EFFECTS OF SPAWNING BEHAVIOR AND ENVIRONMENTAL FACTORS ON ADULT REPRODUCTIVE ECOLOGY AND LARVAL DISPERSAL OF LAKE STURGEON (*ACIPENSER FULVESCENS*)

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ABSTRACT

EFFECTS OF SPAWNING BEHAVIOR AND ENVIRONMENTAL FACTORS ON ADULT REPRODUCTIVE ECOLOGY AND LARVAL DISPERSAL OF LAKE STURGEON (*ACIPENSER FULVESCENS*)

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Adult reproductive behavior plays an important role in population growth and genetic structuring, which is less well understood at the individual level for long-lived fish species like lake sturgeon (*Acipenser fulvescens*). Using genetic determination of parentage, I examined the effects of adult spawning behavior and environmental factors on the species' mating system, genetic structure within a population and on offspring survival and timing to dispersal at the individual level. Analyses were based on adults and larvae captured during 7 years (2001-2007) in the Upper Black River, Black Lake, Michigan. First, I quantified the degree of temporal variation in aspects of the lake sturgeon mating system. The species is polygamous, where males and females mate with a large number of individuals. Mate number and reproductive success (RS, the number of larvae) varied between sexes. However, the linear positive relationship between RS and mate number was similar in males and females. Female RS and mate number was independent of body size though RS and mate number for males slightly increased with increasing body size. Females spawning at different times during the season and locations did not differ in RS or mate number. Mating pairs between males and females that arrived at spawning areas $\geq$8 days apart accounted for 30% of total mating pairs averaged across years, suggesting that temporally discrete spawning groups identified based on direct observations were not reproductively isolated. Second, I tested for evidence of genetic

differentiation among groups of adults spawning at different times in the season over 9 years (2001-2009). Although significant genetic differences were observed between early and late groups of adults captured during ≥ 2 years, evidence of isolation by time was lacking based on low variance in allele frequency (F_{ST}) between early and late spawning groups and lack of correlation between pairwise relatedness and pairwise differences in spawning time. Third, I estimated effective population size (N_e), effective number of breeders (N_b) and degrees to which inter-annual variation in N_b was correlated with adult demographic characteristics. Estimates of N_b and adult census sizes were fairly constant among years. The N_b/N ratios varied 0.26 - 0.61 among years, consistent with low standardized variance (variance/(mean)^2) in RS across years. N_e per generation (132, 95% CI: 104-167) was close to the estimates of annual N_b (harmonic mean: 88). Fourth, I evaluated the relative importance of environmental factors (water temperature and discharge) and maternal effects (female body size, location and timing of spawning) on the embryonic and larval developmental time until dispersal (ELDTUD). Both environmental variables and maternal effects of female and spawning time were equally important predictors of ELDTUD. Finally, I evaluated differences in relative larval loss among females when larvae dispersed a short distance (from two sites 0.15 and 1.5 km from spawning areas) during two consecutive nights. Larval collections were composed of unequal proportions of offspring from females but there was no significant difference in relative larval loss among females. Overall, the study greatly improves understanding of adult reproductive ecology, the roles of adult spawning behavior and environmental factors on timing to dispersal and survival of lake sturgeon at critical early life stages.

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INTRODUCTION

Reproductive variables including body size, spawning time and spawning location together with environmental factors affect variation in recruitment of fish populations (Van Winkle *et al.,* 1997). Reproductive variables can vary among individuals within a spawning group, among groups within a year, and among years. Most studies have investigated how these variables affect recruitment variation at the population level. Recently, with the availability of molecular genetic markers, these effects can be analyzed at the individual level based on data obtained using genetic determination of parentage (DeWoody and Avise, 2001; Garant and Kruuk, 2005; Pemberton, 2008). For long-lived species with small population sizes, individual contributions to population recruitment can affect population levels of genetic diversity and are important to the long term viability of a population. However, the availability of such information is often limited. I used genetic approaches, specifically parentage analysis based on microsatellite loci, to study the effects of environmental factors and reproductive variables on adults' mate number and reproductive success as well as on offspring's timing to dispersal in a threatened fish species, the lake sturgeon *Acipenser fulvescens*. The study was conducted in Black Lake, Cheboygan County, Michigan, USA.

Lake sturgeon have an interesting life history characterized by extreme longevity (potential > 100 years), iteroparity (Auer, 1999) and polygamous mating by both sexes (Bruch and Binkowski, 2002). Males mature earlier (12-15 years) and spawn at shorter inter-annual intervals (1-4 years) than females (> 18 years and every 2-7 years, respectively) (Auer, 1999; Forsythe, 2010), resulting in male-biased sex ratios during the reproductive period in many populations. Lake sturgeon spawn in groups where one

female can be surrounded by several males and one male can mate with multiple females (Bruch and Binkowski, 2002). The spawning season usually begins at the end of April and extends through early June during a period characterized by a wide range of temperatures (9^{o}C - 21^{o}C, Bruch and Binkowski, 2002). Spawning activity occurs in bimodal or multimodal peaks, typically 7-15 days apart (Forsythe, 2010). Adults have a tendency to spawn at the same time in the season over multiple years (*i.e.*, spawning time is repeatable, Forsythe, 2010). Lake sturgeon do not provide parental care post-hatch, which contributes to extremely high mortality during early life stages (Caroffino *et al.*, 2010; Forsythe, 2010). Low survival rates together with delayed maturity partly contribute to low natural recruitment into lake sturgeon adult populations.

Numerous lake sturgeon populations have been severely depleted over the past 100 years throughout the Great Lakes (Houston, 1987; Hay-Chmielewski and Whelan, 1997). Therefore, lake sturgeon has been a species of conservation concern (Holey *et al.*, 2000; Peterson *et al.*, 2007). Despite decades of conservation actions, abundance of many lake sturgeon populations has remained low, in part due to anthropogenic factors resulting in fragmented habitats and degraded water quality (Peterson *et al.*, 2007). Rehabilitation programs have been impeded by lack of knowledge regarding the species reproductive ecology (Holey *et al.*, 2000). Although the species has been studied extensively regarding spawning habitat, population abundance and distribution, knowledge with regards to factors contributing to behavior and survival during early life stages and aspects of adult behavioral ecology during reproduction is less well understood. Information with respect to the species mating system, effective population

size and roles of adult spawning behavior to offspring behavioral traits at the individual level has been limited. These topics are difficult to study by direct observations alone.

Indirect methods, using information of pedigrees based on molecular genetic markers, have been powerful tools and widely used to study evolutionary biology and behavioral ecology (Avise, 2004; Garant and Kruuk, 2005; Pemberton, 2008). Applications include estimation of effective population size, characterization of mating systems and of parental effects on offspring phenotype, behavior and survival in natural populations. Parentage analysis and genetic relatedness are important approaches that facilitate pedigree reconstruction (Pemberton, 2008). Different statistical methods and software employing multi-locus genotypes have been developed to identify parentage (review in Jones *et al.*, 2010) and sibship reconstructions (Wang, 2004; Wang, 2007a; Wang and Santure, 2009a). In my dissertation research, I used genetic approaches based on microsatellite markers to study the effects of reproductive variables (*i.e.*, body size and spawning behavior, which included choice of the time and location for spawning) and environmental factors on adult reproductive ecology and larval dispersal of lake sturgeon.

My dissertation is in five parts. Chapter 1 examines temporal and spatial variation in the mating system of lake sturgeon across seven years (2001-2007). A mating system is characterized by linear regression of the number of offspring (reproductive success) and the number of mates (Bateman, 1948; Arnold and Duvall, 1994; Levitan and Petersen, 1995). Lake sturgeon have been known to be polygamous based on direct observations (Bruch and Binkowski, 2002). However, the number of mates and reproductive success can vary due to spatial and temporal synchrony of spawning, variation in adult

abundance, and operational sex ratios (Emlen and Oring, 1977; Arnold and Duvall, 1994; Levitan and Petersen, 1995), which is known to vary year-by-year in lake sturgeon populations (Auer, 1999; Forsythe, 2010). I quantified how the relationship of reproductive success and mate number fluctuated across years. I also tested whether reproductive success and mate number were independent of body size, spawning time and spawning location. Findings under this objective are important to further studies testing possibility of reproductive isolation by time within this lake sturgeon population. If variation in individual reproductive success was a function of spawning time, which has been shown to be repeatable (Forsythe, 2010), natural selection acting differently during breeding seasons could result in reproductive isolation (Hendry *et al.*, 1999; Hendry and Day, 2005; Tomaiuolo *et al.*, 2007). Estimating reproductive success also provides parameters to facilitate estimation of effective number of breeding adults.

In chapter 2, I tested if levels of genetic differentiation between early and late spawning adults existed as predicted based on observations of adult spawning time over 9 years (2001-2009). Forsythe (2010) suggested that the population was composed of different reproductively isolated groups. This suggestion was based on the observations that spawning activity was bimodal or multi-modal and that spawning time of individuals repeatedly captured in two or more years was highly repeatable. Measures of genetic differences and relatedness among groups spawning at different times within a year and across years provided a quantitative means of evaluating this hypothesis.

In chapter 3, I estimated effective number of breeders (N_b), effective population size (N_e), and degrees to which inter-annual variation in N_b correlated with adult demographic characteristics. N_e and N_b are important parameters describing evolutionary

processes of finite populations (Palstra and Ruzzante, 2008; Charlesworth, 2009), especially small and isolated populations such as the Black Lake lake sturgeon population. In this population, larval collection, a surrogate measure of total annual larval production, varied 40-fold across 7 years (2001-2007) but the number of adult was fairly constant (115-223 individuals). Based on literature that N_b is sensitive to adult census size (Frankham, 1995; Nunney, 1996) but varies less due to standardized variance (*e.g.*, variance/(mean)^2) in female fecundity (Nunney, 1996) , and given available information of adults and larvae of the lake sturgeon population in Black Lake, I predicted that N_b and N_b/N of the population would be relatively stable and would not vary proportionally with the inter-annual variation of offspring collected at the larval stage. I also estimated contemporary N_e and compared N_e and N_b. This study is one of only a few empirical studies showing the relationship of N_e and N_b of a long-lived iteroparous species.

In chapter 4, I examined the relative importance of environmental factors (water temperature and flow) and maternal effects (female body size, location and timing of spawning) on embryonic and larval developmental time until dispersal (ELDTUD). ELDTUD includes three periods: (i) embryonic development, (ii) yolk sac absorption; and (iii) emergence and dispersal from spawning areas. The first two periods are likely dependent on water temperature (Wang *et al.*, 1985; Pepin *et al.*, 1997; Kamler, 2002). The third period may depend more on other environmental factors (*e.g.*, river discharge, food, and predators), larval age or size (Elliott, 1987; Day and Rowe, 2002) and larval behavior (Shanks, 2009), lunar cycle effects on larval concealment (Hernandez-Leon 2008), and female spawning behavior (Copp *et al.*, 2002; Hogan and Mora, 2005;

Shanks, 2009). If effects of environmental factors on ELDTUD outweighed maternal effects, the latter would not significantly contribute to the explanation of variation in ELDTUD. If these effects were relative important, they both would be in the best set of models explaining ELDTUD.

The last chapter tests a hypothesis that environmental factors randomly affected offspring survival among families. I examined whether larvae from different females exposed to the same environmental conditions during dispersal differed in relative levels of mortality. We estimated proportional contributions of females to larval lake sturgeon collections and relative larval loss among females as larvae dispersed downstream during two consecutive nights. Analyses were based on genetically determined parentage. Data pertaining to inter-family variation in contributions to larval dispersal and the relative roles of maternal and stream environmental effects on larval survivorship during dispersal can lead to greater understanding of inter- and intra-annual variation in fish recruitment.

Findings from this work will greatly improve understanding adult reproductive ecology, roles of parental behavior (timing and location of spawning) under interactions with environmental factors on timing to dispersal, and survival of lake sturgeon at critical early life stages. The information will be also useful for restoration programs for this threatened species.

Chapter 1

MATE NUMBER AND REPRODUCTIVE SUCCESS OF MALE AND FEMALE LAKE STURGEON IN BLACK LAKE, MICHIGAN

ABSTRACT – Mating systems of polygamous fishes are dynamic and often difficult to study based on direct observation alone. We used genetic parentage assignment to examine temporal variation in the reproductive success and effective mate number in lake sturgeon (*Acipenser fulvescens*) in a closed population in Black Lake, Michigan. Adults (N= 611 individuals captured 1,024 times) and larvae (N=3,566) were sampled during the entire spawning season during each of 7 consecutive years (2001-2007). Reproductive success (RS) and effective mate number (EMN) differed between sexes and varied greatly among individuals of each sex across years. However, in all years, we observed a similar linear positive relationship between RS and EMN for males and females. RS and EMN were independent of body size in females but RS and EMN of males increased with increasing body size. No evidence of assortative mating by size was observed. Spawning time and location did not affect female RS or mate number. Mating pairs between individuals that arrived at spawning areas $\geq$8 days apart accounted for 30% of the total number of mating pairs on average across years. These data suggest that direct observations documenting the existence of discrete and temporal isolated spawning groups inaccurately represent the degree of gene flow among spawning groups. Collectively, data provide insights regarding factors including male mating behavior that contribute to inter-individual variance in RS the degree of sub-population genetic structure, and effective population size of this lake sturgeon population.

INTRODUCTION

Polygamous mating occurs in many taxa including mammals, birds, reptiles, amphibians, fish and insects (Briton *et al.*, 1994; Hernaman and Munday, 2007). Multiple mating could be adaptive for both females and males (Verner and Willson, 1966). Males that mate with a large number of females have more offspring than males that mate with comparatively fewer females (Slagsvold and Lifjeld, 1994; Hasselquist, 1998). Multiple matings by females may increase genetic diversity and genetic quality of offspring (Jennions and Petrie, 2000; Neff and Pitcher, 2005). Non-genetic benefits such as parental care, promoting egg maturation and oviposition, decreasing the risk of predation, and increasing access to mates thereby increasing probabilities of egg fertilization could be important factors favoring females that mate with multiple males (Reynolds, 1996; Jennions and Petrie, 2000). For broadcast-spawning species, sperm limitation is the major factor affecting female reproductive success (Levitan and Petersen, 1995; Franke *et al.*, 2002; Marshall and Evans, 2005).

In complex mating systems of polygamous mating fish species (Kokita and Nakazono, 1998; Avise *et al.*, 2002), inter-individual variation in mate number and reproductive success (RS) can be affected by many factors. Within a population, the number of mates can be altered by distribution and abundance of potential mates and availability of essential resources (*e.g.*, food and spawning locations) (Verner and Willson, 1966; Emlen and Oring, 1977; Hernaman and Munday, 2007). The distribution and abundance of one sex can vary over time and space (Shuster and Wade, 2003) due to differences in timing of arrival between males and females (*e.g.*, longnose filefish, *Oxymonacanthus longirostris* (Kokita and Nakazono, 1998); steelhead *Oncorhynchus*

mykiss (Seamons *et al.*, 2004)) or due to differences in spawning intervals between males and females. As a result, prior to fertilization, temporal and spatial variation in sex ratios and abundance can influence the number of mates and reproductive success.

During and after fertilization, factors that influence probabilities of egg and offspring survival also affect adult reproductive success, including parental effects and environmental conditions (Kamler, 2005). Maternal effects such as phenotypic traits (*e.g.* body size) and female age on offspring growth and survival have been documented (Chambers and Leggett, 1996; Trippel *et al.*, 1997; Heins *et al.*, 2004a; Kamler, 2005). For example, in numerous fish species where larvae rely on endogenous yolk reserves for growth and survival immediately after hatch, female body size is positively related to egg size, larval size and larval resistance to starvation and predation (Kamler, 2005). Paternal effects also contribute to offspring survival during embryo development stage (Trippel and Neilson, 1992; Kamler, 2005). For instance, positive relationships between sperm density and/or motility and egg fertilization rates were reported in several fish species such as bluehead wrasse *Thalassoma bifasciatum* (Petersen *et al.*, 2001) and bluegill sunfish *Lepomis macrochirus* (Neff *et al.*, 2003). Moreover, choice of spawning time and spawning locations by females will determine the environmental conditions that eggs and larvae initially experience (Einum and Fleming, 2000; Hendry and Day, 2005; Jorgensen *et al.*, 2008), and therefore can affect RS.

In broadcast-spawning species, the number of mates and reproductive success of males and females indicated by fertilization success are strongly density-dependent (Moller and Legendre, 2001; Rowe and Hutchings, 2003). When a population is at low abundance, male RS will likely decrease due to reduced mating opportunities (Allee

effects) (Moller and Legendre, 2001; Rowe and Hutchings, 2003; Levitan, 2004). Female RS is also expected to decrease due to sperm limitation (Levitan and Petersen, 1995; Levitan, 2004; Marshall and Evans, 2005). At high population densities, female reproductive success can also decrease due to polyspermy (eggs simultaneously fertilized by more than one sperm become inviable) as a result of sperm competition (Franke *et al.*, 2002; Levitan, 2004). Furthermore, environmental factors, especially water current, strongly influence fertilization rates of broadcast spawning species (Coma and Lasker, 1997; Petersen *et al.*, 2001; Marshall and Evans, 2005). Spawning synchrony and availability of mates are important for these species to increase fertilization rates and ultimately reproductive success (Emlen and Oring, 1977; Levitan and Petersen, 1995; Coma and Lasker, 1997).

Lake sturgeon *Acipenser fulvescens* is a broadcast-spawning species characterized by extreme longevity (potential > 100 years), iteroparity (Auer, 1999) and polygamous mating by both sexes (Bruch and Binkowski, 2002). Observational studies describing spawning behavior of this species indicated that lake sturgeon spawn in groups where one female can be surrounded by several males and each male can mate with multiple females (Bruch and Binkowski, 2002). Spawning activity occurs during the day or night shortly after ovulating females arrive at spawning sites. During a spawning bout, eggs and sperm are released simultaneously into the water column. Egg deposition by each female typically lasts only 8-12 hours (Bruch and Binkowski, 2002). Similar to other broadcast-spawning species (DeWoody and Avise, 2001), lake sturgeon do not provide post-ovulatory parental care (Bruch and Binkowski 2002). Egg and larval lake sturgeon experience high rates of mortality (*e.g.*, survival rates from eggs to age-0 juvenile stage <

0.1%) (Caroffino *et al.*, 2010; Forsythe, 2010). Extremely high mortality may result in high variation in RS among individuals (Caroffino *et al.*, 2010).

Other aspects of lake sturgeon reproductive ecology can contribute to the variation in mating behavior over time and space. Males mature earlier (12-15 years) and exhibit shorter inter-spawning intervals (1-4 years) than females (> 18 years and every 2-7 years, respectively) (Auer, 1999; Forsythe, 2010). Accordingly, sex ratios of spawning groups of lake sturgeon are usually male-biased, and sex ratios can vary by year and among spawning locations within a population (Auer, 1999). In addition, spawning aggregations of lake sturgeon entering spawning areas during early and late times of the spawning season correspond to different water temperatures and discharge. Adults captured during >2 years exhibit high repeatability in spawning time (Forsythe, 2010). Therefore, differences in spawning adult abundance, operational sex ratios, and individual composition together with differences in environmental factors during a spawning season may result in within-year variation in the number of mates and reproductive success. Differences in spawning adult abundance and sex ratios characterizing early and late spawning groups may be consistent across years, potentially resulting in predictable variation in the number of mates and reproductive success across years.

Mating systems of fish species are difficult to study using direct observations alone, especially in species without parental care (DeWoody and Avise, 2001; Avise *et al.*, 2002; Serbezov *et al.*, 2010), like lake sturgeon. DNA-based parentage inferences provide an opportunity to increase understanding of reproductive strategies and aspects of the mating system of fishes (DeWoody and Avise, 2001; Avise *et al.*, 2002). Different

statistical methods employing multi-locus genotypes have been developed to provide inferences about maternity, paternity (DeWoody and Avise, 2001; Jones *et al.*, 2010) or sibship reconstruction (Wang, 2004, 2007b; Wang and Santure, 2009b).

Our first objective was to estimate and compare variance in male and female RS (the number of larvae assigned to each individual) across years and determine whether RS was positively related to effective mate number, EMN (the number of mates which an individual was assigned the same offspring with). The second objective was to examine whether male and female RS and EMN varied as a function of body size, spawning time, and spawning location. The magnitude of inter-individual variation in RS would provide evidence for reinforcement of reproductive isolation among early and late spawning groups (Hendry *et al.*, 1999; Hendry and Day, 2005; Tomaiuolo *et al.*, 2007). The last objective was to test whether lake sturgeon exhibited random mating with regards to body size and capture time.

METHODS

Study site

Our study was conducted in the Upper Black River (UBR), the largest tributary to Black Lake, Michigan, USA (latitude 45^{o} 43'N, longitude 84^{o} 15'W; Figure 1.1a) and only location used for spawning by lake sturgeon in the drainage. The lake sturgeon population in Black Lake is isolated from other populations in adjacent lakes by dams blocking immigration and emigration from Lake Huron (Smith and Baker, 2005). Adults spawn over a 1.5 km section of the UBR. This section can be divided into six locations of spawning activity that were used across years (Figure 1.1b; Forsythe, 2010). Shallow spawning areas (~1 m in depth) and low turbidity allowed most adults to be observed and

captured (Crossman, 2008; Forsythe, 2010) and larvae dispersing from all spawning areas to be collected (Smith and Baker, 2005).

Sample collection

We collected adults and larvae throughout the spawning season during 7 consecutive years (2001-2007). Sampling for adults was conducted by wading the entire length of the stream encompassing all spawning sites one or more times per day during the entire spawning seasons. We captured spawning adults using long-handled nets. A dorsal fin clip (~ 1 cm^2) was taken for genetic analysis. Sex of adults was determined by extruding gametes, and all individuals were measured for weight (kg) and fork length (cm). We also recorded date and location of capture. Based on observations that lake sturgeon females spent only a few days on the spawning grounds (Forsythe, 2010), date and location of capture were assumed to be the date and location of spawning for females. For males, spawning behavior varied in response to the number and location of spawning females (Forsythe 2010). Accordingly, the time and location of male capture were not considered to accurately represent the time and location of spawning.

Larval sampling was conducted each year starting approximately 10 days after the first spawning event and lasted for 25-40 days, until no larvae were captured for 2 consecutive nights. Larval sampling was conducted at night (2100 to 0200 hrs) when the vast majority of larvae disperse (Auer and Baker, 2002). The sampling site was approximately 2 km downstream from the spawning areas (Figure 1.1a). Five D-frame larval nets were evenly spaced across the river channel (description in Smith and King, 2005) and were checked hourly from 2100-0200 hrs. Net locations remained consistent throughout the sampling period. In some years (2005 - 2007), larvae collected were

transferred to a streamside hatchery where they were reared for several months. Before the fish were released, a fin clip was collected from each individual for genetic analysis. Mortalities during the rearing periods were also preserved in ethanol 95%. Samples used for microsatellite genotyping were selected proportionally by capture night from the total number of preserved fin clips and mortalities of each year. The proportion of the total number of larvae captured for microsatellite genotyping varied across years (Table 1.1). However, during each year the larvae genotyped represented proportionally a random subset from the collection of entire larval collection for a given year.

Genetic analysis

DNA was extracted from fin clips using the QIAGEN DNeasy$^{(R)}$ kit (QIA Inc.). DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc.). All samples were genotyped at 12 tetra-nucleotide microsatellite loci including Spl 120 (McQuown *et al.*, 2000); AfuG 68B (McQuown *et al.*, 2002). 2002); Aox 27 (King *et al.*, 2001); AfuG 68, AfuG 9; AfuG 63, AfuG 74, AfuG 112, AfuG 56, AfuG 160, AfuG 195 and AfuG 204 (Welsh *et al.*, 2003). Using polymerase chain reaction (PCR), 100 ng DNA was amplified in 25 μl reaction mixtures containing 2.5 μl of 10 X PCR buffer (1 M tris-HCl, 1.5 M $MgCl_2$, 1 M KCl, 10% gelatin, 10% NP-40, and 10% triton X); additions of 1 mM $MgCl_2$ (1.5 mM $MgCl_2$ for AfuG 9) for all reactions but no additional $MgCl_2$ for AfuG 63, Aox 27 and AfuG 74; 0.8 mM deoxy-nucleotide-triphosphate (dNTP); 10pmol fluorescently labeled forward and unlabeled reverse primers and 0.5 U of Taq polymerase.

All PCR reactions were conducted using a Robocyler 96 thermal cycler (Stratogene). The PCR conditions were 94^{o}C for 2 minutes, followed by 30 cycles of 1

minute for primer-specific annealing temperatures (48^{O}C for AfuG 9, AfuG 63 and AfuG 112; 50^{O}C for AfuG 74; 53^{O}C for Aox 27; 56^{O}C for AfuG 68 and AfuG 68B; 58^{O}C for AfuG 56, AfuG 160 and AfuG 195; and 62^{O}C for Spl 120 and AfuG 204), 72^{O}C for 1 minute, and the final extension for 2.5 minutes at 72^{O}C. PCR products were run on 6% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO II scanner. Allele sizes were determined independently by two experienced personnel based on commercially available standards (MapMarker^{TM}, Bioventures Inc.) and samples of known genotype. Errors in genotyping were empirically checked by blindly re-genotyping a random 10% of all samples. Reported genotyping error is the ratio between observed number of allelic scoring differences and total number of alleles compared (Bonin *et al.*, 2004).

Parentage analysis

There are numerous programs that utilize multi-locus genetic data to estimate parentage (*e.g.*, CERVUS (Kalinowski *et al.*, 2007); PASOS (Duchesne *et al.*, 2005); COLONY (Jones and Wang, 2010)), and the efficacy of use of different programs or combinations of programs has been widely debated (Christie, 2010; Jones *et al.*, 2010; Walling *et al.*, 2010). Use of multiple programs which are based on different statistical properties to determine parentage has been advocated (Lee, 2008; Jones *et al.*, 2010). We used complimentary aspects of two programs, (1) the Parentage Allocation of Singles on Open Systems (PASOS) program, version 1.0 (Duchesne *et al.*, 2005) and (2) CERVUS version 3.0 (Kalinowski *et al.*, 2007) to conduct parentage analysis. Output of putative

parent-offspring allocations from the two programs were jointly used to increase parental assignment accuracy.

We used program PASOS to estimate the proportion of adults captured and parentage allocation correctness. PASOS can detect missing parents when they have not been collected based on multi-locus genotypes of both parents and offspring. We set the maximum number offset tolerance (MOT), the maximum number of offsets between a parental and an offspring allele that PASOS accepts as possibly due to a scoring error, of 2 and the error model (0.002, 0.008, 0.98, 0.008, 0.002 – this is the assumed transmission probabilities from a parental allele to an offspring allele with –2, –1, 0, +1, + 2 offsets due to scoring errors) for simulations in PASOS (Duchesne *et al.*, 2005). We conducted simulations over 5 iterations of 1000 pseudo-offspring to estimate the allocation correctness. Under the same MOT (MOT = 2) as conducting simulations, we used the allocation function in PASOS to assign each offspring to two collected parents.

CERVUS is the most commonly used categorical parentage technique (Christie, 2010; Jones *et al.*, 2010) and assigns progeny to a particular non-excluded parent based on likelihood scores (LOD scores obtained by taking the natural log of the ratio of likelihoods) derived from the genotypes of all offspring and parents sampled (Marshall *et al.*, 1998; Jones and Ardren, 2003; Kalinowski *et al.*, 2007). Simulations conducted within CERVUS to determine power and likelihood of assignment were based on empirical estimates of population allele frequency, the proportion of adults captured (PASOS output) and an empirical estimate of genotyping error (1.04%). Most likely candidate parent pairs of offspring from the assignment output were accepted as "true parent pairs" based on the criteria of ≥ 70% trio confidence with zero or 1 mismatch

between parents and offspring genotypes. Assignment outputs from the two programs based on criteria above were then compared. Male parent-female parent-offspring triplets that were consistently assigned by both programs were used in further analysis.

The allocation correctness (the probability that each allocation is correct) from PASOS was almost constant across 7 years, ranging between 80 – 83%, and standard deviation among iterations simulated was small (~1%). Meanwhile, CERVUS program provides a confidence level for each allocation based on user-defined confidence levels for cut off values. In order to choose a cut off value for 7- year data, we compared the concordance in assignment between PASOS and three levels of confidence levels in CERVUS including "the most likely", 80 and 95% using parent and larval genotypes in 2001 as an example. The result showed the "most likely" confidence provided the highest assignment rate and the percentage of assignment concordance (between PASOS and CERVUS) was as high as the 95% confidence. Therefore, we used "the most likely" criterion for other year data sets.

Statistical analysis

Effects of body size, spawning time and spawning location on reproductive success (RS) and effective mate number (EMN)

We first tested whether RS and EMN were different between males and females using ANOVA tests. Relative magnitude of variance compared to the mean of RS between males and females was evaluated based on standardized variance (variance/(mean)^2; Nunny 1996). We used a Wilcoxon rank sum test to compare standardized variance between males and females in 7 years. If RS and EMN differed between sexes, further analysis was conducted separately for males and females except

for the test of relationship between RS and EMN (below). We used linear mixed models and linear models to examine the relationship between RS and EMN and the relationship of each of these variables with body size (*i.e.*, fork length, FL), spawning time, and spawning location for females. For males, effects of spawning time and location on RS and EMN were not tested because of male behavior of moving different locations and spending long periods of time in the river (Forsythe 2010).

In the models examining factors contributing to RS and EMN, the effect of years in which sample sizes and parentage assignment rates were different was taken into account and treated as a fixed effect. Adult individuals were treated as a random effect because the same individual could be repeatedly sampled in several years. However, random effect of repeated spawning individuals was not significant and therefore individual was removed from all models that describe the relationship of RS (and the number of mates) and body size, spawning time, spawning location and the number of times of repeated spawning. Generally, models were as follows:

$$Y_{iyj} = \mu + \alpha_y + \gamma X_{iyj} + \beta_y X_{iyj} + \varepsilon_{iyj} \qquad (1)$$

where Y_{iyj} is the response variable (RS or EMN) of the i^{th} individual in year y and at the value j^{th} of another predictor; μ is the population mean (overall intercept) of a response variable; α_y is offset to overall intercept due to year, γ is the overall slope, β_y is the offset to the slope for a particular year.

The standardized spawning date (SSD) of an adult in a given year is the number of days its first capture was from the first day of the spawning season divided by the total duration of spawning activity in that year. SSD accounts for different durations of

spawning activity across years ($0 < SSD \leq 1$). Spawning locations were classified into 6 zones (Figure 1.1a; Forsythe 2010). All spawning zones were not used by adults in every year. Because lake sturgeon had a tendency to use upstream zones at early spawning season and downstream at late season (Forsythe, 2010), spawning location was treated as a continuous variable to evaluate the relative effects of upstream compared to downstream spawning location on the number of mates and RS. Effects of spawning locations on RS and EMN could differ during a spawning season; therefore, we also examined whether the slopes of the interaction between spawning date and spawning differed among years (3-way interaction of year-spawning date-spawning location was added in (1)).

We also tested whether the magnitude of difference in RS and EMN among individuals captured during $\geq$2 years was consistent across year (2001-2007). We used likelihood ratio tests (LRT) to compare the better of fit to the data between two models, (i) a random intercept model:

$$Y_{iy} = \mu + \beta_y X_y + \delta_i + \varepsilon_{iy} \qquad (2)$$

and (ii) a random slope model:

$$Y_{iy} = \mu + (\beta_y + \gamma_i) X_y + \varepsilon_{iy} \qquad (3)$$

where Y_{iy} is the response variable (RS or EMN) of the i^{th} individual in year y; μ is the overall intercept of a response variable; δ (in model 2) is random effect ($\delta \sim N(0, \sigma^2_\delta)$) of individuals that were captured during $\geq$2 years. β_y is the overall slope relating RS (or EMN) with year (X_y). In model (3), this slope could vary by the normally distributed random effect γ ($\gamma \sim N(0, \sigma^2_\gamma)$) of individuals captured during $\geq$2 years.

The better fit of model (3) compared to model (2) to the data would indicate inconsistency of differences in reproductive success among individuals across years.

Values of RS and the number of mates have skewed distributions that violate an assumption of normal distribution of model residuals and therefore can influence inferences on linear relationship between two variables. We transformed the response variables (Y_{ln}) by taking natural log of RS and the number of mates ($Y_{ln} = Ln(Y) + 0.5$). Residuals of models with log-transformed data were nearly normally distributed. Most analyses were consistent between transformed and original data. When the results were different between transformed and original data, the significance of a predictor was reported based on log-transformed data.

