

INVESTIGATIONS INTO LAKE STURGEON REPRODUCTIVE EFFORT: TRADE-OFFS
BETWEEN CURRENT AND FUTURE REPRODUCTION

By

Douglas L. Larson, Jr.

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Fisheries and Wildlife—Master of Science

2023

ABSTRACT

Organisms often vary resource expenditures devoted to reproduction in response to environmental conditions and to biotic conspecific and heterospecific interactions. Resource allocation decisions can also affect future reproductive effort and success. In Chapter 1, I examined how intra- and inter-sexual behavior, biotic variables, and environmental conditions affected individual investments in current year reproductive effort. Results indicated that male lake sturgeon inter-spawning interval decreased as body size (a surrogate of age) increased. In Chapter 2, I examined the role of ovarian fluid in lake sturgeon egg fertilization success and post-fertilization embryo survival when eggs are fertilized with sperm from related and unrelated males. Eggs fertilized by males not related to a female showed higher proportional egg survival than half-sibling males 72-hours following fertilization. No interaction of the unrinsed ovarian fluid and relatedness was observed, suggesting that higher egg survival associated with unrelated males was not attributed to ovarian fluid. The lack of rinsing of ovarian fluid during sperm activation resulted in reduction of sperm velocity and motility, but results were unaffected by the relatedness of the mated pair. Results demonstrated that ovarian fluid may alter sperm velocity and quality, but the reduction in survival of progeny from half-sibling matings did not result from ovarian fluid on the egg surface. Collectively, findings presented provide greater understanding of how behavioral, biological, and environmental factors affect reproduction in lake sturgeon. Studies highlight the value of long-term collection of data to inform species management. Results generated from these experiments contribute to the understanding of how long-lived species plastically respond to current environmental conditions and how effort toward reproductive success varies with age and size at the individual and to population levels.

ACKNOWLEDGEMENTS

First, I'd like to acknowledge and thank the organizations that provided funding support for this project: Michigan Department of Natural Resources (MDNR) and the Black Lake Chapter of Sturgeon for Tomorrow.

I'd next like to thank my advisor Dr. Kim Scribner for his unwavering support during my seven years with the Black River Sturgeon Facility, and the leap of faith given when he agreed to take me on as a project graduate student and project supervisor. The support he provided was invaluable and I can't adequately be put into words. I wish to thank Dr. Edward Baker as well for his support and confidence during my tenure. Thanks also to my committee members, Dr. Travis Brenden and Dr. Brian Roth.

During my time at Michigan State University, I supervised 35 technicians and worked with five graduate students for whom I am eternally grateful. I would like to specifically acknowledge Shaley Valentine for the push to go back to school after being so long removed. Special thanks also to Dr. Lydia Wassink, Dr. Joe Riedy, Jake Kimmel, Amber Johnston, and Max Majinksa, who provided endless help, counseling, and support during this project. Beyond your contributions to the experiments herein, you are all excellent scientists, and genuinely amazing people, and I could not have done this without your support.

Most importantly, I'd like to thank and acknowledge the sacrifices and support from my family during the last two years. Thank you to my son, Henry Larson, my daughter, Madeleine Larson, and my partner, Marissa Larson. Balancing work, school, and life has been challenging, and I simply could not have accomplished this work without your love and support. I can never thank you enough, though a post-graduation trip to Disney World should do the trick. I love you!

TABLE OF CONTENTS

CHAPTER 1: Lake Sturgeon inter-spawning interval is associated with prior reproductive expenditures including spawning behaviors and environmental conditions		1
1. ABSTRACT		1
2. INTRODUCTION.....		2
3. METHODS.....		7
3.1 Study site		7
3.2 Adult capture and handling		7
3.3 RFID data collection.....		8
3.4 Behavioral variables calculated from RFID data		8
3.5 Environmental data collection.....		9
3.6 Analysis of inter-spawning interval.....		10
4. RESULTS.....		11
4.1 Estimates of spawning adult composition.....		11
4.2 Variability of environmental conditions.....		12
4.3 Demographic and behavioral variability		14
4.4 Inter-spawning interval.....		15
5. DISCUSSION		17
5.1 Inter-spawning interval		18
5.2 Environmental conditions and spawning run variability.....		21
5.3 Conclusion		22
REFERENCES		25
APPENDIX		31
CHAPTER 2: Investigations of ovarian fluid as a means of cryptic female choice including effects of mate relatedness on egg fertilization success in Lake Sturgeon (<i>Acipenser fulvescens</i>).....		49
1. ABSTRACT		49
2. INTRODUCTION.....		50
3. METHODS.....		54
3.1 Study site		54
3.2 Sperm volume experiment.....		55
3.2.1 Sperm volume statistical analysis.....		57
3.3 Fertilization experiment.....		58
3.3.1 Fertilization experiment statistical analysis.....		59
3.4 Sperm motility experiment.....		60
3.4.1 Sperm motility experiment statistical analysis		61
4. RESULTS.....		62
4.1 Sperm volume experiment results		62
4.2 Fertilization experiment results		64
4.2.1 Relatedness		64
4.2.2 Fertilization and survival		64
4.3 Sperm motility experiment results		66
4.3.1 Principal components analysis.....		66

4.3.2 Sperm quality in the presence or absence of ovarian fluid	67
4.3.3 Velocity straight line.....	67
4.3.4 Curvilinear velocity	68
4.3.5 Velocity average path	69
4.3.6 Motility and linearity	69
5. DISCUSSION	70
5.1 Egg fertilization and survival following consanguineous mating.....	71
5.2 Sperm quality and the role of ovarian fluid.....	72
5.3 Conclusion.....	74
6. REFERENCES.....	77
7. APPENDIX	84

CHAPTER 1:
Lake Sturgeon inter-spawning interval is associated with prior reproductive effort including responses to biotic interactions and environmental conditions

1. ABSTRACT

Organisms often vary resource expenditures toward reproduction in response to environmental conditions and to biotic conspecific and heterospecific interactions which can affect future reproductive effort and success. However, investigations of resource expenditures reflected by reproductive behaviors have largely focused on current-year investments in gamete quantity and quality (for females) and mate number and quality (for males) rather than trade-offs associated with costs to future reproduction, including reproductive interval and lifetime reproductive success. In lake sturgeon (*Acipenser fulvescens*), a species of conservation concern, variation in timing and duration of spawning migrations and duration of occupancy of river spawning areas can be attributed to behavioral plasticity in response to temperature and discharge and levels of inter- and intra- sex interactions. We used Passive Integrated Transponder (PIT) tags and passive antennas to monitor spawning migration behavior, including upstream migration time, number of spawning migrations, river residence time, and levels of inter- and intra-sexual interactions from male and female lake sturgeon over seven consecutive years (2016 through 2022) in the Black River, MI. We used generalized linear mixed models to evaluate whether behaviors and environmental conditions which were consistent with resource expenditures toward reproduction in the current year predicted inter-spawning interval. For male lake sturgeon inter-spawning interval decreased with increasing body size. Female inter-spawning interval was not associated with measures of current or future reproduction, potentially due to proportionally fewer female than male observations. Time spent in the river was associated with behavior in previous reproductive efforts for males, but not influenced by past effort from females. Results

indicated that current reproductive effort may be influenced by environmental variability and that investment in reproductive opportunities increases as male lake sturgeon age.

2. INTRODUCTION

Iteroparous organisms must weigh the probability of future reproduction when deciding to allocate resources to current reproduction (Fisher 1930, Williams 1966). Reproductive value (expected reproductive contributions of individuals to future population growth; Stearns 1976) generally declines with increasing age (Williams 1966, Emlen 1970, Pianka 1976). Investments in current reproduction may incur costs by depleting resources available for future reproduction (Williams 1966; Bell and Koufopanou 1986). Life history theory predicts the current reproductive effort as a portion of an individuals' total energy budget should increase as residual reproductive value decreases (Williams 1966, Pianka and Parker 1975, Caswell 1982). Generally, the trade-off between costs associated with resource investment in current year reproduction relative to the ability to invest in future survival and reproduction (Williams 1966) likely has a strong influence on reproductive behavior of long-lived iteroparous species (Stearns 1989).

The period between spawning events is associated with resource acquisition and may be important to allocation and future expenditure of resources. When resource acquisition after a spawning season is insufficient for immediate production of gametes, fish may skip a spawning season, or lengthen inter-spawning interval (Walsh et al. 1986, Schwalme and Chouinard 1999, Jørgensen et al. 2006, Rideout et al. 2005, Rideout and Tomkiewicz 2011). Skipped spawning is common in young adult fish (Jørgensen et al. 2006), and in long-lived large-bodied species (Rideout et al. 2005), for which proportional allocations of resources to reproduction relative to somatic growth increases with increasing age (Williams 1966, Schaffer 1974). Sitar et al. (2014)

documented skipped spawning in lean and siscowet lake trout (*Salvelinus namaycush*) in Southern Lake Superior, likely due to food availability. Martin (1970) similarly documented skipped spawning of lake trout in Opeconogo Lake, Ontario, also inferring that the lack of resource availability lengthened the inter-spawning period. Other species where skipped spawning has been described and were linked to low resource levels, include bull trout *Salvelinus confluentus* (Johnston and Post 2009) and white crappie *Pomoxis annularis* (Bunnell et al. 2007). Prior study has consistently shown skipped spawning to be associated with body condition and resource availability and acquisition during the period between reproductive episodes (Kjesbu et al. 1991, reviewed in Rideout et al. 2005, Kennedy et al. 2008, Shaw and Levin 2013), though comparatively few studies have characterized how individual behavior, body size, and environmental conditions (including temperature and discharge) (Somarakis et al. 2019) may affect inter-spawning interval across multiple spawning seasons in a long-lived species.

Environmental conditions during the spawning period can be highly variable and has been shown to influence behavior during reproduction in the Black River lake sturgeon population and elsewhere. Forsythe et al. (2012b) demonstrated that in the Black River, multi-day lagged effects of increasing temperature and decreasing discharge, coupled with lunar phase are cues that initiate spawning. Moreover, when temperature decreases and discharge increases rapidly, female spawning may cease (Dammerman et al. 2019, Figure 2; Larson et al. 2020, Figure 3a-d). For example, Kootenai River white sturgeon (*Acipenser tranmontanus*) are spawning increases rapidly with temperature, however, daily temperature decreases of $\geq 0.8^{\circ}\text{C}$ can interrupt spawning. In addition, when flows exceeded $630 \text{ m}^3 \cdot \text{sec}^{-1}$, spawning slowed substantially (Paragamian and Wakkinen 2002). Water temperature and discharge are strong

predictors of spawning migration in both Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*) (Vine et al. 2019). Other reproductive behaviors are also variable. Some fish species migrate between lake and river systems for reproduction to enhance opportunities to acquire mates and based on availability of habitats conducive to survival of offspring during early life stages (Dingle 1996). Reproductive migrations often are initiated and coincide with environmental cues indicating suitable environmental conditions to meet adult energetic demands of migration, and to increase the likelihood of progeny survival (Hodgson and Quinn 2002). Concordantly, variability of environmental conditions could potentially alter the duration and modality of the reproductive period of migratory fishes, which may present a tradeoff between current and future reproductive potential. However, the degree to which environmental conditions and behavior during a single reproductive event can affect future reproductive effort remains unclear.

Despite equal contribution to offspring and the commonality of most life history traits, factors affecting male and female RS are inherently different. In general, female RS is affected by optimal sex ratio and mate quality. To that end, females can exhibit mate selectivity by delaying reproduction until the optimal number of mates are available (Jirotkul 1999) and by preferentially reproducing with mates possessing desirable traits or behavior (Fleming and Gross 1994 Andersson and Iwasa 1996). Conversely, male RS increases as a function of the number of mates encountered (Bateman 1948, Arnold and Duvall, 1994), which can increase with increased investment in a single reproductive period (Larson et al. 2020). Additionally, larger male body size is important during intra- and inter-sexual interactions and thus contributes to RS (Bose et al. 2018).

Reproductive behavior is highly variable between males and females (Henson and Warner 1997) or polygynous, promiscuous spawning fishes. In general, selection is expected to favor male reproductive behaviors that increase access to mates, whereas females invest more heavily in offspring number and quality (Arnold and Duvall, 1994). Inter-sex differences in reproductive behavior may result in different energetic costs during the spawning period, especially those associated with environmental conditions. As a result, factors which affect current and future reproduction may be different for males and females, particularly in long-lived iteroparous species, necessitating sex-specific evaluation. Male walleye (*Sander vitreus*) arrive on the spawning grounds earlier than females and typically remain in the spawning area longer. As a result, sex ratio was skewed toward males. Male Atlantic cod (*Gadus morhua*) (Morgan and Trippel 1996) and brown-marbled grouper (*Epinephelus fuscoguttatus*) (Rhodes et al. 2012) also arrive early to the spawning grounds and remain after females depart. Despite male behaviors expressed during the spawning period, including longer and energetically costly spawning ground residence relative to females, females to require longer inter-spawning periods resulting from the larger energetic and nutrient investment required to produce eggs compared to the production of sperm (Rideout et al. 2005, Rideout and Tomkiewicz 2011).

Lake sturgeon is a potamodromous, long-lived, iteroparous fish species and is a species of conservation concern throughout its native range (Hay-Chmielewski and Whelan 1997). Lake sturgeon is a highly fecund, broadcast-spawning species that aggregates in large mixed-sex groups to increase probability of fertilization. Adult behavior during the spawning season is highly variable for males and females, including variability in river residence time, upstream migration time, number of complete river migrations during a single season (Larson et al. 2020), and inter-spawning interval (Forsythe et al. 2012a). Female RS has been associated with ordinal

date of arrival at spawning areas, temperature, discharge, and number of mates (Dammerman et al. 2019), whereas male RS is strongly associated with increased river residence time and the resulting increase in intra- and inter-sex interactions (Larson et al. 2020). Longer residence time can reduce gamete quality (Dammerman et al. 2019, Larson et al. 2020) and may present trade-off between current and future reproductive investment and success. Because lake sturgeon are long-lived, highly fecund, iteroparous, and often experience variability in spawning biotic and environmental conditions within and between years, and because of the length and breadth of detailed studies on the Black Lake, MI population, these fish are well suited to critically evaluate the relative contributions of factors associated with current and future reproduction.

The general objective of this study was to determine whether behavioral (river residence time, upstream swimming time, number of annual migrations), biological (inter- and intra-sex interactions, body size) and environmental variables (discharge, temperature, 5% exceedance flow days) were associated with variability in inter-spawning interval for male and female lake sturgeon, a surrogate of lifetime reproductive output for long-lived iteroparous species. We based observations on lake sturgeon for which inter-spawning interval is known for nearly all adult males and females in the population during multiple spawning seasons. We hypothesized that inter-spawning interval in lake sturgeon would decrease with increasing body size (a surrogate of age) (Parker and Pianka 1976). We further hypothesized that variability in behavior and environmental conditions during the current reproductive period would be associated with inter-spawning interval.

3. METHODS

3.1 Study site

This study was conducted during the spring spawning season (late April through early June) in seven consecutive years (2016 – 2022) in the Upper Black River (UBR), a fourth-order tributary of Black Lake in Cheboygan County, MI, USA (Figure 1.1). Spawning areas in the UBR are limited to 11 km of river upstream of Black Lake and downstream of Kleber Dam (Figure 1.1). Alverno Dam impounds the Lower Black River (LBR) 8 km downstream of Black Lake (Hay-Chmielewski 1987, Baker and Borgeson 1999, Smith and Baker 2005), effectively isolating the Black Lake lake sturgeon population. Because the population is reproductively isolated, it is well suited for reproductive ecology studies. Passive PIT antennae arrays based on radio frequency identification (RFID) technology can be employed because of the river's narrow width (~25 meter) and shallow depth (~1-3 meter) and accessibility of all spawning areas (Larson et al. 2020). Physical stream features also allow daily physical capture of ~70-85% and RFID capture of >95% of the known spawning population (Larson et al. 2020) annually.

3.2 Adult capture and handling

Lake Sturgeon were physically captured on spawning areas in the UBR (Figure 1.1) using long handled dip nets by personnel in scuba suits, masks and snorkel. Fork length (cm, FL, Table 1.1), mass (kg), girth (cm) and sex were recorded for all individuals along with date of capture. As FL, mass, and girth were correlated (data not shown), fork length was the only body size variable used in analyses. Sex was determined by expressing gametes at the time of capture or genetically (Scribner and Kanefsky 2021). The Black Lake sturgeon population was estimated to be 1,183 individuals in 2022 based on an open population model estimate described in Pledger et al. (2013), of which 536 were female, and 647 were male (Michigan State University (MSU) and Michigan Department of Natural Resources (MDNR), unpublished data).

3.3 RFID data collection

Passive PIT arrays have been widely used to monitor activities of potadromous fishes during spawning migration (Kusnierz et al. 2009, Thiem et al. 2011, Larson et al. 2020, Swanson et al. 2021). RFID serves as a low-cost alternative to acoustic telemetry technology and allows a proportion of the population to be tracked for long periods of time (Gibbons and Andrews 2004). In the UBR, 1,132 adult lake sturgeon (95.7% of the population) have been tagged with a 23mm (0.6g) or 32 mm (0.8g) half-duplex 134.2 kHz Radio Frequency Identification (RFID) tag (Oregon RFID, Inc.) beginning in 2012 and continuing for the duration of this study. A 22-year spawning run capture-mark-recapture database (2001 to 2022) is maintained based on physical captures of lake sturgeon. Eight RFID antenna arrays are maintained in the UBR. Three antennas were installed 0.5 km upstream of the mouth of the river at FO5 bridge (Figure 1.1). Two antennas were installed immediately downstream of the known spawning areas ~7km upstream of the mouth of the UBR (Figure 1.1). Single antennas were installed at the mid-point of the spawning ground, at the upstream most point of the known spawning areas, and 200 meters downstream of Kleber Dam.

