important for the development of future streamside hatchery programs and will help to improve culture techniques for larval lake sturgeon.

Methods: Larvae were reared in circular tanks (diameter = 1.2m) that were each divided to include 2 replicates of each of the three collection methods described above. Tanks were replicated ten times at the streamside hatchery and six times at the traditional hatchery. Larvae were fed live brine shrimp nauplii (*Artemia* spp) beginning five days following hatch. Brine shrimp were hatched in multiple cone shaped tanks (19 liters) over a 24 hour period set to be harvested at different times. Brine shrimp were harvested by stopping the aeration to the hatching cone and affixing a light source to the bottom of the cone. Brine shrimp are positively phototactic and migrate towards the light source while empty casings and un-hatched eggs floated to the surface. Brine shrimp were then harvested through a valve at the bottom of the tank. We concentrated the brine shrimp using a plankton screen bag (100 μm) and transferred them into an aerated bucket of fresh water. Brine shrimp were fed in 200ml aliquots once every two hours from dawn (0700) until dusk (2100). Tank sections were cleaned daily by purging all the water in the tank to remove excess food and waste and the tank sides were wiped with providone-iodine (Betadine) for disinfection.

Analysis: All analyses were conducted using program R (Version 2.1.1). Differences in the number of larvae that hatched between the two rearing environments in each of 2005 and 2006 were examined using a 2-way ANOVA. Furthermore, we used a 2-way ANOVA within each year to examine if the number of hatched larvae differed

between individual females reared in equal initial numbers between the two hatchery environments. We calculated daily larval survival for a period of 14 days at which time the yolk-sac had been absorbed and the fish were actively feeding. We used a GLM to test for differences in daily survival between hatcheries with day and hatchery location as fixed effects. Daily measures of temperature were used as a covariate. The effects of female could not be incorporated into the analysis of daily larval survival because families had to be mixed at the traditional hatcheries due to a smaller number of rearing tanks. Data were tested for normality and homogeneity of variances by comparing residuals versus fitted values and using a Shapiro-Wilk test in program R. Analysis were run on arcsine-square-root transformed data in cases of non-normality or heterogeneity of variance.

Larval Stage: The effects of within hatchery habitat complexity on larval growth and yolk-sac utilization.

Rationale: Starvation is viewed as a significant source of mortality following absorption of endogenous reserves during the yolk-sac stage of development in larval fishes (Folkvord and Hunter 1986). Mortality has been attributed to patchy distributions of food resources available during period when larvae switch from endogenous reserves to exogenous feeding (Letcher et al. 1996). Resource limited larvae can either decrease metabolic expenditures (reduced activity) (Wieser et al. 1992) or increase activity until suitable resources are located (Mehner and Wieser 1994). It is unknown how larval lake sturgeon utilize yolk-sac reserves when they are in the presence of refuge or resources. It has been noted that larval sturgeon will utilize refuge when presented in an aquaculture

setting (Aloisi et al. 2006). The refuge is subsequently abandoned when endogenous reserves become low to search for resources. In a typical hatchery setting larval sturgeon are not presented with available refuge and food is administered to the tank approximately two weeks following hatch. Information on differential growth and utilization of yolk-sac reserves as a function of refuge and resource availability is lacking for larval lake sturgeon. We examined four treatment groups including; 1) tanks with no refuge, 2) tanks with simulated refuge (shag carpet), 3) tanks with no refuge but presence of a food resource, and 4) tanks with simulated refuge and presence of a food resource. Results from this work address concerns of how artificial rearing conditions within a hatchery influence variation in individual size and will help to improve future lake sturgeon culture techniques in conservation hatcheries.

Methods: Each of the four treatment groups was replicated across paternal half-sib families (n = in 2006; n = 2007). Larvae in two treatments including the presence of resources were fed live brine shrimp every two hours from 0700 until 2100. Larvae that hatched from family groups were measured (n=10) for total length (TL; mm), yolk-sac length (YSL; mm), yolk-sac height (YSH; mm), and body area (BA; mm^2) and then allocated to experimental tanks. Treatment groups were stocked with 100 larvae at the start of the experiment. We then removed 10 random individuals every 2 days for measurements. Individuals were only used once and were not returned to the tank. Tanks were cleaned each day to remove mortalities and uneaten food. We used Image J analysis software (Version 1.34, freeware) to conduct all digital measurements. Information recorded from each larva included: YSL, YSH, YSA, BA, and TL. Total length was measured from the anterior most part of the developing rostrum to the posterior most part

of the notochord. We calculated elliptical yolk-sac volume (YSV in mm^3) following the formula of Blaxter and Hempel (1963),

$$YSV = (\pi/6)*L*H^2$$

where L = yolk-sac length and H = yolk-sac height. Prior to image analysis larvae were anesthetized with 25mg/L MS222.

Analysis: All analyses were conducted using program R (Version 2.1.1). We used a one-way analysis of variance to test for differences in both total length and yolk-sac area between treatment groups on the day of hatch. Furthermore, we also used a one-way ANOVA to test for differences in total length and yolk-sac area for larvae produced from different females at hatch. We used a mixed effects model to examine differences in the response variables (growth and yolk-sac utilization) between the four treatment groups over time. Fixed effects of the model included treatment, day since hatch, and year. Female was included as a random effect to account for variation between females and because we used a limited number of females representing a large population of breeding adults. Female was also nested within year as different females were used in 2006 and 2007 so comparisons between years as a function of female could not be conducted. Data were tested for normality and homogeneity of variances by comparing residuals versus fitted values and using a Shapiro-Wilk test in program R. Analysis were run on log10 transformed data in cases of non-normality or heterogeneity of variance.

Juvenile Stage: The effects of rearing environment on juvenile growth and survival

Rationale: The environments in which a fish is reared and into which it will be released are very strong determinants of a restoration programs success (Travis et al.

1998). Ideally, we want to minimize the possibility that, between hatching and final release, the hatchery is selecting for distinct alleles or allelic combinations that would be disadvantageous in the natural environment. Phenotypic plasticity in response to variations in environmental conditions occurs frequently in a natural setting but may be more critical in an aquaculture setting (Brokordt et al. 2006). This may be particularly evident at early life history stages and can affect abilities to respond to environmental cues following release into the natural environment (Olla et al. 1998; Travis et al. 1998). Streamside hatcheries offer a more natural rearing environment. However, they have gone into operation without research evaluating their effects on juvenile growth and survival relative to more traditional production hatcheries. We evaluated growth and survival of juvenile lake sturgeon collected using three gamete/larval collection methods in two different hatchery environments in each of two years. Results from this work will help to refine culture methods for this species and provide guidelines for the use of streamside hatcheries as a management option for the restoration of lake sturgeon populations.

Methods: The experiment was initiated after all gamete/larval collection methods had been distributed evenly between the two hatchery environments. Fish in both hatchery environments were reared in circular tanks (diameter=1.2m) divided into six to include two replicates of each of the three gamete/larval collection methods. Each collection method was randomly assigned to two of the six slots located within each tank. Tanks were replicated ten times at the streamside hatchery and six times at the traditional hatchery. Water volume within each section was 90 liters. Maximum rearing densities were held to a maximum of 400 fish/m^2 of tank space. Fajfer et al. (1999) found no

significant difference in growth between three different juvenile lake sturgeon rearing densities and concluded that densities of 150–450 fish/m^2 are equally acceptable for rearing lake sturgeon. Juvenile lake sturgeon were transitioned from brine shrimp onto live cultured blackworms (tubificid annelids; J.F. Enterprises, CA, USA) starting at day 21 following hatch. Blackworms were fed for approximately one week prior to the start of feeding frozen bloodworms (chironomid midge larvae). A 1kg sheet of frozen blood worms was fed per 1000 juvenile sturgeon per day. The worms were thawed and divided evenly (~15grams per section) among all tank sections. The amount of bloodworms fed increased approximately .5kg every 2 weeks for the duration of rearing. Tanks were cleaned once daily in both environments to remove waste and excess food. Protocols in both hatcheries involved purging the water volume in each tank through the removal of a central stand pipe. The tank walls were disinfected using betadine and the stand pipe replaced and the tank refilled. At the streamside hatchery all fish were given a salt bath once per week as a preventative measure because the streamside hatchery water source was untreated. Salt was administered at 5ppt for 15 minutes to the entire system simultaneously by adding salt to the final settling tank. Tank sections with fish exhibiting signs of stress (i.e. reduced feeding and movement or increased respiration) were treated with additional salt baths. Mortalities were recorded and removed daily at both hatchery environments. A subset of fish (n=25) from each tank section and corresponding collection method were measured for total length (mm) weekly throughout the rearing period. Daily measurements of temperature (^{o}C) and dissolved oxygen (mg/L) were recorded using data loggers and a handheld YSI instrument, respectively.

Analysis: We examined two response variables, survival and growth, as a function of hatchery rearing environment and different collection methods for gametes/larvae. All analyses were conducted using program R (Version 2.1.1). We used a GLM to examine daily survival of juvenile lake sturgeon as a function of both fixed (hatchery, day, year, treatment) and random effects (tank section). Temperature was included as a covariate. Insignificant factors were eliminated and the model was rerun. We used the same model to examine differences in weekly growth (total length). Data were tested for normality and homogeneity of variances. In cases of non-normality or heterogeneity of variances, analyses were run using arcsine-square-root-transformed survival data and log transformed growth data.

Juvenile Stage: The effects of within hatchery habitat complexity on juvenile survival, growth, and body coloration.

Rationale: Cultured and wild fish grow in different environments and different experiences are likely to produce behavioral and physiological differences at all life history stages (Huntingford 2004). Providing fish in hatcheries with simulated habitat within rearing tanks has been shown to increase post-release survival (Naslund 1992; Tipping 1998; Berejikian et al. 2000; Tipping 2001; Maynard et al. 2004) and influence morphological expression by producing fish with the appropriate camouflage colorations for the habitat backgrounds found in natural habitats. We were interested in examining the effects of three levels of habitat complexity on juvenile lake sturgeon growth. These three treatment groups included 1) tanks with no cover, 2) tanks with 50% cover (Simulated with large rocks), and 3) tanks with 100% cover.

Methods: This experiment was conducted entirely at the streamside facility. Juvenile lake sturgeon were reared in circular tanks (diameter=1.2m) divided into six to include two replicates of each of the three treatment groups we evaluated. We replicated each treatment group six times. A total of 25 fish were stocked into the experiment and were reared for a total of six weeks. We recorded weekly measurements of total length for all individuals within each tank section. Tanks were cleaned and mortalities removed and recorded daily. Juvenile lake sturgeon were fed bloodworms as previously described.

Analysis: We examined growth and survival of juvenile lake sturgeon reared in the three different treatment groups. All analyses were conducted using program R (Version 2.1.1). Survival was examined using a GLM with day tank, treatment, and the interactions as fixed factors. We examined weekly differences in growth using a GLM with week, tank, treatment, and the interactions fixed factors. Data were tested for normality and homogeneity of variances. In cases of non-normality or heterogeneity of variances, analyses were conducted using arcsine-square-root-transformed survival data and log transformed growth data.

RESULTS

Egg Stage

There was no significant difference in the number of eggs collected from females for incubation (mean ± 1SD = 11,157 7883) between 2005 and 2006 (df = 16, t = 2.12, P = 0.99). Overall egg survival to hatch was low in 2005 and did not differ significantly ($F_{1,20}$ = 0.12, P = 0.73) between the streamside hatchery (mean ± SE: 0.17 ± 0.02) or the traditional hatchery (0.15 ± 0.02). Egg survival was significantly different among females

in 2005 ($F_{9,20} = 3.52$, $P = 0.009$; Fig.6), though the interaction between female and hatchery environment was not significant ($F_{1,20} = 0.69$, $P = 0.71$). In 2006, egg survival was significantly higher at the streamside hatchery compared to the traditional hatchery ($F_{1,20} = 11.37$, $P = 0.003$). Egg survival was twice as high at the streamside hatchery (0.35 ± 0.05) compared to the traditional hatchery (0.17 ± 0.04). As in 2005, egg survival differed significantly among females ($F_{7,20} = 2.83$, $P = 0.03$; Fig. 6) but was not a result of the interaction with hatchery environment ($F_{1,20} = 0.47$, $P = 0.85$). The number of egg incubation days varied considerably at the streamside hatchery over the two years (7-14 days) and was a constant 19 days at the traditional hatchery in both years. Temperature was a significant predictor ($F_{1,84} = 334.2$, $P < 0.001$, $r^2 = 0.843$) for egg incubation time (Fig. 7) and may explain inter-female differences.

We compared the daily survival of eggs incubated between hatcheries in 2006. Daily survival rates of eggs reared at the streamside hatchery in 2006 were significantly higher than eggs reared at the traditional hatchery ($F_{1,354} = 6.52$, $P = 0.011$) in the same year. We found a significant difference between groups of eggs within different heath trays ($F_{22,354} = 2.63$, $P < 0.001$) but the significance was not attributed to individual females ($F_{1,354} = 0.42$, $P = 0.52$). Furthermore, the interaction between egg group and hatchery environment was not significant ($F_{17,354} = 0.42$, $P = 0.98$). Microbial infection resulted in a large portion of daily egg mortality in both hatchery environments though the prevalence of infection was significantly lower at the traditional hatchery ($F_{1,321} =$

18.24, $P < 0.001$; Fig. 8). Several factors influenced the occurrence of microbial infection in both hatchery environments. As observed with daily survival, groups of eggs between heath trays differed significantly in infection rates ($F_{22,321} = 3.39$, $P < 0.001$) which again was not driven by either individual females ($F_{1,321} = 1.45$, $P = 0.23$) or the interaction between egg group and hatchery rearing environment ($F_{16,321} = 2.82$, $P < 0.001$). Eggs at the streamside hatchery that experienced higher mean temperatures during incubation had significantly higher rates of microbial infection ($F_{1,321} = 27.06$, $P < 0.001$).

We tested daily survival and the proportion of dead eggs with microbial infection between years at the streamside hatchery. Year was not significant ($F_{1,514} = 0.01$, $P = 0.95$) and was dropped from the model. Daily survival was significantly different ($F_{15,514} = 10.89$, $P < 0.01$) among different incubation times (Fig. 9). For example, significant peaks in mortality were observed at day 3 for eggs incubating for 10 days, and at day 4 for eggs incubating for 11 and 12 days. There was no difference in mean daily temperatures over the entire period of incubation between years ($F_{1,514} = 0.77$, $P = 0.40$). Microbial infection was not different between years at the streamside hatchery ($F_{1,514} = 0.68$, $P = 0.41$) and similar to above, was found to be positively influenced by temperature ($F_{1,514} = 32.17$, $P <0.001$).

Larval Stage

Hatchery Environments: The number of larvae at hatch was significantly higher for the streamside hatchery compared to the traditional hatchery in 2006 ($F_{1,20} = 26.76$, $P < 0.001$), but was not significantly different in 2005 ($F_{1,20} = 1.73$, $P = 0.20$). Larval hatch was significantly different between females (Fig. 10) in both 2005 ($F_{9,20} = 4.28$, $P = 0.003$) and 2006 ($F_{7,20} = 11.99$, $P < 0.001$). Furthermore, in 2006 the interaction between female and hatchery environment was significant ($F_{7,20} = 3.52$, $P = 0.01$) with certain females having higher numbers of larvae at the streamside hatchery compared to the traditional hatchery (Fig. 10).

Larval survival was followed over a period of 14 days at which time the yolk-sac had been absorbed and feeding commenced. Larval survival in 2005 did not differ significantly between hatchery environments ($F_{1,516} = 0.68$, $P = 0.41$) with daily survival rates being 0.957 ± 0.002 at the streamside hatchery and 0.970 ± 0.010 at the traditional hatchery. Survival differed significantly by day ($F_{19,516} = 4.31$, $P < 0.01$) with mortality occurring in the first three days following hatch. In 2006, larval survival was high at the traditional hatchery (0.966 ± 0.001), however larval survival was significantly higher ($F_{1,918} = 30.32$, $P < 0.01$) at the streamside hatchery (0.982 ± 0.001). The interaction between hatchery location and day was marginally significant ($F_{18,918} = 1.52$, $P = 0.073$) with survival at the traditional environment lower in the first three days following hatch.

Hatchery Complexity: A total of 3088 and 3887 individual larvae were measured for total length and yolk-sac area in 2006 and 2007, respectively. We found a significant difference in total length between females at hatch in both 2006 ($F_{5,478} = 21.4$, $P < 0.001$) and 2007 ($F_{7,850} = 106.92$, $P < 0.001$). Since all families were represented in all treatment groups there was no difference in total length between treatments group at the start of the experiment. In both years the experiment was terminated after 15 days due to complete absorption of the yolk-sac. Larval lake sturgeon grew rapidly in total length in both 2006 and 2007. However, differences were found between the four treatment groups. There was a significant interaction between the treatments over time between the two years ($F_{21,6545} = 9.72$, $P < 0.001$) with larvae in the open treatment group being smaller in total length at the end of the experiment compared to the treatment group with available refuge. In 2006, both the open treatment and the open treatment with resources were significantly smaller compared to the treatment with refuge (Fig. 11). In 2007, the difference was only observed between the treatment with refuge and the open treatment without food. In 2007 larvae in the treatment with refuge grew 9.5mm in total length over the course of the experiment while larvae in the treatment with no refuge grew 8.6mm over the same period. Differences between the two years were attributed to differences between treatment groups on certain days but did not differ in their final result. For example, in 2006 the treatment group with refuge was significantly different than the treatment group without refuge at day 9 ($F_{31,6545} = 2.02$, $P = 0.04$) while the same result was not found until day 11 in 2007 ($F_{31,6545} = 2.32$, $P = 0.03$). We found a significant

difference between females in total length over time in both years ($F_{366, 6545} = 13.67$, $P < 0.001$; Fig. 12).

We found a significant difference in yolk-sac volume between females at hatch in both 2006 ($F_{5,478} = 10.93$, $P < 0.001$) and 2007 ($F_{7,850} = 48.8$, $P < 0.001$) with earlier spawning females having larger yolk-sac reserves at hatch. Since treatment groups were replicated across families there was no difference between treatments at the outset of the experiment. There was a significant effect of treatment over time between years on larval yolk-sac volume ($F_{31,6545} = 6.89$, $P < 0.001$). As with total length, differences were attributed within different days but yolk-sac area was not significantly different between the treatment groups at the termination of the experiment ($F_{31,6545} = 0.331$, $P = 0.74$). In 2006, both the open treatment and the open with food treatment had significantly smaller yolk-sac volumes on days 5 ($F_{31, 6545} = 2.58$, $P = 0.01$; Fig. 7) and 7 ($F_{31,6545} = 3.052$, $P = 0.002$; Fig 11) implying greater expenditures (allocation) of resources related to metabolic activity. We found a significant difference between females in yolk-sac volume over time in both years ($F_{366, 6545} = 7.53$, $P < 0.001$; Fig. 12).

Juvenile Stage

Hatchery Environments: We tested for differences in daily survival of juvenile lake sturgeon from three different gamete/larval collection method reared in two different environments in each of two years. Both tank within location ($F_{59,7255} = 0.79$, $P = 0.43$) and the interaction of collection methods over time across years ($F_{638,7255} = 0.01$, $P =$

0.90) were not significant and were removed from analyses. Subsequently, we found a significant difference in survival between collection methods over time at the two different hatchery environments ($F_{284,7671} = 1.72$, $P < 0.001$). In both cases, offspring from the dispersing larvae collection method had the lowest mean daily survival (Fig. 13). Daily survival was significantly lower at the traditional hatchery for the first week of juvenile rearing ($F_{284,7671} = 2.992$, $P = 0.003$) and then mortalities decreased (Fig. 13). Mortalities approached zero rapidly at the traditional hatchery following day 50 post hatch while we still observed minor fluctuations in survival at the streamside hatchery in both years (Fig. 13). Mean daily temperature did not significantly influence survival as a function of hatchery environment ($F_1=0.1840$, $P = 0.67$). Mean daily temperature was 22.01 ± 0.3 and 21.57 ± 0.23 at the streamside hatchery in 2005 and 2006 respectively. Mean daily temperature was 21.4 ± 0.1 and 21.1 ± 0.03 at the traditional hatchery in 2005 and 2006 respectively.

We evaluated juvenile growth for each of three different methods of gamete/larval collection reared in two different hatchery environments in each of two years. The effects of tank and tank sections within tank were dropped from the final model due to insignificance. Average rearing densities per tank slot were 390.6 ± 4.2 fish/m^2 at the streamside hatchery and 362.3 ± 5.4 fish/m^2 at the traditional hatchery. Juvenile lake sturgeon reared at the traditional hatchery in 2006 grew significantly faster among all three treatment groups compared to individuals reared at the streamside hatchery in the same year and all individuals from both hatchery environments in 2005 ($F_{98, 29494} =$

85.7, P < 0.001). For example, in 2006 juvenile sturgeon produced as a product of direct gamete takes grew 7.29cm per week at the streamside hatchery and 11.96cm per week at the traditional hatchery. There was no difference in growth between treatment groups at the traditional hatchery in 2006 ($F_{95, 29493} = 9.62$, P = 0.31). However, juveniles in the NPE treatment group were significantly larger than DL juveniles at the end of the experiment at the streamside hatchery ($F_{95, 29494} = 27.2$, P = 0.008). There was no difference between the growth of juvenile lake sturgeon between the three treatment groups at either hatchery environment in 2005 ($F_{95,29493} = 2.89$, P = 0.18). Weekly growth of juvenile sturgeon in 2005 was 7.21cm at the streamside hatchery and 7.30cm at the traditional hatchery. Weekly growth varied significantly between treatments over time in both hatchery environments in both years ($F_{95,29493} = 67.4$, P <0.001). Variation in total length within a treatment increased over time.

