

STUDY FINAL REPORT

State: Michigan

Project No.: F-80-R-8

Study No.: 230732

Title: Factors affecting lake sturgeon recruitment: a model system for species recovery in Michigan waters of the Great Lakes

Period Covered: October 1, 2003–September 30, 2007

Study Objective: The objectives of this study are to (1) develop models that relate timing of spawning to environmental conditions, (2) build on demographic and genetic data collected in 2001 and 2002 to determine contributions from individual adults to annual recruitment, and to estimate adult spawning efficiency and effective population size, (3) estimate fertilization rate as a function of spawner number and sex ratio, (4) determine sources of egg predation and its magnitude prior to larval emergence, (5) determine factors that influence larval survival during downstream drift from spawning sites to rearing habitats, (6) develop a system for assessing juvenile survival and growth in river and lake systems that will be applied to potential restoration sites in Michigan, (7) test predictions of timing, sources and magnitude of impediments to recruitment in other streams in Michigan using eggs and larvae of different sizes that are obtained from Black Lake, and (8) use data from Black Lake to parameterize models to predict the efficacy of alternative hatchery supplementation strategies in future restoration activities in Great Lakes tributaries.

Summary: Our research addresses critical questions needed to maintain viable populations of lake sturgeon throughout the Great Lakes. Under Objective 1, we collected temperature and stream flow data as variables that may predict initiation of lake sturgeon spawning, duration of spawning, and the interval between early and late spawning periods. We found that spawning occurs in discrete early and late bouts coincident with temporal changes in stream temperature. Spawning typically begins when water temperatures sharply increase above a threshold of 9–12°C and again later in the spring when temperatures again sharply increase. To achieve goals under Objective 2, we used genetic determination of parentage to genotype juveniles that were dispersing from stream areas where spawning occurs. We documented evidence for multiple polyandry and polygyny. Variance in reproductive success was high and resulted in decreased effective population size relative to estimates of the total estimate of spawner abundance. To achieve goals of Objective 3 we deployed several egg collection devices to gather evidence for or against denaturation effects on egg fertilization rate that could be tied to spawner abundance, sex ratio, or stream characteristics. Quantification of fertilization rates was difficult. We observed a high rate of fertilization for groups of eggs collected using each collection method. Further research is necessary to adequately tie demographic and stream physical characteristics to rates of egg fertilization. We conducted a series of experiments under Objective 4 to estimate the magnitude and source of egg mortality following deposition. Egg survivorship was extremely low (<10%). Experimental treatments that differed in size of enclosure openings that precluded different predators from preying on eggs showed that egg survival decreased with increasing mesh size. Results indicate several stream predators of different size and de-adhesion were responsible for high rates of egg loss. To achieve goals of Objective 5, we sampled larvae that dispersed downstream from spawning areas. Over the period of study, the number of larvae captured per year varied from 16,417 to 431 revealing high inter-annual variance in recruitment to the larval stage. Date of initiation of larval dispersal and the duration of dispersal also varied

among years. To achieve goals of Objective 6 we estimated rates of juvenile survival at the time of dispersal and through the first winter using repeated drift net sampling and acoustic telemetry, respectively. We estimated high rates of juvenile mortality in during larval dispersal. We estimated minimum levels of overwinter mortality to be 40%. Under Objective 7, we evaluated several other tributaries within the Cheboygan River drainage and elsewhere in Michigan for future restoration. For the purposes of this study, we felt that releases should be restricted to the upper Black River. However, based on findings and ongoing work across the Great Lakes, lake sturgeon from the upper Black River could be used for future restoration elsewhere in Michigan. To achieve goals of Objective 8 we collected gametes and larvae using several strategies. Juveniles were reared in different hatchery environments and released at three different ages. We found that juveniles produced at a streamside facility had significantly higher rates of recovery than did juveniles reared in a traditional hatchery when released at young ages (8 and 12 weeks). There was no effect of hatchery rearing when releases occurred at 17 weeks of age. We interpret recovery rates as surrogate measures of survival. Controlled replicated experiments simulating larval mortality using tanks and different predators showed strong direct (mortality) and indirect (shifts in movements) effects in response to crayfish. Lake sturgeon juveniles at or greater than 8 weeks of age suffer mortality by other teleost fishes but at a low rate. Mortality rates at younger ages (i.e., at the time of dispersal) are extremely high.

Findings: As findings will not be formally published through the Fisheries Division, this Final Report is submitted.

Job 1. Title: Determine rates of egg fertilization and sources/rates of egg predation.—Results from this job are presented in Appendix 1.

Job 2. Title: Identify release streams, stream characteristics and species composition.—We worked with Michigan DNR Basin Teams and fish management biologists from other resource agencies over the last several years to identify suitable release streams and release and rearing site characteristics for larval lake sturgeon. After consultation with these groups and the Lake Michigan Lake Sturgeon Task Group it was determined that, for the purposes of this research effort, any release of lake sturgeon from the Black Lake population should occur only within the Cheboygan River Watershed, and even more specifically, within the Black Lake drainage including the Upper Black River. The primary reason for keeping released fish within the Upper Black River was concern over the possibility that stocked fish may not imprint to the river where stocked. Recent genetic analyses of lake sturgeon populations from around the Great Lakes have indicated that remnant populations are genetically distinct (Bott 2006; DeHaan et al. 2006; Welsh 2006). Based on these data the Lake Michigan Lake Sturgeon Task Group has recommended extreme caution in the development of stocking plans for lake sturgeon in the Lake Michigan basin. The recommendations specify that stocked lake sturgeon be raised in streamside hatcheries using ambient river water to raise fish (Elliott et al. DRAFT plan). The intent of this recommendation is to maximize the likelihood that the fish will imprint to the stocked river and return there when sexually mature. As a result we concluded that, for the purposes of this research project, we could only release fish back into the Upper Black River. It is likely that in the future the Black Lake population may be used as a source of gametes for restoration stocking in other Great Lakes waters.

Fish species composition data from Michigan DNR files indicated that the Upper Black River in the reaches to be stocked had a diverse fish community. In addition to lake sturgeon, the fish community includes native species in the families Petromyzontidae, Catostomidae, Percidae, Centrarchidae, Cyprinidae, Salmonidae, Esocidae, and Cottidae at least seasonally. Additionally, crayfish (*Orconectes* sp.) are abundant throughout the Upper Black River.

In addition to the Upper Black River, we evaluated the Pigeon and Sturgeon rivers (within the Cheboygan River watershed) as potential release sites. However, for the above stated reasons we determined that these rivers also were unsuitable for an experimental release, but may be suitable for future restoration effects.

Job 3. Title: Record stream environmental data.—Results of work performed under this job are presented in Appendix 2.

Job 4. Title: Estimate larval abundance for lake sturgeon and other species.—Results of work performed under this job are presented with results for jobs 5 and 6 in Appendix 3.

Job 5. Title: Estimate sources and rates of larval lake sturgeon mortality.—Results of work performed under this job are presented with results for jobs 4 and 6 in Appendix 3.

Job 6. Title: Estimate sources and rates of mortality for other larval fishes.—Results of work performed under this job are presented with results for jobs 4 and 5 in Appendix 3.

Job 7. Title: Rear larval lake sturgeon for release.—Results of work performed under this job are presented together with results from Job 8 in Appendix 4.

Job 8. Title: Estimate rates of larval mortality in stocked sites.—Results of work conducted under this job are reported in Appendix 4.

Job 9. Title: Determine movements of lake sturgeon using telemetry (03/04 locales).—Results of work conducted under this job are reported in Appendix 5.

Job 10. Title: Conduct genetic analyses.—Results of work conducted under this job are reported in Appendix 6.

Job 11. Title: Supervise staff.—We have supervised four graduate students, one full time technician, and 12 additional seasonal undergraduate students who have worked on this project during the funding period.

Job 12. Title: Write annual performance report.—Annual reports were completed as scheduled.

Job 13. Title: Write final report.—This report was prepared.

References

- Bott, K. J. 2006. Genetic analyses of dispersal, harvest mortality, and recruitment for remnant populations of lake sturgeon, *Acipenser fulvescens*, in open-water and riverine habitats of Lake Michigan. Master's thesis. Michigan State University, East Lansing.
- DeHaan, P. W., S. V. Libants, R. F. Elliott, and K. T. Scribner. 2006. Genetic population structure of remnant lake sturgeon populations in the upper Great Lakes basin. Transactions of the American Fisheries Society 135:1478–1492.
- Elliott, R. E., B. Eggold, M. Holtgren, and E. A. Baker. Draft. Lake Michigan Lake Sturgeon Task Group rehabilitation plan. Great Lakes Fishery Commission, Lake Michigan Lake Sturgeon Task Group, Ann Arbor, Michigan.
- Welsh, A. B. 2006. Lake sturgeon conservation in the Great Lakes: Scaling it up from genetics to policy. Doctoral dissertation. University of California Davis, Davis.

Appendix 1

Rates of Lake Sturgeon Egg Fertilization Success and Rates and Sources of Egg Predation

Rates of Egg Fertilization Success

Introduction

Reduced intraspecific competition and increased rates of egg and juvenile survival are thought to be several compensatory effects arising from reductions in stock size (Ricker 1954). Traditional stock-recruitment models are based on this assumption; specifically that reductions in density will lead to increased per capita growth rates and increased stock abundance. Alternatively, positive compensatory responses may be limited, and low stock size can result in lower average recruitment (Myers et al. 1999). Reduced per-capita growth rate when population densities are low is commonly referred to as the Allee effect or depensation. Mechanisms contributing to depensatory population dynamics include reduced probabilities of fertilization, impaired group dynamics, inability to find mates, and increased predation rates (Liermann and Hilborn 2001).

Depensation in natural fish populations is difficult to detect but is believed to have led to sudden collapses of numerically depressed fish populations around the world (Hutchings and Reynolds 2004). Density-dependant mechanisms including depensation have been empirically tested. Rowe et al. (2004) used Atlantic cod to provide evidence to support the hypotheses that fertilization rates decline with abundance and that variance in fertilization rate increases as population size declines. The negative genetic consequence of a declining effective population size (N_e) was concomitant with a decline in the ratio of N_e to census population size (N_e/N_c). Declining spawner abundance was evident. However, the relationships between fertilization rate and spawner number and sex ratio are unknown for broadcast-spawning fish species. Slow rates of recovery and continuing declines in population abundance for lake sturgeon *Acipenser fulvescens* may be attributed to depensation due to low fertilization rates. We designed and tested methods to document how fertilization rates vary with spawner abundance and sex ratio.

Methods

Egg Catchment Method 1

Eggs were collected on circular porous filters attached to metal poles positioned in several locations and at multiple depths downstream from spawning adults. Filters passively sampled drifting eggs for approximately 12 h. Filters were taken at a streamside rearing facility and then placed in heath trays fed with water taken directly from the Black River until fertilization success (indicated by a developing egg) could be determined. Before removing the filters, however, we documented the total number and sex of spawning adults (indicated by external tag color) and the position of each egg collector relative to the spawning group. Fertilization rates were estimated as the proportion of developing eggs on each porous filter after 12 h of incubation. We collected approximately 200 eggs from the water column using catchment devices deployed at one spawning location for five consecutive days during peak spawn.

Egg Catchment Method 2

We modified our experimental design to increase the probability of collecting an unbiased sample of drifting eggs from a distinct spawning group, and to increase our ability to document fertilization success under natural conditions. We also wanted to account for fluctuations in the total number of spawners. We constructed portable catchment devices using small plankton nets and metal frames. Catchment devices were placed directly downstream from spawning aggregations once spawning activity was observed. Eight to ten plankton nets were deployed. We were able to maximize the number of eggs collected by adjusting plankton nets according to vertical movements of the fish in the water column or the horizontal movements across the stream channel. We were also able to conduct a more extensive sampling of eggs from all sections of the river and from more than one group of spawners as compared to the previous design.

Several spawning groups were chosen and data on the number of male and female spawners and observations of egg deposition were collected. We conducted several preliminary trials and collected approximately 500 eggs from two spawning aggregations. Samples were immediately transported back to a streamside rearing facility and placed in heath trays fed with natal stream water for incubation. Formalin baths were used during egg incubation to minimize mortality due to confounding environmental and biotic factors such as microbial infection. After 24 h, eggs were subsampled and the fertilized and unfertilized eggs were counted.

Results

We concluded that fertilization rates estimated using Method 1 were high (~100%). However, a high incidence of fungus infection and the amount of silt collected by the porous filters confounded accurate interpretation of fertilization rates. We were also unable to directly tie spawner number and sex ratio to fertilization rate because of frequent temporal shifts in both number of individuals spawning and the sex ratio of the group. Fertilization rates for eggs collected using Method 2 were also high (~100%). However, a high incidence of fungus infection and a large amount of sand collected by the collection nets confounded accurate interpretation of fertilization rates.

Discussion

Preliminary results from both catchment methods indicated fertilization rates near 100%. Our ability to determine the probability of fertilization as a function of adult number and sex ratio remains confounded due to small sample sizes, frequent shifts in adult number (Method 1), high egg mortality during initial egg incubation (Methods 1 and 2), and our inability to assess stages of embryogenesis due to adhesion of eggs to each other and to stream debris. We were unable to show depensation in the Black River. However, given extremely low spawner densities in other streams, depensation likely governs spawning dynamics in some Great Lakes tributaries, and may occur among low-density spawning aggregations in the Black River. The lake sturgeon mating system is characterized by aggregate spawning and most Michigan streams that support breeding populations have been significantly reduced. The impact of depensatory population dynamics in lake sturgeon should be considered and long-term population forecasts should be developed based on empirical data collected from either field or laboratory conditions.

Estimate Rates of Egg Predation by Invertebrates and Fishes

Introduction

Egg mortality has been empirically linked to physical attributes of stream environments including current (Marsden et al. 1991), the timing of ice cover (Freeberg et al. 1990) and the permeability of substrate to water flow providing oxygen for development during the incubation period (Kondou et al. 2001). Predation also has been recognized as an important source of mortality during the egg stage because of overall vulnerability of embryos and the large number of egg predators (Bailey and Houde 1989). Extensive predation on lake trout eggs has been implicated in the lack of natural recruitment in some Great Lakes populations and is often cited as a significant barrier in rehabilitation of the species (Savino et al. 1999). Lake sturgeon eggs are also vulnerable to mortality after they are deposited. It is speculated that predation is the primary mechanism of egg loss in this species. Crayfish *Orconectes* sp., mudpuppies *Necturus maculosus*, redhorse *Moxostoma* sp., common carp *Cyprinus carpio* and adult lake sturgeon *Acipenser fulvescens* have all been observed feeding directly on lake sturgeon eggs shortly after deposition (Kempinger 1988). With the exception of common carp, each predator species is commonly found in the Upper Black River (UBR). Abiotic mechanisms must also be considered as a source of egg loss. Lake sturgeon egg losses have been linked to: increasing amounts of sediment deposition preventing proper incubation; fluctuations in the stream water levels that expose some eggs to desiccation; microbial infections *Saprolegnia* sp.; and increasing water velocity that dislodges eggs from the substrate. Each factor can cause substantial egg mortality (Kempinger 1988). Less than 1% of all the eggs produced during a spawning bout will survive to hatch (Kempinger 1988). Rates of egg predation are therefore potentially driving levels of annual recruitment. We used a field-based experimental design to obtain quantitative estimates of predation on lake sturgeon eggs and to estimate the survival of eggs over the duration of incubation.

Field Methods

We used replicated experimental enclosures representing four treatments to test hypotheses regarding the sources and magnitude of predation. Enclosure treatments were chosen based on research that identified lake sturgeon egg predators, egg predators of other demersally-spawning fish species, and suspected predators in the UBR system. Treatments were designed to incorporate both epibenthic and interstitial predator guilds that forage on eggs on the substrate surface and within interstitial spaces. Treatment were: 1) Open where all predators had access to the eggs, 2) Large Mesh (5.08 cm steel wire cloth) that allowed access of benthic macro-invertebrates such as crayfish, small species of fish and drifting macroinvertebrates, 3) Small Mesh (1.27 cm steel hardware cloth) that allowed colonization by drifting macro-invertebrates and 4) Full Mesh (2-mm steel window screening) serving as a control where all potential predators were excluded. Current velocity was also reduced within replicates of Treatment 4

The type of stream predators primarily responsible for consuming eggs at the highest rate were identified based on statistically significant differences between the four treatment groups. For example, significant differences in egg loss between the large (open and chicken wire) and small (window screen and hardware cloth) mesh size treatments would indicate the effects of small fish and larger macroinvertebrates such as crayfish on egg survival. The proportion of eggs remaining on filters open to all predators will provide a strong indication of the magnitude of egg loss from all combined sources of mortality.

Eggs were divided into lots of 50 based on a standardized volume (2 ml). Two hundred eggs were then fertilized and deposited over brown circular porous filters (3M Worldwide, Inc.; 0.073 m²). Simulated egg densities fell well within the range commonly observed in the UBR under natural

spawning conditions (Forsythe, unpublished data). Filters were randomly assigned to an exclusion treatment and secured to the bottom of the stream using a pre-fabricated base. Four different treatments were randomly placed on a base (block) and eight replicates of each base were deployed perpendicular to the water flow. Flow conditions for one treatment were not affected by an adjacent cage. Stream sampling locations exhibited the range of conditions in water depth, discharge and substrate size quantified at natural spawning and depositional areas in the UBR (Forsythe, unpublished data).

The number of eggs remaining on each filter within each replicate treatment was enumerated once every 24 h. Once the enclosure was removed, we counted egg numbers, recorded developmental stage and noted egg condition (i.e., developing vs. non-developing vs. dead due to microbial infection). All cages were cleaned of stream debris during each assessment period so that flow rates were consistent across replicates within treatments. After the last assessment was conducted, we recorded stream depth and flow conditions inside each treatment replicate using a Marsh-McBirney flow meter.

Statistical Methods

A linear mixed-effects model with randomized complete block design and repeated measures was used to determine enclosure (and presumably predator) effects on the proportion of eggs lost. Proportions were arcsine square root transformed in order to meet assumptions of normality. Fixed factors included in the analysis were Treatment (Control, Small Mesh, Large Mesh, and Open), Time (five 24h intervals), and the interaction term between main effects (Treat \times Time). The autoregressive error, AR(1), was used to model temporal autocorrelation among time periods. The AR(1) models a covariance structure for observations made at equal distances over time. Results indicate that observations that are made further apart will be larger for adjacent times than observations that are made closer together. Block (grouping of four treatments) was treated as a random term in the model. Pair-wise comparisons of significant fixed effects and interactions terms were investigated using LSMEANS ($\alpha = 0.05$).

Results

Pair-wise comparisons of egg abundance revealed a significantly higher proportion of exogenous removal in the Large and Open Mesh treatments relative to conditions where the access by predators was minimized (Small Mesh and Fine Mesh). Increases in egg loss due to removal resulted in a significant main effect of Day ($F_{4, 108} = 32.48$, $p < 0.001$). A significant Day \times Treatment interaction indicated that the rate of loss due to predation differed among treatments and variations in mortality varied daily ($F_{12, 108} = 1.98$, $p = 0.032$). Interpretation of significant interactions were based upon significant variations among treatments examined each day using pair-wise treatment contrasts (Figure 1.1).

A significant increase in the number of eggs removed by predators occurred 24 h after the experiment began. By day 3, mortality due to loss was significantly higher in the three treatments open to predation (i.e., Open, Large Mesh, Small Mesh treatments; Figure 1.1) relative to the Fine Mesh treatment. Significant differences in the rate of removal between treatments also corresponded to observations of predator presence on the egg pads (e.g., stoneflies, caddis flies, crayfish and minnows; data not shown). On day 5 of incubation, 16% of eggs were lost with the greatest proportion of eggs lost in the Open (26%) and Large Mesh (20%) treatments (Small Mesh = 9%, Fine = 7%).

Rapid declines in survival resulted in a significant main effect of Day ($F_{3, 101}$, $p < 0.001$). The mixed model procedure also revealed a statistically significant Day \times Treatment interaction indicating time-dependant differences in survival among treatments and that differences varied as the experiment progressed in time ($F_{12, 101} = 5.97$, $p < 0.001$). To simplify interpretation of the Day \times Treatment interaction, variations among treatments were analyzed separately for each day (Figure 1.2).

Survival remained consistent for approximately 48 h after simulated deposition. By day 3, survival had declined significantly and disproportionately with respect to enclosure treatment. After 96 h, survival had declined by 80% with significant pair-wise differences between the Fine and Small Mesh treatments and those with greater exposure to predation (Large Mesh and Open). A total of 590 eggs survived across all treatment groups after 5 days representing 9% (average) of the initial abundance (Fine Mesh = 11%, Small Mesh = 7%, Large Mesh = 10%, Open = 9%). At the end of incubation, no significant differences in survival were found between treatment groups.

Discussion

This study represents the first quantitative assessment of the magnitude of mortality due to predation on lake sturgeon eggs. We found that predation during incubation could account for as high as 26% of the total mortality depending on the predator environment. However, this probably underestimates rates of predation, which were increasing by the end of the experiment. Significant differences in predator removal between treatment groups (e.g., Large Mesh vs. Small Mesh) also indicate the greater effect of larger macro-invertebrate and fish predators (e.g., crayfish, darters, minnows) on egg survival relative to smaller aquatic predatory species. Even though predation is a major component of mortality for lake sturgeon eggs in natural conditions, this mechanism could not account for all mortality. This experiment also demonstrated that fungal infections of eggs imposed substantial mortality during incubation and may even exceed 80% in certain situations (data not shown).

Results confirm previous observations that lake sturgeon eggs are extremely vulnerable to microbial infection and may even serve as hosts to various aquatic microbial pathogens in the wild (Keppinger 1988). For example, oomycetic fungi colonizing developing lake sturgeon eggs could be from multiple genera (Kitancharoen et al. 1997) but have not been identified to date. Our results indicate that the probability of mortality due to both predation and pathogenic infection in natural conditions can be significant factors in loss throughout embryonic development. Because mortality is strongly tied to predation and egg death attributed to microbial infection, variation in predator and microbe abundance, type and distribution could have substantial consequences for recruitment success and may contribute to the variability commonly observed in lake sturgeon larval production. Extending this experiment to assessing survival across natural habitat types will be critical to rehabilitation programs for this species.

References

- Bailey, K. M., and E. D. Houde. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. *Advances in Marine Biology* 25:1–82.
- Freeburg, M. H., W. W. Taylor, and R. W. Brown. 1990. Effect of egg and larval survival on year-class strength of lake whitefish in Grand Traverse Bay, Lake Michigan. *Transactions of the American Fisheries Society* 119:92–100.
- Hutchings, J. A., and J. D. Reynolds. 2004. Marine fish population collapses: Consequences for recovery and extinction risk. *Bioscience* 54:297–309.
- Kempinger, J. J. 1988. Spawning and early life history of lake sturgeon in the Lake Winnebago system, Wisconsin. *American Fisheries Society Symposium* 5:110–122.
- Kitancharoen, N., K. Hatai, and A. Yamamoto. 1997. Aquatic fungi developing on eggs of salmonids. *Journal of Aquatic Animal Health* 9:314–316.
- Kondou, T., N. Takeshita, A. Nakazono, and S. Kimura. 2001. Egg survival in fluvial population of Masu Salmon in relation to intragravel conditions in spawning redds. *Transactions of the American Fisheries Society* 130:969–974.
- Liermann, M., and R. Hilborn. 2001. Depensation: evidence, models and implications. *Fish and Fisheries* 2:33–58.
- Marsden, J. E., C. C. Krueger, and H. M. Hawkins. 1991. An improved trap for passive capture of demersal eggs during spawning: An efficiency comparison with egg nets. *North American Journal of Fisheries Management*. 11:364–368.
- Myers, R. A., K. G. Bowen, and N. J. Barrowman. 1999. Maximum reproductive rate of fish at low population sizes. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2404–2419.
- Ricker, W. E. 1954. Stock and recruitment. *Journal of the Fisheries Research Board of Canada* 11:559–623
- Rowe, S., J. A. Hutchings, D. Bekkevold, and A. Rakitin. 2004. Depensation, probability of fertilization, and the mating system of Atlantic cod (*Gadus morhua* L.). *ICES Journal of Marine Science* 61:1144–1150.
- Savino, J. F., P. L. Hudson, M. C. Fabrizio, and C. A. Bowen II. 1999. Predation on lake trout eggs and fry: a modeling approach. *Journal of Great Lakes Research* 25:36–44.