3.4 Behavioral variables calculated from RFID data

Data collected from the RFID antennas were used to calculate variables that characterize migratory and spawning behavior, including inter- and intra-sexual interactions, and river residence time, which are collectively associated with individual intra-annual reproductive investment. River residence time (hours, RRT, Table 1.1), or the cumulative time spent in the river during the spawning season, was calculated as all time spent above the RFID antenna 0.5-km upstream of the river mouth (Figure 1.1). Upstream migration time (hours, UST, Table 1.1) was calculated by subtracting the last date and time at which an adult sturgeon passed the river

mouth (FO5) antenna from the first time a fish was detected at the start of the known spawning area (Figure 1.1). The number of intra-annual migrations (MIG, Table 1.1) was calculated by determining the number of complete up- and down-stream river migrations during the spawning season. Fish for which a detection was missed were excluded from analyses. Male x male (intra-sex competition, IAA, Table 1.1), female x female (intra-sex competition, IAA, Table 1.1) and male x female (intra-sex competition, IEA, Table 1.1) interactions were calculated based on the count of known co-occurrence in spawning areas. Fish co-occurred in the river when RFID detections placed both fish in the known spawning area on a given date. The sum of all possible inter-sex and intra-sex interactions during river occupancy were considered for both males and females. Finally, inter-spawning interval (the number of years between reproductive efforts) was calculated as the number of years between RFID detections, conditional on the individual being detected at both FO5 Bridge and at the downstream end of the spawning grounds (Table 1.1).

3.5 Environmental data collection

Temperature ($^{\circ}\text{C}$) and discharge ($\text{m}^3\text{sec}^{-1}$, Q, Table 1.1) data were collected hourly using an Onset HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA) deployed in the most upstream segment of the spawning grounds (Figure 1.1). Previous work indicated that discharge in the upper Black River (UBR) was correlated with discharge collected from the Pigeon River, a nearby tributary of comparable size (Forsythe et al. 2012b). The known spawning area in the UBR is narrow, shallow, and has consistent substrate throughout, and most fish were captured in a 1.5 km stretch of river. We therefore monitored discharge at one site in the known spawning area. To estimate the effect of periods of high discharge, data retrieved from the U.S. Geological Survey (USGS) National Streamflow Information Program (<http://water.usgs.gov/nsip>) from 1942 until it was removed from the UBR

in 2000 were used to generate a flow duration curve for the UBR. A flow duration curve estimates the probability that any measured flow rate will be equaled or exceeded.

Cumulative thermal units (CTU, Table 1.1) were calculated by subtracting Kempinger's (1988) constant of 5.8°C from the daily average temperature. CTU was calculated as the total CTU for each fish summed for all days the fish was detected above the RFID antenna at the mouth of the river, provided a full river migration could be determined. A full list of independent and dependent variables with explanations and abbreviations can be found in Table 1.1.

3.6 Analysis of inter-spawning interval

To evaluate the potential for trade-offs between current and future reproduction, we used RFID data to determine the number of years between river spawning migrations (Inter-spawning interval, ISI, Table 1.1). We evaluated if measures of current reproductive effort (MIG, UST, RRT), environmental conditions (EF, CTU, Q), demographic differences (IEA, IAA) or measures of life-long reproductive effort (FL) were associated with variability in ISI (Table 1.1). Differences in male and female reproductive behavior (Trivers 1972, Oliveira et al. 2008) led to development of separate male and female ISI models. Inter-spawning interval was treated as Poisson distributed with possible under-dispersion due to the small variability in ISI across individuals. To account for the possibility of detecting the same fish in multiple years during the study period, the random effect of individual id (ID, Table 1.1), which was the unique RFID tag number for each fish, was used. Because inter-spawning interval may vary resulting from annual variation not considered in the fixed effects evaluated, the random effect of year (Table 1.1) was also considered. To determine which random effect(s) should be considered in a mixed effects model, we tested the fully parameterized fixed effects generalized linear model with each possible combination of random effects and used Akaike's information criterion (AIC) to

determine the best model. In the event the best model did not include a random effect, only the fixed effects model was considered. Here, we used AIC to determine the fixed effects model of best fit based on the fully parametrized model. Independent variables describing all possible combinations of variables, including the full and null model, were fit. AIC values and weights were calculated for each model using the dredge function of the MuMIn library (Bartón 2017). Model averaging was performed for all models for which the $\Delta AICc < 2$ (Burnham and Anderson 2002, Bunnell et al. 2012, Larson et al. 2020) using the model.avg function of the MuMIn library (Bartón 2017).

Nearly all individuals in the Black Lake population were tagged with RFID tags. Therefore, we calculated return probability based on passive detections at RFID antennas. Differences in the number of years until an additional spawning event when the first spawning year was 2016, 2017, or 2018 was evaluated using a Fisher's Exact Test. To evaluate the difference in spawning behavior and environmental conditions between years because annual capture numbers differed by year, we used a non-parametric Kruskal-Wallis Test with a post-hoc Dunn's Test of Multiple Comparisons (Dunn 1964). In all analyses, males and females were evaluated separately. Finally, as significantly more males than females were present during the spawning period, differences in environmental and behavior variables between sexes were evaluated using non-parametric Mann-Whitney tests.

4. RESULTS

4.1 Estimates of spawning adult composition

Between 2016 and 2022, 1,738 lake sturgeon were physically captured during the annual adult survey of which 453 were female, and 1,285 were male. During the same period, 2,614 lake sturgeon, of which 683 were female, and 1,931 were male, were detected on the passive

RFID antennas (Table 1.2). The number of annual RFID detections ranged from 292 in 2016 to 462 in 2021. Additionally, RFID efficiency ranged from 94.24% in 2020, when monitoring was interrupted by the Covid-19 pandemic, to 98.48% in 2016. Annual capture totals, RFID detection totals, and survey efficiency data are presented in Table 1.2.

4.2 Variability of environmental conditions

Variable environmental conditions can alter the timing of reproduction (date of initiation and duration of spawning), as well as behavior (e.g., river residence time and number of intra- and inter-sexual encounters) during the spawning period. During the seven-year period of study, the earliest date a fish was detected in the spawning area was 9 April in 2021. In 2018 the first fish was not detected until 2 May. The latest fish was recorded in the spawning grounds was 17 June 2019. In 2022 all fish left the spawning area by 3 June. The spawning period ranged from 35 days in 2018 to 60 days in 2019, lasting 42 ± 9 (mean \pm std) days on average (Table 1.2, Figures 1.2 – 1.8). In general, lake sturgeon abundance in the spawning grounds increased following a period when discharge decreased, and temperature increased over a 72-hour period. Variability in discharge and temperature appeared to shift the number of fish in the river during the spawning period. In 2017, a mid-season increase in discharge coupled with warmer water temperatures appeared to initiate movements of fish out of the river (Figure 1.3). Extremely high discharge in 2018 delayed adult arrival on the spawning grounds more than two weeks (Figure 1.4). In 2019 consistently high discharge and temperature variability lengthened the duration of the spawning season, as evidenced by multiple peaks in spawning activity (Figure 1.5). Notably, a large change in temperature was associated with a large decrease in female spawner abundance in 2021 (Figure 1.7). In contrast, low discharge and early-warming resulted in a shortened spawning period (e.g., in 2022; Figure 1.8).

Lake sturgeon response to environmental conditions varied between sexes and across years, largely due to sex differences in river residence time (males longer). Male lake sturgeon experienced greater (MannU, $p = 0.034$) discharge across the duration of the study. Mean (\pm std) cumulative daily discharge units experienced by male lake sturgeon was $98.26 \pm 94.18 \text{ m}^3 \cdot \text{sec}^{-1}$ while female lake sturgeon experienced $88.49 \pm 83.74 \text{ m}^3 \cdot \text{sec}^{-1}$ (mean \pm std) discharge (Figure 1.9). Discharge experienced varied significantly by year for male (Kruskal-Wallis, $p < 0.001$) ranging from $47.32 \pm 47.06 \text{ m}^3 \cdot \text{sec}^{-1}$ in 2016 to $207.18 \pm 136.37 \text{ m}^3 \cdot \text{sec}^{-1}$ in 2020. Similarly, female discharge experienced varied by year (Kruskal-Wallis, $p < 0.001$), ranging from $43.35 \pm 42.39 \text{ m}^3 \cdot \text{sec}^{-1}$ in 2016 to $183.20 \pm 105.86 \text{ m}^3 \cdot \text{sec}^{-1}$ in 2020 (Figure 1.9).

Discharge data from the period the gauging station was present on the upper Black River (October 1942 – September 2000) were available to characterize periods of abnormal discharge that may have influenced behavior and adult resource expenditures (Figure 1.10). Discharge values above $13.08 \text{ m}^3 \cdot \text{sec}^{-1}$ exceeded the upper 10% of all recorded discharge in the UBR, while discharges exceeding $16.37 \text{ m}^3 \cdot \text{sec}^{-1}$ exceeded the upper 5% of all recorded discharge. The maximum recorded discharge in the UBR was $52.67 \text{ m}^3 / \text{sec}$ (Figure 1.2), while the mean (\pm std) discharge was $7.71 (\pm 4.59) \text{ m}^3 \cdot \text{sec}^{-1}$ (Figure 1.10). Days in the river during which the discharge exceeded 5% of all historical flows did not differ between male and female lake sturgeon during the study period (MannU, $p = 0.259$, Figure 1.11). The number of days a male lake sturgeon was in the river when discharge was above the 5% exceedance flow level was 2.32 ± 3.37 days (mean \pm std), while females experienced 1.93 ± 2.60 days (mean \pm std). 5% exceedance flow days experienced varied significantly by year for male (Kruskal-Wallis, $p < 0.001$) ranging from 0.03 ± 0.26 days in 2022 and 7.41 ± 4.95 days in 2020. Similarly, 5% exceedance flow days

experienced varied significantly by year for female (Kruskal-Wallis, $p < 0.001$) ranging from 0.14 ± 0.69 days in 2022 and 5.87 ± 3.19 days in 2020 (Figure 1.11).

Males experienced significantly higher CTUs in the spawning grounds during the study period (MannU, $p < 0.001$, Figure 1.12) than did females, associated with longer periods of river occupancy. Males experienced $60.10 (\pm 48.63)$ (mean \pm std) CTU while females experienced $42.22 (\pm 39.11)$ CTU. CTU varied significantly by year for male lake sturgeon (Kruskal-Wallis, $p < 0.001$) ranging from 41.95 ± 33.54 units in 2017 and 89.11 ± 66.65 units in 2016.

Additionally, CTU varied significantly by year for female lake sturgeon (Kruskal-Wallis, $p < 0.001$) ranging from 29.68 ± 30.22 units in 2019 and 75.05 ± 77.94 units in 2016 (Figure 1.16).

4.3 Demographic and behavioral variability

Inter-annual variability in river environmental conditions resulted in significant differences in adult river migration behavior and spawning area residence time across years and between sexes. Male river residence time was significantly longer than female residence time across all years (MannU, $p < 0.01$). Mean (\pm std) male residence time was $244.38 (\pm 163.12)$ (Range: 9.92 – 961.52) hours, while females were in the river for $169.53 (\pm 141.55)$ (Range: 9.87 – 1,421.47) hours. Residence time varied significantly across years (Kruskal-Wallis, $p < 0.01$, Figure 1.13).

Based on PIT array data on the number of intra- and inter-sexual co-occurrences on the spawning areas, female lake sturgeon experienced significantly more inter-sex interactions than males from 2016 – 2022 (MannU, $p < 0.001$, Figures 1.14, 1.15) due to consistent male-biased spawning sex ratios (Table 1.2). During spawning periods in all seven years, male lake sturgeon experienced 33 ± 19 (mean \pm std) inter-sex interactions, while females interacted with 111 ± 45

(mean \pm std) males. Intra-sex interactions (co-occurrences on the spawning areas) which is a surrogate for access to mates, varied significantly between males and females (MannU, $p < 0.001$), also attributed largely to male-biased sex ratios (Figures 1.2. through 1.8). During the duration of the study, male lake sturgeon encountered (mean \pm std) 131 (± 53) males while females encountered (mean \pm std) 33 (± 17) females (Figures 1.14, 1.15). The number of male intra-sex interactions varied significantly by year (Kruskal-Wallis, $p < 0.001$) across the sampling period, ranging from 97 (± 41 , mean \pm std) in 2016 to 159 (± 53 , mean \pm std) in 2019 (Figure 1.15). Female intra-sex interactions also varied significantly by year (Kruskal-Wallis, $p < 0.001$) ranging from 22 (± 14 , mean \pm std) in 2019 to 44 (± 20 , mean \pm std) in 2017 (Figure 1.14). is

Body size (fork length) varied between male and female lake sturgeon (MannU, $p < 0.001$) across all years of this study. Females were significantly larger (mean \pm std: 152.70 \pm 14.54 cm, range: 101 cm – 189 cm) than males (mean \pm std: 131.20 \pm 13.81 cm, range: 87 cm – 169 cm) (Figure 1.16). Fork length was consistent across years for both females (Kruskal-Wallis, $p = 0.301$), and males (Kruskal-Wallis, $p = 0.362$, Figure 1.16).

4.4 Inter-spawning interval

The period between reproductive seasons (inter-spawning interval; ISI) is important for acquisition and storage of resources needed for growth and reproduction. We evaluated three random effects, ID (RFID identification number), OBS (within year river occupancy), and year (year of initial spawning effort) (Table 1.1) with a fully parametrized mixed effects model to determine if variability in ISI was best explained by inter-year and individual differences. The model of best fit did not include random effects both for males and females (Table 1.3). In both cases, we used only fixed effects to evaluate variability associated with years between spawning seasons.

Inter-spawning interval was highly variable from year-to-year. The average male in the Black Lake population had an inter-spawning interval of 1.61 ± 0.68 years (mean \pm std). Female inter-spawning interval during the study period was 3.19 ± 0.61 years (mean \pm std). Of male lake sturgeon which spawned first in 2016 (K), 52.7% returned in the successive year, 2017 (K+1), 38.4% returned in 2018 (K+2), 4.5% returned in 2019 (K+3), 1.8% returned in 2020 (K+4), and 2.7% took five or more years to return to spawn ($K \geq 5$) (Figure 1.17). Of the males which spawned first in 2017, 40.5% returned in year K+1, 50.6% in year K+2, 8.2% in year K+3, and 0.6% in year K+4. When first spawning occurred during 2018, 52.4% returned in year K+1, 42.1% in year K+2, 5.5% in year K+3. Finally, when first spawning occurred in 2019, 34.4% returned in year K+1, 49.4% in year K+2, 16.1% in year K+3 (Figure 1.17).

The model of best fit for male lake sturgeon inter-spawning interval included positive associations with the fixed effects of CTU and the number of intra-sex interactions as well as negative association with both the number of inter-sex interactions and fork length (cm) (Table 1.4). Variability between the ΔAIC selected models (resulting in 14 candidate models) was minimal, so we averaged all models with a $\Delta AIC < 2$. The resulting composite model demonstrated significant negative associations between male fork length (cm) (GLM, $z = 2.196$, $p = 0.028$, Table 1.5) and inter-sex interactions ($z = 2.786$, $p = 0.005$, Table 1.5). For every one-centimeter increase in fork length, male inter-spawning interval decreased by 0.5%. Over the full range of male fork length (min = 87cm, max = 169 cm) male inter-spawning interval dropped by 41%. For every increase of one inter-sex interaction, inter-spawning interval decreased by 0.7%.

The fixed effect model of best fit for female lake sturgeon was the null model. Ten candidate models with a $\Delta AIC < 2$ were considered, each with the univariate fixed effect of each independent variable (Tables 1.1, 1.6). As variability among models was small, we again averaged

all models with a $\Delta AIC < 2$. Neither the full nor the conditionally averaged model demonstrated significant associations between measures of spawning behavior, body size, or environmental conditions with inter-spawning interval (Table 1.7) indicating variability of inter-spawning interval is likely not best determined by individual variability in behavior or body size.

Female lake sturgeon return probability was evaluated for years 2016-2018, as female inter-spawning interval prevents more than four years of evaluation from 2019 through the end of the study period. Of female lake sturgeon which spawned first in 2016 (K), 0.0% returned in 2017 (K+1), 11.8% returned in 2018 (K+2), 70.6% returned in 2019 (K+3), 17.6% returned in 2020 (K+4), and no females took five or more years to return to spawn ($K \geq 5$) (Figure 1.17). Of the females which spawned first in 2017, 0.0% returned in year K+1, 7.3% in year K+2, 58.5% in year K+3, 31.7% in year K+4 and 2.4% took five or more years to return. Finally, when first spawning occurred during 2018, 0.0% returned in year K+1, 3.4% in year K+2, 51.7% in year K+3, and 44.8% took at least four years to return (K+4) (Figure 1.17).

5. DISCUSSION

There is considerable variability in reproductive behavior among individuals of iteroparous species annually and by the same individual inter-annually due to differences in exposure to variable stream environmental conditions (e.g., temperature and discharge), in concert with demographic variation in adult abundance and sex ratios that affect inter- and intra-sexual encounter levels (mating opportunities and competitive interactions, respectively). Individual plasticity in migratory behaviors exhibited by spawning males and females in response to sources of variability likely led to current year decisions that represented trade-offs between current and future reproductive investments. For long-lived iteroparous species such as

lake sturgeon, these data are particularly relevant to life-time RS (Clutton-Brock 1988) and to forecasting future changes in recruitment in the current era of climate variability and change.

This study provided a unique opportunity to characterize spawning behavior of individuals during an entire reproductive season over seven years, allowing quantification of associations between spawning behavior and potential sources of variability. Passive PIT arrays situated throughout the river allowed us to record activities that constituted measures of current ‘reproductive effort’. Collectively, data revealed that male inter-spawning interval decreased with increasing body size, which is associated with age. Additionally, between year variation in inter-spawning interval for both males and females was associated with variability in environmental conditions during the spawning period.