The relationship between RS and EMN of lake sturgeon as well as other polygamous species was predicted to be positively correlated (Arnold and Duvall, 1994; Andersson and Iwasa, 1996). We tested whether the slopes of this relationship differed between females and males using the data set of two sexes combined and individuals with zero offspring excluded. Similar to other analyses mentioned above, year effect was included in the model:

$$RS_{iys} = \mu + \alpha_y + \beta_s + \gamma M_{iys} + \eta_y M_{iys} + \delta_s M_{iys} + \lambda_{ys} M_{iys} + \varepsilon_{iys} \qquad (4)$$

where i is used as subscript for individual, y for year, and s for sex. RS_{iys} is reproductive success of the i^{th} individual from year y and sex s and $M_{iys}$is the number of mates for that individual. μ is the overall intercept, α is offset to overall intercept due to year, β is offset to intercept due to sex, γ is the overall slope, η is the offset to the slope for a

particular year, δ is the offset to the slope for a particular sex, and λ is the offset to the slope for the particular year and sex combination.

Testing assortative mating by size and by spawning time

We tested whether lake sturgeon had a tendency to choose mates of comparable size (evidence for assortative mating by size) or closer in spawning time (evidence of assortative mating by time) compared to random pairs. Random pairs included all possible pairs between males and females captured each year (*e.g.*, in 2001, there were 3,124 pairs from 71 males and 44 females). To test the null hypothesis of random mating with respect to body size, we first computed the ratio of fork length between female and male of each pair (L_f/L_m). We then compared the observed frequency distribution of L_f/L_m from mating pairs inferred from parentage assignment with the null frequency distribution of random pairs, using a G-test. We also tested the correlation of L_f/L_m and reproductive success of males and females using Pearson correlations.

We also used a G-test to test other hypotheses of random mating with respect to spawning time by comparing the observed frequency distributions of (absolute) differences in capture dates between females and males of mating pairs (identified from parentage assignment) with those from random pairs. In addition, we estimated the proportion of mating between and within groups of adults based on their spawning dates. We classified two individuals of a mating pair from within or between groups using two ways. First, based on spawning intervals among groups within a year (Figure 1.2), an individual was assigned as an “early” or “late” spawner. A mating pair was from a “within group” if males and females were captured at similar times (*e.g.*, early-early or

late-late). Secondly, based on differences in spawning dates between mates, we grouped a mating pair as "within group" if absolute difference in spawning date < 8 days. In 2001-2005, it was clear that two individuals spawning at 8 days apart were from different groups. In 2006-2007, although spawning activity was longer and comprised of less discrete groups, we also used the same criterion (shorter or longer than 8 days difference between two partners) as in the other years.

Because the time and location of the first capture of an individual was considered to represent spawning time and spawning location and given our observations that females spent few days at the spawning ground while male may remain for longer periods, differences in spawning dates between female and males were based on plasticity in male behavior.

RESULTS

Parentage assignment

Proportions of larvae assigned to captured adults from program CERVUS each year (69.9% - 91.2%) were comparable to larvae assigned from PASOS (64.3 -82.3) across 7 years (Table 1.2). Concordance in parentage assignment between the two programs ranged from 69.2% to 85.2% of larvae assigned based on a single program across 7 years. The percentage of larvae genotyped assigned concordantly from the two programs in 7 years was 46.5% to 67.6%. A high proportion of captured males (57-94%) and captured females (83-98%) was assigned offspring based on the two programs (Table 1.2).

Individual reproductive success and effective mate number across years

The number of offspring assigned (reproductive success, RS) to females (mean ±SE: 6.27 ± 0.94 across years) was significantly higher than that of males (mean ±SE:

2.75 ± 0.33) ($F_{1, 1017}$ = 123, P <0.001, Figure 1.3, top). However, standardized variance of RS was not significantly different between males and females (Wilcoxon rank sum test, n= 7, P =0.8). Within a year and for each sex, RS and EMN were highly skewed and varied widely among individuals as shown for 2007 (Figure 1.4).

Reproductive success for males and females varied across years (Figure 1.3, top). Effects of "year" explained 23.6% variation in RS for females and 25.6% for males. We evaluated relative success among adults captured more than 1 year to test whether adults that had higher RS in a given year also had more offspring in other years. The interaction between years and individuals resulted in better fit of model 3 compared to model 2 (LRT, df = 27, P <0.001), indicating that the difference in RS among individuals that were captured during ≥2 years was not consistent across years. Results were comparable for males and females.

Similar to results of variation in RS, variation in EMN was mainly explained by the effect of year (30.3% and 33.4% for females and males, respectively, Figure 1.3, bottom). The significant interaction between individuals and years (LRT, df = 27, P <0.001) indicated that differences in EMN among individuals were not consistent across years.

Relationship between reproductive success and effective mate number

Reproductive success of males and females was highly positively related to effective mate number (P<0.001). There was no significant interaction of sex and EMN on RS ($F_{1,1003}$ = 0.1, P = 0.76), indicating the relationship of RS and EMN for males and females was similar (slope ±SE: 1.38 ± 0.08 for female, 1.30 ± 0.08 for males) but intercepts differed. For each sex, the slope of RS and EMN relationship varied by year

(Mate-Year interactions: $F_{6, 302} = 14.9$, $P<0.001$ for females, $F_{6, 695} = 4.8$, $P<0.001$ for males). EMN, year and their interaction together explained 93.1% and 83.9% variation in RS, in which EMN alone explained 90.2 and 82.6% variation in RS of females and males respectively (Figure 1.5), indicating a strong relationship between RS and EMN.

Relationships of reproductive success and effective mate number and body size, spawning time and spawning location

Relationships between EMN and other variables including body size, spawning time and spawning location were similar to relationships of RS with these variables due to EMN and RS were highly related. Effects of body size on RS and EMN did not vary significantly among years in either sex (*e.g.*, year-fork length interaction for female RS: $F_{6,302} = 0.7$, $P =0.7$; for male RS: $F_{6, 695} =1.7$, $P = 0.1$). Larger adults had slightly higher RS (Figure 1.6) but this relationship was not significant in females (both original and transformed data, $P > 0.11$) but was significant for males (slope ± SE = 0.016 ± 0.009, $t_{1, 701} = 1.85$, $P =0.06$, transformed data $P <0.01$).

Similarly, larger males had higher EMN (slope ± SE = 0.017 ± 0.006, $t_{1, 701} =2.5$, $P =0.02$, transformed data $P <0.01$) than smaller males. For females, the positive relationship of EMN and body size was not significant (slope ± SE = 0.04 ± 0.03, $t_{1, 308} =1.54$, $P = 0.12$).

Female RS was not significantly related to either the 3-way interaction of spawning time, spawning location and year (transformed data, $F_{6, 287} =1.1$, $P=0.37$) or the interaction between spawning time and location ($F_{1, 287} =2.8$, $P=0.09$). Neither was the interaction predictive of EMN. There were no general patterns of female RS and EMN

during the spawning seasons and among locations (main effects of spawning time and location, all tests $P > 0.10$). Although the effects of spawning time and location on female RS and EMN varied among year (*e.g.*, year interaction with spawning time on EMN: $F_{6, 302} = 6.4$, $P < 0.01$ and with spawning location $F_{6, 302} = 3.5$, $P < 0.01$), RS and EMN were not significantly different among females spawning at different times (Figure 1.7) or locations in most years. Except in 2007, females spawning early and at upstream locations had higher RS and EMN than females spawning late in the season (for both RS and EMN: $F_{1, 61} \sim 20$, $P<0.01$) and at downstream ($F_{1, 61} \sim 7$, $P<0.01$), respectively (Figure 1.8).

Assortative mating by captured time

To test whether adults were more likely to mate with individuals in the same spawning groups relative to different groups, we compared the observed frequency distribution of (absolute) differences in capture dates between partners of mating pairs (inferred from parentage) to that of random pairs (random combination of males and females captured). The results revealed that the two frequency distributions were significantly different in 5 of 7 years studied (G-test, $P <0.01$, Table 1.3). Differences in capture dates between males and females of mating pairs ranged 0 – 24 days (Figure 1.9). Based on classifications of males and females into groups assigned based on date of capture, mating occurred between individuals of the same within spawning groups on average 69.4% of the time (range 53.6% – 92.3% over 7 years), and mating among individuals from different spawning groups occurred on average 30.6% of the total mating pairs each year (Table 1.3).

Assortative mating by size

Females are usually larger than males (Table 1.4). Therefore, the ratio of fork length between females and males of mating pairs were greater than 1, ranging 1.12 – 1.23. The observed frequency distributions of L_f/L_m for mating pairs was not significantly different from expected by chance in 5 of 7 years (G-test, Table 1.4), indicating that there was no trend of nonrandom mating with respect to body size. In 2001 and 2005, where L_f/L_m of mating pairs was significantly higher than expected by chance ($P < 0.01$ and $P = 0.012$, respectively; Figure 1.10), we tested whether this difference was related to reproductive success of males and females. However, individual reproductive success did not vary as a function of body size in either males ($F_{1, 69} = 0.89$, $P = 0.34$ in 2001, and $F_{1, 104} = 0.50$, $P = 0.48$ in 2005) or females ($F_{1, 42} = 0.003$, $P = 0.95$ in 2001, and $F_{1, 45} = 2.65$, $P = 0.11$ in 2005), and thus was not correlated with L_f/L_m ($r = -0.002$, $df = 256$, $P = 0.97$ for females, $r = 0.02$, $P = 0.74$ for males in 2001; $r = 0.10$, $df = 53$, $P = 0.47$ for females, $r = -0.07$, $P = 0.61$ for males in 2005). Given the lack of correlation between observed L_f/L_m of mating pairs and individual RS, differences in frequencies of L_f/L_m between mating pairs random pairs did not provide evidence for assortative mating.

DISCUSSION

Polygamous mating of lake sturgeon can vary temporally and spatially within a population. Using DNA-based inference of parentage based on data from 7 years, we could quantify the variation of and factors affecting reproductive success and mate number. Our findings showed a similar strong positive relationship between RS and mate

number for males and females. Within each sex, the most reproductively successful individuals in one year were not consistently more successful than other individuals. No consistent patterns emerged suggesting that RS and mate number of individuals spawning early in the season and at upstream locations differ from individuals spawning late in the season and at downstream locations. We also found that mating could occur between individuals from different spawning groups, indicating the Black Lake population was not composed of reproductively isolated groups as previously thoughts (Forsythe, 2010).

Relationship between reproductive success and effective mate number

The relationship between RS and EMN in polygamous species was predicted to be positively related in both sexes because of low reproductive investment (Arnold and Duvall, 1994; Andersson and Iwasa, 1996). This prediction was observed in lake sturgeon. For species with external fertilization like lake sturgeon, sperm availability affects egg fertilization and thus greatly contributes to female reproductive success (Levitan and Petersen, 1995; Franke *et al.*, 2002; Levitan *et al.*, 2004). Lake sturgeon females release eggs as sperm is released from multiple males (Bruch and Binkowski, 2002). Females which spawn when surrounded by a large number of males are likely to realize higher fertilization rates than females spawning in the presence of fewer males. Meanwhile, males increased their reproductive success by mating with as many females as possible (Levitan *et al.*, 2004).

Based on Bateman's principle (Bateman 1948), larger variance in RS and the stronger relationship between RS and mate numbers would be expected in the sex that invests less energy into reproduction compared to the other sex (Bateman, 1948; Arnold and Duvall, 1994; Andersson and Iwasa, 1996). In lake sturgeon, we did not observe

different slopes of RS on EMN between males and females, which was consistent with similar standardized variance of reproductive success between the sexes.

Factors affecting RS and EMN

Spawning synchrony and availability of mates in terms of abundance and operational sex ratios are important factors contributing to fertilization success and thus to the number of mates and reproductive success in both sexes of lake sturgeon as well as other broadcast spawning species (Emlen and Oring, 1977; Arnold and Duvall, 1994; Levitan and Petersen, 1995). Therefore, spawning time and spawning location could be related to RS and EMN. Lake sturgeon spawning early in the season were usually more numerous and spawned over longer periods compared to individuals spawning late in the season (Figure 1.2). RS and EMN were expected to differ by spawning time and spawning location. Specifically, RS and EMN were predicted to be higher for individuals spawning early in the season and at upstream locations compared to individuals spawning late in the season and downstream locations. This trend was observed only in 2007 but was not consistent in other years.

In addition to differences in the number of adults and the length of spawning periods between early and late portions of the season, environmental factors associated with spawning time and spawning location of adults can affect the probability of survival of fertilized eggs to larval stages, and thus could contribute to variation in adult RS and EMN at different spawning locations and during a spawning season. Environmental factors that can cause mortality of lake sturgeon in these early stages include biotic factors such as predation, microbial infection, food availability, *etc.*, and abiotic factors,

for example, water temperature and flow (direct or indirect effects via oxygen supply) (Kempinger, 1988; Kamler, 1992; Caroffino *et al.,* 2010; Forsythe, 2010).

Temperature and water flow in the Upper Black River, where lake sturgeon spawn, follow a seasonal pattern in which lower temperature and higher flow occur early in the spawning season compared to later in the season (Forsythe, 2010). Seasonal patterns of the number of adults and two important environmental factors (temperature and flow) was not reflected in variation in RS and mate numbers during the spawning season, implying that RS and EMN likely result from environmental stochasticity, as observed in other species (e.g, brown trout *Salmo trutta* (Serbezov *et al.*, 2010).

Adult body size is another attribute that was predicted to contribute to variation in RS and EMN. Similar to other fish species, body size and fecundity of lake sturgeon females are positively related (Bruch *et al.,* 2006). But high reproductive potential may not alone predict high RS. We found that RS and EMN of lake sturgeon females were independent of body size. A weak positive relationship between RS and body size was observed in lake sturgeon males. There could be two possible explanations for this result. First, larger males might have higher sperm quantity or/and quality (*e.g.,* guppy *Poecilia reticulata* (Skinner and Watt, 2007); lake whitefish *Coregonus clupeaformis (Blukacz et al., 2010),*). Alternatively, larger males might compete better for mates, leading to higher EMN and RS (*e.g.*, in Atlantic cod *Gadus morhua* (Rowe *et al.*, 2007); leopard gouper *Mycteroperca rosacea* (Erisman *et al.*, 2007)). We did not have evidence to distinguish these or other possiblities. In addition, because the relationship of body size and RS as well as EMN was weak in males and females given a large sample size across years, size was not a dominat factor in determining RS.

However, based on spawning behavior of lake sturgeon and findings from other similar group-spawning species we believe that male competition might be present among lake sturgeon males. For such species, larger males often have a better ability to position themselves in proximity to females during spawning, and thus may increase EMN and also RS (Petersen and Warner, 1998; Bekkevold *et al.*, 2002; Erisman *et al.*, 2007). In leopard groupers (*Mycteroperca rosacea*) for example, male - male competition occurs, where dominant males who occupy the closest position to females can fertilize more eggs and therefore increase higher reproductive success than peripheral males (Erisman *et al.*, 2007). In addition, male-biased of spawning groups observed in lake sturgeon (Figure 1.2) suggested an opportunity for male competition (Sadovy *et al.*, 1994; Hall and Hanlon, 2002).

Assortative mating by size and by capture time

Weak assortative mating by size occurs commonly in many species (Blachford and Agrawal, 2006). For broadcast spawning species, evidence of both nonrandom mating (*e.g.*, Atlantic cod *Gadus morhua* (Rowe *et al.*, 2007)) and random mating (*e.g.*, steelhead trout *Oncorhynchus mykiss* (Seamons *et al.*, 2004) with respect to body size has been reported. For lake sturgeon, we did not find correlations between male and female body size for mated pairs. Although in two years (2001 and 2005), the ratios of female fork length to male fork length (L_f/L_m) significantly differed between mating pairs (inferred from parentage) and random pairs (all possible pairs of males and females captured in a given years), the L_f/L_m from mating pairs was not correlated with male and female reproductive success, indicating no evidence of assortative mating to increase reproductive success. The difference in L_f/L_m between mating pairs and random pairs in

2001 and 2005 was not due to sex ratios or distribution of fork length of males and females because sex ratios (Table 1.1) fork length of adults (Table 1.4) and in 2001 and 2005 were similar with the other years.

Mating among individuals might be not random with respect to arrival time, especially for broadcast spawning species where spawning synchrony is an important determinant of RS and mate number (Emlen and Oring, 1977; Levitan and Petersen, 1995). We found that for lake sturgeon, the majority (~70%) of mating pairs were from males and females that were captured less than 8 days apart. Because ovipision of female typically lasts for only 8 – 12 hours (Bruch and Binkowski, 2002) or a few days (by observation of the time most females stayed in the spawning ground, Forsythe, 2010), mating between members from different groups is likely due to male behavior. The date of first capture was thus not representative of the spawning date for males. Males were frequently observed to remain at the spawning areas over prolonged periods, potentially increasing opportunities to mate with females arriving later in the season. We observed that 14.5% of males were recaptured in 2007, 7-16 days following the first capture. In other lake sturgeon populations, the same male behavior of prolonged occupancy of spawning areas facilitating mating opportunities with multiple females was also observed (Bruch and Binkowski, 2002).

Quantification of RS and EMN in this study was based on genetically identified parentage at the larval stage and different sample sizes across years. Thus, the slopes of RS on EMN may depend on sample sizes and life stages of offspring. Survival of offspring from larvae to later stages (e.g., juvenile, (Nichols *et al.*, 2003; Caroffino *et al.*, 2010)) can be very low, resulting in fewer offspring per family and EMN can be detected.

We acknowledged that the larger of sample sizes, the more offspring would be assigned and therefore the higher EMN would be detected (Figure 1.3). Accordingly, slopes and intercepts of the linear regression of RS on EMN for each sex would vary with different sample sizes. However, the differences in slopes of RS on EMN between two sexes, which imply levels of reproductive investment (Bateman, 1948; Arnold and Duvall, 1994; Andersson and Iwasa, 1996), were more interested. The relationship between RS and EMN for males and females was strong and the same slopes for both sexes were consistent even though sample size varied across years, indicating that estimates of RS and EMN and their inter-relationship in males and females was not influenced by sample sizes. Because the number of unassigned larvae varied across years (32 – 53%), results could be biased. However, based on more relaxed criteria of parentage assignment in which far greater proportions of larvae were assigned (60-80% across years), the general results did not change (data not shown). Thus, we felt that the proportion of unassigned larvae did not affect our conclusion.

Implications

Components of the mating system such as mate number, reproductive success, and mating behavior play an important role in population growth (Rowe and Hutchings, 2003) and population genetic structure (Johannesen and Lubin, 1999; Fagan *et al.*, 2010). Two important implications can be drawn from our findings quantifying aspects of the lake sturgeon mating system. First, RS among individuals was highly skewed, which likely resulted in lower effective population size compared to population census size. The magnitude of differences in RS among individuals captured during $\geq$2 years, however, was inconsistent across years, indicating that cumulative differences among individuals

would be decrease disproportionally with mean lifetime RS. Thus, there is an advantage of longevity and iteroparity with respect to maintaining higher effective population size. Compared to other species, variation in RS of lake sturgeon was similar. As summarized by Clutton-Brock (1988) and Nunney (1996), standardized variance in reproductive success from several examples of insect, amphibians, birds, mammals ranged from 0.08 – 1.35 (mean ± SD: 0.44 ±0.32), compared to that range of lake sturgeon, from 0.38 – 3.08. In fish (*e.g.*, steelhead *Oncorhynchus mykiss)*, variability index from several year-runs ranged from 1.2 – 6.5 (Araki *et al.*, 2007), compared to that of lake sturgeon from 1.1 - 10.8.

Second, male behavior leads to high incidence of mating between individuals from different groups. Further, we found no significant effects of spawning time on RS and EMN across years. These findings have important implications to the genetic structure of this lake sturgeon population. Matings between individuals in different spawning groups serves to maintain gene flow among groups spawning at different times, thereby decreasing the potential for genetic differentiation among groups within this population. Given that spawning time of lake sturgeon in this population is repeatable (Forsythe, 2010), if matings between members of different groups were limited, and if RS and EMN had consistently differed by spawning time, selection associated with environmental conditions at the time of reproduction could reinforce temporal isolation within this population (Hendry and Day, 2005). We found that no consistent trends of differences in RS and MS among females spawning at different times during the season across years were observed, supporting the idea that RS and EMN partly resulted from stochasticity of environmental conditions (Serbezov *et al.*, 2010).

This study increases our understanding of the degree of variation in aspects of the polygamous mating system of a broadcast-spawning species of conservation concern. Estimates of the proportion of adults contributing offspring to the larval stage and quantifying RS provides parameters necessary for the estimation of the effective number of breeders (N_b) and helps explain temporal patterns of N_b. Plasticity in male behavior with respect to duration of time spent in and near spawning areas is important for interpretation of degree of sub-population structuring in this lake sturgeon population.

Table 1.1. Sample sizes of adults captured, adult sex ratios and the number of lake sturgeon larvae captured and larvae genotyped in 2001 – 2007

Year	Number of adults captured				Number of larvae collected	Larvae genotyped	
	Male	Female	Total	Sex ratio (M:F)		Number	(%) of collection
2001	71	44	115	1.61	1,691	553	32.7
2002	71	33	104	2.24	1,320	136	10.3
2003	80	40	120	2.00	16,417	488	3.0
2004	76	25	101	3.04	437	241	55.2
2005	106	47	153	2.26	7,800	362	4.6
2006	162	63	225	2.57	5,587	342	6.1
2007	143	63	206	2.27	1,444	1,444	100

Table 1.2. Summary of parentage assignment estimated for single programs and based on concordance between two programs PASOS and CERVUS

Year	% adults assigned		% offspring assigned by			Final assignment	
	Male	Female	PASOS	CERVUS	Concordance (%)*	Number	% of larvae#
2001	90.1	90.9	78.7	87.9	73.6	320	57.9
2002	76.1	93.9	79.4	78.7	85.2	92	67.6
2003	80.0	87.8	73.4	69.9	69.2	236	48.4
2004	71.1	88.0	64.3	78.4	72.3	112	46.5
2005	57.5	83.0	82.3	89.5	72.1	225	62.2
2006	65.4	98.4	80.1	91.2	73.0	212	62.0
2007	93.7	88.9	74.7	85.5	81.5	879	60.9

Note: (*) % concordance was calculated as the percentage of the female-male-offspring triplets that were concordantly assigned by the two programs compared to the lower number of larvae assigned from one program.

(#) % of larvae finally assigned concordantly from the two programs.

Table 1.3. Percentage of mating pairs (inferred from parentage) within and between groups of adults spawning at different times of spawning seasons (2001-2007) based on two ways groups were classified. Frequency distributions of difference in spawning date between male and female of a pair were compared between mating pairs and random pairs (all possible pairs between males and females captured in a given year) using G-test (P-value provided)

Year	By group[a]		By spawning date[b]		P-value
	Within	Between	Within	Between	
2001	69.1	30.9	64.5	35.5	< 0.01
2002	61.3	38.7	61.3	38.7	< 0.01
2003	56.6	43.4	56.6	43.4	0.65
2004	83.3	16.7	92.3	07.7	0.17
2005	89.8	10.2	89.8	10.2	< 0.01
2006	50.3	49.7	53.6	46.4	< 0.01
2007	89.3	10.7	67.4	32.6	< 0.01

Note: (a) based on discrete spawning intervals among groups within a year (Figure 2.2), an individual was assigned as "early" or "late" spawner.

(b) based on differences in spawning dates between two partners of a pair, a mating pair was a"within" group" if absolute difference in spawning date < 8 days.

Table 1.4. Number of adults captured, fork length (mean ± SD (range)) and percentage of adults that were assigned to larvae sampled in 2001 -2007. Difference in L_f/L_m between mating pairs and random pairs was compared using G-test

Year	Males captured		Female captured		L_f/L_m of a pair		P-value
	n	FL (cm)	n	FL (cm)	Random pairs	Mating pairs	
2001	71	139 ± 13	44	156 ± 10	1.16 ± 0.14	1.23 ± 0.14	**<0.001**
2002	71	137 ± 11	33	153 ± 8.2	1.12 ± 0.12	1.12 ± 0.14	0.52
2003	80	136 ± 13	40	159 ± 10	1.19 ± 0.14	1.17 ± 0.13	0.21
2004	76	136 ± 14	25	156 ± 12	1.18 ±0.15	1.18 ± 0.13	0.54
2005	106	131 ± 14	47	157 ± 13	1.20 ± 0.16	1.23 ± 0.15	**0.024**
2006	162	136 ± 15	63	160 ± 11	1.19 ± 0.15	1.18 ± 0.15	0.95
2007	143	135 ± 14	63	158 ± 10	1.19 ± 0.15	1.18 ± 0.14	0.51

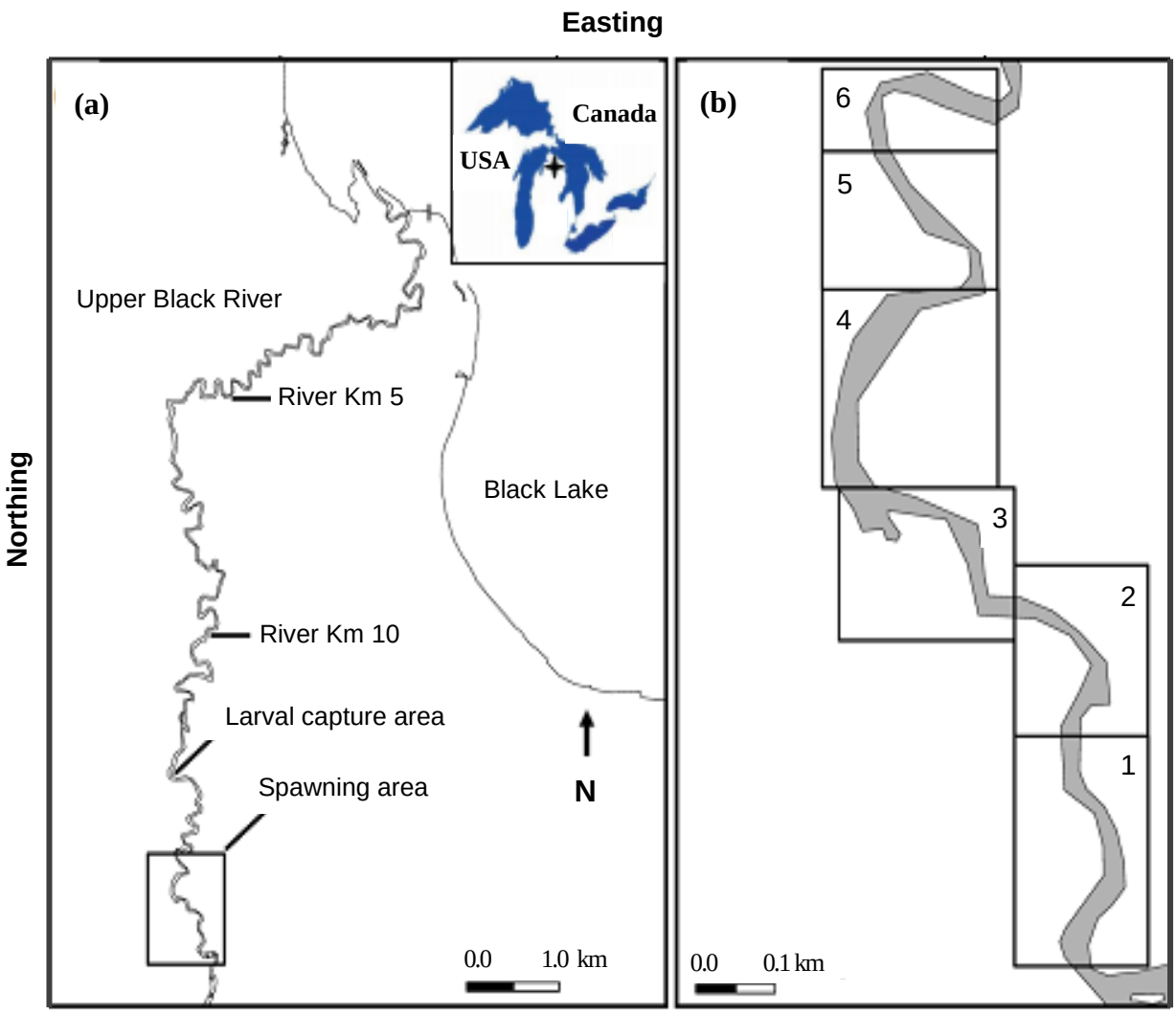

Figure 1.1. Study site on the Upper Black River (Michigan, USA), showing positions of adult lake sturgeon spawning areas and larval collection sites (a), and an enlarged view of the six spawning areas (b). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

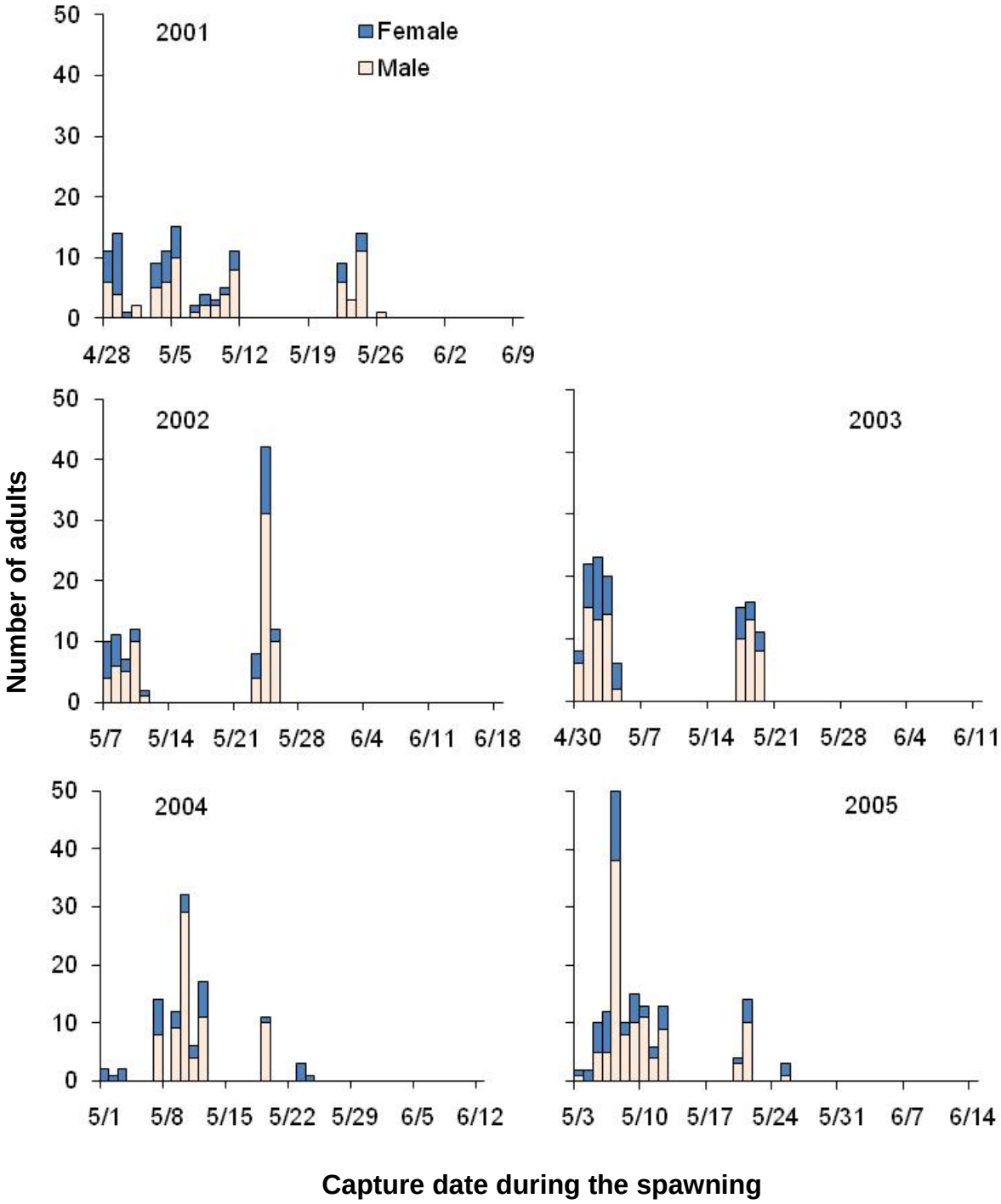

Figure 1.2. Number of adult males and females captured by day of the spawning season during 9 years (2001 -2009)