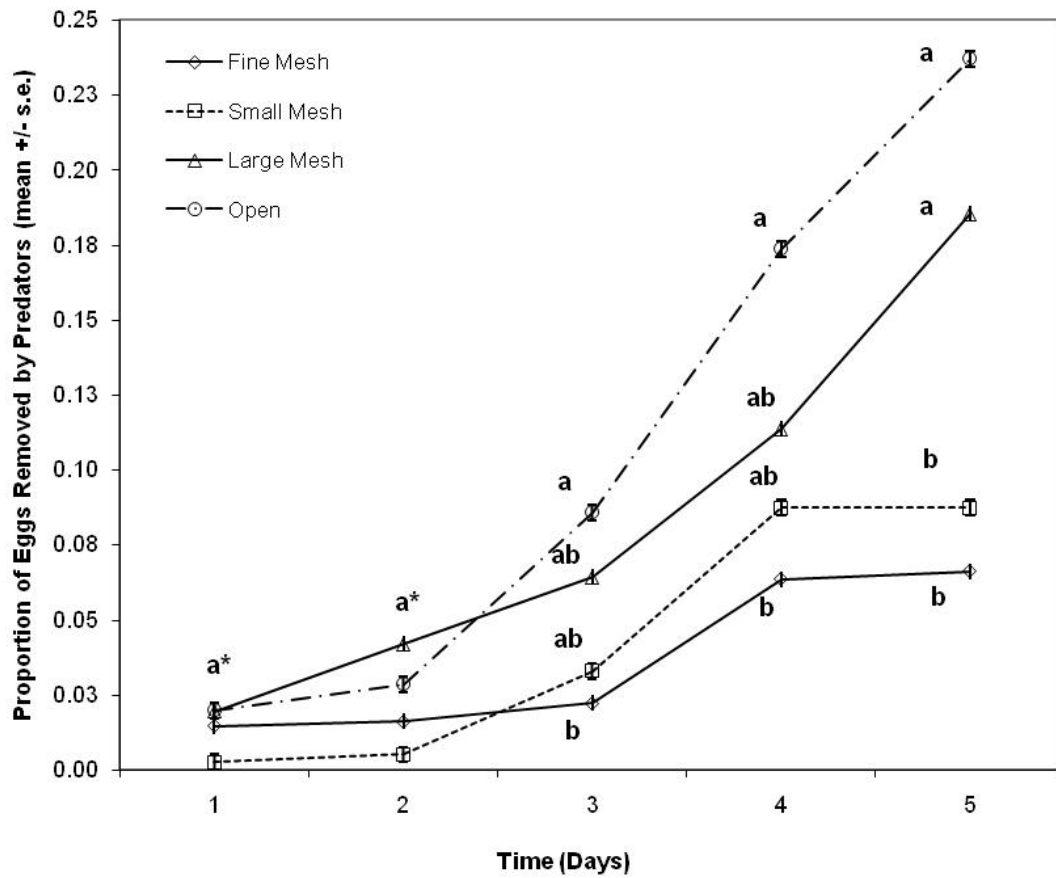


Figure 1.1.—The proportion of eggs removed by predators made each day for 5 consecutive days of incubation in natural conditions. Bars or data points labeled with the same lower-case letter indicate contrasts among treatments that do not differ significantly (LSMEANS; $\alpha = 0.05$).

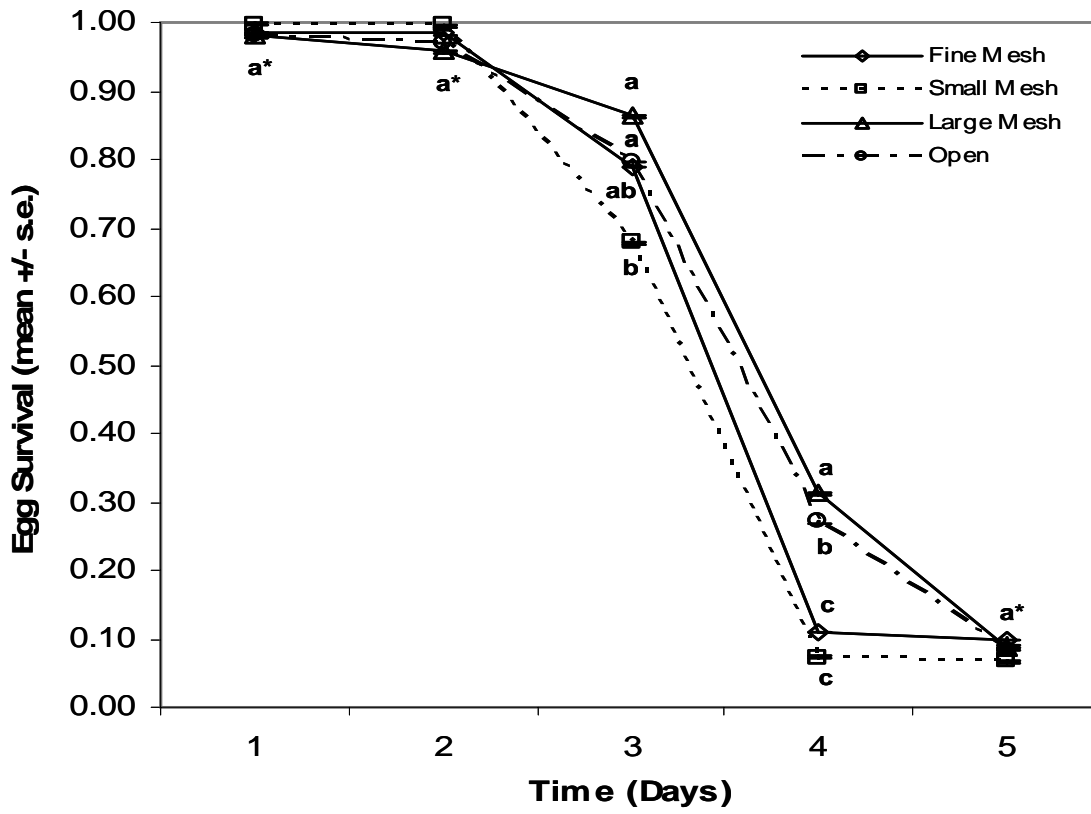


Figure 1.2.—Estimates of egg survival made each day for five consecutive days of incubation in natural conditions. Data points labeled with the same lower-case letter indicate contrasts among treatments that do not differ significantly (LSMEANS; $\alpha = 0.05$).

Appendix 2

Demography and Environmental Cues Associated with Spawning Lake Sturgeon in the Black River

Introduction

Demographic and morphological attributes of migrating fishes (abundance, sex ratio and body size) are often associated with environmental stream conditions (Mitchell and Cunjak 2007). Jonsson (1991) concluded that the number of Atlantic salmon spawning adults and river discharge were positively correlated. Others have speculated that low river flows may impede the access of larger bodied individuals to spawning locations thereby increasing the proportion of smaller fish in a run (van den Berghe and Gross 1989). Discharge is only one of several factors found to influence fish migratory behavior (Workman et al. 2002). Others factors include temperature (Erkinaro et al. 1999), light cycles, and celestial phase (Saulamo and Lappalainen 2007).

The spawning migrations of North American sturgeons are often strongly associated with water temperatures in the spring, with the initiation of upstream migration occurring between 8 and 25°C depending on the species (Hall et al. 1991; Chapman and Carr 1995; Fox et al. 2000). River velocity was not found to influence onset of migration or the intensity of spawning activity (Fox et al. 2000). However, once fish begin spawning, even slight variation in current velocity can influence spawning activity. Stream discharge can affect duration of spawning, the timing of arrival to spawning locations, and demographic characteristics of the spawning group and spawning site selection (Auer 1996).

Environmentally-induced timing of spawning migrations can play a major role in dynamics of fish populations through differential survivorship of eggs, larvae and embryos. Accurate and reliable predictions of migration timing may help managers mitigate factors that may impede natural migration, change spawning behavior, reduce fertilization efficiency or increase the magnitude of mortality. Our objectives were 1) to describe the timing, duration and demographic composition of adult lake sturgeon spawning over multiple years and determine environmental correlates that predict when and where spawning occurs, and 2) determine inter-spawning interval and whether males and females spawn during the same segment of the spawning period during each spawning year's episode.

Methods

Over five consecutive field seasons (2003–07) we used large trapezoidal landing nets to capture male and female lake sturgeon during their spring migration from Black Lake into the Upper Black River (UBR). All individuals collected were tagged with PIT tags. Colored Floy tags were implanted externally to permit visual identification of sex and spawning period (early or late). Total length, fork length, girth and sex were recorded. We also recorded the location of capture to one of six designated river zones. River temperature was monitored continuously during the spawning period using temperature loggers deployed at several locations along the Upper Black River. Loggers were deployed several days before the onset of migration and were programmed to record hourly water temperature.

To examine periodicity for individual adult lake sturgeon we compiled all recapture observations on unique females and males since the beginning of our tagging program, including up to the current year (2003–07). To determine estimates of periodicity for males and females and to test for differences in periodicity estimates between genders we ran a one-way analysis of variance

(ANOVA) using gender as the factor and the periodicity value associated with each individual as the response. We also examined the propensity for males and females to spawn within the same spawning run (early or late) in different years. Using the recapture data for both males and females we scored each spawning observation for all recaptures as either an early or late spawning run. To examine the probability of spawning in the same run over multiple spawning seasons we conducted a chi-square test separately for each sex. Expected results for the chi-square test were assumed to be random, or 0.5.

Results

We made a total of 817 observations of male and female lake sturgeon during the 2003–07 spawning seasons. The total number of spawning adults captured each year varied from 100 to 234 (Figure 2.1) as did adult sex ratio (1.95–3.0 M:F; Figure 2.2). The date of first capture ranged from April 20 in 2006 to May 3 in 2005. The annual duration of spawning activity ranged from 19 to 40 days. We observed two to five periods of spawning activity depending on the year (Figures 2.3–2.7). Average river temperatures at the time of first observation also varied substantially over the five years of this study (8.1–13.3°C). The timing of migration and the duration of spawning activity for the first and subsequent spawning pulses appeared to be correlated with a consistent and rapid increase in water temperature.

Since the start of our tagging research in 2001, we have recaptured 49 unique females and 166 unique males. From these individuals we have a total of 58 observations of repeat spawning for females and 302 observations for males. Minimum and maximum number of years between spawning events was 2 and 6 for females and 1 and 6 for males. Estimates of periodicity, or years between spawning events, were found to be 3.31 ± 0.135 (± 1 SE) for females and 1.91 ± 0.147 for males (Figure 2.8). Females spawned significantly less frequently than males ($df=2,358$ t -value=24.529, $P \leq 0.05$). The propensity to spawn within the same spawning run time in different years (Figure 2.9) was found to be significant for females ($df=1, 105$, $F=11.27$, $P=0.001$) and marginally significant for males ($df=1,517$, $F=3.86$, $P=0.056$).

Discussion

Black Lake sturgeon exhibited intra- and inter-annual variation in spawning stock abundance, spawning frequency, sex ratios, spawning duration and spawning site selection. These characteristics of the spawning population may be directly tied to migratory behavior as a function of different environmental cues. In this population, migration does not appear correlated with specific water temperatures in this system given the range of conditions observed on the first date of capture. Rather, individuals appear to actively respond (by migrating or out-migrating) to both sudden and dramatic increases or decreases in water temperature. For this report, we have provided data on one of several potential environmental cues likely to initiate the migratory movements of adult lake sturgeon from Black Lake to spawning areas in the Upper Black River. In future analyses, we will incorporate information on discharge and lunar cycles to determine if migratory responses are a result of the interaction between several cues. These models will be used to predict spawning activity and can aid in management by reducing the magnitude of mortality on eggs and larvae that may be related to environmental conditions at specific spawning times both within and across reproductive seasons.

References

- Auer, N. A. 1996. Response of spawning lake sturgeon to change in hydroelectric facility operation. *Transactions of the American Fisheries Society* 125:66–77.
- Chapman F. A., and S. H. Carr. 1995. Implications of early life stages in the natural history of Gulf of Mexico sturgeon, *Acipenser oxyrinchus desotoi*. *Environmental Biology of Fish* 43:407–413
- Erkinaro, J., and F. Okland, K. Moen, E. Niemelae, and M. Rahiala. 1999. Return migration of Atlantic salmon in the River Tana: the role of environmental factors. *Journal of Fish Biology* 55:506–516.
- Fox D. A., J. E. Hightower, and F. M. Parauka . 2000. Gulf sturgeon spawning migration and habitat in the Choctawhatchee River system, Alabama–Florida. *Transactions American Fisheries Society* 129:811–826
- Hall, J. W., T. I. .J, Smith, and S. D. Lamprecht. 1991. Movements and habitats of shortnose sturgeon, *Acipenser brevirostrum* in the Savannah River. *Copeia* 199:695–702.
- Jonsson, N. 1991. Influence of water flow, water temperature and light on fish migration in rivers. *Nordic Journal of Freshwater Research* 66:20–35.
- Mitchell, S. C., and R. A. Cunjak. 2007. Relationship of upstream migrating adult Atlantic salmon (*Salmo salar*) and stream discharge within Catamaran Brook, New Brunswick. *Canadian Journal of Fisheries and Aquatic Sciences* 64:563–573.
- Saulamo, K., and J. Lappalainen. 2007. Effects of abiotic factors on movements of pikeperch during pre-spawning and spawning season in a Baltic archipelago. *Hydrobiologia* 579:271–277.
- Van den Berghe, E. P., and M. R. Gross. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). *Evolution*, 43:125–140.
- Workman, D. R., D. B. Hayes, and T. G. Coon. 2002. A model of steelhead movement in relation to water temperature in two Lake Michigan tributaries. *Transactions of the American Fisheries Society* 131:463–475.

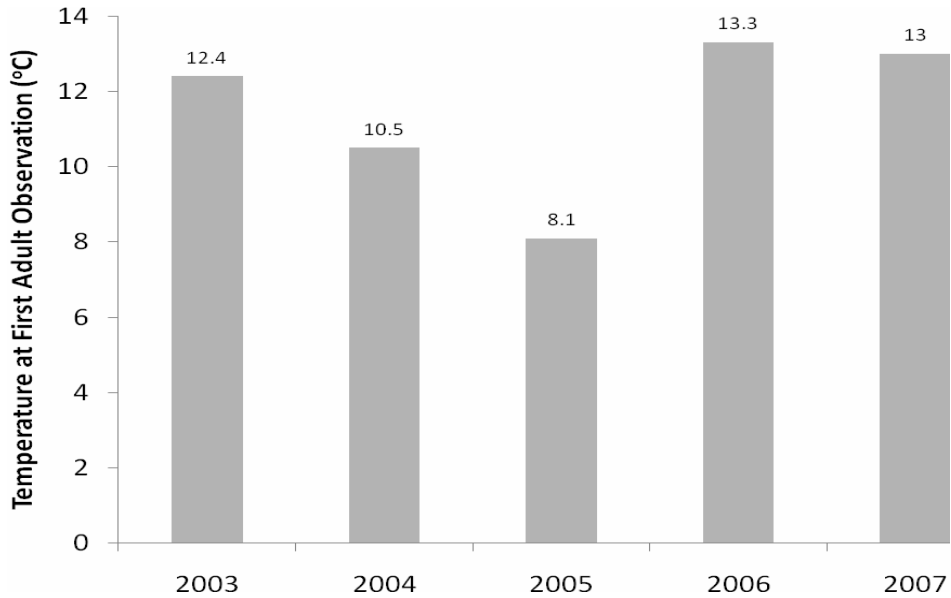


Figure 2.1.—River water temperature at first observation of adult lake sturgeon moving into spawning areas of the Upper Black River.

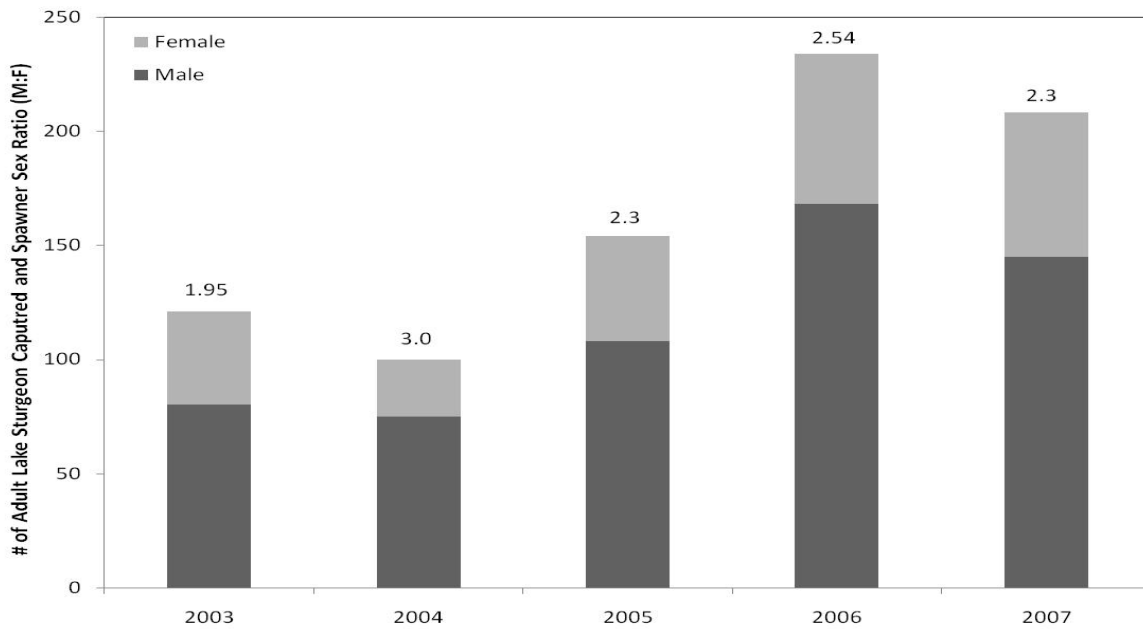


Figure 2.2.—The total number of male and female adult lake sturgeon captured on the Upper Black River for five consecutive years 2003–07. The sex ratio (M:F) is provided on top of each bar.

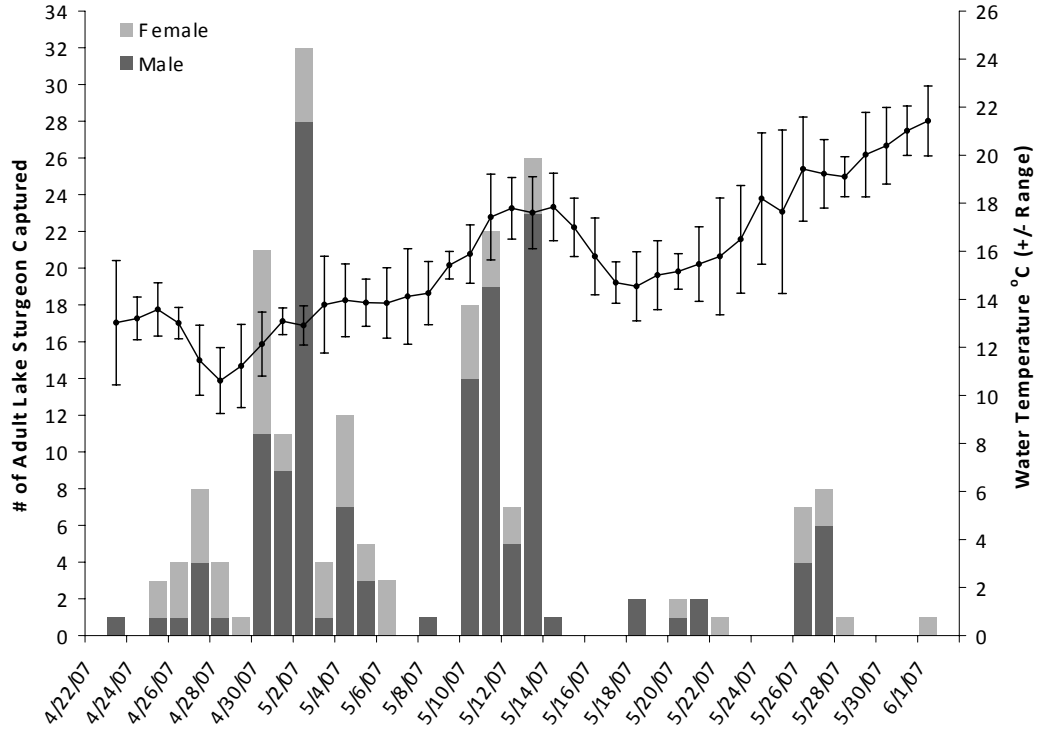


Figure 2.3.—The number of new fish captured each day on the Upper Black River during spawning 2007 in relation to water temperature at Kleber Dam.

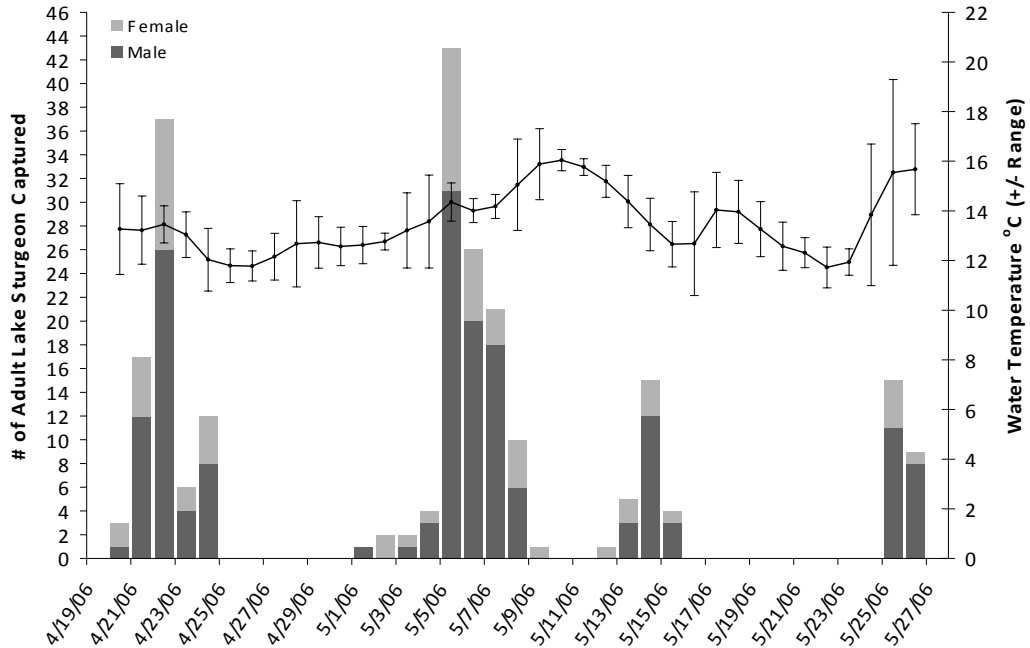


Figure 2.4.—The number of new fish captured each day on the Upper Black River during spawning 2006 in relation to water temperature at Kleber Dam.

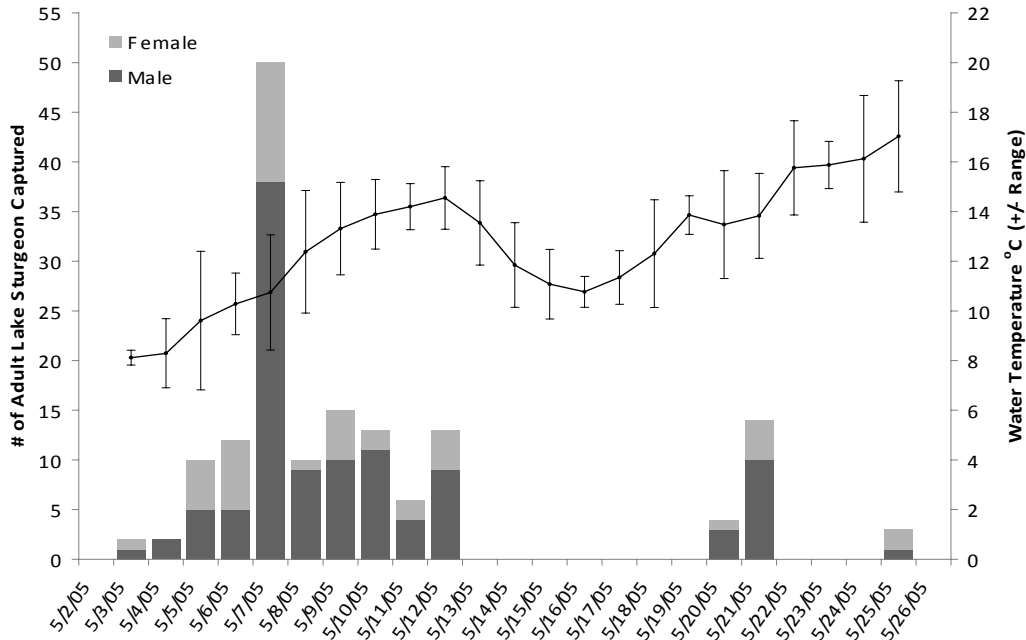


Figure 2.5.–The number of new fish captured each day on the Upper Black River during spawning 2005 in relation to water temperature at Kleber Dam.

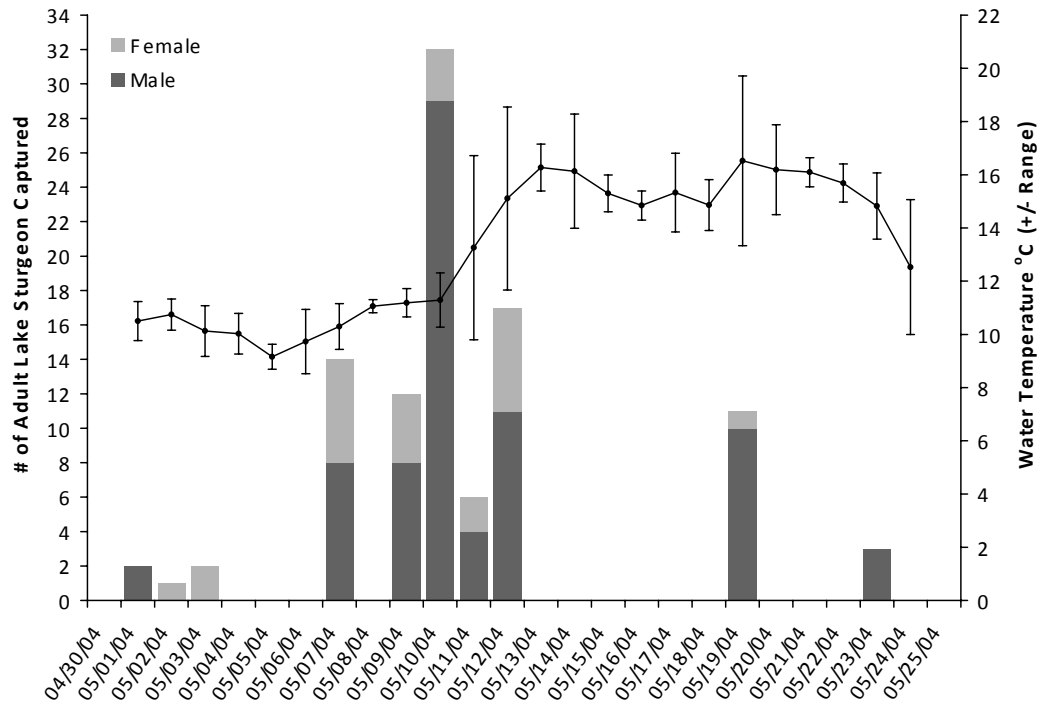


Figure 2.6.–The number of new fish captured each day on the Upper Black River during spawning 2004 in relation to water temperature at Kleber Dam.