5.1 Inter-spawning interval

Results provided evidence of a negative association between male body size (and therefore age) and inter-spawning interval. Over the range of sizes observed during the study period, we observed a 41% decrease in male ISI with increasing size (age), indicating that older males attempted to reproduce more frequently. Though there was some year-to-year variability in male inter-spawning interval (Figure 1.17), variation was not associated with individual id, suggesting that yearly variability in ISI resulted from fixed effects characterizing conditions experienced by the individuals spawning at specific times and locations. Findings of lower inter-spawning interval with increasing body size is consistent with Jørgensen et al. (2006), who predicted that inter-spawning interval should be longest immediately following first maturity, and decreasing with increasing age. Evidence exists suggesting that inter-spawning interval decreases with body size, and/or age in other species. Morbey and Shuter (2013) demonstrated that the period between lake trout (*Salvelinus namaycush*) spawning events decreased as fish

aged. Additionally, Skjæraasen et al. (2012) observed shorter inter-spawning periods in cod (*Gadus morhua*) as age increased. Collectively, results indicated that future male ISI may be influenced by conditions during current reproduction, in accordance with theory (Williams 1966), which predicts increased reproductive investment as residual breeding opportunities decrease.

Several measures of reproductive effort have been used to evaluate trade-offs between conditions favoring current vs residual breeding investment in fishes, including juvenile survivorship, clutch size, adult mortality, and reproductive effort (Stearns 1992). We focused specifically on associations between current reproductive effort (defined by time spent in the river during the spawning period) and future reproductive opportunities (probability of returning in successive years). Years when river residence time in spawning areas was shortest coincided with the highest next year (K+1) return probability for male lake sturgeon (52.7% in 2016, 52.4% in 2018). Conversely, years when residence time was long (e.g., 2019) resulted in significantly lower first year return probability for male lake sturgeon (34.4%, Figure 1.17). Finally, male ISI decreased as inter-sex interactions increased. Likely, males increase inter-sex interactions as reproductive effort increased with size (and therefore age). Results demonstrated that male lake sturgeon behavior during the spawning season and variable environmental conditions may be associated with differences in probability of return in subsequent spawning years. Differences in the shape and duration of the spawning run were associated with differences in river residence time, which increases annual RS (Larson et al. 2020), but may also result in a trade-off reducing the probability of returning to the river in a successive year (Figure 1.17). Individual lifetime RS is dependent on the number of recruits produced by an individual during its entire lifespan that survive to reproduce in the following generation (Clutton-Brock

1988, Newton 1989). Spawning in successive years would be skipped when expected loss in reproductive output from year K+1 would be balanced with the expected RS in year K (Jørgensen et al. 2006).

Female ISI was found to be associated with environmental conditions, body size, or potential mate presence, and was significantly longer than in males; however, there was evidence that female ISI was variable between spawning years (Figure 1.17). In general, iteroparous females take longer than males to recover following reproduction (Fleming and Reynolds 2004, Morbey and Shuter 2013, Baron et al. 2013), and the length of the inter-breeding period is associated with the cost of increasing fecundity and the availability of resources during the inter-breeding period (Bull and Shine 1979, McNamara and Houston 2008). Additionally, because females have higher energetic demands associated with reproduction, breeding period can be affected by environmental stochasticity (Shaw and Levin 2013, Morbey and Shuter 2013), which may limit the ability of females to acquire resources during the non-breeding period. The lack of associations between female ISI and the variables evaluated could also be due to the proportionally lower sample size for females relative to males. As neither body size, nor environmental conditions were associated with ISI for female sturgeon in the UBR, ISI was likely associated with food availability or costs of gamete production. Also of note, the average female inter-spawning interval during this study was 3.19 ± 0.61 years. As a result, most females in this study were captured initially with only one additional capture event during the study period. It is possible that the lack of multiple spawning events may have contributed to the lack of significant associations with body size and environmental conditions.

5.2 Environmental conditions and spawning run variability

The timing of initiation and duration of the spawning period varied considerably over the course of the study. The initiation of spawning in the UBR is associated with multi-day periods of decreasing discharge, increasing temperature and by lunar phase (Forsythe et al. 2012b).

While these cues are typically predictive of lake sturgeon river entry across years, and the standardized day of spawning is highly repeatable by individual, the ordinal day of spawning initiation varied from year-to-year. During the study period, the first day of the spawning run varied from 9 April to 2 May. Spawning period duration was also highly variable ranging from 35 days to 60 days (Figures 1.2-1.8). Duration of the spawning season is likely consequential. Male lake sturgeon, for example, are likely to increase inter-sex interactions as residence time increases. As male RS is heavily dependent on female availability (Larson et al. 2020), years with longer spawning runs may result in greater within year RS but may result in a trade-off in the reduction of sperm quality or greater resource expenditures. Female lake sturgeon RS is also associated with male availability (operational sex ratio, Dammerman et al. 2019) though the degree to which a longer season may affect female river residence time remains unclear.

The date of spawning initiation did not impact the duration of the spawning period nor the modality of adult arrival and daily sex ratio. Instead reduced environmental variability both lengthened the spawning run and increased the number of days spawner density was high. Spawning run modality and, to some extent, spawning season duration was associated with the magnitude, number, and duration of changes in discharge and temperature. Cumulative discharge (Figure 1.9), days when river discharge exceeded 5% of all recorded values (Figure 1.11) and CTUs (Figure 1.12) all varied significantly by year. Consequently, it is likely that spawners experienced differences in resource expenditures resulting from variable environmental

conditions across years. Additionally, while female standardized spawning day is highly repeatable across years (Forsythe et al. 2012a), female residence time was significantly shorter than males in all years (Figure 1.13). When considered in combination with differences in male and female ISI, it is likely that both within- and between- year adult spawning demography were highly variable.

Annual variability in the onset and duration of spawning as presented in Figures 1.2-1.8 may highlight future challenges in lake sturgeon recruitment. Migratory fishes are reliant on external cues which signal the onset of spawning migration (Gilhousen 1980, Quinn and Adams 1996). Sturgeon species rely on the lagged effect of increasing temperature and decreasing discharge (Goodman et al. 2012, Vine et al. 2019) along with lunar phase (Forsythe et al. 2012b) to initiate spawning migration. Stable and predictable environmental conditions benefit long-lived species with delayed sexual maturity, whereas stochastic conditions typically favor short-lived species with high fecundity (Pianka 1970). Generally, migration occurs when environmental conditions are likely to be most suitable for offspring survival (Heath 1992, Hodgson and Quinn 2002). As climate change is predicted to increase environmental stochasticity, cues to spawning migration are likely to become less reliable predictors of conditions during early ontogeny. Wassink et al. (2020) indicated that this mismatch between maternal and offspring experiences may alter offspring behavior and ultimately has population-level consequences for recruitment.

5.3 Conclusion

This study contributes to the understanding of the complex conditions that lead to decisions associated with current and future reproduction, which vary considerably among individuals and between males and females. Annually, the initiation of spawning in the Black

River is associated with phase of the lunar cycle (initiated on a new moon), and multi-day periods of decreasing river discharge and increasing water temperature (Forsythe et al. 2012b). While these cues to initiate spawning are repeatably followed by spawning lake sturgeon across years, the ordinal day of spawning initiation varies from year to year. Additionally, variability in environmental conditions can alter the length of the spawning season, for which date of initiation of spawning is not a reliable predictor. The resulting differences in spawning duration can result in major differences in adult demography (e.g., sex ratio and the number of intra- and inter-sexual interactions), which may affect current RS both for males (Larson et al. 2020) and females (Dammerman et al. 2019), and likewise may be responsible for variability of inter-spawning interval.

Of particular interest in this study was the finding that male lake sturgeon inter-spawning interval increased with increasing body size, while female inter-spawning interval could not be explained by behavior during the proceeding reproductive period, body size, environmental variability, between year variation or individual variability. While a link between body size/age and increasing ISI has been hypothesized (Forsythe et al. 2012a), this study offers the first evidence of this link in a sturgeon species. The reduction of inter-spawning interval in males with increasing body size is likely a response to the reduced number of residual breeding opportunities as an individual ages. Conversely, female inter-spawning interval may be explained by factors not evaluated in this study, including the time needed to acquire and store resources necessary for gamete production. Alternatively, because the inter-spawning interval of females exceeded three years, most females in this study were only captured twice, including the initial time of capture. These contrasts with males, which could be captured annually, providing

far more resolution over a seven-year study period. Future work should evaluate female inter-spawning interval with multiple recapture events.

Collectively, results demonstrate that reproductive behavior exhibits some degree of plasticity from year-to-year and that some behaviors (i.e., inter-spawning interval) are associated with reproduction over long periods of time, as exemplified over our seven-year study. This study further demonstrated the value of continued monitoring of populations of long-lived fish on an individual basis. From a management perspective, individual-based monitoring is a tool to predict annual recruitment variability more accurately and continues to build our understanding of the complex interplay of spawning effort, reproductive success and recruitment, particularly as climate change increases environmental stochasticity thereby reducing the reliability of cues to spawning migration in long-lived fishes.

REFERENCES

- Andersson, M., and Iwasa, Y. 1996. Sexual Selection. *Trends in Ecology and Evolution*, 11: 53-58.
- Arnold, S.J., and Duvall, D. 1994. Animal mating systems: a synthesis based on selection theory. *Amer. Nat.*, 143: 317-348.
- Báez-Revueltas, FB. 2009. "Residual Logistic Regression." New York, NY, USA: Stony Brook University. <https://ir.stonybrook.edu/xmlui/handle/11401/72390>.
- Baker, E. A., and Borgeson, D. J. 1999. Lake Sturgeon Abundance and Harvest in Black Lake, Michigan, 1975-1999. *North American Journal of Fisheries Management*, 19: 1080 – 1088.
- Baron, J., Gilliard, J., Ferrière, R., and Tully, T. 2013. Intermittent breeding and the dynamics of resource allocation to reproduction, growth and survival. *Functional Ecology*, 27: 173-183.
- Bartón, K. 2017. MuMIn, Multi-model inference. R package version 1.40.0 [online]. Available from www.CRAN.R-project.org/package=MuMIn
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity*, 2: 349-368.
- Bell, G. and Koufopanou, V. 1986 The cost of reproduction. In *Oxford surveys in evolutionary biology* (ed. R. Dawkins and M. Ridley), pp. 83-131. Oxford University Press.
- Bull, J. J. and Shine, R. 1979. Iteroparous animals that skip opportunities for reproduction. *The American Naturalist*, 114, 296–303.
- Bunnell, D. B., Thomas, S. E., and Stein, R. A. 2007. Prey resources before spawning influence gonadal investment of female, but not male, white crappie. *Journal of Fish Biology*, 70: 1838–1854.
- Bunnell, D. B., Eshenroder, R. L., Krause, A. E., and Adams, J. V. 2012. Depth segregation of deepwater ciscoes (*Coregonus* spp.) in Lake Michigan during 1930–1932 and range expansion of *Coregonus hoyi* into deeper waters after the 1990s. *Adv. Limnol.* 63: 3–24. doi:10.1127/advlim/63/2012/3.
- Bose, A. P. H., Cogliati, K. M., Luymes, N., Bass, A. H., Marchaterre, M. A., Sisneros, J. A., Bolker, B. M., and Balshine, S. 2018. Phenotypic traits and resource quality as factors affecting male reproductive success in a toadfish. *Behavioral Ecology*, 29: 496-507.
- Burnham, K. P. P., and Anderson, D. R. R. 2002. *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach*. *Ecological Modelling*, 172, Springer. <https://doi.org/10.1016/j.ecolmodel.2003.11.004>

- Caswell, H. 1982. Optimal life history and the age-specific costs of reproduction. *Journal of Theoretical Biology*, 98: 519-529.
- Clutton-Brock, T. H. 1988. *Reproductive Success. Studies of Individual Variation in Contrasting Breeding Systems.* The University of Chicago Press.
- Dammerman, K., Webb, M., and Scribner, K. T. 2019. Riverine characteristics and adult demography influence female lake sturgeon (*Acipenser fulvescens*) spawning behavior, reproductive success, and ovarian quality. *Canadian Journal of Fisheries and Aquatic Sciences*, 76: 1147-1160.
- Dingle, H. 1996. *Migration: The biology of life on the move.* New York: Oxford University Press.
- Dunn, O. J. 1964. Multiple comparisons using rank sums. *Technometrics*, 6(3): 241–252. doi:10.1080/00401706.1964.10490181.
- Emlen, J. M. 1970. Age specificity and ecological theory. *Ecology*, 51: 588-601.
- Fenkes, M., Shields, H. A., Fitzpatrick, J. L., and Nudds, R. L. 2016. The potential impacts of migratory difficulty, including warmer waters and altered flow conditions, on the reproductive success of salmonid fishes. *Comparative Biochemistry and Physiology Part A. Molecular and Integrative Physiology*, 193: 11-21.
- Fisher, R. A. 1930. *The genetical theory of natural selection.* Oxford University Press.
- Fleming, I. A., and Gross, M. R. 1994. Breeding competition in a pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution*, 48(3): 637–657.
- Fleming, I. A., and Reynolds, J. D. 2004. Salmonid breeding systems. Pages 264–294 in A. P. Hendry and S. C. Stearns, editors. *Salmon illuminated: salmon and their relatives.* Oxford University Press, New York, New York, USA.
- Forsythe, P. S., Crossman, J. A., Bello, N. M., Baker, E. A. and Scribner, K. T. 2012a. Individual-based analyses reveal high repeatability in timing and location of reproduction in Lake Sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences*, 69: 60-72.
- Forsythe, P. S., Scribner, K. T., Crossman, J. A., Ragavendran, A., Davis, C., Baker, E. A., and Smith, K. K. 2012b. Environmental and lunar cues are predictive of timing of river entry and spawning site arrival in Lake Sturgeon. *Journal of Fish Biology*, 81: 35-53.
- Gibbons, J. W., and Andrews, K. M. (2004). PIT Tagging, Simple Technology at Its Best. *BioScience*, 54(5): 447–454.

- Gilhousen, P. 1980. Energy sources and expenditures in Fraser River sockeye salmon during their spawning migration. *International Pacific Salmon Fisheries Commission Bulletin* 22.
- Goodman, B. J., Guy, C. S., Camp, S. L., Gardner, W. M., Kappenman, K. M., and Webb, M. A. H. 2012. Shovelnose Sturgeon Spawning in Relation to Varying Discharge Treatments in a Missouri River Tributary. *River Research and Applications*, 29: 1004-1015.
- Hay-Chmielewski, E. M. 1987. Habitat Preference and Movement Patterns of the Lake Sturgeon (*Acipenser Fulvescens*) in Black Lake, MI. Michigan Department of Natural Resources Fisheries Division Research Report No. 1949.
- Hay-Chmielewski, E.M., and Whelan, G.E. 1997. Lake sturgeon rehabilitation strategy. Special Report 18, Michigan Department of Natural Resources, Ann Arbor, Mich.
- Heath, M. R. 1992. Field investigations of the early-life stages of marine fish. *Advances in Marine Biology*, 28:1–174.
- Henson, S. A., and Warner, R. R. 1997. MALE AND FEMALE ALTERNATIVE REPRODUCTIVE BEHAVIORS IN FISHES: A New Approach Using Intersexual Dynamics. *Annual Review of Ecology and Systematics*, 28: 571-592.
- Hodgson, S., and Quinn, T. P. 2002. The timing of adult Sockeye Salmon migration into fresh water: adaptations by populations to prevailing thermal regimes. *Canadian Journal of Fisheries and Aquatic Sciences*, 80:542– 555.
- Jirotkul, M. 1999. Operational sex ratio influences female preference and male– male competition in guppies. *Animal Behavior*, 58: 287–294.
- Johnston, F. D., and Post, J. R. 2009. Density-dependent life-history compensation of an iteroparous salmonid. *Ecological Applications*, 19: 449–467.
- Jørgensen, C. Ernande, B., Fiksen, Ø., and Dieckmann, U. 2006. The logic of skipped spawning. *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 200–211.
- Kempinger, J. J. 1988: Spawning and early life history of lake sturgeon in the Lake Winnebago System, Wisconsin. In: 11th Annual Larval Fish Conference. R. D. Hoyt (Ed.). American Fisheries Society, Symposium 5, Bethesda, Maryland, USA, pp. 110–122.
- Kennedy, J., Witthames, P. R., Nash, R. D. M., and Fox, C. J. 2008. Is fecundity in plaice (*Pleuronectes platessa L.*) down-regulated in response to reduced food intake during autumn? *Journal of Fish Biology*, 72: 78–92.
- Kjesbu, O. S., Klungsoyr, J., Kryvi, H., Witthames, P. R., and Walker, M. G. 1991. Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. *Canadian Journal of Fisheries and Aquatic Sciences*, 48: 2333–2343.

- Kusnierz, P. C., Stimmell, S. P., and Leonard, J. B. K. 2009. Movement and growth indicators in resident and adfluvial coaster brook trout (*Salvelinus fontinalis*) in the Hurricane River, Lake Superior, Michigan, USA. *Journal of Great Lakes Research*, 35: 385–391.
- Larson, D. L., Kimmel, J. G., Riedy, J. J., Hegna, J., Baker, E. A., and Scribner, K. T. 2020. Male Lake Sturgeon (*Acipenser fulvescens*) migratory behavior associated with intra-annual variability in sperm and reproductive success. *Canadian Journal of Fisheries and Aquatic Sciences*, 77: 1943-1959.
- Martin, N. V. 1970. Long-term effects of diet on the biology of the Lake Trout and the fishery in Lake Opeongo, Ontario. *Journal of the Fisheries Research Board of Canada*, 27:125–146.
- McNamara, J. M. and Houston, A. I. 2008. Optimal annual routines: behaviour in the context of physiology and ecology. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 363, 301–319.
- Morbey, Y. E., and Shuter, B. J. 2013. Intermittent breeding in the absence of a large cost of reproduction: evidence for a non-migratory, iteroparous salmonid. *Ecosphere*, 4: 1-18.
- Morgan, M. J., and Trippel, E. A. 1996. Skewed sex ratios in spawning shoals of Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Science*, 53: 820–826.
- Newton, I. 1989. Lifetime reproduction in birds. Academic Press, San Diego, CA.
- Oliveira, R. F., Taborsky, M. and Brockman, H. K. 2008. Alternative Reproductive Tactics: An Integrative Approach. Cambridge University Press, Cambridge, UK.
- Paragamian, V. L., and Wakkinen, V. D. 2002. Temporal distribution of Kootenai River white sturgeon spawning events and the effect of flow and temperature. *Journal of Applied Ichthyology*, 18: 542-549.
- Pianka, E. R. 1970. On r- and k-selection. *The American Naturalist*, 104:592-597.
- Pianka, E. R., and Parker, W. S. 1975. Age-specific reproductive tactics. *The American Naturalist*, 109: 453-464.
- Pianka, E. R. 1976. Natural selection of optimal reproductive tactics. *American Zoologist*, 16: 775-784.
- Pledger, S., Baker, E. A. and Scribner, K. T. 2013. Breeding return times and abundance in capture-recapture models. *Biometrics*, 69: 991-1001.
- Quinn, T. P., and Adams, D. J. 1996. Environmental changes affecting the migratory timing of American Shad and Sockeye Salmon. *Ecology*, 77:1151– 1162.