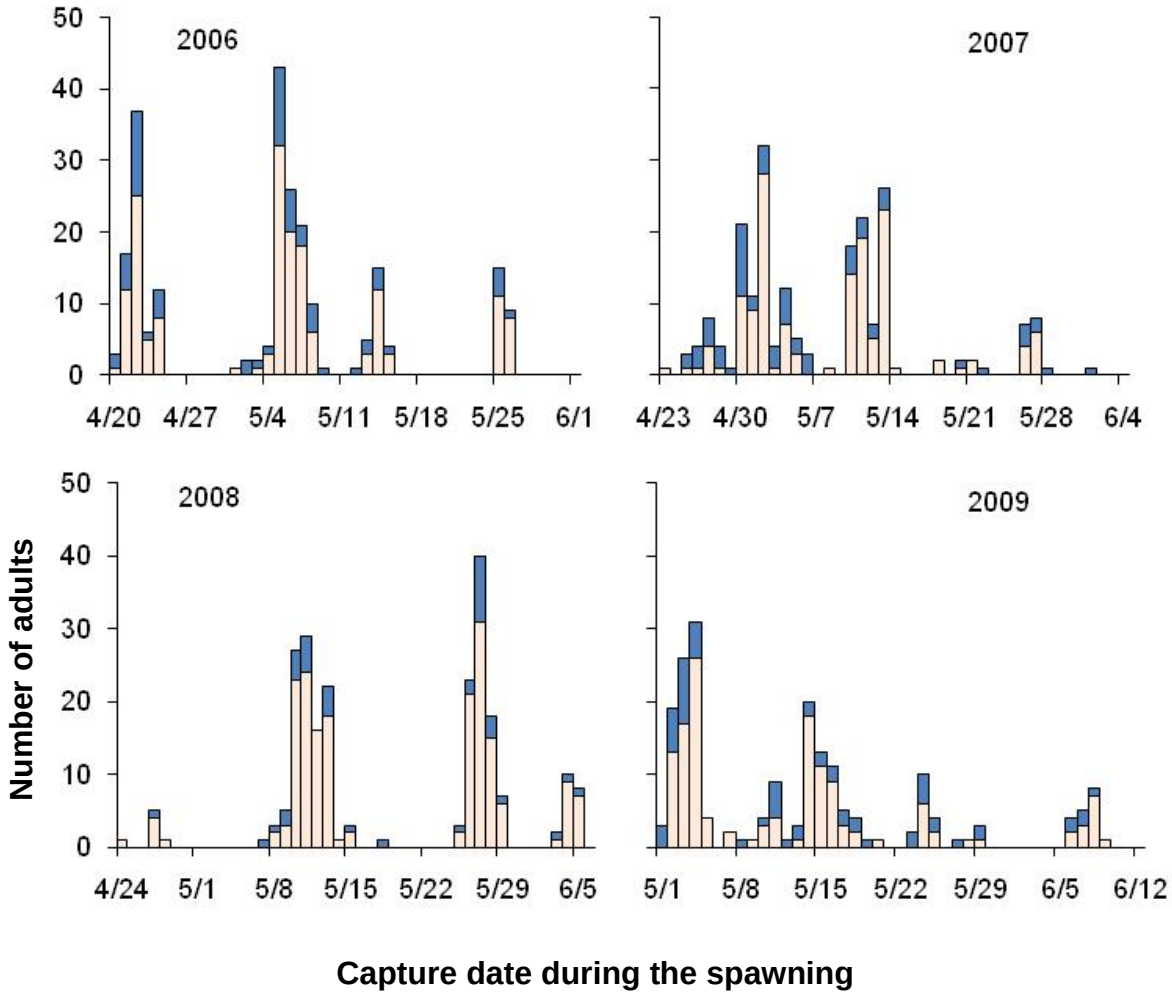

Figure 1.2. (cont'd)

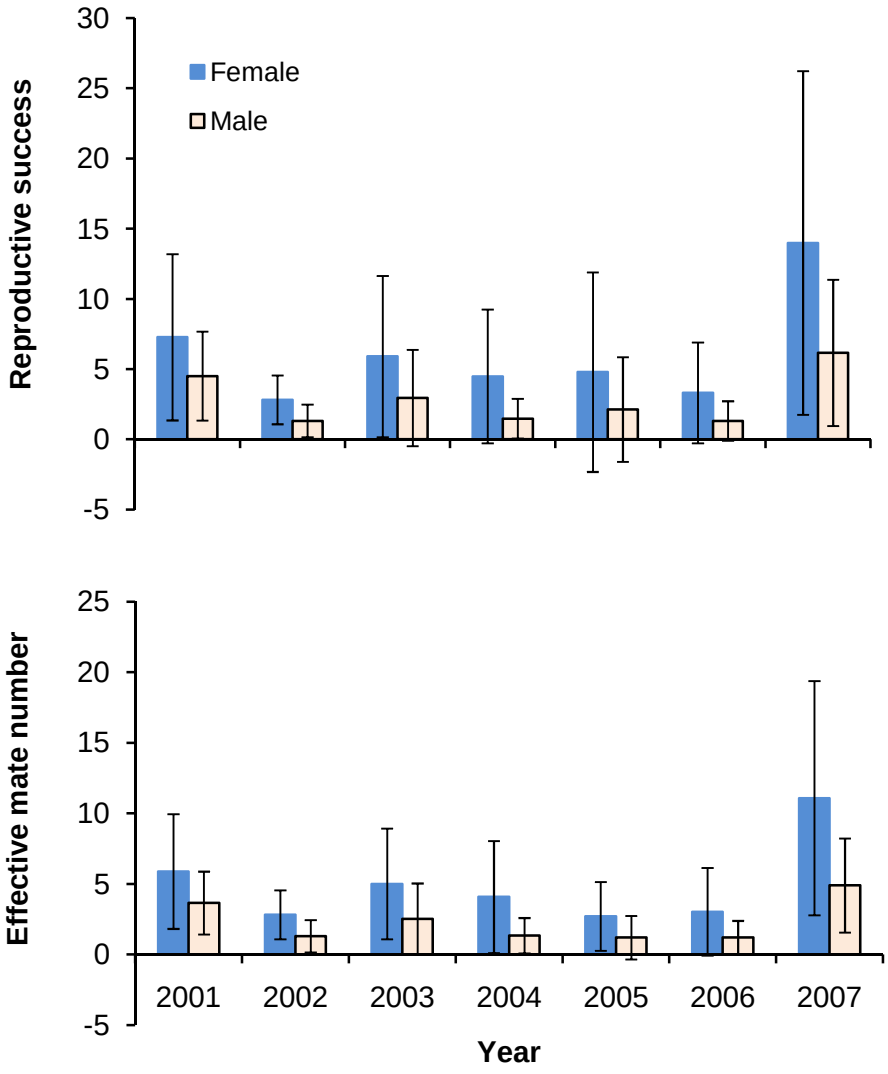

Figure 1.3. Reproductive success (the number of offpsring; top) and effective mate number (EMN; bottom) of males and females in each of 7 consecutive years (bars represent 1 standard deviation, SD).

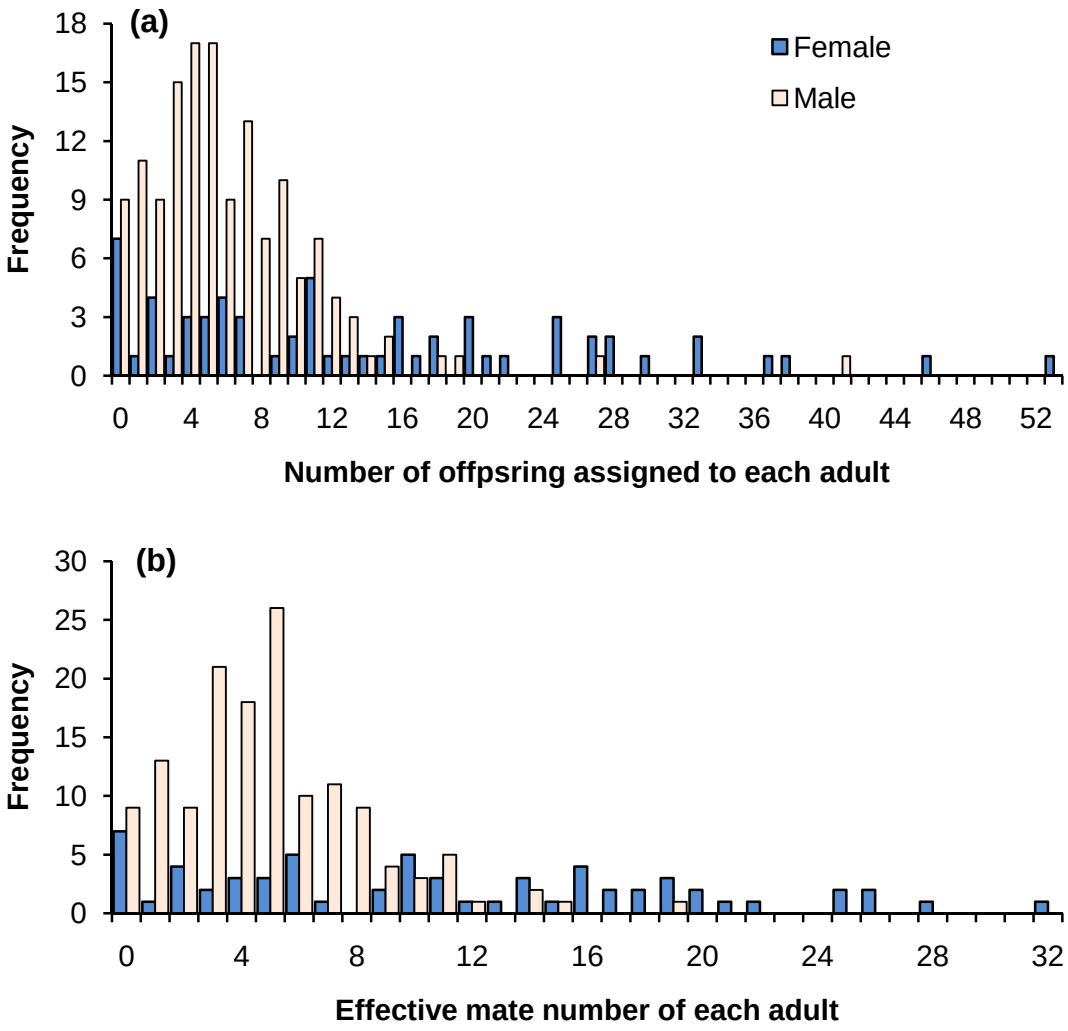

Figure 1.4. Distribution of the number of offpsring assigned to each individual (a) and effective mate number (b) of male and female lake sturgeon captured in 2007. Larvae were collected during the time larvae dispersed approximately 1.5 km downstream from spawnign areas (Figure 1.1a).

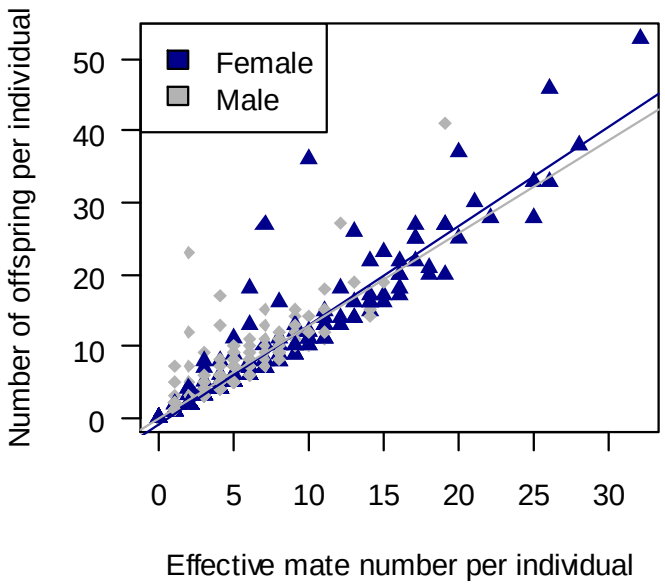

Figure 1.5. Relationship between number of offspring and effective mate number of males and females captured in 7 years (2001-2007)

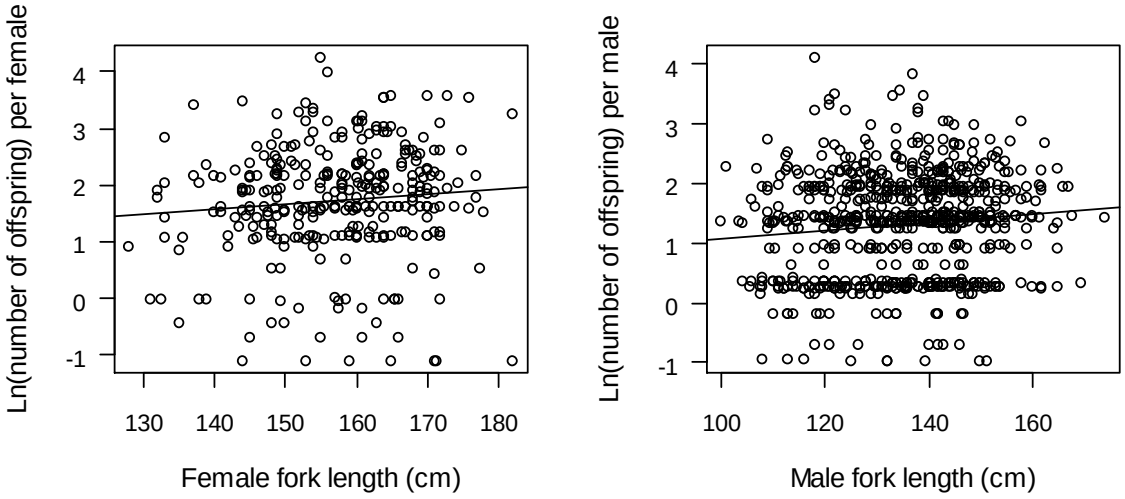

Figure 1.6. Relationship between measures of reproductive success and body size of males and females after adjusting for year effects.

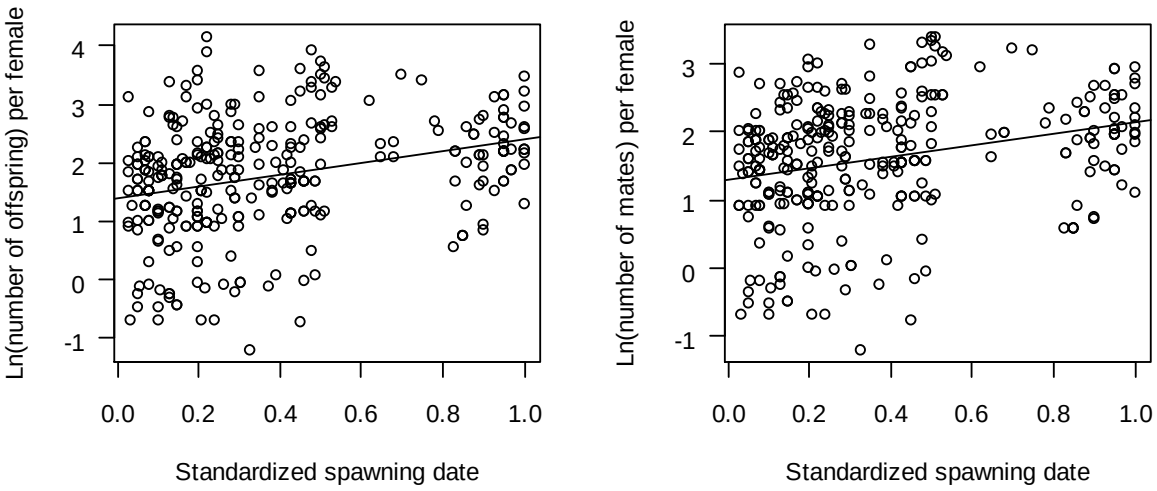

Figure 1.7. Relationship between reproductive success (left) and effective mate number number of mates (right) of females (expressed as natural logarithm) and standardized spawning dates across years, after adjusting for year and year-spawning date interaction (slope ± SE for reproductive success: 0.99 ± 0.53, P= 0.06; for effective mate number: 0.84 ± 0.49, P=0.08).

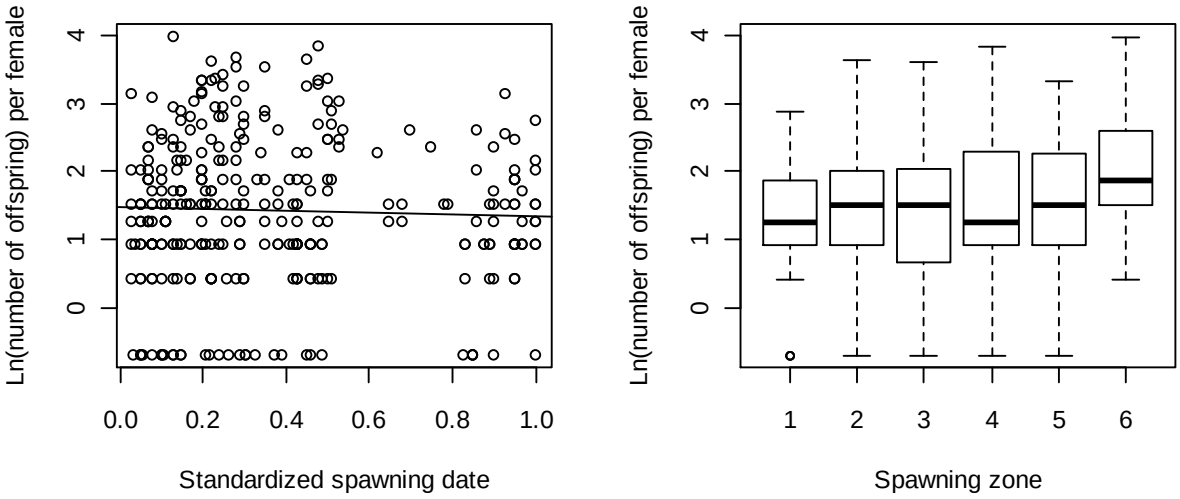

Figure 1.8. Reproductive success of females spawning at different times (left) and locations (right, bars represent the range excluding potential outliers) in 2007.

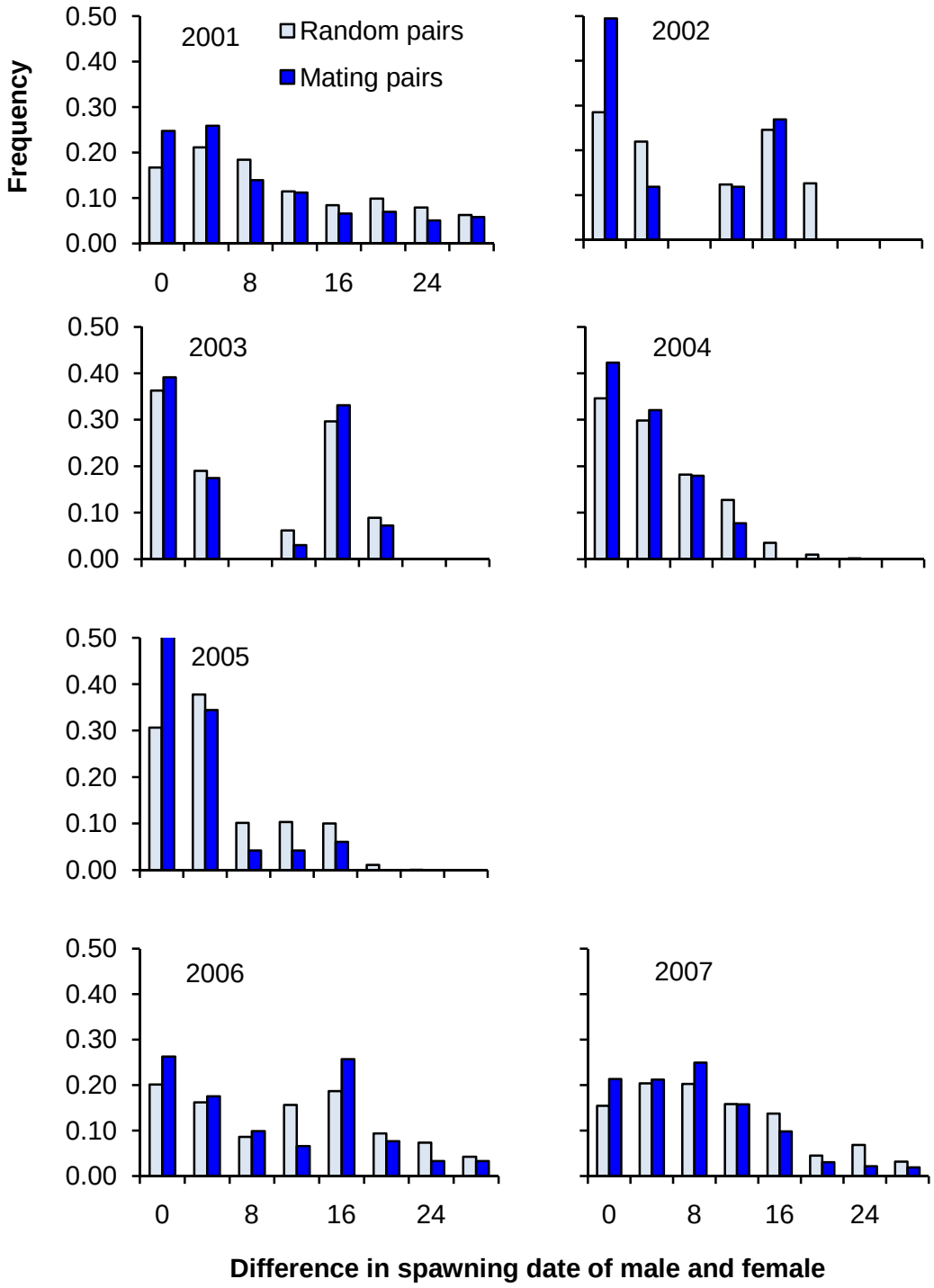

Figure 1.9. Frequencies of absolute pairwise difference in spawning date of random pairs and mating pairs in 2001-2007

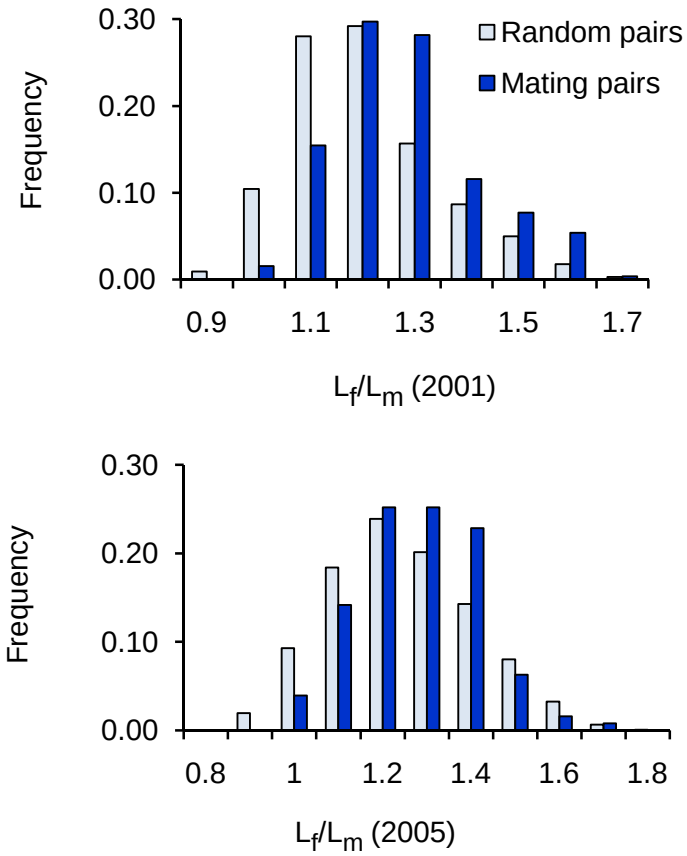

Figure 1.10. The ratios of fork length female to fork length male (L_f/L_m) of random pairs and mating pairs in 2001 and 2005.

Chapter 2

POPULATION GENETIC STRUCTURE AND RELATEDNESS OF LAKE STURGEON POPULATION IN BLACK LAKE, MICHIGAN

ABSTRACT – Lake sturgeon (*Acipenser fulvescens*), a long-lived iteroparous fish species, exhibit a multimodal temporal distribution of spawning activity, in which individuals repeatedly spawn at the same time across spawning seasons. If repeatability in spawning date has endured over generations, reproductive isolation within the population may occur, leading to high genetic correlation among individuals within relative to among members of different spawning groups. We tested this prediction for adults captured in the same year and for adults that were captured during more than one year over 2001-2009 in a closed population in Black Lake, Michigan, using genetic relatedness r_{xy} and variance in allele frequency (F_{ST}). We found that the population exhibited a low level of genetic structure associated with spawning time, as evidenced by significance of genetic differences and differences in mean relatedness between early and late spawning groups of adults captured ≥ 2 times over 9 years. However, evidence of isolation by time was lacking based on (i) small magnitude of F_{ST} and small differences in r_{xy} between groups of adults captured ≥ 2 years, (ii) no genetic differences among groups within 5 of 7 years; and (iii) no relationship between inter-individual genetic relatedness and inter-individual differences in spawning time. Based on male mating behavior inferred from genetically identified parentage for 7 years (2001-2007), we suggested that prolonged occupancy of spawning areas by males and plasticity in individual spawning time increased mating opportunities among individuals from early and late groups, thereby retarding temporal reproductive isolation.

INTRODUCTION

Groups of individuals within a population can become reproductively isolated and genetically structured as a result of two major mechanisms including isolation by distance (IBD) and isolation by time (IBT) (Hendry and Day, 2005; Maes *et al.*, 2006; Doctor and Quinn, 2009). Compared to IBD, which has been well-studied in species exhibiting homing behavior to natal areas (Taylor, 1991; Quinn *et al.*, 2000), IBT was less commonly reported. Most empirical examples of IBT have been shown in plant and salmonid species whose breeding times are heritable (Hendry and Day, 2005). Heritability of spawning time can limit gene flow between individuals that spawn at different times, especially early and late in the spawning season, leading to genetic differentiation within a population even when spawning activity is unimodally distributed (Hendry and Day, 2005; Doctor and Quinn, 2009). In addition, differences in spawning time can result in nonrandom mating or temporal assortative mating (Fox, 2003; Weis and Kossler, 2004; Devaux and Lande, 2008). Depending on the degree and duration that reproductive isolation is maintained, increases in genetic relatedness among individuals within groups can develop.

Degree of genetic structure within a population is influenced by interactions of factors associating with spawning time such as natural selection (Hendry *et al.*, 1999; Hendry and Day, 2005; Casagrande *et al.*, 2006; Tomaiuolo *et al.*, 2007), spawning behavior (Johannesen and Lubin, 1999; Fagan *et al.*, 2010), and variation in individual reproductive success (Maes *et al.*, 2006; Pujolar *et al.*, 2006). These factors can vary by time and interact with environmental conditions at spawning time generating underlying temporal genetic differences within populations (Calderon and Turon, 2010).

When environmental conditions associated with adult spawning time affect offspring fitness (Einum and Fleming, 2000), selection regimes that vary during the spawning season can result in differences in adaptation among temporal spawning groups within a population (adaptation by time, Hendry *et al.*, 1999; Hendry and Day, 2005). For example, seasonal variation in reproductive success has been reported in numerous species (*e.g.*, birds (Verhulst *et al.*, 1995); fish (Schultz, 1993)). Selection can also reinforce differences in spawning time based on adaptations of individuals to conditions at the time of spawning in sub-populations, thus further limiting effective gene flow among subpopulations (Fraser and Bernatchez, 2005).

In addition, individual movements during the spawning season can result in mating between individuals from different groups and likely plays an important role in determine the degree of genetic population structure (Johannesen and Lubin, 1999; Fagan *et al.*, 2010). In mammals, male-mediated gene flow resulting in genetic structure within and between populations has been well-documented (*e.g.*, mammals (Crawford *et al.*, 2009; Pilot *et al.*, 2010). In fish, especially for species with polygynous mating, male fitness is limited by mate availability and males can employ different strategies to find mates (Fagan *et al.*, 2010). In brook charr *Salvelinus fontinalis*, for example, males disperse among tributaries decreasing the degree of spatial isolation (Fraser *et al.*, 2004). Meanwhile, in other species such as lake sturgeon (*Acipenser fulvescens*), males can remain at spawning locations, thereby increasing opportunities for mating with females from different spawning groups (Bruch and Binkowski, 2002). Such male behavior facilitates opportunities for gene flow among spawning groups spatially or temporally distributed segregated.

While evidence of ITB has been reported in several fish species including salmonids (review in Hendry and Day, 2005), Eels (Maes *et al.*, 2006), there is a lack of information on IBT in long-lived iteroparous species whose generation time can take one to several decades. Heritability of spawning time in such species has not been available. However, some species such as lake sturgeon *Acipenser fulvesnces* (Forsythe, 2010) have been shown to spawn at similar times during multiple years. If repeatability of spawning time in these iteroparous species has a genetic basis, IBT can exist in populations composed of discrete multiple spawning groups. Such information is important for conservation and management of threatened species with small population sizes because limited gene flow together with genetic drift would further reduce genetic variation within a small population.

The lake sturgeon have a unique life history characterized by extreme longevity (potential > 100 years), iteroparity (Auer, 1999) and polygamous mating by both sexes (Bruch and Binkowski, 2002). Males mature at younger ages (12 -15 yrs.) and spawn at shorter intervals (1-3 yrs.) compared to females (18-27 yrs. and 3-7 yrs., respectively) (Auer, 1999; Bruch, 1999; Forsythe, 2010). The species exhibits reproductive characteristics which can lead to IBT and development of sub-population genetic structure within the population. Spawning activity typically occurs in bimodal or multimodal peaks. Like salmonids (review in Doctor and Quinn, 2009), lake sturgeon individuals repeatedly spawn at similar times and locations (Auer, 1999; Forsythe, 2010). Data collected over eight years in Black Lake, (MI) shown that repeatability (the ratio of among-individual variance to the total variance for a given trait) in spawning time of individuals captured in two or more years was high for both females (0.56) and males

(0.42) (Forsythe, 2010). These results suggest that different segments of the population may be reproductively isolated as a function of spawning time. Moreover, effects of environmental conditions (*e.g.,* water temperature, discharge) which are correlated with spawning time contribute to temporal variation in phenotypic traits of offspring and of early and late spawning adults, possibly leading to different selection regimes through the spawning season. Previous studies in this lake sturgeon population have documented temporal variation in traits including egg incubation time, yolk-sac size at hatch (Crossman, 2008), and embryonic and larval developmental time until dispersal (Duong *et al.,* in press), which may lead to reinforcement of difference in spawning time.

The goal of this study was determine whether levels of genetic differentiation between early and late spawning adults in this population exited as predicted based on high repeatability in spawning times observed over eight-year period in Black Lake, Michigan. Our objectives were to (i) estimate genetic difference among adults from different spawning groups within a year and across years; and (ii) quantify the relationship between inter-individual genetic relatedness and inter-individual differences in spawning time.

METHODS

Study site

The study was conducted in the Black Lake, Michigan. This lake sturgeon population is isolated by dams blocking immigration and emigration from other populations in adjacent lakes (Smith and King, 2005). Lake sturgeon adults spawn in the Upper Black River (UBR), the largest tributary to Black Lake and the sole spawning locations for the population. Adults spawn in multiple locations over a period of 19-42

days (Forsythe, 2010), extending from late April through early June (Figure 1.2). Spawning occurs within a short 1.5 km-section of the river, about 9 km upstream from the river mouth (Smith and King, 2005; Forsythe, 2010). The small, shallow and wadable river section permits sampling of nearly all spawning adults each year.

Adult sampling

We sampled adults each year for 9 consecutive years (2001-2009). There were 1,448 captures of 760 individuals including 460 males and 300 females. During each spawning season, we collected lake sturgeon adults daily by walking the entire spawning area of the stream one or more times per day. Adults were captured as they arrived at the spawning areas using long-handled nets. Upon capture, sex was determined and biological information including, body weight (kg), fork length (cm), capture date and location were recorded. A small fin clip (~1 cm^2) was collected from each adult for genetic analysis.