Hatchery Complexity: We evaluated the effects of three different levels of tank complexity (open, half cover, total cover) on daily survival and weekly growth of juvenile lake sturgeon at the streamside hatchery in 2005. There was no significant difference in daily survival among the three treatment groups over the course of the experiment ($F_{82,504} = 0.96$, P = 0.57). Survival was high among all treatments with total survival at the end of the experiment being 0.952, 0.968, and 0.968 for open, 50% covered, and 100% covered treatment groups respectively. There was no significant difference in the total length among the three treatment groups at the outset of the experiment ($F_{2,222} = 1.89$, P = 0.15). We found a significant effect of treatment over time

($F_{10,1253} = 1.86$, $P = 0.04$) with juvenile lake sturgeon in the open treatment group reaching a larger size by the end of the experiment (Fig. 14).

DISCUSSION

For recruitment-limited and numerically depressed populations such as the lake sturgeon, conservation hatcheries are an important technique in the return of populations to sustainable levels. Furthermore, conservation hatcheries for sturgeon species will need to employ management techniques that maximize the probability of imprinting to natal streams following release. We evaluated the effects of two different hatchery rearing environments on growth and survival. Results are discussed on a stage by stage basis.

Egg Stage

Egg survival was relatively low in both years in both hatchery environments with the highest egg to hatch survival being 0.35 at the streamside hatchery in 2006. We documented significantly higher survival of lake sturgeon eggs reared at the streamside hatchery in 2006 compared to the traditional hatchery in the same year. This also translated to higher daily survival at the streamside hatchery compared to the traditional hatchery. Stream environmental conditions including temperature, stream physical and biotic conditions vary temporally within a season and between years and are expected to affect embryonic development and survival in fishes (Houde 1987). Higher egg survival at the streamside hatchery is likely attributed to differences in incubation temperatures as all other rearing methods and females used were consistent between the two hatcheries.

Eggs incubating at the two different hatchery environments incubated for different lengths of time. Eggs incubating in 11^{o}C water at the traditional hatchery did not hatch until day 19 while a much broader range was seen at the streamside hatchery where the embryos were subject to more natural temperature regimes. Within the streamside hatchery we documented minimum and maximum incubation times of 7 and 14 days respectively. Temperature profiles did not differ between years within each hatchery environment. Furthermore, daily egg survival was different across different incubation lengths. Experiencing natural variations in water temperatures, within tolerable limits, may have both beneficial and deleterious effects on survival. Previous research on sturgeon species has shown that rates of development at the embryonic stage are temperature dependant (Wang et al. 1985). Eggs incubating at lower temperatures may benefit by larvae being bigger at hatch but suffer a tradeoff by being exposed to higher levels of predation at the egg stage. Eggs incubating at higher temperatures will hatch rapidly avoiding additional risks of predation at the egg stage but will produce larvae of smaller size. For conservation purposes it is critical that hatcheries capture this diversity during the incubation stage. If spawning time is repeatable for adult female lake sturgeon (Forsythe, unpublished data) then this would suggest that females choose when to deposit eggs and collecting eggs from only one of multiple spawning groups would limit overall diversity captured. Furthermore, the impacts of collecting eggs from females spawning in late periods with high water temperatures and rearing them in conditions reflective of earlier spawning females is still unknown in lake sturgeon culture. Rearing lake sturgeon eggs under constant and cool water conditions eliminates natural variability in incubation observed in both the river and the streamside hatchery environment. Constant hatchery

environments create a logistically efficient hatchery environment where eggs hatch more synchronously and on a predicted date. However, if timing of spawning is further influenced by the conditions the egg is reared in then preference for spawning time could be shifted in one direction with eggs incubated in constant traditional hatchery conditions. This has yet to be evaluated in lake sturgeon.

We also documented differences in egg survival between different females but the interaction between hatchery environments was not significant. Maternal effects have become increasingly recognized (Mousseau and Fox 1998) as having significant effect on survival during early life stages including the egg stage (Heath et al. 1999). These effects are still relatively understudied in sturgeon. Differences in egg survival could have been due to differences in egg quality between females spawning at different times. Egg quality is an important component for fertilization and egg survival (Bromage et al. 1994; Brooks et al. 1997). Differences between female lake sturgeon eggs have been documented in the levels of total lipids and thiamine (K. Debrowski, personal communication), and yolk-sac reserves (this study).

Microbial infection was found to be significantly higher at the streamside hatchery compared to the traditional hatchery in 2006. Furthermore, incidences of microbial infection increased with water temperature. Microbial infection, primarily the fungal order saprolegniales and other aquatic fungi, are widespread in water supplies of fish hatcheries and often result in elevated mortality during the egg stage (Marking et al. 1994). Microbial infection has been noted in studies on sturgeon (Smith et al. 1980; Dettlaff and Goncharov 2002) and research has been conducted on developing efficient methods to treat eggs prior to and during incubation (Rach et al. 1997; Rach et al. 1998;

Bouchard and Aloisi 2002). We used heath trays as incubation vessels in both hatcheries. However, MacDonald jars are typically used for sturgeon egg incubation as the eggs remain suspended and rolling which helps to reduce fungal infection. We used heath trays because they provided an efficient way to quantify egg mortality and microbial infection on a daily basis. Furthermore, we were using fairly small numbers of eggs which were reared separately by family. Small volumes of eggs typically do not remain suspended as easily in MacDonald jars as large batches of eggs. It was not surprising that microbial infection was higher at the streamside hatchery. Direct river water was minimally treated before entering the incubation system and might expect to have higher levels of prokaryotic and eukaryotic egg pathogens relative to cooler ground water. Finally, different batches of eggs composed of eggs taken from females spawning at different time periods differed in the time it took for microbial infection to occur (Fig. 8). Later batches of eggs had earlier incidences of microbial infection. Differences might be expected at the streamside hatchery where different batches of eggs throughout the season will be subject to different daily mean temperatures. However, even within a constant temperature profile at the traditional hatchery we saw microbial infection arrive earlier in one batch then the other. One previously mentioned cause may be related to egg quality between females spawning at different times (Bromage et al. 1994; Brooks et al. 1997). Future research designed to identify egg quality between females as a function of reproductive timing is warranted.

Larval Stage

Larval hatch was higher at the streamside hatchery in 2006 compared to both the traditional hatchery in the same year and both hatcheries in 2005. This corresponds to the increase in egg survival observed over the same time period. Daily larval survival was high at both hatcheries in 2006 but was significantly higher at the streamside hatchery. In both years we saw the bulk of the larval mortalities in the three days immediately following hatch at both hatchery environments. Larval survival was then very high for the remainder of the period in which they had endogenous reserves. We did not observe a significant difference in survival at the end of the larval stage when the yolk-sac was completely absorbed. This is not surprising as other sturgeon species have been shown to resist starvation for 18 days (Hardy 2000).

Larvae grown in different levels of rearing habitat complexity responded differently in growth and utilization of yolk-sac reserves. We found that larvae in tanks with refuge reached larger total lengths by the end of the experiment compared to those that were reared in open tank environments. In natural conditions, larvae hatch and immediately burrow into the stream substrate. They utilize their endogenous reserves during this period. When endogenous reserves get low larvae then emerge from the substrate at night and drift passively downstream into feeding areas. This hiding phase is likely a critical stage in the development of a larval lake sturgeon. We showed a difference in size of larvae that were provided with available refuge. Larvae reared in open treatments remained active during the daylight hours, apparently searching for refuge. The presence of resources (food) in the tanks only differed between as a function of available refuge. Our initial hypothesis was that if a larval sturgeon could perceive a resource rich environment then they may utilize yolk reserves at a faster rate. The timing

of when a larval sturgeon can perceive this type of variable in the environment it still relatively unknown. We also found a difference in the size at hatch and growth rates between families, irrespective of treatment groups. Females that had larvae which hatched at smaller sizes remained smaller throughout the course of the experiment (Fig. 12). The effects of refuge on phenotypic expression should be considered by managers conducting conservation hatchery programs for lake sturgeon.

Larval lake sturgeon utilized endogenous yolk-sac reserves over a period of 15 days. Yolk-sac reserves between treatments did not differ in the initial or final days of the experiment. We did find however that yolk-sac reserves were used at a faster rate during days 5 and 7 in the open treatments. The efficiency of yolk utilization depends on the relationship of the rate at which yolk reserves are used in metabolic processes with the rate at which they are incorporated into body tissue (Blaxter and Hempel 1966). Larval lake sturgeon reared in tanks without cover spent a large majority of their time during the day actively swimming which likely resulted in higher amounts of yolk reserves put towards metabolic processes. This was observed up until day 7 which at that time the majority of the larvae in the open tanks remained on the bottom and clustered in the corners of the tanks. As previously mentioned, other species of sturgeon have been shown to be able to resist starvation for long periods. Therefore, this delay in growth, caused by increased use of yolk reserved in metabolic processes, may result in a reduction in survival in later stages (Bailey and Houde 1989).

Juvenile Stage

We examined differences in daily survival of juvenile lake sturgeon as a function of year, method of gamete/larval collection, and hatchery environments. One important result was that survival of offspring collected as dispersing larvae was significantly lower during the first week of rearing in both hatchery environments and years compared to the other two collection methods (Fig. 13). Dispersing larvae were captured when yolk-sac reserves were diminished and they were passively dispersing downstream. These individuals could have been feeding on natural food sources and the transition onto hatchery feed (brine shrimp) was not as easy compared to individuals obtained using the other collection methods that were exposed to only brine shrimp since hatch. DiLauro et al. (1998) found that once larval lake sturgeon had imprinted on brine shrimp they would not consume a different and formulated diet. Future programs interested in utilizing this collection method may need to explore different mixtures of natural and artificial food sources in order to wean a greater proportion of dispersing larvae onto suitable hatchery diets. Anderson (1984) found high survival using a larval lake sturgeon diet consisting of natural sources (mixed zooplankton species and aquatic annelid worms). Differences in daily survival between hatchery environments occurred in the first week with streamside hatchery fish having higher daily survival. We were limited to feeding during the daylight hours at both hatchery environments when small hourly feedings over a 24 hour period using automatic feeders would likely have been favorable (Cui et al. 1997). Water at the streamside hatchery may have provided an additional and natural source of food. However we did not quantify natural sources of food. One observation to support this hypothesis was four larval lake sturgeon that were found in a heath tray several weeks after hatch that had never been offered brine shrimp but were equal in size to individuals

that had been on food for weeks. An analysis of the potential larval food sources (zooplankton) contained in filtered river water supplying streamside hatcheries would be an important future research.

Once all juvenile lake sturgeon transitioned to blood worms (~day 50) survival was very high and close to 1 for the remaining rearing period. We observed minor peaks of mortality at the streamside hatchery in both years following this period. These mortalities occurred in seemingly healthy fish which may indicate the potential for either parasitic or disease issue at the streamside hatchery. If these mortalities were indeed a function of natural parasites, then weekly salt bath treatments may have helped reduce the occurrence of parasite-mediated mortality to a minimum. At the end of the rearing period and prior to release into the wild, a subset of juvenile lake sturgeon from both hatchery environments were required by the Michigan Department of Natural Resources to undergo routine disease testing. Results of these tests were inconclusive. Programs utilizing streamside hatcheries for lake sturgeon culture may need to have more frequent testing done to ensure fish quality and reduce mortalities.

Juvenile lake sturgeon grew rapidly in all years at both hatchery environments. Growth in both hatcheries was a minimum of 7.2mm per week which is similar to estimates from other studies on lake sturgeon (Fajfer et al. 1999). Holtgren et al. (2007) also reared juvenile lake sturgeon in a streamside hatchery and experienced higher growth compared to the streamside hatchery used in this study with a limited number of fish being fed continuously with automated feeders. Growth was significantly higher at our traditional hatchery in 2006 compared to the streamside hatchery in the same year and both hatcheries in 2005. Fish from all collection methods grew approximately the

same within a hatchery environment. Inter-individual variation in growth increased throughout the rearing period in both years. Differences may be attributed to increased competition within tank sections as certain individuals grow faster than others. In most production hatchery settings these fish would be graded in order to keep growth rates of all fish as close to maximum as possible. Our goal was not to maximize growth but to compare growth and survival across two hatchery environments and three different collection methods.

Juvenile lake sturgeon grown under different levels of habitat complexity exhibited growth trends that were opposite from those observed during the larval stage. Fish in the open treatments grew significantly more over a six week period than those reared in tanks with available cover. Observation of juvenile feeding behavior from this experiment indicated that fish actively searched for food for extended periods of time between feedings. Fish reared in the open treatments were able to find and consume 100% of the provided food while lake sturgeon grown in treatments with cover may not have been successful in finding food. Other studies have found no difference in growth between traditional and semi-natural rearing conditions (Berejikian et al. 2001; Maynard et al. 2004). However, Berejikian et al. (2000) found that steelhead fry reared in an enriched hatchery environment grew faster than those from a conventional environment when the two treatments were stocked together but not when they were stocked separately. Results from this and other studies suggest that individuals reared in semi-natural hatchery environments may not exhibit a growth advantage until stocked into the wild. Unfortunately we could not evaluate juvenile lake sturgeon used in this study beyond the hatchery environment. Studies that have used a more semi-natural rearing

approach suggest that factors other than growth may be important for supplemental fish following release. For example, available structure and habitat in rearing tanks has been shown to influence coloration patterns in fish (Ellis et al. 1997). Lake sturgeon have evolved patterns of body coloration that reflect the benthic (sand) environment that they live in. If hatchery produced juveniles exhibit different coloration patterns than this may increase probabilities of survival. This research question is one that will be important for conservation hatchery programs to evaluate.

CONCLUSIONS

We documented differences in growth and survival of lake sturgeon between two different hatchery environments. Survival from the egg stage until the end of the rearing process was highest at the streamside hatchery (7.7%) in 2006 and lowest at the traditional hatchery in 2005 (4.8%). The majority of mortality was incurred during the egg stage at both hatchery environments. The main difference between the two hatchery environments was that offspring reared at the streamside hatchery experienced a natural temperature regime. We found that this natural temperature regime resulted in maintaining natural variation in egg incubation time and larval size at hatch. The traditional hatchery environment minimized this variation. Increasing temperature had negative effects on egg incubation and larval size at hatch. Rearing eggs across a more natural temperature regime is important for hatchery programs wanting to maximize survival and natural variation in offspring phenotype. Repeatability for spawning time in

adult lake sturgeon may be influenced if eggs that are deposited in late conditions are reared only in early environmental conditions. Stocked offspring may all return at the same spawning time and diminish any adaptations that had evolved due to reproductive isolation between spawning groups. Production of lake sturgeon in hatcheries has been limited in certain years due to poor success obtaining eggs from the wild breeding population. We demonstrated that rearing offspring from three collection methods results in similar levels of survival and growth. However, natural food sources need to be evaluated for offspring collected as dispersing larvae. Conventional hatchery practices purposely reduce individual size variability in juveniles and we found that by rearing different groups of offspring produced from different reproductive periods we were able to maintain variation in fish size which increased over time. The natural variability in development and timing characteristic of wild fish may be inherent factors which enable them to adapt to changing conditions.

Results from this study provide the first comparison between streamside and traditional hatchery rearing environments. Streamside rearing and the addition of habitat complexity within rearing tanks produce lake sturgeon offspring that are morphologically similar to their wild counterparts. Future research directed at identifying the effects of different rearing environments on behavior following release as well as the mechanisms and timing of imprinting would be important next steps in evaluating streamside rearing for lake sturgeon.

Chapter 3

HATCHERY REARING ENVIRONMENT AND AGE AFFECT SURVIVAL AND MOVEMENTS OF STOCKED JUVENILE LAKE STURGEON.

INTRODUCTION

Hatchery programs have been widely used as both an enhancement and management tool to address declines in wild fish populations (Waples 1999). More recently, hatchery programs are receiving increased scrutiny because of the potential negative impacts of captive reared individuals to natural populations (Ford 2002; Araki et al. 2007a; Miller et al. 2004). Criticisms have been raised based on empirical evaluations of the relative effects of different management options including; hatchery rearing environments (Berejikian et al. 2000), age of release into the natural environment (Paragamian and Kingery 1992), and gamete, juvenile, or broodstock collection and maintenance protocols (Flagg and Nash 1999).

Stocking protocols developed from hatchery programs are used to increase wild population abundance and probabilities of survival during critical life history stages (Alverson 2002; Brown and Day 2002). Furthermore, they have the potential to supplement recruitment when unfavorable environmental conditions result in high embryo and larval mortality (Secor and Houde 1998). Although stocking programs have been used to establish and enhance many fisheries, programs have failed to increase the numerical abundance of others (Secor et al. 2000; Brown and Day 2002; Myers et al. 2004). General conclusions concerning the effectiveness of stocking programs have not been reached and challenges remain to reconcile benefits and potential costs to

population dynamics, genetic integrity of resident populations, and to ecosystem processes and resource economics (Travis et al. 1998; Utter 1998). Supplementation programs are becoming increasingly important for conservation efforts of threatened and endangered species (Brown and Day 2002), including regionally imperiled lake sturgeon (*Acipenser fulvescens)*.

The lake sturgeon is considered as threatened throughout much of its range. This species has experienced dramatic declines in both numerical abundance and in distributional range, and numbers have been projected to be less than 1% of historic levels (Hay-Chmielewski and Whelan 1997). Current impediments to lake sturgeon restoration include the sensitivity of adults to anthropogenic factors such as over-harvesting, degradation in water quality and spawning habitat and loss of connectivity due to impoundment (Holey et al. 2000). In recent years, lake sturgeon rehabilitation through stocking has become a high priority throughout the Great Lakes (Peterson et al. 2007). Despite this, considerable uncertainty remains regarding the efficacy of different egg and larval collection methods, and the appropriate age of fish to stock. Success of supplementation programs is typically based on contributions of stocked fish to older age classes (Li et al. 1996) or return to post stocking assessments (Walters et al. 1997). However, these methods are not amenable to lake sturgeon as they are not widely subject to harvest and younger age classes are not efficiently captured in surveys using standard sampling methods (Benson et al. 2005). Late age at maturity, infrequent spawning, low recruitment rates (Nilo et al. 1997), and occupancy of geographically separated breeding and nonbreeding areas also confound interpretations, dictating that other methods be employed to evaluate supplementation programs. Stocking prescriptions have typically

been based on a large salmonid literature though species such as lake sturgeon have very different ecologies, mandating that research investigating the success of alternative methods of culture and release be conducted on this species explicitly.

A number of factors contribute to the success of hatchery based stocking programs. Stocking of lake sturgeon throughout the Great Lakes region has employed numerous techniques for collecting progeny (e.g., larval collection (Auer and Baker 2002) and direct gamete takes), and juveniles have been stocked at different ages (fry, fall fingerling, and yearlings; Schramm et al. 1999). Stocking studies that have attempted to identify optimal ages for release into natural environments have reported mixed results with respect to survival based on age at stocking (Amtstaetter and Willox 2004; Elrod et al. 1988; Margenau 1992; Secor and Houde 1998). Studies have indicated that rearing hatchery fish to larger sizes leads to increased survival upon release (Gunn 1987; McKeown et al. 1999; Yule et al. 2000). Recently streamside hatcheries have been advocated as a restoration tool for lake sturgeon throughout the Great Lakes (Holtgren et al. 2007). Streamside hatcheries use water from streams targeted for release to maximize probabilities of imprinting and to help reduce domestication selection. Conservation hatcheries have been used for trout and salmon and have attributed positive results to more natural rearing conditions (Maynard et al. 1996; Berejikian et al. 2000). In this study, we examined the effects of different hatchery environments by rearing lake sturgeon in two different hatchery settings, a streamside hatchery and a traditional hatchery, in order to quantify differences in post stocking success.

We tested the null hypothesis that hatchery rearing environment and age at release are not significant predictors of post-stocking survival for juvenile lake sturgeon. Studies

identifying the success of stocking strategies typically define success by the proportion of fish recaptured in subsequent assessments (Leber et al. 2005). We adopted this criteria and directed research objectives to: 1) determine contributions of fish stocked from two different rearing environments and three different collection methods on the rate of recapture using in-stream assessment, 2) determine which stocking age results in higher rates of capture (a surrogate measure of survival), and 3) quantify rates of downstream dispersal as a function of age and size. Results of this study demonstrate how lake sturgeon rearing conditions, collection methods, and size and age at stocking contribute to the variance in probability of survival, and movements after release into the natural environment.

METHODS

Study Site

Research was conducted on the Black Lake system, Michigan (Fig 15, see details in Smith and Baker 2005; Smith and King 2005). Fish were stocked into the Upper Black River, a fourth order stream located in the northeastern corner of the state of Michigan that is highly influenced by dams. The hydrology of the Upper Black River provides a unique opportunity to enumerate a large proportion of the annual adult spawning run as well as collect gametes and juveniles. Physical variables (water depth and flow; Table 7) provide wadeable conditions for several kilometers downstream of the first impoundment allowing for instream experimental work.

Rearing environment

Juvenile lake sturgeon were reared in two hatchery environments. Half of all fish were reared at a streamside hatchery using water from the natal stream on the Upper Black River, Michigan. The remaining fish were reared using ground water at a state hatchery in southwestern Michigan, representing a traditional hatchery environment. The streamside hatchery used a flow-through design where water was pumped directly from the Upper Black River. Water was mechanically filtered to remove sediments. Flow rates were kept constant and at a level that resulted in approximately two complete water turnovers every hour. Cleaning, feeding, lighting, and water flow protocols were consistent between the two hatchery environments following common lake sturgeon rearing methods (Ceskleba et al. 1985; Fajfer et al. 1999). Fish in both hatcheries were kept in 1.22m diameter tanks (0.5m in depth) divided to include 2 replicates of three different gamete/juvenile collection methods (discussed below).