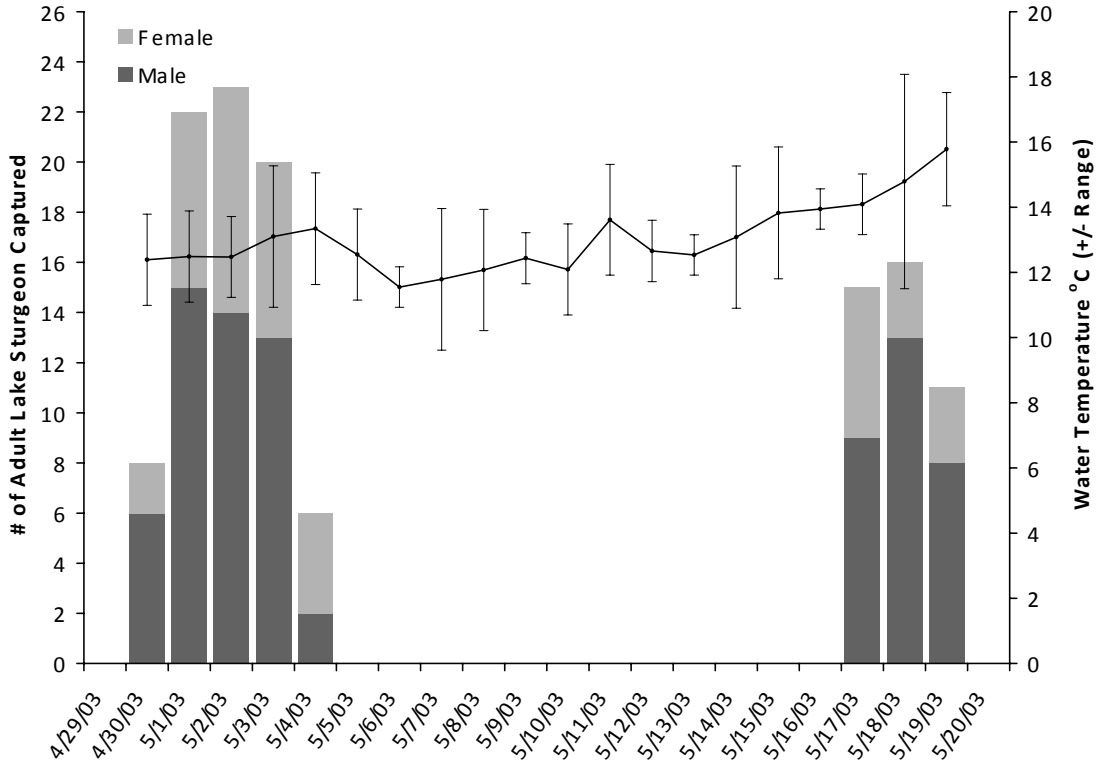


Figure 2.7.—The number of new fish captured each day on the Upper Black River during spawning 2003 in relation to water temperature at Kleber Dam.

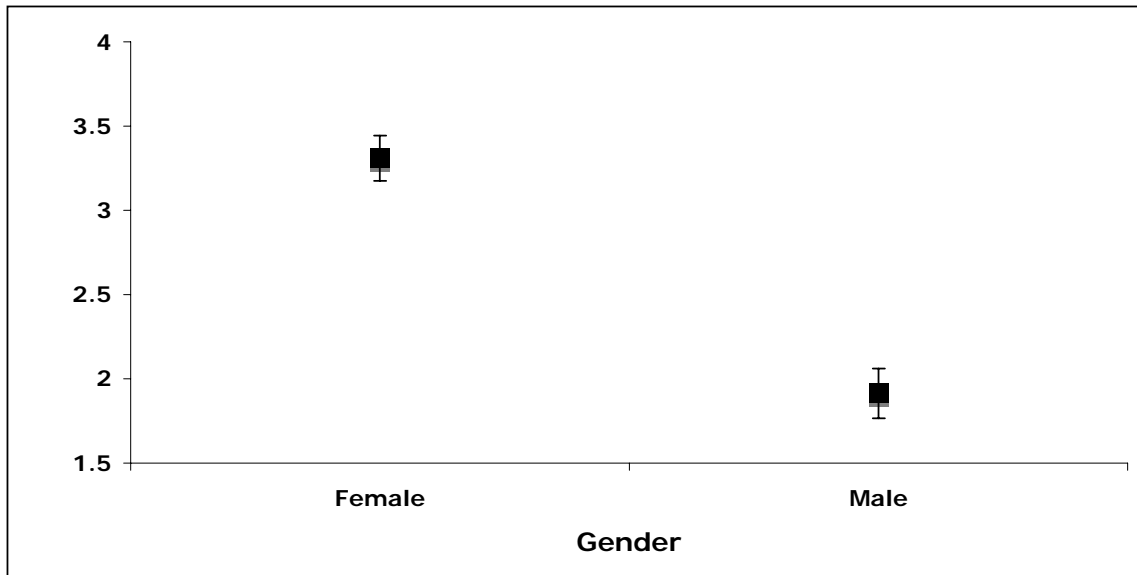


Figure 2.8.—Periodicity estimates for female and male lake sturgeon based upon recapture data from the Upper Black River, MI (2001–07).

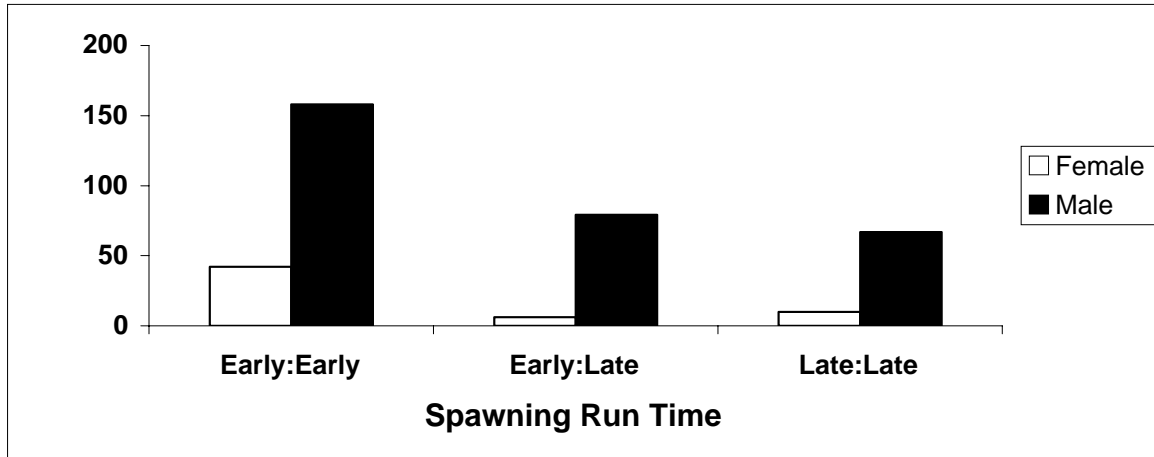


Figure 2.9.—The frequency of recaptured female and male lake sturgeon spawning on a consistent (early:early, late:late) or inconsistent (early:late) reproductive time scale.

Appendix 3

Estimates of Dispersing Lake Sturgeon Larval Abundance, Abundance of Other Larvae Species and Rates of Larval Lake Sturgeon Mortality

Introduction

Lake sturgeon larvae hatch approximately 5–18 days after fertilization depending on the water temperature after egg deposition. After hatching, the larvae remain in the stream substrate until the yolk sack is absorbed. Once exogenous feeding begins the larvae drift downstream to find habitat that is suitable for growth and survival. At this time the larvae are approximately 17.5 to 21 mm in total length and vulnerable to a variety of fish predators (Kempinger 1988, LaHaye et al. 1992). Studies have documented the onset, duration, and periodicity of larval lake sturgeon dispersal from natural spawning areas. Larval sturgeon drift primarily on the stream bottom and in the central portion of the stream channel (Kempinger 1988; LaHaye 1992; D'Amours et al. 2001; Auer and Baker 2002; Smith and King 2005). Nocturnal dispersal may be an anti-predator defense that is common in a variety of fish species and invertebrates. However, there is disagreement regarding degree of synchrony of larval dispersal (Auer and Baker 2002, Smith and King 2005).

Mortality during the larval stage of fishes has been empirically linked to starvation, predation and abiotic mechanisms such as river discharge (Bailey and Houde 1988). It is likely that mortality of larvae during downstream drift is a significant barrier to lake sturgeon recruitment and reproductive success in this species. Studies have demonstrated a marked decline in the numbers of lake sturgeon larvae collected from the most upstream sampling sites compared to downstream locations (D'Amours et al. 2001, Auer and Baker 2002). Declines in larval abundance documented over sequential downstream sampling locations has been attributed to a number of factors. First, downstream sections of rivers are used by larval sturgeon as nursery habitat. Declines in numbers could result from larvae settling between locations. However, attempts to locate individuals have failed (D'Amours et al. 2001). This suggests that the majority of the river is not being used as nursery habitat at least in some systems. Second, predation could also be responsible for the downstream decline in larval sturgeon. Larval lake sturgeon, however, have not been found in the stomachs of predators where directed sampling has taken place in the wild (D'Amours et al. 2001). If predation rates are high and variable across years, it is likely that mortality of larvae during downstream drift is a significant barrier to lake sturgeon recruitment and reproductive success.

Methods

Estimates of Larval Sturgeon Abundance and Other Species in Drift

We used D-frame drift nets 76 cm across the base and 54cm high with a knotless 1,600 μM mesh nylon bag 317.5 cm long with a detachable collection cup on cod-end to sample dispersing larval sturgeon in each of five consecutive years. To standardize our assessment of larval production, 4–5 drift nets were deployed at the same location on the Upper Black River and positioned approximately two meters apart along the center of the stream channel. Larval sampling was conducted for 5 h (1000–0300). Three velocity readings (left, center, right) were taken in front of the drift net openings with a Marsh–McBirney Flo-Mate 2000 (Marsh–McBirney Inc., Fredrick, MD, USA). To calculate discharge each night of sampling, the cross-sectional area of the channel was measured using a standardized transect and the mean velocity was recorded at 1m intervals. We used a formula

developed by Veshchev et al. (1994) for dispersing sturgeon larvae in large rivers to estimate the absolute abundance of larvae collected during 2005 and 2006:

$$P = \frac{(q \times N)}{O} \times K \quad (\text{e.q. 1})$$

where P is the number of larvae passing sampling site, q is the flow volume passing the site ($\text{m}^3 \text{h}^{-1}$), N is the number of larvae collected in the net after 1 h, O is the volume of water sampled ($\text{m}^3 \text{h}^{-1}$) determined by the formula: $V \cdot S$, V is the current velocity in the net, S is the sectional area of the net (m^2), and K is a collection coefficient determined by comparing the difference in velocities in front of the net and beside the net.

The composition of other fish and invertebrate species in drift was also noted during each field season.

Estimated Rates of Larval Sturgeon Mortality

Repeated drift net sampling was used to estimate rates of larval lake sturgeon mortality in 2005 and 2006. Sampling nets were simultaneously deployed at two locations on the Upper Black River for 10 consecutive days during the peak period of larval dispersal. Nets were placed approximately 2 km downstream of the furthest downstream spawning ground and at the traditional larval drift sampling site. At each location, five larval drift nets were deployed two meters apart in a straight line across the center of the stream channel perpendicular to the flow. Stream flow and depth were measured at meter intervals along a standardized transect at each site. We also recorded three measurements of water velocity at each net. Sampling was conducted at night over a 5-h time period, beginning at dusk and ending in the early morning (2100 and 0300 h). We began sampling for newly hatched and out-migrating larval lake sturgeon directly below the spawning grounds approximately 5 to 12 days after spawning activity was observed. All sturgeon larvae collected at this site were counted and released at the end of each sampling hour. The total number of larval lake sturgeon produced was calculated for each site. Estimates were compared to determine rates of larval sturgeon mortality within this 2 km stretch of the UBR.

Estimate the Magnitude of Predation on Larval Sturgeon and Other Species by Fish Predators

The magnitude of predation on larval lake sturgeon and other larval species by fish predators downstream was assessed by passively sampling the stream community using traditional fyke and drift nets and electroshocking. Passive sampling was conducted in 2003 and resulted in small sample sizes which precluded our ability to accurately quantify sources of predation, although we were able to document the presence of abundant stream predators (see findings for Job 2). In 2005, predation on newly hatched and dispersing larval lake sturgeon was assessed by electroshocking the Upper Black River using a barge shocker. Shocking occurred several times during the peak of dispersal at the two sampling locations described above. This method of stream sampling resulted in a much greater sample size ($N > 200$) than the passive sampling technique. Once target species were captured, we examined their stomach contents to quantify the extent of in-stream predation on larval sturgeon and other species. Because sampling was conducted during the peak of evening larval drift, we assumed that larval sturgeon would be distinguishable from other prey that may have been consumed (i.e., full digestion should not have taken place). For the majority of potential predators, stomach contents were examined non-lethally by using gastric lavage. Predators were measured for total length (mm) and weight (0.1 g) before release. Stomach contents were preserved in 95% ethanol. A subsample of

potential predators that could not be examined using gastric lavage was preserved in 95% ethanol and their stomach contents were analyzed in the lab at a later date.

Results

We sampled 31,682 lake sturgeon larvae dispersing from spawning areas on the UBR over the past five years. Substantial inter-annual variation was observed in the total number of larvae captured (e.g., 437 in 2005 and 16,417 in 2003; Figure 3.1). Larval abundance did not correlate with estimates of adult spawning number (see results for Job 3). Temporal estimates of larval dispersal within years revealed two, three or four peaks of abundance during each of the 5-year sampling periods (Figure 3.2), presumably corresponding to the number of temporally distinct adult spawning periods.

The temporal succession of other insect (Ephemeroptera sp., Plecoptera sp.) and larval fish species (*Catostomus* sp., *Moxostoma* sp., *Ichthyomyzon* sp.) co-dispersing with lake sturgeon was consistent between all years of this study as noted in previous studies (D'Amours et al. 2001; Smith and King 2005).

Hourly capture of lake sturgeon at the upstream location showed a 1-h delay in drift abundance relative to what was observed at the downstream sampling site (Figure 3.3). In 2005 and 2006, we estimated a total larval production of 268,338 and 167,927 individuals, respectively. In 2005 and 2006, we estimated a total mortality rate of 0 and 8% based on differences in the absolute number of larvae calculated between the upstream and downstream sampling locations (Figures 3.4 and 3.5). We were unable to detect predation based on stomachs of any predator collected from our stream sampling. Results are significantly downwardly biased because Equation 1 fails to account for inter-location variation in capture probabilities owing to variation in gear efficiency and characteristics of the stream locations sampled.

Discussion

Several studies have demonstrated that dispersal of lake sturgeon larvae is not uniform in time and the longitudinal and cross-sectional space of a river (D'Amours et al. 2001; Smith and King 2005). These studies have also described patterns of lake sturgeon larval dispersal in relation to other co-occurring species in drift. Furthermore, the absolute abundances of larvae in drift have been calculated from currently available techniques based on captures from discrete locations on the Upper Black River. Our long-term evaluation of lake sturgeon larval drift confirms the findings of these studies. First, larvae disperse episodically. Periods of high larval dispersal correspond with previous peaks of spawning activity. Secondly, lake sturgeon larvae exhibit strong diel patterns in drifting activity with peak drift occurring between 11am and 1pm.

We provide quantitative estimates of mortality based on simultaneous sampling of larvae dispersing between two locations in the same system. Using currently available methods, we estimate a larval mortality rate between 0 and 8% over a two kilometer stretch of stream. Mortality could reach as high as 40% if this rate is extrapolated to the entire river. Given our failure to document any larval sturgeon in the stomachs of potential stream predators, we cannot conclude that predation is responsible for a decline in abundance. It is clear from this exercise that new methods for determining the absolute number of larvae in drift need to be developed for site specific locations. Estimators need to incorporate site-specific drifting behavior, the spatial distribution of dispersing larvae and the efficiency of nets placed in different areas of the stream channel. Minor fluctuations in lake sturgeon larval mortality during drift, especially when numbers are low, could cause substantial variation in recruitment.

References

- Auer, N. A., and E. A. Baker. 2002. Duration and drift of larval lake sturgeon in the Sturgeon River, Michigan. *Journal of Applied Ichthyology* 18:557–564.
- Bailey, K. M., and E. D. Houde. 1988. Predation on eggs and larvae of marine fishes and the recruitment problem. *Advances in Marine Biology* 25:1–82.
- D'Amours, J., S. Thibodeau, and R. Fortin. 2001. Comparison of lake sturgeon (*Acipenser fulvescens*), *Stizostedion* spp., *Catostomus* spp., *Moxostoma* spp., quillback (*Carpionodes cyprinus*), and mooneye (*Hiodon tergisus*) larval drift in Des Prairies River, Quebec. *Canadian Journal of Zoology* 79:1472–1489.
- Kempinger, J. J. 1988. Spawning and early life history of lake sturgeon in the Lake Winnebago system, Wisconsin. *American Fisheries Society Symposium* 5:110–122.
- LaHaye, M., A. Branchaud, M. Gendron, R. Verdon, and R. Fortin. 1992. Reproduction, early life history, and characteristics of the spawning grounds of the lake sturgeon (*Acipenser fulvescens*) in Des Prairies and L'Assomption rivers, near Montreal, Quebec. *Canadian Journal of Zoology* 70:1681–1689.
- Smith, K. M., and D. K. King. 2005. Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology* 21:161–168.
- Veshchev, P. V., A. P. Slivka, A. A. Novikova, and K. L. Shekhodanov. 1994. Guidelines for counting sturgeon eggs and migrating larvae in rivers. *Hydrobiological Journal* 30:5-13.

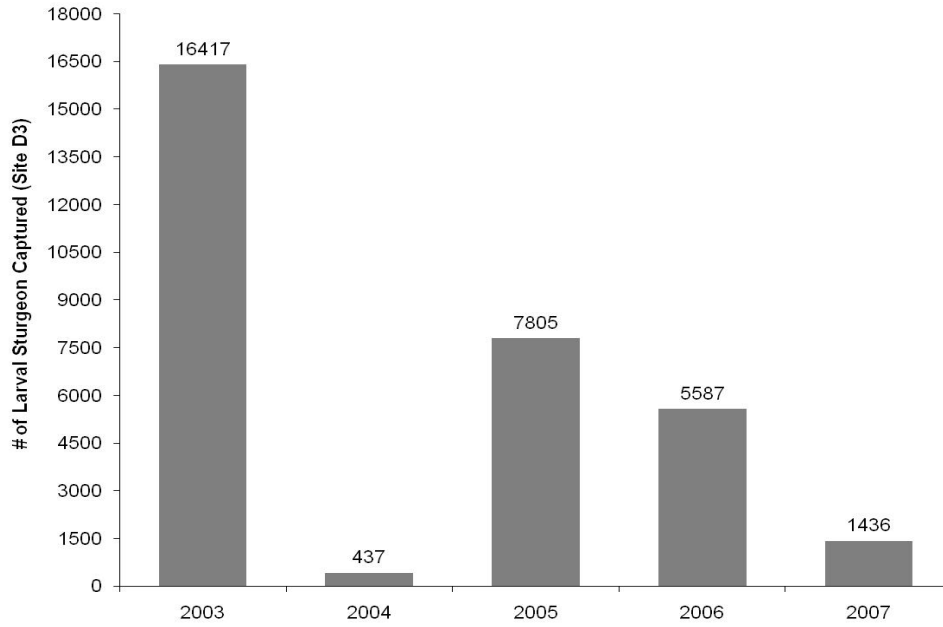


Figure 3.1.—Number of larval sturgeon captured at the downstream sampling location during each of five consecutive years.

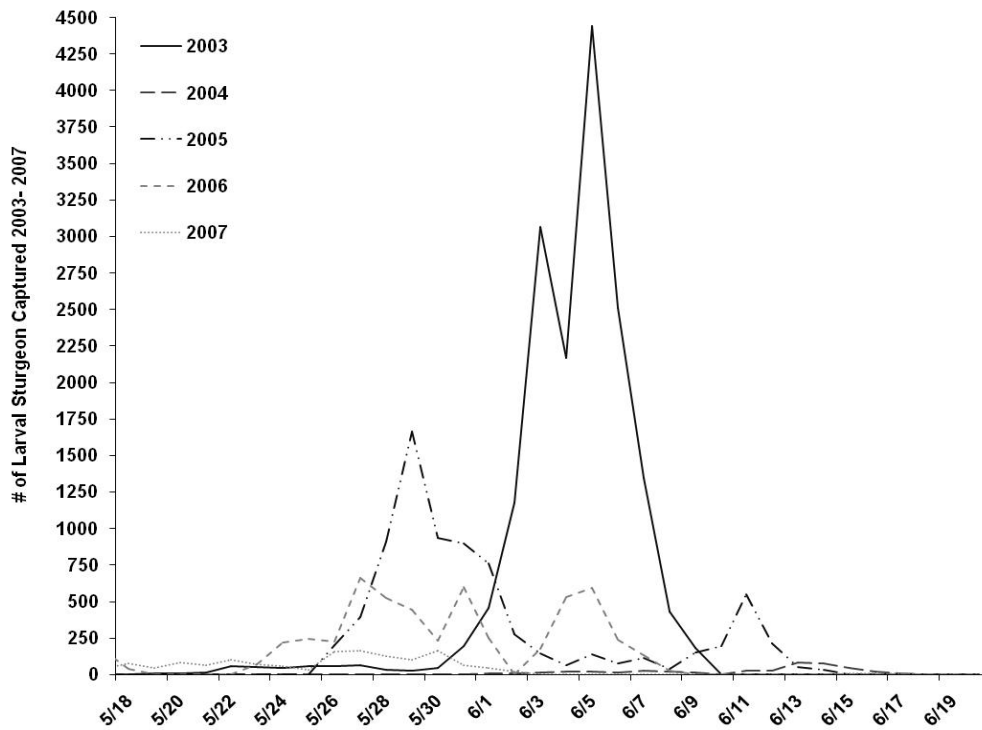


Figure 3.2.—Chronology of larval lake sturgeon dispersal for each of five consecutive years (2003–07).

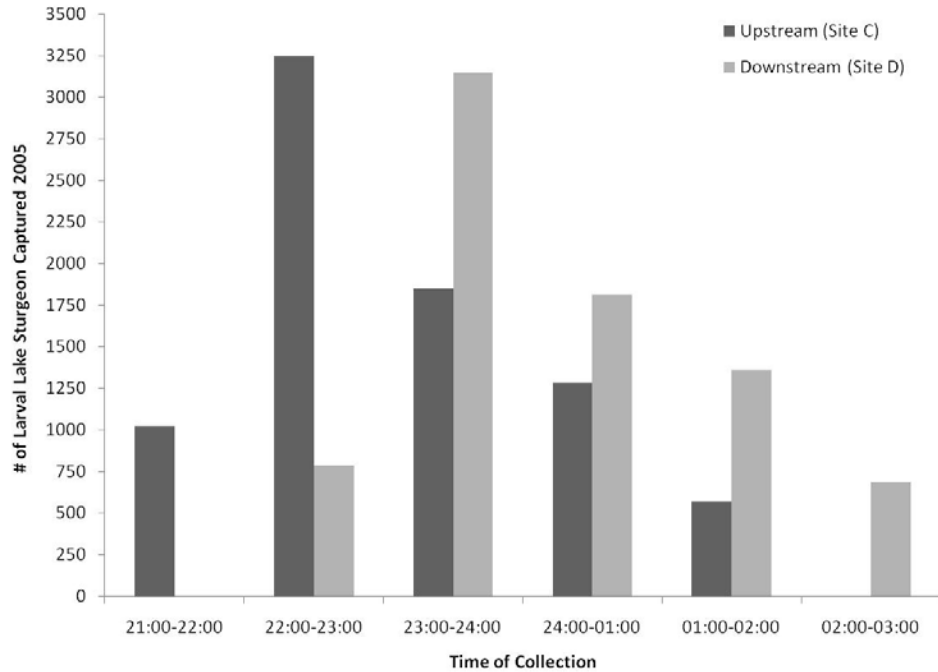


Figure 3.3.—Number of lake sturgeon captured in 2005 during an evening at two sampling locations on the Black River.

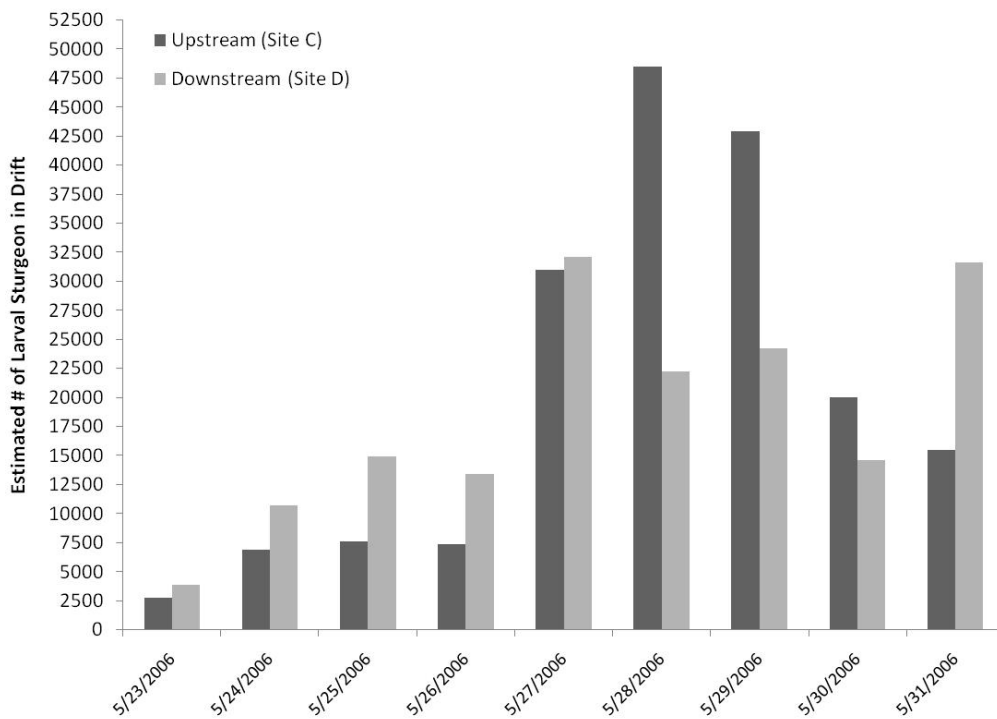


Figure 3.4.—Estimated number of larval lake sturgeon at both upstream and downstream sampling locations in 2006.