We used only information of date of capture to classify an individual as early or late spawner in order to examine genetic differences among groups of adults (a group was defined as those individuals that had similar dates of first capture) spawning at different times. We have observed that lake sturgeon females spent one or few days at the spawning areas whereas males remain on spawning areas for longer periods and early-spawning males could leave the areas but return subsequently with late-spawning females.

Microsatellite DNA analysis

DNA was extracted from fin clips of adult samples using a QIAGEN DNeasy$^{(R)}$ kit (QIA Inc.). DNA concentration was measured using a Nanodrop 1000

spectrophotometer (Thermo Fisher Scientific Inc.). All samples were genotyped at 12 tetra-nucleotide microsatellite loci including Spl 120 (McQuown *et al.*, 2000); AfuG 68B (McQuown *et al.*, 2002). 2002); Aox 27 (King *et al.*, 2001); AfuG 68, AfuG 9; AfuG 63, AfuG 74, AfuG 112, AfuG 56, AfuG 160, AfuG 195 and AfuG 204 (Welsh *et al.*, 2003). Using polymerase chain reaction (PCR), 100 ng DNA was amplified in 25 μl reaction mixtures as described in the above references. All PCR reactions were conducted using a Robocyler 96 thermal cycler (Stratagene). PCR products were run on 6% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO II scanner. Allele sizes were scored independently by two experienced personnel. Genotyping errors checked by blindly re-genotyping a random subset 10% of all samples.

Data analysis

A previous study showed that high repeatability in spawning times of lake sturgeon was observed over eight-year period in Black Lake, Michigan (Forsythe, 2010). If repeatability in spawning date has endured over generations, reproductive isolation may lead to high genetic correlations among individuals within relative to among members of different spawning groups (as evaluated by r_{xy}) and accrual of variance in allele frequency (F_{ST}). We tested this prediction for adults captured in the same single years and for adults that were captured ≥ 2 years during 2001-2009.

Genetic differences among groups of adults spawning during early and late season

Analyses were based on adults captured in each of 9 years and for adults that were captured ≥ 2 years over 9 consecutive years (2001-2009). For a single year, adults were classified into early or late spawning groups based on discrete spawning dates between groups (temporal spawning groups). In years 2006-2009, the interval among spawning

groups was short and there were more than two peaks of spawning activity. In these years, spawning groups were classified as early, middle and late.

Over 9 years of sampling, there were a total of 349 individuals that were captured ≥ 2 years (92 females and 257 males). We classified an individual as an early, middle or late spawners based on the mean of "standardized spawning dates" across years that the individual was captured. The standardized spawning date (SSD) of an adult in a given year is the number of days of first capture from the first day of the spawning season to the day the individual was captured divided by the total duration of spawning activity in that year ($0 < SSD \leq 1$). SSD thus accounted for different durations of spawning activity across years. The early spawning group was defined as individuals with 30% smallest SSD, and late spawning group were composed of individuals with 30% highest SSD. With this classification, the ranges (mean ± standard deviation) of SSD of early and late groups did not overlap (Figure 2.1). Because spawning date of an individual could vary across years, groups classified based on mean SSD better represent temporal spawning groups of the whole population compared to groups in a single year.

We tested the null hypothesis that there was no genetic differentiation (*i.e.*, degree of population structuring) either among spawning groups of adults each year or among groups of adults captured during ≥ 2 years. First, we tested whether genotypic frequencies deviated from Hardy-Weinberg expectations for spawning adults each year. Deviations were tested using a Fisher's exact test in program GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008). The exact P-value of the tests were estimated based on the Markov chain method (Guo and Thompson, 1992). The Markov chain method was also used to estimate P-values for contingency tests of genotypic differences between

early and late spawning groups within a year. Intra-annual population structure measured as the variance in allele frequency among individuals within and among groups (F_{IS} and F_{ST}, respectively) (Weir and Cockerham, 1984) was quantified using program GENEPOP.

Relatedness

Estimates of genetic relatedness among individuals from the same and different groups can be used to test whether spatial or temporal reproductive isolation has occurred within a population (Rousset, 2000; Hendry and Day, 2005). Based on observations of repeatable spawning times (Forsythe, 2010), we predicted that genetic relatedness among individuals spawning at similar times would be greater than relatedness among individuals spawning at different times.

Genetic relatedness (r_{xy}) or the coefficient of relationship defined by Sewall Wright is the probability that the alleles present at a random locus will be identical by descent (Hamilton, 1963). More formally, r_{xy} is a measure of genetic similarity between two individuals relative to the relatedness between two random individuals from the same population (Blouin, 2003). There are several commonly used estimators of relatedness, which can be grouped into two classes, including maximum likelihood (ML) estimators and various methods of moment (MOM) estimators (review in (Ritland, 2000; Van de Casteele *et al.*, 2001; Blouin, 2003; Csillery *et al.*, 2006). The rank order of estimator performance varies by research scenario, generally is population-specific, depending on the frequency distribution of relatedness in the population and properties of markers used (*e.g.*, allele frequency distribution, number of allele per locus (Van de Casteele *et al.*, 2001; Csillery *et al.*, 2006; Oliehock *et al.*, 2006).

To choose a relatedness estimator for lake sturgeon population, we evaluated the correlation of estimator-based r_{xy} values and true values that are simulated from allele frequencies of the population (Wang, 2010). We used program COANCESTRY (Wang, 2010), which allows simultaneous estimation of 2 ML estimators (Triadic likelihood [TriML] (Wang, 2007b) and Dyadic likelihood [DyadML] (Milligan, 2003)) and 5 MOM estimators (proposed by Queller and Goodnight, 1989 [QG]; Lynch and Ritland 1999 [LynchR]; Wang, 2002; Lynch 1988 and Li *et al.*, 1993; and Ritland 1996). Simulations were conducted using 100 reference individuals as recommended (Wang, 2010) for each type of dyad relationships (*e.g.*, unrelated, parent-offspring, ful-sib, half-sib, and first cousins). The QG, TriML and DyadicML were more highly correlated (0.74 – 0.75) with true values compared to other estimators (0.46 – 0.70), and they were highly correlated to each other (0.89 – 0.99). Similarly, the QG-based estimates were also highly correlated (0.81 – 0.86) with estimates based on Konovalov and Heg (Konovalov and Heg, 2008a) and ML (Konovalov and Heg, 2008b) estimators using program KINGROUP, version v2_09050 (Konovalov *et al.*, 2004). Thus, we chose to use the Queller and Goodnight (QG) method based on 1) the high degree of correlation with true values and other estimators; 2) the high frequency of the QG method in the literature (Csillery *et al.*, 2006); and 3) that the QC method has been described as being robust in different conditions (Van de Casteele *et al.*, 2001). Queller and Goodnight relatedness values were generated using the program RELATEDNESS 5.0 (Goodnight and Queller, 2001). Pairwise relatedness among spawning adults was estimated based of allele frequencies of the whole population (each individual that spawned during 9 years, 2001-2009, was included, N = 722).

We estimated pairwise relatedness for adults in 9 single years and for adults captured during ≥ 2 years. First, for single years, we examined the genetic relatedness among individuals within a year in regard to their spawning time. Specifically, we used the difference in SSD as a measure of the proximity in spawning time, and tested whether individuals spawning at similar times were more closely related than individual spawning at different times of a spawning season. Mantel tests implemented in PASSAGE (Rosenberg, 2001) were conducted to test for significance of the correlation between matrices of pairwise relatedness and pairwise difference in SSD, as suggested by Hendry and Day (2005).

For 349 individuals captured during ≥ 2 years, we compared mean relatedness within (early, middle and late) and between groups. Because each individual was used to compute pairwise relatedness with multiple individuals, we used a permutation approach to test for significance of differences in mean relatedness (r_{xy}) within and between spawning groups using an SAS-based program (A. Saxton, University of Tennessee, unpublished software) (Ratnayeke *et al.*, 2002). The program was set to compute the observed difference between the means of the two groups first. Then, data from the two groups were pooled, and the pooled data were randomly sub-sampled 1000 times. Each time, the difference between the observed and theoretical mean calculated from the pooled data was computed and the one-tailed probability that mean r_{xy} of within groups is greater than r_{xy} between groups was tested. The absolute differences in distribution of cumulative frequencies of r values between groups were tested using non-parametric Kolmogorov-Smirnov tests conducted in R (R-Development-Core-Team, 2009).

RESULTS

Data from 9 years of adult capture and recapture show that adults consistently spawn over a range of time (Figure 1.2) and that the time of individual spawning is repeated across years. Degree of repeatability, as evidenced by the standard deviation of standardized spawning date is particularly small in the earliest and latest spawning adults (Figure 2.1). The possibilities of genetic differentiation within this population were examined at two temporal scales, (i) among groups of adults captured in the same single years and (ii) among groups of adults that were captured ≥ 2 years during 2001-2009.

Genetic differentiation and mean relatedness among groups of adults captured ≥ 2 years

Evidence for genetic differences among adults with regard to spawning time (early vs. late) was examined across years. During 9 years, 349 individuals that were captured ≥ 2 years were grouped as early-, middle- and late-repeated spawning groups. The overall F_{ST} for these three groups of adults (both males and females) was low (mean ± SE = 0.004 ± 0.001) but was significant (P <0.01). Genotypic differences (chi-square tests) and F_{ST} were significant between early- and middle-, and early- and late- repeated spawning groups of males, and when both sexes were combined but was not significantly different among groups of females. Meanwhile, there were no genetic differences between middle- and late- spawning groups of males or females (Table 2.1).

We compared mean pairwise relatedness (r_{xy}) among repeated spawning individuals from within early-, middle- and late-spawning groups and among individuals from different groups for females and males and when both sexes were combined. Mean pairwise relatedness (r_{xy}) of females was not significantly different among groups (P >

0.10). Mean r_{xy} of males as well as estimates for all adults (both sexes) was significantly higher in the early spawning groups compared to the middle or late spawning groups ($P < 0.01$). Mean r_{xy} of individuals within middle and within late groups was similar and not significantly different from mean r_{xy} between groups, either sexes were separately analyzed or when combined ($P < 0.01$) (Table 2.2).

We further compared the differences in frequency distribution of r_{xy} values between early spawning group (the group characterized by the highest mean r_{xy}) and the other spawning groups. The absolute differences in cumulative frequency distribution of relatedness values from early spawning group compared to middle and late spawning groups were 5.9% ($P <0.01$) and 4.4% ($P <0.01$), respectively. These differences were small (Figure 2.2) and thus may not be biologically relevant.

Genetic differentiation and mean relatedness among groups of adults in single years

Within single years, genetic differences among groups were also evaluated based on genotypic difference (Chi-square tests) and the significance of F_{ST} estimated. In years 2006 – 2009, spawning time was not clearly distinct among groups. At first, we divided 3 groups in each of these years and tested genetic differences among three groups. Because there was no genetic difference between middle and late groups (non-significant difference in genotypic frequencies between the two groups and non-significant difference from zero of F_{ST}), individuals from middle and late groups were pooled as "late" spawning group. Genetic differences between early and late adult groups were

inconsistent among years (Table 2.3). Estimates of F_{ST} between early and late spawning groups was significant (P <0.01) only in 2007 (0.0048) and 2006 (0.0044).

To examine genetic relatedness among individuals in the same year, we tested the significance of correlations between pairwise relatedness and pairwise difference in SSD among individuals. A significantly negative correlation would indicate that individuals spawning at a similar time were more genetically related than individuals spawning at different times in a spawning season. However, correlations were not significantly different from 0, indicating that pairwise relatedness was independent of pairwise difference in spawning date among individuals captured within the same year (Table 2.4).

DISCUSSION

Limited gene flow among adults that spawn at different times can result in genetic structure within populations (Hendry and Day, 2005; Doctor and Quinn, 2009). Genetic differences among groups of adults spawning at different times if at low levels do not provide evidence of IBT (Hendry and Day, 2005). Evidence of IBT would be stronger if a low level of genetic differences among groups had a consistent pattern across years. Our nine-year data on spawning time and genotypes for each individual of almost all lake sturgeon adults in Black Lake population revealed important insights into the evidence for IBT in this closed population.

We found that the lake sturgeon population in Black Lake exhibited a low level of subpopulation structure associated with spawning time, as evidenced by significance of genetic differences and differences in mean relatedness between early and late groups of adults captured ≥ 2 years. However, there was lack of evidence of isolation by time in this population given (i) low observed values of F_{ST} between early and late groups of

adults captured ≥ 2 years (across years); (ii) non-significance of intra-annual F_{ST} in 5 of 7 years; and (iv) no correlation between pairwise relatedness and pairwise difference in spawning time.

Grouping an adult into early or late spawning groups based on mean standardized spawning date better reflected the timing of spawning of each individual. Accordingly, genetic differences were observed between early and late groups of adults captured ≥ 2 years but not between groups within a year suggesting that repeatability in spawning date represented some level of heritability. Individuals that spawned early and late in the seasons repeatedly across years are more likely to be progeny of adults from separate groups from the parental generation that spawned early and late, respectively. Limited gene flow could occur between groups of individuals spawning earliest and latest in the season, while gene flow could be mediated by individuals spawning at middle season and/or by individuals exhibiting greater plasticity in spawning time (based on larger variation in standardized spawning date). As a result, genetic differentiation could be initiated at very low levels and accumulate slowly over generations, resulting in the heterogeneous genetic composition observed in the population at present. In a given year, individual spawning time was plastic (as shown by standard deviation in SSD, Figure 2.1), thus a spawning group (*i.e.*, early or late) could include individuals from different groups in which they actually belonged to. Mixing individuals from different temporal spawning groups that were genetically differentiated a low levels contributed to the non-significant genetic differences between early and late groups in a given year.

Levels of genetic differentiation within the lake sturgeon population could be influenced by the behavior of males during spawning season, the plasticity in spawning

time of adults, and the comparatively short spawning season. These factors could vary temporally in response to fluctuations environmental factors (Forsythe, 2010). In several lake sturgeon populations (*e.g.*, Black Lake, Michigan (Smith and Baker, 2005); Wolf and upper Fox rivers, Wisconsin (Bruch and Binkowski, 2002), males have been observed to spend long periods of time at the spawning areas, which could result in matings with multiple females including females from early and late groups. Concordantly, based on genetically identified parentage, we have found that mating of individuals from different groups (early vs. late) comprised from 10 - 40% of total mating pairs from 2001-2007 (see chapter 1). Therefore, gene flow among spawning groups occurred frequently and varied yearly. Data revealed that "distinct" groups of adults spawning early and late in the season based on direct observations were not reproductively isolated. The behavior of males appears to play an important role in retarding temporal reproductive isolation.

Plasticity in spawning time of repeated spawning individuals may also contribute to the lack of evidence of reproductive isolation. Individuals that repeatedly spawn late in the seasons had larger variation in spawning time compared to individuals spawning early in the seasons (Figure 2.1). Plasticity in spawning time commonly observed in numerous species (*e.g.*, birds (Reed *et al.*, 2009); fish (Valiente *et al.*, 2010) has been reported as a result of the interaction between age, experience and environment (Wilson *et al.*, 2007). In lake sturgeon, variation in spawning time was observed to be associated with inter-annual fluctuation of stream environmental factors such as water temperature and discharge (Forsythe, 2010).

Spawning duration of lake sturgeon might also explain low levels of genetic differentiation within the population and the lack of evidence of reproductive isolation. The spawning season of lake sturgeon is short (19 -42 days in Blake Lake population, similar to other populations (Bruch and Binkowski, 2002)). Short spawning duration and thus a short temporal distance (the period separating spawning groups) provides opportunities for gene flow within the population. Temporal distances of lake sturgeon spawning groups were about 7- 15 days (4 days apart in some years). We have found fewer mating pairs occurred when temporal distance between males and females increased (see chapter 1). In many salmonids populations, temporal distance between groups was about 1 month apart, and genetic difference was positively correlated with temporal distance among groups (for review on salmonids, see Hendry and Day, 2005). In two sockeye salmon populations in Tustumena Lake, Alaska, for example, genetic differences between early and late groups were not significant (F_{ST} = 0.003) in one population with temporal distance of 13-15 days, and significant (F_{ST} = 0.006) within the other population with temporal distance between groups of 21-25 days (Woody *et al.*, 2000).

The values of F_{ST} found between early and late repeated spawning lake sturgeon are comparably lower than F_{ST} reported from different groups within populations of other species (*e.g.*, sockeye salmon *Oncorhynchus nerka* (Fillatre *et al.*, 2003); European Eel *Anguilla anguilla* (Maes *et al.*, 2006; Pujolar *et al.*, 2006); red sea urchin *Strongylocentrotus franciscanus* (Moberg and Burton, 2000); purple sea urchin, *Strongylocentrotus purpuratus (Edmands et al., 1996)*; Pacific oysters *Crassostrea gigas*

(Li and Hedgecock, 1998)). In sockeye salmon (*Oncorhynchus nerka*) in Klukshu River (Yukon, Canada), for example, F_{ST} between runs (spawning groups) within years (1994-2000) were significantly greater than zero (range: 0.018–0.041) except one year F_{ST} =0.004 (Fillatre *et al.*, 2003).

In addition to testing genetic difference at the group-level, we also examined pairwise relatedness as a measure of IBT at the individual level. Although mean relatedness of repeated spawning individuals from the early group was significantly higher than that of individuals from middle and late groups, the magnitude of differences was small, and thus the differences were not likely biological relevant. Moreover, in each year, estimates of pairwise relatedness among adults were found to be independent of difference in spawning time. Therefore, at the individual level, there was no evidence of high genetic correlations among individuals spawning at similar time during spawning seasons.

There are several possible biological and statistical explanations for this result. First, no correlation of pairwise relatedness and difference in spawning time despite heritable spawning time could be due to polygamous mating system and straying of lake sturgeon males. Polygamous mating helps reduce pairwise relatedness among adults compared to monogamous mating with the same number of breeders (Woxvold and Mulder, 2008) due to lower probability of shared alleles among siblings. Further, male lake sturgeon prolong their stay in the spawning areas to increase opportunities to mate with early and late females (see chapter 1), which increases gene flow between early and late spawning groups. Mean pairwise relatedness in the population also depends on effective population size, where small effective population size can increase the

probability of mating among closely relatedness individuals (Falconer, 1989; Launey *et al.*, 2001). With N_e estimated at ~132 (see chapter 3), inbreeding will increase only slightly each generation. Finally, relatedness estimates have large variance (Ritland, 2000; Van de Casteele *et al.*, 2001; Csillery *et al.*, 2006), there would be low power to detect significance of differences in mean relatedness among groups (Csillery *et al.*, 2006).

In sum, based on the low signal of genetic differentiation and difference in relatedness between early and late groups of individuals captured ≥ 2 years, sub-population structuring has developed but at a low level in this lake sturgeon population. If spawning time is heritable, low genetic differentiation within this population can be maintained despite the plasticity in spawning time of both males and females and male behavior during the spawning season, which increases gene flow among spawning groups.

ITB would evolve if gene flow among temporal spawning groups was limited and if natural selection on adults and/or offspring acted differently during different periods of the spawning season, initiating the process of temporal lineage segregation (Tomaiuolo *et al.*, 2007). In the lake sturgeon population in Black Lake, the conditions (*i.e.*, limited gene flow, differential selection) promoting ITB have not been observed. We have found that the number of offspring produced did not vary as a function of spawning time, indicating no differential selection across years during the spawning season (see chapter 1). Moreover, the time required for evolution of spatial reproductive isolation is highly variable and usually takes long periods (Nei *et al.*, 1983), from several decades (introduced sockeye salmon in Lake Washington for 13 generations (Hendry *et al.*,

2000); Chinook salmon in Newzerland for 30 generations (Quinn *et al.*, 2000) to thousand years (Schluter, 1996; Taylor, 1999). The time required for evolution of temporal reproductive isolation could take longer because factors such as environmental conditions that contribute to temporal reproductive barriers are more dynamic. Therefore, given the species' prolonged inter-generation interval, genetic differentiation could develop at low levels but would not exhibit temporal reproductive isolation for a long period of time.

Table 2.1. Pairwise differences in genotypic frequency (Chi-square test, df = 24) and variance in allele frequency (F_{ST}) among groups of individuals that were captured ≥ 2 years during 2001-2009

Group	Female		Male		Sexes combined	
	X^2	F_{ST}	X^2	F_{ST}	X^2	F_{ST}
Early-Middle	21.0	-0.0001	56.2***	0.0070**	56.7***	0.0053**
Early-Late	24.6	0.0019	57.0***	0.0065***	65.8***	0.0063***
Middle-Late	21.2	0.0008	22.2	-0.0009	22.0	0

Note: Asterisks indicate the significance level of a test (** when P <0.01, and *** when P <0.001)

Table 2.2: Mean (± SD) of estimated inter-individual relatedness within and between members of early and late spawning groups based on adults captured ≥ 2 years, as indicated in Figure 2. 1

Spawning group	Male	Female	Both sexes
Within early	$0.0228^{b} \pm 0.2316$	$0.0092^{a} \pm 0.2191$	$0.0200^{b} \pm 0.2272$
Within middle	$-0.0107^{a} \pm 0.1965$	$0.0152^{a} \pm 0.2123$	$-0.0047^{a} \pm 0.2117$
Within late	$-0.0001^{a} \pm 0.2171$	$-0.0193^{a} \pm 0.2118$	$0.0019^{a} \pm 0.2136$
Between groups	$-0.0011^{a} \pm 0.2087$	$-0.0092^{a} \pm 0.2083$	$-0.0028^{a} \pm 0.2105$

Note: Values within a column with the same letter are not significantly different (P >0.05), using permutation tests.

Table 2.3. Differences in genotypic frequency (Chi-square test) and variance in allele frequency (F_{ST}) between early and late spawning groups within each of 9 years 2001-2009

Year	Genotypic difference		F_{ST}
	X^2	P-value	
2001	32.7	0.11	0.0069
2002	14.9	0.92	-0.0038
2003	37.2	0.042	0.0025
2004	18.8	0.76	-0.0013
2005	28.2	0.24	0.002
2006	42.9	0.01	0.0044*
2007	45.2	0.006	0.0048*
2008	23.3	0.49	0.0002
2009	34.5	0.08	0.0015

Note: An asterisk indicates the significance level (P <0.05) of a test

Table 2.4. Results of Mantel tests of correlations between inter-individual relatedness (r_{xy}) and inter-individual differences in standardized spawning dates between individuals within a year (2001-2009)

Year	Correlation coefficient	P-value
2001	-0.0178	0.688
2002	0.0208	0.220
2003	-0.0214	0.984
2004	-0.0075	0.425
2005	-0.0151	0.519
2006	0.0040	0.810
2007	-0.0227	0.424
2008	0.0175	0.465
2009	-0.0002	0.838

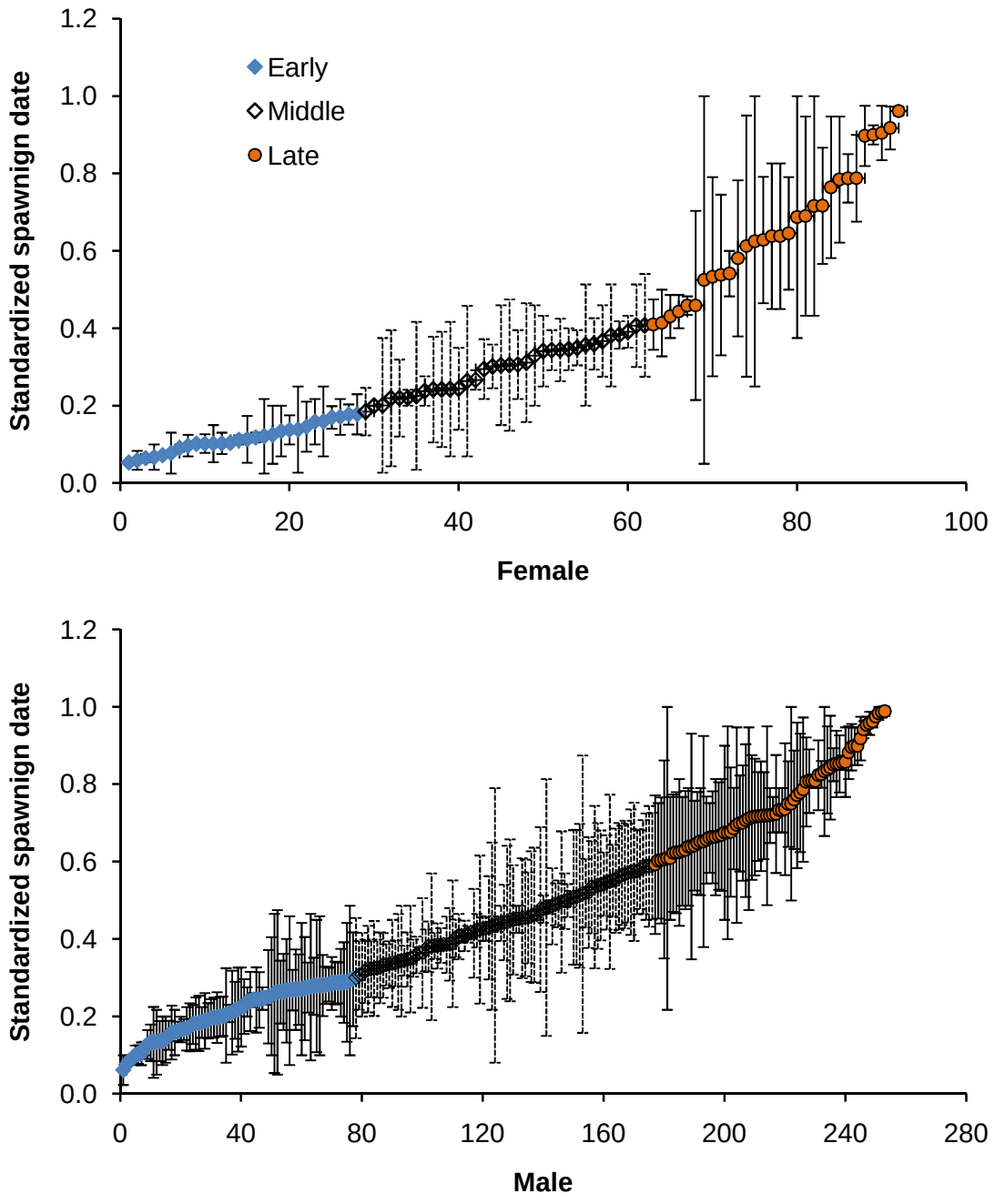

Figure 2.1. Standardized spawning date (SSD) of 92 females (top) and 257 males (bottom) that re-spawned more than 1 time during 2001-2009. Individuals were ordered left to right by ascending values of mean SSD.

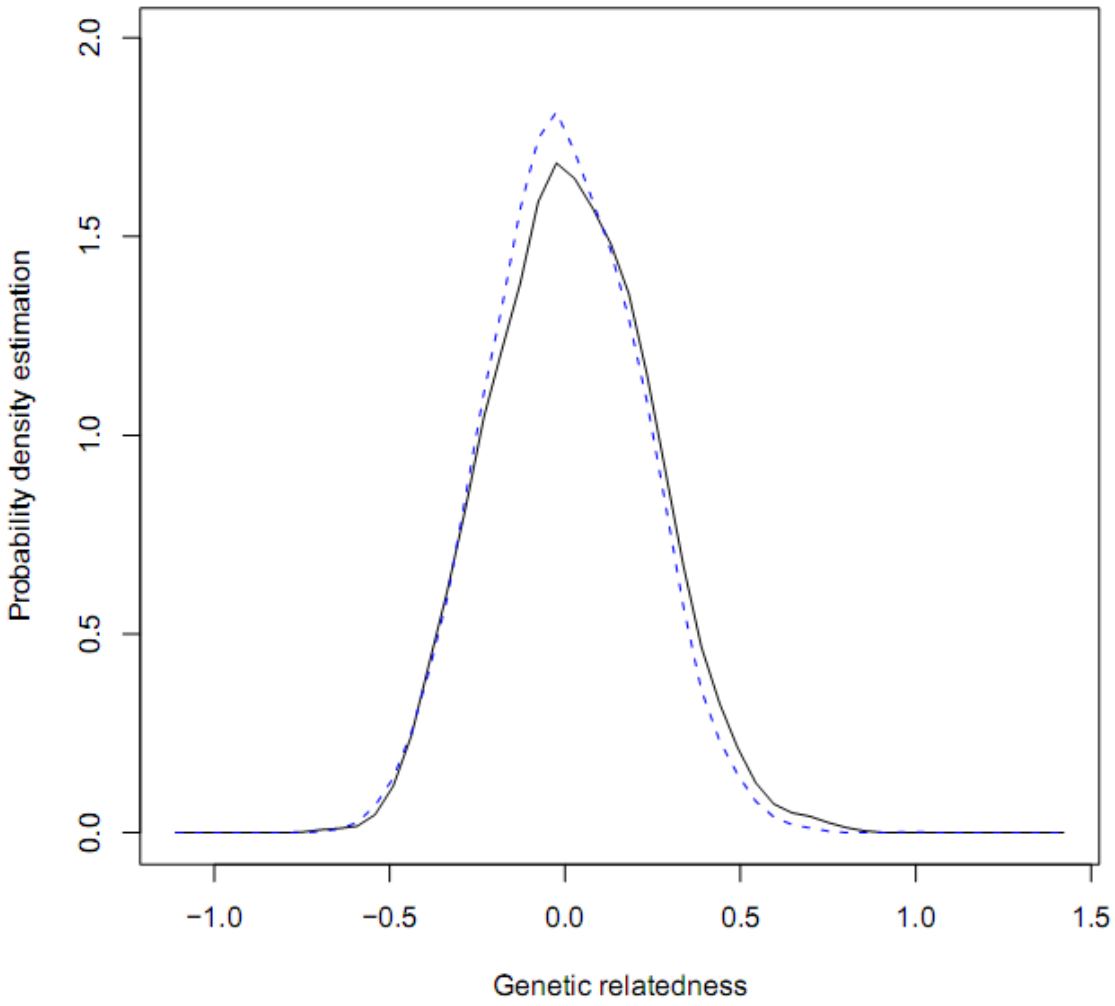

Figure 2.2. Probability density estimation of estimated inter-individual genetic relatedness among adults from early spawning groups (solid line) and from late spawning groups (dash line) based on males and females that were captured ≥ 2 years during 2001-2009.

Chapter 3

EFFECTIVE POPULATION SIZE AND TEMPORAL VARIATION IN EFFECTIVE NUMBER OF BREEDERS FOR THE LAKE STURGEON POPULATION IN BLACK LAKE, MI

ABSTRACT – Estimating effective population size (N_e) and quantifying temporal variation in effective number of breeders (N_b) and the relationship between N_b and adult census size (N) are important to predict genetic changes in small and isolated populations, especially for threatened species like lake sturgeon (*Acipenser fulvescens*). Estimates of N_b were computed using (1) demographic data inferred from genetically identified parentage and (2) linkage disequilibrium (LD) based on 12 microsatellite loci for larval samples collected in each of seven years, 2001-2007. The two methods yielded similar estimates of N_b. Estimates of N_b were fairly constant as were adult census sizes among years (mean of LD-based N_b ranged 63-126, 95% CI ranged 39-197). Low inter-annual variation in N_b/N ratios (0.26 - 0.61) resulted from consistently low standardized variance of relative reproductive success across years. Consistency of N_b/N was realized even though the number of larvae collected, a surrogate measure of total annual larval production, varied 40-fold across years (437 – 16,417). N_e per generation (mean: 132, 95% CI: 104-167) estimated based on a mixed sample of lake sturgeon adults captured during 2001-2007 (N = 612) was close to annual estimates of N_b. Therefore, for lake sturgeon generational estimates of N_e should not be estimated as a product of the harmonic mean of effective number of breeders and generation time as has been

suggested for semelparous species. That approach would also probably be inappropriate for other long-lived iteroparous species.

INTRODUCTION

Effective population size (N_e) is an important parameter describing evolutionary processes of finite populations, and therefore has been an important conceptual focus in evolutionary studies and in conservation biology (Palstra and Ruzzante, 2008; Charlesworth, 2009; Waples, 2010). N_e allows predictions of generational rates of change in allele frequencies and of inbreeding occurring in a finite population due to genetic drift and interactive effects of drift with mutation, migration, recombination and selection (Charlesworth, 2009). Since Frankham's review (1995) revealing that the ratio of effective to census population size (N_e/N) across 102 wild species was smaller (median of 0.1) than previously perceived, more attention has been focused on investigations of N_e and causal factors affecting N_e for natural populations (Mackay, 2007). Small and fragmented populations can be exposed to greater risk of extinction due to low N_e (Palstra and Ruzzante, 2008). Therefore, estimation of N_e and N_e/N is especially important to elucidate conservation issues for small and isolated populations.