Collection of progeny

We used three methods to collect lake sturgeon progeny. The first two methods are commonly used for many sturgeon species and included directed egg takes from spawning females (Birstein 1993), and collection of recently hatched larvae dispersing downstream of the spawning grounds (Auer and Baker 2002). The third method involved collecting eggs naturally fertilized and deposited on the stream substrate by spawning adults.

1) Direct take spawning: Lake sturgeon were captured on the spawning grounds using large dip nets. Eggs were removed by hand stripping females captured in the act of

spawning. Eggs were placed in zip lock bags and transferred to a cooler to maintain ambient river temperatures. Milt was collected using a 30ml syringe and was immediately placed on ice. Fertilizations were conducted within 12 hours of egg collection. Egg volumes from each female were measured, divided into two equal lots, and fertilized separately with milt from two randomly selected males to create paternal half-sib family groups (n=26 in year 1, n=25 in year 2). Half of the eggs from each half-sib family were transported to the traditional hatchery and half were maintained at the streamside hatchery. Eggs were incubated and hatched out in heath trays at both hatcheries.

2) Drift Larvae: Sampling for larval lake sturgeon passively dispersing downstream has been shown to be a viable collection strategy (Auer and Baker 2002, Smith and King 2005). Larval sampling was conducted during a 5-hour period, beginning at dusk (21:00) and ending in the early morning (03:00) at a sampling location approximately 2 km downstream (Figs 1,15) from the primary spawning areas on the Upper Black River. Five D-framed drift nets were set across the stream channel to capture dispersing larval sturgeon. This design was replicated nightly each year for the entire drifting period. Nets were checked hourly and the larvae within each net were enumerated. Larval lake sturgeon captured each evening were reared at the streamside hatchery until the end of the drifting period. The larvae were then divided between the two hatchery environments. Minimum number of adults contributing to larval drift based on capture data was 154 and 234 for year one and two respectively.

3) Naturally fertilized and deposited eggs: Systematic kick-net sampling was conducted below observed spawning locations to collect eggs that were naturally

fertilized and deposited in the stream substrate. Transects were run across the stream at 1 meter intervals. At each interval we conducted kick-net sampling for 10 seconds. Transects continued downstream in intervals of 5 meters until no eggs were collected in consecutive transects. Eggs were incubated and hatched at the streamside rearing environment because of pathogen concerns of the traditional hatchery. Larvae were then divided between the two rearing environments.

Fish stocking and assessment

We used the Upper Black River as an experimental release site to test the hypothesis that hatchery rearing environment and age were not significant predictors of post stocking survival. The release and assessment area encompassed a stream region of approximately 4 km (Fig. 15). Three age classes were stocked into the Upper Black River. Fish at 8 and 13-weeks of age were released during 2005 and 17-week old fish were released in 2006. Prior to release, all individuals were measured (total length (cm)), weighed (g) and tagged with a colored implant elastomer dye (Northwest Marine Technology, WA, USA) unique to their rearing environment and gamete/juvenile collection method. Elastomer was injected on the ventral side of the rostrum where the colors were most easily distinguished. Juvenile lake sturgeon were transported from the traditional hatchery to the streamside hatchery one day prior to each release. All age classes were acclimated to ambient stream conditions within the streamside hatchery for >12hours prior to being transported to the release site. All fish were released simultaneously at the same location on the Upper Black River.

We chose a region of the river because of increased catchability and ease of replication of methods across the different stocking ages. The release location did not represent a stream region where juvenile sturgeon would naturally be present at the three ages of release. Prior to release, four capture locations were established downstream of the release site (Fig. 15). Within each site four D-framed drift nets were deployed across the stream at equal intervals. Net position was recorded using a GPS unit. Water velocity (m/s) and depth were recorded at the mouth and corners of each net. Nets were checked and emptied at hourly intervals following release. Sampling persisted for approximately 24hours following release. Sampling at the most upstream assessment site was discontinued for all release ages after no juvenile sturgeon were captured in consecutive hours. Each captured individual was examined for elastomer marks indicating collection method, rearing location, and total length (cm) was recorded. Fish were then released immediately downstream of the drift nets.

Block nets were deployed downstream of the third site assessment site for both the 13 and 17-week release ages. During the 17-week release a second block net was also deployed below the fourth downstream site. The block nets consisted of 0.32cm^2 mesh leaders and large mesh (0.32cm^2) fyke nets. Leaders were used to guide dispersing lake sturgeon into fyke nets. Block nets were deployed to estimate drift net capture efficiency, and to use as a second gear type to assess downstream dispersal. Block nets were not installed during the 8-week release. Sampling protocols for the block nets were consistent with those for the drift nets.

A DC tow-barge shocker was used to survey the stream predator community to assess sources of mortality for released juvenile lake sturgeon. Shocking was conducted 1

hour following release. We conducted a single pass starting at the release location and proceeded downstream past all assessment sites. Predators were captured and held in an aerated container until the end of the pass. We used gastric lavage to expel the stomach contents of all captured fish. All predators containing juvenile sturgeon were measured for total length and gape width and height.

Statistical Analysis

All statistical analyses were performed using program R (R Development Core Team 2007, http://www.rproject.org). Data were tested for normality and homogeneity of variances using a Shapiro-Wilk test and by examining residuals versus fitted values in R. In cases of non-normality or heterogeneity of variances, analyses were run using arcsine-square-root-transformed survival data and log transformed length data. For analyses within and among release ages we only included data from the drift net captures. Two dependant variables, the proportion of fish recaptured and the total length of recaptured fish were examined. The proportion of fish from each collection method and rearing environment captured was calculated for each hourly sampling interval at each assessment site for all release ages. All recaptured proportion values were weighted by the total number released from each hatchery environment and the different collection methods within each release age. We used a general linear mixed effects model to examine the effects and interactions of six independent variables on the proportion of fish recaptured. Fixed effects included assessment site of capture, gamete/juvenile collection method, rearing environment, net location, physical stream variables (depth and flow measured at the net), and the age of release. Time of capture was included as a random

variable in the model. We also estimated interactions among age, rearing environment, time of capture, and assessment site. Data collected from each block net used in the 13 and 17 week releases were analyzed separately due to lack of comparability to the drift nets and because we were unable to replicate the sampling strategy across all release ages. The same model was used for the block net as the drift nets except for the assessment site variable. Efficiency estimates for the drift nets were calculated by dividing the total amount of fish recaptured in the drift nets by the total amount of fish recaptured within the same time period (both gear types).

For all stocking events we analyzed the response variable total length of recaptured juvenile lake sturgeon using a general linear mixed effects model. In this model the response was the observation of length for a specific fish at a distinct sampling time and at a specific sampling site. Fixed effects included capture site, collection method, and rearing environment. Time of capture was included as a random effect in the model. We also examined the interaction effects between all independent variables. The independent variable age was not examined in our analysis of total length because there were significant differences between ages in terms of total length at the time of release. The block net data was analyzed using the same models.

We tested for mean differences in the total length of recaptured fish in relation to the total lengths at the time of release for each treatment group at each release age using a 2 factor ANOVA. The response variable, length, was predicted by period (prior to release or recapture), time (recapture hour), physical stream variables, net location, and we examined the interaction between the independent variables. Analyses were conducted separately for fish captured in the block nets and drift nets for each release age. To test

for biases in sized based catchability between the two gear types we used an unpaired t-test to test for mean differences in the total lengths of recaptured fish from each gear type. This was conducted separately for data from the 13 and 17 week releases.

RESULTS

Results are presented for dependant variables, recapture rates and size, as they related to age, hatchery rearing environment, and gamete/juvenile collection method. Stream depth and flow as well as net position were removed from the final model due to insignificance. Though sampling was conducted for 24 hours, no juvenile lake sturgeon were captured beyond 15 hours following release. Physical conditions, including water depth and velocity were not statistical predictors of capture rates but varied between the release ages (Table 7).

Variation in Recapture Rates

Based on 11,721 juvenile lake released across each collection method, hatchery rearing environment, and age class (Table 8) we documented significantly higher recapture rates for fish released at 17 weeks of age ($F_{21,148} = 6.45$, $P < 0.01$) compared to fish released at 8 and 13 weeks of age (Fig 16). During the 8 and 13-week releases we observed a significant main effect of hatchery rearing environment on the proportion of fish recaptured. Significantly fewer fish ($F_{21,148} = 6.45$, $P = 0.03$) recaptured were reared in the traditional hatchery environment relative to the streamside hatchery (Fig. 16). Significantly fewer fish were captured at increasing downstream distances from the

release site ($F_{21,148} = 6.45$, $P = 0.03$) relative to the most upstream assessment sites (Fig. 16). We recaptured significantly more fish at downstream assessments sites in the 13-week release when compared to the 8-week release and in the 17-week release when compared to both the 8 and 13 week releases (Fig. 16). There was a significant effect of time following release on the proportion of fish recaptured ($F_{21,148} = 6.45$, $P < 0.01$). At 8 and 13 weeks of age we captured fish moving downstream rapidly after release during the daylight hours (Fig. 17). At 17-weeks of age we captured very few fish during the daylight hours immediately following the release (Fig. 17). A large proportion of total captures occurred during evening hours >8 hours following the release (Fig. 17). There was no main effect of treatment group on recapture success among release ages. Distinct differences among treatment groups (Table 8) occurred within sites at distinct time periods but no overall trends emerged from the analysis.

The block net deployed during the 13-week and 17-week release provided estimates of the efficiency of the drift nets as well as a second gear type for assessing downstream dispersal. Within the 13-week stocking event the block net below the third downstream site recaptured 10.3% of the total number of fish released. There were significantly more fish moving downstream of this assessment site that were from the streamside hatchery environment ($F_{1,27} = 3.04$, $P < 0.01$). Efficiency of the drift nets varied hourly with a mean (± 1.SE) of 12.6 ± 2.8% during the 13-week release. There were no distinct trends in treatment differences among sampling times. Within the 17-week stocking event the block net below the third downstream sampling location resulted in recapturing 15.01% of the total number of fish released. Drift net efficiency was 14.3 ±

5.2%. There was no effect of rearing environment on the proportion of fish moving through this site at any time during the sampling period. As with the drift nets for this age class, significantly more fish were captured at later sampling hours ($F_{7,11} = 38.52$, $P < 0.01$). The block net below the fourth downstream site produced recaptures totaling 19.26% of the fish released. Drift net efficiency was 6.2 ± 2.1%. There was no effect of rearing environment on the proportion of fish recaptured. Again, we caught significantly more fish at later sampling hours compared to hours immediately following release at this downstream site ($F_{6,8} = 11.82$, $P < 0.01$). Even though the drift net efficiency varied hourly, results from the block nets are consistent with those from the drift nets.

Variation in Size

Documenting differences in length within and among release ages is important for determining size based downstream dispersal and as a factor affecting differential survival. We observed no significant differences between rearing environments in the total length of fish prior to release for the 8 and 13-week release ages (Table 8). At 17 weeks of age fish from the traditional rearing environment were significantly larger ($F_{1,609} = 11.52$, $P < 0.01$) than those reared at the streamside hatchery at the time of release (Table 8). There was no main effect of rearing environment or treatment type on the size of recaptured fish in both the 8 and 13-week releases. There was a significant effect of rearing environment in the 17-week release with recaptured fish from the traditional hatchery being significantly larger than those from the streamside hatchery ($F_{26,583} = 22.32$, $P < 0.01$). In all release ages there was a significant positive effect of

time following release on the size of recaptured fish (Fig. 18). Fish captured in later post-release were significantly larger than fish captured soon after release in the 8-week ($F_{17,202} = 4.06$, $P < 0.01$), 13-week ($F_{18,302} = 7.46$, $P < 0.01$), and 17-week ($F_{26,583} = 22.32$, $P < 0.01$) release (Fig. 18).

Sizes of fish captured in the block nets at both 13 and 17-weeks revealed trends consistent with those from the drift nets. There was a significant increase in the length of fish captured in successive sampling periods post-release in the block during the 13-week release. The same results were found for both block nets during the 17-week release ($F_{7,621} = 23.97$, $P < 0.001$).

During the release at 8 weeks of age the mean total length of recaptured fish was significantly smaller ($F_{5,589} = 11.50$, $P < 0.01$) than the mean length prior to release with the exception of the final recapture period. This was consistent in the 13 week release with fish lengths prior to release being significantly larger than recaptured individuals at all hours except the final net check ($F_{6,1596} = 65.82$, $P < 0.01$). The mean total length of fish captured in the block net at 13 weeks of age was significantly smaller compared to mean total length prior to release for all hourly checks ($F_{7,1597} = 56.91$, $P < 0.01$). At 17 weeks of age, juvenile lake sturgeon recaptured in the drift nets were significantly smaller on average than prior to release for all hourly checks except the final recapture period ($F_{4,1191} = 7.07$, $P < 0.01$). Fish recaptured in the block nets at 17 weeks of age were significantly smaller for all recaptured periods except the final two net checks for both the third ($F_{6,916} = 16.53$, $P < 0.01$) and fourth most ($F_{6,2859} = 17.59$, $P < 0.01$)

downstream sites. There was no significant difference in the size of juvenile sturgeon recaptured in the block net compared to the drift nets ($df = 392$, $t = 1.293$, $P = 0.197$) during the 13 week release. During the 17 week release there was no significant difference in sizes between the gear types at the third most downstream site ($df = 691$, $t = 0.092$, $P = 0.93$) but a significant difference was found at the fourth most downstream assessment site ($df = 848$, $t = 3.51$, $P < 0.01$) with fish in the block and drift nets averaging 153.47 ± 0.97mm and 138.35 ± 3.80mm respectively.

Evidence of mortality

We were unable to quantify sources or rates of mortality by fish predators following release using the tow barge shocker. During the release at 8-weeks of age two rock bass were identified as having juvenile lake sturgeon in their stomachs. Further attempts to quantify predation at later release ages proved unsuccessful. At both 8 and 13-weeks of age we visually observed crayfish preying upon juvenile lake sturgeon after release.

DISCUSSION

Sampling protocols providing quantifiable and comparable results are currently lacking for age-0 lake sturgeon (Holey et al. 2000), despite recent advances in visual surveys (Benson et al. 2005). Using this natural stream as an experimental tool was positive for attempting to provide quantified estimates of survival and downstream movement for a species that is otherwise difficult to capture and enumerate. Assessments

of juvenile lake sturgeon released at 8, 13, and 17 weeks of age revealed that there were low rates of recovery and inferentially high rates of mortality. Rates of recovery increased with increasing age. We demonstrated an effect of hatchery rearing environment on juvenile lake sturgeon survival and movements when juveniles were stocked at 8 and 13 weeks of age. Our ability to detect this difference is an indication that even moderate differences in hatchery rearing environments potentially related to domestication selection (e.g. Lynch and O'Hely 2001) can play an important role in post release movements and survival. The effects of rearing environment immediately following release may be reduced by releasing fish at older ages as evident by the lack of significant differences attributed to hatchery environment for 17 week old fish. However, theoretical (Lynch and O'Hely 2001) and empirical (Araki et al. 2007a) data on the effects of hatchery rearing environment on reproductive success of hatchery reared fish dictates that further research be conducted for sturgeon.

Significant differences in recapture rates between different hatchery environments indicates the need to tailor culture methods that maximize probabilities of survival through important life-history transitions. This is especially important when dealing with threatened or endangered species such as lake sturgeon. Modifying juvenile rearing environments to approximate natural conditions is increasingly used to minimize the degree of domestication for captive animals (Flagg and Nash 1999). Studies of fish reared in different environments have documented differences in behavior (Olla et al 1998; Berejikian et al. 1999; Flagg and Nash 1999), post stocking survival (Wiley et al. 1993; Maynard et al. 1996), growth rates (Mesick 1988), social rankings (Berejikian et al. 2000), and development of appropriate body camouflage coloration (Maynard et al.

1996). Olla et al. (1998) suggested that fish reared in a sensory deprived hatchery environment have poor feeding efficiency and are less capable of avoiding predators. Differences between the streamside hatchery and the traditional hatchery used in this study were likely sensory because physical rearing conditions such as tank size, shape, flow rate, and feeding regimes were consistent between the two environments. Juvenile lake sturgeon reared in the streamside hatchery may have benefited from fluctuations in temperature mimicking natural environmental conditions. Mechanical filtration within the streamside hatchery, though effective at removing larger particulates, likely allowed exposure to stream organic material and biological organisms. Furthermore, the water at the streamside hatchery potentially contained natural stream chemical cues from either conspecifics, predators, or other species.

We observed a significant effect of time on fish capture. During releases conducted at 8 and 13 weeks of age, we found that fish were captured at significantly earlier times following release relative to recoveries at 17 weeks of age (Fig. 17). Fish released at 17 weeks dispersed downstream after sunset. This negative photo-tactic or nocturnal behavior has been noted with lake sturgeon larvae dispersing downstream of spawning grounds (Auer and Baker 2002) and similar behavioral trends have been noted in field (Chiasson et al. 1997; Benson et al. 2005) and hatchery studies on juveniles (Peake 1997). This is the first documentation of nocturnal behavior patterns in hatchery reared juvenile lake sturgeon immediately following release into the natural environment. It has been shown that nocturnal behavior of other sturgeon species (Kynard et al. 2005; Kynard and Parker 2005) as well as other fish species (Bradford and Taylor 1997; Crisp 1991) is a dominant feature of migration and foraging in the first year of life. Variability

in the proportion of juveniles recaptured was high during the early hours following release at the 8 and 13 week age classes. This is likely due to the fact that most individuals moving passively in the current were unable to control their rate of downstream dispersal. We saw a noted decrease in the variability in sizes of fish dispersing downstream later in the day at 8 and 13 weeks which can be attributed to the movement of primarily larger fish (Fig. 18). There was a significant effect of fish size on dispersal at 17 weeks of age as well. However the variation surrounding the estimates was noted in later hours, opposite to that of the earlier age classes (Fig. 18). This increase in variation at later time periods following release indicates that the majority of juveniles had an ability to choose dispersal time. Size-based downstream dispersal has been noted with other species of fish. Bradford and Taylor (1997) found that larger Chinook salmon fry had a higher probability of dispersal during nighttime hours than smaller individuals of the same cohort. Age dependant differences in physical abilities, as suggested by differences in timing and size distributions of dispersing juveniles should be used to develop release strategies for juvenile lake sturgeon.

Our estimates of recapture success and stream movements among fish from different collection method within each release age are robust because of the large number of fish released (Table 8). However, comparisons of results across age classes should be interpreted in the context of limitations imposed by our sampling design. Catchability has been shown to increase with increases in body size of fish (Borgstroem and Skaala 1993) and models assuming equal catchability typically underestimate the actual number of fish in the population (Mäntyniemi et al. 2005). Juvenile lake sturgeon were stocked over two years due to a combination of rearing limitations and the need for

large sample sizes for release. The use of the block nets as a means of assessing the drift net efficiency and as second gear type was important. Consistency of results between the two gear types was encouraging because drift net efficiency was generally low. Furthermore, we only documented differences in size of fish between the two gear types at one assessment site. This was at the most downstream assessment site and slower water velocities (Table 8) likely allowed the juvenile lake sturgeon to elude this passive sampling gear. Finally, transport of juveniles reared at the traditional hatchery to the streamside hatchery may have posed increased levels of stress. However, fish were acclimated at the streamside hatchery overnight (>12hrs) prior to release and we don't believe transport affected our results.

River conditions varied between release events with the lowest water velocities occurring during the 17 week release. Lower drift net efficiency at this site can likely be attributed to the decrease in water velocities. Studies have indicated that higher water velocities can strongly influence downstream displacement of young fish (Daufresne et al. 2005). This may have been the case for juvenile lake sturgeon at 8 and 13 weeks of age however, we documented a subset of larger fish being able to control their rate of dispersal in each release.

It is imperative that well defined species specific protocols be developed for stocking programs (Cowx 1999). Supplementation protocols should be tailored to species specific ecologies and behaviors, are assessments should be made to quantify estimates of survival following release. Protocols have been developed for many recreationally and commercially important species that incorporate ecologically important information. Largemouth bass (*Micropterus salmoides*) have been shown to have low dispersal

following release indicating that localized supplemental stocking in distinct areas of complex reservoirs may increase the benefits on the population level (Copeland and Noble 1994). Ellison and Franzin (1992) indicate that matching stocking times and locations to appropriate food resources are important in walleye (*Stizostedion vitreum*) introductions. Our results indicate that supplementation strategies should focus on night releases and in areas of moderate flow. These parameters ensure that rates of dispersal are high immediately following release and will likely have an indirect effect on levels of predation by reducing mortality incurred by visual predators. It is critical that release sites are evaluated on a system by system basis with emphasis placed on determining areas that maximize suitable juvenile nursery habitat. For lake sturgeon, natural juvenile rearing habitat has been defined as predominantly shallow riverine habitat consisting mostly of sand (Benson et al. 2005). Our research addresses the importance of tailoring stocking programs to individual species and systems rather than focusing on numbers and sizes of fish.

CONCLUSIONS

In conclusion, our study addresses the need for further empirical and experimentally rigorous evaluations of hatchery rearing and stocking programs for little studied species such as lake sturgeon. Streamside rearing may be advantageous to small/young lake sturgeon by exposing them to an enriched environment prior to release. However there may be an age/size threshold where the effects of rearing environment on movements and survival immediately following release are diminished. Further work that characterizes mechanisms influencing differences among hatchery rearing environments

for lake sturgeon is important to the development of system specific management prescriptions.