- Rhodes, K. L., McIlwain, J., Joseph, E., Nemeth, R. S. 2012. Reproductive movement, residency and fisheries vulnerability of brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775). *Coral Reefs*, 31:443–453.
- Rideout, R.M., Rose, G.A., and Burton, M.P.M. 2005. Skipped spawning in female iteroparous fishes. *Fish and Fisheries*, 6: 50–72.
- Rideout, R. M., and Tomkiewicz, J. 2011. Skipped Spawning in Fishes: More Common than You Might Think. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, 3: 176-189.
- Scribner, K. T., and Kanefsky, J. 2021. Molecular sexing of lake sturgeon. *Journal of Great Lakes Research*, 47: 934-936.
- Schaffer, W. M. 1974. Optimal reproductive effort in fluctuating environments. *American Naturalist*, 108: 783-790.
- Schwalme, K., and Chouinard, G. A. 1999. Seasonal dynamics in feeding, organ weights, and reproductive maturation of Atlantic cod (*Gadus morhua*) in the southern Gulf of St Lawrence. *ICES Journal of Marine Science*, 56: 303–319.
- Shaw, A. K., and Levin, S. A. 2013. The evolution of intermittent breeding. *Journal of Mathematical Biology*, 66:685–703.
- Sitar, S. P., Jasonowicz, A. J., Murphy, C. A., and Goetz, F. W. 2014.) Estimates of Skipped Spawning in Lean and Siscowet Lake Trout in Southern Lake Superior: Implications for Stock Assessment. *Transactions of the American Fisheries Society*, 143(3): 660-672.
- Skjæraasen, J. E., Nash, R. D. M., Korsbrekke, K., Fonn, M., Nilsen, T., Kennedy, J., Nedreaas, K. H., Thorsen, A., Witthames, P. R., Geffen, A. J., Høie, H., and Kjesbu, O. S. 2012. Frequent skipped spawning in the world’s largest cod population. *Proceedings of the National Academy of Sciences USA*, 109: 8995– 8999.
- Smith, K. M. and Baker, E. A. 2005. Characteristics of spawning lake sturgeon in the Upper Black River, Michigan. *North American Journal of Fisheries Management* 25: 301–307.
- Somarakis, S., Tsoukali, S., Giannoulaki, M., Schismenous, E., Nikolioudakis, N. 2019. Spawning stock, egg production, and larval survival in relation to small pelagic fish recruitment. *Marine Ecology Progress Series*, 617: 113-136.
- Stearns, S. C. 1976. Life-history tactics: a review of the ideas. *Biological Reviews*, 51(1):3-47
- Stearns, S. C. 1989 Trade-offs in life-history evolution. *Functional Ecology* 3, 259–268.
- Stearns, S. C. 1992. *The Evolution of Life Histories*. The Oxford University Press.

- Swanson, R. G., McCann, E. L., Johnson, N. S. and Zielinski, D. P. 2021. Environmental factors influencing annual sucker (*Catostomus sp.*) migration into a Great Lakes tributary. *Journal of Great Lakes Research*, 47: 1159-1170.
- Thiem, J. D., Binder, T. R., Dawson, J. W., Dumont, P., Hatin, D., Katopodis, C., Zhu, D. Z., and S. J. Cook. 2011. Behaviour and passage success of upriver-migrating lake sturgeon *Acipenser fulvescens* in a vertical slot fishway on the Richelieu River, Quebec, Canada. *Endangered Species Research*, 15(1): 1-11.
- Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136-179. in B. Campbell (ed.). *Sexual selection and the descent of man. 1871-1971*. Aldine, Publ. Chicago, IL.
- Vine, J. R., Holbrook, S. C., Post, W. C., and Peoples, B. K. 2019. Identifying Environmental Cues for Atlantic Sturgeon and Shortnose Sturgeon Spawning Migrations in the Savannah River. *Transactions of the American Fisheries Society*, 148: 671-681.
- Walsh, S. J., Wells, R., and Brennan, S. 1986. Histological and visual observations on oogenesis and sexual maturity of Flemish Cap female cod. *NAFO Sci. Council Res. Doc. No. 86/111*.
- Wassink, L., Huerta, B. Li, W. and Scribner, K. T. 2020. Interaction of egg cortisol and offspring experience influences stress-related behaviour and physiology in lake sturgeon. *Animal Behaviour*, 161: 49-59.
- Williams, G. C. 1966. *Adaptation and natural selection*. Princeton University Press.
- Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist*, 100:687-690.

APPENDIX

Table 1.1 List of variable names, acronyms, variable definitions, and corresponding tables for analysis of behavioral, biological, and environmental variables associated with inter-spawning interval.

	Variable	Unit	Acronym	Estimated
Dependent Variable	Inter-spawning Interval	Years	ISI	Years between RFID detections
Independent Variable	Previous Migrations	Count of Total Migrations	MIG	Number of times in a season a fish was detected migrating into and out of the known spawning area
	Upstream Migration Time	Hours	UST	Time between detection at the mouth of the UBR and detection at the start of the known spawning area
	River Residence Time	Hours	RRT	Time between first detection at the mouth of the UBR and last detection leaving the UBR if fish was detected in the known spawning area
	5% Exceedance Flow	Count of Days	EF	Number of days an individual fish was in or above the known spawning area and for which the total daily average discharge exceeded the 5% exceedance flow ($16.37 \text{ m}^3 \cdot \text{sec}^{-1}$)
	Fork Length	Centimeters	FL	Fork length measured at the most recent time of capture
	Inter-sex Interactions	Total Count	IEA	Count of the number of fish of the opposite sex in the known spawning area overlapping spawning ground occupancy by an individual
	Intra-sex Interactions	Total Count	IAA	Count of the number of fish of the same sex in the known spawning area overlapping spawning ground occupancy by an individual
	Cumulative Thermal Units	°C	CTU	Sum of the daily total cumulative thermal units for days when an individual fish occupied the known spawning area
	Cumulative Discharge	$\text{m}^3 \cdot \text{sec}^{-1}$	Q	Sum of the daily total average discharge for days when an individual fish occupied the known spawning area
	Random Effects	Year	Year in Date Format	---
Observation		Ordinal Entry Position	OBS	Random effect for individual observations on the spawning grounds during the last recorded event leading up to the return migration
Identity		RFID Identifier	ID	Individual fish RFID identification number

Table 1.2 Number of male and female lake sturgeon captured by hand and documented to be in the river using a passively monitored RFID antenna array in the upper Black River, MI from 2016-2022. Hand capture efficiency calculated as the total number of hand captured fish relative to the total number of RFID captured fish (excludes untagged, non-handled fish). RFID capture efficiency calculated as the total number of fish which were captured in the hand capture survey but were not captured on RFID antenna (excludes untagged, non-handled fish).

Year	Hand Capture			RFID Capture			Spawning Period			Efficiency	
	Male	Female	TOTAL	Male	Female	TOTAL	Date of First Detection	Date of Final Detection	Spawning Period Duration (Days)	Hand Capture Efficiency	RFID Capture Efficiency
2016	139	59	198	220	72	292	16-Apr	9-Jun	54	67.81%	98.48%
2017	168	70	238	257	92	349	16-Apr	3-Jun	48	68.19%	96.64%
2018	156	44	200	265	100	365	2-May	6-Jun	35	54.79%	96.50%
2019	233	60	293	329	96	425	21-Apr	17-Jun	57	68.94%	95.56%
2020*	106	33	139	247	89	336	12-Apr	6-Jun	55	41.37%	94.24%
2021	250	101	351	333	129	462	9-Apr	8-Jun	60	75.97%	95.73%
2022	233	86	319	280	105	385	25-Apr	3-Jun	39	82.86%	96.87%

Table 1.3 AIC difference for models including combinations of within-year and inter-year random effects associated with male and female lake sturgeon inter-spawning interval including year (2016-2021), order of river entry, and unique fish identifier (PIT Tag number). Fixed effects (number of migrations, upstream swimming time (hrs), river residence time (hrs), cumulative discharge ($\text{m}^3 \text{sec}^{-1}$), 5% exceedance flow days, cumulative critical temperature units, fork length (cm), inter-sex interactions, and intra-sex interactions) were included in all models. DeltaAICc scores are relative to the model with the lowest AICc score.

Number	Model	Male		Female	
		AIC	DeltaAIC	AIC	DeltaAIC
8	No Random Effects	2302.31	0.00	407.03	0.00
4	YEAR	2303.11	0.80	409.03	2.00
2	ID	2304.31	2.00	409.03	2.00
3	OBS	2304.31	2.00	409.03	2.00
5	ID + YEAR	2305.11	2.80	411.03	4.00
7	OBS + YEAR	2305.11	2.80	411.03	4.00
6	ID + OBS	2306.31	4.00	411.03	4.00
1	ID + OBS + YEAR	2307.11	4.80	413.03	6.00

Table 1.4 AIC selected fully parameterized fixed effects models excluding random effects for inter-spawning interval for male lake sturgeon.

Model	AIC	Delta AIC	Weight
CTU + Intra-sex Interactions - Inter-sex Interactions - Fork Length	2297.101	0.000	0.035
CTU + Migrations + Cumulative Discharge - Inter-sex Interactions - Fork Length	2297.145	0.044	0.034
CTU + Intra-sex Interactions + Migrations - Inter-sex Interactions - Fork Length	2297.642	0.541	0.027
CTU + Migrations - Inter-sex Interactions - Fork Length	2297.950	0.849	0.023
CTU + Intra-sex Interactions + Migrations + Cumulative Discharge - Inter-sex Interactions - Fork Length	2297.968	0.867	0.023
CTU + Intra-sex Interactions + Cumulative Discharge - Inter-sex Interactions - Fork Length	2298.105	1.004	0.021
CTU + Intra-sex Interactions + River Residence Time - Inter-sex Interactions - Fork Length	2298.539	1.438	0.017
CTU + Migrations + Cumulative Discharge - 5% Exceedance Flow - Inter-sex Interactions - Fork Length	2298.584	1.483	0.017
CTU + Migrations + Cumulative Discharge + Upstream Time - Inter-sex Interactions - Fork Length	2298.687	1.586	0.016
CTU + Intra-sex Interactions - 5% Exceedance Flow - Inter-sex Interactions - Fork Length	2298.725	1.624	0.016
CTU + Migrations + Cumulative Discharge + River Residence Time - Inter-sex Interactions - Fork Length	2298.829	1.728	0.015
CTU + Intra-sex Interactions + Upstream Time - Inter-sex Interactions - Fork Length	2298.898	1.797	0.014
CTU + 5% Exceedance Flow + Migrations - Inter-sex Interactions - Fork Length	2298.977	1.876	0.014
CTU + 5% Exceedance Flow + Intra-sex Interactions + Migrations - Inter-sex Interactions - Fork Length	2299.056	1.955	0.013

Table 1.5 Full and conditional ($\Delta AIC < 2$) fixed effect model parameter estimates and standard errors of environmental conditions, biological attributes, and migratory behavior on inter-spawning interval for male lake sturgeon. Parameter estimates and standard error are in log scale.

Full Model	Estimate	SE	z-value	Pr(> z)
Intercept	1.036	0.294	3.522	<0.001
Migrations	0.056	0.035	1.618	0.106
Upstream Time	-0.001	0.001	-1.001	0.317
River Residence Time	0.000	0.000	0.834	0.404
Cumulative Discharge	0.001	0.001	0.955	0.339
5% Exceedance Flow Days	-0.007	0.016	-0.425	0.671
Fork Length	-0.005	0.002	-2.267	0.023
Inter-sex Interactions	-0.007	0.002	-3.132	0.002
Intra-sex Interactions	0.001	0.001	0.937	0.349
CTU	0.001	0.001	1.318	0.188

Conditional Model ($\Delta AIC < 2$)	Estimate	SE	z-value	Pr(> z)
Intercept	1.032	0.294	3.507	<0.001
Migrations	0.057	0.033	1.699	0.089
Upstream Time	0.000	0.000	0.588	0.556
River Residence	0.000	0.000	0.684	0.494
Cumulative Discharge	0.001	0.000	1.304	0.192
5% Exceedance Flow Days	0.002	0.014	0.109	0.913
Fork Length	-0.005	0.002	2.196	0.028
Inter-sex Interactions	-0.007	0.002	2.786	0.005
Intra-sex Interactions	0.001	0.001	1.665	0.096
CTU	0.001	0.001	2.460	0.014

Table 1.6 AIC selected fully parameterized fixed effects models excluding random effects for inter-spawning interval for female lake sturgeon.

Model	AIC	Delta AIC	Weight
NULL	392.665	0.000	0.036
-Upstream Time	393.858	1.194	0.020
-Inter-sex Interactions	393.976	1.311	0.019
-Fork Length	394.099	1.434	0.017
Cumulative Discharge	394.099	1.434	0.017
CTU	394.144	1.479	0.017
5% Exceedance Flow Days	394.156	1.491	0.017
River Residence Time	394.479	1.814	0.014
-Migrations	394.495	1.830	0.014
-Intra-sex Interactions	394.495	1.830	0.014

Table 1.7 Full and conditional ($\Delta AIC < 2$) fixed effect model parameter estimates and standard errors of environmental conditions, biological attributes, and migratory behavior on inter-spawning interval for female lake sturgeon. Parameter estimates and standard error are in log scale.

Full Model	Estimate	SE	z-value	Pr(> z)
Intercept	1.698	0.766	2.217	0.027
Migrations	0.025	0.106	0.237	0.813
Upstream Time	-0.002	0.002	-1.013	0.311
River Residence Time	0.000	0.001	0.220	0.826
Cumulative Discharge	0.001	0.001	0.535	0.592
5% Exceedance Flow Days	-0.007	0.043	-0.152	0.879
Fork Length	-0.003	0.005	-0.606	0.545
Intra-sex Interactions	0.002	0.006	0.369	0.712
Inter-sex Interactions	-0.002	0.002	-0.797	0.425
CTU	0.000	0.002	0.215	0.830

Conditional Model ($\Delta AIC < 2$)	Estimate	SE	z value	Pr(> z)
Intercept	1.226	0.299	4.078	0.000
Migrations	-0.046	0.095	0.475	0.635
Upstream Time	-0.001	0.001	0.909	0.363
River Residence Time	0.000	0.000	0.507	0.612
Cumulative Discharge	0.001	0.001	0.795	0.427
5% Exceedance Flow Days	0.018	0.023	0.755	0.450
Fork Length	-0.004	0.005	0.789	0.430
Intra-sex Interactions	-0.001	0.003	0.481	0.630
Inter-sex Interactions	-0.001	0.001	0.862	0.388
CTU	0.001	0.001	0.770	0.441

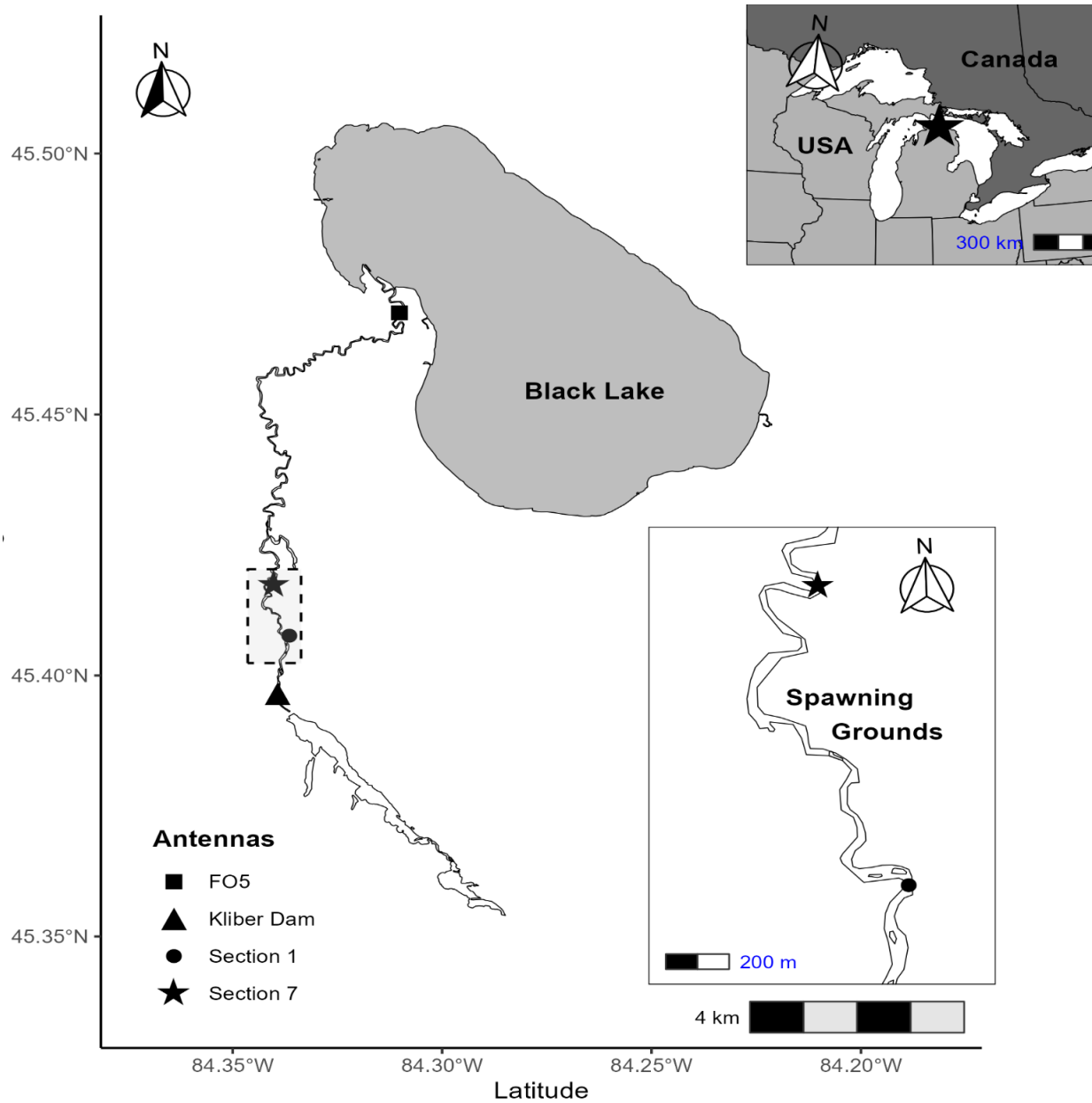


Figure 1.1 Map of the Upper Black River and Black Lake in Cheboygan County, MI, USA. Dashed box indicates the historical known spawning area. Shapes indicate an RFID antenna location including the mouth of the river (■), start of the known spawning area (★), end of the known spawning area (●), and Kliber Dam (▲), the upstream most point available for sturgeon in the upper Black River.