Effective population size (N_e) as first defined by Wright (1931) is the number of individuals in a theoretically "ideal" population that has the same magnitude of effects due to genetic drift and inbreeding as a real population of size N (Nunney, 1993; Charlesworth, 2009). In an ideal population, mating is assumed to be random and each individual has an equal probability of contributing gametes to the next generation. In natural populations, numerous factors can cause unequal probabilities of genetic

contributions among adults to progeny, resulting in smaller N_e than N (Frankham, 1995; Charlesworth, 2009; Waples, 2010). Major factors that can lower N_e include fluctuations in adult population size over time, skewed sex ratios and non-random variation in lifetime reproductive success (Charlesworth, 2009) (Frankham, 1995; Charlesworth, 2009; Waples, 2010). In addition, N_e is usually smaller than N in populations which are structured by age or stage-classes, or which experience inbreeding, selection or limited migration (Charlesworth, 2009). Population structuring which results from spatial and temporal reproductive isolation among sub-populations (Hendry and Day, 2005; Doctor and Quinn, 2009) can also reduce N_e (Chesser *et al.*, 1993; Waples, 2010). Moreover, biological characteristics of a species such as attributes of the mating system (Nunney, 1991, 1993; Pearse and Anderson, 2009), generation time, life span and levels of iteroparity (Waples, 1990; Nunney, 1996) also influence N_e and N_e/N in different species and populations.

Different methods have been used to estimate N_e. Because of the difficulties in estimating demographic parameters necessary for estimation of N_e in natural populations, indirect estimation of N_e using genetic data is commonly applied (Waples, 1989; Luikart and Cornuet, 1999). Several approaches have been used including one – sample based methods such as linkage disequilibrium (Hill, 1981; Waples, 1991), heterozygote excess (Pudovkin *et al.*, 1996), approximate Bayesian computation (Tallmon *et al.*, 2008), and sib-ship method (Wang, 2009). Two – sample based methods such as temporal variance in allele frequency (Krimbas and Tsakas, 1971; Waples, 1989), and loss of

heterozygosity (Harris and Allendorf, 1989) (review in Luikart and Cornuet, 1999; Nunney, 2000; Luikart *et al.*, 2010) have also been employed. Genetic data can also be used to determine parentage in natural populations (Pemberton, 2008), allowing estimations of the mean and variance in male and female reproductive success that can be used for estimation of N_e (Crow and Denniston, 1988; Caballero, 1994).

Most genetic estimators have been developed for semelparous species (*e.g.*, Pacific salmon *Oncorhynchus spp*.) based on key assumptions of the Wright's (1931) ideal population, including population closure and discrete and non-overlapping generations. For long-lived and iteroparous species, if samples are obtained from a single cohort, standard methods for N_e estimation mentioned above can be used to estimate the effective number of breeders (N_b, number of breeding adults that produced the cohort) (review in Waples, 2010). However, N_b is difficult to relate to N_e in long-lived and iteroparous species because breeders in a year include a mixture of adults from different age classes including representatives from different generations (Luikart *et al.*, 2010; Waples, 2010). Long-term data from a closed population in which samples can be collected from multiple cohorts may help resolve relationships between N_e and N_b for long-lived and iteroparous species.

Lake sturgeon (*Acipenser fulvescens*) is an important species to study N_e, relationship of N_e to N_b and ecological variables that affect effective sizes. The species is a target of conservation (Holey *et al.*, 2000; Peterson *et al.*, 2007) because lake sturgeon numbers have been severely depleted over the past 100 years throughout their native range (Houston, 1987; Hay-Chmielewski and Whelan, 1997). Abundance of many lake

sturgeon populations has remained low due to anthropogenic factors resulting in fragmented habitats and degraded water quality (Peterson *et al.*, 2007). Aspects of the species life history and reproductive ecology including longevity and low natural recruitment rates of lake sturgeon also influence relationships among N_e, N_b and adult census size.

Lake sturgeon are characterized by a long generation time (15-20 years), extreme longevity (potential > 100 years), iteroparity (Auer, 1999) and polygamous mating by both sexes (Bruch and Binkowski, 2002). Each of these characteristics can influence N_e/N. In species with overlapping generations like lake sturgeon, the ratio of N_e/N is expected to increase as generation time increases (Felsenst.J, 1971; Hill, 1972; Waples, 2010). Iteroparity of lake sturgeon can result in increasing variance in life time reproductive success over time, which reduces N_e/N, if individuals with higher fecundity are consistently more successful than other individuals in producing offspring that survive to reach sexual maturity. In contrast, if differences in reproductive success among individuals were not consistent across their lifetime, iteroparity would result in comparatively higher N_e/N (Nunney, 1996; Turner *et al.*, 2002). The broadcast spawning behavior of lake sturgeon can also result in high variance in reproductive success. Lake sturgeon exhibit aggregate mating, where negatively buoyant eggs and sperm are released by groups of females and males over large areas of rock and gravel in the absence of nest preparation and without post-ovulatory parental care. Because of the species' mating behavior and exposure of eggs to environmental conditions and to predation, juvenile lake sturgeon experience extremely high mortality early in life (Kempinger, 1988).

However, the prediction of high variance in reproductive success on N_e/N may be countered by the species promiscuous mating system where males and females can have multiple mates within a year (DeHaan, 2003; Duong, chapter 1). Promiscuity should decrease male and female variance in lifetime reproductive success, acting in an opposite manner compared to effects of variance in reproductive success on N_e/N (Nunney, 1993; Sugg and Chesser, 1994; Pearse and Anderson, 2009).

Infrequent spawning intervals and repeatedly spawning at the same time and the same location observed in lake sturgeon (Auer, 1999; Forsythe, 2010) can also influence N_e and N_e/N. Lake sturgeon males mature at younger ages (12 -15 yrs.) and spawn at shorter intervals (1-3 yrs.) compared to females (18-27 yrs. and 3-7 yrs., respectively) (Auer, 1999; Bruch, 1999; Forsythe, 2010). Accordingly, many populations are male-biased and sex ratios can vary across years. In addition, the tendency of adults to spawn at the same time and the same location, similar to other fish species (*e.g.*, salmonids, review in Doctor and Quinn 2009), can result in within-population structuring that generally reduces N_e/N (Chesser *et al.*, 1993; Caballero, 1994; Turner *et al.*, 2002).

A small self-sustaining population of lake sturgeon in Black Lake, Michigan, USA allows studies of N_b, N_e and the relationship between population effective size and census size. A closed population helps avoid inaccuracy in N_e estimation due to immigration (Waples, 2010). The Black Lake population has been isolated from gene flow from other Great Lakes populations due to dam construction since 1903 (Baker and Borgeson, 1999). The population has been well studied estimating adult numbers and annual larval production as well as reproductive features including sex ratios and patterns

of spawning time and spawning location (DeHaan *et al.*, 2006; Crossman, 2008; Forsythe, 2010). The number of adults captured, when adjusted for inter-annual variability in sampling effort, was similar across years. However, there was a high inter-annual variation in the number of larvae sampled, a surrogate of total larval production, which varied by 40 fold over the 7 years of study. Long-term sampling of adults and larvae in this population helps quantify demographic factors that affect inter-annual variation in effective number of breeders. Moreover, large sample sizes of adults collected across multiple years allows estimation of N_e and N_e/N, and comparison of the estimates of N_e and N_b.

Our first objective was to estimate annual N_b of the lake sturgeon population in Black Lake over 7 consecutive years in the current generation and qualitatively examine the relationship of N_b and N_b/N to population demographic data. We proposed two predictions of patterns of variation in N_b and N_b/N across 7 years. First, N_b and N_b/N of the population were expected to be relatively stable and would not vary proportionally with the inter-annual variability in larval collections. This prediction was based on literature that N_b is sensitive to the number of census adults (Frankham, 1995; Nunney, 1996) but is expected to vary less due to standardized variance (*e.g.*, variance/(mean)^2) in female fecundity (Nunney, 1996), and on our observation that the number of spawning adults was relatively consistent across years. Alternatively, if inter-annual variation in larval production reflected high standardized variance in reproductive success among males and females (the number of offspring per individual), a large inter-annual variation in standardized variance would result in fluctuation of N_b across years. The second

objective was to estimate contemporary N_e and compare values of N_e and annual N_e in order to understand the relationship between N_e and N_b in a long-lived and iteroparous species.

METHODS

Study site

The study was conducted in the Black Lake system in Michigan. Lake sturgeon spawn only in the Upper Black River, the largest tributary to Black Lake (Smith and King 2005). Adults spawn in multiple locations over a period of 28-43 days (Forsythe, 2010), extending from late April through early June. Spawning occurs within a shallow 1.5 km-section of the river, about 9 km upstream from the river mouth (Smith and Baker, 2005; Forsythe, 2010). The small, shallow and wadable river allows us to sample nearly all spawning adults each year.

Sample collection

Adult and larvae samples were collected for 7 years (2001-2007, Table 1.1). During each spawning season, we collected lake sturgeon adults daily by walking the entire length of stream used for spawning one or more times per day and using long-handled nets to capture fish. Sex was determined by cloacal examination and extruding gametes. Biological data including, body weight (kg), fork length (cm), and total length were recorded along with capture date and location. A dorsal fin clip (~1 cm^2) was taken from each individual for genetic analysis to genetically assign parentage.

Larval sampling was conducted each year, starting 10 days after the first spawning event and lasting for 25-40 days, until no larvae were captured for 2 consecutive nights. Five D-frame larval nets were spaced across the river channel at a site

about 2 km downstream from the spawning areas (Figure 1.1). We checked nets hourly, from 2100 to 0200 hrs, when the vast majority of larvae dispersed (Auer and Baker, 2002). Sampling was consistent throughout the larval sampling period and among years. In 2005 - 2007, larvae collected were transferred to a streamside hatchery where they were reared for several months. Before the fish were released, a fin clip was collected from each individual for genetic analysis. Fish that died in the hatchery were preserved in 95% ethanol. Samples used for microsatellite genotyping were taken proportionally from the total preserved fin clips and mortalities from each year. The proportion of the total larval catch sampled for microsatellite genotyping was not constant across years (Table 1.1). However, during each year the larvae genotyped represented proportionally a random subset from the entire collection of larvae for a given year.

Genetic analysis

DNA was extracted from samples using the QIAGEN DNeasy(R) kit (QIAGEN Inc.) and concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc.). All samples were genotyped at 12 tetra-nucleotide microsatellite loci including Spl 120 (McQuown *et al.*, 2000); AfuG 68B (McQuown *et al.*, 2002). 2002); Aox 27 (King *et al.*, 2001); AfuG 68, AfuG 9; AfuG 63, AfuG 74, AfuG 112, AfuG 56, AfuG 160, AfuG 195 and AfuG 204 (Welsh *et al.*, 2003). Using polymerase chain reaction (PCR), 100 ng DNA was amplified in 25 μl reaction mixtures as described in the above references. All PCR reactions were conducted using a Robocyler 96 thermal cycler (Stratagene). Then, PCR products were run on 6% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO II scanner. Allele

sizes were scored independently by two experienced personnel. Genotyping errors checked by blindly re-genotyping a random subset 10% of all samples.

Data analysis

Testing assumptions on marker loci used in the study

Most genetic marker-based methods (including linkage disequilibrium estimating effective number of breeders and parentage analysis presented below) assume loci are unlinked and under Hardy-Weinberg equilibrium (HWE). We tested these assumptions for adult samples each year using program GENEPOP 4.0 (Raymond and Rousset, 1995; Rousset, 2008). Deviations of observed genotypic frequencies from HWE were tested using the exact test option. One (AfuG 68) of 12 loci departed from HWE, possibly due to null alleles. We chose the log likelihood ratio statistic to test for linkage disequilibrium among loci. No significant linkages among loci were detected.

Estimating effective number of breeders

Effective number of breeders was estimated using methods for calculating N_e based on samples from the same cohort (Luikart *et al.*, 2010). Then, N_e was replaced by N_b, the number of breeders that produced progeny.

Performance of each N_b (and N_e) genetic estimator can vary among species and markers used; therefore, using a combination of methods has been recommended (Luikart *et al.*, 2010; Waples, 2010). We used two methods: (1) linkage disequilibrium and (2) estimation based on demographic data. The former estimates N_b based on genotype data of offspring (single sample) without prior knowledge of parentage or demographic data. Meanwhile, the latter requires information on the number of males and females that

produce offspring and estimates of mean and variance of the number of offspring per individual (reproductive success), which was inferred based on genetically identified parentage. Estimates of N_b based on demographic data may be overestimated if variance in reproductive success is underestimated for natural populations (Turner *et al.*, 2002). Given the differences in methods used, similarities in N_b estimates based on two methods (called N_b when based on LD and N_{bV} when based on reproductive success) in each of 7 years should provide an indicator of reliability of each estimator.

To estimate N_b and calculate N_b/N each year (2001-2007), we used genotype data from larvae randomly sampled from the entire larval collection each year (Table 1.1). The number of adults captured each year (N) was adjusted based on catch per effort using our records indicating that the effort (number of person-days sampling) in years 2004-2007 was approximately 1.5 times the effort expended in years 2001-2004. Therefore, $N_{adjusted}$ was estimated to be $1.5*N_{captured}$ in each of years 2001-2004.

Linkage disequilibrium (LD) method

The LD method of effective population size estimation has been the most commonly used among single-sample estimators (England *et al.*, 2006; Luikart *et al.*, 2010). Compared to the temporal method (two- sample estimator) that has been widely applied, LD provides greater precision with microsatellite data (Waples, 2010). We used the program LDNE (Waples and Do, 2008) to estimate the effective number of breeders using multi-locus genotype data of larval lake sturgeon. LDNE was developed based on the LD method proposed by Hill (1981) and adjusted for bias when the sample size is less than the true unknown effective size (England *et al.*, 2006; Waples, 2006; Waples and

Do, 2010). The method is based on the principle that in a finite population, genetic drift generates the nonrandom association (linkage disequilibrium) among alleles at different loci (Hill, 1981). This theoretical relationship between effective size and the correlation of allele frequencies at pairs of loci is expressed as:

$$N_b = 1/[3(r^2 - 1/S)] \qquad (1)$$

(Hill, 1981, modified by Waples, 2006), where r^2 is a measure of linkage disequilibrium, the mean squared correlation of allele frequencies at pairs of loci, and S is the number of individuals sampled for genetic analysis. Several methods have been used to estimate r^2, in which Burrows' Delta is the most commonly used (England *et al.*, 2006; Waples, 2006; Waples and Do, 2010). LDNE uses the unbiased Burrow's Delta estimator (Waples, 2006) to compute r^2. The jackknife method implemented in LDNE used a leave-one-out re-sampling procedure over loci to estimate 95% confidence interval for N_b (Waples and Do, 2008).

Rare alleles can increase the precision but also upwardly bias N_b estimated using the LD method due to the increased number of allelic combinations (Waples, 2006). Simulations performed by Waples and Do (2010) have shown that a trade-off between bias and precision of LD was realized when alleles with frequencies of less than 0.02 were excluded. We compared N_b estimates when alleles with frequencies less than 0.05, 0.02 and 0.01 were eliminated. We found that excluding alleles with frequencies less than 0.01 and 0.02 yielded similar estimates of N_b (the difference of the two estimates averaged 1.4% compared to the estimates obtained with allele frequency ≤ 0.02), which

are 24.5% lower than N_b estimates when all alleles were included. Results of N_b reported are based on LD estimates when allele frequencies less than 0.02 were excluded.

Estimation of N_b using reproductive success

To better interpret demographic factors (*e.g.*, sex ratios and variance in reproductive success) affecting N_b, we provided another estimate of N_b based on the mean and variance in reproductive success as described by Crow and Denniston (1988) and Caballero (1994). First, the effective numbers of males (N_{bm}) and females (N_{bf}) were calculated as

$$N_{bm} = \frac{k_m N_m - 1}{k_m - 1 + V_m/k_m} \qquad (2)$$

and

$$N_{bf} = \frac{k_f N_f - 1}{k_f - 1 + V_f/k_f} \qquad (3)$$

respectively, where N is the number of males (N_m) and females (N_f) captured; k is the mean number of offspring produced by males (k_m) or female (k_f), and V is the variance in the number of offspring for males (V_m) or females (V_f). The effective number of breeders was then computed as:

$$N_b = \frac{4N_{bm}N_{bf}}{N_{bm} + N_{bf}} \qquad (4)$$

Mean and variance in reproductive success (the number of offspring assigned to each parent) for males and females were estimated based on results from parentage assignment using adult and offspring samples each of seven years 2001-2007.

We acknowledge that because larvae were sampled shortly after hatch (< 30 days, Duong *et al.*, in review) and given that significant mortality occurs between the time of larval capture and the time larvae recruit to the adult breeding populations, estimates of N_b for larval stages may differ from N_b that produce a cohort of adult recruits.

Parentage analysis - There are numerous programs that utilize multi-locus genetic data to estimate parentage (*e.g.*, CERVUS (Kalinowski *et al.*, 2007); PASOS (Duchesne *et al.*, 2005); COLONY (Jones and Wang, 2010)), and the efficacy of different programs or combinations of programs has been widely debated (Christie, 2010; Jones *et al.*, 2010; Walling *et al.*, 2010). Use of multiple programs which are based on different statistical properties to determine parentage has been advocated (Lee, 2008; Jones *et al.*, 2010). We used complimentary aspects of two programs, the Parentage Allocation of Singles on Open Systems (PASOS) program, version 1.0 (Duchesne *et al.*, 2005) and CERVUS version 3.0 (Kalinowski *et al.*, 2007) to conduct parentage analysis. Output of putative parent-offspring allocations from the two programs were jointly used to increase the allocation accuracy.

We used PASOS to estimate the proportion of adults captured and parentage allocation correctness. PASOS can detect missing parents when they have not been collected based on multi-locus genotypes of both parents and offspring. We set the maximum number offset tolerance (MOT), the maximum number of offsets between a parental and an offspring allele that PASOS accepts as possibly due to a scoring error

(Duchesne *et al.*, 2005), of 2 and the error model (0.002, 0.008, 0.98, 0.008, 0.002) for simulations in PASOS. We conducted simulations over five iterations of 1000 pseudo-offspring to estimate the allocation correctness. Under the same MOT (MOT = 2) as conducting simulations, we used the allocation function in PASOS to assign each offspring to two collected parents and estimate the proportion of adults captured from the natural population. The proportion of captured adults was used to parameterize analysis in CERVUS program.

CERVUS is the most commonly used categorical parentage technique (Christie, 2010; Jones *et al.*, 2010) and assigns progeny to a particular non-excluded parent based on likelihood scores (LOD scores obtained by taking the natural log of the ratio of likelihoods) derived from the genotypes of all offspring and parents sampled (Marshall *et al.*, 1998; Jones and Ardren, 2003; Kalinowski *et al.*, 2007). Simulations conducted within CERVUS to determine power and likelihood of assignment were based on empirical estimates of population allele frequency, the proportion of adults captured (PASOS output) and an empirical estimate of genotyping error (1.04%). Most likely candidate parent pairs of offspring from the assignment output were accepted as "true parent pairs" based on the criteria of ≥ 70% trio confidence with zero or 1 mismatch between parents and offspring genotypes. Assignment outputs from the two programs based on criteria above were then compared. Male parent-female parent-offspring triplets that were consistent between the two programs were used for further analysis to determine N_{bm} and N_{bf}.

The allocation correctness (the probability that each allocation is correct) from PASOS was almost constant across 7 years, ranging 80 – 83%, and standard deviation

among iterations simulated was very small (~1%). Meanwhile, CERVUS program provides a confidence level for each allocation based on user-define confidence levels. In order to choose a confidence value for all 7 years of data, we compared the concordance in assignment between PASOS and three confidence levels in CERVUS including "the most likely", 80 and 95% using parent and larval genotypes in 2001 as an example. The result showed the "most likely" confidence provided the highest assignment rate with the percentage of PASOS concordance as high as the 95% confidence. Therefore, we used the criterion for other year data sets.

Relative reproductive success of males and females

Relative reproductive success used in this study was defined as the number of offspring produced by each adult, which was the number of larvae genotyped and assigned to each individual. Mean (k_m and k_f) and variance (V_m and V_f) in reproductive success among individuals were calculated for all adults captured in the same year. Variation in N_b across years depends on differences in the magnitude of variance compared to the mean of reproductive success among years (equation 2). Therefore, we used standardized variance (variance/(mean)^2) in reproductive success for males and females to compare the magnitude of variance compared to the mean of reproductive success among years. We tested the correlation of mean and variance in reproductive success among years by using Spearman correlation (rho) because of small sample size (n = 7). The tests were conducted using R (R-Development-Core-Team, 2009).

Estimating contemporary N_e

Relationships between N_e and N_b estimated using single-sample methods including LD for a long-lived iteroparous species have rarely been investigated (Waples and Do, 2010). Luikart et el. (2010) suggested that if samples included multi-age classes, a LD-based estimate would provide values between N_b and N_e. Similarly, Waples and Do (2010) proposed that if the number of age-classes represented in a sample is approximately equal to the length of generation time, a LD-based estimate may approximate N_e per generation. Our adult data meet this criterion. During 7 years (2001-2007), most adults in the population were captured, consisting of a wide range of age-classes (15 – 52 yrs for males and 28-57 yrs for females, Figure 3.1, age was estimated from age-length relationship provided by Baker, unpublished). The LD-based estimate using genotypes of all adults captured in 2001-2007 (612 individuals genotyped) was interpreted as N_e per generation. Because samples collected from a single year were large (n =115 – 225, Table 1.1) and also contained the same range of age classes, single-year samples could also be used to estimate N_e. The age range of adults captured in a single year was as large as the entire adult sample captured in 7 years, the lower and upper ranges for females 26 - 32 yrs. and 57 – 60 yrs., for males 13 – 17 yrs. and 51 – 59 yrs., respectively (based on age-length relationships provided by Baker, unpublished) . We predicted that N_e estimated using adult samples from a single year would be similar to the N_e estimated based on all adults over 7 years.

RESULTS

Demographic parameters

Demographic parameters including adult sex ratios and male and female reproductive success were estimated to examine the relationship of these parameters and effective sizes. Sex ratios (males: females) were male-biased in all years, ranging from 1.61 -3.01. Results from parentage analysis revealed that high proportions of males (57-94%) and females (83-98%) over 7 years were assigned offspring (Table 3.1). Estimated mean and variance in reproductive success of males and females were highly positively correlated (rho = 0.89, P = 0.012 for females and rho = 0.85, P = 0.016 for males, n = 7), implying that both mean and variance in reproductive success were higher in years with higher larval collection compared to years with lower larval collection. Standardized variance across years varied 5.8-fold (between 0.38 – 2.20) and 6.2-fold (between 0.49 – 3.08) for females and males, respectively. Meanwhile, larval production varied approximately 40-fold across years (437 -16,417).

Annual estimates of N_b (2001-2007)

Annual N_b estimates based on the LD method ranged from 47 to 167 (95% CI 39 – 425, Figure 3.2). N_b estimated in 2002 was unusually high (mean: 167, 95% CI: 95-425) compared to other years. Except for 2002, the ratios of N_b to census adult numbers N were relatively stable across years and averaged 0.47 (0.31 – 65) (Table 3.2). Estimates of effective number of breeders using demographic data (N_{bV}) were close to but consistently lower than N_b estimated based on LD, ranging from 41 (in 2005) to 106 (in 2007) (Table 3.2, Figure 3.2). The ratios N_{bV}/N were relatively constant, ranging from

0.27 to 0.55. The small range of N_b/N obtained from the two methods indicated similar patterns of N_b and N across years. N and N_b varied 1.5-fold and 3.5-fold, respectively, during 7 years. Both methods consistently revealed that N_b and N_b/N was smallest in 2005 compared to the other years. In 2002, LD-based estimate of N_b (167) was much higher than that from demographic method (86), leading to an unusual ratio of N_b/N (> 1).

Effective population size per generation

Based on genotypes of all unique adults (612 individuals) collected in 7 years, N_e estimate per generation was 132 (95% CI: 104 – 167) (Table 3.3). This estimate is similar to the harmonic mean (133) of N_e estimated based on adults captured each year from 2001-2007 (Table 3. 1). Surprisingly, the estimate of N_e was close to annual N_b values. The harmonic mean of N_b of the current population (82) was 38% smaller than N_e (N_e = 132). Given the number of adults (N) in the Blake Lake population was estimated as approximately 750 - 1,100 (Baker, unpublished), the ratio N_e/N was approximately 0.12 - 0.18.

DISCUSSION

Using long-term data and applying a single sample method to estimate the effective number of breeders (N_b) and effective population size (N_e), we quantified inter-annual variability of N_b and N_b/N over 7 years for a closed lake sturgeon population characterized by high inter-annual variation in larval production. The number of

spawning adults each year and N_b were relatively stable although the number of larvae collected varied 40-fold across years. Therefore, stability of annual N_b/N may be explained by low yearly estimates of standardized variance (variance/(mean)^2) in relative reproductive success of lake sturgeon adults.

Relationship of N_b and aspects of the species biology

Effects of demographic factors such as reproductive success, sex ratios and adult census size on N_b can differ among species (Osborne *et al.*, 2010). The inverse relationship between variance in reproductive success and N_b has led to the hypothesis that high female fecundity and concomitantly high reproductive variance can result in proportionally lower N_b/N (Hedgecock *et al.*, 1992; Hedrick, 2005). Alternatively, N_b/N should vary as a function of the standardized variance in relative reproductive success (Nunney, 1996). Small and consistent standardized variance in relative reproductive success resulted in relatively constant N_b/N over 7 years in this lake sturgeon population. Given the fluctuation in numbers of spawning adults across years was small, years of higher larval production were characterized by higher relative reproductive success and also higher variance in relative reproductive success compared to years of lower larval production. Estimates of mean and variance in individual reproductive success inferred from parentage may not capture actual inter-annual trends in lake sturgeon reproduction because unequal proportions of larvae collected were genotyped in each year. However, standardized variance, which is independent from the mean and thus is not influenced by sample size, can represent inter-annual variation in reproductive success. In addition, estimates of standardized variance in reproductive success based on parentage were not

expected to differ from actual estimates based on production. This prediction was based on the high observed correlation between mean and variance in reproductive success, and on our sampling design that randomly sampled with proportional numbers from all larvae collected. Differences in standardized variance in relative reproductive success among years were small, resulting in small inter-annual variation in N_b. Therefore, variation in relative reproductive success across years was not predictive of temporal variation in N_b/N. Nunney (1996) demonstrated that among three sources of variation in relative reproductive success including individual differences, seasonal differences and age-related variation in relative reproductive success, individual differences most affected N_e (or N_b), and age-related variation in relative reproductive success had minor effects on N_e. Standardized variance of individual differences should be greater than 10 to reduce N_b/N <0.1, and that value is unusual in natural populations (Nunney, 1996). In natural populations of many taxa, standardized variance in female fecundity averaged 0.441 ± 0.322 (Clutton-Brock 1988). Similar values of standardized variance in relative reproductive success were observed in our lake sturgeon population.

Lake sturgeon female are highly fecund (mean: 323,684 eggs per fish, range: 53,672 - 460,270 eggs, range of weight 21.32 - 37.65 kg, n =14 (Bruch *et al.*, 2006)). However, eggs and larvae experience extremely high rates of mortality (Caroffino *et al.*, 2010; Forsythe, 2010), including the period between hatch and the time larvae were sampled in our study. Accordingly, the species may be susceptible to a “Hedgecock Effect” (Hedrick, 2005), a phenomenon whereby species characterized by high fecundity and extremely high variance in reproductive success due to progeny surviving from a few

parents will result in small within-generation N_e and a low N_e/N ratio (Hedgecock *et al.*, 1992). However, our data showed most adults captured each year contributed offspring to larval collections. Therefore, the "Hedgecock Effect", a scenario observed in some marine fish (Hedrick, 2005; Hauser and Carvalho, 2008) and invertebrate species (Levitan, 2005) may not occur commonly in lake sturgeon until the larval stage. Given the species has a long generation time (~ 20 years), the "Hedgecock Effect" may be observed at sexual maturity if survival rates vary widely among families. Information on inter-family variation in survival from the larval stage to the adult stage is not available for lake sturgeon.

In addition to variance in relative reproductive success, highly skewed sex ratios can reduce N_b/N (Frankham, 1995). For lake sturgeon, however, the moderate inter-annual variation in sex ratios observed over 7 years (1.61 -3.04) did not contribute to temporal variation in N_b/N (Figure 3.3, right). The non-effect of observed sex ratios on N_b/N could be explained based on the species mating system. Lake sturgeon exhibit polygyny and polyandry. The polygamous mating system reduces variance in reproductive success in both males and females, and thus maintains moderate N_b/N and compensates for the effect of sex ratios on N_b/N (Nunney, 1996; Pearse and Anderson, 2009).

Reliability of N_b estimates for the lake sturgeon population in Black Lake

Comparisons of different estimators provide better understanding of how different factors contribute to N_b (Waples and Do, 2010), and allow evaluation of reliability of

each method (Beebee, 2009)**.**The LD and demographic methods used in our study yielded comparable estimates and a relatively consistent pattern of inter-annual variation in N_b, indicating the estimates were reliable. Small confidence intervals of N_b estimated using LD method are also indicators of precision of the estimates (Beebee, 2009).

The ranges of N_b estimated were relatively small compared to other studies on different species using LD temporal methods (*e.g.*, Atlantic salmon *Salmo solar* (Palstra *et al.*, 2009); Pecos bluntnose shiner *Notropis simus pecosensis* (Osborne *et al.*, 2010)). However, in some cases the LD method yielded N_e estimates of infinity for several populations, and therefore the results were difficult to interpret (Palstra *et al.*, 2009; Osborne *et al.*, 2010). N_e estimated using the LD method may be biased when sample sizes are smaller than N_e (England *et al.*, 2006; Waples, 2006; Waples and Do, 2010). A higher range of confidence interval for N_b in 2002 compared to the other years (from 2001-2007) could be attributed to smaller sample size. Except for 2001, sample sizes in the other years were large (Table 1.1) enough to minimize bias in N_b estimates.

Ratios of the effective number of breeders to adult census size for this population are in the range commonly found in other species. Nunney (1993, 1996) reported that N_e/N commonly varied between 0.25 and 0.75. In salmonids species (*e.g.*, steelhead trout), N_e/N ranged 0.1-0.3 (Heath et al 2002) or 0.2-0.4 (Araki *et al.*, 2007). Meta-analyses from numerous species by Frankam (1995) and recently by Palstra and Ruzzante (2008) showed that median of N_e/N was 0.11 and 0.14, respectively. Reviews of theoretical and empirical studies from different species and characterized by different

mating systems suggest that a lower threshold of N_e be greater than 50 and the N_e/N be in the range 0.3 – 0.5 to buffer from genetic and demographic stochasticity (Frankham, 1995; Palstra and Ruzzante, 2008).

Contemporary N_e and the relationship between N_e and N_b

The LD estimator of contemporary N_e derived using adult samples provides an empirical example applying Waples and Do's (2010) suggestion that a LD-based estimate using a mixed sample including the number of age-classes as equal to generation time can be interpreted as N_e per generation. Luikart *et al.* (2010) also suggested that LD-estimates using samples composed of multiple cohorts represent values between N_b and N_e. Although the conjecture of these authors has not been quantitatively evaluated (Waples, 2010), our 7-year dataset including a larger range of age classes than the generation time of lake sturgeon would be expected to produce a reliable estimate of N_e. In addition, the similarity in age ranges of samples pooled across years versus yearly samples yielded similar estimates of N_e based on pooled samples and the harmonic mean of N_e based on a single year sample.

The relationship of N_b and N_e is complicated for long-lived and iteroparous species (Waples, 2010). In this study, we have found that N_e was comparable to N_b of a single larval cohort. We proposed two alternative predictions based on this result. First, the survival of larvae from the time sampled to the time larvae reach sexual maturity is likely very low and non-random among families. In this case, N_b estimated for a cohort of mature progeny could be much smaller than N_b estimated using samples at the larval

stage. If N_b/N was assumed to be similar to the observed N_e/N, approximately 0.12, the observed estimated N_e was smaller than the product of assumed N_b (~ 20) and generation time, as suggested for semelparous species with constant census sizes (Waples, 1990). Second, if mortalities were random among families, the correlation of allele frequencies at pairs of loci (r) based on genotypes of larvae would not vary when larvae mature. As a result, the observed N_b would be stable at different stages of a cohort. In sum, both scenarios suggested that N_e be smaller than the product of N_b and generation time. In other iteroparous species, (*e.g.*, natterjack toad (*Bufo calamita*)), N_b was also found to be similar to N_e in several populations (Beebee, 2009).