Chapter 4

OVERWINTER SURVIVAL OF STOCKED AGE-0 LAKE STURGEON REARED IN NATAL AND NON-NATAL ENVIRONMENTS

INTRODUCTION

Survival through the first winter of life is commonly viewed as a critical determinant of year class strength (Oliver et al. 1979). Despite this, quantified estimates of overwinter mortality are lacking for many species. Survival throughout early life history stages, including the first winter of life, is mediated by a number of selective processes that operate as fish experience elevated rates of size dependant mortality due to both predation and energy depletion (Miller et al. 1988). Physiological changes (e.g., metabolic rates, hematological parameters) during the winter months result in decreasing condition and a depletion of energy reserves, the effects of which differ among individuals from the same cohort (Cunjak, 1988). Overwinter energy reserves are highly correlated with the length of the growing season, which is reduced for temperate species (Post and Parkinson 2001). Furthermore, fish produced in later reproductive events have even greater constraints with the shortened growing season acting as a strong selective pressure against overwinter survival (Biro et al. 2003). Knowledge of age-specific survivorship through this critical stage is particularly important as a measure of the success of hatchery programs, which are increasingly used as a part of recovery programs for imperiled species.

Populations of lake sturgeon (*Acipenser fulvencens*), a native fish of the Great Lakes, have been numerically depressed throughout their range due to anthropogenic factors and are believed to be at less than 1% of historical numbers (Hay-Chmielewski and Whelan, 1997). Restoration activities have been initiated, largely in the absence of data regarding their effectiveness, focusing on hatchery supplementation (Holey et al. 2000). Recently, there has been an interest in the use of streamside hatcheries (Holtgren et al. 2007) as a restoration tool. Streamside hatcheries use water from streams targeted for release, potentially reducing the degree of domestication and increasing the likelihood of imprinting. Assessing the overall impact of stocking juveniles reared in streamside versus traditional hatchery environments is important to project the effects of supplementation on long-term population abundance. This assessment is complicated by this species' unique life history characteristics (e.g., delayed sexual maturity), and inefficient collection methods for young age classes (Benson et al. 2005a). Low sampling efficiency affects accuracies of survival estimates which impedes evaluations of post-release survival of juveniles reared in different hatchery environments. Most lake sturgeon culture and stocking efforts in the Great Lakes have reared fish to fall fingerling size (~4 months) under the assumption that larger fish survive better following release into the natural environment (Schram et al. 1999). No quantitative data are available on age-specific lake sturgeon survival, including survival through the first winter.

Ultrasonic telemetry is a valuable fisheries technique used to estimate parameters such as mortality, distance traveled, and range size (Flavelle et al. 2002; Taverny et al. 2002; Zamora and Moreno-Amich 2002). Despite some limitations (e.g. sample size, battery life, and handling-induced mortality), telemetry allows the collection of multiple

observations from a single individual, which is an advantage over passive capture techniques such as gillnetting (Zamora and Moreno-Amich 2002). Recent advancements in telemetry technology, particularly decreasing transmitter size, have increased opportunities to study species over a greater range of ages. This is especially important for studies interested in assessing the survival of fish released during their first year of life. Telemetry studies on sturgeon have generally been conducted in the spring and summer months (Hay-Chmielewski 1987; Holtgren and Auer 2004; Smith and King 2005). Research through the winter months has described overwinter movement patterns and identified habitats that serve as both important nursery grounds for subadults and staging areas for adults (Quist 1999; Sulak and Clugston 1999; Li et al. 2007).

There is a critical need for determining over-winter survival of age-0 sturgeon. Our overall goal was to provide estimates of overwinter survival, however our specific objectives were to, 1) estimate overwinter survival of hatchery reared juvenile lake sturgeon released at a traditional (fall fingerling) age and 2) determine differences in survival between juvenile lake sturgeon reared in two different hatchery environments, a streamside hatchery on the natal river and a traditional non-natal hatchery environment. This study provides both the first quantitative estimate of overwinter survival and the first attempt at assessing the effects of hatchery rearing environment on overwinter survival for juvenile lake sturgeon.

METHODS

Study Site

Juvenile lake sturgeon were collected from a population located in Black Lake, Michigan (See Smith and King 2005 for site description). Black Lake encompasses approximately 4,000 hectares. Black River, the primary habitable tributary to Black Lake provides spawning habitat for adults and nursery habitat for juveniles. The population of lake sturgeon is closed to immigration by dams on both the Lower Black River and the Upper Black River.

Rearing Environment

Twenty juvenile lake sturgeon were reared from eggs to six months of age in each of two environments, a streamside hatchery using water from the natal Upper Black River, and a traditional hatchery (The Michigan Department of Natural Resources (MDNR) Wolf Lake hatchery) using a non-natal (ground water) source. Rearing conditions (circular fiberglass tanks 1.22m in diameter) and feeding regimes were consistent between the two hatcheries. Fish were reared separately for three months at which time all individuals from the streamside facility were transported to the traditional hatchery and retained for an additional three months. Fish were relocated from the streamside hatchery to the traditional hatchery for several reasons, the first being that the streamside hatchery could not be operated during the fall. Secondly, we needed to increase growth to a size (~approximately 25 cm total length) that would allow insertion of the ultrasonic transmitters.

Transmitter Implantation and Monitoring

Juvenile lake sturgeon were surgically implanted with small coded ultrasonic transmitters; 1.7 ± 0.01 cm (mean ± 1 S.E) in length and 0.7 ± 0.001 cm in diameter with a mass of 1.95 ± 0.01 g in water (Vemco, model V7, Nova Scotia, Canada). Prior to surgery, streamside hatchery fish had a mean ± SE total length (TL) of 31.05 ± 0.34cm and a weight (W) of 106.39 ± 2.59g. Traditional hatchery fish were 31.45 ± 0.37cm in TL and 106.44 ± 3.25g in W. All surgeries were conducted between December 8th and December 12th, 2005. The weight of the transmitter did not exceed 2% of the fish weight, which fell well within the generally accepted 2% rule (Winter 1996). Fish were anesethized using tricaine methane sulfonate (MS222; 125 mg/l; Summerfelt and Smith 1990) in an aerated container prior to surgery. Sedation was maintained with a constant dose of MS222 (50 mg/l) recirculating through a portable surgical table (LaVigne 2002). Transmitters were anchored to the wall of the peritoneal cavity using non-absorbable sutures to reduce movements of the transmitter in the body cavity. Incisions were closed using 3-0 gauge non-absorbable monofilament nylon sutures (Ethicon) in an interrupted pattern. Ovadine antiseptic was applied to the wound to promote rapid healing. Following surgery, fish were monitored for a 3-day period for complications from the surgical procedure. Juvenile lake sturgeon were transported to the release site in an aerated stocking trailer. Fish were acclimated into the wild using a net pen for a 3 hour period prior to release into Black Lake on December 16th 2005. Sonic transmitters were set at an operating frequency of 69 kHz and programmed to emit a coded pulse randomly between 40s and 120s.

Manual tracking excursions followed transects that spanned the length of the lake. Manual tracking was conducted on successive days during which time we attempted to cover the total area of Black Lake. Attempts were also made to track upstream and downstream in the Black River. When possible, tracking was conducted on a weekly schedule. Movements of sonically tagged fish were monitored from a boat using a Vemco receiver (Model VR100) equipped with both omni-directional (VH65) and directional (V10) hydrophones. The directional hydrophone had a horizontal beam width of 22 degrees and a vertical beam width of 150 degrees. Transmitters had a potential detection range of 1 km. Hydrophones were deployed every 500 m on calm days and every 250 m or less during days with moderate waves. Recorded positions of detected fish were made using a handheld WAAS enabled GPS receiver and bearings were taken using a magnetic compass adjusted to obtain true magnetic north. Fish locations were triangulated with a minimum of three geographic points. Three automated hydrophone receivers (Vemco, Model VR2) were strategically placed within Black Lake to detect movement between distinct zones. Collected positioning data were managed and analyzed using geographic information system (GIS) software (ESRI, ArcMap v. 8.3). GIS data were managed in the form of Universal Transverse Mercator (UTM) units. Temperature was monitored near the release site for the duration of the observational period at 1-hour intervals using an Onset HOBO Pro underwater temperature logger (Onset Computer Corp., Bourne, Massachusetts).

Data Analysis

Juvenile lake sturgeon locations were calculated using the bearing angle and the GPS coordinates from that observation using the telemetry software Locate II (Nams 2001), which uses the method of least squares to estimate the error associated with the predicted location. Juvenile lake sturgeon exhibiting active movement patterns after ice-out were assumed to have survived. Average inter-observational distance traveled between tracking events was calculated for each individual as further evidence of survival. This was calculated by taking straight line measurements between successive detection points. We also calculated the inter-observational distance of each individual from the release site by taking straight line measurements to each detection point from the release location.

We used a chi-square test to determine significance of differences in survival attributed to rearing location. We used a t-test to examine differences in the size at stocking for fish between the two hatchery environments, to test for the sizes of surviving fish relative to undetected fish, and to test for differences between hatchery environments in distance traveled.

RESULTS

There were no significant differences in length or weight of fish prior to release between hatchery rearing environment (TL: $df = 19$, $t = 0.42$, $P = 0.68$; Weight: $df = 19$, $t = -0.29$, $P = 0.78$). Tracking was conducted from April 15th until June 1st 2006. Sixteen juvenile lake sturgeon were detected and determined to be actively moving during the

period of observation (Table 9). This corresponds to a minimum survival of 40%. Seven of these individuals were reared at the streamside hatchery environment and nine individuals were from the traditional hatchery environment. A chi-square test revealed no significant difference in the number of fish surviving between the two rearing environments (df = 1, χ^2 = 0.25, P = 0.62). Of the remaining 24 fish that were released, only two other fish were located but were assumed to be dead after several consecutive tracking excursions identified them to be in the same location. This location was close to the release site and a stationary automated receiver recorded these individuals over an extended time period. All detected and surviving individuals remained within 1 km of the release location (Table 9) until the end of May 2006. Manual tracking and automated receivers documented a number of fish moving extensively within this zone. There was no significant difference between fish from the different hatchery environments in inter-observational distance traveled (df = 14, t = -0.24, P= 0.81) or in inter-observational distance from the release site (df = 14, t = -0.96, P = 0.351). Average distance traveled between detection events was 0.19 ± 0.14 km (Table 9). Water temperatures ranged between 1.4 and 22.3^{o}C (mean ± SE, 14.4 ± 0.5^{o}C) during the observational period.

The size distribution at the time of release of fish identified as surviving was not significantly different from fish that were not detected for TL (df = 38, t = -1.63, P = 0.11) and W (df = 38, t = -1.29, P = 0.20). A t-test revealed no significant difference attributed to hatchery environment in TL (df = 14, t = -0.88, P = 0.39) or W (df = 14, t = 0.86, P = 0.40) of surviving fish.

DISCUSSION

This quantitative estimate of overwinter survival is the first recorded for hatchery reared juvenile lake sturgeon. Our study was not exhaustive and the estimate presented (40%) should be considered as a minimum value of survival. This result is encouraging considering the number of deleterious factors associated with long-term hatchery rearing, including elevated levels of domestication (Huntingford 2004). Our survival estimate for age-0 lake sturgeon is comparable to estimates produced for other non-Acipenseriform species. For example, Biro et al. (2004) estimated overwinter survival for age-0 rainbow trout (*Oncorhynchus mykiss)* stocked into two different lakes to be 27% and 33% respectively. Overwinter survival of wild juvenile coho (*Oncorhynchus kisutch*) salmon has been documented as high as 46% (Quinn and Peterson 1996) while monthly survival estimates for juvenile largemouth bass (*Micropterus salmoides*) have been found to be as low as 54% during the winter months (Miranda and Hubbard 1994). Finally, Jonas et al. (1996) found that survival of fall stocked muskellunge (*Esox masquinongy*) was low (2.3%) and attributed results to a significant loss in energy reserves through the winter.

Greater stored energy reserves of larger age-0 individuals of some fish species increase probabilities of over-winter survival relative to smaller individuals (Shuter et al. 1980; Miranda and Muncy 1987). We did not document a significant difference in the sizes of fish detected versus not detected in our study however it is important that we compare the size of fish released in this study to wild fish of similar age. Often, estimates of survival of hatchery stocked fish might be inflated because hatchery fish are generally larger and in better condition than their wild counterparts. This is attributed to a longer

hatchery growing season. Hatchery reared juvenile lake sturgeon in our study were approximately 31.23 cm in total length and 106.41 g in wet weight at the time of release in early December. Wild juvenile lake sturgeon captured in the fall months as late as November in the Peshtigo River, Wisconsin, were as large as 31.6 cm in total length and weighed as much as 134 g (Benson et al. 2005b) indicating that they were in comparable, if not better, condition relative to the hatchery produced fish. Even though hatcheries provide progeny for stocking that are of good condition at the time of release, tradeoffs in elevated levels of domestication and reduced foraging ability might reduce probabilities of survival if monitored over a longer period.

One observation from detected individuals was that juvenile lake sturgeon maintained activity throughout the observational period. However individuals remained within a very small area close to the release location. Smith and King (2005) found that yearling sturgeon in Black Lake had individual areas of activity that were likely associated with preferred habitats. Restricted movements have also been documented by Fox et al. (2002), who found that Gulf sturgeon (*Acipenser oxyrinchus desotoi*) remained in localized areas for extended time periods before rapidly dispersing due to competition, food, or physical parameters. Taverny et al. (2002) also documented similar restricted movement behaviors when tracking juvenile European sturgeon (*Acipenser sturio)*. Juvenile lake sturgeon in our study could have preferred habitats adjacent to the river mouth because of potentially higher productivity (e.g. ice free earlier). Furthermore, dispersal following release in December might not have been very high as colder water temperatures reduced activity.

We failed to detect 55% of released fish. The non-detection of the remaining fish could be attributed to several factors. First, equipment failure associated with the ultrasonic transmitters could explain our inability to locate fish. Secondly, fish could have passed the hydroelectric dam on the outlet from Black Lake in the Lower Black River. Survival rates of fish attempting this is unknown but if there is a natural propensity to disperse downstream to more productive feeding areas then this could have occurred. We did not survey downstream of the dam on the Lower Black River or in adjoining Lake Huron. Mortality due to predation is likely and has been noted with other hatchery stocked fish (Olla et al. 1998). However, transmitters should have been identified either within the predator itself or on the substrate after expulsion. Furthermore, if fish died near the release location at the mouth of the river the transmitter could become buried in sediment exiting the Upper Black River rendering the signal undetectable. This was unlikely because the signal strength should penetrate through moderate amounts of bottom substrate (Vemco, personal communication). Finally, we did not document a significant difference in survival due to hatchery rearing environment though there are several explanations for the lack of differences. First, streamside reared fish were transported and kept at the traditional hatchery for approximately three months. This movement back to traditional rearing conditions may have diminished any potential fitness advantage incurred at the streamside hatchery, like exposure to fluctuating temperature regimes and natural river odors. Secondly, data collected by Crossman (chapter 3) suggests that the effects of different hatchery rearing environments have a size/age threshold beyond which the effects of hatchery environment may not be important. Our results support and extend this finding.

CONCLUSIONS

In conclusion, our minimum estimate of over-winter survival (40%) is important for the designs of restoration programs since it has been identified that survival of juveniles past the young of the year stage is high and similar to adult stages (Gross et al. 2002). It is generally accepted that mortality rates of young the year sturgeon are high (Nilo et al. 1997) with the young of the year age class having the strongest effect on overall population growth (Gross et al. 2002). Hatchery restoration programs have the ability to target these early age classes in an attempt to increase survival and augment population abundance. Hatchery rearing environment did not affect survival probabilities of age-0 lake sturgeon. However, further research is needed identifying the contribution of hatchery reared sturgeon to population growth. Finally, future studies aimed at identifying overwinter survival of wild or hatchery reared juvenile lake sturgeon should incorporate finer scale monitoring immediately following release and assessments should continue for as long as possible thereafter.

Chapter 5

DIRECT AND INDIRECT EFFECTS OF PREDATION ON SURVIVAL AND HABITAT USE OF JUVENILE LAKE STURGEON

INTRODUCTION

The ecological importance of predator-induced indirect effects is widespread and represents a significant component of a predator's impact on prey communities (Pecor and Werner 2004; Werner and Anholt 1996), thus influencing community and ecosystem level processes (Walsh and Reznick, 2008). Predators can influence the behavior of prey species which in turn modifies effects incurred from other predators (Miller and Kerfoot 1987). These indirect/non-lethal effects of predators may be as important as the direct mortality of prey in understanding the outcome of species interactions (Mittelbach 1986; Werner and Anholt 1996; Werner and Pecor 2003). Predation risk and effects on foraging have been shown to influence habitat choice across a number of species (Lima 1998; Belk et al. 2001; Byström et al. 2003; Kneib 1987; Winkelman and Aho 1993). Furthermore, alteration of prey behavior can affect probabilities of detection or capture (Lima 1998). Estimating behavioral responses related to ontogenetic changes in body size and morphology during early life history stages is particularly relevant to understanding the ecology of predator-prey interactions (Werner and Gilliam 1984; Werner and Anholt 1993).

Documenting sources of mortality, as well as behavioral or phenotypic traits associated with mortality, is complex in natural settings (Houde 1987) due to several interacting factors of both the prey and predators (e.g. morphology, size, behavior, and

distribution; Schlosser 1987). Laboratory mesocosm studies have provided a successful arena to study direct and indirect effects on prey as a function of habitat types and predator species (Bernot and Turner 2001; Stunz and Minello 2001; Werner and Hall 1988; Werner et al. 1983). However, the complexity of experimental conditions likely plays a large role in the outcomes of predator-prey interactions. This is particularly relevant for hatchery-raised fish, which are commonly used as prey in experimental predation studies. The types or complexity of habitats simulated in mesocosm studies can affect the outcomes of predator-prey interactions. Increasing the complexity of rearing environments, including the addition of habitat, has been shown to improve survival (Maynard et al. 1996) and can influence morphology and color patterns (Fuji 1993) compared to fish grown in tanks devoid of cover. Furthermore, fish subjected to chemical and extracted predator odorants prior to release exhibit antipredator responses (Berejikian et al. 1999; Gazdewich and Chivers 2002), which subsequently increase survival in the wild. Understanding how rearing environment and age interact to influence both predation risk and anti-predator behavior is important for describing phenomenon in natural systems and for improving restoration efforts of numerically depressed species.

Estimating the direct effects of different predators on juvenile lake sturgeon survival and the indirect effects on behavior are critical for the regionally imperiled lake sturgeon (*Acipenser fulvescens*) as they are the center of extensive restoration work throughout the Great Lakes. Sources of natural mortality, particularly mortality attributed to predation, for juvenile lake sturgeon are currently unknown, despite recent work conducted on the vulnerability of sturgeon eggs and larvae (Miller and Beckman 1996;

Gadomski and Parsley 2005a), and juvenile sturgeon of other species (Gadomski and Parsley 2005b; Gadomski and Parsley 2005c). Accordingly, we designed experiments to test several hypotheses to determine, 1) how habitat use by juvenile lake sturgeon of different age classes and body sizes varied with and without the presence of predators, 2) the effects of age and body size on levels of predation by different predator species, 3) the effects of hatchery rearing environment on predation vulnerability, and 4) rates of predation on juvenile lake sturgeon in the presence of an alternate prey species. Results define important mechanisms associated with predation risk in juvenile lake sturgeon during the first three months of life.

METHODS

Juvenile lake sturgeon were from hatched from paternal half-sib family groups that were collected and produced from a population in Black Lake, Michigan. Families were reared separately in two hatchery environments. Half of all fish used in predation trials were reared at a streamside hatchery in water from the natal Upper Black River. The remaining fish were reared using ground water at a state hatchery in southwestern Michigan, representing a traditional hatchery environment. The streamside rearing facility used a flow-through design where water was pumped directly from the river. Water was mechanically filtered to remove sediments, and returned directly to the river after passing through the juvenile rearing tanks. Flow rates were kept constant and at a level that resulted in approximately two complete water turnovers every hour for all

rearing tanks (1.22m diameter, 0.5m deep). Cleaning, feeding, and lighting protocols were consistent between the two hatchery environments.

Adult rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieue*), northern pike (*Esox lucius*), and rusty crayfish (*Orconectes rusticus)* were used as predators in experimental trials. Predators were collected from the Upper Black River using fyke nets, backpack electro-fishing, and hook and line fishing. Species were held separately at the streamside hatchery in indoor, circular tanks fiberglass tanks (0.61m diameter 0.7m deep). All indoor tanks were supplied with flow-though river water. Tanks had overhead lighting that mimicked a natural photoperiod. Predators were collected ≤4 days prior to the start of each experimental trial and were not fed during captivity. Total length (T_L; cm; mean ± 1 S.E) and gape width (G_W) and height (G_H) for fish predators used in trials were as follows: Rock Bass: T_L: 17.96 ± 1.92, G_W: 2.75 ± 0.24, G_H: 2.62 ± 0.22. Smallmouth Bass: T_L: 28.04 ± 0.98, G_W: 3.4 ± 0.07, G_H: 3.45 ± 012. Northern Pike: T_L: 51.0 ± 1.29, G_W: 3.9 ± 0.09, G_H: 4.1 ± 0.14. Total carapace length (C_L; mm; mean ± 1 S.E) and pincher width (P_W) for crayfish predators was: C_L: 28.75 ± 2.11, P_W: 13.44 ± 1.0.

Experimental design

All experiments were conducted at the streamside hatchery. Experiments were conducted in a circular (2.44m diameter, 0.6m deep) fiberglass tank that was encircled with a black tarp to reduce human induced behavioral effects. The tank was divided into

two equally sized sections so 2 replicates could be run simultaneously. Each section included three substrate types (sand, small gravel, and large rocks) divided into equal contiguous segments. Substrate types were removed and randomly redistributed prior to the beginning of each trial. Trials were conducted using 30 juvenile lake sturgeon for a duration of 24 hours beginning at 0730. We conducted trials for juvenile lake sturgeon that were 8-9, 11-13, and 15-16 weeks of age. Age classes were incorporated because we could only run two trials simultaneously and several weeks were needed to complete all combinations of juvenile lake sturgeon and the different predators. Fish reared at the traditional hatchery environment were transported to the streamside rearing facility a few days prior to the start of the trials. Fish from the different hatchery environments, and families were marked uniquely with a visible implant elastomer dye (Northwest Marine Technology, WA, USA). Elastomer was injected on the ventral side of the rostrum where the colors were most easily distinguished. All fish were measured for total length before introduction into the tank and surviving fish were re-measured at the termination of the trial.