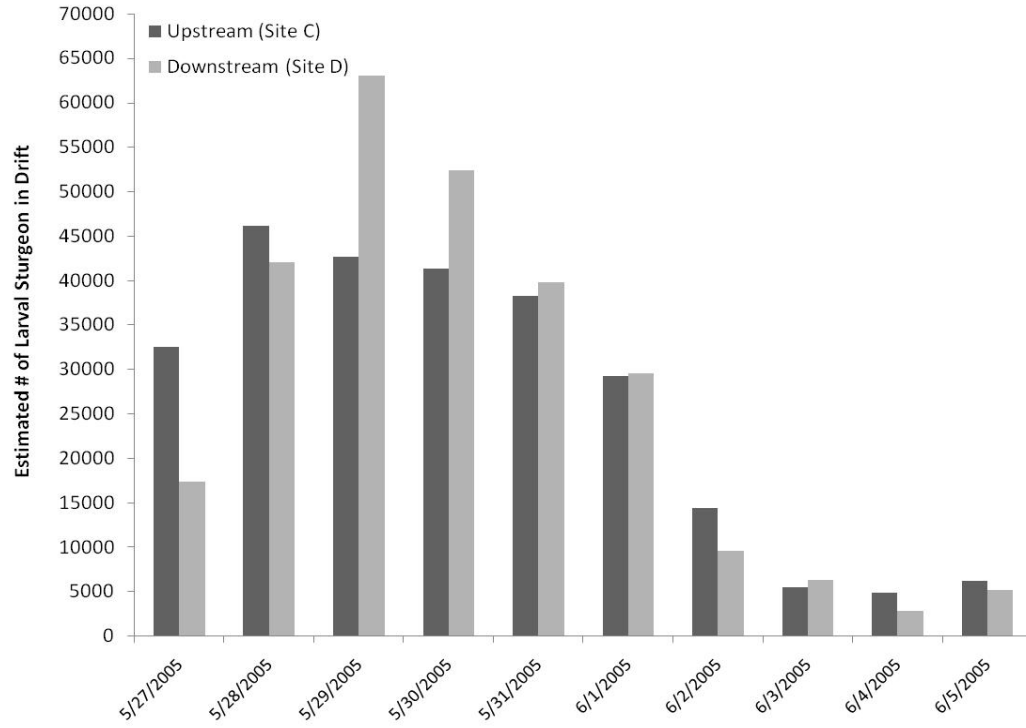


Figure 3.5.—Estimated number of larval lake sturgeon at both upstream and downstream sampling locations in 2005.

Appendix 4

Experimental Releases of Juvenile Lake Sturgeon

Introduction

Stocking programs have been widely used as a resource management tool to reverse declines in wild population abundance and to increase probabilities of survival during critical life history stages (Ross 1997; Alverson 2002). Stocking programs 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 positive and potentially negative impacts to population dynamics, genetic integrity of resident populations, and to ecosystem processes and resource economics (Travis et al. 1998; Utter 1998).

The use of hatchery programs as both an enhancement and management tool for declining wild fish populations has been increasingly used over the last century throughout the world (Waples 1999). More recently, hatchery programs used to produce fish for stocking programs are receiving increased scrutiny regarding the contribution of captive reared individuals to the natural population (Ford 2002; Miller et al. 2004; Araki et al. 2007). This negative attention has promoted calls for empirical evaluations of the relative effects of different hatchery management prescriptions on survival. Factors include hatchery rearing environments (Berejikian et al. 2000), age of release into the natural environment (Paragamian and Kingery 1992), and how gametes, juveniles, or broodstock are collected and maintained (Flagg and Nash 1999). Though both stocking and hatchery programs have typically been used as fisheries management tools, they are becoming increasingly important in the conservation and management 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 extreme sensitivity of adults to anthropogenic factors such as overharvest, degradation in water quality and spawning habitat and loss of connectivity due to impoundment (dams) (Holey et al. 2000). In recent years, lake sturgeon rehabilitation through restocking has become a high priority throughout the Great Lakes (Holey et al. 2000). Despite this, considerable uncertainty remains regarding the effects that straying of stocked fish may have on remnant populations, the efficacy of different egg and larval collection methods, and the appropriate age of fish to stock. Assessment of the success of stocking programs is typically based on rates of recovery of adult fish to the creel. However, these methods are not amenable to lake sturgeon assessments as they are not subject to harvest. Assessment is further complicated because current juvenile collection methods are inefficient and data pertaining to juvenile abundance is not comparable across age classes. Stocking prescriptions have typically been based on a large salmonid literature and species such as lake sturgeon have very different ecologies, mandating that research on alternative methods of culture and release be conducted on them explicitly. The species' unique life history characteristics i.e. late age at maturity, infrequent spawning, and low recruitment rate (Nilo et al. 1997) dictate that other methods be used to evaluate stocking programs.

Stockings of lake sturgeon throughout the Great Lakes region have used a variety of techniques for collecting progeny (larval collection (Auer and Baker 2002), 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 (Elrod et al. 1988; Margenau 1992, Secor and Houde 1998; Amtstaetter and Willox 2004,). Certain studies have indicated that rearing hatchery fish to larger sizes leads to increased survival upon release (Gunn et al. 1987; McKeown et al. 1999; Yule et al. 2000). Schram et al. (1999) stocked lake sturgeon at three different life history stages (fry, fingerlings, yearlings). However, no data were reported on the survival of fish of different ages. More recently there has been considerable interest in the use of streamside hatcheries as a restoration tool for lake sturgeon throughout the Great Lakes (Holtgren et al. 2007). Conservation hatcheries have been used for trout and salmon (Maynard et al. 1996, Berejikian et al. 2000). Improved rearing environments can reduce unnatural selective pressures and increase survival probabilities of fish reintroduced into the natural environment. In this study, we addressed effects of different rearing environments by rearing lake sturgeon in two different hatchery settings to quantify differences in post stocking success.

Post-release survival of hatchery-reared individuals is typically estimated based on increases in spawning stock abundance or creel surveys. Sources of mortality are usually unknown or assumed. Assessments conducted using either method, if conducted after extended periods following release are not capable of documenting sources of mortality or behavioral or phenotypic traits associated with the mortality.

The magnitudes of various sources of sources of mortality 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 2005). Vulnerability to predation is complex and dependent on many factors such as prey and predator morphology, size, behavior, distribution patterns, and environmental conditions (Olla et al. 1998).

Complex rearing environments have been shown to improve post-release survival (Maynard et al. 1996) as well as influence morphological color patterns (Fuji 1993). Furthermore, it has also been found that hatchery fish subjected to chemical and extracted predator odors prior to release exhibit predator avoidance responses (Berejikian et al. 1999; Gazdewich and Chivers 2002), which subsequently increase survival in the wild (Berejikian et al. 1999). Understanding how rearing environments and age at release influence both predation risk and predator avoidance behavior prior to release into the natural environment can be critical for the success of lake sturgeon restoration programs.

We tested two main hypotheses. First, rearing environment and age at release are not significant predictors of post-stocking survival for juvenile lake sturgeon. Secondly, rearing environment and age do not influence predation risk or anti-predator behavior in 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 criterion. Research objectives were to, 1) determine contributions of fish stocked from two different rearing environments and collection methods on the recapture rate using instream assessment, 2) determine which stocking age results in higher rates of recapture which we assumed was a surrogate measure of survival; 3) quantify stream retention time (rate of downstream dispersal). The magnitude of predator-induced mortality is currently unknown for juvenile lake sturgeon. Accordingly, using controlled experiments, we determined 1) how habitat use by juvenile lake sturgeon of different age classes and body sizes varied with and without the presence of a predator; and 2) the effects of age and body size on levels of predation by different predator species. Results provide evidence of how lake sturgeon rearing conditions, collection methods, and size and age at stocking contribute to the variance in probability of survival.

Materials and Methods

Rearing Environment

Juvenile lake sturgeon were reared in two hatchery environments. Half of all fish were reared at a streamside hatchery in water from the natal stream (the 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. Cleaning, feeding, and lighting protocols were consistent between the two hatchery environments. Fish in both hatchery environments 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. Methods included directed egg takes from spawning females, and collection of recently hatched larvae dispersing downstream of the spawning grounds. Both methods are commonly used for many sturgeon species (Birstein 1993; Auer and Baker 2002). The third method involved collecting eggs naturally fertilized and deposited on the stream substrate by spawning adults. More detailed descriptions of the three collection methods are as follows:

- 1) *Direct take spawning*: The hydrology of the Upper Black River provides a unique opportunity to enumerate a large proportion of the annual adult spawning run. 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 and maintained at ambient temperatures. Milt was collected using a 30ml syringe and was immediately placed on ice. Fertilizations were conducted within 12 h of egg collection. Egg volumes from each female were measured, divided into multiple lots, and fertilized with milt from two randomly selected males to create half-sib family groups. Half of the eggs were transported to the traditional hatchery rearing environment. Eggs were incubated and hatched in heath trays at both rearing environments.
- 2) *Drift Larvae*: Drift 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). Sampling was conducted during a 5-h period, beginning at dusk (21:00) and ending in the early morning (03:00) at a traditional sampling location on the Upper Black River (Figure 4.1). Five D-framed drift nets were set across the stream channel to capture dispersing larval sturgeon. This design was replicated from the start of the drifting period to the end. Nets were checked hourly and the larvae within each net were enumerated. Nightly captures of larval lake sturgeon were reared at the streamside hatchery until the end of the drifting period. The larvae were then divided between the two hatchery environments.
- 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.

Stocking and Assessment

Three age classes were stocked into the Upper Black River (Figure 4.1). 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 visible 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. All age classes were acclimated to ambient stream conditions within the streamside hatchery for >12 h and released simultaneously at the same location on the Upper Black River (release site; Figure 4.1).

The release location was not a stream region where juvenile sturgeon would naturally be present at times when the three age classes were released. Sampling protocols provided quantifiable and comparable estimates of survival that are currently lacking for age-0 lake sturgeon (Holey et al. 2000), despite recent advances in visual surveys (Benson et al. 2005). Prior to release, four capture locations were established downstream of the release site. Within each site four D-framed drift nets were deployed across the stream at equal intervals. Net position was recorded with a handheld 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 was conducted for approximately 24 h following release. The most upstream assessment site was discontinued for all releases after no juvenile sturgeon were captured in consecutive hours. Each captured individual was checked for specific elastomer marks indicating collection method and rearing location. We also measured total length (cm). Fish were then released immediately downstream of the drift nets.

Block nets were constructed and deployed downstream of the third assessment site for both the 13- and 17-week release ages. During the 17-week release a second block net was deployed below the fourth downstream site. The block nets consisted of 0.32cm² mesh leaders and large mesh (0.32cm²) 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 serve as a second gear type to assess downstream dispersal. Block nets were not installed during the 8-week release due to high water levels. Sampling protocols for the block nets were consistent with those for the drift nets.

A DC tow-barge shocker was used to assess sources of mortality for released juvenile lake sturgeon. Shocking was conducted 1 h following release. We ran single passes starting at the release location and worked 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). Analyses only included data from individuals captured using drift nets. 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. To account for differences in numbers of fish released from the two rearing environments all recaptured proportion values were weighted by the total number released from each collection method 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 the assessment site of capture, the collection method, the rearing environment, net location, physical stream variables (depth and flow), and the age of release. Time of capture was included as a random variable in the model. Stream depth and flow as

well as net position were removed from the final model due to insignificance. We examined the interactive effects between 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 summing the total amount of fish recaptured in a given time period by the total amount of fish recaptured by the block net within that same time period.

Variation in total length of recaptured juvenile lake sturgeon was analyzed using a general linear mixed effects model. 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 was a significant difference between ages in terms of total length at the time of release.

Experimental Predation Studies

We conducted experimental predation studies in the summer of 2006 to compliment the stream assessment work and to further investigate sources of mortality as a function of juvenile age. We quantified direct effects of different stream predators as sources of mortality and indirect effects associated with behavioral changes in habitat use. Experiments were motivated by preliminary results and visual observations from the first two stocking ages and because we were unable to quantify sources of juvenile mortality following releases in the stream. Extending field based observations with experimentally and hypothesis driven research in the laboratory setting is critical in determining mechanisms behind ecological phenomenon. We replicated across all ages and hatchery environments used in the stocking studies in the predation trials. The overall goals of these experiments were to determine the influence of predator presence on juvenile lake sturgeon habitat choice and to determine rates of predation for a number of different predators.

Predator collection and maintenance.—Adult rock bass *Ambloplites rupestris*, smallmouth bass *Micropterus dolomieu*, northern pike *Esox lucius*, and the rusty crayfish *Orconectes rusticus* were used as predators in experimental trials. Predators were collected from the Upper Black River using fyke nets, backpack electrofishing, and hook and line fishing. Species were held separately in indoor, circular fiberglass tanks (0.61m diameter 0.7m deep). All indoor tanks were supplied with flow-through river water. Tanks had overhead lighting that mimicked a natural photoperiod. Predators were collected a maximum of 4 days prior to the start of each experimental trial and were not fed during captivity. Total length (T_L ; cm; mean \pm 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 ± 0.12 . 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 \pm 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 performed in a circular (2.44m diameter, 0.6m deep) fiberglass tank. The tank was divided into two equally sized sections allowing 2 replicates to be run simultaneously. Each section included three substrate types (sand, small gravel, and large rocks) that were divided into equal contiguous sections within tank partitions. Substrate types were removed and randomly redistributed prior to the beginning of each trial. Trials were conducted using 30 fish for a duration of 24 h beginning at 0730. We conducted trials for age classes 8–9, 11–13, and 15–16 weeks corresponding to stream release

ages. 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 locations were marked with elastomer following methods described above in the stocking strategies. All fish were measured for total length before introduction into the tank.

Experiment 1: The effect of predation risk on lake sturgeon distribution.—This experiment was conducted to test two hypotheses: 1) The presence of predators does not significantly affect the distribution of juvenile lake sturgeon among habitats, and 2) There is no effect 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. 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 h. At that time a new set of sturgeon were introduced into the tank with the predator. The same sets of fish were never used twice for any trial to remove effects of learned behavior towards predators or substrate types. The same sets of observations were taken with the addition of including predator location. The predator was removed immediately after the trial.

We used a multifactor 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 prior to further analysis. 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 analyzed again including 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: Size-dependant predation rates.—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. Trials were run separately using fish from each hatchery environment. We also conducted trials using equal proportions from each hatchery environment marked uniquely with elastomer. Furthermore, we added a genealogical component to the study by using juveniles (N=5) from each of six full-sib families. Family groups were uniquely marked with elastomer. All fish were measured for total length prior to the trial.

We used a general linear model to analyze predation rates. Survival was predicted using several independent variables including: age class, predator species, rearing environment, and family. Survival was square-root transformed to achieve normality. To determine if there was size selective predation with each age class, we used a chi-square test to identify differences in the distribution of lengths at the start and termination of each trial.

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 (N=15; *Notropis atherinoides*) were introduced into a tank containing an acclimated predator [either smallmouth bass (n=1 per trial) or crayfish (n=4 per trial)]. The effects of family and rearing environment were not evaluated in alternate species trials. Total length was estimated for sturgeon and minnows before introduction. Data describing habitat use and mortality were recorded hourly until nightfall and then again the following morning. One-way ANOVA analyses were conducted

separately for each predator species. The proportion of lake sturgeon surviving in each trial was compared between treatments with and without alternate prey species.

Results

Stocking Studies

Based on total numbers of juvenile lake released across each collection method, hatchery rearing environment, and age class (Table 4.1) 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 (Figure 4.2). 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 (Figure 4.2). 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 (Figure 4.2). We recaptured significantly more fish at downstream assessment 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 (Figure 4.2). 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 (Figure 4.3). At 17 weeks of age we captured very few fish during the daylight hours immediately following the release (Figure 4.3). A large proportion of the total captures occurred during nighttime hours >8 h following the release (Figure 4.3). We observed no main effect of treatment group on recapture success among release ages. Distinct differences among treatment groups occurred within sites at distinct time periods but no overall trends emerged from the analysis.

Documenting differences in length within and among release ages is important for determining size based downstream dispersal and as a mechanism driving differential survival. There were no significant differences between rearing environments in the total length of fish prior to release for the 8- and 13-week release ages (Table 4.1). 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 4.1). 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 (Figure 4.4). 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 (Figure 4.4).

The block net implemented 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. Water velocity and depth at this site (mean \pm SE) were 0.59 ± 0.02 m/s and 0.77 ± 0.09 m respectively. There were significantly more fish moving downstream of this assessment site that were from the streamside hatchery environment ($F_{1,27} = 3.04$, $P < 0.01$). Efficiencies of the drift nets during the 13-week release were as high as 19.8% and then decreased throughout the remaining assessment period to a minimum of 5.9%. 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. Water velocity and depth at this site (mean \pm SE) were 0.51 ± 0.003 m/s and 0.66 ± 0.01 m

respectively. Drift net efficiency ranged from 1.9 to 31.4% throughout the sampling period. 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. Water velocity and depth at this site (mean \pm SE) were $0.37 \pm 0.01\text{m/s}$ and $0.91 \pm 0.01\text{m}$ respectively. Drift net efficiency ranged from 4.0% to 15.3% throughout the sampling period. There was no effect of rearing environment on the proportion of fish recaptured. Again, we caught significantly more fish at later sampling hours at this downstream site ($F_{6,8} = 11.82, P < 0.01$).

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 ($F_{7,621} = 23.97, P < 0.001$).

We were unable to quantify sources of mortality (predators) following release using the tow barge shocker. During the release of 8-week juveniles, two rock bass had 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. Significant main effects of hatchery rearing environment during the 8- and 13-week ages combined with difficulties in quantifying predation in the stream motivated experimental research to extend our field observations.

Experimental Predation Studies

Experiment 1: The effect of predation risk on lake sturgeon distribution.—Studies have documented that juvenile lake sturgeon show strong preference for sand when provided with a choice of substrate types (Peake 1999). Using 4 habitat classifications we documented a shift in habitat selection of juvenile lake sturgeon in the presence of two different predator classes. Juvenile lake sturgeon exposed to three different fish predators used sandy substrate at a significantly higher proportion ($F_{59,1220} = 172.6, P = 0.005$) when compared to gravel, larger rocks, and the water column (Figure 4.5). 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 ($F_{59,1220} = 172.6, P = 0.016$; Figure 4.5). We did not find any significant differences attributed to predator type or the main effect of hatchery rearing environment in this analysis. The fish from age class 8–9 weeks spent significantly less time ($13 \pm 4\%$; $F_{59,1220} = 172.6, P = 0.006$) 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.

We documented a habitat shift when crayfish were used as the predator species. Preference for the water column increased significantly ($F_{55,584} = 65.39, P < 0.001$; Figure 4.5) over time following predator introductions, while preference for the sand decreased ($F_{55,584} = 65.39, P < 0.001$; Figure 4.5). In the absence of crayfish the preference for sand was high and increased significantly over time following being introduced ($F_{55,584} = 65.39, P = 0.004$; Figure 4.5). There was no main effect of hatchery environment or age on fish distributions with and without predator presence.

Experiment 2: Size-dependant predation rates.—Survival rates across all age classes of sturgeon were high for all fish predators evaluations. Survival rates ranged from 0.974–1 at 8–9 weeks of age, 0.985–1 at 11–13 weeks of age, and 1.00 for 15–16 weeks of age. Survival was significantly less in trials conducted with 8–9 week old juvenile lake sturgeon ($F_{15,224} = 3.48, P < 0.001$) compared to the two other age classes. 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 grouping was not a significant cause of mortality due to overall low predation rates however it should be noted that in three trials where only 1 fish was consumed the family group was consistent in each one. The probability of this occurring by random chance is 3.7×10^{-5} . The total length of eaten individuals was not significantly different from the surviving fish across all ages.

Survival rates were significantly less for juvenile lake sturgeon exposed to crayfish ($F_{1,286} = 1,393.6$, $P < 0.001$). There was a significant effect of time on juvenile sturgeon survival with a higher proportion of mortality occurring at hours immediately following release ($F_{11,36} = 3.94$, $P < 0.001$). 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. Unfortunately we did not have samples from the 8-9 weeks of age class for the crayfish trials. 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 grouping or size across different ages or trials.

Experiment 3: Alternate species trials.—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 24-h period. Survival of juvenile lake sturgeon was 1.00 in all trials and average survival of minnows was 0.68 ± 0.22 . We found the opposite result with crayfish as the predator species. Significantly more juvenile sturgeon ($F_{1,44} = 457.8$, $P = <0.01$) were consumed during the 24-h period with survival rates being 0.67 ± 0.08 for sturgeon and 1.00 for minnows.

Discussion

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. Survival appeared to increase with increasing age. We demonstrated an effect of 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 deviations between rearing environments can play an important role in post release behavior and survival. Major differences between the streamside hatchery and the traditional hatchery in this study were more sensory than physical as tank size and shape was consistent between the two environments. Juvenile lake sturgeon reared in the streamside hatchery benefited from fluctuations in temperature mimicking that of the natural environment. Mechanical filtration within the streamside hatchery did not remove small particles or organisms from the incoming water and the fish were exposed to stream organics and biological organisms. Furthermore, the water at the streamside hatchery likely contained natural stream pheromones or chemical cues from either conspecifics, predators, or other species.

Significant differences in recapture success between juveniles reared in different environments identifies a need to tailor culture methods to maximize probabilities of survival through important life-history transitions when dealing with threatened or endangered species. Modifications to juvenile rearing environments to approximate natural conditions is increasingly used to minimize the degree of domestication for captive animals (Flagg and Nash 1999). Studies documenting differences between fish reared in different environments have ranged from issues surrounding 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 a lower probability of carrying out basic survival skills, such as feeding and predator avoidance

Estimates of capture success and stream movements at each release age are robust but results across age classes should be interpreted with caution. Catchability has been shown to increase with increases in the total length 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). We can however, examine results from within each stocking age. The use of the block nets as a means of assessing the drift net efficiency and as second gear type was important. The fact that the results were consistent between the two gear types was encouraging because drift net efficiency was low. Large numbers of juvenile lake sturgeon were released at each age, allowing tests for significant differences attributed to age and hatchery environment. 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. Fish released at 17 weeks dispersed downstream after sunset. This negative photo-tactic or nocturnal behavior had been noted with lake sturgeon larvae (Auer and Baker 2002) however this is the first documentation of the same migration patterns in juveniles. It has been shown that nocturnal behavior of other sturgeon species is a dominant feature of migration and foraging in the first year of life (Kynard et al. 2005). This may be an important result for developing release strategies for this species. Night releases would encourage rapid dispersal and could reduce mortality by visual predators during the period immediately following release.

In all release ages we found that larger fish moved downstream at significantly later times following release than did smaller fish. Variability in juvenile proportions recaptured was high during the 8 and 13 week releases, because most individuals appeared unable to control downstream dispersal. Variability in sizes of fish dispersing downstream decreased over time during each release. We found the opposite trend at 17 weeks of age. Variability increased at later time periods following release, indicating that most juveniles had an ability to choose dispersal time. Using the stream as an experimental tool was positive for attempting to provide quantified estimates of survival and downstream movement. Based on our data, release sites for lake sturgeon should include habitats that are critically needed for foraging and refuge. From our predation work and other studies (Peake 1999), release sites should include sandy substrates with moderate flow.

Results from our predation experiments represent the first replicated data on predation rates and avoidance for juvenile lake sturgeon, or any sturgeon species. The most encouraging result from this work was anti-predator behavior of the lake sturgeon. The significant shift in the distribution with and without the presence of a predator suggests that even after lengthy periods of captivity, predator avoidance was still observed. Predator avoidance response was most pronounced in the presence of crayfish. Studies have indicated that fish learn predator avoidance behaviors rapidly after witnessing only a few attacks on conspecifics (Berejikian et al. 1999). Crayfish predation rates were much higher relative to the visual fish predators, which might explain why the distribution shift was much more dramatic in the crayfish trials. We attempted to examine differences between hatchery rearing environments; however, there were no significant results. This is likely attributed to the fact that the experiments were done 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. This would have allowed them time to acclimate to the environment. Furthermore, if incoming water contains chemical odorants or cues from predator species, 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 behavioral type of captive animals, simple pre-release environmental enrichment may be successful in facilitating the expression of differential behavioral types in populations slated for release (Watters and Meehan 2007).

Overall rates of predation on juvenile lake sturgeon by the visual predators were low. One main reason for including the trials run with an alternate prey species was to determine if the hatchery environment, or tank, had any effect on the feeding behavior of the predator species. We had

significantly more minnows consumed in these trials. Observations of the visual fish predators revealed unsuccessful attacks on juvenile lake sturgeon at all ages (J. Crossman, Michigan State University, personal communication). The opposite trend in the crayfish trials was not a surprising result. The crayfish were very efficient at capturing juvenile lake sturgeon at all ages. Predation rates would have been much higher if more than four crayfish had of been included in the trials. Our observations were that each crayfish immediately captured a sturgeon but it took most of the trials time period to consume it due to the size of the sturgeon relative to the crayfish. 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 predation over prolonged periods. The rusty crayfish used in our trials are an invasive species 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. Release locations for lake sturgeon restoration programs should be assessed for crayfish densities prior to stocking.