Implications

Our long-term study in this unique Black Lake system allowed us to quantify annual N_b from progeny that survived to the larval stage and N_e of adults. We were also able to evaluate the relationship of N_b with demographic parameters estimated concurrently over 7 years. The study has important implications for the conservation of lake sturgeon and other fish species. Although the current census size of the population is moderately large (upper limit ~1,100, Baker, unpublished), the ratio of N_e per generation to census size is low (0.12-0.18), suggesting that the population should be closely monitored to ensure retention of diversity. Comparisons of N_e and N_b suggest that for lake sturgeon and perhaps other long-lived, iteroparous species, N_e may not be approximated as the product of N_b and generation time (Waples 2010) as has been

demonstrated for semelparous salmon ($N_e = gN_b$, (Waples, 1990). Estimates of N_b in this study are close to N_e. Therefore, a supplemental breeding program for sturgeon species should not divide the target N_e into an annual target (*e.g.*, if target N_e =200 and generation time g=20, target N_b =10) as suggested previously (Kincaid, 1999; Welsh *et al.*, 2010)Because N_b is positively correlated with adult breeding number, maintaining a sufficient number of adults each year will likely minimize effects of other factors reducing N_b and thus maintain genetic diversity of lake sturgeon populations.

Table 3.1. Sex ratio and estimated individual reproductive success (RS) including mean, variance and standardized variance (SDV) of females and males in 7 years 2001-2007

Year	% adults assigned		Female RS			Male RS		
	Female	Male	Mean	Variance	SDV	Mean	Variance	SDV
2001	90	91	7.27	35.04	0.66	4.51	10.05	0.49
2002	76	94	2.82	3.03	0.38	1.31	1.36	0.79
2003	80	90	5.90	32.96	0.95	2.95	11.79	1.36
2004	71	88	4.48	22.68	1.13	1.47	1.99	0.91
2005	58	83	4.79	50.43	2.20	2.12	13.88	3.08
2006	65	98	3.31	12.88	1.17	1.31	1.99	1.16
2007	94	89	13.98	149.8	0.77	6.16	27.05	0.71

Table 3.2. Effective number of breeders estimated using linkage disequilibrium (N_b) and demographic methods (N_{bV}), and the ratios of N_b and adult census size (N_b/N) in 2001-2007

Year	Number of adults*	N_{bV}	N_b	N_{bV}/N	N_b/N
2001	173	76	63	0.44	0.37
2002	156	86	167	0.56	1.07
2003	182	57	69	0.32	0.38
2004	152	43	77	0.28	0.51
2005	153	41	47	0.27	0.31
2006	225	105	147	0.46	0.65
2007	206	106	126	0.51	0.61

(*) Census adult numbers in 2001-2004 (Table 1.1) were adjusted based on sampling effort ($N_{adjusted} = 1.5 * N_{captured}$).

Table 3.3. Contemporary effective population size (mean and 95% confidence interval, CI) estimated using linkage disequilibrium method based on adult genotypes from single year and pooled unique individuals in 7 years (2001 -2007).

Year	Sample size	Mean	95% CI
2001	114	110	73 – 186
2002	103	122	80 – 220
2003	119	154	93 – 330
2004	100	208	112 – 754
2005	152	126	83 – 220
2006	224	129	92 – 193
2007	205	119	88 – 170
All samples pooled	612	132	104 - 167

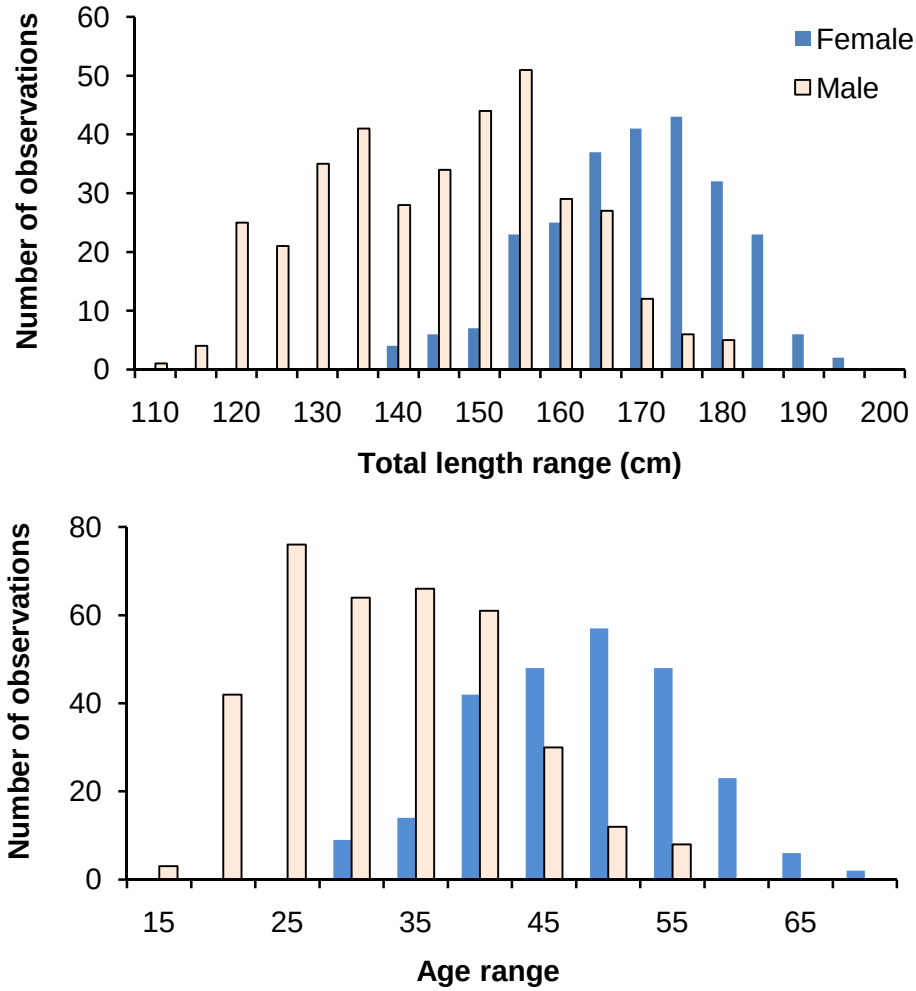

Figure 3.1. Total length (top) and age (bottom) distribution of male (n = 362) and female (n = 249) lake sturgeon (Age data provided by Baker, unpublished).

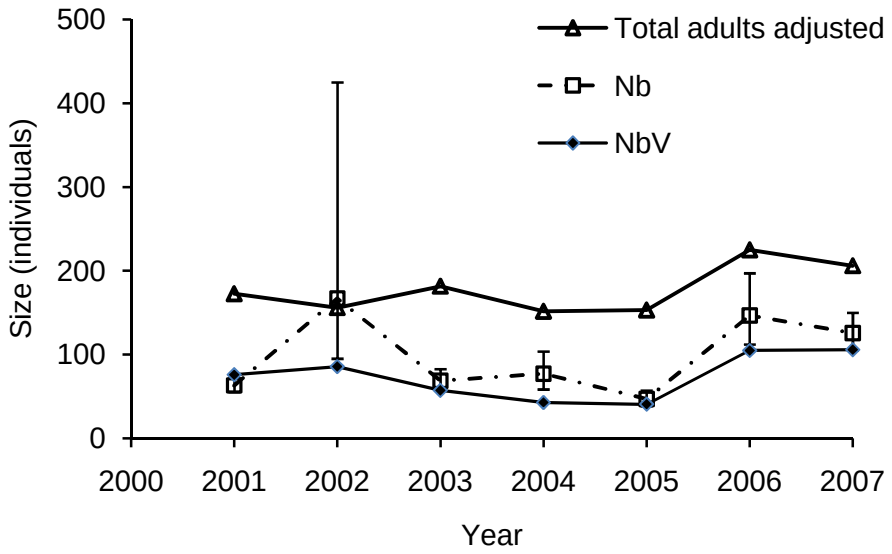

Figure 3.2. Total number of captured adults adjusted for sampling effort and effective number of breeders estimated using linkage disequilibrium (N_b) (bars show 95% confidence interval) and demographic methods (N_{bV}) in 2001-2007.

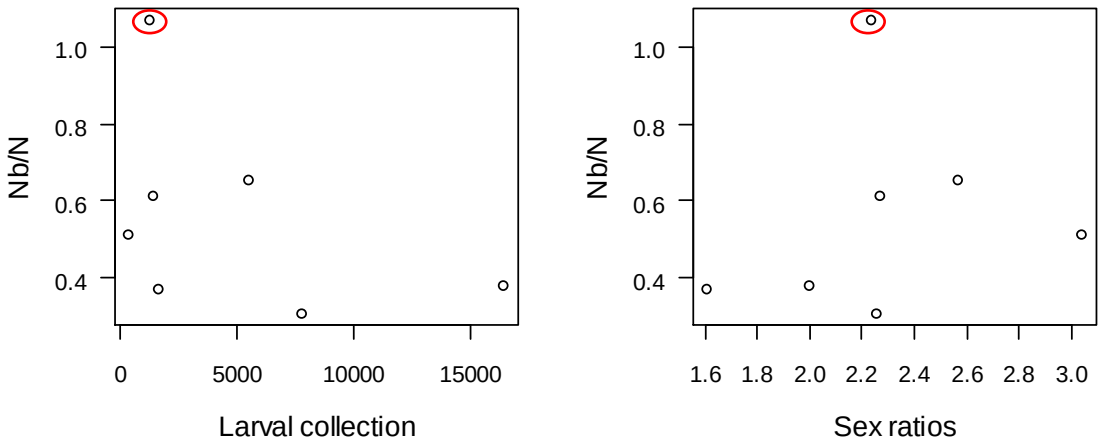

Figure 3.3. Plots showing relationships between the ratio of effective number of breeders to adult census size (N_b/N) and larval collection (left) and adult sex ratios (right) in 7 years (2001-2007). The points circled are data from 2002.

Chapter 4

ENVIRONMENTAL AND MATERNAL EFFECTS ON EMBRYONIC AND LARVAL DEVELOPMENTAL TIME UNTIL DISPERSAL OF LAKE STURGEON

ABSTRACT- For migratory fish like lake sturgeon (*Acipenser fulvescens*), the period from egg deposition through embryonic and larval development until dispersal (ELDTUD) contributes substantially to variation in lifetime survival at the individual level and to population levels of recruitment. Using genetically determined parentage, we examined the relative importance of stream environmental variables (*e.g.*, temperature and discharge) and maternal effects including female body size, spawning time and location to ELDTUD on an individual basis. Lake sturgeon adults (n = 208) spawning in the Upper Black River (Michigan, USA), and larvae (n = 1 444) dispersing downstream were captured during the 2007 spawning season. We used generalized mixed models and multi-model inference based on Kullback-Leibler information-theoretic criteria to demonstrate that environmental variables and maternal effects of female and spawning time were both important predictors of ELDTUD. Decreasing ELDTUD during the season resulted from linearly increasing temperature and nonlinearly decreasing river discharge. Spawning time and individual females explained a large proportion of variation in ELDTUD. The individual-based approach used in this study provided precise estimates of ELDTUD and also facilitated the partitioning of variation in ELDTUD of larvae produced by the same female and among females spawning at different times and different environmental conditions.

INTRODUCTION

Estimating the timing of development and of transitions between ontogenic events during early life stages and determining the relative importance of factors effecting developmental time under natural conditions are important subjects in fish biology. Embryonic and larval developmental time until dispersal (ELDTUD) is a critical period that exposes larvae to high risks of mortality due to biotic (*e.g.*, predation and competition (Garvey *et al.*, 1994; Paradis *et al.*, 1996), and abiotic factors such as temperature, oxygen (Chandler and Bjornn, 1988; Einum and Fleming, 2000)). ELDTUD may vary as a function of environmental factors (*e.g.*, temperature, stream discharge, *etc.*) and maternal effects (*e.g.*, egg size) (Gillooly *et al.*, 2002; Kamler, 2002; O'Connor *et al.*, 2007). Environmental conditions during embryonic and larval development are typically selected by parents (Trippel *et al.*, 1997; Kamler, 2002; Jorgensen *et al.*, 2008). Therefore, maternal effects, which occur when female phenotype or environments experienced at the time and location of spawning influence offspring phenotypic traits (Mousseau and Fox, 1998), and together with environmental variables associated with spawning time and spawning location can collectively contribute to embryonic and larval developmental time (Einum and Fleming, 2000; Kamler, 2002) and dispersal (*e.g.*, in aquatic invertebrates, Reitzel *et al.*, 2004; in fish, Edwards *et al.*, 2007).

Water temperature and stream discharge are two environmental factors that play an important role in the timing of larval hatch and dispersal (Heggberget, 1988; Pepin *et al.*, 1997) (Heggberget, 1988; Pepin *et al.*, 1997; Tetzlaff *et al.*, 2005) and thus ELDTUD. Metabolic rate increases with temperature (Gillooly *et al.*, 2001), which decreases the time required for incubation and yolk absorption. Temperature-dependent

developmental time has been reported in a variety of taxa including insects (Pritchard *et al.*, 1996; Johnson *et al.*, 2007; Arbab *et al.*, 2008), fish and invertebrates (Gillooly *et al.*, 2002; O'Connor *et al.*, 2007), and amphibians (Gillooly *et al.*, 2002). River discharge indirectly influences developmental time via oxygen supply (Kamler, 2002), and directly affects dispersal time by changing drifting and swimming speeds of larvae (Elliott, 1987; Fausch *et al.*, 2001; Siegel *et al.*, 2003).

ELDTUD may also vary due to maternal effects including spawning time and location, and effects associated with female body size or age. Spawning at specific times and locations has been shown to dictate conditions for offspring development and survival (Trippel *et al.*, 1997; Jorgensen *et al.*, 2008). Empirical and modeling studies have shown that adult spawning time and location also affect timing of larval emergence and dispersal (Einum and Fleming, 2000; Reitzel *et al.*, 2004; Edwards *et al.*, 2007). Maternal effects on egg size, where larger females usually produce larger eggs, have been observed in fish (Chambers and Leggett, 1996; Heath *et al.*, 1999; Heins *et al.*, 2004b). Larger eggs may require longer time for embryonic development (Pepin *et al.*, 1997; Gillooly and Dodson, 2000). In addition, for many species, genetic differences among adult groups spawning at different times (isolation by time) may contribute to variation in phenotypic traits of offspring (Hendry *et al.*, 2005).

Studies that simultaneously evaluate environmental and maternal effects may better explain variation in ELDTUD than studies that focus on effects of one set of variables alone. There is a lack of research that simultaneously quantifies effects of environmental factors and maternal effects on ELDTUD at the individual-level under natural conditions. Reasons for the lack of data include difficulties in identifying

genealogical relationships between adults and larvae and the need to collect a large number of parents and offspring from natural populations. The problem can be overcome using genetic makers to genetically determine parentage (Garant and Kruuk, 2005; Pemberton, 2008) of a population reproducing in natural and accessible habitats.

In this study, we used genetically determined parentage to examine effects of environmental factors and maternal effects on ELDTUD in a threatened fish species, the lake sturgeon (*Acipenser fulvescens*). Lake sturgeon exhibit an aggregate mating behavior where eggs and sperm are released by spawning adults over rock and gravel without nest preparation or parental care (Bruch and Binkowski, 2002). Because of the species mating behavior and exposure of eggs and post-hatch larvae to environmental conditions, larval lake sturgeon experience extremely high mortality early in life (Kempinger, 1988; Forsythe, 2010). Therefore, selection of spawning times and locations, which determines environmental conditions that affect eggs and larvae (Mousseau and Fox, 1998), by adult lake sturgeon could play important roles in offspring survival and ELDTUD. Newly-hatched larvae generally remain in the stream substrate until yolk-sac reserves are depleted, and then individuals disperse downstream in the current at night (Auer and Baker, 2002; Kynard and Parker, 2005; Smith and King, 2005). However, factors underlying variation in ELDTUD at the individual level are still unknown.

Similar to heritable spawning time observed in many salmonid species (Siitonen and Gall, 1989; Gall and Neira, 2004) repeatability of spawning time among adult groups in iteroparous species may have a genetic basis. Repeatability has been used as a measure of trait heritability (Boake, 1989). Data collected on spawning lake sturgeon in the Black River, Michigan (USA) over eight years (2001-2008) revealed that repeatability of

spawning time for females and males who have been captured more than two occasions was high (0.56 and 0.42, respectively, Forsythe 2010). We also observed that early-spawning lake sturgeon females produced offspring that had longer incubation times, larger body size, and larger yolk-sac reserves at hatch compared to offspring of late-spawning females (Crossman, 2008), which might lead to longer period from egg deposition to dispersal (ELDTUD). If groups of lake sturgeon spawning at different times are genetically differentiated and if spawning time affects ELDTUD, genetic factors could contribute to differences in ELDTUD.

The main objective of this study was to evaluate the relative importance of environmental factors (water temperature and discharge) and maternal effects (female body size, spawning time and location) and their interactions to ELDTUD of lake sturgeon under natural conditions. We also tested whether different adult groups whose offspring differed in ELDTUD were genetically differentiated. Additionally, we quantified the degree of temperature-dependence (cumulative thermal units or CTU) for ELDTUD to evaluate the practicality of this single variable as a predictor of the timing of larval dispersal for lake sturgeon.

METHODS

Study site

Our study was conducted in Upper Black River (UBR), the largest tributary to Black Lake, Michigan, USA (latitude 45^{o} 43'N, longitude 84^{o} 15'W; Figure 1.1a). The lake sturgeon population in Black Lake is isolated from other populations in adjacent lakes by dams blocking immigration and emigration from Lake Huron (Smith and King, 2005). Adults spawn over a 1.5 km-section of UBR. This section can be divided into six

locations of spawning activity that were used across years (Figure 1.1b). Shallow spawning areas (~1 m deep) and low turbidity allowed most adults to be observed and captured (Crossman, 2008; Forsythe, 2010) and larvae dispersing from all spawning areas to be collected (Smith and King, 2005).

Sample collection

Sampling for adults was conducted daily in 2007 by wading through the entire length of the stream encompassing all spawning sites one or more times per day during the entire spawning season. We captured spawning adults (143 males and 63 females) using long-handled nets. Sex of adults was determined by extruding gametes, and all individuals were measured for weight (kg) and fork length (cm). We also recorded date and location of capture, which were assumed to be the date and location of spawning based on our observation that lake sturgeon females spent only a few days on the spawning grounds (Forsythe, 2010). A dorsal fin clip (~1 cm^2) was taken for genetic analysis.

Larval sampling was conducted at night when the vast majority of larvae disperse (Auer and Baker, 2002). The larval sampling site was about 1.5 km downstream from the spawning areas (Figure 1.1a). Five D-frame larval nets were evenly spaced across the river channel (description in Smith and King, 2005) and were checked hourly from 2100-0200 hrs. Net locations remained consistent throughout the sampling period. Sampling began 10 days after the first spawning event was observed and continued until there were two consecutive nights with no larvae captured. Larvae were transferred to a stream-side hatchery and were reared in different tanks by sampling night (n = 31) until individuals were large enough for dorsal fin clips to be collected non-lethally. Mortality during the

rearing period was recorded and dead larvae were preserved in 95% ethanol by sampling night. In total, 1 444 tissue samples from dead and surviving larvae were available for genetic analysis. Based on the estimates of stream velocity and the proportions of total stream width sampled, juveniles collected were estimated to represent approximately 13% of total larval production (Smith and King, 2005).

Environmental data

Water temperature and river discharge data were collected continuously throughout the season. Water temperatures were recorded hourly using HOBO® data loggers (Onset Computer Corp.) placed at spawning areas. UBR discharge was predicted based on current discharge data obtained from the United States Geological Survey (USGS) gauging station on the Pigeon River, a nearby tributary of Mullet Lake, Michigan. Using UBR and Pigeon River discharge data during April - June for 50 years (1950-2000, USGS National Streamflow Information Program), we found that daily discharge of the UBR could be predicted as a linear function of daily discharge from the Pigeon River ($r^2 = 0.68$, $F_{1, 4\,056} = 9\,355$, $P < 0.001$). We used this relationship to estimate UBR daily discharge during 2007 because there were no discharge data from the UBR that year.

Genetic analysis

DNA was extracted from fin clips using the QIAGEN DNeasy$^{(R)}$ kit (QIA Inc.). DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc.). All samples were genotyped at 12 tetra-nucleotide microsatellite loci including Spl 120 (McQuown *et al.*, 2000); AfuG 68B (McQuown *et al.*, 2002). 2002); Aox 27 (King *et al.*, 2001); AfuG 68, AfuG 9; AfuG 63, AfuG 74, AfuG 112,

AfuG 56, AfuG 160, AfuG 195 and AfuG 204 (Welsh *et al.*, 2003). Using polymerase chain reaction (PCR), 100 ng DNA was amplified in 25 μl reaction mixtures containing 2.5 μl of 10 X PCR buffer (1 M tris-HCl, 1.5 M $MgCl_2$, 1 M KCl, 10% gelatin, 10% NP-40, and 10% triton X); additions of 1 mM $MgCl_2$ (1.5 mM $MgCl_2$ for AfuG 9) for all reactions but no additional $MgCl_2$ for AfuG 63, Aox 27 and AfuG 74; 0.8 mM deoxy-nucleotide-triphosphate (dNTP); 10pmol fluorescently labeled forward and unlabeled reverse primers and 0.5 U of Taq polymerase.

All PCR reactions were conducted using a Robocyler 96 thermal cycler (Stratogene). The PCR conditions were 94^{o}C for 2 minutes, followed by 30 cycles of 1 minute for primer-specific annealing temperatures (48^{o}C for AfuG 9, AfuG 63 and AfuG 112; 50^{o}C for AfuG 74; 53^{o}C for Aox 27; 56^{o}C for AfuG 68 and AfuG 68B; 58^{o}C for AfuG 56, AfuG 160 and AfuG 195; and 62^{o}C for Spl 120 and AfuG 204), 72^{o}C for 1 minute, and the final extension for 2.5 minutes at 72^{o}C. PCR products were run on 6% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO II scanner. Allele sizes were determined independently by two experienced personnel based on commercially available standards (MapMarker^{TM}, Bioventures Inc.) and samples of known genotype. Errors in genotyping were empirically checked by blindly re-genotyping a random 10% of all samples. Reported genotyping error is the ratio between observed number of allelic differences and total number of alleles compared (Bonin *et al.*, 2004).

Parentage analysis

There are numerous programs that utilize multi-locus genetic data to estimate parentage (*e.g.*, CERVUS (Kalinowski *et al.*, 2007); PASOS (Duchesne *et al.*, 2005); COLONY (Jones and Wang, 2010)), and the efficacy of use of different programs or combinations of programs has been widely debated (Christie, 2010; Jones *et al.*, 2010; Walling *et al.*, 2010). Use of multiple programs which are based on different statistical properties to determine parentage has been advocated (Lee, 2008; Jones *et al.*, 2010). We used complimentary aspects of two programs, the Parentage Allocation of Singles on Open Systems (PASOS) program, version 1.0 (Duchesne *et al.*, 2005) and CERVUS version 3.0 (Kalinowski *et al.*, 2007) to conduct parentage analysis. Output of putative parent-offspring allocations from the two programs were jointly used to increase the proportion of offspring allocated to parents and allocation accuracy.

We used PASOS to estimate the proportion of adults captured and parentage allocation correctness. PASOS can detect missing parents when they have not been collected based on multi-locus genotypes of both parents and offspring. We set the maximum number offset tolerance (MOT), the maximum number of offsets between a parental and an offspring allele that PASOS accepts as possibly due to a scoring error (Duchesne *et al.*, 2005), of 1 and the error model (0, 0.01, 0.98, 0.01, 0) for simulations in PASOS. We conducted simulations over 5 iterations of 1000 pseudo-offspring to estimate the allocation correctness. Under the restricted MOT (MOT of 1 is more restricted than MOT of 2), we used the allocation function in PASOS to assign each offspring to two collected parents and estimate the proportion of adults captured from the

natural population. The proportion of captured adults was used to parameterize analysis in CERVUS program.

CERVUS is the most commonly used categorical parentage technique (Jones *et al.* 2010; Christie 2010) and assigns progeny to a particular non-excluded parent based on likelihood scores (LOD scores obtained by taking the natural log of the ratio of likelihoods) derived from the genotypes of all offspring and parents sampled (Marshall *et al.*, 1998; Jones and Ardren, 2003; Kalinowski *et al.*, 2007). Simulations conducted within CERVUS to determine power and likelihood of assignment were based on empirical estimates of population allele frequency, the proportion of adults captured (PASOS output) and an empirical estimate of genotyping error (1.04%). Most likely candidate parent pairs of offspring from the assignment output were accepted as "true parent pairs" based on the criteria of ≥ 65% trio confidence with zero or 1 mismatch between parents and offspring genotypes. Assignment outputs from the two programs based on criteria above were then compared. Each male parent-female parent-offspring triplet that was inconsistent between the two programs was evaluated based on biological information (*e.g.*, proximity of captured time and captured location of both parents).

Genetic differences and relatedness among spawning groups

Repeatability in spawning time and spawning location for individual lake sturgeon males and females that have been captured 2 or more times (Forsythe, 2010) suggests the possibility of genetic differentiation among spawning groups within the UBR population, which may contribute to differences in ELDTUD. Due to higher repeatability in individual spawning time than in spawning location (0.56 and 0.42 vs. 0.16 and 0.04 for females and males, respectively, Forsythe, 2010), we predicted that

genetic differences could be detectable among members of different adult groups spawning at different times but not among groups spawning at different locations. We first estimated genetic differentiation (F_{ST}) among the three temporal spawning groups (early, middle and late) and among six spatial spawning groups of adults using the method of Weir and Cockerham (1984) implemented in the program FSTAT 2.9.3 (Goudet, 2001). If adult groups could be genetically differentiated, we then compared distributions and mean of inter-individual relatedness within and between genetically different groups. Moment-based estimators for pair-wise relatedness, r_{xy} (Queller and Goodnight, 1989) based on 12 microsatellite-locus genotype were obtained using KINGROUP v.2 program (Konovalov *et al.*, 2004). Nonparametric Kolmogorov-Smirnov tests were used to compare distributions of r_{xy} among adult groups. A permutation approach was used to test for difference in mean relatedness within and among spawning groups using a SAS-based program (A. Saxton, University of Tennessee, unpublished software) (Ratnayeke *et al.*, 2002). We also report allelic diversity, heterozygosity, probabilities of false parental exclusion, and tests (with Bonferroni correction) for deviations from Hardy-Weinberg equilibrium using CERVUS.

Data analysis

We defined the embryonic and larval developmental time until dispersal (ELDTUD) as the number of days elapsed between the day of female capture and the day of offspring capture as the larvae dispersed downstream from the spawning site (Figure 1.1a). Accordingly, ELDTUD included periods of incubation, yolk absorption in the stream substrate, and migration over a 2-km distance.

We calculated cumulative thermal units (CTU) and examined the importance of factors affecting ELDTUD. CTU (degree-day in Celsius) was calculated using the method of Kempinger (1988). CTU for each larvae was estimated as the sum of daily temperature (adjusted by substracting a constant 5.8 ^{o}C) during the period from egg deposition until dispersal.

We used analysis of variance (ANOVA) and pairwise t tests with unequal variance to test CTU differences among offspring produced by females that spawned at different times ("early", "middle" and "late"). Females were grouped into three categories based on observed discontinuities in spawning dates among adult groups. Difference in CTU among larval groups would indicate other factors beside temperature affecting ELDTUD (Kamler, 2002).

We used generalized linear and nonlinear mixed effect models to simultaneously examine environmental factors and maternal effects affecting ELDTUD. Environmental factors include mean daily water temperature and daily river discharge that larvae experienced during the period from egg deposition until capture. We defined maternal effects to include female spawning date (Julian date), spawning location (n = 6, Figure 1.1b) and body size (*i.e.,* fork length) and a random effect of females. Females were treated as a random effect to account for non-independence among offspring of the same female.

We initially fit data to the full model including fixed effects of water temperature (Tem_j), river discharge (Dis_l), Julian spawning date ($Date_m$), spawning location (Loc_n) and fork length (Len_o) and possible two-way interactions of fixed effects and random effect of females (Fem_u) using generalized linear mixed effect models. Two-way

interactions of fixed effects were examined based on biological relevance as recommended by Burnham and Anderson (2002). Accordingly, the effect of female body size on ELDTUD would not be expected to vary as a function of the time or location of spawning. Therefore, we excluded interactions with female body size from the initial full model. We also tested for multi-collinearity among independent variables of the model by examining the variance inflation factor (VIF) from the linear model with all fixed effects without interactions and the Pearson correlation of variable pairs (Graham, 2003). There was evidence of multi-collinearity between water temperature and Julian date (VIF of temperature and Julian date was 10.3 and 7.8, respectively; Pearson correlation, $r = 0.91$, $n = 1154$, $P < 0.001$). To isolate confounded effects of these two explanatory variables, we first assumed one variable was more explanatory of observed variance. We then replaced the less important variable by its residuals from the regression against the more important variable (Graham, 1997, 2003); review in (Heikkinen *et al.*, 2006). We reasoned that Julian date representing the conditions including temperature at spawning would be a more important explanatory variable than mean water temperature. The unique contribution of Julian date was disassociated from the shared effects of temperature by adding temperature residuals ($T.res_j$) from regression of water temperature (dependent variable) against Julian date (independent variable) in the full model. We evaluated normality of the data by examining residual plots of response and predicted variables. Based on the plot showing the nonlinear relationship between ELDTUD and river discharge, the quadratic term of river discharge was treated as a fixed effect and was also added to the model:

$$ELDTUD = \mu + T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Len_o + Dis*Date_{lm} + Dis*Loc_{ln} + Date*Loc_{mn} + Fem_u + \varepsilon_{ijlmnou} \quad (1)$$

where μ is the overall mean, (*) represents the interaction of two variables; and $\varepsilon_{ijlmnou}$ is the residual error for each larva i^{th}. In the above model, the random effect of females (Fem_u) was assumed to be normally distributed.

Model selection for the best model from a set of all possible combinations of environmental factors and maternal effects in (1) was based on Akaike Information Criteria (AIC) (Burnham and Anderson, 2002). The inclusion of females as a random effect in the full model was examined first, followed by selecting fixed effects of interactions and main effects (Ngo and Brand, 1997). The random effect was included if the AIC difference (ΔAIC) of the full model without and with random effect of females was greater than 4 indicating the model without random effect was less supported by the data (Burnham and Anderson, 2002). We used restricted maximum likelihood (REML) to fit models differing by the presence of the random effect and maximum likelihood (ML) for competing models differing in fixed effects (Pinheiro and Bates, 2000). The relative importance of environmental factors or maternal effects in ELDTUD was assessed based on ΔAIC (Δ_i =AIC of the i^{th} model - smallest AIC) and Akaike weights ($w_i = \exp(-\Delta_i/2)$). Variables with higher sum w_i (importance weight) from all models containing the variables in question are more important (Burnham and Anderson, 2002). We also used a confidence set for Kullback-Leibler best models (Burnham and Anderson, 2002) to provide the best explanation of variation in ELDTUD. The 95% confidence set represents

the subset of models with the sum of $w_i \geq 0.95$. All statistical analyses were conducted using R (R-Development-Core-Team, 2009).

RESULTS

Adult capture, larval collection and parentage analysis

The 2007 spawning season extended from 23 April to 1 June. Timing of spawning activity was multimodal with three distinct peaks in spawning (Figure 4.1a). More individuals were observed spawning at the beginning ("early" adults, n = 108 individuals) and middle of May ("middle" adults, n = 74) as opposed to later in May ("late" adults, n = 24). Larvae dispersed in two peaks 15 to 20 days following adult spawning (Figure 4.1b). Water temperatures increased throughout the spawning season, from 10.6 ^{o}C to 22^{o}C. River discharge (range 5.71 to 9.61 m^3/s) was higher and more variable during adult spawning than during the larval dispersal period.