Experiment 1: Tests of indirect effects of predation risk on substrate use

This experiment was conducted to test two hypotheses: 1) The presence of predators did not significantly affect the distribution of juvenile lake sturgeon among habitats, and 2) There was no affect of rearing environment on the distribution of juvenile lake sturgeon among habitats with and without the presence of predators. Fish from traditional and streamside hatcheries were used in separate trials because we were unable to follow individual fish during the trial. Within each trial we used juveniles (n=5) from 6

families marked correspondingly. Juvenile lake sturgeon were introduced into tanks without a predator. Observations of fish locations, either on one of three substrate types or in the water column, were taken hourly until nightfall and then again in the morning. Sturgeon were removed and the predator was introduced. The predator was allowed to acclimate to the tank conditions for a period of 24 hours and a new set of sturgeon were introduced into the tank with the predator. The same juvenile lake sturgeon were never used twice. The same sets of observations were taken including predator location. The predator was removed immediately following the termination of the trial.

We used a multifactor analysis of variance (ANOVA) to analyze the data. The response variable was the proportion of juvenile sturgeon observed using each substrate type. The response variable was transformed using a square root transformation to achieve normality. Factors in this analysis included the presence or absence of a predator, the age class, rearing environment, and the trial number. Trial was not a significant factor within either the presence or absence of a predator so all the trial data were pooled. Data were reanalyzed using repeated measures ANOVA to incorporate hour of observation as an additional factor. We conducted separate analyses for the visual water column predators (fish) and the benthic predators (crayfish).

Experiment 2: Direct effects of predation on age-specific survival

This experiment was conducted to test the hypothesis that juvenile lake sturgeon reared in different hatchery environments and at different ages were not differentially vulnerable to predation. Experimental design was consistent with experiment 1 with the exception that we could follow individual survival across hatchery environments and

families. Within each trial we used juveniles (n=5) from 6 families uniquely marked. All fish were measured for total length prior to the trial. Surviving fish were re-measured at the termination of each trial.

We used a general linear model to predict survival using several independent variables including: Age, predator species, rearing environment, and family. Survival was square root transformed prior to analysis to achieve normality. To determine if there was size selective predation within each age class we used a paired t-test to test for mean differences in the total lengths of fish that were killed and those that survived.

Experiment 3: Alternate species trials

This experiment was conducted to test the hypothesis that predation rates on juvenile lake sturgeon were not significantly different with and without the presence of an alternate prey species. An equal mixture of juvenile lake sturgeon (n=15) and emerald shiners (*Notropis atherinoides*; n=15) were introduced into a tank containing an acclimated predator (smallmouth bass (n=1 per trial) and crayfish (n=4 per trial)). Only age classes 11-13 and 15-16 were represented in these trials. Family and rearing environment treatments were not included in alternate species trials. Total length of sturgeon and minnows was measured for prior to the trial. A t-test was conducted to test for differences in the size distributions between sturgeon and minnows for each age class. Substrate use and survival were recorded hourly until nightfall and then again the following morning. A three-factor ANOVA was used to analyze variation in survival as a function of age, alternate prey species presence/absence, and predator species.

RESULTS

Experiment 1: Tests of indirect effects of predation risk on substrate use

Juvenile lake sturgeon strongly prefer sand when provided with a choice of substrate types (Peake 1999). Without the presence of a predator, sand was significantly preferred ($F_{3,1276}$ = 4670, P < 0.01). Proportion of substrate used by juvenile lake sturgeon changed in the presence predators. Juvenile lake sturgeon exposed to three different fish predators used sand substrate at a significantly higher proportion ($F_{59,1220}$ = 172.6, P = 0.005) when compared to gravel, larger rocks, and the water column (Fig 19). Furthermore, juvenile lake sturgeon preference for sand remained significant ($F_{59,1220}$, P < 0.001) following increased time periods after being introduced into the tank while preference for the water column significantly decreased over the same time period ($F_{59,1220}$ = 172.6, P = 0.016; Fig. 19). There were no significant differences between the three predatory fish species or the main effect of hatchery rearing environment in our analysis. Juvenile lake sturgeon at 8-9 weeks of age spent significantly less time ($F_{59,1220}$ = 172.6, P = 0.006) in the water column compared to fish at both 11-13, and 15-16 weeks of age. In general, substrate preference was divided between sand and the water column with gravel and large rocks combining for <15% total preference across all trials containing the three fish predators.

Proportional substrate use of juvenile lake sturgeon was significantly different when crayfish were used as the predator species compared to all three fish predators ($F_{11,755}$ = 373.6, P <0.01). Use of the water column increased significantly ($F_{55,584}$ =

65.39, $P < 0.001$; Fig. 19) over time following introduction, while use of sand decreased ($F_{55,584} = 65.39$, $P < 0.001$; Fig. 19). In the absence of crayfish the preference for sand was high and increased significantly over time following being introduced ($F_{55,584} = 65.39$, $P = 0.004$; Fig. 19). There was no effect of hatchery environment or age on fish distributions with and without crayfish predators.

Experiment 2: Size-dependant predation rates

Survival rates across all age classes were high when exposed to visual fish predators. Survival rates ranged from 97.4 – 100% at 8-9 weeks of age, 98.5 – 100% at 11-13 weeks of age, and 100% for 15-16 weeks of age. Survival was significantly less in trials conducted with 8-9 week old juvenile lake sturgeon ($F_{15,224} = 3.48$, $P < 0.001$). Rock bass had significantly higher rates of predation among the three fish predator types ($F_{2,237} = 12.35$, $P < 0.001$). There were no significant effects of predator size, rearing environment, or time on the survival of juvenile lake sturgeon exposed to the fish predators. Family was not significantly related to mortality, potentially due to overall low predation rates. However in three trials where only 1 juvenile lake sturgeon was consumed all mortalities were from the same family. The probability of this occurring by random chance is 3.7×10^{-5}. The total length individuals that died were not significantly different from the surviving fish across all ages (df = 162, t = 0.468, P = 0.64).

Survival rates were significantly less for juvenile lake sturgeon exposed to crayfish ($F_{1,286} = 1393.6$, $P < 0.001$) compared to the visual fish predators. There was a significant effect of time on juvenile sturgeon survival with a higher proportion of

mortality occurring at hours immediately following release into the tank ($F_{11,36} = 3.94$, $P < 0.001$). There was no effect of age on survival with rates being 0.66 ± 0.01 and 0.65 ± 0.09 for age 11-13 and 15-16 weeks respectively. There was no effect of hatchery rearing environment on survival and we could not test for a relationship with crayfish morphometric data as multiple crayfish were in each trial simultaneously. We did not document any differences attributed to family or size across different ages or trials. The total length individuals that were killed in the crayfish trials were not significantly different from the surviving fish across all ages (df = 100, t = 1.187, P = 0.238).

Experiment 3: Alternate species trials

Age was not a significant factor ($F_{1,138} = 1.41$, P = 0.238) in the analysis so the data were pooled. Predators differed significantly in prey choice ($F_{1,140} = 313.94$, P < 0.01). We documented a significant difference ($F_{1,92} = 94.52$, $P < 0.01$) in smallmouth bass predation rates on sturgeon and minnows with larger proportions of minnows being consumed over the 24hour period (Fig. 20).All juvenile lake sturgeon survived in all trials and average survival of minnows was 0.69 ± 0.21. We found the opposite result with crayfish. Significantly more juvenile lake sturgeon ($F_{1,44} = 457.8$, $P = <0.01$) were consumed during the 24hour period with survival rates being 0.67 ± 0.08 and 1.0 for sturgeon and minnows respectively (Fig. 20). There was no significant difference in the total length of sturgeon compared to minnows at the 11-13 age class (df = 118, t = 0.092, p = 0.927) however sturgeon were significantly larger during the 15-16 age class trials (df = 118, t = 34.04, p < 0.001).

DISCUSSION

We documented a shift in juvenile lake sturgeon habitat use as a result of the indirect effects of two predator types. Indirect effects of predation have been documented to influence habitat shifts in numerous studies (Lima 1998). The shift in habitat preference was most pronounced in the presence of crayfish (Fig 19). Studies have indicated that fish learn anti-predator behaviors rapidly after witnessing only a few attacks on conspecifics (Berejikian et al. 1999). Predation rates for juvenile lake sturgeon exposed to crayfish were much higher relative to the visual fish predators. This result likely explains the dramatic distribution shift in the crayfish trials. Even with the higher survival rates, juvenile lake sturgeon still chose sand at a significantly greater proportion when in the presence of the fish predators (Fig 19). These indirect effects may play a large role in survival in the wild as one predator type might force juvenile lake sturgeon to choose habitat that has a higher risk of exposure to predation by other predator types. Rahel and Stein (1988) showed that a prey darter species (*Etheostoma nigrum*) avoided predatory bass by hiding under provided cover, however became vulnerable when flushed from the cover by a second species, a benthic crayfish. In such instances, selection might be expected to favor alternative avoidance responses such as shifts to other refuge, which would be an important extension of this work. Previously, it has been hypothesized that changing habitat to avoid predation risk is unlikely once juvenile sturgeon obtain a size too large for most predators (Kynard et al. 2000). We observed habitat shifts in each juvenile lake sturgeon age class evaluated.

Documenting the rates of predation by fish predators is important for understanding lake sturgeon ecology and for defining barriers to recruitment. Our results represent the first replicated data on predation as it relates to age and avoidance for juvenile lake sturgeon provided with habitat choices. Efforts have been made to document rates of predation on embryos (Kynard and Horgan 2002; Miller and Beckman 1996; Nichols et al. 2003) and larvae (Gadomski and Parsley 2005a; Kynard and Horgan 2002) but limited work has been conducted on juveniles using age as an experimental factor. Gadomski and Parsley (2005b) found that predation is a likely cause of mortality in age-0 white sturgeon (*Acipenser transmontanus*) and that predation rates decreased with age when exposed to four predator species. They documented higher rates of predation for younger ages however direct comparisons with the results from our study is difficult due to differences in experimental design. Gadomski and Parsley (2005b) used smaller, larval, sized individuals initially, used large prey size ranges within each trail and predators in their study were predominantly benthic feeding fish. We extended their study by providing a heterogeneous tank environment and following antipredator behavior, which are important ecological factors.

There is a lack of knowledge regarding predation mortality for different ages of juvenile lake sturgeon but it is generally accepted that mortality rates of young the year sturgeon are high (Nilo et al. 1997). Rates of predation on juvenile lake sturgeon by the visual fish predators were low. In all trials, none of the predators used were gape limited for the size of juvenile lake sturgeon used. During the 8-9 week age class rates of predation were significantly higher than later ages. Juvenile lake sturgeon responded behaviorally to the presence of predators by reducing activity levels, a behavior that has

been documented in other predator-prey studies (Skelly and Werner 1990; Anholt et al. 2000). Observations taken during trials with the visual fish predators revealed unsuccessful attacks on juvenile lake sturgeon at all age classes by all predator types. We included alternate species trials to help determine if the hatchery environment, or tank, had any effect on the feeding behavior of the predator species. Significantly higher predation rates on the alternate prey species confirmed the fish were actively feeding. Another positive aspect of our experiment was that trials were conducted within a large tank space including only one predator which was never reused, eliminating learned behavior. This reduced issues surrounding small-scale experiments by creating conditions where the rates of interaction between predators and prey were not elevated in confinement, which is a common problem in predation trials. Furthermore, crayfish densities were at a level that reduced aggressive interaction among individuals which is common with higher crayfish densities.

We found significantly different results between predator types in the alternate species trials (Fig. 20). Significantly more minnows were consumed by the visual fish predator compared to the same initial amount of juvenile lake sturgeon. The opposite trend was documented in the crayfish trials and was not a surprising result. Crayfish were very efficient at capturing juvenile lake sturgeon at both 11-13 and 15-16 weeks of age, despite large sturgeon body sizes. Gadomski and Parsley (2005c) found a similar difference in prey choice between two different predators, one preferring juvenile white sturgeon over the alternative prey choice and the opposite with the other predator. Predation rates would have been much higher if more than four crayfish had been used. Predation by crayfish was immediate (Fig 19) and observations were that each crayfish

immediately captured a sturgeon. However the entire trial period was needed for a single crayfish to consume a single juvenile lake sturgeon. The high levels of vigilance, schooling and avoidance behaviors of the minnows precluded their capture by crayfish. Results showing high levels of predation by crayfish regardless of juvenile age suggest that lake sturgeon remain vulnerable to crayfish predation over prolonged periods. The rusty crayfish used in our trials are an invasive species (Olden et al. 2006) that are in high abundance in the Upper Black River system. Crayfish are active predators at night and this could be detrimental for lake sturgeon due to their nocturnal behavior at juvenile ages (Crossman chapter 3). Release locations for lake sturgeon restoration programs should be assessed for crayfish densities prior to stocking to reduce post release mortality.

There were no significant results attributed to the different hatchery rearing environments. This is likely due to the fact that the experiments were conducted at the streamside hatchery. Fish from the traditional hatchery were transported to the streamside facility a few days prior to the start of the predation trials allowing for acclimation to the environment. Furthermore, if incoming water contains chemical odorants or cues from predator species (as suggested by Berejikian et al. 1999), or injured conspecifics, then the fish could have already been slightly conditioned prior to introduction into the trial. This would be another benefit for streamside rearing of lake sturgeon. Because experience influences the behavioral responses to different stimuli and can shape the future behavior of captive animals, pre-release environmental enrichment may be successful in facilitating the expression of adaptive behavioral responses following release (Watters and Meehan 2007). Further research that examines juvenile lake sturgeon mortality within each of the different hatchery environments would be desirable. The significant

shift in habitat use with and without the presence of a predator suggests that even after lengthy periods of captivity, predator avoidance was still observed which is important for lake sturgeon restoration.

CONCLUSIONS

In conclusion, our results revealed a strong anti-predator response in juvenile lake sturgeon when exposed to predators in a heterogeneous environment. Even though overall predation was low for the visual fish predators, this source of mortality cannot be discounted as a significant barrier to recruitment. Furthermore, since many large fish predators (e.g. smallmouth bass) prey upon crayfish, examining rates of juvenile lake sturgeon predation with two predator types simultaneously would be important, even though predation rates on rusty crayfish by fish are lower than native crayfish species (Kuhlmann et al. 2008). Finally, indirect effects are likely to predispose juvenile lake sturgeon to higher rates of predation by alternate species.

FINAL CONCLUSIONS

Results presented in my dissertation address the need for further empirical and experimentally rigorous evaluations of hatchery rearing and stocking programs for little studied species such as lake sturgeon. It is evident based on currently available scientific data that restoration programs could be significantly improved if methods by which lake sturgeon supplemental progeny are acquired, reared, and released are critically evaluated. In order to collect supplemental progeny for hatchery programs I recommend that programs maximize genetic diversity by focusing collection efforts on dispersing larvae because of the collection methods evaluated, they represent the most unbiased sample of progeny from natural adult reproduction. Collection of dispersing larvae may not be possible across all systems given logistics and unknown population parameters such as spawning locations or timing. If programs directly take gametes from adults or collect naturally produced eggs from the streams substrate focus should be directed at incorporating aspects of the lake sturgeon reproductive ecology including sampling across spawning times and locations. Managers should also recognize the variation that exists in growth and survival as a function of family (i.e., variation in important traits is heritable). Quantifying additive genetic variation for adaptive phenotypic traits at the time the progeny enters the hatchery and to the extent possible when they are released into the wild will be important future predictors of future population viability. Furthermore, differences between hatchery rearing environments and relative to natural stream conditions will influence probabilities of survival and thus levels of genetic variation in supplemental progeny.

Streamside rearing may be advantageous to small/young lake sturgeon by exposing them to an enriched environment prior to release. However, data suggest there may be an age/size threshold where the effects of rearing environment on movements and survival are diminished at the time of stocking (chapter 3) and in terms of overwinter survival (chapter 4). I recommend the use of streamside hatcheries for rearing lake sturgeon supplemental progeny up to 13 weeks of age. The benefit of rearing fish to older ages (>13 weeks) and subsequently larger sizes at a traditional hatchery is outweighed by the evidence indicating that lake sturgeon progeny survive better and exhibit natural variation in incubation times and size at hatch when reared at a streamside hatchery. Furthermore, researchers have suggested that streamside rearing may minimize straying following stocking by maximizing probabilities of imprinting on the natal river, as has been observed with other species. Future work that characterizes factors influencing differences among hatchery rearing environments for lake sturgeon are needed to develop system-specific management prescriptions.

My research emphasizes the importance of tailoring stocking programs to individual species and systems rather than focusing on management prescriptions solely based on numbers and body sizes of supplemental fish released. Results indicate that supplementation strategies for juvenile lake sturgeon should focus on night releases in areas of sand and moderate flow. Releases if conducted under these conditions will ensure that rates of dispersal are high immediately following release and will likely have an indirect effect on levels of predation by reducing mortality incurred by visual predators. Results of my predation work (chapter 5) revealed a strong anti-predator response in juvenile lake sturgeon when exposed to predators in a heterogeneous

environment. Because crayfish predation was documented as a significant source of mortality, releases should be conducted in areas of low crayfish densities. It is critical that release sites are evaluated on a system by system basis with emphasis placed on determining areas that maximize suitable juvenile nursery habitat.

Hatchery programs have received increased scrutiny because of the potential negative impacts of captive reared individuals to natural populations. Improving lake sturgeon culture methods should be considered a priority for future management and conservation programs. In long-lived iteroparous organisms such as the lake sturgeon, longevity is required because per capita recruitment is expected to be low during a single reproductive period and rates of mortality after early juvenile stages are expected to be low. Therefore, small improvements in probabilities of individual survival will result in substantial gains in probabilities of population sustainability. Managers must also maintain diversity across reproductive time and locations to not diminish adaptations that have evolved due to reproductive isolation that has likely evolved in response to temporal and spatial environmental heterogeneity. Methods and results described in my dissertation provide a framework for evaluating alternative strategies for managers designing and implementing conservation programs for lake sturgeon. Accordingly, results from this research may be applied to other systems and can be used to develop predictions that can be tested for other species.

APPENDIX I

TABLES AND FIGURES

Table 1. Comparisons of sample size (N) and summary measures of genetic diversity including coancestry (θ) and effective population size for individuals sampled at different developmental stages using each of three methods of gamete/larval collection over each of three years. Effective population size estimates include calculations using the number of males and females (N_e), male and female reproductive variance (N_{ev}), and coancestry ($N_{e\theta}$). Individuals were raised in one of two hatchery environments (streamside or traditional).

Hatchery Environment and Developmental Stage	Year	N	θ	N_e	N_{ev}	$N_{e\theta}$
	2005					
Streamside Hatchery						
Egg Stage[a]		94,856	0.020	38.1	38.1	24.9
Larval Stage[a]		15,383	0.029	29.3	29.4	17.5
56 Days		1,500	0.034	29.3	29.4	14.7
90 Days		1,500	0.040	29.3	29.4	12.5
Traditional Hatchery						
Egg Stage		79,983	0.026	29.3	29.3	19.5
Larval Stage		10,371	0.033	26.7	26.7	15.0
	2006					
Streamside Hatchery						
Egg Stage		77,731	0.024	31.1	31.7	20.9
Larval Stage		19,129	0.030	26.2	26.2	16.8
40 days		1,000	0.032	26.2	26.2	15.9
140 Days		1,000	0.041	26.2	26.3	12.3
Traditional Hatchery						
Egg Stage		47,702	0.037	21.3	21.3	13.6
Larval Stage		9,894	0.043	15.0	15.0	11.6
	2007					
Streamside Hatchery						
Egg Stage		50,170	0.017	38.6	38.6	29.3
Larval Stage		13,243	0.019	38.6	38.6	26.3

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

Table 2. Comparisons of sample size (N) and summary measures of genetic diversity including coancestry (θ) and effective population size for offspring collected as naturally produced eggs in each of two years. Effective population size estimates include calculations using the number of males and females (N_e), male and female reproductive variance (N_{ev}), and coancestry ($N_{e\theta}$). Individuals were raised in one of two hatchery environments (streamside or traditional).

Hatchery Environment	Year	N	θ	N_e	N_{ev}	$N_{e\theta}$
	2005	1,100				
Streamside Hatchery		700	—	—	—	—
Traditional Hatchery		400	—	—	—	—
	2006	1,138	0.008	51.3	125.1	63.4
Streamside Hatchery		772	0.004	42.8	93.7	126.0
Traditional Hatchery		366	0.014	13.7	33.2	36.0
	2007					
Streamside Hatchery		275	0.007	14.9	59.0	72.0

Table 3. Comparisons of sample size (N) and summary measures of genetic diversity including coancestry (θ) and effective population size for offspring collecting as dispersing larvae in each of three years. Effective population size estimates include calculations using the number of males and females (N_e), male and female reproductive variance (N_{ev}), and coancestry ($N_{e\theta}$). Individuals were raised in one of two hatchery environments (streamside or traditional).

Hatchery Environment	Year	N	θ	N_e	N_{ev}	$N_{e\theta}$
	2005	7,800				
Streamside Hatchery		3,000	—	—	—	—
Traditional Hatchery		4,800	—	—	—	—
	2006	5,500	0.004	122.1	214.10	141.3
Streamside Hatchery		3,500	0.004	70.0	190.58	116.7
Traditional Hatchery		2,000	0.004	87.9	188.22	122.6
	2007					
Streamside Hatchery		1,400	0.005	80.1	181.3	105.9

Table 4. Reproductive success of males and females based on known pedigree information for offspring produced from directed gamete takes and reared in two hatchery environments (Streamside and Traditional) during each of 3 years. Variables represent the mean ± 1SD.