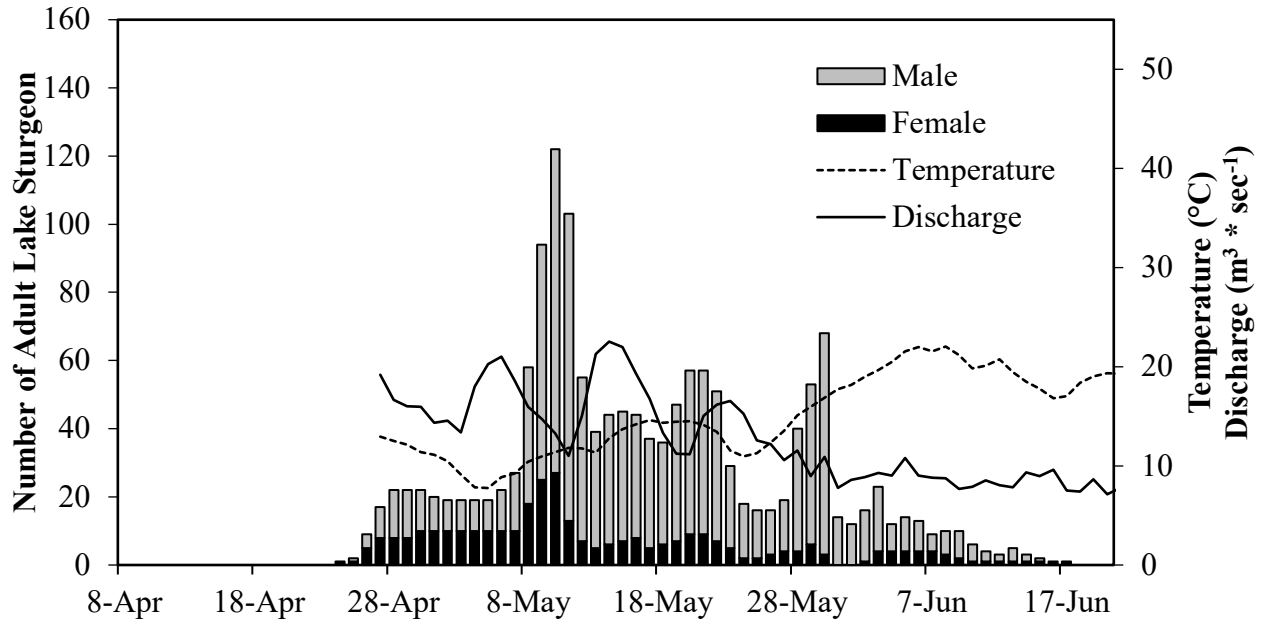


Figure 1.2 Adult lake sturgeon spawning grounds occupancy by day during the 2016 lake sturgeon spawning season. Dashed line represents daily average temperature (°C), solid line represents daily average discharge (m³*sec⁻¹) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

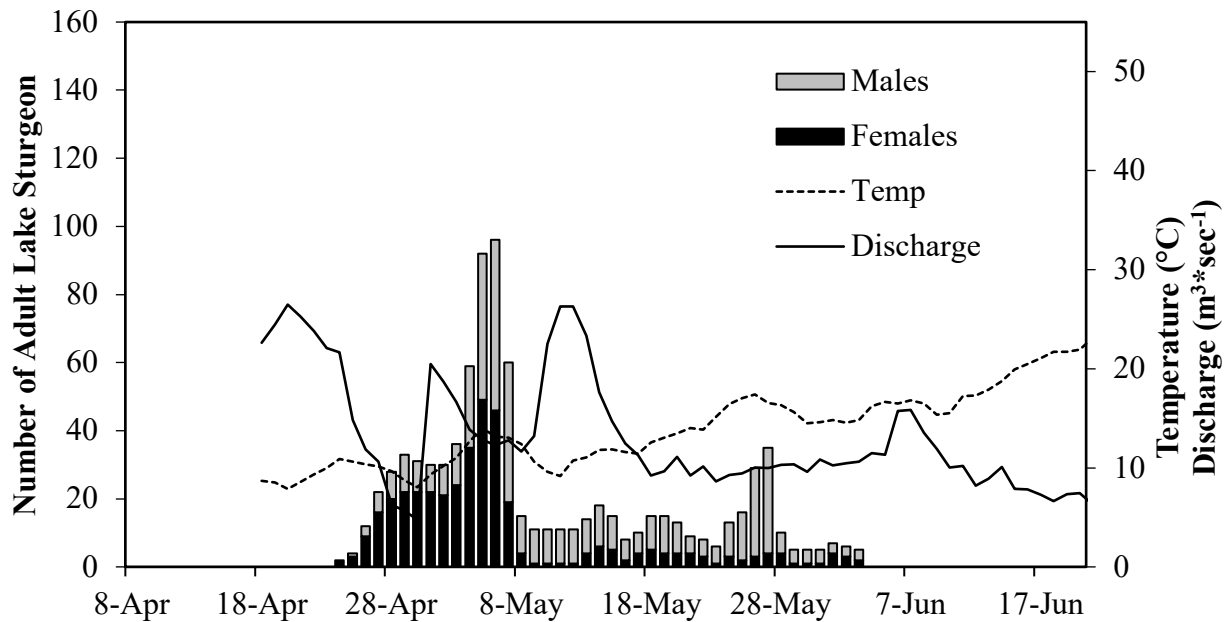


Figure 1.3 Adult lake sturgeon spawning grounds occupancy by day during the 2017 lake sturgeon spawning season. Dashed line represents daily average temperature (°C), solid line represents daily average discharge (m³*sec⁻¹) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

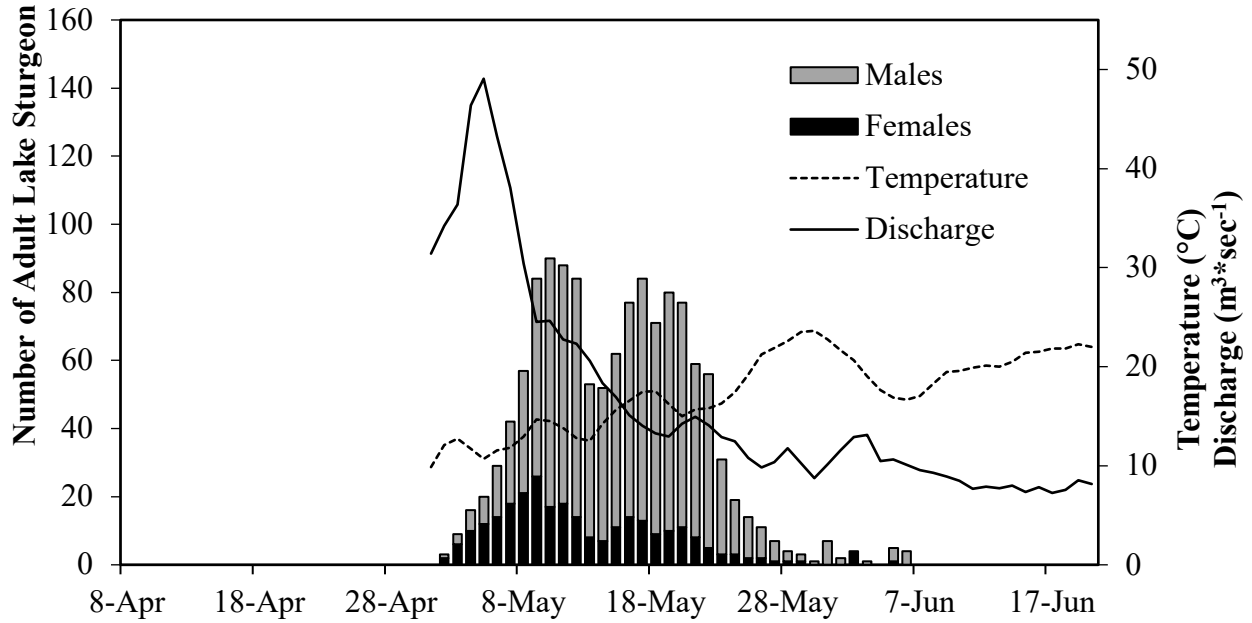


Figure 1.4 Adult lake sturgeon spawning grounds occupancy by day during the 2018 lake sturgeon spawning season. Dashed line represents daily average temperature ($^{\circ}\text{C}$), solid line represents daily average discharge ($\text{m}^3\cdot\text{sec}^{-1}$) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

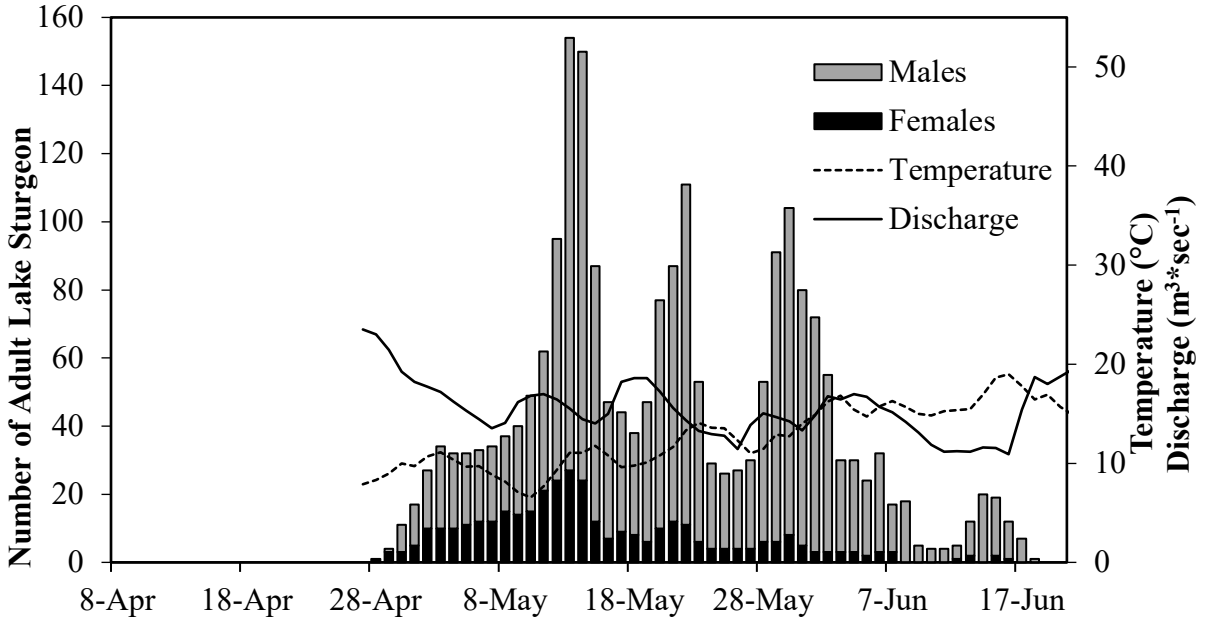


Figure 1.5 Adult lake sturgeon spawning grounds occupancy by day during the 2019 lake sturgeon spawning season. Dashed line represents daily average temperature ($^{\circ}\text{C}$), solid line represents daily average discharge ($\text{m}^3\cdot\text{sec}^{-1}$) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

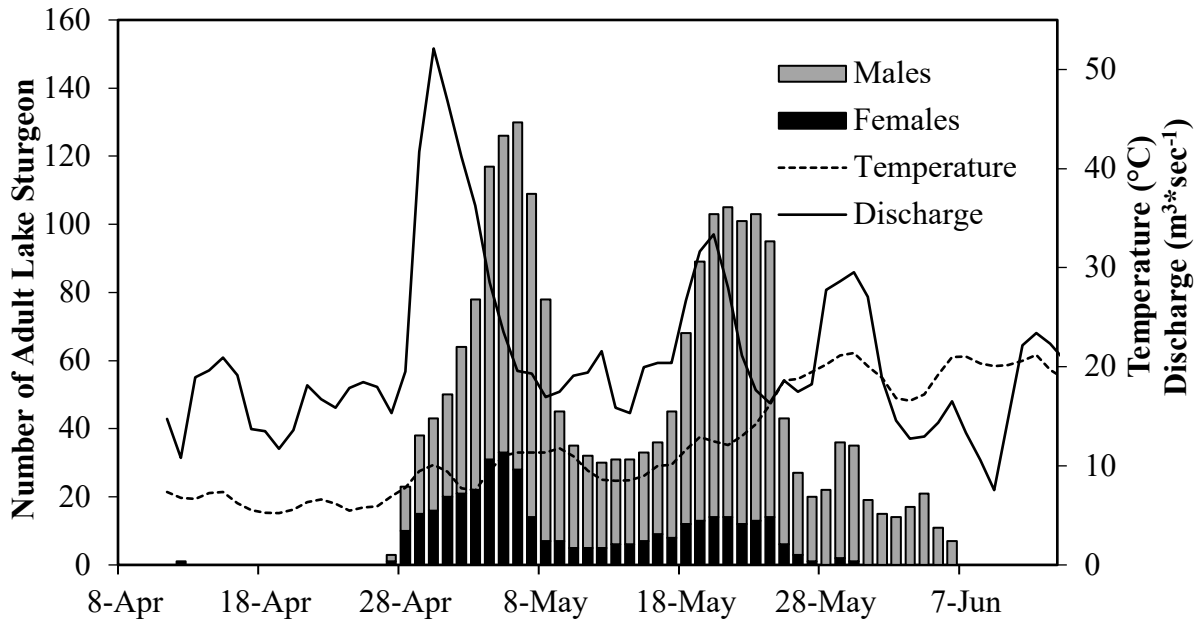


Figure 1.6 Adult lake sturgeon spawning grounds occupancy by day during the 2020 lake sturgeon spawning season. Dashed line represents daily average temperature ($^{\circ}\text{C}$), solid line represents daily average discharge ($\text{m}^3 \cdot \text{sec}^{-1}$) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

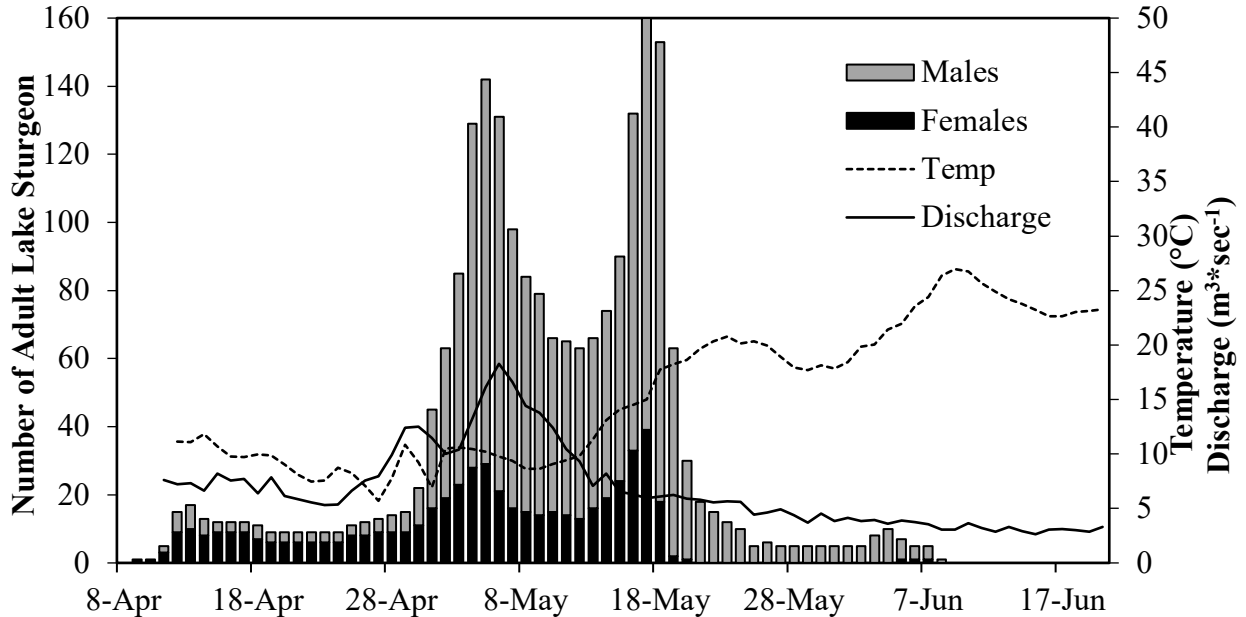


Figure 1.7 Adult lake sturgeon spawning grounds occupancy by day during the 2021 lake sturgeon spawning season. Dashed line represents daily average temperature ($^{\circ}\text{C}$), solid line represents daily average discharge ($\text{m}^3 \cdot \text{sec}^{-1}$) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