Estimates of allele diversity (range from 2 to 11, average 5.3 alleles per locus) and expected heterozygosity (0.59) of lake sturgeon adults were moderate. Genotype frequencies from 11 of 12 loci conformed to Hardy Weinberg expectations for all adults sampled.

Of the total number of captured adults (143 males and 63 females) PASOS estimated that 89.2% of females and 87.2% of males contributed to the larvae collected. The assignment rate of offspring to two collected parents was 74.7% (1 079 parents-offspring allocations) and mean allocation correctness (± standard deviation) was estimated to be 79.1 ± 0.6%. The assignment rate from CERVUS was 85.5% (1 234 allocations). Comparison of parent-offspring triplets assigned from the two programs

revealed assignment consistency to be 78.6%. Exclusion of larvae with allocation discrepancies between the two programs did not affect results (data not shown). Each parent-offspring triplet in PASOS parental allocations was also one of the most likely triplets with positive LOD scores found in CERVUS. Based on concordant assignments from both programs, 1 154 offspring (79.9% of larvae collected) were assigned to collected parents including 137 male parents (94.5% of males captured) and 58 female parents (92.1% of females captured). The average number of offspring collected per male was 8.4 ± 6.5 and per female was 19.9 ± 14.3.

Embryonic and larval developmental time until dispersal

ELDTUD varied from 4-36 days (mean ± SD = 20.3 ± 5.7, n = 1 154). ELDTUD of larvae from the same female parent (the time period from the first to the last offspring collected) was 11.6 ± 4.0 days (n = 58 female parents). ELDTUD was significantly different among larvae whose female parents spawned during different segments (early, middle or late) of the breeding season ($F_{2,96.3}$ = 655, P < 0.001) (Table 4.1). Cumulative thermal units (CTU) averaged 201 ± 53 degree - days and also differed among larval groups (Table 4.1).

Certain combinations of both environmental variables and maternal effects better explained variability in ELDTUD than did variables within only one or the other of the variable categories (Table 4.2). ELDTUD was best explained by model 8 (w_i = 0.66; Table 4.2) including the random effect of females and fixed effects of temperature residuals, river discharge, quadratic effect of discharge, Julian spawning date, spawning location, and the discharge and spawning date interaction. Fixed effects in the model explained most of variation in ELDTUD (88.0%). Of the remaining variation

unexplained by the fixed effects, the random effect representing differences among females (variance 8.77, confidence intervals, CI = 6.05 – 12.71) accounted for 94.5% (CI = 92.8% – 95.8%), which is much greater than differences within females (*i.e.*, residual variance) in average ELDTUD.

Based on Kullback-Leibler criteria, river discharge and spawning date associated with temperature were equally important predictors of ELDTUD. Importance weights for all these factors (sum of w_i for models containing these factors) were close to 1 (Table 4.2). ELDTUD was non-linearly related to river discharge. Coefficient estimates of the linear term (slope ± SE = - 143 ± 3.0) and quadratic term (slope ± SE = 2.58 ± 0.16) of river discharge in the final model (Table 4.3) indicate the nonlinear decrease in ELDTUD with increasing river discharge. Larvae produced by females spawning early in the season when water temperature was comparatively low (14 – 15^{o}C) dispersed after longer periods of time compared to larvae produced by females spawning later in the year (slope ± SE = -6.40 ± 0.12, $t_{1, 56}$ = - 51.9, $P < 0.001$). However, the magnitude of effects of spawning date and river discharge on ELDTUD depended on the interaction between these variables (Table 4.3). Spawning date effects on ELDTUD included the effect of water temperature (Pearson correlation, $r = 0.91$, $n = 1154$, $P < 0.001$). Julian date alone explained 55.8% of the variation in ELDTUD, compared to 20.9% variation explained by water temperature (based on coefficient of determination, r^2, from linear models of ELDTUD with each predictor of Julian date and temperature, respectively). Nevertheless, temperature residuals increased model fit. The full model with temperature residuals had an AIC score lower compared to the same model without temperature residuals

(difference in AIC = 6), indicating the importance of temperature when its effect was disassociated from the shared effect of spawning date on ELDTUD.

Spawning location and female body size were less important predictors of ELDTUD (importance weights were 0.30 and 0.15, respectively; Table 4.2). When the fixed effect of spawning date and random effect of females were included in the model to account for variation in ELDTUD of larvae from different families, spawning location and female body size on ELDTUD no longer improved model predicting (comparing AIC values of model 9 and 10, Table 4.2).

The confidence set for Kullback-Leibler best models included models 8, 7 and 5 (sum $w_i = 0.95$). These nested models supported the hypothesis that a combination of environmental factors and maternal effects best explained variation in ELDTUD. There was no evidence ($w_i \sim 0$) supporting the hypotheses that either environmental factors or reproductive variables alone provided the best prediction of ELDTUD of lake sturgeon.

Genetic differences and relatedness among spawning groups

Genetic differences (F_{ST}) were not significant among females and adult groups spawning at different locations, (95% confidence interval of overall F_{ST} [bootstrapping over loci] for only females ranged from 0.000 to 0.016, and for all adults from 0.000 to 0.002). Genetic differences were observed among adult groups (males and females) spawning at different times but not among different spawning groups of female. Pairwise F_{ST} between early and middle adults groups (0.007) was significant (alpha = 0.05, after standard Bonferroni corrections, P-value adjusted = 0.016). However, no genetic differences were observed between early-late ($F_{ST} = 0.002$) and middle-late ($F_{ST} =$

0.006) adult groups (P = 0.17 and 0.20, respectively). Mean inter-individual relatedness (r_{xy}) among adults of the middle spawning group (mean ± SD; 0.034 ± 0.213) was significantly higher than r_{xy} among members of the early spawning group (-0.015 ± 0.223) and late spawning group (-0.017 ± 0.209). Distributions of cumulative expected frequencies of r_{xy} values were significantly different between middle adult group compared to early and late groups (differences in distribution, Kolmogorov-Smirnov tests, 9.8 and 12.1%, respectively, P < 0.001). No differences in mean (P = 0.45) and distribution (P = 0.81) of r_{xy} were observed between early and late groups. Mean r_{xy} estimated within and between groups of adults were also not significantly different (P = 0.08).

DISCUSSION

Using individual-based measures of embryonic and larval developmental time until dispersal (ELDTUD) based on genetic determination of parentage, we quantified variation in offspring ELDTUD within and among females and evaluated the relative contributions of environmental and maternal factors affecting ELDTUD. Variation in ELDTUD was lower among larvae produced by the same female relative to those produced by different females that spawned during the same period of the spawning season (early, middle and late groups). Similarly, variation in ELDTUD was lower among larvae produced by females spawning within the same group relative to those produced by females that spawned at different times.

Non-genetic components of maternal effects (*e.g.*, spawning time and spawning location) confounded by environmental factors affected ELDTUD. Using statistical

approaches to account for correlations among variables based on Kullback-Leibler multi-model inference (Burnham and Anderson, 2002; Johnson and Omland, 2004), we disassociated confounding effects due to colinearity among predictor variables. Maternal effects of female and spawning date, and environmental factors (water temperature and discharge) were in the confidence set for Kullback-Leibler best models indicating that these variables were of comparable importance to ELDTUD of larval lake sturgeon.

Temperatures during spawning and embryonic and larval development can affect offspring traits, including body size (Fox and Czesak, 2000) and developmental time (Gillooly and Dodson, 2000; Stillwell and Fox, 2005). Although temperature impacts larval developmental time in many species (Gillooly *et al.*, 2001; O'Connor *et al.*, 2007) inter-individual variation in developmental time may be substantial. In our study, mean daily temperature only explained 20.9% of variation in ELDTUD of larval lake sturgeon. The degree of temperature-dependency of ELDTUD could differ during the three consecutive developmental periods represented by this composite variable including (i) embryonic development, (ii) yolk sac absorption; and (iii) emergence and dispersal from spawning areas. The first two periods are likely more dependent on water temperature (Wang *et al.*, 1985; Pepin *et al.*, 1997; Kamler, 2002). The third period may depend more on other environmental factors (*e.g.*, river discharge, food, and predators), larval age or size (Elliott, 1987; Day and Rowe, 2002) and behavior (Shanks, 2009), lunar cycle effects on larval concealment (Hernandez-Leon, 2008), and female spawning behavior (Copp *et al.*, 2002; Hogan and Mora, 2005; Shanks, 2009).

Another environmental factor, river discharge, has been shown to affect timing of larval emergence (Fausch *et al.*, 2001) and movements (Elliott, 1987; Siegel *et al.*, 2003),

and therefore was predicted to affect ELDTUD. Fausch *et al.* (2001) found that emergence of rainbow trout (*Oncorhychus mykiss*) fry occurred during the period of high water velocity. After emergence, larvae can be dislodged from substrate by strong currents (salmonids (Elliott, 1987); pallid sturgeon *Scaphirhynchus albus,* (Kynard *et al.*, 2007)). However, for some species such as reef fishes, larvae can actively adjust their swimming speed. At high river discharge, larvae swim more slowly (Hogan and Mora 2005). Similarly, lake sturgeon larvae might exhibit considerable behavioral plasticity by remaining in substrates longer during times of high river discharge.

The effect of female spawning date on ELDTUD could be due to different environmental conditions (*e.g.*, water temperature and discharge) at spawning and also different adult groups. In many fish species, adult groups spawning early or late in the season might differ in maternal effects (Chambers, 1997; Champer, 1997; Einum and Fleming, 2000). This is also the case for lake sturgeon, evidenced by highly significant differences in ELDTUD among the three larval groups (Table 4.1 and Figure 4.2) and greater among-female than within female variation in ELDTUD (Figure 4.3). Adult groups that spawned at different times exhibited the low level of genetic differentiation, providing a certain degree of support for the hypothesis that maternal effects on ELDTUD could be due in part to genetic factors. Higher F_{ST} among temporal spawning groups than spatial spawning groups is consistent with stronger effects of spawning time compared to spawning location on ELDTUD, which provides further evidence of a genetic component that may partly contribute to differences in ELDTUD. However, given the low variance in frequency of alleles at the microsatellite loci used, gene flow among members of different spawning groups is likely. Further investigation of the

concordance among measures of inter-group variance in additive and neutral genetic traits is warranted.

The importance of spawning date in determining larval developmental and dispersal time observed in lake sturgeon was also reported in marine fish and invertebrates (Reitzel *et al.*, 2004). Edwards *et al.* (2007) used 2-dimensional dispersal kernels to examine factors affecting larval dispersal and found that spawning time and location might be more important than larval behavior in determining larval dispersal time. In our study, the effect of spawning location on larval dispersal time was less important than spawning date. The relatively small spawning areas of the lake sturgeon population covering 1.5 km of UBR may explain comparatively minor effects of spawning locations on ELDTUD.

Incorporating both ecological and genetic data in this study provided a useful tool to explore degrees of, and factors contributing to inter-individual variation in ELDTUD of lake sturgeon. Individual-level measurement of ELDTUD based on the knowledge of parentage was more informative compared to population-based observations. For example, our results of CTU influences on ELDTUD were different from those reported by Smith and King (2005) for the same lake sturgeon population. Smith and King (2005) calculated CTU based on observations from the day of adult spawning to the day of peak larval drift. They reported that CTU for two or three larval groups from each spawning season from the years 2000 through 2002 ranged from 136.2 – 181.2. In their study, CTU and time to dispersal of the early larval group were lower than for the late larval group in the same year, which is opposite of our findings. Water temperature is generally colder early in the spawning season. Consequently, the time from egg deposition to dispersal of

larvae would be expected to be longer due to temperature-dependent development (O'Connor *et al.*, 2007). Importantly, population-based approaches could not detect important relationships between developmental time at early life stages and spawning behavior of individual female parents. Population-based estimates of CTU might introduce considerable bias in estimating ELDTUD in the absence of genetic data that conclusively ties larvae to a specific date and location of a spawning event.

Several factors should be considered when interpreting the data. First, the characterizations of spawning time and spawning locations were based on the time and location of adult capture. This assumption could lead to decreased precision in calculating ELDTUD. However, daily surveys of the spawning sites suggest that few females spent more than 2 or 3 days in the spawning areas (Forsythe, 2010), indicating minor effects on ELDTUD given the duration of spawning activity. Second, data were collected from a single year, while inter-annual variation in environmental conditions can be large and different groups of adults spawn in different years (Forsythe, 2010). Based on Akaike weights of the best model set, we believe that the relative importance of the variables in our model will not vary substantially, though ELDTUD observed as a function of these variables may vary depending on inter-annual variation in environmental conditions realized each spawning season. Finally, the dispersing larvae were collected within 2 km of the spawning area and numbers captured during this critical life stage may not reflect the numbers of larvae that disperse over different ELDTUD periods and survive.

Future research on costs of timing of dispersal is necessary and can be accommodated using genetic methodologies as employed in this study. Rates of mortality may vary as a function of larval body size, whereby selection acts to select individuals

that disperse at different times and concomitantly at different sizes (Sogard, 1997). Predation acting on different size classes of juveniles is likely a significant source of mortality (Mittelbach and Persson, 1998). Spatially complex rearing habitats are expected to contribute to variation in larval growth and dispersal time. Costs of dispersal are expected to be high, and can increase if larvae disperse at certain times or developmental stages in response to stream conditions. Further analyses are warranted to address the costs of dispersal time in terms of probability of mortality during this period.

Our findings have important implications for early life history studies and for species conservation. CTU and timing of adult spawning events should be simultaneously taken into account when predicting larval ELDTUD. Individual-based estimates revealed different levels of variation in ELDTUD within and between female families and among female groups. Such variation likely provides conditions for natural selection to act and may be of importance for retention of levels of viability at the population level. Effects of environmental factors did not outweigh maternal effects including spawning time on ELDTUD. Accordingly, we suggest that the maternal effects may counter the effects of the environmental variables on development, performance, and survival of larval lake sturgeon as well as other fish species.

Table 4.1. Mean (± 1 standard deviation) temperature at spawning and daily temperature over the embryonic and larval developmental time until dispersal (ELDTUD) and cumulative thermal units (CTU) of three larval groups illustrated in Figure 4.2.

Larval group (Female spawning date)	Number of observations	Spawning temperature (^{o}C)	Mean daily temperature (^{o}C)	ELDTUD (days)	CTU (degree-days)
Early (4/25 – 5/6)	792	12.9 ± 1.0	15.4 ± 0.5	22.7 ± 4.6	219 ± 48
Middle (5/10 – 5/13)	329	16.9 ± 0.8	16.6 ± 0.3	15.4 ± 3.4	168 ± 41
Late (5/20 – 5/27)	33	15.9 ± 1.3	18.3 ± 0.7	9.24 ± 2.7	114 ± 33

Note: All variables differed significantly among periods ($P < 0.001$).

Table 4.2. Model structure describing embryonic and larval developmental time until dispersal (ELDTUD) and model selection criteria based on Akaike Information Criteria (AIC), AIC difference (ΔAIC) and Akaike weight (w_i).

No.	ELDTUD model	k	AIC	Δ AIC	w_i
	Including environmental and maternal effects				
1.	$T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Len_o + Dis*Date_{lm} + Dis*Loc_{ln} + Date*Loc_{mn} + Fem_u$	24	2 868	27	0.00
2.	$T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Len_o + Dis*Date_{lm} + Dis*Loc_{ln} + Date*Loc_{mn}$	23	4 678	1 837	0.00
3.	$T.res_j + Dis_l + Date_m + Loc_n + Len_o + Dis*Date_{lm} + Dis*Loc_{ln} + Date*Loc_{mn} + Fem_u$	23	3 061	220	0.00
4.	$T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Len_o + Dis*Date_{lm} + Date*Loc_{mn} + Fem_u$	19	2 849	7	0.02
5.	$T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Len_o + Dis*Date_{lm} + Fem_u$	14	2 844	3	0.15
6.	$T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Len_o + Fem_u$	13	3 980	1 139	0.00
7.	$T.res_j + Dis_l + Dis^2_l + Date_m + Loc_n + Dis*Date_{lm} + Fem_u$	13	2 844	3	0.15
8.	$\mathbf{T.res_j + Dis_l + Dis^2_l + Date_m + Dis*Date_{lm} + Fem_u}$	**8**	**2 841**	0	**0.66**
9.	$Dis_l + Dis^2_l + Date_m + Dis*Date_{lm} + Fem_u$	7	2 847	6	0.02
	Including only maternal effects				
10.	$Date_m + Loc_n + Len_o + Date*Loc_{mn} + Fem_u$	15	6 249	3 408	0.00
11.	$Date_m + Fem_u$	4	6 232	3 391	0.00
	Including only environmental variables				
12.	$Tem_j + Dis_l + Dis^2_l + Tem*Dis_{jl}$	6	6 310	3 469	0.00
13.	$Tem_j + Dis_l + Tem*Dis_{jl}$	5	6 309	3 468	0.00

Note: Models include different combinations of fixed effects of temperature residuals ($T.res_j$, from temperature [Tem_j] regression against Julian date [$Date_m$]), river discharge (Dis_l), location (Loc_n), fork length (Len_o), and interactions of discharge-Julian date ($Dis*Date_{lm}$), discharge-location ($Dis*Loc_{ln}$) and Julian date-location ($Date*Loc_{mn}$), and random effect of females (Fem_u). k = total number of parameters including intercept and error terms. Model 1 and 2 was fit by restricted maximum likelihood (REML) for testing the importance of random effect.

Table 4.3. Coefficient estimates of fixed effects in the best model (model 8 in Table 4.2) representing relationships between model parameters and embryonic and larval developmental time until dispersal (ELDTUD).

Parameters	Coefficient estimate	SE[a]	denDF[b]	t-value	P-value
Intercept	1 072	18.6	1 092	57.6	<0.001
Temperature residual	-0.43	0.16	1 092	-2.62	<0.01
Discharge	-143	3.0	1 092	-48.1	<0.001
Discharge^2	2.58	0.16	1 092	16.06	<0.001
Julian date	-6.40	0.12	56	-51.87	<0.001
Discharge*Julian date	0.72	0.02	1 092	44.08	<0.001

[a] Standard error

[b] Denominator degrees of freedom. The numerator degrees of freedom for all parameters are equal to 1.

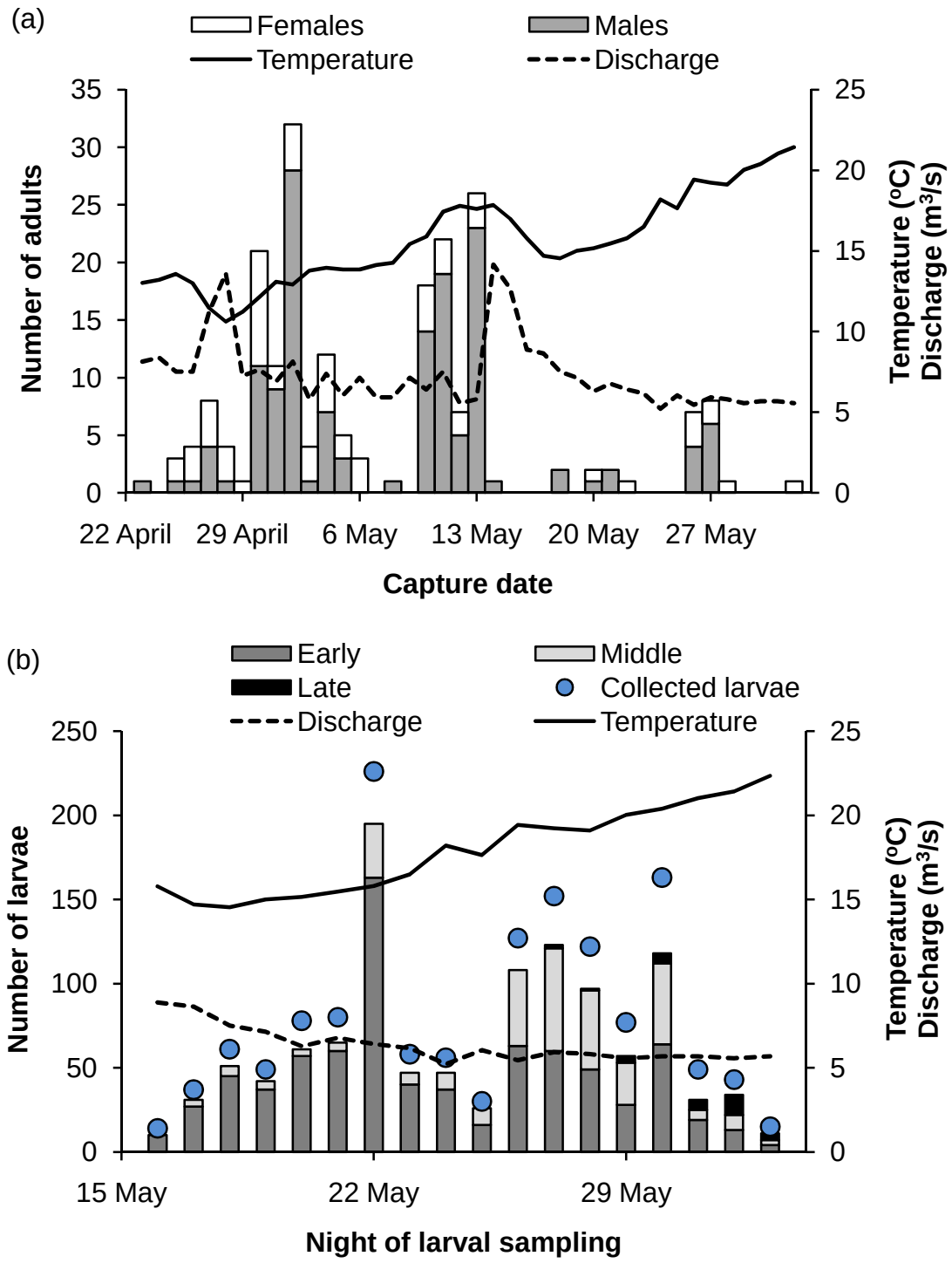

Figure 4. 1. Number of adults captured (a) and number of larvae collected and those assigned to early, middle or late female groups (b), together with mean daily water temperature and river discharge during the periods from egg deposition until larval dispersal.

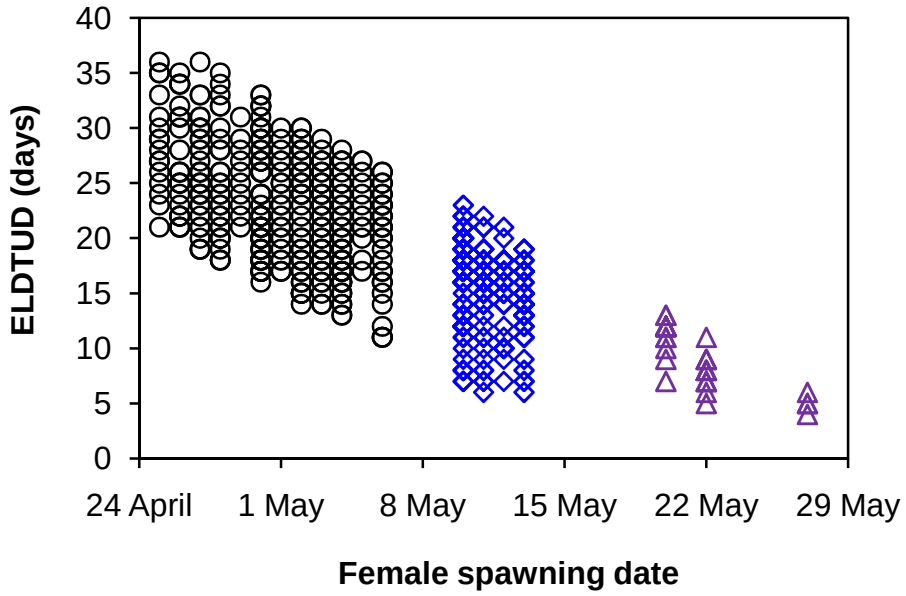

Figure 4.2. Embryonic and larval developmental time until dispersal (ELDTUD) of three groups of larvae whose maternal parents spawned during “early” (circle), “middle” (diamond) or “late” (triangle) periods of the spawning season. Darkened symbols indicate more than one offspring characterized by the same ELDTUD and female spawning date.

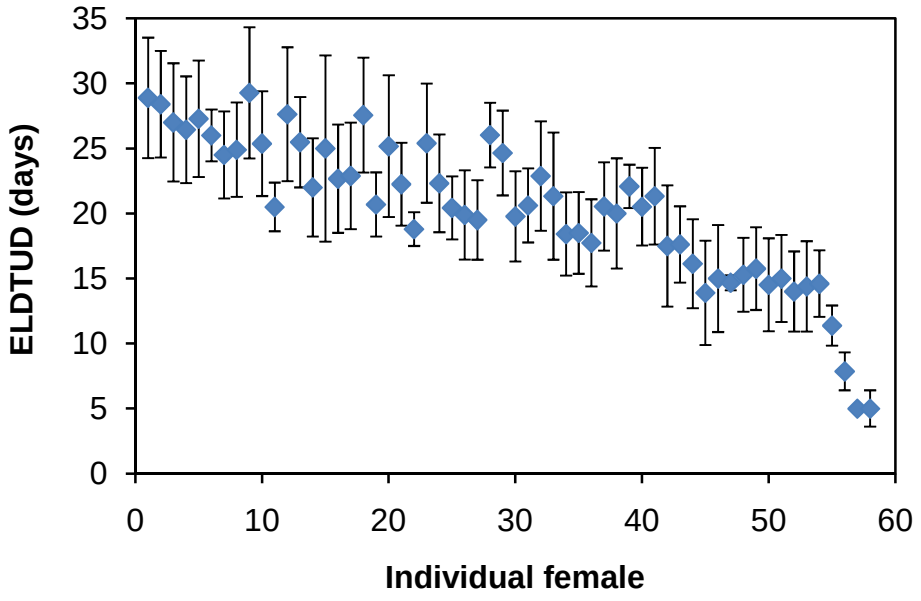

Figure 4.3. Mean and standard deviation of embryonic and larval developmental time until dispersal (ELDTUD) among siblings from the same female parent. Individual females were ordered left to right by spawning date.

Chapter 5

RELATIVE LARVAL LOSS AMONG FEMALES DURING DISPERSAL OF LARVAL LAKE STURGEON

ABSTRACT - Mortality that occurs during larval dispersal as a consequence of environmental, maternal, and genetic effects and their interactions can affect annual recruitment in fish populations. We studied larval lake sturgeon (*Acipenser fulvescens*) drift to examine whether larvae from different females exposed to the same environmental conditions during dispersal differed in relative levels of mortality. We estimated proportional contributions of females to larval collections and relative larval loss among females as larvae dispersed downstream between two sampling sites during two consecutive nights based on genetically determined parentage. Larval collections were composed of unequal proportions of offspring from different females that spawned at upstream and downstream locations (~0.8 km apart). Hourly dispersal patterns of larvae produced from females spawning at both locations were similar, with the largest number of larvae observed during 2200-2300 hrs. Estimated relative larval loss did not differ significantly among females as larvae were sampled at two sites approximately 0.15 and 1.5 km from the last section downstream of spawning locations. High inter- and intra-female variation in larval contributions and relative larval loss between nights may be a common feature of lake sturgeon and other migratory fish species, and likely is a source of inter- annual and intra-annual variation in fish recruitment.

INTRODUCTION

Mortality during early life stages is a major factor affecting recruitment in fish populations (review in Pepin 2009). Quantifying mortality rates of fish at early life stages is important to understand fish population dynamics (Keesing and Halford, 1992; Pepin, 2009). During the larval stage, high mortality occurs at critical periods, for example, when larvae switch from endogenous feeding (yolk absorption) to exogenous feeding (Kamler, 1992; Fuiman and Higgs, 1997), and when individuals disperse from spawning locations to nursing grounds (Bartsch *et al.*, 1989; Kamler, 1992; Fuiman and Higgs, 1997). In many fish species, these two events occur at or near the same time, as larvae often begin feeding prior to or during dispersal (*e.g.,* lake sturgeon *Acipenser fulvescens* (Auer and Baker, 2002)). Therefore, larvae become most susceptible to mortality during the initial period of dispersal when larvae emerge and disperse from the spawning ground (Einum and Fleming, 2000).

Exogenous and endogenous factors can contribute to larval mortality during dispersal (Kamler, 1992; Cowen and Sponaugle, 2009). Major exogenous sources include biotic (*e.g.*, predation, food availability) and abiotic factors (*e.g.*, extreme temperatures, stream flow) (Kamler, 1992; Mion *et al.*, 1998; Cowen and Sponaugle, 2009). Endogenous factors such as egg size, egg energy content (yolk size), larval size, etc., involve female phenotypes as maternal effects (*e.g.*, size, age) (Hutchings, 1991; Keckeis *et al.*, 2000; Kamler, 2005). Spawning time and location, which are also classified as maternal effects (Mousseau and Fox, 1998) also determines the environmental conditions that developing eggs and larvae are exposed to after egg deposition (Trippel *et al.*, 1997; Hendry and Day, 2005; Jorgensen *et al.*, 2008). Therefore, maternal effects can influence

probabilities of survival at embryonic, larval and juvenile stages (Kamler, 1992; Chambers, 1997). Previous studies have emphasized that exogenous factors are dominant sources of larval mortality (Kamler, 1992). Recently, increasing emphasis has been placed on the importance of larval behavior and genetic and maternal effects, which can lead to different probabilities of individual survival even when exposed to similar environmental conditions (Heath and Gallego, 1997; Bolnick *et al.*, 2003; Leis, 2006; Fiksen *et al.*, 2007; Clobert *et al.*, 2009). When larvae are exposed to a common environment, if maternal effects are weak, and differences in probability of individual survival are small compared to strong effects of external environmental factors, the relative rates of offspring mortality among females would be expected to be similar. The opposite prediction would lead to greater inter-family variation compared to intra-family variation, which are two important components of selection, in larval survival. We tested these predictions using a well-studied population of lake sturgeon (*Acipenser fulvescens*).

Lake sturgeon are characterized by a long life span, delayed maturity, iteroparity and high fecundity but low annual recruitment due to high mortality during early life stages (Peterson *et al.*, 2007; Forsythe, 2010). Lake sturgeon do not provide post-ovulatory parental care for eggs or larvae (Bruch and Binkowski, 2002). Deposited eggs adhere to stream substrates and hatch following 5 to 11 days of incubation depending on water temperature (Auer and Baker, 2002; Smith and King, 2005). Newly-hatched larvae remain in the stream substrates until yolk-sac reserves are depleted. Larvae are negatively phototactic and disperse downstream at night (Auer and Baker, 2002; Kynard and Parker, 2005; Smith and King, 2005). This nocturnal behavior is considered to be an adaptive means of predator avoidance (Auer and Baker, 2002), and has been observed in other

sturgeon species (Kynard and Parker, 2005) and other fishes (Crisp and Hurley, 1991; Bradford and Taylor, 1997). Previous studies have investigated spatial and temporal patterns in abundance of dispersing lake sturgeon larvae in relation to environmental conditions (*e.g.*, water temperature and water flow), revealing that dispersing larvae were distributed in a non-uniform manner across the width of stream and position in the water column, and over different hours of the night (Auer and Baker, 2002; Smith and King, 2005). Little information is available about the contributions of different females to dispersing larvae each night. Neither are quantitative estimates available regarding relative larval loss among females during the larval dispersal period for many fish species including lake sturgeon.

The objectives of this study were to (i) quantify proportional contributions of different females to dispersing larvae collected during consecutive nights; (ii) characterize hourly dispersal patterns of larvae from females spawning at different locations; and (iii) quantify relative larval loss among females and among female groups spawning at different locations. Data pertaining to inter-family variation in contributions to larval dispersal and the relative roles of maternal and stream environmental effects on larval survivorship during dispersal can lead to greater understanding of inter-annual and intra-annual variation in fish recruitment.

METHODS

Study site

A well-studied population of lake sturgeon located in Black Lake, northern Michigan was used for this study. In this system, the Upper Black River (UBR) is the largest tributary to Black Lake and the sole location used for spawning (Figure 5. 1). The

Black Lake population is isolated by dams blocking immigration and emigration from other populations (Smith and King, 2005). Adults spawn over ~ 1 km-section of the UBR, classified for the purposes of this study into two areas referred to as upstream (site B) and downstream (site C), which are utilized consistently by spawning females in all years (Forsythe, 2010).