In conclusion, our study addresses the need for further empirical and experimentally rigorous evaluations of both hatchery and stocking programs for lake sturgeon. Streamside rearing may be advantageous to small and young lake sturgeon by exposing them to an enriched environment prior to release. Predation by crayfish may be a significant source of mortality for juvenile lake sturgeon up to three months of age.

References

- Alverson, D. L. 2002. Factors influencing the scope and quality of science and management decisions (The good, the bad and the ugly). *Fish and Fisheries* 3:3–19.
- Amtstaetter, F., and C. C. Willox. 2004. Survival and growth of lake whitefish from two stocking strategies in Lake Simcoe, Ontario. *North American Journal of Fisheries Management* 24:1214–1220.
- Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* 318:100–103.
- Auer, N. A., and E. A. Baker. 2002. Duration and drift of larval lake sturgeon in the Sturgeon River, Michigan. *Journal of Applied Ichthyology* 18:557–564.
- Benson, A.C., Sutton, T.M., Elliott, R.F. and Meronek, T.G. 2005. Seasonal Movement Patterns and Habitat Preferences of Age-0 Lake Sturgeon in the Lower Peshtigo River, Wisconsin. *Transactions of the American Fisheries Society* 134(5): 1400-1409.
- Berejikian, B. A., R. J. F. Smith, E. P. Tezak, S. L. Schroder, and C. M. Knudsen. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of Chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences* 56:830–838.
- Berejikian, B. A., E. P. Tezak, T. A. Flagg, A. L. LaRae, E. Kummerow, and C. V. W. Mahnken. 2000. Social dominance, growth, and habitat use of age-0 steelhead *Oncorhynchus mykiss*. grown in enriched and conventional hatchery environments. *Canadian Journal of Fisheries and Aquatic Sciences* 57:1000–1008.
- Birstein, V.J. 1993. Sturgeons and Paddlefishes: Threatened Fishes in Need of Conservation. *Conservation Biology* 7(4): 773-787.
- Borgstroem, R., and O. Skaala. 1993. Size-dependent catchability of brown trout and Atlantic salmon parr by electrofishing in a low conductivity stream. *Nordic Journal of Freshwater Research* 68:14–20.
- Brown, C., and R. L. Day. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. *Fish and Fisheries* 3:79–94.
- Elrod, J. H., D. E. Ostergaard, and C. P. Schneider. 1988. Comparisons of hatchery-reared lake trout stocked as fall fingerlings and as spring yearlings in Lake Ontario. *North American Journal of Fisheries Management* 8:455–462.
- Flagg, T. A., and C. E. Nash. 1999. A conceptual framework for conservation hatchery strategies for pacific salmonids. NOAA Technical Memorandum NMFS-NWFSC-38.
- Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology* 16:815–825.
- Fuji, R. 1993. Coloration and chromatophores. Pages 535–562 in D.H. Evans, editor. *The physiology of fishes*. Academic Press, New York.
- Gadomski, D. M., and M. J. Parsley. 2005. Effects of turbidity, light level, and cover on predation of white sturgeon larvae by prickly sculpins. *Transactions of the American Fisheries Society* 134:369–374.

- Gazdewich, K. J., and Chivers, D. P. 2002. Acquired predator recognition by fathead minnows: influence of habitat characteristics on survival. *Journal of Chemical Ecology* 28:439–445.
- Gunn, J. M., M. J. McMurtry, J. N. Bowlby, J. M. Casselman, and V. A. Liimatainen. 1987. Survival and growth of stocked lake trout in relation to body size, stocking season, lake acidity, and biomass of competitors. *Transactions of the American Fisheries Society* 116:618–627.
- Hay-Chmielewski, E. M., and G. Whelan, editors. 1997. Lake sturgeon rehabilitation strategy. Michigan Department of Natural Resources, Fisheries Special Report 18, Lansing.
- Holey, M. E., E. A. Baker, T. F. Thuemler, and R. F. Elliott. 2000. Research and assessment needs to restore lake sturgeon in the Great Lakes. Great Lakes Fishery Trust, Workshop Results, Muskegon, Michigan.
- Holtgren, J. M., S. A. Ogren, A. J. Paquet, and S. Fajfer. 2007. Design of a portable streamside rearing facility for lake sturgeon. *North American Journal of Aquaculture* 69:317–323.
- Kynard, B., E. Parker, and T. Parker. 2005. Behavior of early life intervals of Klamath River green sturgeon, *Acipenser medirostris*, with a note on body color. *Environmental Biology of Fishes* 72:85–97.
- Leber, K. M., R. N. Cantrell P. S., and Leung. 1995. Optimizing cost-effectiveness of size at release in stock enhancement programs. *North American Journal of Fisheries Management* 25:1596–1608.
- Mäntyniemi, S., A. Romakkaniemi, and E. Arjas. 2005. Bayesian removal estimation of a population size under unequal catchability. *Canadian Journal of Fisheries and Aquatic Sciences*, 62:291–300.
- Margenau, T. L. 1992. Survival and cost-effectiveness of stocked fall fingerling and spring yearling muskellunge in Wisconsin. *North American Journal of Fisheries Management* 12:484–493.
- Maynard, D. J., T. A. Flagg, C. V. W. Mahnken, and S. L. Schroder. 1996. Natural rearing technologies for increasing post release survival of hatchery-reared salmon. *Bulletin of the National Research Institute of Aquaculture* 2:71–77.
- McKeown, P. E., J. L. Forney, and S. R. Mooradian. 1999. Effects of stocking size and rearing method on muskellunge survival in Chautauqua Lake, New York. *North American Journal of Fisheries Management* 19:249–257.
- Mesick, C. E. 1988. Effects of food and cover on numbers of Apache and brown trout establishing residence in artificial stream channels. *Transactions of the American Fisheries Society* 117:421–431.
- Miller, A. I., and L. G. Beckman. 1996. First record of predation on white sturgeon eggs by sympatric fishes. *Transactions of the American Fisheries Society* 125:338–340.
- Miller, L. M., T. Close, and A. R. Kapuscinski. 2004. Lower fitness of hatchery and hybrid rainbow trout compared to naturalized populations in Lake Superior tributaries. *Molecular Ecology* 13:3379–3388.
- Myers, R. A., S. A. Levin, R. Lande, F. C. James, W. W. Murdoch, and R. T. Paine. 2004. Hatcheries and endangered salmon. *Science* 303:1980.

- Nilo, P., P. Dumont, and R. Fortin. 1997. Climatic and hydrological determinants of year-class strength of St. Lawrence River lake sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences* 54:774–780.
- Olla, B. L., M. W. Davis, and C. H. Ryer. 1998. Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bulletin of Marine Science* 62:531–550.
- Paragamian, V. L., and R. Kingery. 1992. A comparison of walleye fry and fingerling stockings in Three Rivers in Iowa. *North American Journal of Fisheries Management* 12:313–320.
- Peake, S. 1999. Substrate Preferences of Juvenile Hatchery-reared Lake Sturgeon, *Acipenser fulvescens*. *Environmental Biology of Fishes* 56(4): 367-374.
- R Development Core Team. 2005. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Ross, M. R. 1997. Fisheries conservation and management. Prentice Hall, Upper Saddle River, New Jersey.
- Schram, S. T., J. Lindgren, and L. M. Evrard. 1999. Reintroduction of lake sturgeon in the St. Louis River, Western Lake Superior. *North American Journal of Fisheries Management* 19:815–823.
- Secor, D. H., and E. D. Houde. 1998. Use of larval stocking in restoration of Chesapeake Bay striped bass. *ICES Journal of Marine Science* 55:228–239.
- Secor, D. H., V. Arevyev, A. Nikolaev, and A. Sharov. 2000. Restoration of sturgeons: Lessons from the Caspian Sea sturgeon ranching program. *Fish and Fisheries* 1:215–230.
- Smith, K.M., and King, D.M. 2005. Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology* 21(3): 161–168.
- Travis, J., F. C. Coleman, C. B. Grimes, D. Conover, T. M. Bert, and M. Tringali. 1998. Critically assessing stock enhancement: An introduction to the Mote Symposium. *Bulletin of Marine Science* 62:305–311.
- Utter, F. 1998. Genetic problems of hatchery-reared progeny released into the wild, and how to deal with them. *Bulletin of Marine Science* 62:623–640.
- Waples, R.S. 1999. Dispelling Some Myths about Hatcheries. *Fisheries* 24(2): 12-21.
- Watters, J. V., and C. L. Meehan. 2007. Different strokes: Can managing behavioral types increase post-release success? *Applied Animal Behaviour Science* 102:364–379.
- Wiley, R. W., R. A. Whaley, J. B. Satake, and M. Fowden. 1993. An evaluation of the potential for training trout in hatcheries to increase post-stocking survival in streams. *North American Journal of Fisheries Management* 13:171-177.

Yule, D. L., R. A. Whaley, P. H. Mavrakis, D. D. Miller, and S. A. Flickinger. 2000. Use of strain, season of stocking, and size at stocking to improve fisheries for rainbow trout in reservoirs with walleyes. *North American Journal of Fisheries Management* 20:10–18.

Table 4.1.—Stocking numbers and total length averages corresponding to age class, rearing environment, and treatment group for three experimental releases of juvenile lake sturgeon into the Upper Black River.

Age	Rearing location	Collection method	Total released	Proportion recaptured	Total length (cm \pm 1SE)	
8	streamside	artificial ^a	1,257	0.09	7.87 \pm 1.06	
		drift ^b	485	0.05	7.51 \pm 0.82	
		natural ^c	220	0.07	7.68 \pm 1.11	
	traditional	artificial	1,380	0.03	7.38 \pm 0.99	
		drift	725	0.04	7.42 \pm 0.73	
		natural	240	0.02	7.77 \pm 1.22	
		Total	4,307	0.05		
	13	streamside	artificial	999	0.16	10.84 \pm 0.08
			drift	494	0.06	11.70 \pm 0.20
natural			181	0.13	11.98 \pm 0.17	
traditional		artificial	623	0.10	11.20 \pm 0.20	
		drift	692	0.04	11.70 \pm 0.14	
		natural	237	0.10	12.90 \pm 0.21	
		Total	3,226	0.10		
17		streamside	artificial	1,000	0.16	13.85 \pm 0.15
			drift	1,088	0.08	14.81 \pm 0.20
	natural		399	0.26	14.49 \pm 0.15	
	traditional	artificial	1,000	0.17	16.51 \pm 0.19	
		drift	493	0.05	17.19 \pm 0.30	
		natural	208	0.27	15.80 \pm 0.34	
		Total	4,188	0.15		
	Total			11,721	0.10	

^a Artificial: Half-sib families, each created with one female and two males.

^b Drift: Larvae captured dispersing downstream from spawning areas.

^c Natural: Naturally fertilized and deposited eggs that were collected from the stream and incubated in the hatchery.

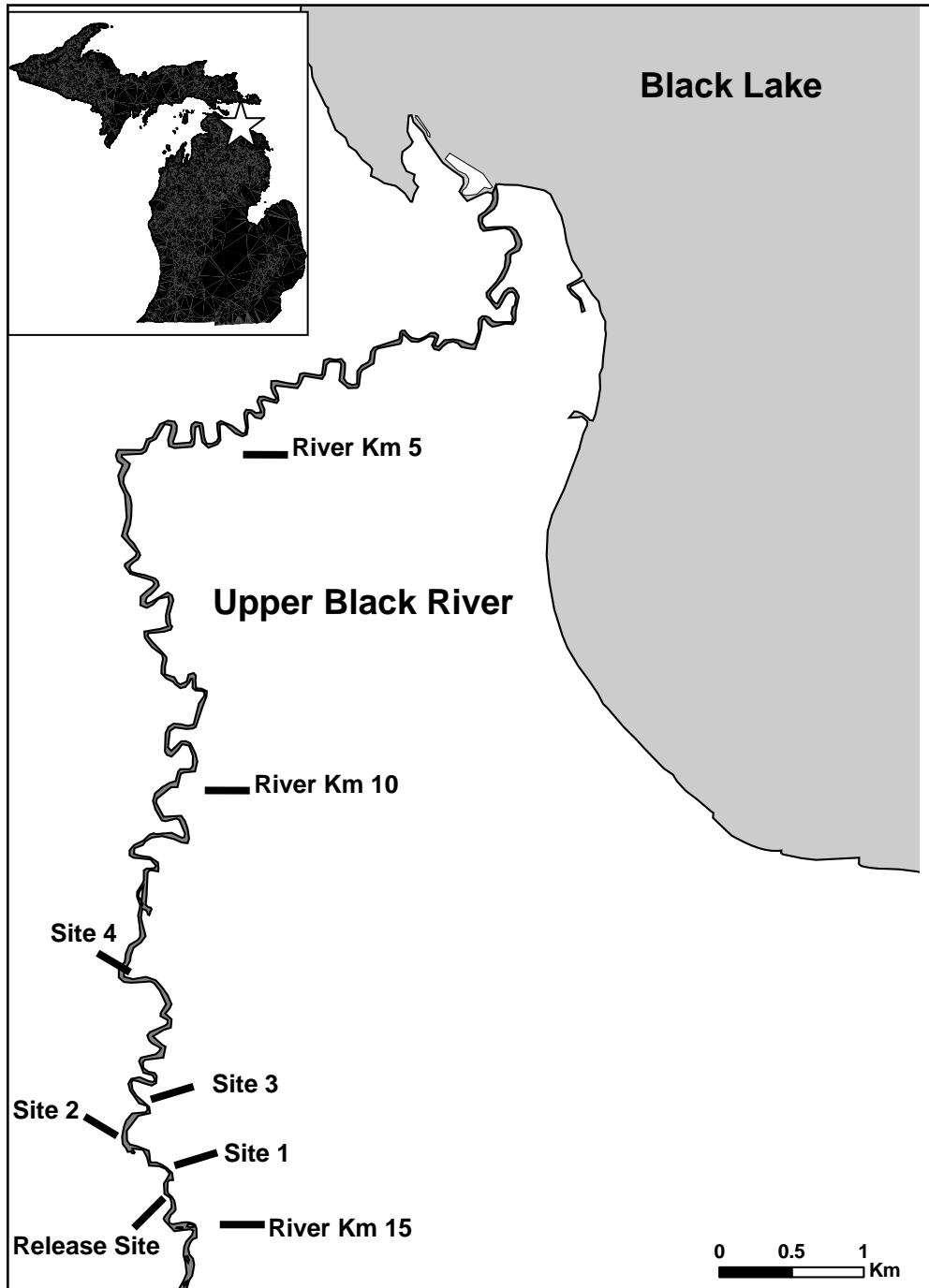


Figure 4.1.—The Black Lake system Michigan, showing the release location and sampling sites (1–4) for release assessments.

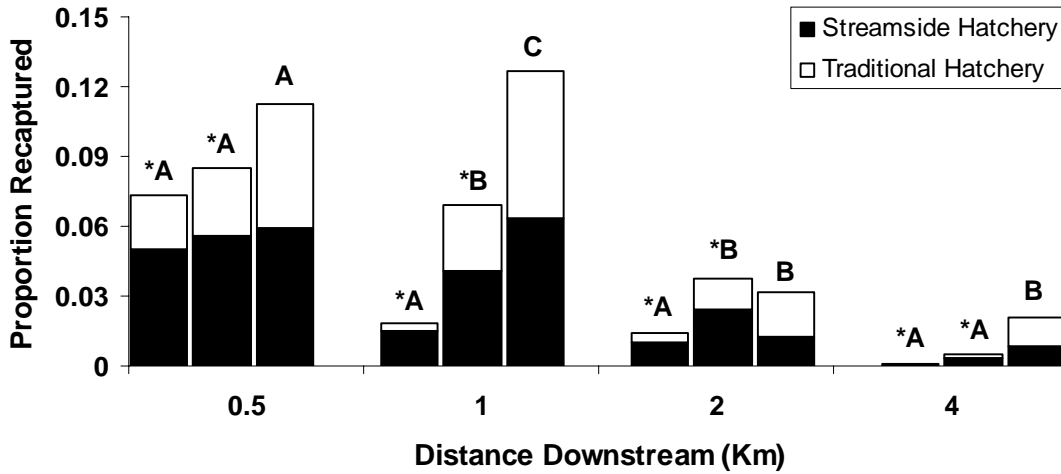


Figure 4.2.—The proportion of juvenile lake sturgeon captured at downstream assessment sites that were reared in different hatchery environments. Bars within a downstream interval represent results of releases at 8, 13, and 17 weeks of age respectively. The asterisks represent significantly greater proportions 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.

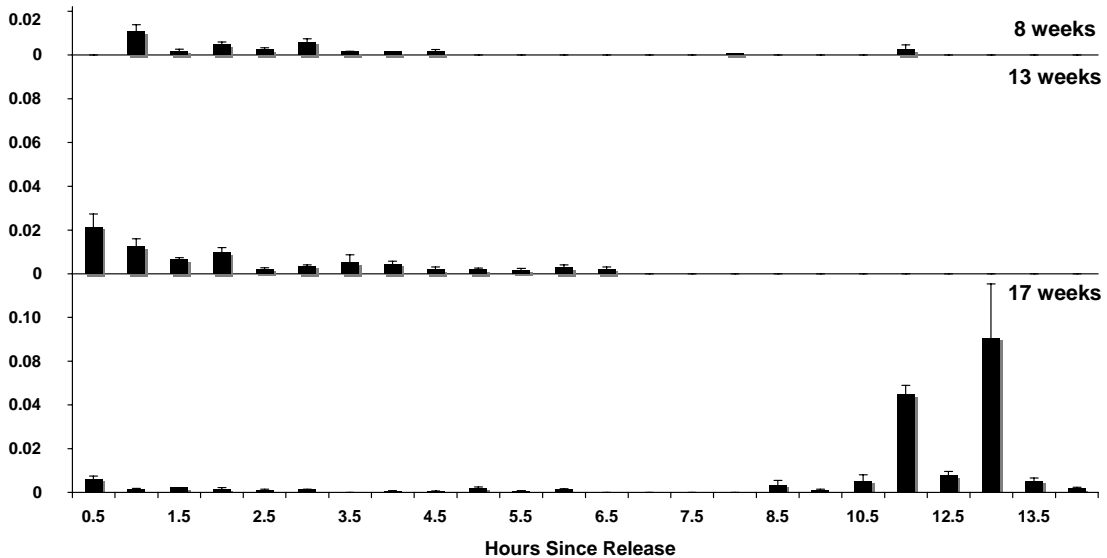


Figure 4.3.—The proportion of juvenile lake sturgeon captured at consecutive hours following release into the Upper Black River at 8, 13, and 17 weeks of age.

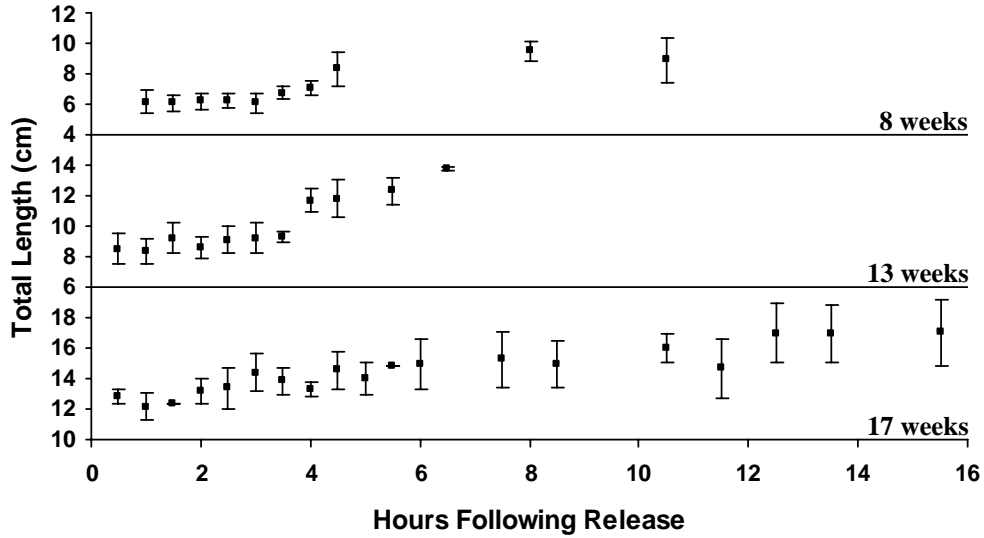


Figure 4.4.—Total length of recaptured juvenile lake sturgeon over time following release at 8, 13, and 17 weeks of age into the Upper Black River. Data presented represent means \pm 1 S.E.

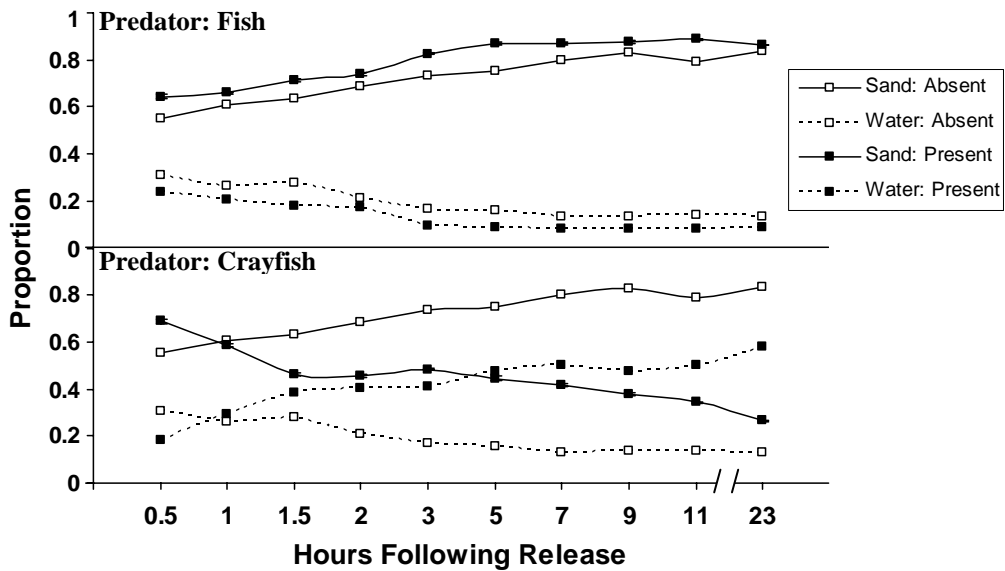


Figure 4.5.—The effect of two predator classes (Fish, Crayfish) on the proportion of juvenile lake sturgeon observed on sandy substrate or that were swimming actively in the water column. Gravel and large rock substrates were also available choices in this experiment but are not included due to insignificant choice for these two habitat types. Predators included rock bass *Ambloplites rupestris*, smallmouth bass *Micropterus dolomieu*, northern pike *Esox lucius* and crayfish *Orconectes rusticus*. Absent and present refer to trials conducted with and without predators.

Appendix 5

Quantification of Overwinter Survival and Habitat Usage of Juvenile Lake Sturgeon Reared in Natal and Non-natal Environments

Introduction

For many fishes, the first winter of life is commonly viewed as a critical period that ultimately determines year class strength (Oliver et al. 1979). In general, studies have shown that a large body size in fall often improves the probability that an individual will survive through the first winter primarily as a function of size-dependent energy depletion and predation (Shuter et al. 1980; Miranda and Muncy 1987). Lake sturgeon in the state of Michigan have been traditionally reared until early–late fall following the premise that larger fish survive better once released back into the natural environment. Unfortunately no information is available on overwinter survival, or any quantified levels of survival, for age-0 juvenile lake sturgeon. This is common across all sturgeon species due to inefficient collection methods for early age classes.

Ultrasonic telemetry has been identified as a valuable technique to collect information on survival, activity levels, habitat preference, and correlations between movements and environmental variables for a number fish species. Despite some limitations (e.g. sample size, battery life, and post-manipulation mortality), telemetry allows multiple behavioral observations to be collected, which is an advantage over passive capture techniques (e.g. gillnetting) (Zamora and Moreno-Amich 2002) and can be used to estimate mortality, distance traveled, and range size (Taverny et al. 2002; Zamora and Moreno-Amich 2002) as well as identifying critical habitats such as spawning grounds (Flavelle 2002). Recent advancements in telemetry technology, particularly with regards to decreasing tag size, have increased opportunities to study species over a greater range of ages. This is especially important for studies 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; Quist et al. 1999; Holtgren and Auer 2004; Smith and King 2005). There is a critical need for determining overwinter survival of age-0 sturgeon. Mortality rates over the first year of life are believed to be considerably higher than for older age groups. Estimates of age-specific mortality through critical ages are particularly important as a measure of the success of hatchery stocking programs which are increasingly used as a part of comprehensive species recovery programs. Success of most hatchery programs can be defined by measurable increases in the spawning stock biomass. However, long-lived iteroparous species such as the lake sturgeon exhibit delayed sexual maturity. Estimates of year class survival, derived from telemetry studies can be used to project future spawning adult abundance.

The primary goal of our study was to examine overwinter survival of juvenile lake sturgeon reared in two different hatchery environments and released at a traditional (fall fingerling) hatchery age using implanted ultrasonic transmitters. A secondary objective of this work was to determine differences in movement patterns between streamside-reared and traditional hatchery-reared individuals following release into the natural environment. This study provides the first quantified estimate of overwinter survival for both hatchery-reared juvenile lake sturgeon and for fish reared in different environments.