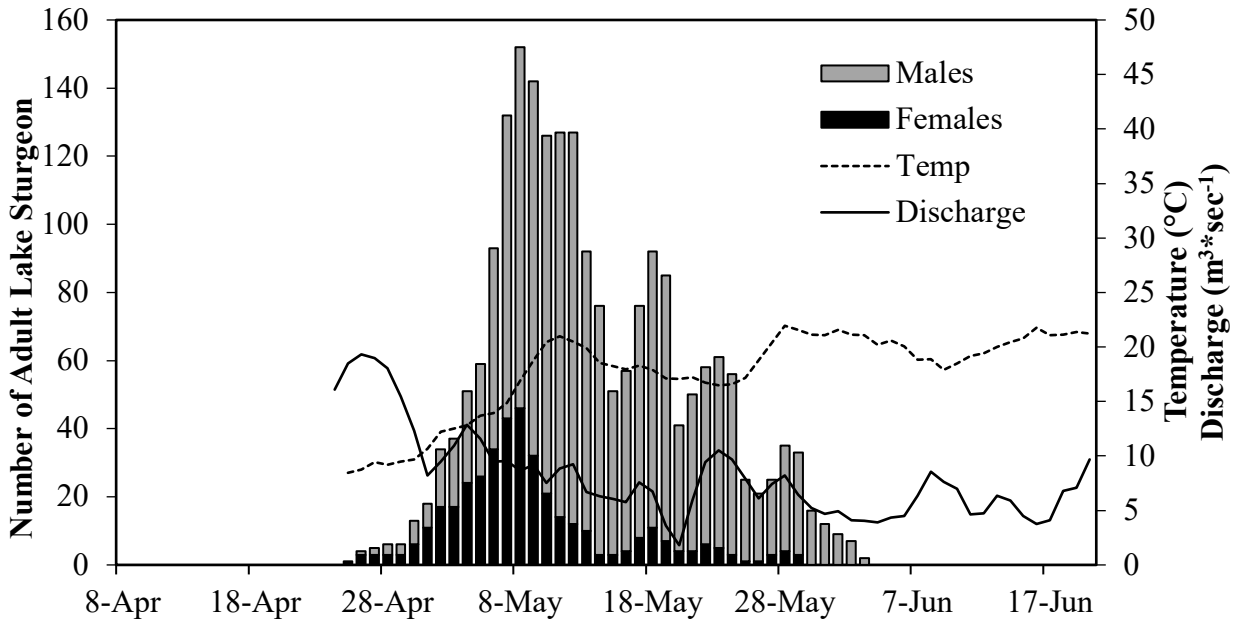


Figure 1.8 Adult lake sturgeon spawning grounds occupancy by day during the 2022 lake sturgeon spawning season. Dashed line represents daily average temperature ($^{\circ}\text{C}$), solid line represents daily average discharge ($\text{m}^3\cdot\text{sec}^{-1}$) measured by a HOBO pressure and temperature logger (Onset Computer Corp, Cape Cod, Massachusetts, USA).

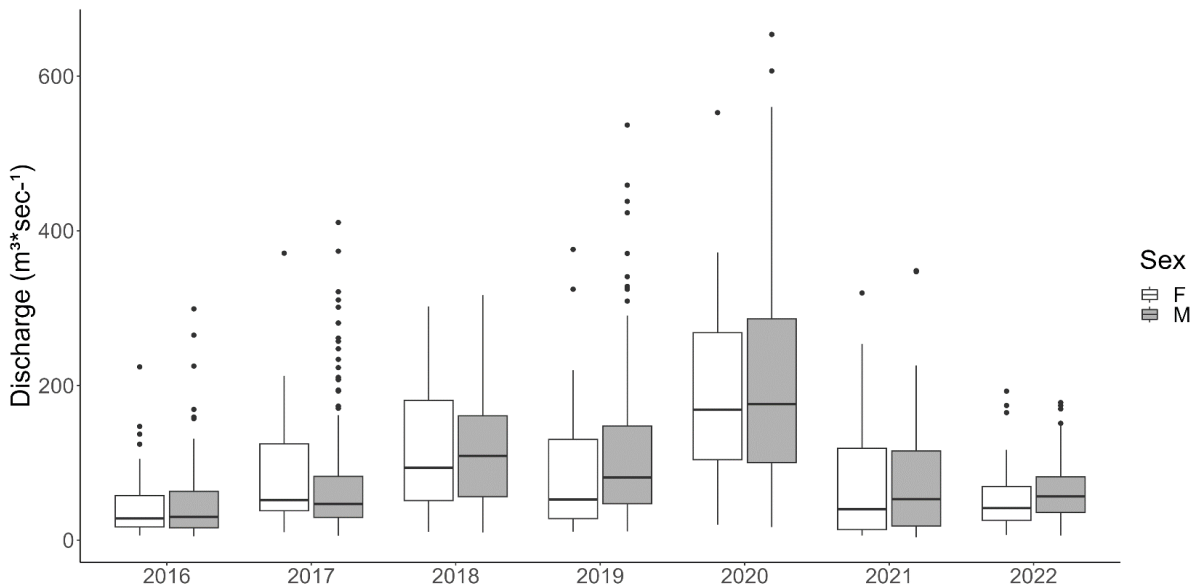


Figure 1.9 Boxplot of the distribution of cumulative discharge experienced in the river by male and female lake sturgeon during the spawning period from 2016 – 2022.

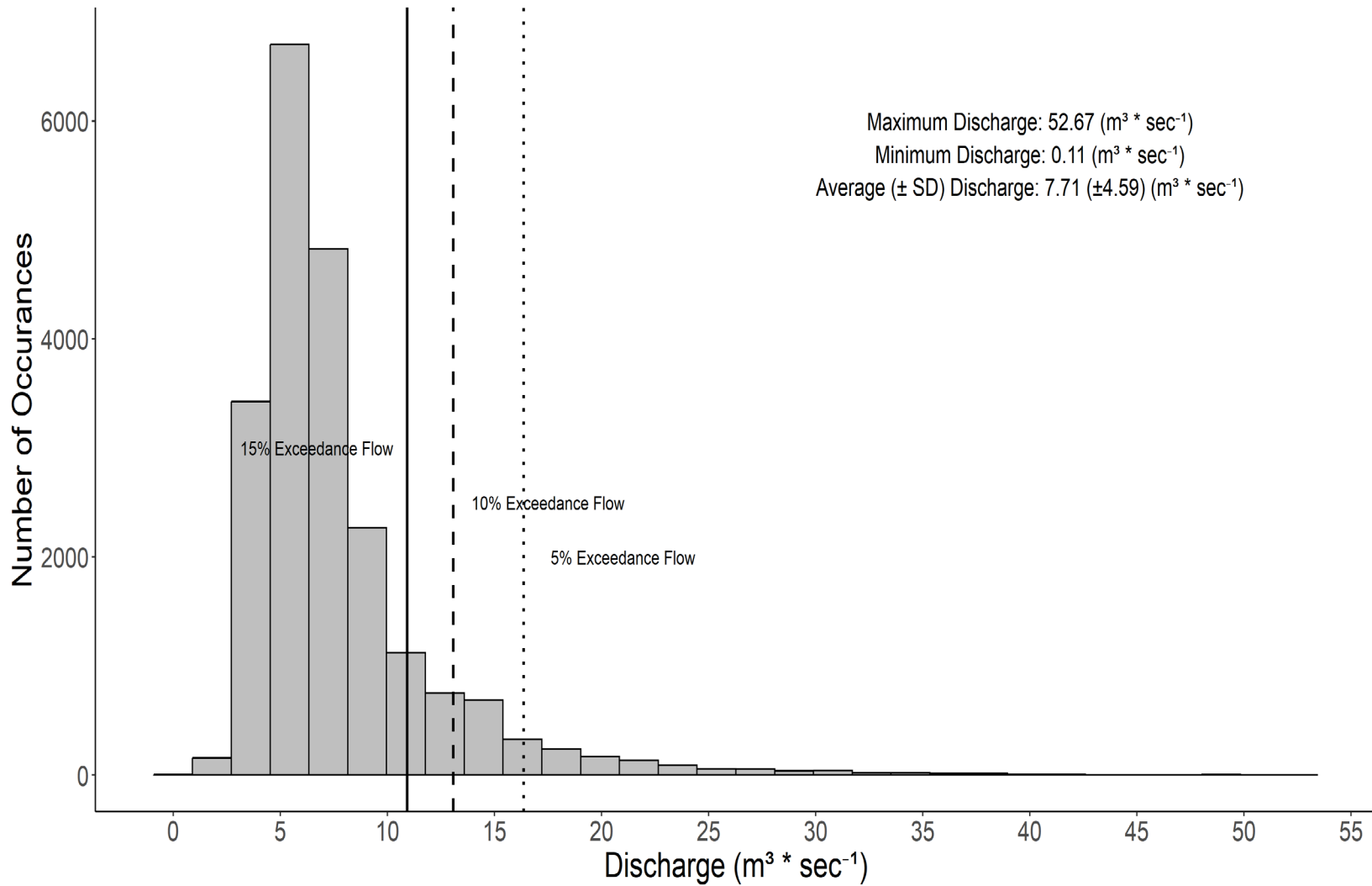


Figure 1.10 Distribution of all USGS measured discharge values from October 1942 to September 2000. Values used to calculate the number of days in the river in which an individual experienced discharge which exceeded the upper 5% of all

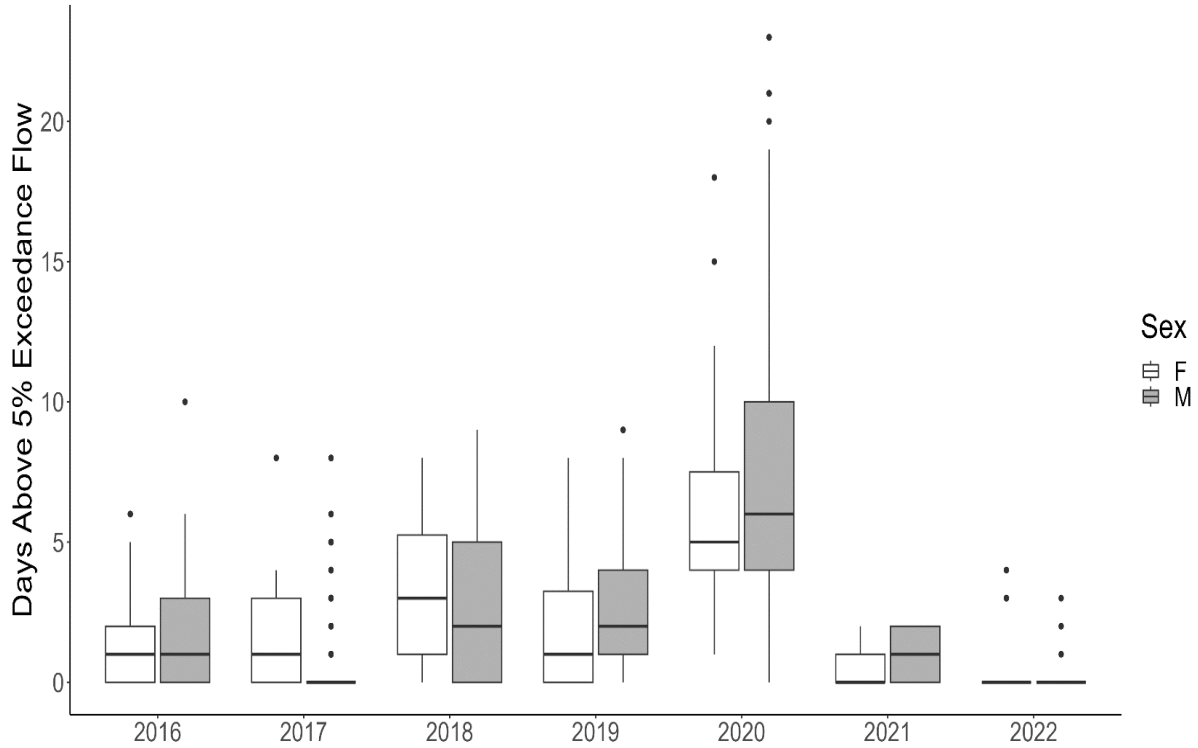


Figure 1.11 Boxplot of the distribution of the number of days spent in the river when discharge was in the upper 5% of all historical recorded discharges by male and female lake sturgeon during the spawning period from 2016 – 2022.

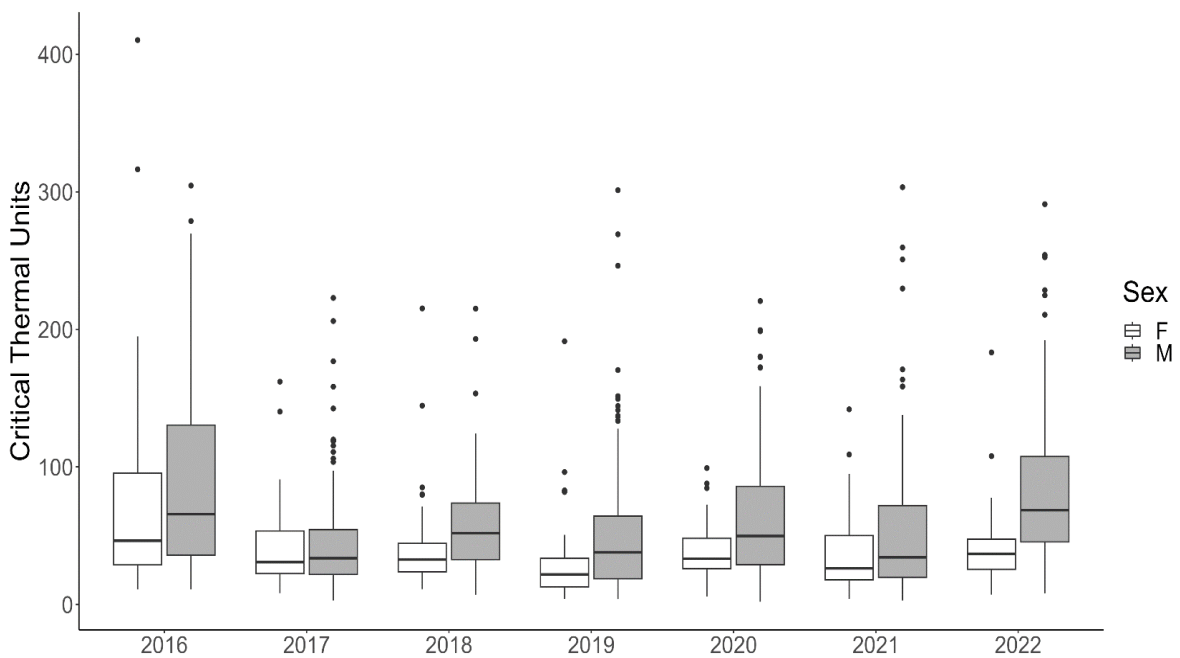


Figure 1.12 Boxplot of the distribution of the critical thermal units experienced in the river by male and female lake sturgeon during the spawning period from 2016 – 2022.

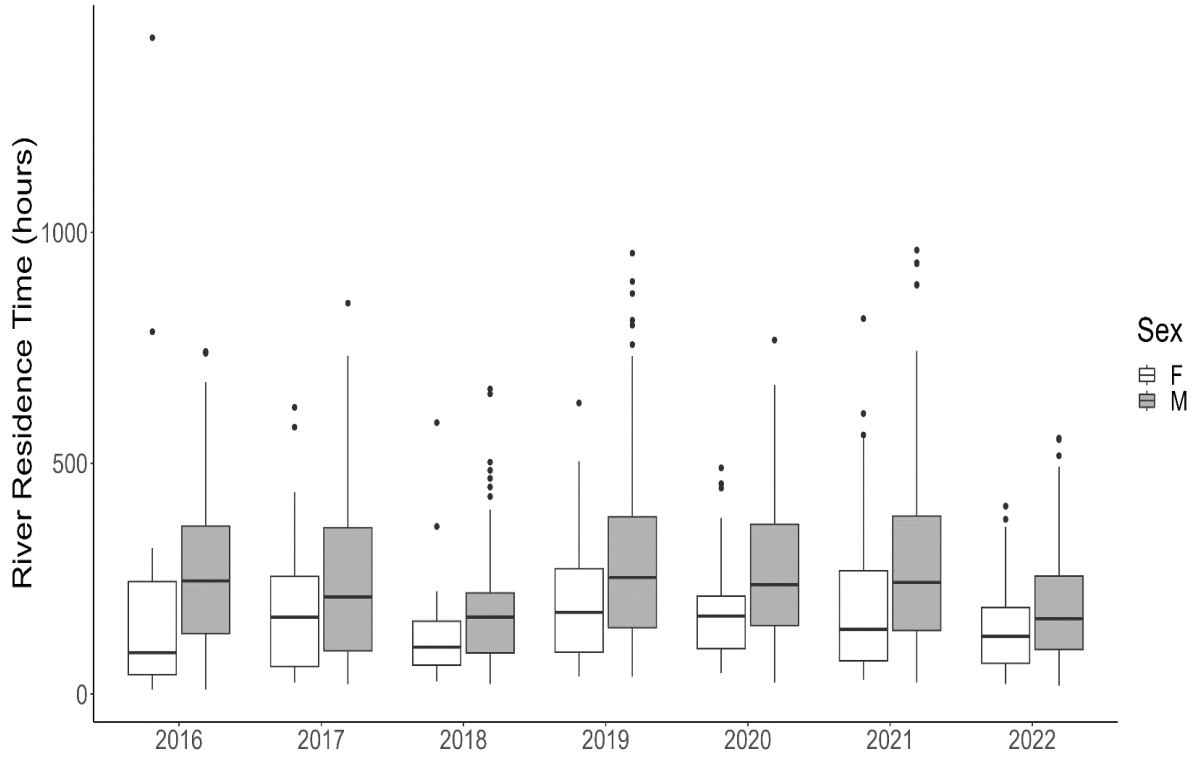


Figure 1.13 Boxplot of the distribution of time spent in the river by male and female lake sturgeon during the spawning period from 2016 – 2022.