Sampling design, sample collection and measurement of environmental factors

Lake sturgeon adults were sampled daily as they arrived at spawning locations during the 2006 spawning season that occurred from April 20 to May 26. We walked the entire spawning area one or more times per day using long handled nets to capture spawning adults. Biological information including sex, capture date and location was recorded for all individuals. A dorsal fin clip (~1 cm^2) was taken from each individual for genetic analysis.

Larvae were sampled at 2 sites located 0.15 (D1) and 1.5 (D2) km downstream from the most downstream zone where adults spawned (Figure 5. 1). Larval sampling was conducted during two nights, May 28 and 29, 2006. At each site, five D-frame larval nets were placed across the river channel (description in Smith and King, 2005) and checked hourly from 2200 to 0200 hrs. Larvae captured were kept separate by hour of capture and site and were reared in a streamside hatchery until they were large enough (for 3 months) for fin clips to be taken. Then, 30% of all the fish were sub-sampled for genetic analysis. Samples genotyped included 831 larvae including 283 and 145 larvae collected at sites D1 and D2 on May 28, and 271 and 132 larvae at sites D1 and D2 on May 29, respectively.

We measured water temperature and water velocity every day of the spawning season through the larval sampling period. Water temperature was recorded hourly using HOBO data loggers (Oneset Computer Corp.) at three locations: the upstream spawning location, upstream of, and downstream of larval collection sites. Three water velocity readings (left, center, right) were taken in front of the drift net openings (0.6 of the stream depth measured downward from the surface) with a Marsh–McBirney Flo-Mate 2000 (Marsh–McBirney Inc., Fredrick, MD, USA). Measurements of water velocity were also recorded from the entire cross-sectional area of the channel at 1m intervals across a standardized transect. In this study, we reported water temperature and water velocity in two days of larval sampling. Average (±SD) temperatures and velocity among locations measured on May 28 and 29 were 20.33± 0.69ºC and 20.72 ± 0.05ºC; and 0.36± 0.11 m/s and 0.47 ± 0.12 m/s, respectively.

Genetic analysis

DNA was extracted from larval tissue samples using a QIAGEN DNeasy$^{(R)}$ kit (QIA Inc.). DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Inc.). All samples were genotyped at 12 tetra-nucleotide microsatellite loci including Spl 120 (McQuown *et al.*, 2000); AfuG 68B (McQuown *et al.*, 2002). 2002); Aox 27 (King *et al.*, 2001); AfuG 68, AfuG 9; AfuG 63, AfuG 74, AfuG 112, AfuG 56, AfuG 160, AfuG 195 and AfuG 204 (Welsh *et al.*, 2003). Using polymerase chain reaction (PCR), 100 ng DNA was amplified in 25 μl reaction mixtures as described in the above references. All PCR reactions were conducted using a Robocyler 96 thermal cycler (Stratagene). PCR products were run on 6% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO II scanner. Allele sizes were

scored independently by two experienced personnel. Genotyping errors were checked by blindly re-genotyping a random subset of 10% of all samples.

Parentage analysis

The number of candidate parents affects the success rate of and confidence in parentage assignment (Marshall *et al.*, 1998). We used a subset of all captured spawning adults for parentage analysis based on empirical estimates of the cumulative temperature units from the period from egg deposition to larval dispersal (Smith and King, 2005; Duong *et al.*, in press), and estimated spawning date (based on date of capture) of adults. Using these criteria larvae collected on May 28-29 were expected to be offspring of adults captured from May 1 to May 15 (35 females and 91 males, Figure 5. 2). The offspring of these adults were predicted to disperse on average 17 days following the female spawning date under mean water temperature (from May 1, the first day of spawning by these adults, to May 29, the last day of collecting larvae) of 14.7±2.4°C. Meanwhile, offspring of females spawning earlier (from April 20-24) were predicted to disperse approximately19 days post-spawning, and offspring of females spawning later (May-25-26) predictably dispersed 13 days post-spawning. Thus, at the time of larval sampling, larvae from early spawning females had already dispersed, and larvae from late spawning adults had not hatched or dispersed yet.

Two software programs, the Parentage Allocation of Singles on Open Systems (PASOS), version 1.0 (Duchesne *et al.*, 2005) and CERVUS 3.0 (Kalinowski *et al.*, 2007) were used for parentage analysis. Parental assignments for individual larvae from the two programs were compared, and larvae that were concordantly assigned from the two programs were used for further analysis.

We used two functions in PASOS including the allocation function for assigning offspring to collected adults and estimating missing parents that were not collected, and the simulation function for estimating the probability that any assignment is correct (correctness probability). Simulations to determine accuracy (correctness) of parentage assignments were conducted over 5 iterations using 1,000 simulated offspring. Using CERVUS, we first simulated 10,000 offspring to determine the likelihood of assignment and confidence levels of parental assignment based on population estimates of allele frequencies (N=126 spawning adults), captured adult proportions estimated from PASOS, and the empirical estimate of genotyping errors (0.67%). From the assignment output, a "true parent pair" of an offspring was chosen from the most likely candidate parents. We then compared the concordance of parental assignment outputs from the two programs. Inconsistent assignments of offspring to two collected parents between the two programs were evaluated based on biological information (*e.g.*, proximity of captured time and location of both parents).

Data analysis

We first tested the hypothesis that females contributed the same proportion of larvae to collections at both sampling sites (upstream D1 and downstream D2) during each of 2 nights (May 28 and 29). Proportional contributions of larvae from each female were estimated as the ratio of the number of offspring per female to the total number of offspring collected at each larval sampling site each night (hereafter called standardized offspring). We used a general linear mixed model approach to quantify differences in standardized offspring among females as a fixed effect at two larval sampling sites and at two nights. Because sites and nights of sampling were not independent, they were treated

as random effects (Burnham and Anderson, 2002; Bolker *et al.*, 2009). We used likelihood ratio tests to determine significance of random effects and Wald tests for fixed effects.

A mixed model was also used to compare proportions of larvae from the upstream and downstream spawning locations, which contributed to the total number of larvae collected at sites D1 and D2. In this model, spawning location was treated as a fixed effect and female as a random effect.

Hourly dispersal patterns of larvae from females spawning at upstream (site B) and downstream (site C) across five consecutive hours of sampling at site D1 during each of two nights were also characterized. We used nonparametric Mann-Whitney Wilcoxon tests to compare the number and proportion of larvae collected by hours from two female groups spawning at upstream and downstream sites.

We then estimated and compared the relative loss of larvae among females between upstream and downstream collection sites. Relative loss by a female was defined as the ratio of the number of larvae that were collected at the downstream site and assigned to each female to the total number of the same female's offspring collected at both upstream and downstream sites (D1 and D2, respectively). Differences in relative loss among females were tested using a general linear mixed model including the fixed effect of female and the random effect of sampling dates. Similarly, relative loss for female groups spawning at upstream and downstream locations was also compared with a mixed model including spawning locations as a fixed effect and females as random effects.

RESULTS

Parentage assignment

Based on program PASOS, 741 larvae (89% of the total number of larvae genotyped) were assigned to two collected parents with allocation correctness estimated to be 0.82±0.01. Male and female adults captured were estimated (by the program) to represent 89.3 and 91.8%, respectively, of the total number of adults estimated to have contributed to the larvae sampled. The assignment rate obtained from CERVUS was 91.8%. Comparing parental assignment outputs from the two programs revealed that 72.4% (602 larvae) of all sampled larvae were concordantly assigned to the same two collected parents. Based on biological information regarding timing of spawning observed for putative male-female pairs to evaluate discordance in assignment of the two programs, we finally selected 738 larvae for further analysis.

Hourly pattern of larval dispersal

The number of larvae collected revealed an hourly pattern of larval dispersal from each spawning area. Hourly patterns were consistent across the 2 nights and between female spawning locations (Figure 5. 3). Based on stream velocity (0.36 and 0.47 m/s on May 28 and 29, respectively), the time necessary to disperse passively from the upstream spawning location (site B) to the upstream larval collection site (D1) was about 35-45 minutes. Genetic data revealed that when checking nets hourly, larvae from both spawning sites were present in the upstream larval collection. We observed an approximately one hour lag between the peaks of larvae captured at upstream and downstream locations. The highest number of larvae from both spawning sites was observed from 2200-2300 hrs at site D1 and 2300-2400 hours at site D2. The number and

proportion of larvae collected by hour at both larval collection sites were not significantly different between the two groups of females that spawned at locations B (upstream) and C (downstream) (Wilcoxon tests, $P > 0.5$ for all tests).

Proportional contributions of females to larval collections at two sampling sites and nights

The number of larvae captured during 2 nights and assigned to a female varied from 1 to 140 individuals (mean = 21, median = 11, mode = 5). Standardized offspring also varied significantly among females ($F_{34, 104} = 32.0$, $P < 0.001$). Four females consistently had higher standardized offspring at both sampling locations and dates (Figure 5. 4). Random effects of sampling night and sampling site were not significant (LRT ≈ 0, df =1, $P = 0.99$). Therefore, the rank order of standardized offspring among females was consistent between two nights and also between the two larval sampling sites.

The number of larvae produced by females from the upstream location B comprised a higher proportion of larvae captured relative to larvae from females spawning at the downstream spawning location C ($F_{1, 137} = 14.4$, $P < 0.01$). However, in terms of larval proportion (standardized offspring) contributed by each female, standardized offspring per female spawning at location B was not significantly higher than that of females spawning at location C ($F_{1, 104} = 1.90$, $P = 0.17$). There was a large variation in standardized offspring among females within each spawning location.

Relative larval loss

Differences in the number of offspring between upstream and downstream larval sampling sites provided a measure of relative larval loss among females between the

sampling sites. Relative larval loss was not significantly different among females ($F_{34, 34}$ = 1.36, P = 0.19). This result indicates that females with higher numbers of offspring lost proportionally more offspring than did females contributing comparatively fewer offspring, when larvae from all females dispersed through a common environment from site D1 to site D2. However, large variation in relative larval loss within female (the same female between nights) and among females was observed (Figure 5. 5). Partitioning components of variance in relative larval loss revealed that a small proportion (3%) of total variance was attributed to random effects of sampling nights (across females), and a larger proportion (97%) was attributed to variance in relative loss of the same female in two nights (residual error). When females were grouped by spawning site, estimated relative larval loss from females spawning at the upstream location (0.25±0.20) was not significantly different from females spawning at the downstream location (0.29±0.22) ($F_{1, 34}$ = 0.55, P = 0.46). The non-significance in relative loss of females from two spawning locations was likely attributed to the large degree of variation among females within each spawning location.

DISCUSSION

Differences in trait expression among individual larvae, which affect survival, can occur as a result of maternal and genetic effects, even when individuals are exposed to the same environmental conditions (Heath and Gallego, 1997; Bolnick *et al.*, 2003; Leis, 2006; Fiksen *et al.*, 2007; Clobert *et al.*, 2009). Further, inter- and intra-family variation in phenotypes at larval stages can affect traits and survival at later life stages, providing the opportunity for the evolution of maternal traits and behavior (*e.g.*, spawning time) in natural populations (Einum and Fleming, 2000). One important question is whether

larvae produced by different females are differentially susceptible to mortality when exposed to a common environment, for example, when larvae disperse from spawning grounds to areas utilized during subsequent early life stages. Such a question cannot be addressed without genetic data or other measures establishing pedigree relationships and estimates of reproductive success and survival. Variation in relative larval mortality among females would imply that maternal effects and individual variability outweigh the effects of stream environmental factors.

Using genetically determined parentage, we quantified the intra- and inter-female variability of larval loss during dispersal through a common natural stream environment over a distance of 1.5 km during two consecutive nights. A considerable portion of mortality during early life stages occurs at emergence and during the initial period of dispersal (Einum and Fleming, 2000). Therefore, the time and distance over which we examined larval loss was predicted to be an important period when larvae experienced high mortality. Results showed that lake sturgeon females differed in both absolute numbers and relative proportions of offspring contributed to larval collections each night. However, no significant difference in relative larvae loss among females or between groups of females spawning at upstream and downstream locations was detected. This result indicates stronger effects of exogenous factors (*e.g.*, predation, food availability, temperature, water flow, etc.,) than endogenous factors (*e.g.* maternal effects and larval behavior) on mortality of larvae from all females and spawning locations. However, variation in relative larval loss between the two sampling nights for each female (within female) was as high as variation among females (Figure 5. 5). Such large intra-female variation can mask the significant effects of inter-female variability in larval loss. On the

other hand, daily mortality at larval stages can vary greatly, and is a common phenomenon across species (*e.g.*, walleye *Stizostedion vitreum* (Mion *et al.*, 1998); Pepin 2009). Accordingly, the large degree of intra-and inter-female variation in larval loss observed in lake sturgeon may be a normal feature in stream environments.

Estimates of larval loss between two sampling sites can likely be attributed to natural mortality (*e.g.*, due to starvation or predation), differences in capture efficiencies at the two larval sampling sites or differences in movement behavior among larvae. We believe the latter two hypotheses are unlikely. First, if we assumed that all larvae had an equal probability of capture, differences in capture efficiencies between sampling sites would not bias estimates of relative larval loss among females. Second, it is possible that downstream movement may not be consistent among larvae (Siegel *et al.*, 2003; Leis, 2006; Shanks, 2009). For example, some larvae could settle in substrates between the two sampling sites or slow their movement. However, based on our observation of stream conditions over the stream area surveyed and consistent patterns of larval dispersal by hour each night (Figure 5. 3), variation in larval movement behavior was likely minor and did not contribute to differences in relative larval loss among females. In sum, the lack of differences in relative larval loss among females was likely due to similar susceptibility to natural mortality during the two nights.

The main sources of natural mortality during the initial period of larval dispersal can be predation, starvation, and physical injure due to water velocity (Kamler, 1992; Mion *et al.*, 1998; Cowen and Sponaugle, 2009). Water velocity (Mion *et al.*, 1998) and other exogenous abiotic factors such as oxygen concentration and temperature (Kamler, 2005) are unlikely to cause larval loss in this study because the stream conditions were

characterized by intermediate levels of water velocities (0.36 – 0.47 m/s) and stable temperatures (20 – 21ºC) during two nights. Predation is one of the most significant causes of larvae mortality during dispersal for numerous fish species (Paradis *et al.*, 1996; Cowen and Sponaugle, 2009). Predators of lake sturgeon larvae in the Upper Black River included crayfish (*Orconectes rusticus*) and rock bass (*Ambloplites rupestris*) (Crossman, 2008; Forsythe, 2010). Larvae are not able to escape predators during dispersal until the juvenile stages where the total length is > 40 cm (Auer and Baker, 2002). Therefore, the effects of predation on larval mortality are expected to be random among females. Starvation can cause substantial mortality of larvae in several fish species (*e.g.*, trout *Salmo trutta* (Elliott, 1986); plaice *Pleuronectes platessa* (Fox *et al.*, 2007)). In lake sturgeon, larvae emerge from the substrate and disperse after depleting yolk-sac reserves (Auer and Baker, 2002; Kynard and Parker, 2005; Smith and King, 2005). Accordingly, starvation likely results in mortality during the initial period of larval dispersal. Given starvation mortality may be related to egg size and egg energy content (Keckeis *et al.*, 2000), which are components of maternal effects (Mousseau and Fox, 1998; Kamler, 2005), larger inter-female variation compared to intra-female variation in relative larval loss would be expected if starvation or decreased energy levels due to food limitation was a main source of mortality. However, our study showed that intra- and inter-female variation were approximately equal, suggesting predation mortality was dominant over starvation mortality.

Biologists have examined whether larvae dispersing in groups consisted of offspring from different families, in order to understand schooling behavior of fish during dispersal (Avise *et al.*, 2002). We found that collections of dispersing lake sturgeon

larvae each night were comprised of offspring from the majority of females sampled in spawning locations. However, proportional contributions from different females varied greatly (Figure 5. 4). Different proportional contributions of females to larval drift could be explained due to variability in female fecundity (Bruch *et al.*, 2006), hatching rate (Nichols *et al.*, 2003; Crossman, 2008), and survival from egg deposition to larval dispersal (Caroffino *et al.*, 2010). These factors could result in different reproductive success of individual females. Although this study was not designed to disentangle the causes of differences in proportional contribution of females to larval collections, a large range in the numbers of offspring captured and the consistent magnitude of differences in standardized offspring among females between two sampling nights is indicative of high variation in reproductive success among females.

Our study also revealed similar hourly dispersal patterns of larvae produced from females spawning at upstream and downstream locations in two nights, with the peak of larval drift occurring between 2200 and 2300 hrs. Under average water velocities of 0.36 – 0.47 m/s, similar patterns of dispersal across hours (nets were checked hourly) observed in larvae from two spawning locations implies that movements of larvae downstream over the distance investigated were likely passive. These patterns were observed consistently for 2 consecutive nights in this study and also in other studies conducted in different years (*e.g.*, from 2000-2003, Smith and King 2005).

The short sampling period might have limited our ability to detect differences in relative larval loss among females. We did not collect larvae produced from females representing the entire spawning period. Given that daily mortality of larvae within and among families is highly variable (Mion *et al.*, 1998; Pepin, 2009), many days of

sampling may reduce intra-family variation and thus allow detection of inter-family variation in larval loss. In addition, because larvae produced from the same spawning event may disperse over many days (Smith and King, 2005; Duong *et al.*, in press) proportional contributions of larvae from females and relative loss interpreted based on two sampling nights and a short distance may not reflect differences in numbers of offspring produced by females and relative larval loss among females over the entire period of larval dispersal.

Further studies that use genetic markers to quantify relative larval loss by families could profitably explore the effects of timing, duration and distance of dispersal on larval loss. Severe environmental conditions such as high river discharge, high predation pressure, extreme temperatures, and other exogenous factors may accentuate differences in rates of mortality among families.

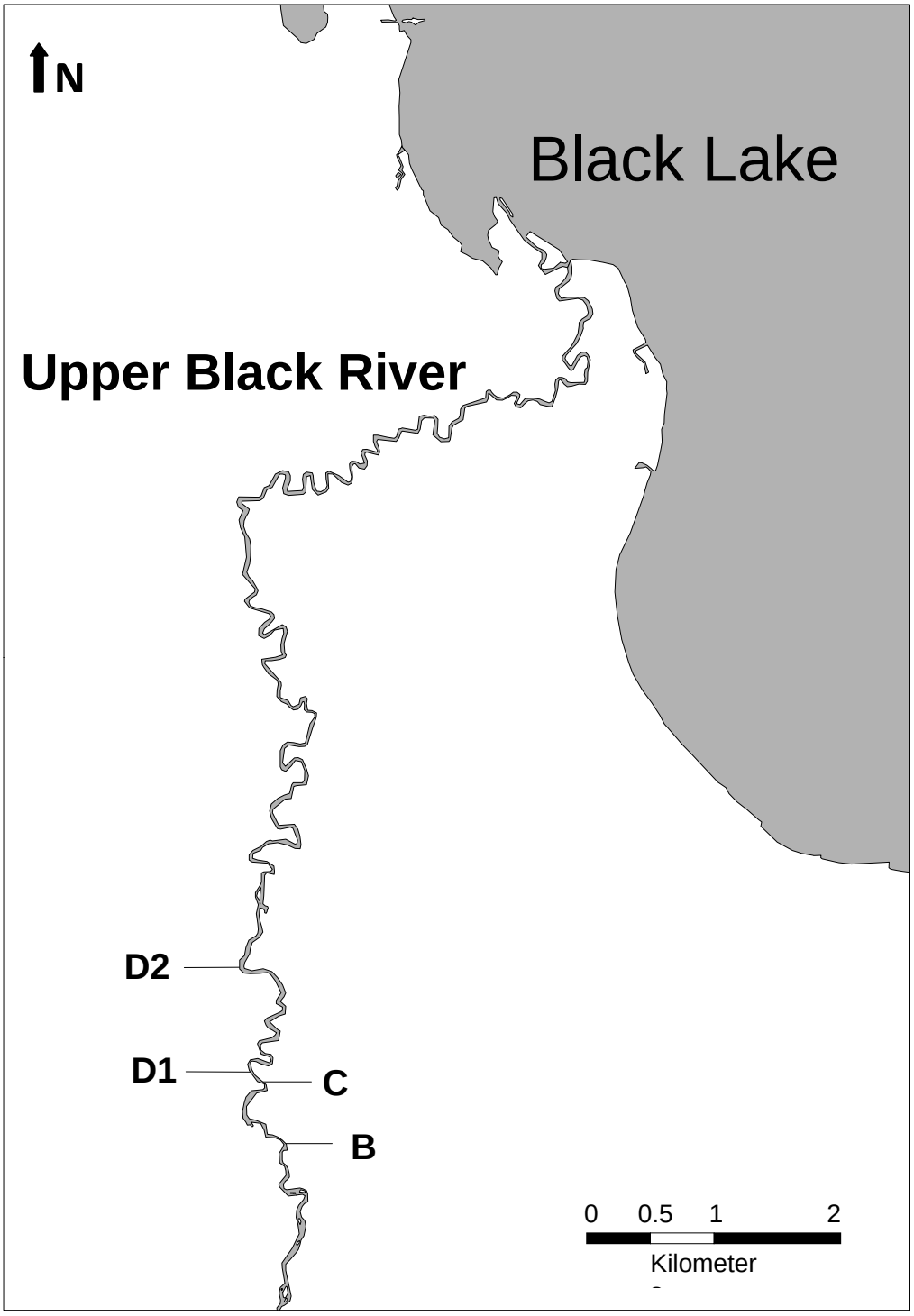

Figure 5.1. The study site in the Upper Black River, Michigan, showing upstream and downstream adult spawning locations (B and C, respectively); and upstream and downstream larval collection sites (D1 and D2, respectively).

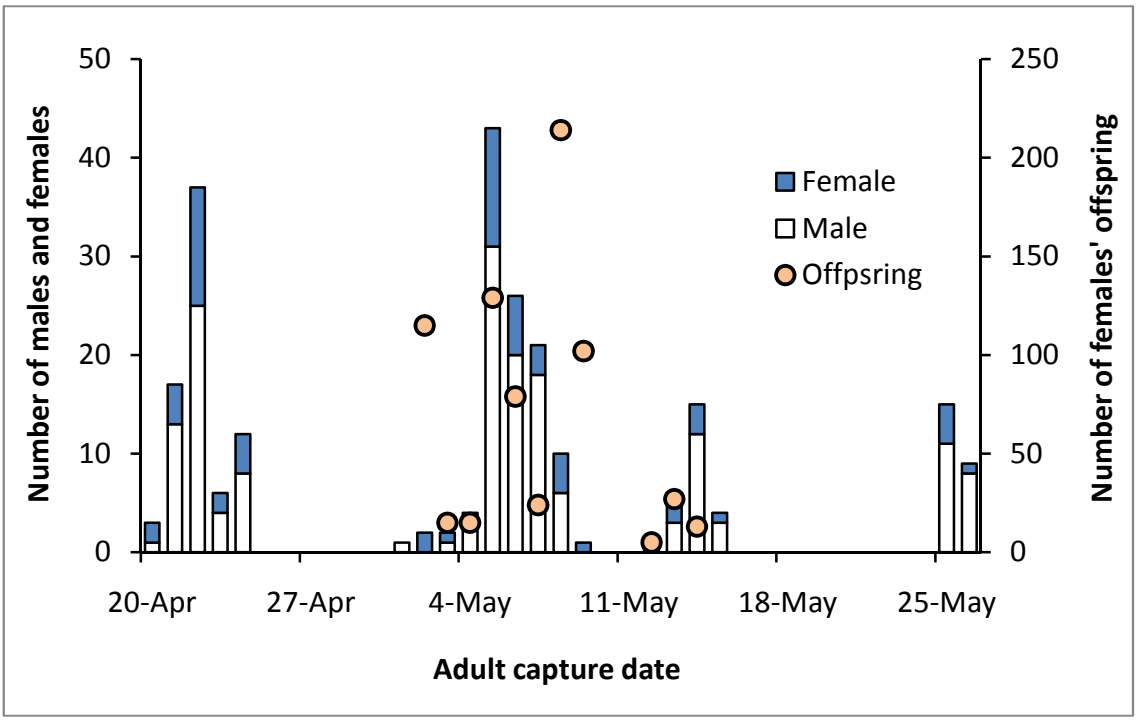

Figure 5. 2. Numbers of adults captured each day of the 2006 spawning season and total number of offspring collected on two nights (May 28 and 29) that were assigned to captured females using genetically determined parentage.

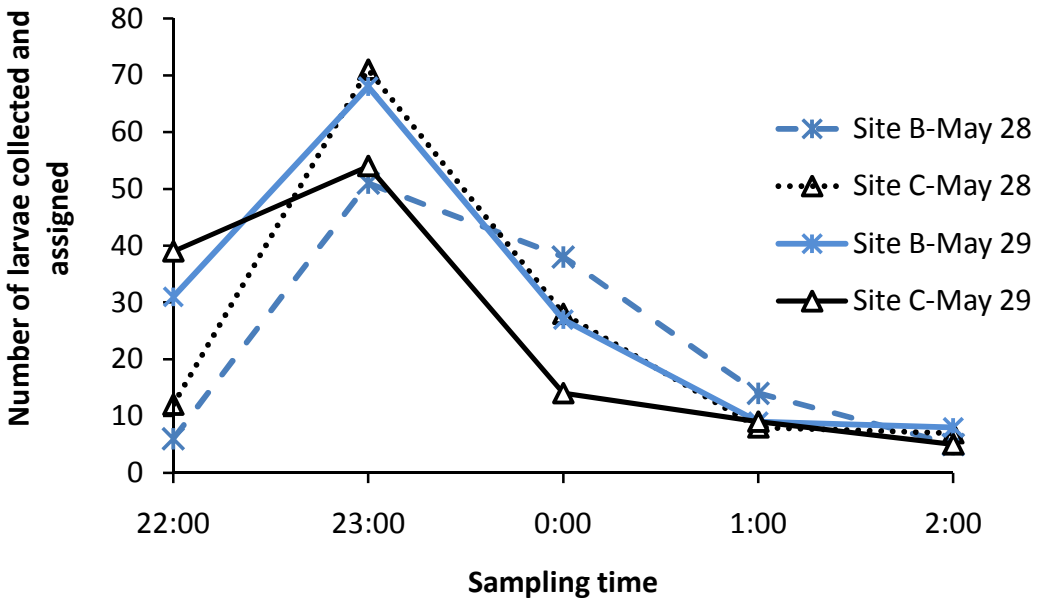

Figure 5.3. Hourly pattern of larval dispersal based on collections at sampling site D1 on 2 nights (May 28 and 29). Larval numbers are cumulative totals over all females spawning at upstream (Site B) and downstream (Site C) locations (Figure 5. 1).

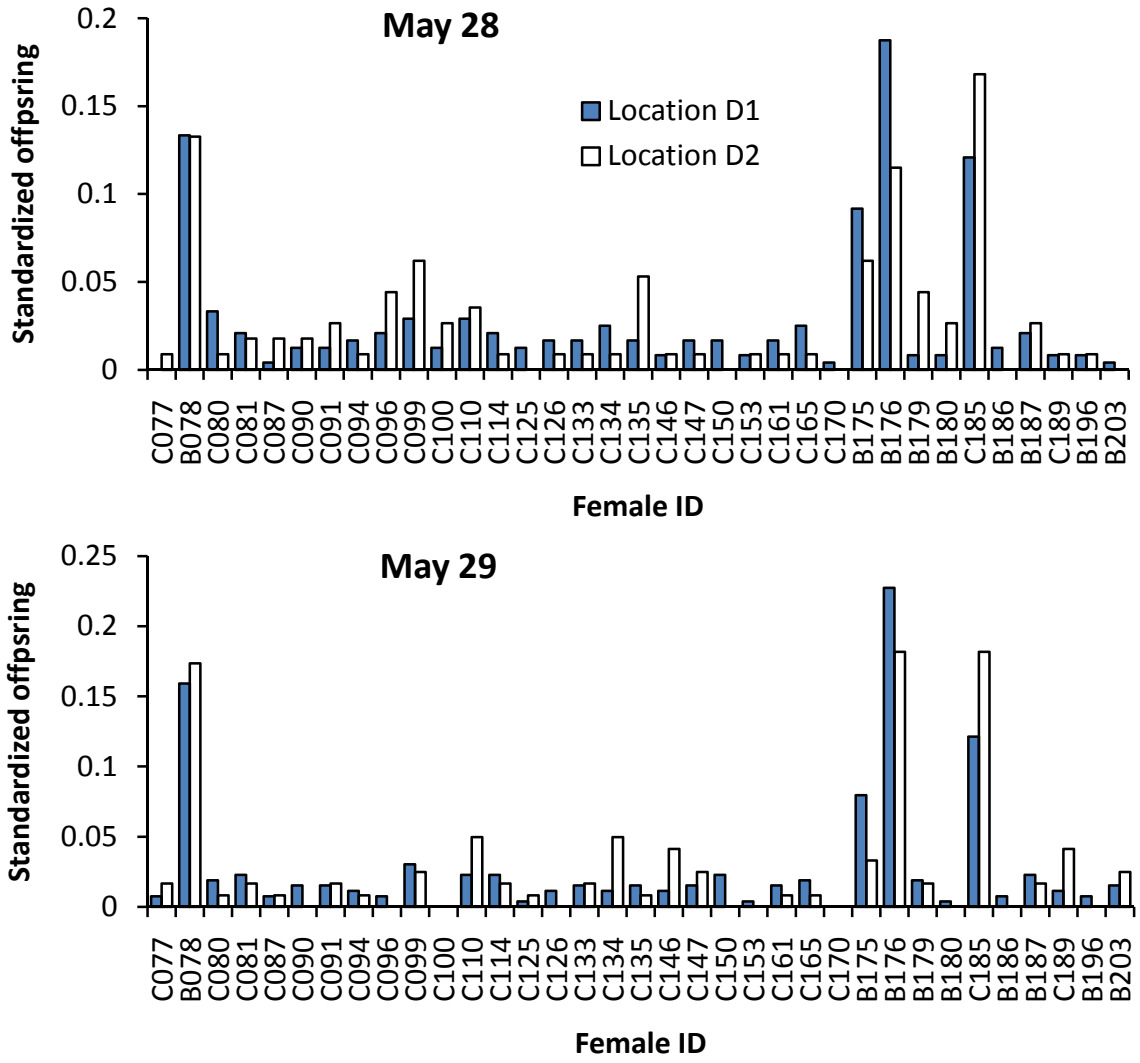

Figure 5.4. Standardized offspring produced by each female estimated based on collections at two larval sampling sites on two consecutive sampling nights. Female ID includes reference to spawning locations (B vs. C) and are presented in order of spawning date (May 1-15, Figure 5. 2).

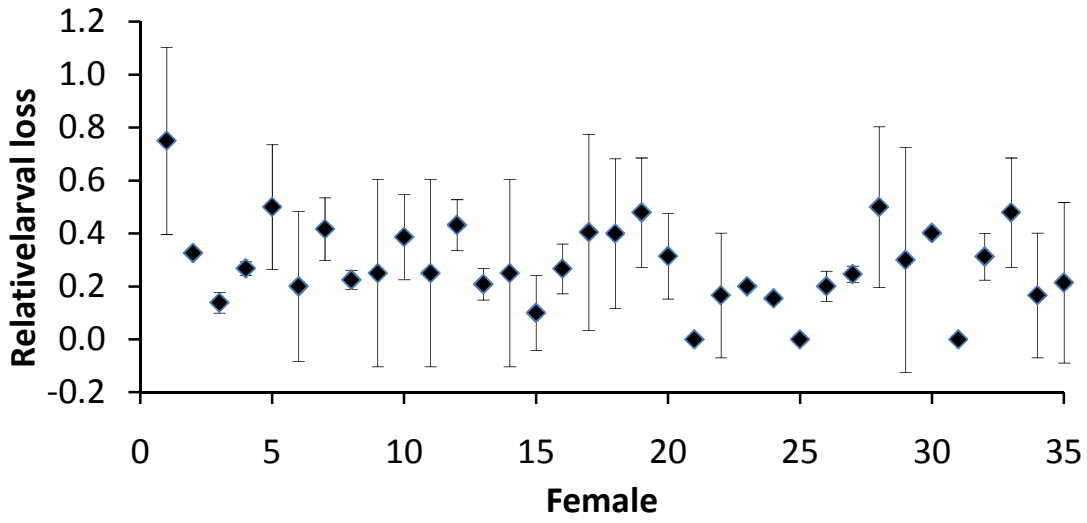

Figure 5. 5. Relative larval loss among females (bars represent 1SD between two nights). The order of females was as the same as Figure 5. 4.

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