Methods

Rearing Environment

Juvenile lake sturgeon were reared in the two separate environments, a streamside hatchery using water from the natal river and a traditional hatchery using a non-natal (ground water) source. Fish were reared separately for three months and then all individuals from the streamside facility were transported to the traditional hatchery. This was conducted to accomplish 2 goals; (1) overcome logistical constraints of keeping our stream side facility operating and (2) to increase total length to a size (~approximately 25cm total length) that would support the insertion of the ultrasonic transmitters. When lake sturgeon grew to approximately this size class they were transported to holding tanks at Michigan State University.

Transmitter Implantation

Forty juvenile lake sturgeon, 20 reared at our stream side facility and 20 reared in the traditional hatchery setting, were surgically implanted with small coded ultrasonic tags; 1.7 ± 0.01 cm (mean \pm 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). Fish were 31.23 ± 0.25 cm in total length and 106.41 ± 2.01 g in weight on average prior to surgery. There was no significant difference in fish size attributed to rearing environment prior to release (Figure 5.1). All surgeries were conducted between December 8 and December 12, 2005. Sonic transmitters were set at an operating frequency of 69 kHz and programmed to emit a coded pulse randomly between 40s and 120s. The weight of the tag did not exceed 2% of the fish weight, which fell well within the generally accepted 2% rule (Winter 1996). Fish were anesthetized using tricaine methane sulfonate (MS222; 125 mg/l; Summerfelt and Smith 1990) in an aerated container prior to surgery. For the duration of the surgery sedation was maintained with a maintenance dose of MS222 (50 mg/l) recirculating through a portable surgical table. Transmitters were anchored to the wall of the peritoneal cavity using non-absorbable sutures to reduce movements of the tag in the body cavity. Incisions were closed using 3.0 gauge non-absorbable monofilament nylon sutures (Ethicon) in an interrupted pattern. Iodine antiseptic was applied to the wound to promote rapid healing. Following surgery fish were monitored for a 3-day period prior to their release into Black Lake on December 16 2005.

Fish Monitoring

Black Lake was divided into eight quadrants. We chose quadrants in random order prior to the start of each tracking event. When possible, tracking was conducted on a weekly schedule. Movement and activity levels 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 for transmitters operating at 69KHz. Tracking was done manually with transmitters that 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. Fish locations were triangulated with a minimum of three geographic points. 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 were tracked during the winter by drilling holes in the ice and deploying the hydrophones a meter under the ice to eliminate broken sonic signals caused by ice interference. Automated hydrophone receivers (Model VR2) were strategically placed within Black Lake to monitor approximate fish locations.

Telemetry and Geographic Data

Collected positioning data were managed and analyzed using geographic information system (GIS) software (ESRI, ArcMap v. 8.3, Arcview v. 3.3, and Arcview spatial analyst v. 2.0a). GIS data were managed in the form of Universal Transverse Mercator (UTM) units.

Data Analysis

Triangulations of detection points were calculated using program Locate II (Nams 2006), which uses the method of least squares to estimate the error associated with the predicted location. The bearing angle error and the number of bearings used in each triangulation were used to calculate the error ellipse surrounding each estimated location. Juvenile lake sturgeon exhibiting active movement patterns after ice-out were assumed to have survived. We used a paired t-test to examine differences in the size at stocking of surviving fish relative to those presumed to be dead.

Results

Tracking efforts were conducted in the winter, spring, and summer months during 2006. A total of 16 juvenile sturgeon, or a minimum of 40%, survived the winter months. Seven of these fish were hatched at the streamside hatchery environment and 9 were from the traditional environment. Two other fish were located but were assumed to be dead after consecutive tracking excursions identified them at the same location. This location was close to the release location and an automated receiver recorded these individuals over an extended time period. All located individuals were within 5 km of the release location until the end of May 2006. Automated receivers documented that a number of fish moved extensively within this zone (Table 5.1). There was no significant difference in the size at stocking of surviving fish compared to those presumed to have died. After the end of May no fish were located throughout Black Lake, despite extensive tracking surveys in the lake, the Upper Black River, and the Lower Black River.

Discussion

This is the first quantified result for overwinter survival of hatchery reared juvenile lake sturgeon. The minimum survival value of 40% is encouraging despite a number of factors associated with long term hatchery rearing including the inability to successfully forage and elevated levels of domestication. Unfortunately, our tracking efforts did not result in enough information to examine movement patterns in relation to environmental variables or rearing environment. We failed to detect 55% of released fish. The total disappearance from the system could be attributed to several factors, the first being an error with the ultrasonic transmitters. Secondly, smaller fish can pass through the hydroelectric dam on 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 outside of the immediate watershed connected to Black Lake. Mortality of all fish due to predation is likely. However, transmitters should have been identified either within the predator itself or on the substrate after being expelled by. Furthermore, if fish died near the mouth of the river the transmitter could become buried in the sediment exiting the Upper Black River to a level that would make the signal undetectable. This was deemed as highly unlikely by the manufacturing company as the signal strength should operate through a moderate amount of sand cover. Fish size prior to release was fairly large for juvenile sturgeon at age-0. However, larger age-0 individuals of some fish species have better overwinter survival than smaller individuals

because of greater stored energy reserves (Shuter et al. 1980; Miranda et al. 1987). Surviving lake sturgeon released into Black Lake were not significantly larger.

One observation was that juvenile lake sturgeon maintained activity through monitoring periods in the early spring. However individuals remained within a very small area before signal was lost. Movement behaviors could be related to foraging, as suggested in other acoustic telemetry studies (McCleave et al. 1977), who found that shortnose sturgeon *Acipenser brevirostrum* displayed similar foraging behavior regardless of the time of day. Taverny et al. (2002) found similar restricted 24 h movement behaviors when tracking juvenile European sturgeon *Acipenser sturio*. 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 moving rapidly (>20km) to a different location. Furthermore, short-term movement for feeding purposes has also been observed with juvenile and adult Atlantic sturgeon (Smith 1985; Moser and Ross 1995; Armstrong and Hightower 2002), white sturgeon *Acipenser transmontanus* (Haynes et al. 1978), and different populations of gulf sturgeon (Zehfuss et al. 1999). Juvenile lake sturgeon probably foraged over the productive area near the mouth of the Upper Black River and when the competition, food, or physical parameters changed to no longer benefit them they moved on. This is typical of sturgeon in general, as individuals often range widely to take advantage of scattered and seasonally abundant resources (Beamesderfer and Farr 1997). Unfortunately we did not detect them after their dispersal from the mouth of the Upper Black River.

In conclusion, this is the first study attempting to quantify the survival of hatchery reared juvenile lake sturgeon. Our estimate of a minimum level (40%) of overwinter survival is critical for the designs of restoration programs for this imperiled species.

References

- Armstrong, J. L., and J. E. Hightower. 2002. Potential for restoration of the Roanoke River population of Atlantic sturgeon. *Journal of Applied Ichthyology* 18:475–480.
- Beamesderfer, R. C. P., and R. A. Farr. 1997. Alternatives for the protection and restoration of sturgeons and their habitat. *Environmental Biology of Fishes* 48:407–417.
- Flavelle, L. S., M. S. Ridgway, T. A. Middel, and R. S. McKinley. 2002. Integration of acoustic telemetry and GIS to identify potential spawning areas for lake trout (*Salvelinus namaycush*). *Hydrobiologia* 483:137–146.
- Fox, D. A., J. E. Hightower, and F. M. Parauka. 2002. Estuarine and nearshore marine habitat used by Gulf sturgeon from the Choctawhatchee River System, Florida. Pages 111–126 in W. Van Winkle, P. J. Anders, D. H. Secor, and D. A. Dixon, editors. *Biology, Management, and Protection of North American Sturgeon*. American Fisheries Society Symposium 28, Bethesda, Maryland.
- Hay-Chmielewski, E. M. 1987. Habitat preferences and movement patterns of the lake sturgeon (*Acipenser fulvescens*) in Black Lake, Michigan. Michigan Department of Natural Resources, Fisheries Research Report 1949, Ann Arbor.
- Haynes, J. M., R. H. Gray, and J. C. Montgomery. 1978. Seasonal movements of white sturgeon (*Acipenser transmontanus*) in the mid-Columbia River. *Transactions of the American Fisheries Society* 107:275–280.
- Holtgren, J. M., and N. A. Auer. 2004. Movement and habitat of juvenile lake sturgeon (*Acipenser fulvescens*) in the Sturgeon River/Portage Lake system, Michigan. *Journal of Freshwater Ecology* 19:419–432.
- McCleave, J. D., S. M. Fried, and A. K. Towt. 1977. Daily movements of shortnose sturgeon, *Acipenser brevirostrum*, in a Maine estuary. *Copeia* 1977:149–157.
- Miranda, L. E., and R. J. Muncy. 1987. Recruitment of young-of-year largemouth bass in relation to size structure of parental stock. *North American Journal of Fisheries Management* 7:131–137.
- Moser, M. L., and S. W. Ross. 1995. Habitat use and movements of shortnose and Atlantic sturgeons in the Lower Cape Fear River, North Carolina. *Transactions of the American Fisheries Society* 124:225–234.
- Nams, V.O. 2006. Locate II. Radiotelemetry Triangulation Program. Freeware, Ver. 3.21. Available online at: <http://www.locateiii.com/>
- Oliver, J. D., G. F. Holeton, and K. E. Chua. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores and environmental temperature. *Transactions of the American Fisheries Society* 108:130–136.
- Quist, M. C., J. S. Tillma, M. N. Burlingame, and C. S. Guy. 1999. Overwinter habitat use of shovelnose sturgeon in the Kansas River. *Transactions of the American Fisheries Society* 128:522–527.
- Shuter, B. J., J. A. MacLean, F. E. J. Fry, and H. A. Regier. 1980. Stochastic simulation of temperature effects on first year survival of smallmouth bass. *Transactions of the American Fisheries Society* 109:1–34.

- Smith, K. M., and D. K. King. 2005. Movement and habitat use of yearling and juvenile lake sturgeon in Black, Michigan. *Transactions of the American Fisheries Society* 134:1159–1172.
- Smith, T. I. J. 1985. The fishery, biology, and management of Atlantic sturgeon, *Acipenser oxyrinchus*, in North America. *Environmental Biology of Fishes* 14:61–72.
- Summerfelt, R.C. and Smith, L.S. 1990. Anesthesia, surgery, and related techniques. *Methods for fish biology*. American Fisheries Society, Bethesda, Maryland.
- Taverny, C., M. Lepage, S. Piefort, P. Dumont, and E. Rochard. 2002. Habitat selection by juvenile European sturgeon *Acipenser sturio* in the Gironde estuary (France). *Journal of Applied Ichthyology* 18:536–541.
- Winter, J. 1996. Advances in underwater biotelemetry. Pages 555-585 in B. R. Murphy and D. W. Willis, editors. *Fisheries techniques*, 2nd edition. American Fisheries Society, Bethesda, Maryland.
- Zamora, L., and Moreno-Amich, R. 2002. Quantifying the activity and movement of perch in a temperate lake by integrating acoustic telemetry and a geographic information system. *Hydrobiologia* 483:209–218.
- Zehfuss, K. P., J. E. Hightower, and K. H. Pollock. 1999. Abundance of Gulf sturgeon in the Apalachicola River, Florida. *Transactions of the American Fisheries Society* 128:130–143.

Table 5.1.—Automated receiver detection results from the period of December 15, 2005 until May 2, 2006. Detection results include only those individuals that were detected using automated receivers.

Sonic ID	First date detected	Last date detected	# of days detected	Largest # of consecutive days detected
2524	2-19-2006	4-17-2006	34	32
2529	12-28-2005	5-2-2006	91	91
2532	12-30-2005	1-1-2006	3	3
2537	12-22-2005	3-9-2006	9	2
2538	12-24-2005	5-2-2006	90	90
2548	4-5-2006	4-14-2006	4	2
2549	1-9-2006	3-3-2006	7	5

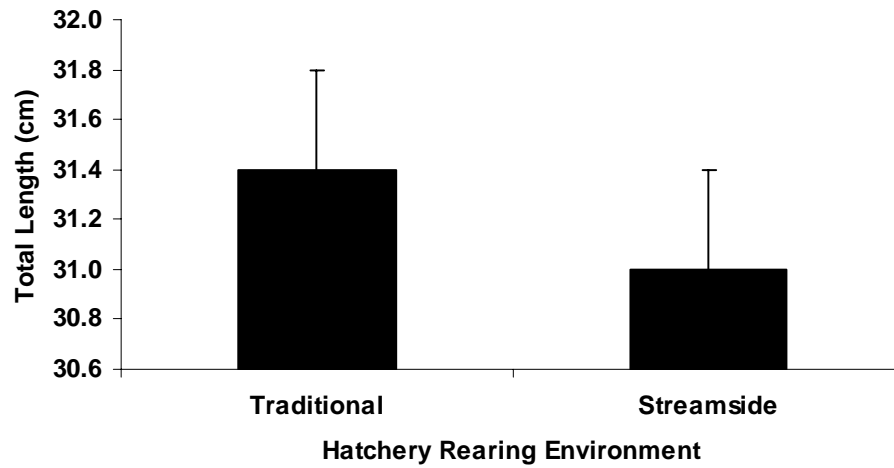


Figure 5.1.—Total length of juvenile lake sturgeon prior to release from both the traditional rearing environment and the streamside hatchery environment.

Appendix 6

Reproductive Ecology and Conservation Status of Lake Sturgeon, *Acipenser Fulvescens*: Inferences Based on Genetic Determination of Parentage

Introduction

Genetic markers are used for studies of parentage and mating systems in many species including fishes (see review in Avise et al. 2002). Genetic studies have provided insights into many aspects of fish mating systems including incidence of multiple paternity (DeWoody et al. 1998; Moran and Garcia-Vazquez 1998; Jones et al. 2001; Mackiewicz et al. 2002), sex role reversals (Jones and Avise 2001) and ontogenetic shifts in mating strategies (Moran et al. 1996) that were difficult to assess based on observational data alone. Studies of mating systems that combine genetic and life history data can also provide valuable information for conservation efforts including data on numbers of spawning adults, skews in sex ratios, variance in reproductive success and the phenotypic and environmental factors underlying variance in reproductive success. Such data are critical for estimating effective population sizes as well as propensities for population declines in genetic diversity (Moran and Garcia-Vazquez 1998; Garant et al. 2001; Bekkevold et al. 2002; Fiumera et al. 2002).

Populations that have experienced severe declines in abundance and have low effective sizes may be susceptible to Allee effects, where aspects of the species ecology lead to a decrease in population growth rate when population size becomes low (Saether et al. 1996). When population sizes are low, fishes that exhibit group spawning behavior may experience difficulties finding mates or may be forced to pair with poor mates, resulting in lowered reproductive success and ultimately a reduction in population growth rate (Saether et al. 1996; Stephens and Sutherland 1999). Additionally, broadcast spawning fishes may also experience reduced fertilization rates as density declines and spawning group sizes become smaller (Lundquist and Botsford 2004). Myers et al. (1995) documented these effects for a stock of herring *Clupea harengus*, an aggregate spawning fish subject to intensive commercial harvest. Another group of fishes that may be subject to Allee effects due to their life history characteristics and dramatic population declines are the sturgeons (family *Acipenseridae*).

Sturgeons are long-lived chondrosteian fishes that have recently experienced rapid population declines throughout the world (Birstein 1993). The lake sturgeon *Acipenser fulvescens* is a freshwater species native to the Great Lakes, Mississippi River and Hudson Bay drainages of North America (Houston 1987). Current population estimates for lake sturgeon in the Great Lakes are believed to be less than 1% of historic estimates (Hay-Chmielewski and Whelan 1997). These severe declines have occurred within approximately the past 100 years, and are largely the result of overharvest, blockage of spawning habitat by dams and loss of spawning habitat (Houston 1987; Auer 1999a). Because of these dramatic declines, the lake sturgeon has become a species of conservation concern throughout its native range.

Lake sturgeon share life history traits common to other members of the family *Acipenseridae*. Lake sturgeons have delayed sexual maturity. Individuals typically mature at 15 to 25 years of age (Houston 1987; Auer 1999a). During spring, mature adults migrate to natal rivers to spawn (DeHaan et al. 2006). Lake sturgeon do not spawn annually; males may spawn every 1 to 4 years, whereas females may spawn every 3 to 7 years (Houston 1987; Auer 1999a). The tendency for males to spawn more frequently than females leads to male-biased sex ratios during spawning periods (Kempinger 1988). During spawning single female lake sturgeon have been observed to spawn with multiple males simultaneously (Kempinger 1988; Bruch and Binkowski 2002). As females release their eggs,

males compete to position themselves as close to the female as possible to increase the likelihood of fertilizing the eggs (Bruch and Binkowski 2002).

Lake sturgeon are broadcast spawners and do not construct any type of nest prior to releasing their gametes (Kempinger 1988; Bruch and Binkowski 2002). Spawning is temperature dependent, with spawning observed primarily between 10 and 15° C. Spawning activity typically lasts between one and three weeks (Houston 1987; Kempinger 1988; LaHaye et al. 1992; Auer 1996; Bruch and Binkowski 2002). Often, a drop in temperature during the spawning period will cause individuals to cease spawning and leave spawning areas until water temperatures increase, at which time spawning will resume (Kempinger 1988; Bruch and Binkowski 2002). Adults typically leave spawning grounds shortly after spawning. Adults make no attempt to protect eggs nor do they provide any post-ovulatory parental care for offspring following egg deposition (Kempinger 1988; Bruch and Binkowski 2002). Eggs incubate rapidly (5 to 14 days) and after hatching, larval lake sturgeon drift downstream to larger bodies of water (Houston 1987; Kempinger 1988; LaHaye et al. 1992). Because lake sturgeon make no parental investment in their offspring (other than maternal effects associated with provisioning of eggs), recruitment during a single year is likely to be low due to predation on the unprotected eggs and offspring (Kempinger 1988). Life history traits including rapid incubation time, high fecundity and longevity likely co-evolved as means to compensate for high predation on gametes and juveniles and concomitantly, low annual recruitment.

Variation in reproductive success may be influenced by both biotic and abiotic factors. Female lake sturgeon can produce over 100,000 eggs in a single spawning season with larger females presumably producing larger numbers of eggs (Houston 1987). Polygynous matings and competition among males during spawning are likely to result in variance in reproductive success as well (Schuster and Wade 2003). Previous work has suggested that environmental factors may play a larger role in reproductive success. However, in a four year study, Kempinger (1988) found that reproductive success, as determined by numbers of larval lake sturgeon captured downstream of spawning grounds, was greatest during the year with the lowest number of eggs deposited. During this year, the author observed a much more gradual, consistent increase in water temperature as opposed to other years of the study when water temperatures and water levels fluctuated considerably during the spawning and larval drift period. The author also observed a strong correlation between water temperature and the amount of time required for eggs to hatch. In addition, Auer (1996) found that numbers of spawning adults varied year to year in response to varying stream flow produced by a hydroelectric dam. Kempinger (1988) also observed that survival was approximately 42 percent higher in protected (covered) versus non-protected lake sturgeon eggs, presumably due to predation.

Our objective was to use microsatellite markers to determine parentage in a remnant lake sturgeon population. Based on parentage data, inferences can be made regarding many aspects of the lake sturgeon mating system about which little information is currently available as well as the potential for this population to retain genetic diversity. Specifically, we enumerated the number of mates per male and female and we estimated variance in reproductive success and the phenotypic and environmental factors responsible for estimated variance in reproductive success. Parentage data was also used to estimate the effective number of breeding adults. Data regarding the lake sturgeon mating system and effective numbers of breeders in the Black Lake population can serve as a model for other, much smaller, remnant populations of lake sturgeon and other related species when establishing conservation and management goals.

Materials and Methods

Study Location

This study focuses on a remnant lake sturgeon population in Black Lake, Michigan, USA (Figure 6.1), one of the largest remnant populations in the Great Lakes basin (Holey et al. 2000). Historically lake sturgeon could migrate from Lake Huron to Black Lake and then to the upper Black River to spawn. However, this population has been landlocked since 1903 due to construction of a dam on the lower Black River (Baker and Borgeson 1999). This population declined substantially over approximately 25 years due to low recruitment and overharvest. Recent population estimates suggest that there are approximately 1,250 lake sturgeon in Black Lake, with about 550 of those being fish of reproductive age (Baker and Borgeson 1999). Each spring adult sturgeon in Black Lake spawn in the upper Black River downstream of a dam. Because spawning areas in the upper Black River are well documented and easily accessible, spawning adults can easily be enumerated each spring. Inferences regarding the lake sturgeon mating system made based on this relatively large population can be used as an indicator of how the lake sturgeon mating system evolved as well as the mating system of other sturgeon species prior to recent declines.

Sample Collection

Field sampling was conducted during the spring and summer of 2001. Adult sturgeon were captured between April 28 and May 26, 2001 during spring spawning migrations in the upper Black River (Figure 6.1) using large dip nets (see Smith and Baker 2005). Total length, fork length, girth and weight were recorded for all adult fish captured. Sex was determined based on the presence of eggs or milt. A small tissue sample was taken from the caudal or dorsal fin and dried and stored at ambient temperatures.

Sampling for out-migrating larval sturgeon was conducted from May 9 to June 14, 2001. D-frame drift nets measuring 85cm across the base and 55cm high were used to capture drifting larval lake sturgeon 1.14km downstream of spawning grounds. Sampling occurred during peak drift h, between 22:00 and 02:00 (see Smith and King 2005). All larval lake sturgeon captured were held in on-site holding tanks for a period that enabled us to enumerate three separate larval drift periods and were then transported to the Michigan Department of Natural Resources Wolf Lake State Fish Hatchery. Larval sturgeon were divided into three groups at the hatchery corresponding to time of downstream migration.

Juveniles were reared in the hatchery and released the following fall into the Upper Black River. Hatchery mortalities (n = 779) were preserved in 95% non-denatured ethanol for genetic analyses. Prior to their release into the Upper Black River, fin clips were taken from all surviving juveniles (n = 912) and dried and stored at ambient temperatures.

Genetic Analyses

DNA was extracted from all adult tissue samples and 576 juvenile tissue samples using QIAGEN DNeasy® kits (QIAGEN Inc.) according to the manufacturers' protocol. 192 juvenile lake sturgeon were selected from each of three out-migrating groups for genetic analyses. Juvenile lake sturgeon were sampled proportionally from the hatchery mortalities and from the fish that were released back into the Upper Black River. DNA was quantified using a Beckman DU® 7400 spectrophotometer.

Individuals were genotyped at eight disomically inherited microsatellite loci including *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001) *AfuG9*, *AfuG63*, *AfuG74* and *AfuG112* (Welsh et al. 2003). PCR reactions were conducted in

25µl volumes containing 100ng DNA, 10X PCR Buffer (1M Tris-HCl, 1M MgCl₂, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), 2mM of each dNTP, 10pmol of forward and reverse primer and 0.3µl Taq polymerase. PCR conditions were as follows; 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, 1 minute at primer specific annealing temperatures, and 72° C for 1 minute, and a final extension at 72° C for 2.5 minutes. PCR products were run on 6% denaturing polyacrylamide gels and visualized on a Hitachi FMBIO II scanner. Allele sizes were determined using commercially available size standards (MapMarker™, BioVentures Inc.) and based on standard samples of known genotype.

Statistical Analyses

Estimates of allele frequencies, mean number of alleles per locus, and observed and expected heterozygosities were calculated using the computer program CERVUS v2.0 (Marshall et al. 1998). 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 8 loci.

Parentage assignments were made using a maximum likelihood-based approach (Thompson 1975; Meagher 1986) employed by the program CERVUS v2.0 (Marshall et al. 1998). All adults that were captured in the Upper Black River during the spawning period were considered potential parents of each offspring. Using CERVUS we first assigned the female parents and then the male parents to each offspring. We used CERVUS to analyze adults in the reverse order (males first, then females) in order to validate our results. For each offspring we examined the output for all genotyped parental pairs who matched the offspring at all loci.

Parental assignments were made for each offspring according to several criteria. First, we attempted to assign a single parental pair for each offspring based on total exclusion of all other male and female pairs. In cases where a single parental pair matched the offspring at all loci, parentage was assigned to that male and female. In cases where more than one parental pair matched an offspring at all loci, the parental pair with the greatest likelihood at either 95% (strict) or 80% (relaxed) probabilities relative to other parental pairs was assigned to an offspring. Confidence levels associated with each parental pair were derived based on population allele frequencies and representative levels of tolerance of false parentage (see Marshall et al. 1998 for details). In cases where all possible parental pairs were excluded for a particular offspring, we examined the adult males and females separately to determine if we could assign either a single male or a single female parent to an offspring based on total exclusion of all other males or females.

Mean and variance in estimates of the numbers of mates and numbers of offspring produced were estimated from the sample of offspring with assigned parents. To test for evidence of size-related assortative mating, Pearson correlation coefficients between the total length of males and females that spawned with one another were estimated using SAS (SAS Institute Inc.). To determine if mating between closely related individuals (i.e. inbreeding) was occurring, we used the program Kinship v1.3.1 (Goodnight and Queller 1999) to estimate coefficients of relatedness (R_{xy} ; Queller and Goodnight 1989) among pairs of individuals that spawned with one another and pairs of individuals that did not spawn with one another.

To determine whether body size was a significant predictor of reproductive success, the Pearson correlation coefficient between male and female total length and the number of offspring produced was estimated using SAS. Correlations between the number of mates and the number of offspring produced were also estimated using SAS to examine the number of mates as another predictive factor underlying variance in male and female reproductive success. Relationships were considered significant at the $\alpha < 0.05$ level.