Hatchery	Variable	2005	2006	2007
Streamside	Eggs/Female	6323.7 ± 5056.2	6477.6 ± 4857.2	3345.0 ± 2030.4
	Larvae/Family	669.2 ± 666.2	863.4 ± 820.1	343.8 ± 214.8
	N_m:N_f[a]	26:15	22:12	27:15
	Mates/Female	1.7 ± 0.5	2.1 ± 0.7	1.8 ± 0.4
	Mates/Male	1	1.1 ± 0.3	1
Traditional	Eggs/Female	7271.1 ± 5415.2	5962.8 ± 4812.4	—
	Larvae/Family	518.6 ± 475.6	549.7 ± 420.1	—
	N_m:N_f[a]	22:11	16:8	—
	Mates/Female	2	2	—
	Mates/Male	1	1	—

a: Ratio of the number of males (N_m) to females (N_f).

Table 5. Reproductive success of males and females based on genetically determined pedigree information for offspring collected using two collection methods and reared in two hatchery environments (Streamside and Traditional) during each of 3 years. Variables represent the mean ± 1SD.

Collection Method and Hatchery Environment	Variable	2005	2006	2007
Naturally Produced Eggs				
Streamside	Offspring/Female	—	1.9 ± 1.1	1.1 ± 0.4
	Offspring/Male	—	1.4 ± 0.7	1.1 ± 0.4
	N_m:N_f[a]	—	23:20	8:7
	Mates/Female	—	1.4 ± 0.8	1.1 ± 0.4
	Mates/Male	—	1.2 ± 0.5	1.1 ± 0.4
Traditional	Offspring/Female	—	1.50 ± 0.8	—
	Offspring/Male	—	1.13 ± 0.4	—
	N_m:N_f[a]	—	8:6	—
	Mates/Female	—	1.50 ± 0.8	—
	Mates/Male	—	1.13 ± 0.4	—
Dispersing Larvae				
Streamside	Offspring/Female	—	1.67 ± 1.1	2.2 ± 1.3
	Offspring/Male	—	1.19 ± 0.4	1.5 ± 0.8
	N_m:N_f[a]	—	42:30	51:33
	Mates/Female	—	1.63 ± 1.0	2.2 ± 1.3
	Mates/Male	—	1.17 ± 0.4	1.5 ± 0.8
Traditional	Offspring/Female	—	2.1 ± 1.4	—
	Offspring/Male	—	1.3 ± 0.5	—
	N_m:N_f[a]	—	59:35	—
	Mates/Female	—	2.0 ± 1.2	—
	Mates/Male	—	1.3 ± 0.4	—

a: Ratio of the number of males (N_m) to females (N_f).

Table 6. Estimates of mean relatedness (r_{xy}) of parent pairs for offspring collected using three different gamete/larval collection methods and the adult spawning population (SP) during 2005-2007. Collection methods included direct gamete takes (DGT), naturally produced eggs (NPE) and dispersing larvae (DL). Mean r_{xy} (± 1SE) is presented along the diagonal. Uncalculated values are represented with —.

Collection Method	2005	2006	2007
DGT	0.014 ± 0.033	-0.084 ± 0.06	0.034 ± 0.04
NPE	—	0.013 ± 0.04	-0.013 ± 0.08
DL	—	0.021 ± 0.02	0.007 ± 0.03
SP	0.001	0.004	-0.004

Table 7. Physical conditions of the Upper Black River during stocking events of three different age classes of juvenile lake sturgeon. Physical variables including water depth (m) and velocity (m/sec) are included for each downstream assessment site during each release age.

Age	Site	Water Depth	Water Velocity
8	1	0.62 ± 0.05	0.79 ± 0.04
	2	0.52 ± 0.06	0.73 ± 0.03
	3	0.61 ± 0.06	0.62 ± 0.04
	4	0.74 ± 0.01	0.32 ± 0.02
13	1	0.62 ± 0.13	0.74 ± 0.03
	2	0.68 ± 0.05	0.71 ± 0.10
	3	0.77 ± 0.09	0.59 ± 0.03
	4	0.99 ± 0.07	0.40 ± 0.01
17	1	0.65 ± 0.06	0.48 ± 0.16
	2	0.66 ± 0.03	0.45 ± 0.18
	3	0.68 ± 0.03	0.46 ± 0.18
	4	0.93 ± 0.05	0.37 ± 0.24

Table 8. Numbers released and total length (cm, mean ± 1SE) corresponding to age, rearing environment, and gamete/juvenile collection method for three experimental releases of juvenile lake sturgeon into the Upper Black River, Michigan. Letters (A, B, C) correspond to statistically greater proportions of fish recaptured between the different collection methods within each release age.

Age (weeks)	Hatchery Environment	Collection Method	Total Released	Proportion Recaptured	Total Length
8	Streamside	Artificial*^{A}	1257	0.09	7.87 ± 1.06
		Drift†^{B}	485	0.05	7.51 ± 0.82
		Natural‡^{B}	220	0.07	7.68 ± 1.11
	Traditional	Artificial^{B}	1380	0.03	7.38 ± 0.99
		Drift^{B}	725	0.04	7.42 ± 0.73
		Natural^{C}	240	0.02	7.77 ± 1.22
		Total	4307	0.05	
13	Streamside	Artificial^{A}	999	0.16	10.84 ± 0.08
		Drift^{B}	494	0.06	11.70 ± 0.20
		Natural^{A}	181	0.13	11.98 ± 0.17
	Traditional	Artificial^{A}	623	0.10	11.20 ± 0.20
		Drift^{B}	692	0.04	11.70 ± 0.14
		Natural^{A}	237	0.10	12.90 ± 0.21
		Total	3226	0.10	
17	Streamside	Artificial^{A}	1000	0.16	13.85 ± 0.15
		Drift^{B}	1088	0.08	14.81 ± 0.20
		Natural^{C}	399	0.26	14.49 ± 0.15
	Traditional	Artificial^{A}	1000	0.17	16.51 ± 0.19
		Drift^{B}	493	0.05	17.19 ± 0.30
		Natural^{C}	208	0.27	15.80 ± 0.34
		Total	4188	0.15	
		Grand Total	11721	0.10	

*Artificial: Paternal half-sib families, each created with one female and two males (n = 26 and 25 for two years).
†Drift: Larvae captured dispersing downstream from spawning areas.
‡Natural: Naturally fertilized and deposited eggs that were collected from the stream.

Table 9. The number of observations (Obs), average inter-observational distance traveled (Mean ± 1 SD), and average inter-observational distance traveled (Mean ± 1 SD) from the release site for juvenile lake sturgeon surgically implanted with ultrasonic transmitters and released into Black Lake, Michigan. Other characteristics of the experimental fish represented include: individual fish ID number, hatchery rearing location (Loc: S: streamside; T: traditional), total length (T_L) and weight (W).

ID	Loc	T_L (cm)	W (g)	Obs	Distance Traveled (Km)	Distance From Release Site (Km)
36	S	28.58	102.10	4	0.46 ± 0.03	0.12 ± 0.01
42	S	29.21	93.06	5	0.11 ± 0.01	0.03
44	S	29.21	92.90	6	0.08 ± 0.01	0.04 ± 0.01
49	S	31.75	101.80	3	0.14 ± 0.10	0.08 ± 0.02
50	S	33.66	118.60	4	0.21 ± 0.05	0.03 ± 0.01
55	S	28.80	90.00	2	0.16 ± 0.02	0.06 ± 0.03
56	S	31.75	112.60	7	0.10 ± 0.10	0.13 ± 0.01
Mean ± SE		30.42 ± 0.74	101.58 ± 4.06	4.43	0.18 ± 0.05	0.07 ± 0.02
22	T	31.43	110.50	8	0.04 ± 0.02	0.1 ± 0.02
24	T	30.32	101.60	4	0.55 ± 0.15	0.05 ± 0.01
27	T	32.39	116.90	3	0.14 ± 0.04	0.06 ± 0.01
28	T	30.80	99.10	5	0.09 ± 0.03	0.09 ± 0.01
29	T	29.21	94.60	4	0.25 ± 0.11	0.11 ± 0.02
30	T	30.48	97.70	2	0.17 ± 0.08	0.07 ± 0.04
31	T	30.62	103.00	5	0.10 ± 0.05	0.07 ± 0.02
51	T	31.75	105.00	6	0.32 ± 0.10	0.09 ± 0.03
52	T	33.02	126.60	3	0.12 ± 0.04	0.11 ± 0.01
Mean ± SE		31.11 ± 0.39	106.11 ± 3.41	4.44	0.20 ± 0.05	0.08 ± 0.01

Figure 1. The Black Lake study site in Michigan, showing the adult spawning grounds, the collection sites for naturally produced eggs (*), and the collection site for dispersing larvae.

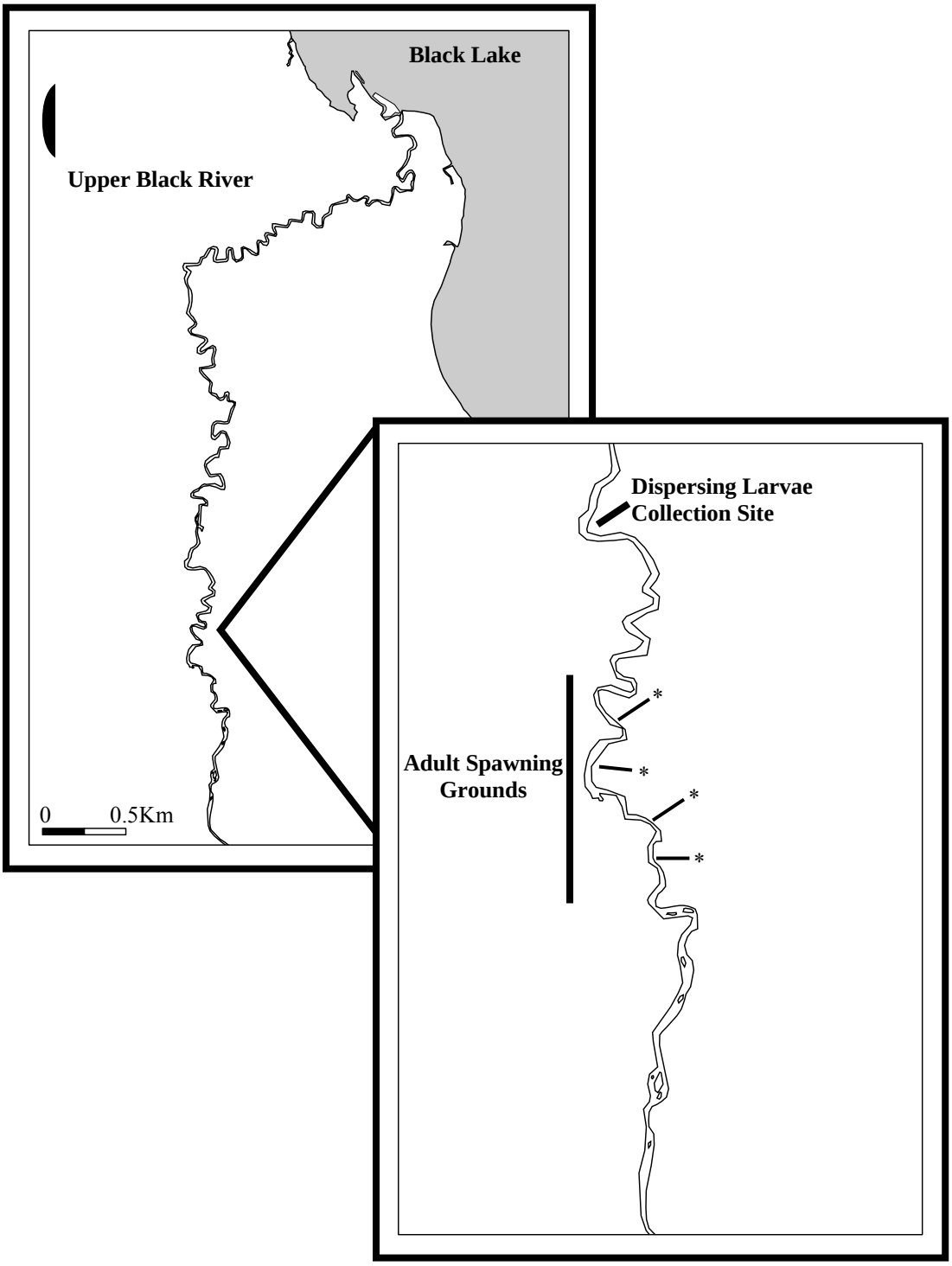


Figure 2. Total number of adult lake sturgeon captured by day on the spawning grounds of the Upper Black River from 2005 to 2007.

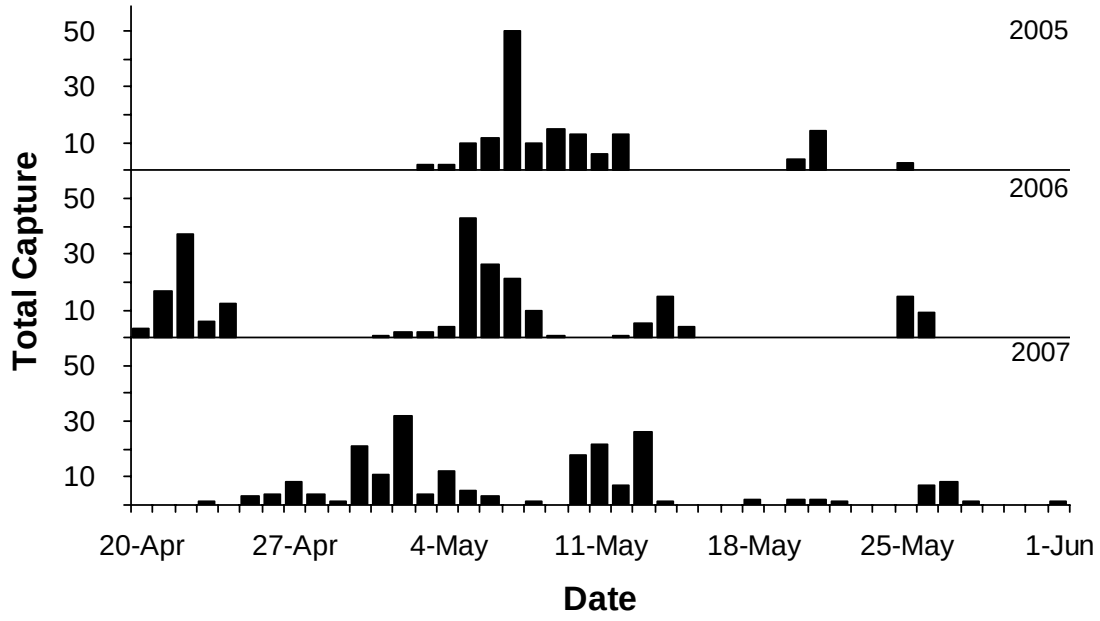


Figure 3. Frequency distributions of inter-individual relatedness values (r_{xy}) values for adults producing offspring from A) each of three different gamete/larval collection methods (Naturally Produced Eggs (NPE), Directed Gamete Takes (DGT), Dispersing Larvae (DL)) and B) the total adult spawning population (SP) over three years (2005-2007)

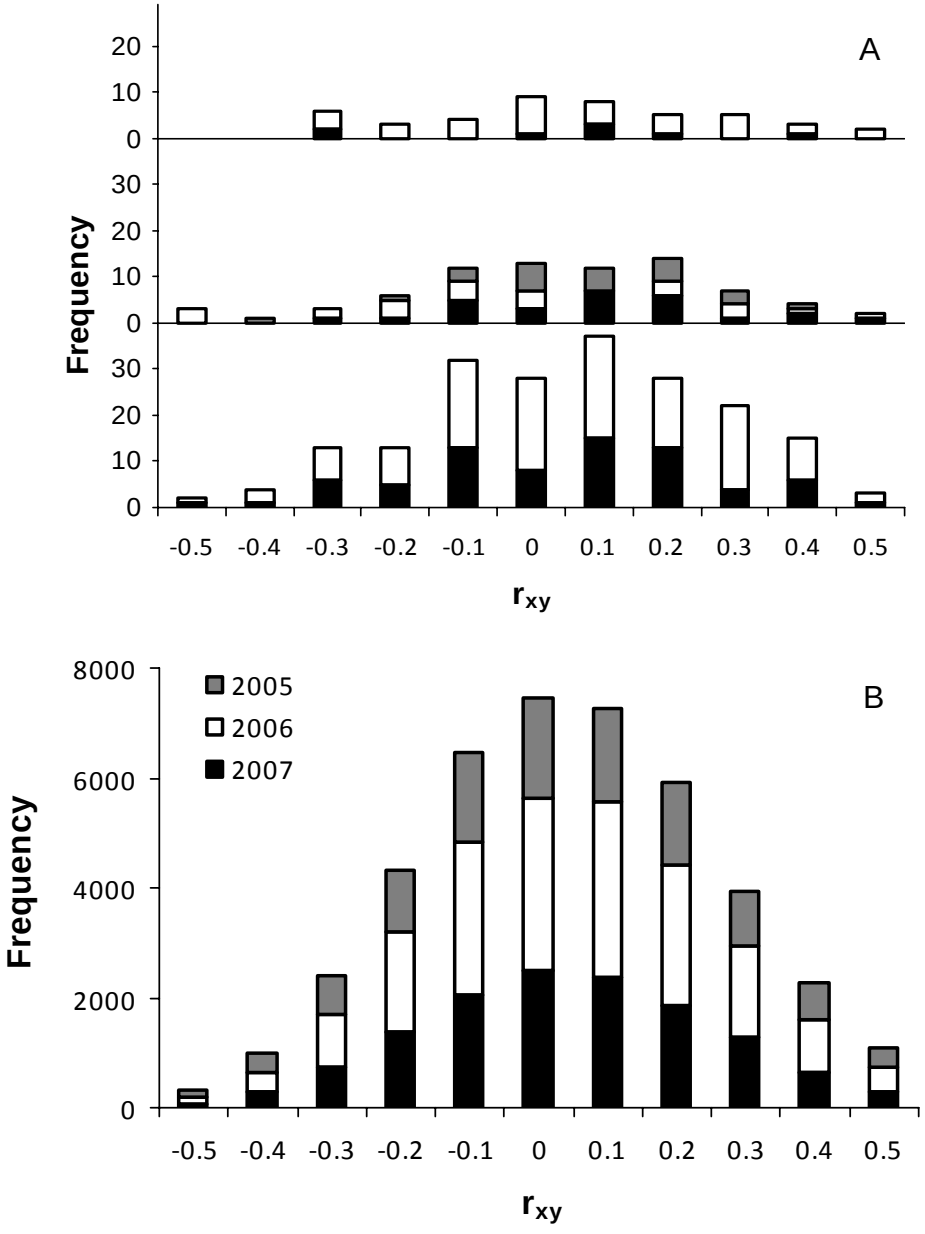


Figure 4. Estimates of inter-individual relatedness (r_{xy}) values (Mean ± 1SE) between adult female lake sturgeon concurrently breeding within distinct spawning zones in the Upper Black River during each of three years (2005-2007). Numbers refer to the total number of pairwise comparisons used to calculate the mean

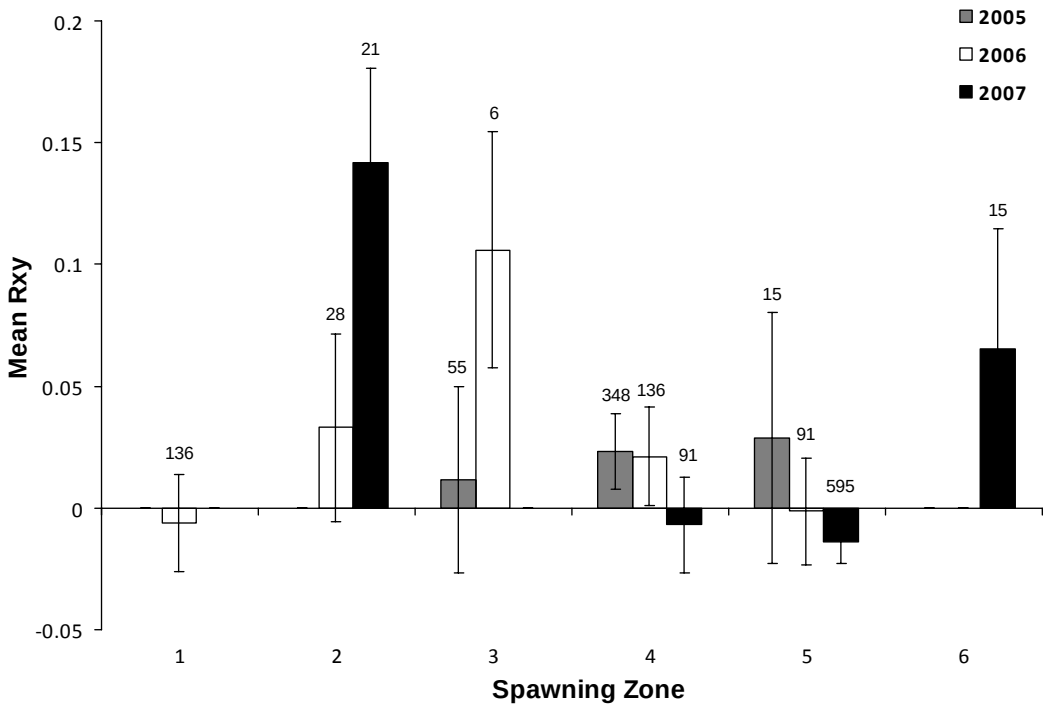


Figure 5. Estimates of inter-individual relatedness (r_{xy}) (mean ± 1SE) between adult female lake sturgeon concurrently breeding within distinct spawning runs in the Upper Black River during each of three years (2005-2007). Numbers refer to the total number of pairwise comparisons used to calculate the mean