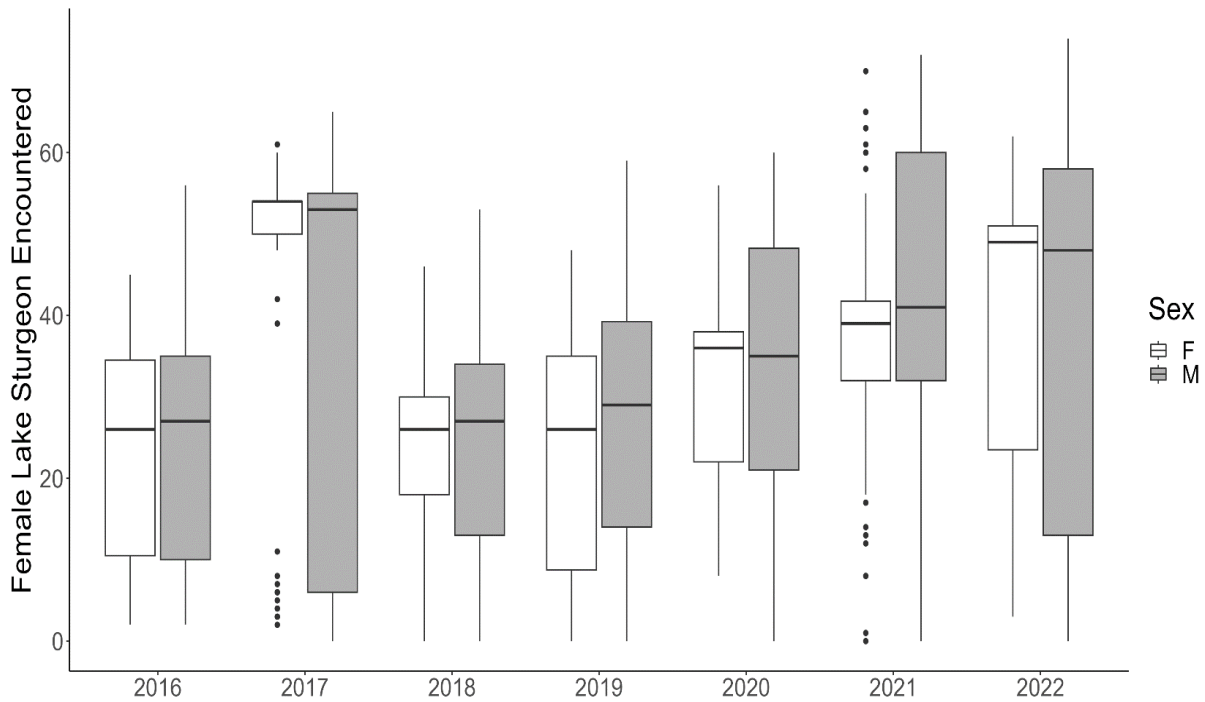


Figure 1.14 Boxplot of the distribution of the number of female lake sturgeon encountered in the river by male and female lake sturgeon during the spawning period from 2016 – 2022.

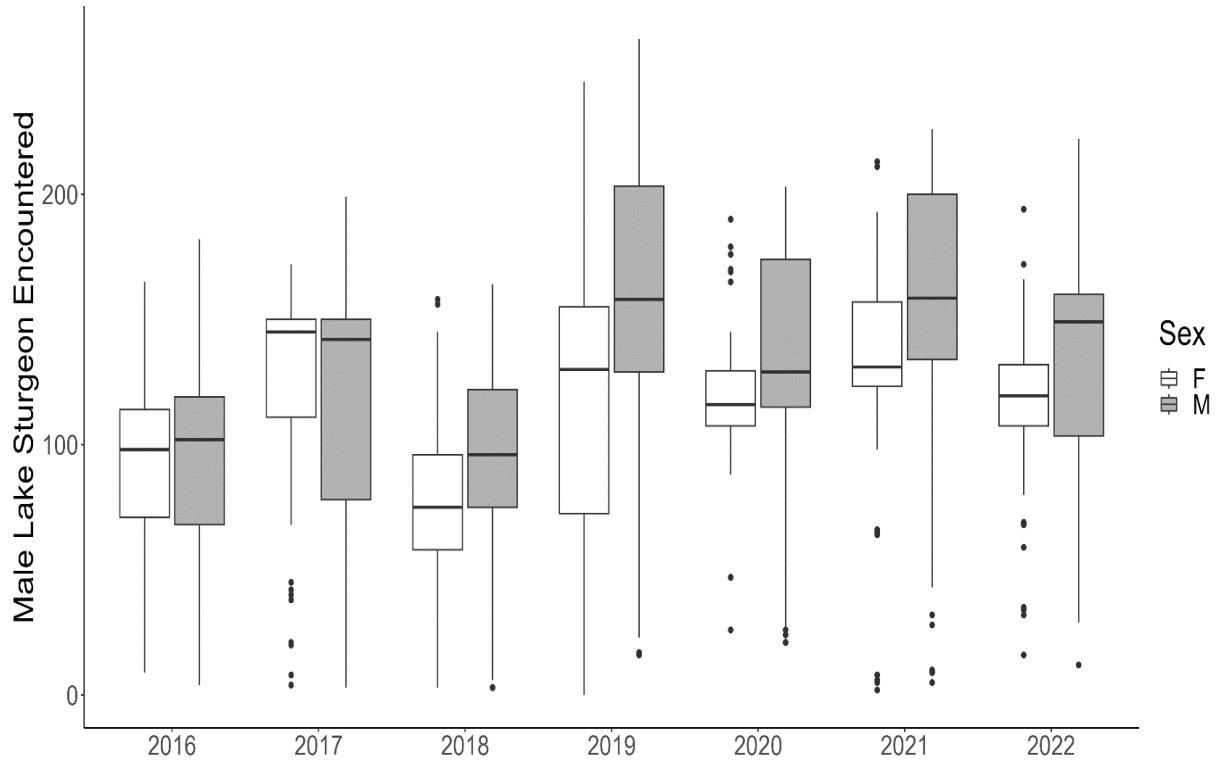


Figure 1.15 Boxplot of the distribution of the number of male lake sturgeon encountered in the river by male and female lake sturgeon during the spawning period from 2016 – 2022.

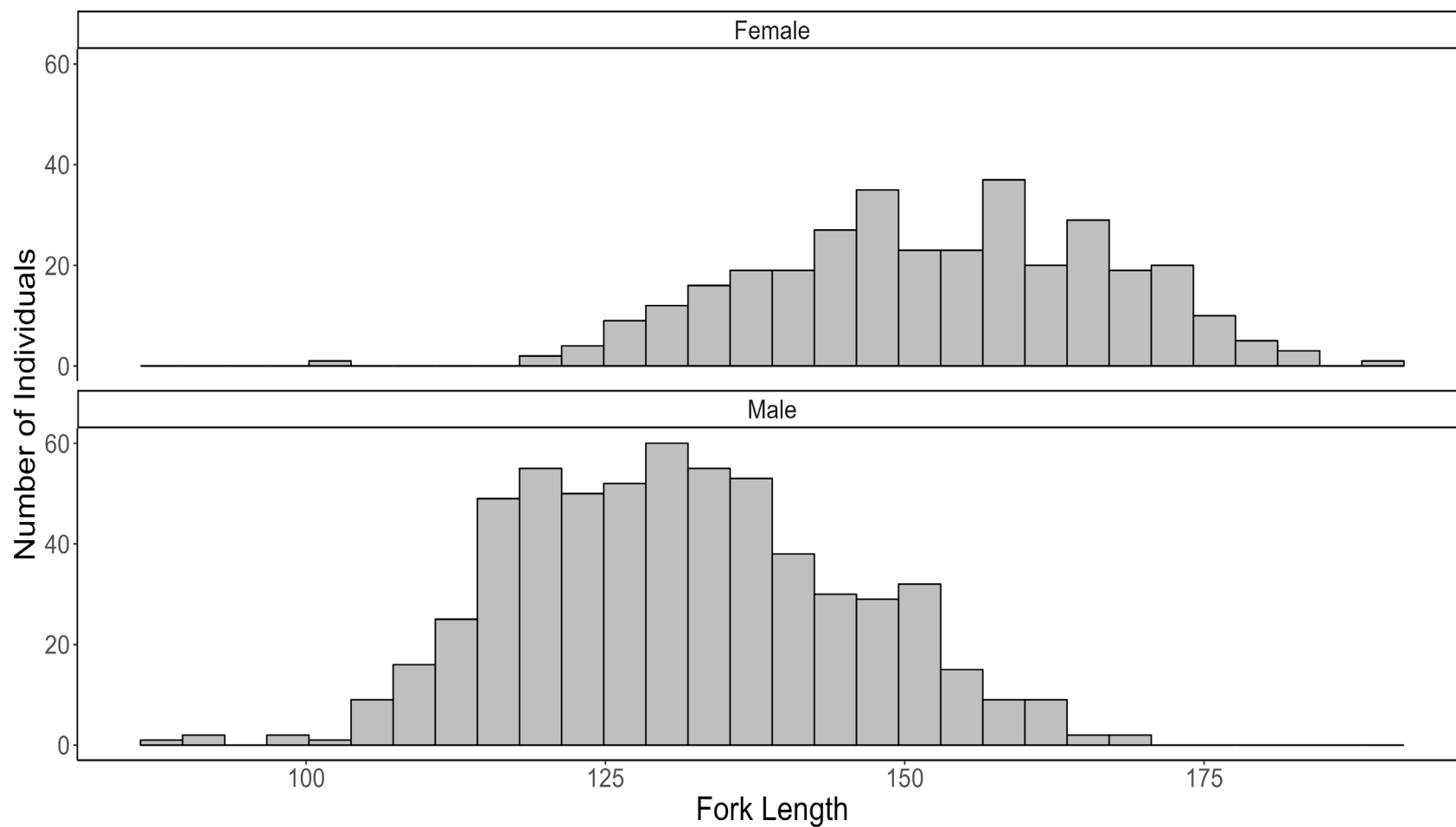
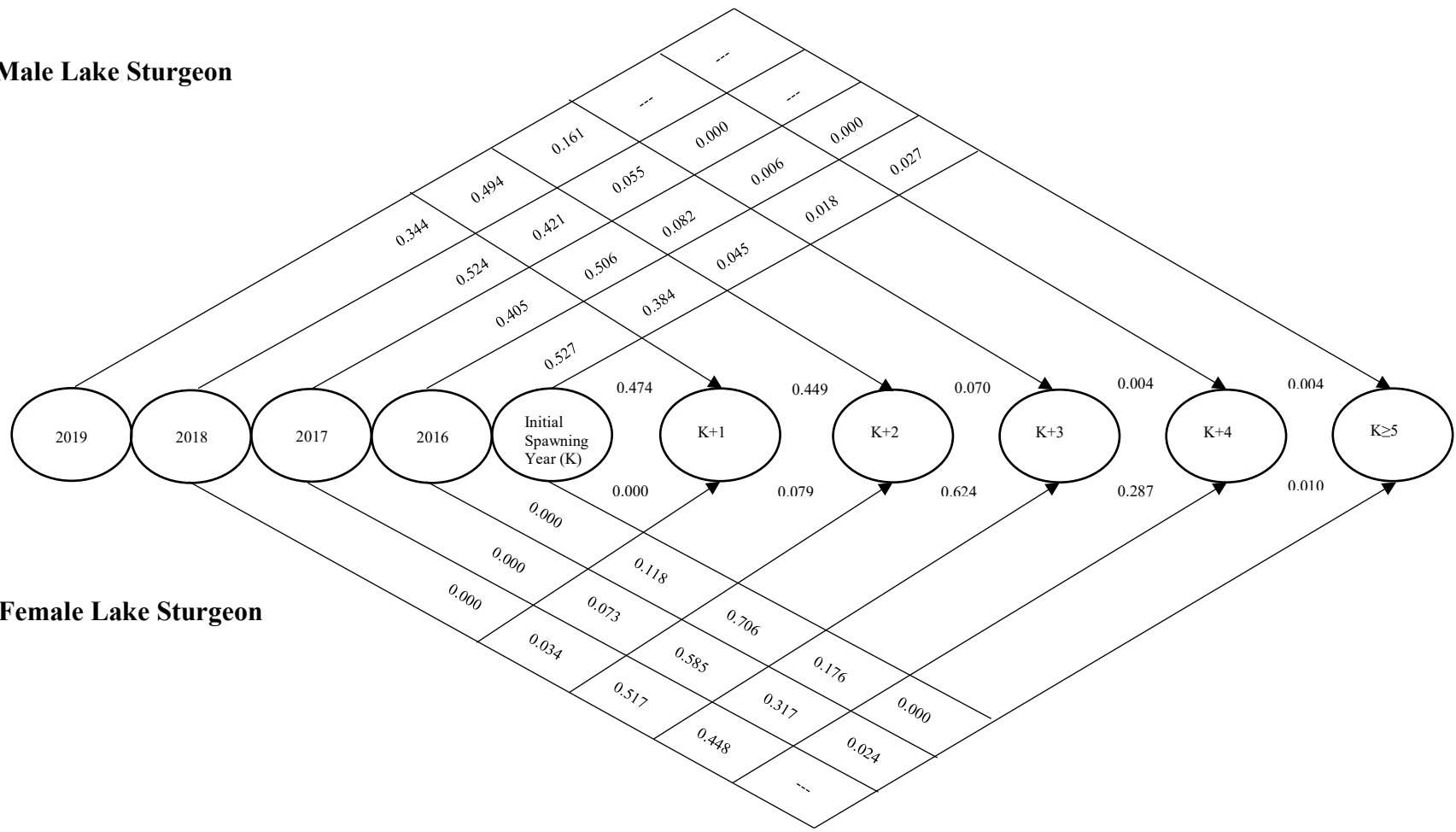


Figure 1.16 Distribution of all fork lengths (cm) of male and female lake sturgeon physically captured in the Upper Black River from 2016 – 2022.

Male Lake Sturgeon



Female Lake Sturgeon

Figure 1.17 Probably of return to the river to spawn after observed spawning in year K for male and female lake sturgeon in the upper Black River, Michigan based on RFID data collected from 2001-2022. Numbers on each axis represent the return year proportion based on each initial year of spawning for males and females. Numbers listed horizontally represent the mean (±SD) proportional return across the duration of the study period for males and females.

CHAPTER 2:
Experimental evaluations of ovarian fluid ability to mediate Lake Sturgeon (*Acipenser fulvescens*) cryptic female choice via reduced sperm motility and fertility success: implications for probabilities of consanguineous matings

1. ABSTRACT

Gamete fertilization success in broadcast-spawning fish is largely mediated by properties of sperm, including sperm quantity (concentration and volume), and measures of quality (e.g., velocity and motility duration). In some species, reduction of sperm motility by ovarian fluid may contribute to cryptic female choice for or against males of specific phenotypes or genetically related males. To evaluate the role of ovarian fluid to alter male fertilization success in lake sturgeon (*Acipenser fulvescens*) we conducted experimental fertilizations with males differing in levels of relatedness to focal females when ovarian fluid was present and absent. We further evaluated sperm quality traits of related and unrelated males when ovarian fluid is present or removed using computer assisted sperm analysis (CASA). Generalized linear models using a beta distribution were used to evaluate proportional egg fertilization and survival. Eggs fertilized by unrelated males had a 17% higher proportional embryonic survival than those fertilized by related males 72-hours following fertilization. There was no significant interaction between ovarian fluid and relatedness, suggesting that high egg survival in unrelated males was not related to ovarian fluid. CASA demonstrated that the presence of lake sturgeon ovarian fluid during sperm activation results in a reduction of sperm quality (velocity and motility) unaffected by the relatedness of a male to a female. Results contribute to greater understanding of lake sturgeon reproductive biology in complex riverine environments.

2. INTRODUCTION

Understanding how long-lived species limit inbreeding is vital for critical management efforts (Hedrick and Kalinowski 2000; Brook et al. 2002). In many species, ejaculate from multiple males is present when eggs are released, limiting pre-zygotic mate selection (Eberhard 1996). Cryptic female choice offers a mechanism by which female of a species bias the outcome of sperm competition toward sperm from a selected mate (Eberhard 1996, Birkhead 1998). Selection occurs through mediation of sperm velocity based on favored breeding strategy (Lehnert et al. (2017), coloration (Pilastro et al. 2004), or genetic compatibility (Liljedal et al. 2008, Gasparini and Pilastro 2011), potentially limiting inbreeding in small, reproductively isolated populations.

Following egg release, sperm characteristics are thought to affect male reproductive success. Sperm traits such as velocity, motility, morphology, and longevity are associated with increased successful fertilization during sperm competition in many species with external fertilization (Gage et al., 2004, Beausoleil et al. 2012, Simmons and Fitzpatrick 2012, Evans et al. 2013). However, ovarian fluid, which is concurrently released with eggs and covers the outer egg surface, can increase, or decrease sperm velocity. In ocellated wrasse (*Symphodus ocellatus*), sperm concentration was less important to fertilization success than increased sperm velocity when in the presence of ovarian fluid (Alonzo et al. 2016). For selected males, sperm velocity, motility, and linear movement all increased in vitro in the presence of ovarian fluid. While ovarian fluid is offered as a mechanism by which compatible males are selected and has been demonstrated to alter sperm velocity resulting in female selection in teleost species, previous research has demonstrated the importance of ovarian fluid to sturgeon reproduction (Kholodnyy et al. 2021), as a sperm preservative, but not as a mechanism of cryptic female

choice. Additionally, no research has evaluated if ovarian fluid is a medium by which sturgeon limit consanguineous matings.

Selection against inbreeding can occur either pre-zygotically through spatial or temporal variation in male and female location or sexual readiness or via mate selection, including through mediation of sperm performance immediately prior to fertilization. Post-zygotic reproductive isolating mechanisms includes genetic incompatibilities and elevate embryo death or reduced offspring reproductive competence. Cryptic female choice is an example of pre-reproductive isolation and occurs when a female exerts control over the probability of fertilization by certain sperm either before or after intromission (Eberhard 1996). In broadcast spawning fishes, female choice can occur prior to egg release where a female extrudes eggs in response to physical stimulation by several males (Bruch and Binkowski 2002). Females respond positively to male-biased operational sex ratio to assure higher rates of egg fertilization (Dammerman et al. 2019). Conversely, in some externally fertilizing teleost species females rely on ovarian fluid, which coats the egg surface and is the mechanism by which females cryptically select for a mate following egg release (Alonzo et al. 2016).