Because only a subset of the adult lake sturgeon population in Black Lake spawns each year, it was not possible to estimate effective population size based on data from only one spawning season. Instead, we estimated the effective number of breeding adults (N_b) based on the parentage data from 2001. We calculated our estimates of effective number of breeders using two different methods. First, estimates of variance in reproductive success were used to estimate the effective number of female (Equation 1) and male (Equation 2) breeders as in Kimura and Crow (1963). We then used these estimates to estimate the effective number of breeders for 2001 taking unequal sex ratio into account (Equation 3) (Kimura and Crow 1963):

$$N_{ef} = \frac{N_{fk} - 1}{[k - 1 + (V_k / k)]} \quad (1)$$

$$N_{em} = \frac{N_{mk} - 1}{[k - 1 + (V_k / k)]} \quad (2)$$

$$N_b = \frac{4N_{ef}N_{em}}{(N_{ef} + N_{em})} \quad (3)$$

where N_{ef} represents the estimate of effective number of females, with mean family size k and variance in family size V_k and N_{em} represents the estimate of effective number of males with mean family size k and variance in family size V_k .

We also estimated N_b based upon temporal changes in allele frequencies (Waples 1989). We first estimated the temporal variance in allele frequency between adults and juveniles (F_c) as follows:

$$F_c = \frac{1}{k} \sum \frac{(x_i - y_i)^2}{(x_i + y_i)/(2 - x_i y_i)} \quad (4)$$

where k represents the number of alleles per locus, x_i represents the frequency of allele i in the adult population and y_i represents the frequency of allele i in the progeny. We estimated F_c for each individual locus and then used the mean F_c over all loci to estimate N_b as follows:

$$N_b = \frac{t}{2[F_c - 1/(2S_o) - 1/(2S_t) + 1/N]} \quad (5)$$

Where S_o represents the number of adults sampled, S_t represents the number of juveniles sampled, N represents the total adult population size sampled and t represents the number of generations between the adults and progeny.

Results

We obtained tissue samples from a total of 114 adult lake sturgeon (70 males and 44 females) captured in the Upper Black River during multiple periods of spawning activity between 28 April and May 26, 2001. We observed multiple peaks in the numbers of drifting larvae, presumably coinciding with the peaks in adult spawning activity (Figure 6.2, see also Smith and King 2005).

Genotypic frequencies at all 8 loci did not deviate from Hardy-Weinberg expectations. The mean number of alleles per locus was 6.63 (range 3–11) and observed heterozygosity ranged from 0.448 to 0.818. The overall exclusion probability was estimated to be 0.943 when neither parent was presumed known. A single parental pair was assigned to 146 of the offspring. 103 of these assignments (70.5%) were at the 95% confidence level, 33 of the parentage assignments (22.6%) were at the 80% confidence level and 10 of the parentage assignments (6.9%) were below the 80% confidence level. The 10 cases where parentage assignments were made below 80% confidence represented situations where parentage was assigned based on total exclusion of all other potential parents. A single parent was assigned for an additional 139 offspring based on total exclusion of all but a single male or female. Of the offspring that we successfully assigned parentage for, 138 of them were fish that were re-released into the upper Black River and the other 146 were fish that died while in the hatchery.

Mating System

Multiple incidences of polygyny and polyandry were observed. Estimates of the number of males spawning with each female ranged from 0 to 11 (mean = 3.10; variance = 6.19) (Figure 6.3). The number of females spawning with each male ranged from 0 to 5 (mean = 1.93; variance = 2.20) (Figure 6.3). Three of 44 females and eight of 70 males were not assigned as parents to any of the offspring sampled. Low correlation (0.068; $P > 0.436$) was observed between the total length of males and females that spawned with one another, suggesting that assortative mating based on body size was not occurring.

Females whose offspring were found in only one of the groups of out-migrating larvae spawned with an average of 1.86 males, whereas females whose offspring were found in more than one group spawned with an average of 4.16 males. Similarly, males whose offspring were found in only one of the groups of drifting larvae spawned with an average of 1.25 females and males whose offspring were found in multiple groups spawned with an average of 2.74 females.

Our mean estimate of relatedness (R_{xy}) for all male and female pairs was 0.0056. Mean estimates of R_{xy} for pairs that did spawn and pairs that did not spawn were 0.0224 and 0.0050 respectively and the distribution of relatedness values between those pairs that did spawn and those pairs that did not spawn were similar (Figure 6.4). Of all the male and female pairs in the 2001 spawning population (i.e. those that did spawn and those that did not spawn), 14.6% had pairwise levels of relatedness characteristic of half-siblings or greater ($R_{xy} \geq 0.25$; Queller and Goodnight (1989)). Of the pairs that did spawn in 2001, 17.8% had pairwise relatedness estimates of 0.25 or greater.

Reproductive Success

Estimates of reproductive success varied considerably among males and females. Estimates of the number of offspring produced per female based on total captures of larvae in the drift nets ranged from 0 to 22 (mean = 5.05; variance = 21.02) (Figure 6.5). The number of offspring produced per male (also based on drift net captures) ranged from 0 to 17 (mean = 3.16; variance = 8.02) (Figure 6.5). Estimates of the number of offspring each adult produced in each of three out-migrating groups of juveniles also varied considerably (Figure 6.6). Fourteen females (34%) and 24 males (39%) produced offspring in a single out-migrating group. The mean number of juveniles produced by adults whose offspring out-migrated during one time period was 2.21 for females and 1.75 for males. Adults with offspring that out-migrated in multiple groups appear to be more successful, however. Twenty-five females (66%) and 38 males (61%) produced offspring in two or three groups of out-migrating juveniles. The mean number of offspring produced by adults whose offspring out-migrated over multiple time periods was approximately three times greater than the estimates for individuals that produced offspring in only 1 group (7.24 for females and 4.71 for males).

Overall, no relationship was observed between body size (total length) and number of offspring produced ($r = -0.093$ ($P > 0.05$) and 0.180 ($P > 0.05$) for males and females, respectively). In contrast, there was a high correlation between the estimated number of mates and the number of offspring produced ($r = 0.810$ ($P < 0.0001$) and 0.927 ($P < 0.0001$) for males and females respectively) (Figure 6.7).

Effective Number of Breeders

Estimates of mean and variance for numbers of offspring that males and females produced were used as estimates of mean family size (k) and variance in family size (V_k) in Equations 1 and 2 above. Based on Equations 1 and 2, the effective number of females (N_{ef}) and males (N_{em}) was estimated to be 20.71 and 31.72 respectively. We used these values in Equation 3 to produce an estimate of 50.12 effective breeders for 2001, representing 43.96% (N_b/N) of the adult spawning population during spring 2001. When we estimated N_b based on temporal shifts in allele frequencies, our mean estimate of F_c over all loci calculated using Equation 4 was 0.0058. When this value was substituted into Equation 5 using 114 adults and 576 offspring, we estimated N_b to be 53.73. This value represents 47.13% (N_b/N) of the total adult spawning population.

Discussion

To date, studies of the lake sturgeon mating system have been based exclusively on observational data (see Kempinger 1988; Bruch and Binkowski 2002). Observational studies have provided insight into some spawning behaviors lake sturgeon exhibit (e.g. group size during spawning and the potential for polygyny and polyandry). However, many questions regarding the lake sturgeon mating system remain. Estimates from this study represent the first attempt to examine lake sturgeon reproductive ecology using genetic markers.

In this study we genotyped a large number of offspring ($n = 576$), yet we were only able to assign a parental pair to 146 juveniles and a single parent only to an additional 139 juveniles. The majority (60.8%) of the individuals that we were unable to assign parentage for were due to low confidence levels (low power) associated with parentage assignments. While all of the markers we used were polymorphic, our mean number of alleles was rather low (mean = 6.63) and three of the loci we used only had three alleles in this population. Further investigations of the lake sturgeon mating system would benefit from markers with higher levels of variability. Since there was no physical barrier in the upper Black River (e.g. weir) where we could sample all adults prior to spawning, there likely were adults that we did not sample, and thus we could not assign a small portion of the offspring to their true parents. Because we chose to only assign parentage in cases where offspring matched to parents at all loci (i.e. total exclusion), we may have been overly stringent and in some cases excluded parents in cases when mis-matches were due to genotyping errors or mutations between parents and offspring. Because we were only able to assign parentage to a subset of the offspring we genotyped, estimates presented in this study (i.e. number of mates, reproductive success, etc.) likely represent the lower bounds of the actual values. Due to these caveats these results represent a preliminary examination of the lake sturgeon mating system and should be interpreted as such.

Mating System

Because lake sturgeon spawn in groups, and are broadcast spawners, it is difficult to assess visually which males and females are spawning with one another. The fact that adults often leave spawning grounds in response to environmental conditions such as changes in water temperature and

then return later to resume spawning (Kempinger 1988; Auer 1999b; Bruch and Binkowski 2002), further complicates observational studies. Ease of access to adults and large numbers of offspring combined with genetic parentage data allowed a much more accurate means of assessing the species mating system.

Aggregate group spawning results in high levels of polygyny and polyandry. Kempinger (1988) observed six to eight males spawning with one female and Bruch and Binkowski (2002) observed between two and eight males spawning with a single female and also observed males spawning with multiple females. Whereas females in Lake Winnebago, Wisconsin (USA) were observed spawning with an average of 5–6 males (Bruch and Binkowski 2002), based on genetic analyses we estimated that females in the Black River successfully spawned with an average of 3.10 males. Discordance in observed sex ratios during single spawning events between studies is likely due to more heavily skewed sex ratios during spawning periods in the Winnebago system (1:5.7 females to males (Bruch and Binkowski 2002) vs. 1:1.6 females to males in this study) and the fact that our estimates were based on assignment of adults that successfully spawned rather than observed spawning. The low correlation between the total length of males and females that spawned with one another in Black Lake indicates that size-based assortative mating is not occurring in this population.

Eleven of the adults in this study, three females and eight males, were not assigned as potential parents to any of the 576 offspring. These individuals either did not attempt to spawn, they may have had low reproductive success and their offspring were too few to be represented in our sub-sample or we may not have been able to correctly assign their offspring. Because adults were sampled on spawning grounds and the gender of these fish was determined based on the presence of eggs or milt (indicative of fish in spawning condition), it is highly likely that these fish did attempt to spawn in 2001. Therefore a lack of offspring assigned to these adults can be attributed either to the fact that these individuals had lower reproductive success than other adults and none of their offspring were in the sample of fish we genotyped or that we were unable to correctly assign parentage for the offspring of these 11 adults.

When population sizes become small, individuals have fewer choices for mates and may be forced to pair with inferior mates (i.e., Allee effects; Stephens and Sutherland 1999). For example, a lack of mate choices may cause individuals that have an increased level of relatedness (e.g. full or half siblings) to pair with each other because few other unrelated mates are available. Concerns about mating between closely related individuals are especially pertinent for remnant lake sturgeon populations as many of them have very few spawning individuals in any given year (Holey et al. 2000). While we did identify some closely related individuals that spawned with one another in Black Lake, overall, levels of relatedness we observed among individuals that did spawn were not significantly different than those that would be expected if individuals mated randomly. Black Lake still supports a relatively large lake sturgeon spawning population with over 100 spawning adults observed in recent years (Smith and Baker 2005). The relatively high number of spawning adults likely buffers the population from increased mating among close relatives.

Reproductive Success

We observed a high level of variation in reproductive success among male and female lake sturgeon, a trend previously observed in other fish species as well (Moran et al. 1996; Gross and Kapuscinski 1997; Garant et al. 2001; Bekkevold et al. 2002; Fuimera et al. 2002; Seamons et al. 2004). One explanation for variation in reproductive success in fishes is body size. Body size often confers different reproductive advantages for males and females. In some species, larger females are capable of producing larger and greater numbers of eggs (Beacham and Murray 1993). In species in which males compete for access to spawning females, larger males may be better suited to fend off smaller competing males, and thus may have higher reproductive success. Bekkevold et al. (2002)

found that for cod *Gadus morhua*, a species that also spawns in groups, on average larger males sired higher numbers of offspring.

We did not observe a significant relationship between body size (length) and reproductive success for male or female lake sturgeon in this study ($r = -0.093$ ($P > 0.05$) for males and 0.180 ($P > 0.05$) for females). Because adults were sampled at different stages of the mating period (i.e. pre-spawning vs. post spawning), we did not test the relationship between body weight and reproductive success. Studies of Atlantic and Pacific salmonids similarly observed a low correlation between body size and number of offspring produced for males and females (Garant et al. 2001; Seamons et al. 2004). These species exhibit similar mating tactics to lake sturgeon including polygyny and polyandry. A significant correlation was observed however, between the numbers of mates a male or female had and the number of offspring produced ($r = 0.810$ ($P < 0.0001$) and 0.927 ($P < 0.0001$) for males and females, respectively). Our data suggest that for lake sturgeon, distributing gametes among several mates rather than concentrating reproductive efforts on just one or two mates offers one means of increasing reproductive success. Similarly, a high correlation between the number of mates and the number of offspring produced was observed in Atlantic salmon (Garant et al. 2001). Sexual selection theory suggests that in species such as lake sturgeon that exhibit no parental care for offspring, individuals that have greater numbers of mates show increased reproductive success (Arnold and Duvall 1994).

Males and females appear to be more successful when they distribute their gametes among multiple mates. There are multiple mechanisms that may account for this increase in reproductive success. We observed multiple peaks in the numbers of new lake sturgeon captured in spawning areas followed by multiple peaks in the number of larval fish captured downstream, suggesting that multiple peaks in spawning activity may have occurred, a trend suggested in previous studies (Kempinger 1988; Bruch and Binkowski 2002). These data, coupled with data on the numbers of mates, suggests that two different mating strategies exist. One strategy is for fish to spawn during a single time period and then return to Black Lake. Another segment of the population in Black Lake spawns over multiple time periods, thereby distributing their gametes over multiple spawning events with multiple groups of potential mates. Adults whose offspring were found migrating downstream over multiple time periods (presumably due to repeat spawning) produced significantly greater numbers of offspring than those whose offspring were found migrating downstream during a single time period (t -test, $P < 0.05$). Given that environmental variables appear to play a significant role in reproductive success (Kempinger 1988), those individuals that spawn repeatedly and apportion their gametes over multiple time periods increase their likelihood of encountering optimal larval incubation and rearing conditions. Additionally, apportioning gametes over multiple time periods may help to increase avoidance of predation on developing eggs and larvae.

An alternative hypothesis is that adults spawn over a single, more protracted time period and deposit their gametes among different locations within the stream when spawning with different mates. Habitat characteristics important for larval sturgeon development including substrate, dissolved oxygen concentrations and temperature regimes can vary significantly within a stream over spatial and temporal scales (Allan 1995). The location of egg deposition may cause differential rates of egg incubation and hatching due to variation in environmental characteristics among locations. Differences in incubation time and hatching date may thus account for the multiple peaks we observe in the number of offspring captured downstream. There are also likely to be differences in predation rates at different locations within a stream as potential predators have preferred habitats which influence their spatial distribution within a stream. Additionally there may be genetic factors that influence the rate at which eggs and larvae develop (Crossman, unpublished data). Further studies that link differences in habitat characteristics among sites of egg deposition and genetic data with incubation and development time will help to further clarify the factors that influence reproductive success.

Effective Number of Breeders

Estimates of effective population size can provide valuable information for developing conservation strategies including information regarding rates of genetic drift and levels of inbreeding in a population. We estimated N_b based on both demographic data and temporal shifts in allele frequencies and found similar values for both estimates (50.12 and 53.73 respectively). Both of our estimates were found to be lower than the census number of breeding adults observed. Frankham (1995) reviewed reported estimates of effective population size for 102 different species from several taxa and found that the three most important factors influencing effective population size were fluctuations in population size, variance in family size, and skews in sex ratio. As a result of increased energetic demand on females associated with egg production, females spawn less frequently than males resulting in male-biased sex ratios during spawning periods. Lake sturgeon in Black Lake exhibited male biased sex ratios during spawning periods with males out-numbering females nearly two to one. Our estimates also showed a high degree of variance in reproductive success among both males and females. In addition, this population has experienced a 66% reduction in size in a span of 25 years (Baker and Borgeson 1999). The combination of these factors coupled with a lack of immigration from other populations is likely the cause for the reductions in effective numbers of breeders we observed.

Estimates of effective population size have been calculated for many different taxa and range widely. Frankham (1995) found that the mean N_e/N ratio for 102 different species was estimated to be 0.11. A variety of methods have been used to estimate effective population size of fish species and reported estimates vary widely (Jorde and Ryman 1996; Allendorf et al. 1997; Miller and Kapuscinski 1997; Fiumera et al. 2002; Heath et al. 2002; Ardren and Kapuscinski 2003). The majority of these estimates of N_e/N for fish species were much lower than our estimate for lake sturgeon, initially implying that this particular population may not be at as great a risk of generational loss of genetic diversity as other populations/species of fishes.

Because sturgeons do not spawn annually, our estimates of N_b do not represent a true effective population size and should not be interpreted as such. Both estimates of N_b were based on data sampled from a very early life stage (immediately post hatching). Waples (2002) demonstrates that sampling at various life stages may significantly affect estimates of effective population size due to differences in stage-specific survivorship. Given that mortality is highest during the first year of life for lake sturgeon and recruitment during any given year is likely to be low (Houston 1987), we would expect our estimates of N_b to be considerably lower if estimates were calculated from data based on numbers of offspring that survived to adulthood. Due to the fact this population has been isolated for approximately 100 years (roughly five sturgeon generations) and has declined rapidly in that time, initial reductions in N_b raise conservation concerns for this remnant population. Populations that experience reduced effective population sizes are likely to experience increases in mean coancestry as well as greater losses of genetic diversity, ultimately leading to increased probability of extinction (Newman and Pilson 1997).

Conclusions

These data represent the first analysis of aspects of lake sturgeon reproductive ecology using genetic markers. Data presented in this study extend the results of previous observational studies and provide new insights into aspects of lake sturgeon reproductive ecology that have conservation relevance. Spawning behaviors such as polygyny and polyandry as well as spawning over a protracted period of time combined with the species long life span helped to maximize reproductive success and ensure population viability for lake sturgeon in the past. When species with group spawning behaviors experience reductions in population size, there are likely to be negative effects on

population growth rates (Allee effects). Lake sturgeon populations range wide, including the population in Black Lake, have experienced severe declines in abundance within relatively few generations. Given these rapid declines, the initial reductions we observe in effective numbers of breeders should raise concern for this isolated population. Conservation measures aimed at limiting harvest and increasing reproductive success have recently been implemented for this population (Baker and Borgeson 1999) and represent effective means to help maintain the viability and genetic diversity in this relatively large remnant population.

Expansion of Analyses to Additional Years

All adults captured over the period 2001–07 (N=660) have been genotyped for all eight microsatellite loci. An additional four microsatellite loci have been used to genotype adults and juveniles. In addition, 147 larvae from the 2002 year-class (10% of the total larvae collected) were genotyped. Parentage analyses for juveniles obtained during 2002 and subsequent years is ongoing.

References

- Allan, J. D. 1995. Stream ecology: structure and function of running waters. 3rd edition. Chapman and Hall, London.
- Allendorf, F. W., D. Bayles, D. L. Botton, K. P. Currens, C. A. Frissell, D. Hankin, J. A. Lichatowich, W. Nehlsen, P. C. Trotter, and T. H. Williams. 1997. Prioritizing Pacific salmon stocks for conservation. *Conservation Biology* 11:140–152.
- Ardren, W. R., and A. R. Kapuscinski. 2003. Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. *Molecular Ecology* 12:35–49.
- Arnold, S. J., and D. Duvall. 1994. Animal mating systems: a synthesis based on selection theory. *The American Naturalist* 143:317–348.
- Auer, N. A. 1996. Response of spawning lake sturgeons to change in hydroelectric facility operation. *Transactions of the American Fisheries Society* 125:66–77.
- Auer, N. A. 1999a. Lake Sturgeon: A unique and imperiled species in the Great Lakes. Pages 515–538 in W. Taylor and C. P. Ferreri, editors. *Great Lakes Fisheries Policy and Management: A Binational Perspective*. Michigan State University Press, East Lansing.
- Auer, N. A. 1999b. Population characteristics and movements of lake sturgeon in the Sturgeon River and Lake Superior. *Journal of Great Lakes Research* 25:282–293.
- Avise, J. C., A. G. Jones, D. Walker, J. A. DeWoody and collaborators. 2002. Genetic mating systems and reproductive natural histories of fishes: Lessons for ecology and evolution. *Annual Review of Genetics* 36:19–45.
- Baker, E. A., and D. J. Borgeson. 1999. Lake sturgeon abundance and harvest in Black Lake, Michigan, 1975–1999. *North American Journal of Fisheries Management* 19:1080–1088.
- Beacham, T. D., and C. B. Murray. 1993. Fecundity and egg size variation in North American Pacific salmon (*Onchorhynchus*). *Journal of Fish Biology* 42:485–508.
- Bekkevold, D., M. M. Hansen, and V. Loeschcke. 2002. Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). *Molecular Ecology* 11:91–102.
- Birstein, V. J. 1993. Sturgeons and paddlefishes: Threatened species in need of conservation. *Conservation Biology* 7:773–787.
- Bruch, R. M., and F. P. Binkowski. 2002. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). *Journal of Applied Ichthyology* 18:570–579.
- DeHaan, P. W., S. T. Libants, R. F. Elliott, and K. T. Scribner. 2006. Genetic population structure of remnant lake sturgeon populations in the upper Great Lakes Basin. *Transactions of the American Fisheries Society* 135:1478–1492.
- DeWoody, J. A., D. E. Fletcher, S. D. Wilkins, W. S. Nelson, and J. C. Avise. 1998. Molecular genetic dissection of spawning, parentage, and reproductive tactics in a population of redbreast sunfish, *Lepomis auritus*. *Evolution* 52:1802–1810.

- Fiumera, A. C., B. A. Porter, G. D. Grossman, and J. C. Avise. 2002. Intensive genetic assessment of the mating system and reproductive success in a semi-closed population of the mottled sculpin, *Cottus bairdi*. *Molecular Ecology* 11:2367–2377.
- Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: A review. *Genetical Research* 66:95–107.
- Garant, D., J. J. Dodson, and L. Bernatchez. 2001. A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *Journal of Heredity* 92:100–110.
- Gross, M. L., and A. R. Kapuscinski. 1997. Reproductive success of smallmouth bass estimated and evaluated from family-specific DNA fingerprints. *Ecology* 78:1424–1430.
- Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology* 8:1231–1234.
- Hay-Chmielewski, E. M., and G. E. Whelan. 1997. Lake sturgeon rehabilitation strategy. Michigan Department of Natural Resources, Fisheries Special Report 18, Ann Arbor.
- Heath, D. D., C. Busch, J. Kelly, and D. Y. Atagi. 2002. Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Molecular Ecology* 11:197–214.
- Holey, M. E., E. A. Baker, T. F. Thuemler, and R. F. Elliott. 2000. Research and assessment needs to restore lake sturgeon in the Great Lakes: Results of a workshop sponsored by the Great Lakes Fishery Trust.
- Houston, J. J. 1987. Status of the lake sturgeon, *Acipenser fulvescens*, in Canada. *The Canadian Field-Naturalist* 101:171–185.
- Jorde, P. E., and N. Ryman. 1996. Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics* 143:1369–1381.
- Jones, A. G., and J. C. Avise. 2001. Mating systems and sexual selection in male-pregnant pipefishes and seahorses: Insights from microsatellite-based studies of maternity. *Journal of Heredity* 92:150–158.
- Jones, A. G., D. Walker, K. Lindstrom, C. Kvarnemo, and J. C. Avise. 2001. Surprising similarity of sneaking rates and genetic mating patterns in two populations of sand goby experiencing disparate sexual selection regimes. *Molecular Ecology* 10:461–469.
- Kimura, M., and J. F. Crow. 1963. The measurement of effective population number. *Evolution* 17:279–288.
- King, T. L., B. A. Lubinski, and A. P. Spidle. 2001. Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Conservation Genetics* 2:103–119.
- Kempinger, J. J. 1988. Spawning and early life history of lake sturgeon in the Lake Winnebago system, Wisconsin. Pages 110–122 in R. D. Hoyt, editor. *Proceedings of the 11th Annual Larval Fish Conference*. American Fisheries Society Symposium 5. American Fisheries Society, Bethesda, Maryland.

- LaHaye, M., A. Branchaud, M. Gendron, R. Verdon, and R. Fortin. 1992. Reproduction, early life history, and characteristics of the spawning grounds of the lake sturgeon (*Acipenser fulvescens*) in Des Prairies and L'Assomption Rivers, near Montreal, Quebec. *Canadian Journal of Zoology* 70:1681–1689.
- Lundquist, C. J., and L. W. Botsford. 2004. Model projections of the fishery implications of the Allee effect in broadcast spawners. *Ecological Applications* 14:929–941.
- Mackiewicz, M., D. E. Fletcher, D. Wilkins, J. A. DeWoody, and J. C. Avise. 2002. A genetic assessment of parentage in a natural population of dollar sunfish (*Lepomis marginatus*) based on microsatellite markers. *Molecular Ecology* 11:1877–1883.
- Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639–655.
- May, B., C. C. Krueger and H. L. Kincaid. 1997. Genetic variation at microsatellite loci in sturgeon: Primer sequence homology in *Acipenser* and *Scaphirhynchus*. *Canadian Journal of Fisheries and Aquatic Sciences* 54:1542–1547.
- McQuown, E. C., G. A. E. Gall, and B. May. 2002. Characterization and inheritance of six microsatellite loci in lake sturgeon. *Transactions of the American Fisheries Society* 131:299–307.
- McQuown, E. C., B. L. Sloss, R. J. Sheehan, J. Rodzen, G. J. Tranah, and B. May. 2000. Microsatellite analysis of genetic variation in sturgeon: New primer sequences for *Scaphirhynchus* and *Acipenser*. *Transactions of the American Fisheries Society* 129:1380–1388.
- Meagher, T. R. 1986. Analysis of paternity within a natural population of *Chamaelirium Luteum*. 1. Identification of most-likely male parents. *The American Naturalist* 128:199–215.
- Miller, L. M., and A. R. Kapuscinski. 1997. Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics* 147:1249–1258.
- Moran, P., A. M. Pendas, E. Beall, and E. Garci-Vazquez. 1996. Genetic assessment of the reproductive success of Atlantic Salmon precocious parr by means of VNTR loci. *Heredity* 77:655–660.
- Moran, P., and E. Garcia-Vazquez. 1998. Multiple paternity in Atlantic Salmon: A way to maintain genetic variability in relict populations. *Journal of Heredity* 89:551–553.
- Myers, R. A., J. A. Barrowman, J. A. Hutchings, and A. A. Rosenberg. 1995. Population dynamics of exploited fish stocks at low population levels. *Science* 269:1106–1108.
- Newman, D., and D. Pilson. 1997. Increased probability of extinction due to decreased genetic effective population size: Experimental populations of *Clarkia pulchella*. *Evolution* 51:354–362.
- Queller, D., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- Saether, B. E., T. H. Ringsby, and E. Roskaft. 1996. Life history variation, population processes and priorities in species conservation: Towards a reunion of research paradigms. *Oikos* 77:217–226.
- SAS Institute Inc. 1989. *SAS/STAT® Users Guide, V6, 4th Edition, Volume 1*. Cary, NC: SAS Institute Inc.