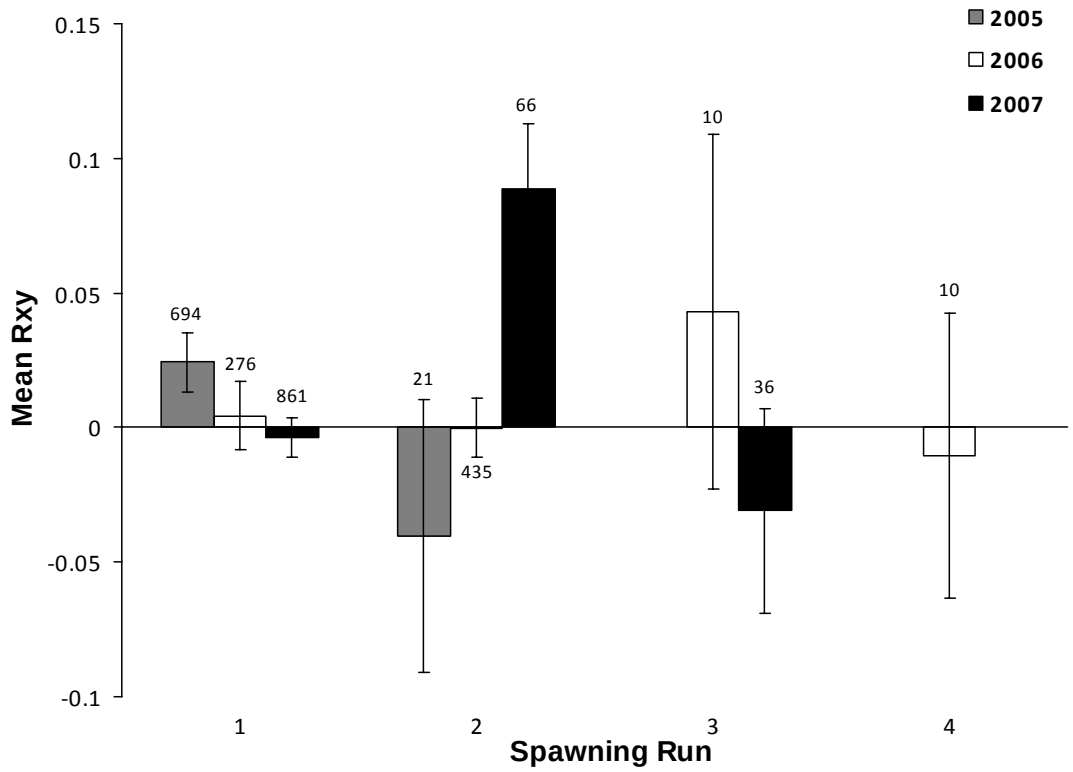


Figure 6. Overall egg survival (mean ± 1SE) to hatch for females incubated in two different hatchery environments across two different years. Different sets of females were used between years

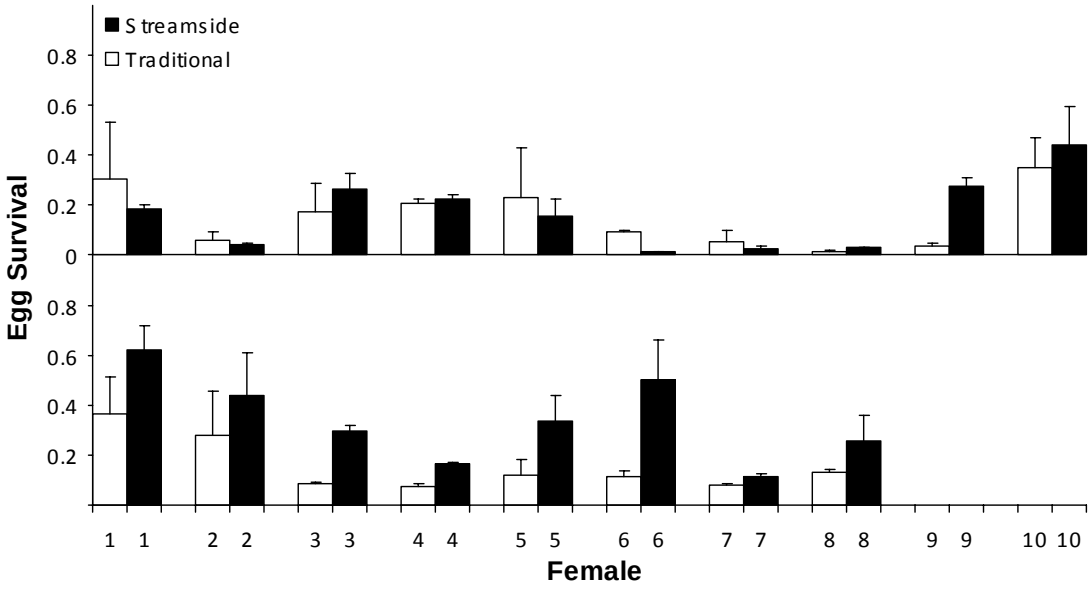


Figure 7. The effect of temperature on egg incubation time for eggs reared at a streamside hatchery in 2005 and 2006

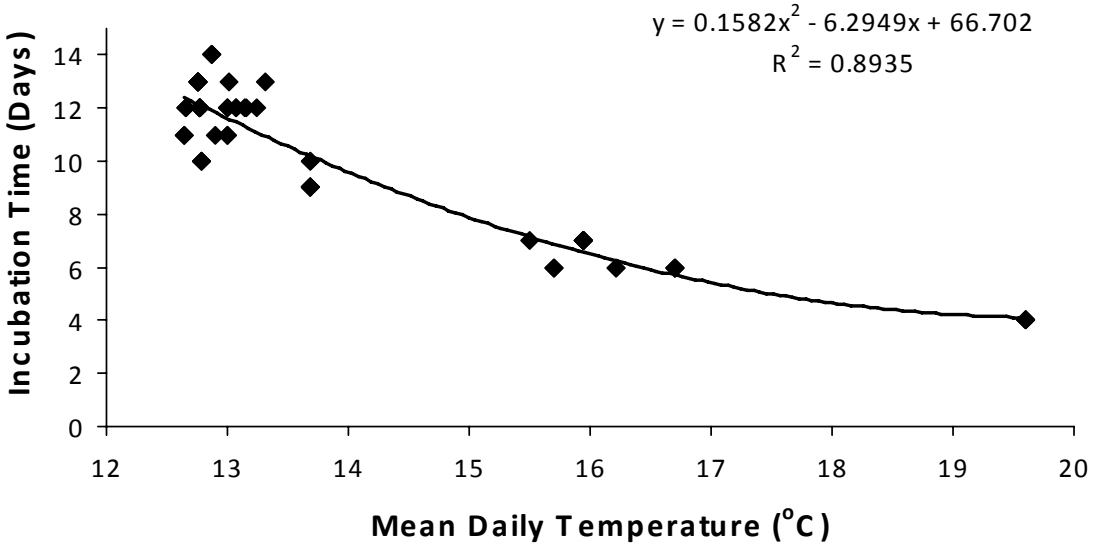


Figure 8. Daily proportions of dead eggs infected by microbes (mean ± 1SE) for 2 different lots of eggs (Early (E) and Late (L)) reared in streamside and traditional hatcheries

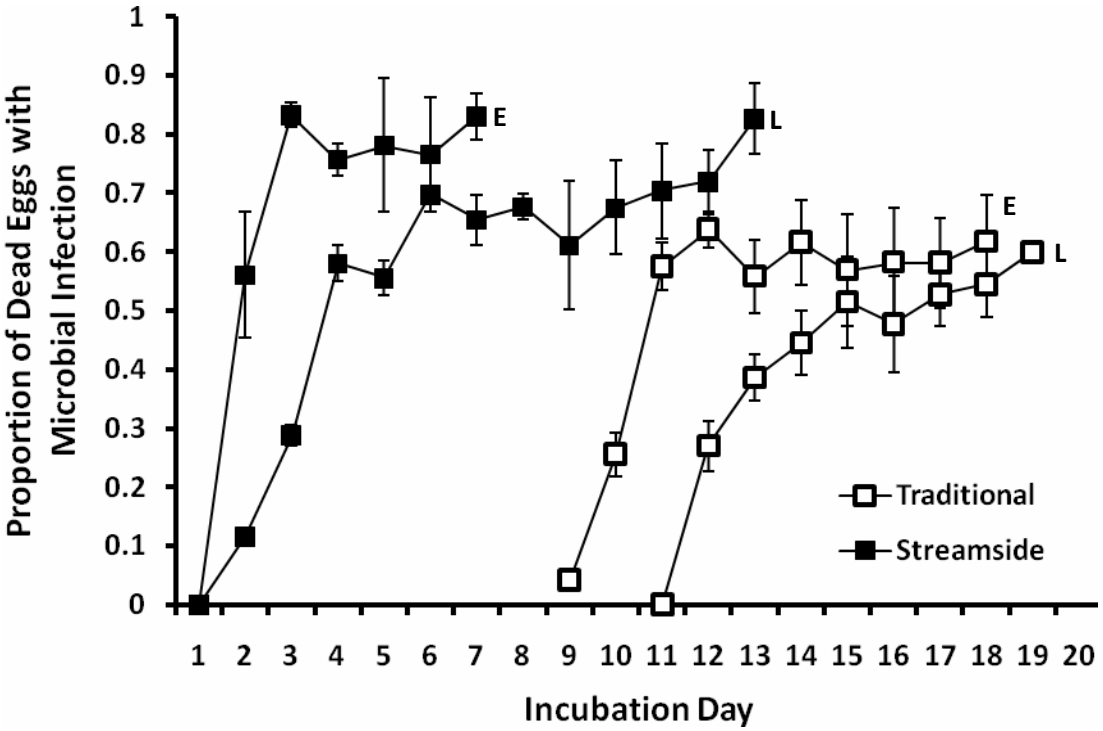


Figure 9. Daily survival rates for lake sturgeon eggs incubating for a different number of days until hatch

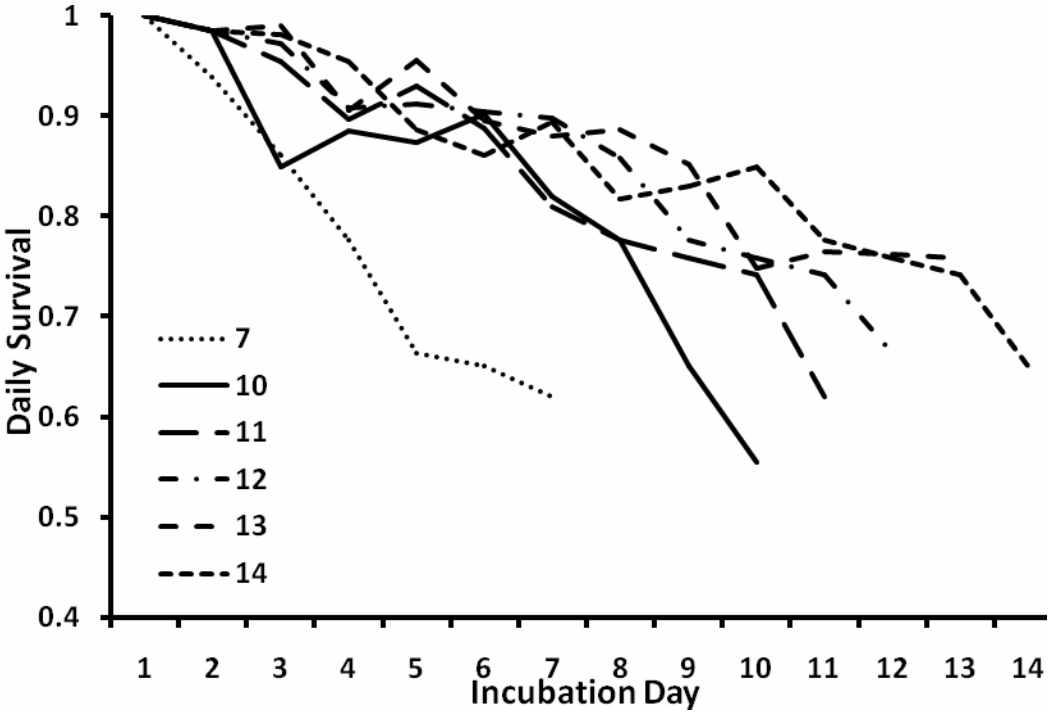


Figure 10. Total numbers of larvae hatched (mean ± 1SD) from females in two different hatchery environments in A) 2005, and B) 2006. Different sets of females were used between years

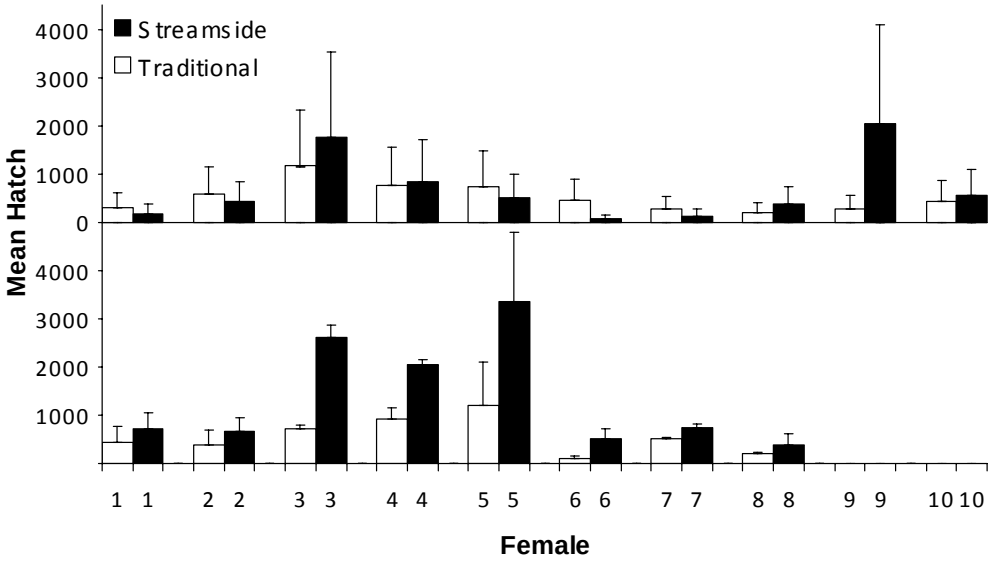


Figure 11. The effects of four treatments and time since hatch (days) on the change in larval lake sturgeon total length (A) and yolk-sac utilization (B). Treatments correspond to tanks that include refuge (cover), refuge and resources (cover-food), totally open tank (open), and an open tank with resources (open-food)

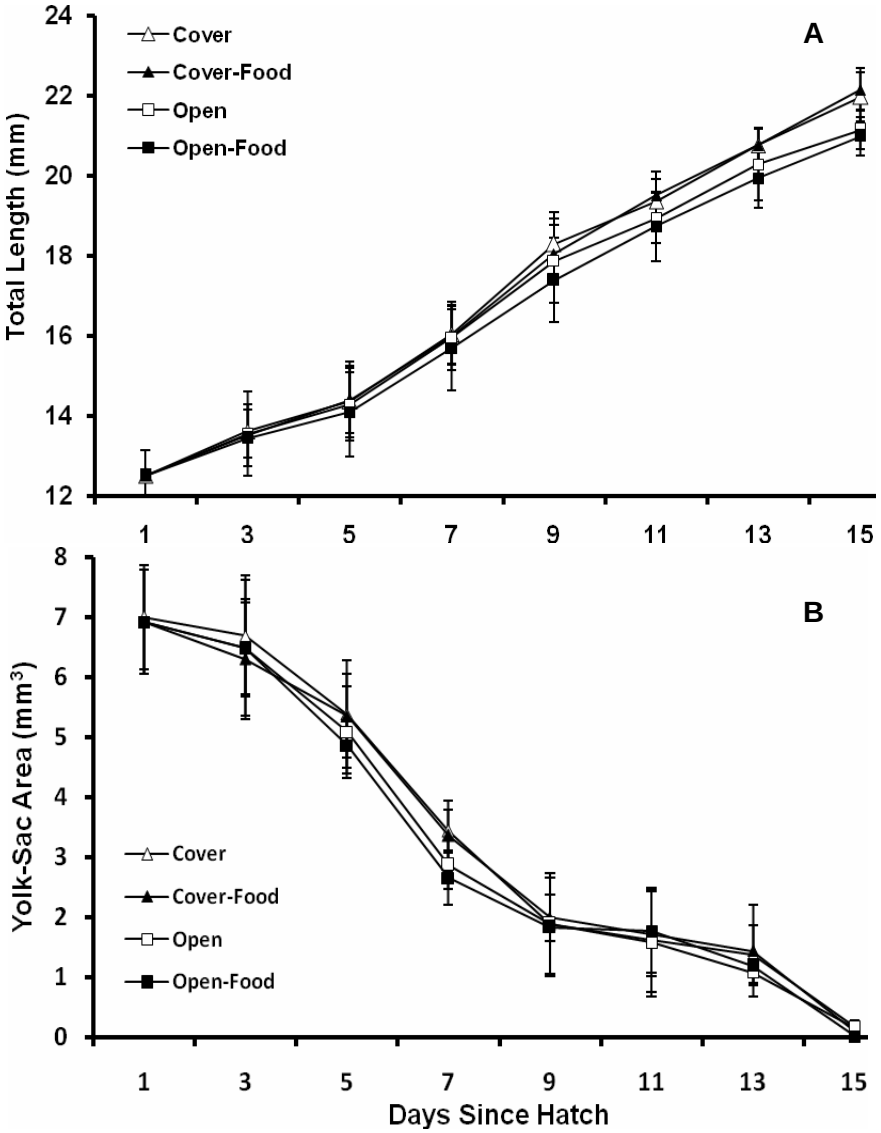


Figure 12. Differences in total length (A) and yolk-sac utilization (B) over time among larval lake sturgeon from six females reared at a streamside hatchery in 2006

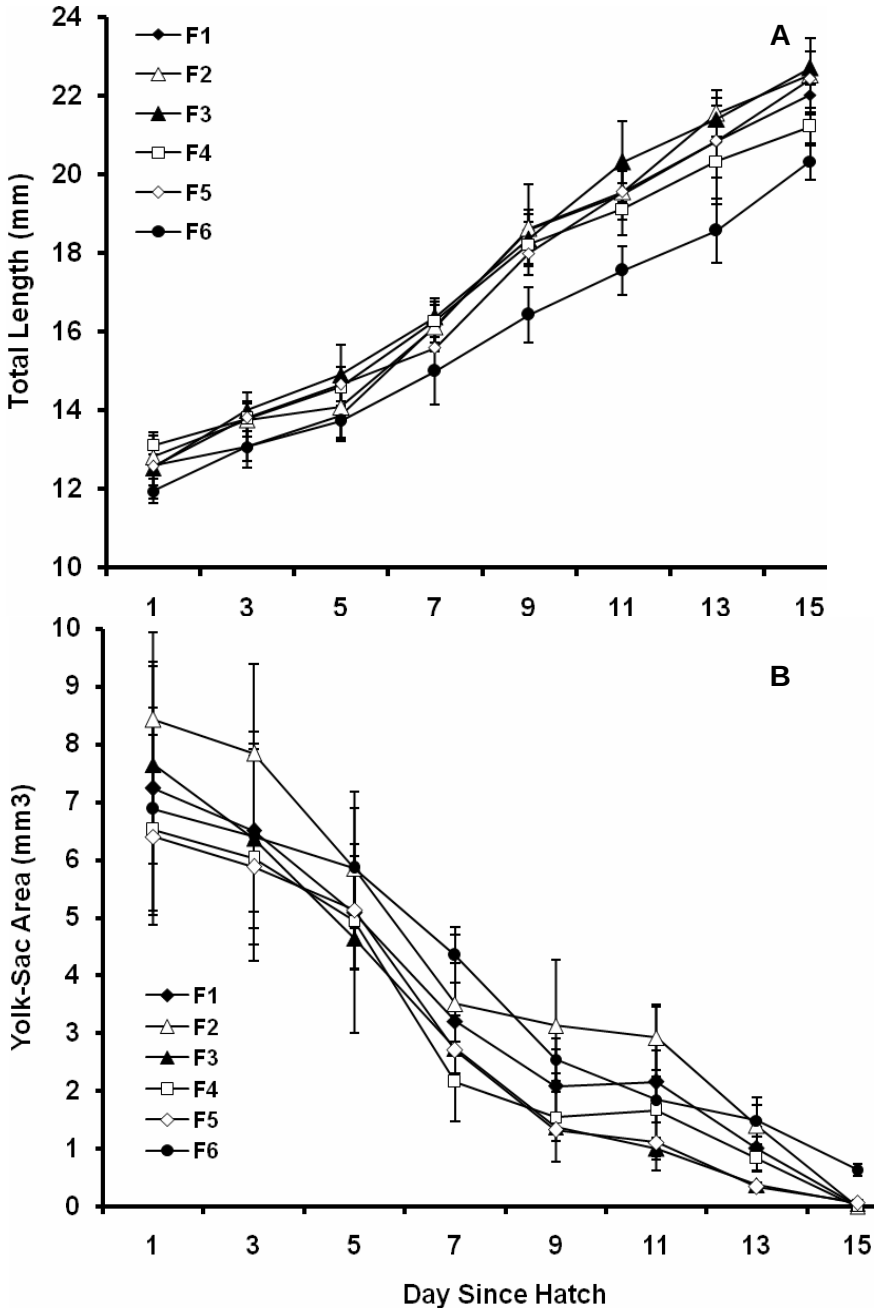


Figure 13. Daily survival of juvenile lake sturgeon collected using three gamete/larval collection methods (dispersing larvae (DL), naturally produced eggs (NPE), and direct gamete takes (DGT)) reared in two different hatcheries (streamside and traditional) in 2005