- Schuster, S. M., and M. J. Wade. 2003. *Mating Systems and Strategies*. Princeton University Press. Princeton, New Jersey.
- Seamons, T. R., P. Bentzen, and T. P. Quinn. 2004. The effects of adult length and arrival date on individual reproductive success in wild steelhead trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:193–204.
- Smith, K. M., and E. A. Baker. 2005. Characteristics of spawning lake sturgeon in the Upper Black River, Michigan. *North American Journal of Fisheries Management* 25:301–307.
- Smith, K. M., and D. K. King. 2005. Dynamics of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology* 21:161–168.
- Stephens, P. A., and W. J. Sutherland. 1999. Consequences of the allee effect for behaviour, ecology and conservation. *Trends in Ecology and Evolution* 14:401–405.
- Thompson, E. A. 1975. The estimation of pairwise relationships. *Annals of Human Genetics* 39:173–188.
- Waples, R. S. 2002. Evaluating the effect of stage-specific survivorship on the N_e/N ratio. *Molecular Ecology* 11:1029–1037.
- Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121:378–391.
- Welsh, A. B., M. Blumberg, and B. May. 2003. Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, *A. medirostris*. *Molecular Ecology Notes* 3:47–55.

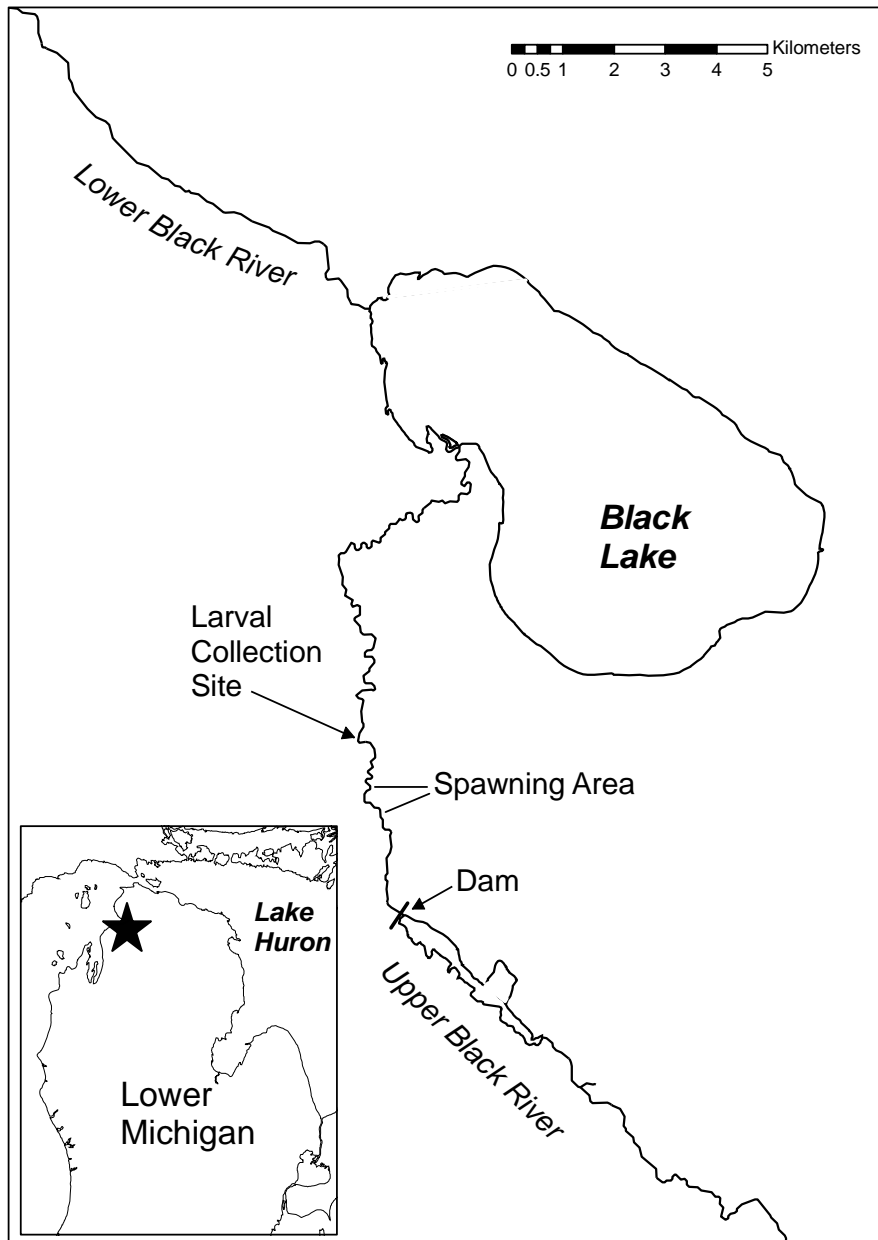


Figure 6.1.—Map of the study site including Black Lake and sections of the lower and upper Black Rivers. Adult sturgeon were captured near spawning areas in the Upper Black River and larval sturgeon were collected immediately after hatching in the Upper Black River.

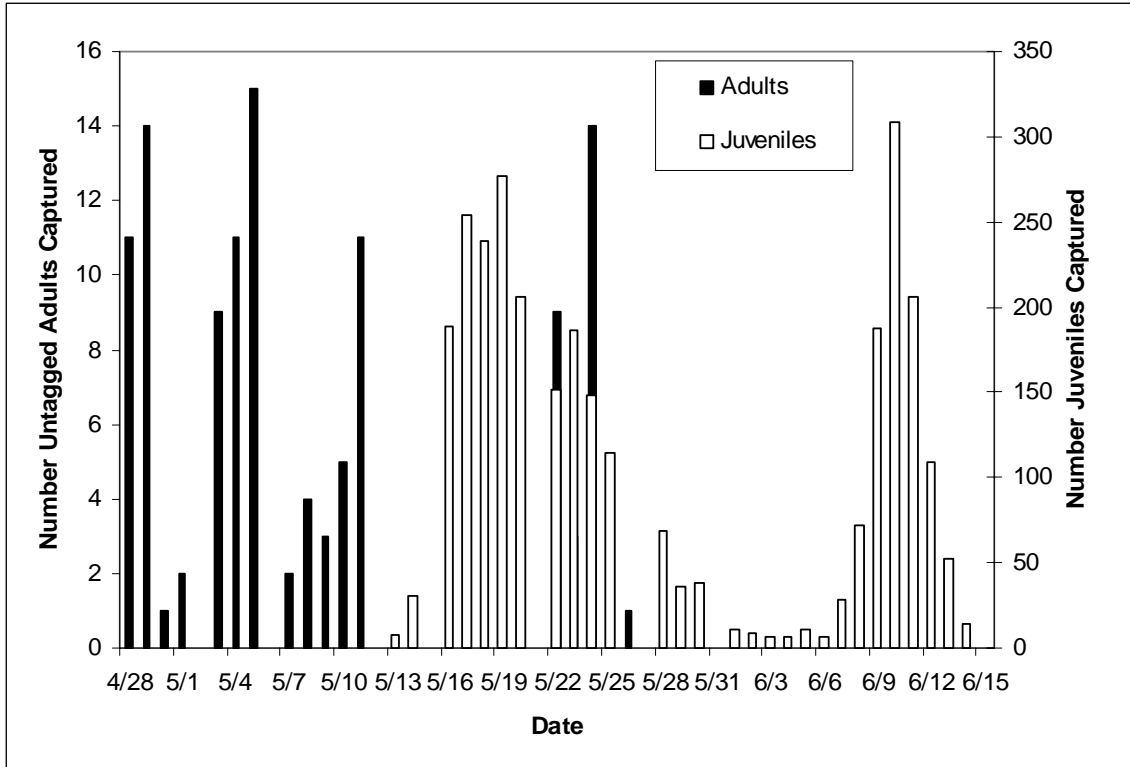


Figure 6.2.—Number of adult and juvenile lake sturgeon captured each day during spring 2001 in the upper Black River. It is important to note that values for adults represent the number of untagged adults captured on a particular day and does not necessarily represent the total number of spawning adults in the upper Black River for a given day.

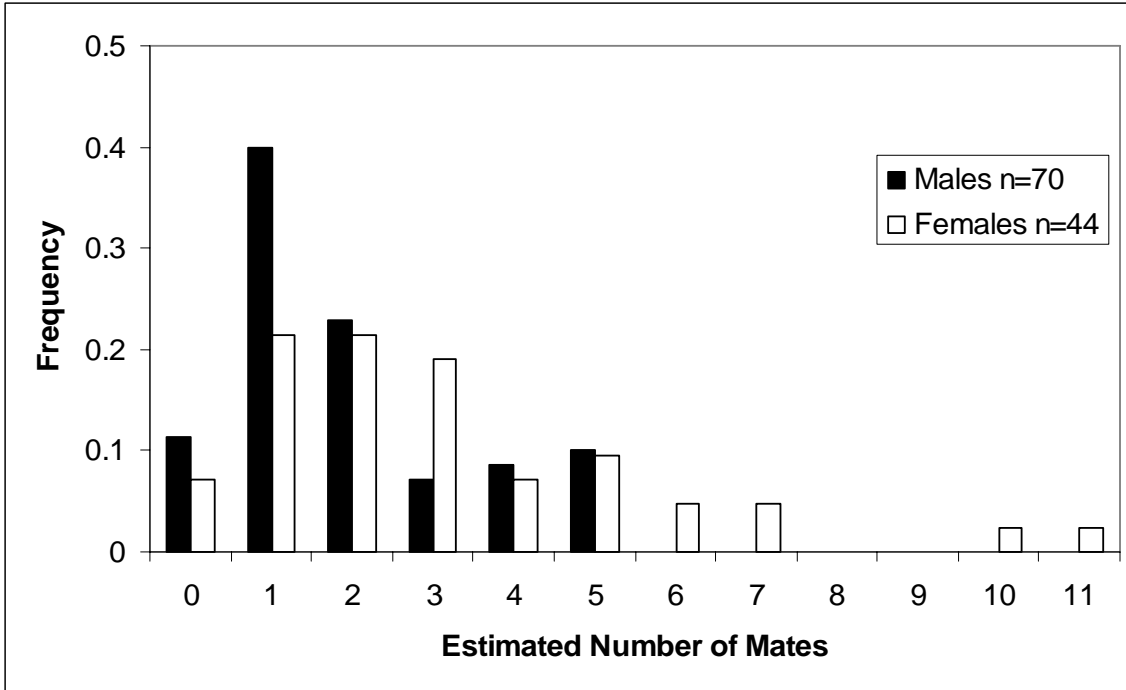


Figure 6.3.—Estimates of the number of mates per female (mean = 3.10; $\sigma^2 = 6.19$) and the number of mates per male (mean = 1.93; $\sigma^2 = 2.20$) for the 114 adult lake sturgeon captured in the Black River during the spring 2001 spawning period.

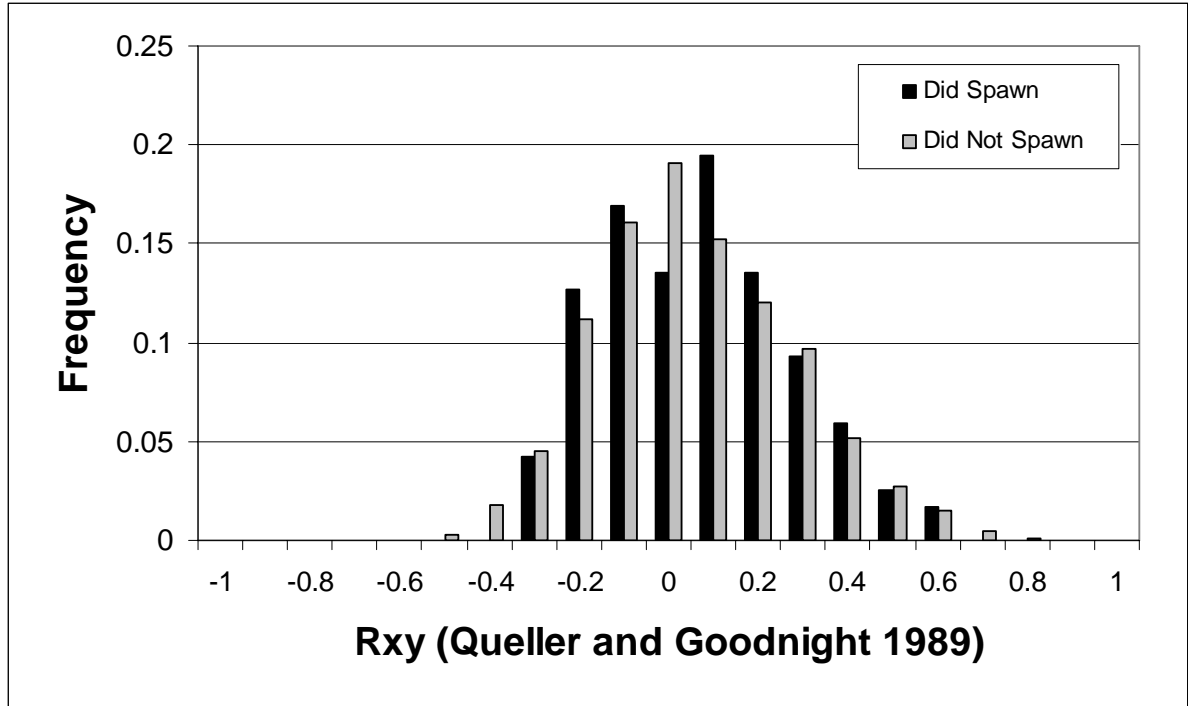


Figure 6.4.—Queller and Goodnight’s (1989) pairwise estimates of relatedness for male and female pairs of lake sturgeon that did spawn (black bars) and did not spawn (grey bars) in 2001. A Wilcoxin test showed that there was no significant difference between the two distributions (Still need to test this to be sure it is true).

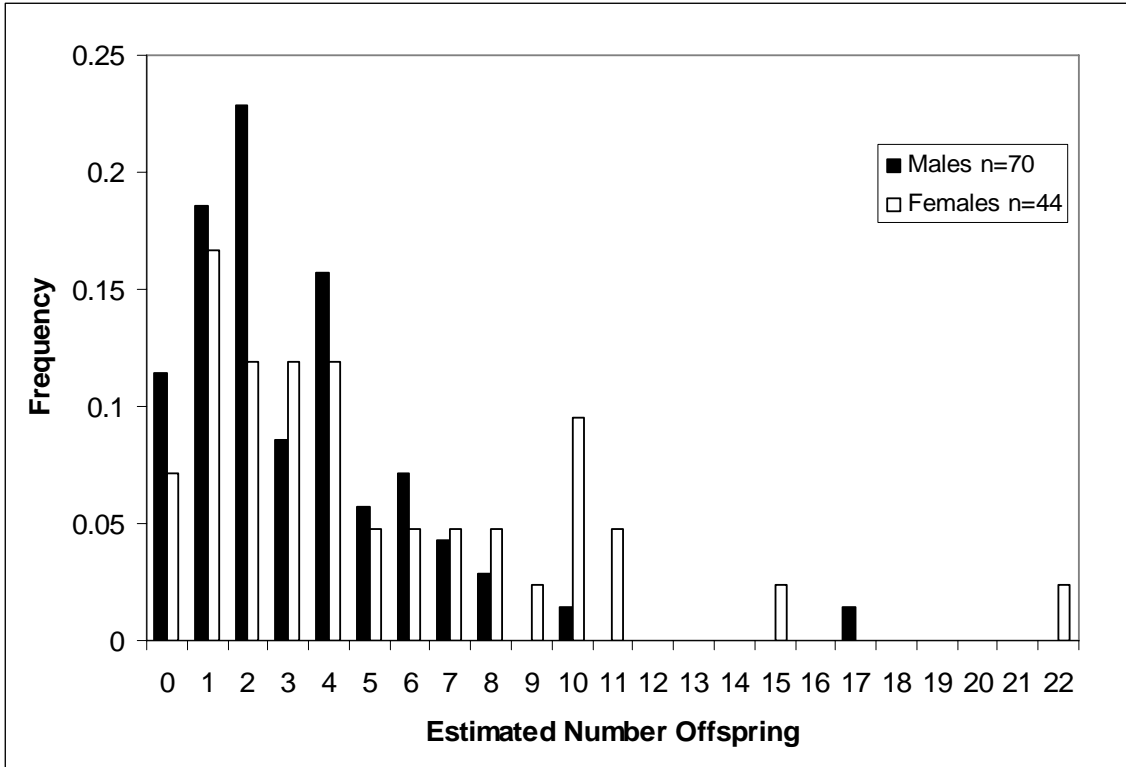


Figure 6.5.—Estimated numbers of offspring produced for females (mean = 5.05; $\sigma^2 = 21.02$) and males (mean = 3.16; $\sigma^2 = 8.02$) for the 114 lake sturgeon captured in Black Lake during the spring 2001 spawning period.

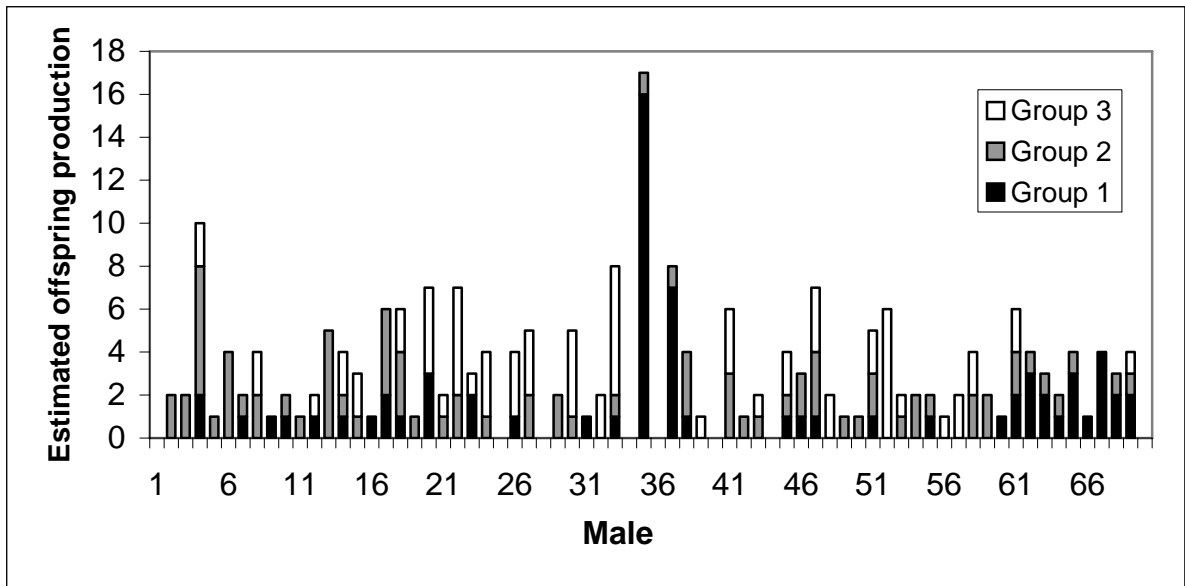
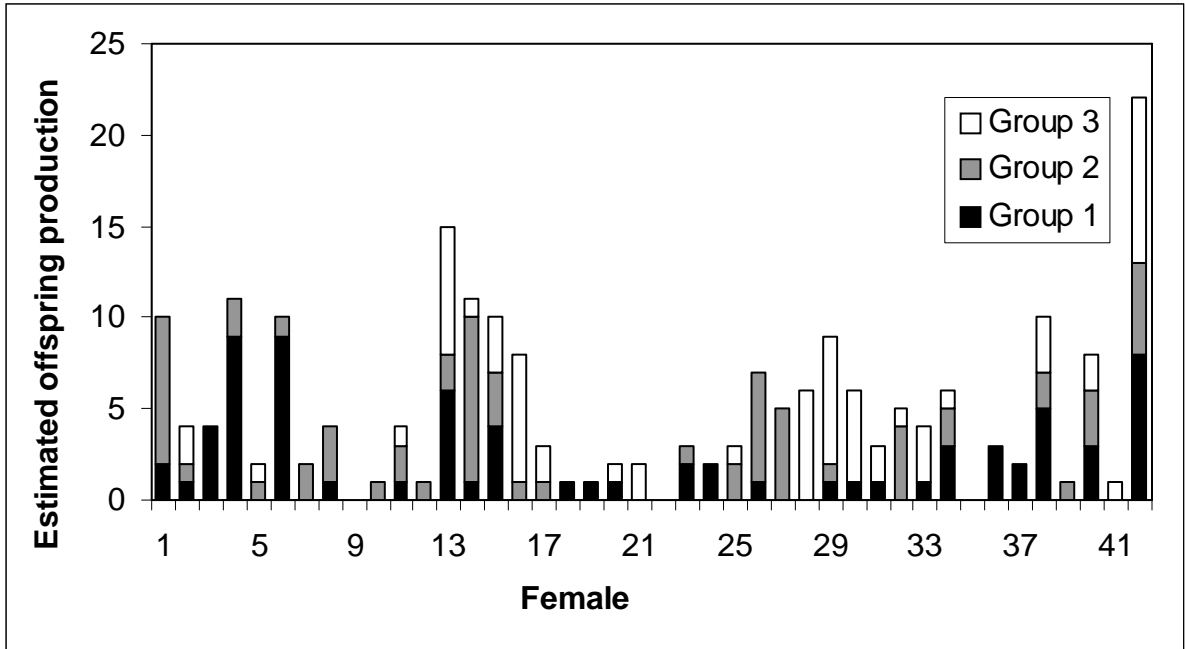


Figure 6.6.—Estimates of the number of offspring each female and male produced in each of the three groups of out-migrating juveniles. For each individual male and female the black area represents the number of offspring collected in the first emigrating group, the gray area represents the number of offspring collected in the second emigrating group and the white area represents the number of offspring collected in the final emigrating group.

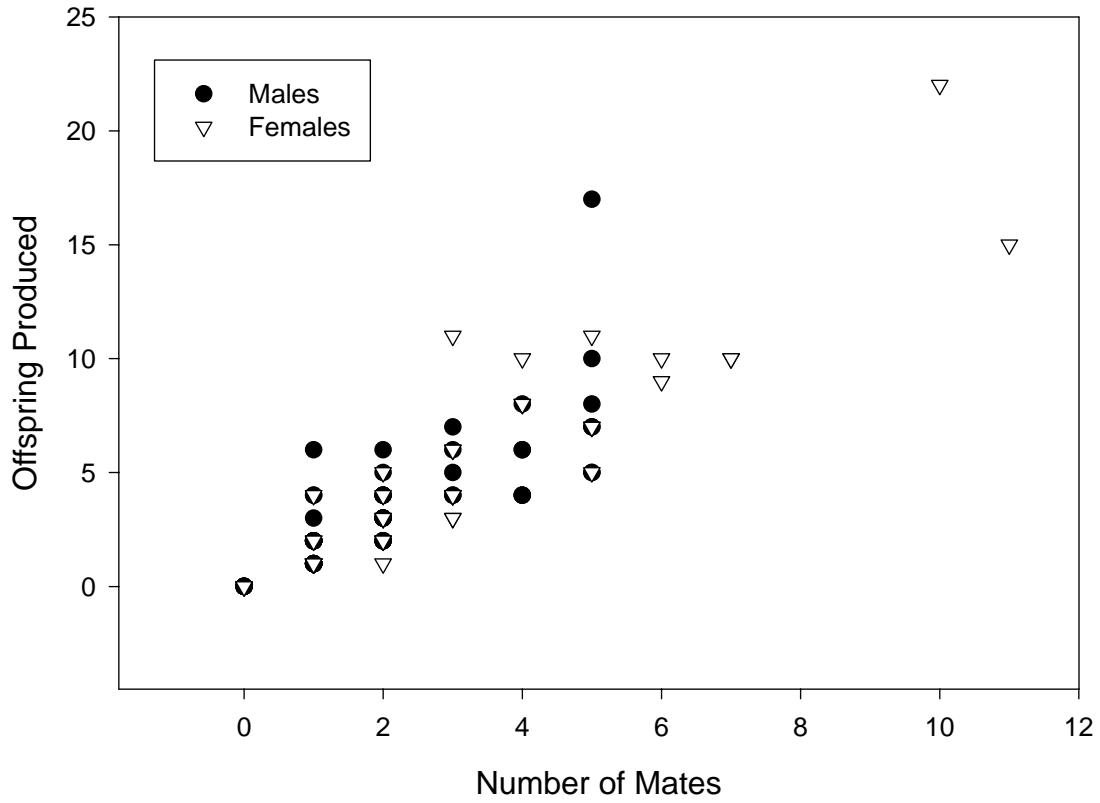


Figure 6.7.—Relationship between the number of mates and the number of offspring produced for males ($r = 0.810$; $P < 0.0001$) and female lake sturgeon ($r = 0.927$; $P < 0.0001$).