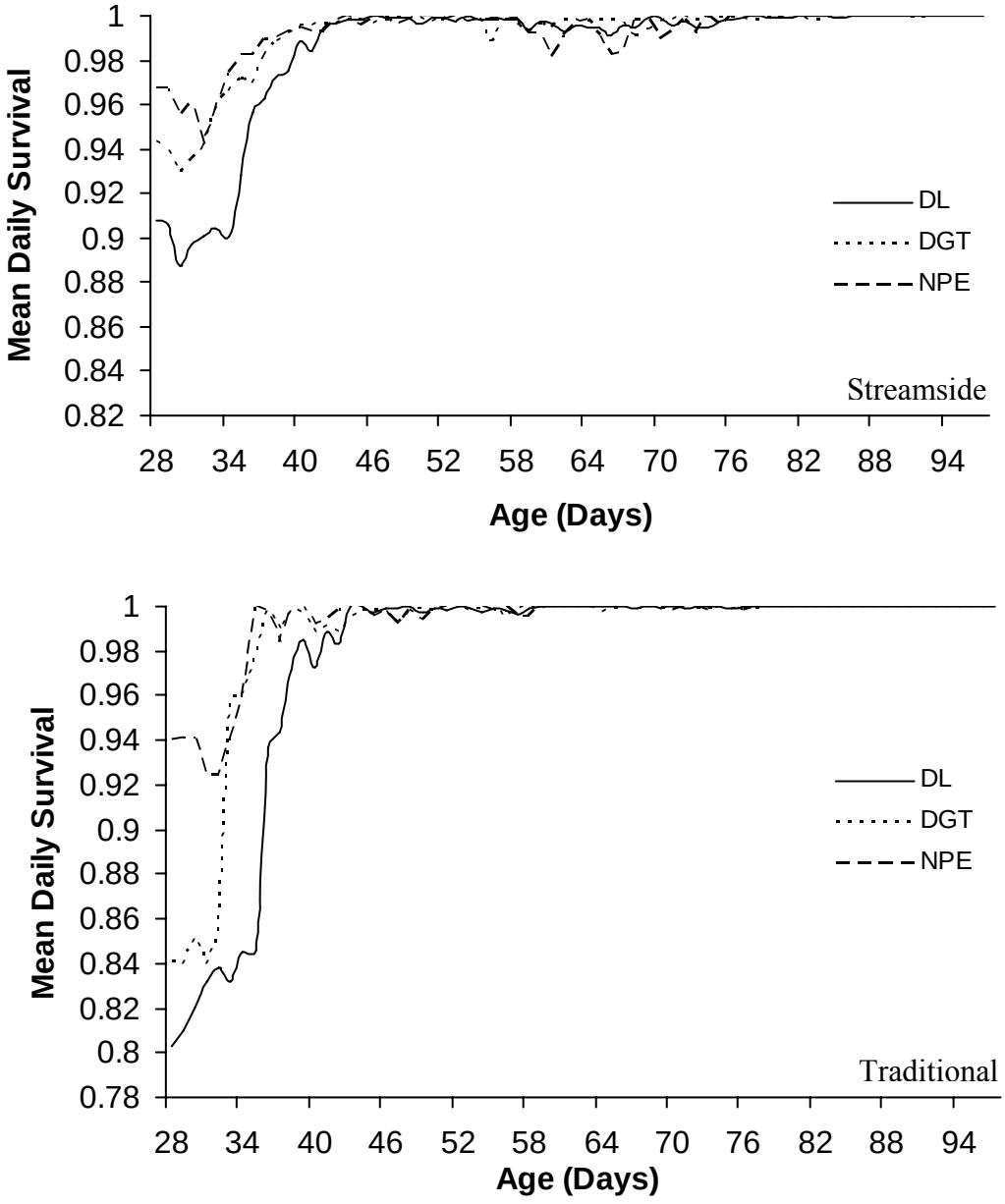


Figure 14. Growth of juvenile lake sturgeon reared in three different levels of habitat complexity. Habitat complexity consisted of completely open, filled half way with large rocks to simulate cover, and finally tanks that were completely covered

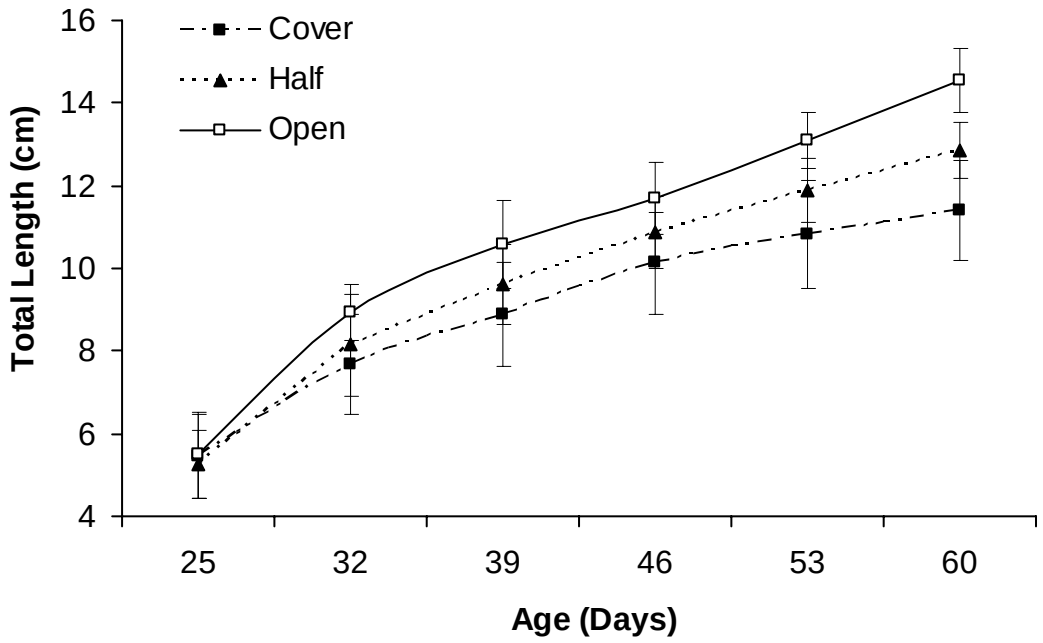


Figure 15. The Black Lake study site in Michigan, showing the release location and sampling sites (1-4) for release assessments in the Upper Black River. Site 4 also corresponds to the larval sampling site

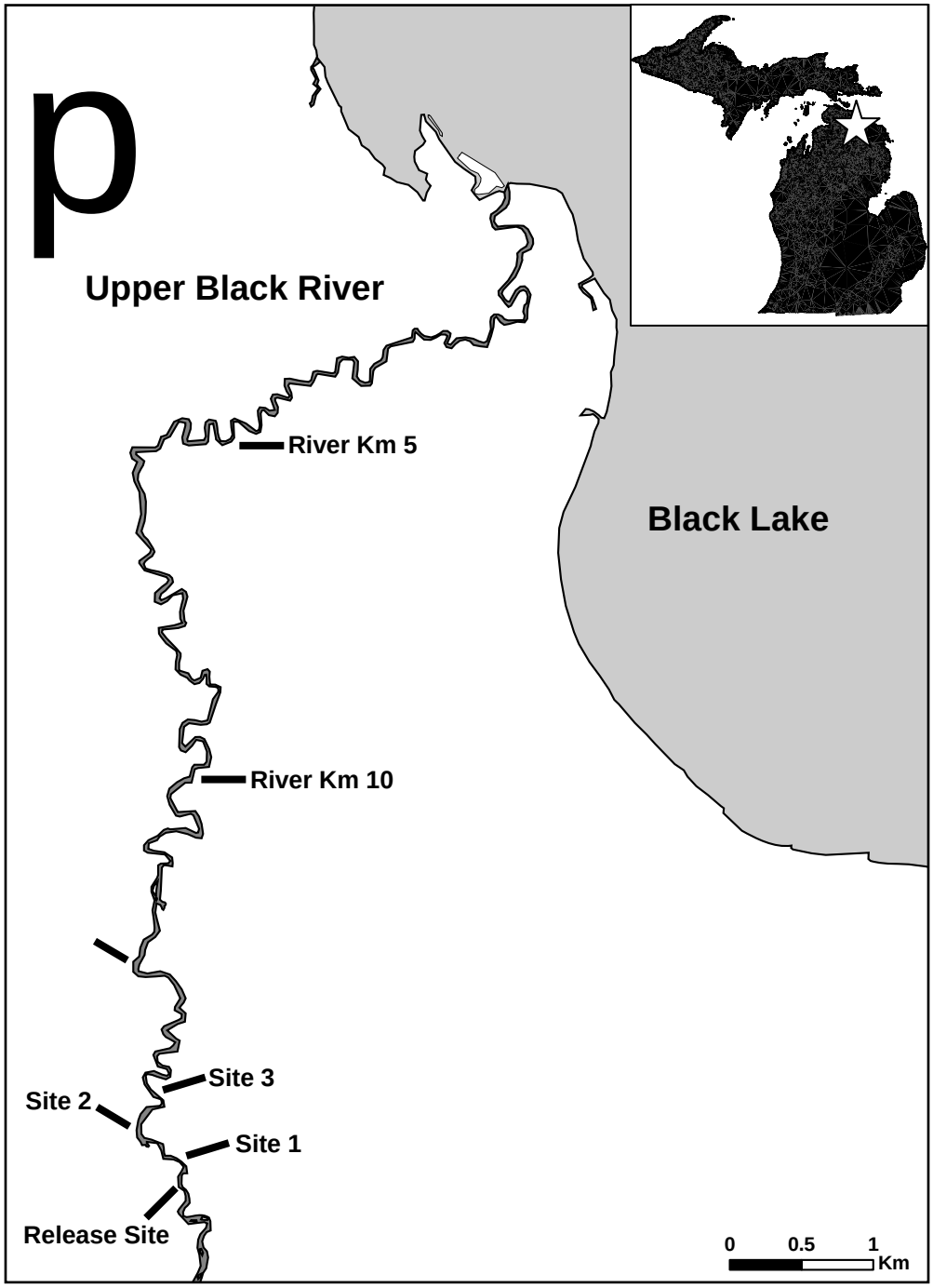


Figure 16. The proportion of juvenile lake sturgeon recaptured at downstream assessment sites (Sites 1-4; Fig 1) that were reared in different hatchery environments. Ages 8, 13, and 17 weeks are represented within a downstream interval respectively reading left to right. Asterisks represent significantly greater proportions of recaptured fish that were reared at the streamside hatchery versus the traditional hatchery. Letters (A, B, C) correspond to statistically greater proportions of fish recaptured at the different release ages

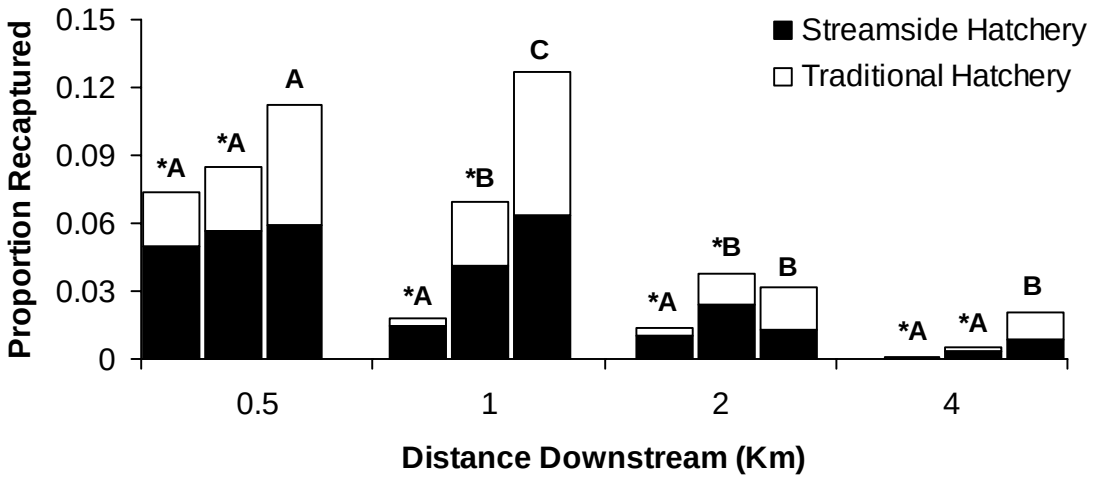


Figure 17. The proportion of juvenile lake sturgeon captured at consecutive hours following release into the Upper Black River at 8, 13, and 17 weeks of age

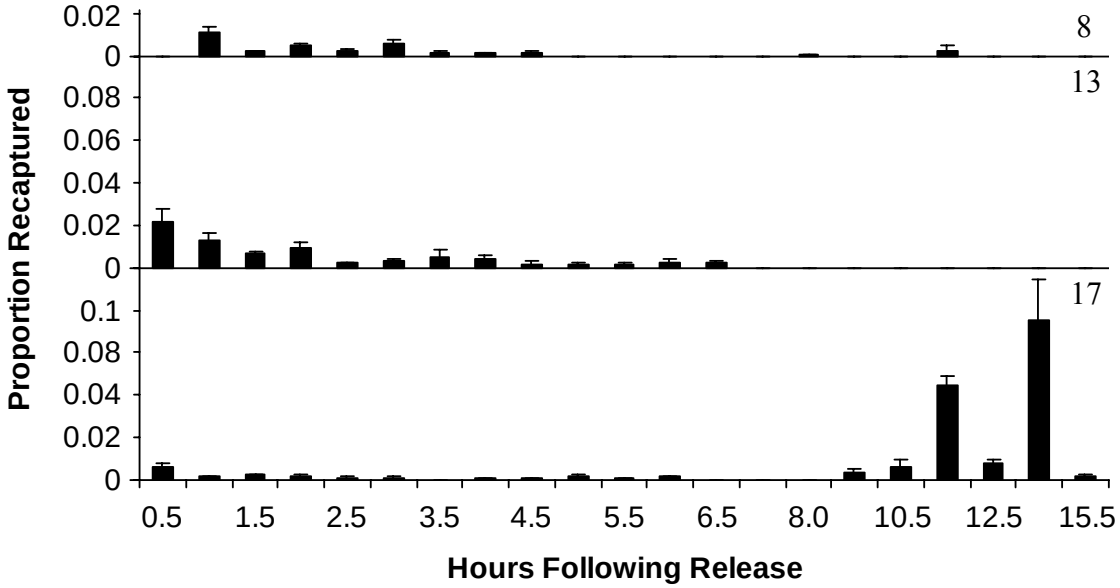


Figure 18. Total length (means ± 1 S.E) of recaptured juvenile lake sturgeon over time following release at 8, 13, and 17 weeks of age into the Upper Black River

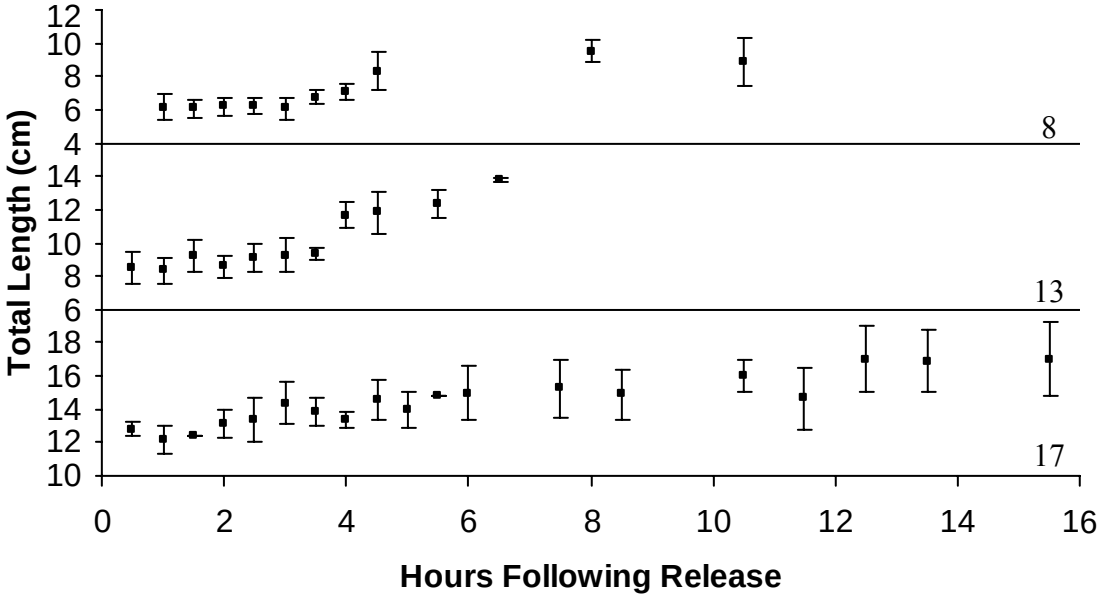
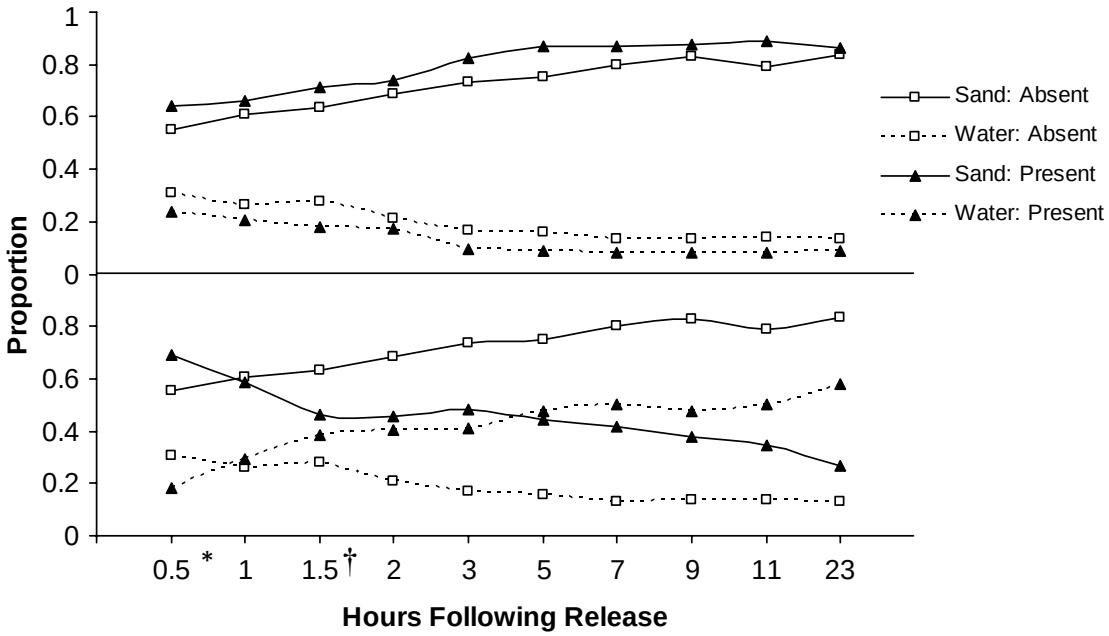


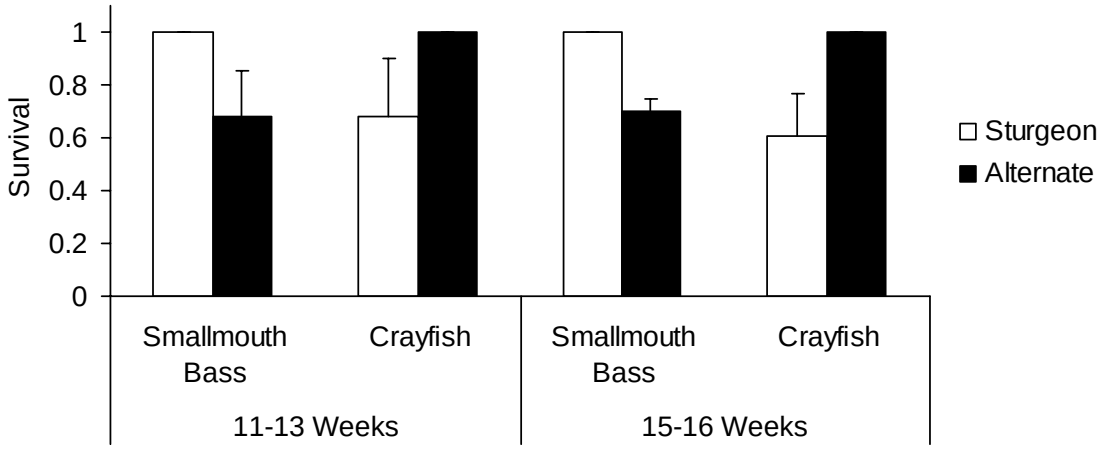
Figure 19. The effect of two predator classes (Fish, Crayfish) on the proportion of juvenile lake sturgeon observed on sandy substrate or that were swimming in the water column. Gravel and large rock substrates were also available choices in this experiment but are not included due to insignificant choice for these two habitat types. Predators included rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieue*), northern pike (*Esox lucius*) and the rusty crayfish (*Orconectes rusticus*). Absent and present refer to trials conducted with and without predators



* First observation of predation by crayfish predators.

† First observation of predation by fish predators.

Figure 20. Survival (Mean ± 1 SE) of juvenile lake sturgeon and an alternate prey species exposed to two predator species at two ages (smallmouth bass, *Micropterus dolomieue,* and crayfish, *Orconectes rusticus*)



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