Prior research has demonstrated that females can cryptically select for males and have suggested ovarian fluid as the medium of cryptic female selection in chinook salmon (*Oncorhynchus tshawytscha*) (Rosengrave et al. (2008). Rosengrave et al. (2016) further demonstrated that when two competing males attempted to fertilize a female chinook salmon, ovarian fluid mediated sperm swimming speed, allowing the faster sperm to fertilize proportionally more eggs. Lehnert et al. (2017) also demonstrated that chinook salmon ovarian fluid increases sperm velocity of unrelated males and altered the outcome of sperm competition between different male fertilization tactics, equalizing fertilization rates between jacks (sneaker

males) and hooknoses (large guarding males). In addition to mediating cryptic female selection, ovarian fluid differentially affected male fertilizations of eggs as a function of relatedness between the male and female. In guppy, (*Poecilia reticulata*) ovarian fluid affected sperm competition by slowing sperm movements from a closely related male, increasing the probability of fertilization by an unrelated male. Both sperm velocity and fertilization rates were higher for the unrelated male guppy (Gasparini and Pilastro 2011). Butts et al. (2012) further demonstrated differences in lake trout (*Salvelinus namaycush*) sperm velocity on the basis of male:female relatedness resulting from the presence of ovarian fluid in a computer assisted sperm analysis system. Little is currently known about the mechanism by which ovarian fluid selects sperm based on relatedness. Skarstein et al. (2005) offers that membrane-bound proteins found on the surface of sperm and eggs are rapidly evaluating to promote gamete recognition and encourage fertilization. Butts et al. (2012) offers that this may result in differential fertilization by related males. Gasparini and Pilastro (2011) agree that proteins on the surface of the sperm and egg may be important, offering that signaling peptides may have chemokinetic effects of sperm, operating prior to fertilization when found both on the surface of the sperm membrane and in ovarian fluid.

Lake sturgeon exhibit both polyandry and polygyny, as well as promiscuity (Bruch and Binkowski 2002, Dammerman et al. 2019). Spawning typically occurs in short bouts initiated by females over an 8–12-hour period and spawning timing and location is repeatable across spawning seasons (Forsythe et al. 2012a). Females release many eggs in the presence of multiple males during a single reproductive event, and fertilization by more than one male is possible (Dammerman et al. 2019), which can result in polyspermy (Marble et al. 2011). Although there are similarities between characteristics of the teleost egg chorion, unlike teleost fishes, which

have a single micropyle for sperm to enter the egg (Hart 1990), sturgeon have several micropyles (Ciereszko et al. 1996, Siddique et al. 2014), and a complex double-tapered entry canal (Cherr and Clark 1985). Micropyles likely aid in the fertilization process and may reduce polyspermy, however, nothing in the egg structure is known to limit consanguineous matings. Other female-provisioned compounds extruded with eggs into the aquatic environment may have important functions associated with fertilization, particularly for broadcast spawning species like lake sturgeon.

This study was designed to determine whether ovarian fluid was a determinate of cryptic choice for female lake sturgeon, by affecting fertilization success of males, and to quantify whether the mechanism for differential fertilization success could be explained by alteration of traits characterizing sperm quality with and without the presence of ovarian fluid. The objective of experiment one (*Sperm Volume Experiment*) was to determine if the volumes and concentrations of sperm previously described (Bruch and Binkowski 2002, Dammerman et al. 2016, Tucker et al. 2020) would differentially fertilize lake sturgeon eggs in vitro and to avoid sperm swamping in fertilization success trials (Rosengrave et al. 2016). In experiment two (*Fertilization experiment*), the objective was to determine if proportional fertilization and survival was altered by the degree to which a male is related to a female, with and without ovarian fluid present on the egg surface. In experiment three (*Sperm Motility Experiment*) the objective was to determine the degree to which ovarian fluid altered the motility of sperm when present, particularly for males related to the female.

In experiment one, we hypothesized that proportional egg fertilization (24-hours) and proportional survival of eggs (48-, 72-hours) would not differ by volume of sperm used to fertilize eggs. In experiment two, we hypothesized that proportional egg fertilization (24-hours)

and proportional survival (48-, 72-hours) would vary based on hours evaluated post-fertilization, by rinsed/unrinsed ovarian fluid, and by the unrelated/related treatment. Specifically, we hypothesized that the two-way interaction between ovarian fluid and relatedness would be significant indicating that eggs fertilized by a half-sibling male had lower proportional fertilization or survival when ovarian fluid was not rinsed from the egg surface. Finally, for experiment three, we hypothesized that sperm quality and velocity would vary based on ovarian fluid presence/absence, and by the relatedness of the male and female paired. Specifically, we hypothesized a significant two-way interaction between relatedness and ovarian fluid presence would result in significantly lower sperm quality and velocity from sperm from half-sibling males when ovarian fluid was present.

Understanding the effect of ovarian fluid during fertilization provides insight on the effects of female choice on sperm behavior and will inform future supplementation and conservation efforts for lake sturgeon.

3. METHODS

3.1 Study site

Experiments were conducted during the spring (April-June) 2021 and 2022 lake sturgeon spawning period in the upper Black River (UBR), a fourth-order tributary of Black Lake, located in Cheboygan County, MI (Fig. 1.1). The Black Lake lake sturgeon population is isolated by Kleber Dam upstream of Black Lake and Alverno Dam, downstream of Black Lake, restricting the upstream spawning migration to 11 km of free-flowing river from Kleber Dam downstream to Black Lake (Baker and Borgeson 1999). Spawning habitat is limited to the upstream four kilometers of river below Kleber Dam.

The Black Lake population had an estimated 1,183 adult lake sturgeon in 2022, of which 647 were male, and 536 were female during the sampling period (Pledger et al. 2013, Michigan State University (MSU) and Michigan Department of Natural Resources (MDNR), unpublished data). The timing of spawning by individual males and females in the UBR is repeatable across years (Forsythe et al. 2012b), although males exhibit considerable plasticity in migratory behavior and residence time in spawning areas (Larson et al. 2020). The duration of the spawning season on the UBR during 2021 and 2022 was 60 days (9 April 2021 – 8 June 2021) and 39 days (25 April 2022 – 3 June 2022), respectively. Adults engage in polygynous and polyandrous matings in spawning groups that vary in size and of different duration.

3.2 Sperm volume experiment

Eggs from three lake sturgeon were collected during the 2021 lake sturgeon spawning period in the Upper Black River consistent with methods described in Crossman et al. (2011). Eggs were stored in a 500mL Whirl-Pac in river water at ambient temperature. Sperm from a single male who was estimated to be unrelated to each female was collected on the same day as eggs, using methods described in Crossman et al. (2011). The collected sperm was stored on ice in a cooler and returned to the hatchery.

Experimental sperm volumes were selected based on sperm concentrations described in Bruch and Binkowski (2002), Dammerman et al. (2016), and Tucker et al. (2020). Bruch and Binkowski (2002) estimated that the ratio of eggs to sperm from a natural fertilization pairing is 1,000-1,400 eggs to 100 billion sperm (1×10^{11}) per reproductive encounter (average 50 mL sperm released). Sperm concentration in the Upper Black River varied between 2.0 billion (2.00×10^9) and 3.82 billion (3.82×10^9) sperm per mL of milt in 2017 and 2018, respectively (Larson et al. 2020), thus 1 mL (~ 52 eggs per mL, Michigan State University, unpublished data)

of eggs may mimic optimal natural fertilization conditions as estimated when fertilized with 74.0 million (7.4×10^7) sperm, or between approximately 19.4 μL and 37.0 μL of milt. Dammerman et al. (2016) reported fertilizing 1,600 eggs with 0.5 mL of milt, or 16.3 μL of milt per 1 mL of eggs (between 32.6 million and 62.3 million sperm). Tucker et al. (2020) described fertilizing 1mL of eggs with 2 billion (2.0×10^9), 4 billion (4.0×10^9) and 6 billion (6.0×10^9) total sperm across three experimental volumes. Based on published estimates of sperm concentration from the UBR, between 500 μL and 3,000 μL of milt were used to fertilize eggs across treatments. To determine the best ratio of sperm to egg volumes based on fertilization rate, we fertilized 1mL eggs with sperm from a single unrelated male at five different milt volumes: 20 μL , 100 μL , 500 μL , 750 μL , and 1000 μL , which represents the range of milt volume and total sperm concentration between Dammerman et al (2016) and Tucker et al. (2020). We conducted this experiment using three females, each with a separate unrelated male. Crosses with unrelated males were conducted in six replicates per female. Egg plus ovarian fluid volumes were measured in a graduated cylinder for each replicate and treatment for consistency. Prior to fertilization, ovarian fluid was rinsed from eggs to provide an estimate of fertilization success not influenced by ovarian fluid. Rinsed/unrinsed treatments were selected randomly. Eggs from which ovarian fluid was rinsed were gently rinsed with river water from a hatchery water line under minimal pressure. Water passed over the surface of each egg sample until eggs were completely submerged three times (~15 seconds) so as not to not completely hydrate the follicular epithelium preventing fertilization through the release of sialic acid (Siddique et al. 2014). Fertilization methods were as described in Crossman et al. (2011). Fertilized eggs from each replicate were adhered to a 4-inch (10.16 cm) diameter coupling made of PVC plastic and mesh

then randomly assigned to trays in the heath tray stack as described by Wassink et al. (2019). All fertilizations occurred within six hours of collection.

Eggs were assessed using a Nikon SMZ-800 dissecting microscope on 6x magnification at 24-, 48-, and 72-hours post fertilization for visual confirmation of cellular division to determine successful fertilization as described by Dettlaff et al. (1993). The number of successfully fertilized eggs (cellular division present) and failures (no cellular division) were recorded for each treatment and replicate. Eggs were only removed from a replicate coupling if signs of *Saprolognia sp.* infection were observed and removed eggs were counted as failures in the successive time replicates.

3.2.1 Sperm volume statistical analysis

To evaluate the effects of sperm concentration on egg fertilization success, we used a generalized linear mixed model (GLMM) treating female ID as a random effect (Dammerman et al. 2015). We considered the additive and interactive fixed effects of sperm volume (20 μ L, 100 μ L, 500 μ L, 750 μ L, and 1000 μ L) and time post fertilization (24, 48, and 72-hrs post fertilization) using the beta distribution where the proportion of eggs successfully fertilized was the number of successfully fertilized eggs at the evaluated time period divided by the total number of eggs in each replicate. All statistical analyses were performed in R (4.2.2) (www.r-project.org), using the `glmmTMB` function (Brooks et al. 2017).

Results from this experiment were used to determine the volume of milt used in experiments to evaluate the role of ovarian fluid in fertilization success in subsequent experiments. Should no differences be found across milt volume treatments, milt volume of 20 μ L would be used, consistent with Bruch and Binkowski (2002), and Dammerman et al. (2016).

3.3 Fertilization experiment

We tested whether removing ovarian fluid altered proportional fertilization rates of eggs by sperm from either related or unrelated males. Michigan State University Black River Streamside Rearing Facility (MSU-BRSRF) maintains a genetic database of adult lake sturgeon captured during annual surveys from 2001-2022 in which >95% (n = 1,398) of the spawning population has been genotyped. The entire Black River adult population was genotyped at 13 disomic microsatellite loci (Larson et al. 2020): AfuG68B (McQuown et al. 2002), Spl 120 (McQuown et al. 2000), Aox 27 (King et al. 2001), AfuG68, AfuG74, AfuG56, AfuG195, AfuG9, AfuG63, AfuG204, AfuG160, AfuG112 (Welsh et al. 2003), and Atr113 (Rodzen and May 2002) using conditions described in Dammerman et al. (2019).

The program ML-Relate (Kalinowski et al. 2006) was used to calculate a maximum likelihood estimate of pairwise inter-individual relatedness, and to discriminate among general pedigree categories: parent-offspring (PO; $R_{xy}=0.50$), full-sibling (FS; $R_{xy}=0.050$), half-sibling (HS; $R_{xy}=0.25$) and unrelated (U; $R_{xy} = 0.00$) based on likelihood ratio tests. For purposes of this experiment, we used ML-Relate to test the putative relationship between two individuals (unrelated) against the alternative relationship (half-sibling) using likelihood ratio tests, based on a 0.05 significance level. We used the 1,320 individuals that have been genotyped since 2000 to generate all possible male:female relatedness combinations. Male:female pairings, R_{xy} values for each relationship and log likelihood values are listed in Table 2.1. With these data, any two possible mates captured while spawning could be queried for relatedness with the focal female. We conducted *in vitro* fertilization comparisons to determine whether differences in proportional fertilization success were evident based on visual evidence of fertilization (cellular division) after 24-hours when eggs from an individual female were fertilized by an unrelated vs related male,

with and without the presence of ovarian fluid. Additionally, we visually evaluated embryo survival after 48- and 72-hours to determine if eggs developmentally arrested after fertilization.

To conduct this experiment, eggs from four unique females (Table 2.1) to account for hatch variability which could be ascribed to parentage. Eggs were each fertilized with sperm from an estimated unrelated male or an estimated half-sibling male as determined by the maximum likelihood estimate of relatedness from ML-Relate. In each of six replicates of each cross, 1 mL of eggs from a single female from which ovarian fluid was removed was fertilized with 20 μ L sperm from the unrelated male. Further, six 1 mL replicates of eggs were fertilized with 20 μ L of sperm from the same unrelated male with ovarian fluid present on the egg surface. Additionally, six replicates of 1 mL of eggs were fertilized with 20 μ L of sperm from a half-sibling male with ovarian fluid removed. Finally, in six replicates, 1 mL of eggs with ovarian fluid present were fertilized with 20 μ L of sperm from the same half-sibling male. For every replicate, sperm was active in a 200 μ L of river water.

3.3.1 Fertilization experiment statistical analysis

The dependent variables in this experiment were proportional fertilization rate 24-hours post-fertilization and proportional survival of eggs evaluated at 24- and 48- hours post-fertilization. Eggs were fertilized by unrelated or related males with or without ovarian fluid present. Fertilization success (24-hours) and survival (48-, 72-hours) were determined by visual evaluations based on presence of cellular division viewed through a Nikon SMZ-800 dissecting microscope on 6x magnification 24, 48, or 72-hrs post-fertilization as described by Dettlaff et al. (1993). We used a generalized linear mixed model (GLMM) treating female id as a random effect (Dammerman et al. 2015) and using the beta distribution where the proportional fertilization success rate was defined as the number of successfully fertilized eggs at the

evaluated time period divided by the total number of eggs in each replicate. All statistical analyses were performed in R (4.2.2) (www.r-project.org), using the `glmmTMB` function (Brooks et al. 2017).

3.4 Sperm motility experiment

We studied lake sturgeon sperm activated in water, and when activated in a 50:50 dilution of water and ovarian fluid from a female that was either estimated to be unrelated to the male or related as a half sibling. Eggs from six lake sturgeon (Table 2.1) were collected during the 2022 adult lake sturgeon spawning run in the upper Black River consistent with methods described in Crossman et al. (2011). Eggs were stored in a 500mL Whirl-Pac in water consistent in temperature with river conditions. As sturgeon can maintain post-vitellogenic ovary for extended periods before spawning (Webb et al. 1999, Dammerman et al. 2019), eggs collected from each female in the field were isolated by female and combined into separate graduated cylinders, from which ovarian fluid was collected from a homogenized sample by female, individually. For each female, sperm was collected from two males (one estimated to be unrelated, and one estimated to be half-sibling) on the same day. The collected sperm was stored in a cooler and returned to the hatchery. All sperm activation was completed within six hours of collection. Sperm was activated in six replicates in each of four treatments: Experimental treatment 1. Sperm from an estimated unrelated male activated in water only; Experimental treatment 2. Sperm from an estimated unrelated male activated in water and ovarian fluid; Experimental treatment 3. Sperm from an estimated half-sibling male activated in water only; Experimental treatment 4. Sperm from an estimated half-sibling male activated in water and ovarian fluid. A dilution of 5 μ L of sperm to 200 L of river water or river water/ovarian fluid (1:40) was used as a baseline dilution for activation. In cases where the concentration of sperm

exceeded 5.00×10^8 sperm·mL⁻¹ and the sample could not be effectively counted, a dilution of 0.5 µL of sperm to 200 L of river water river water/ovarian fluid (1:400) was used. To account for possible variability in sperm samples associated with time from collection, trials (half-sibling / unrelated; river water only / river water/ovarian fluid) were conducted at random.

Sperm samples from the 2022 field season were assessed using a computer assisted sperm analyzer system with the ImageJ CASA plugin (1.0) described in Wilson-Leedy and Ingermann (2007) and Larson et al. (2020). Using the 40× objective of a Nikon Eclipse E100 compound microscope with a Nikon 0.7× DXM relay lens and an optiMOS 16-bit monochrome camera, 16-bit 480 x 270-pixel image stages were created using the Multi-Dimensional Acquisition tool collected at 100 frames*s⁻¹. In total, 3000 frames representing 30 seconds of sperm video were collected for each replicate sample. Ten seconds of sperm movement (1000 frames) was analyzed for each sample. Settings for threshold values used and CASA sperm quality parameters can be found in Table 2.2.

3.4.1 Sperm motility experiment statistical analysis

CASA software produced five correlated measures of sperm quality, including motility (MOT), curvilinear velocity (VCL), velocity along the average path (VAP), straight line velocity (VSL), and linearity (LIN). To evaluate variability in sperm quality across males both with and without ovarian fluid, a principal components analysis was used to generate composite variables from orthogonal axes which best encompassed the variability in the entire set of CASA variables, as described in Larson et al. (2020). Axes of significance were selected using a Scree test (Cattell 1966), where significant axes were those with combined variability explaining >80% of the total variability in all sperm samples. We used Pearson correlations to determine which variables were significantly directionally correlated to each principal component. Factor loadings

for each sample were extracted using the Factoextra library (Kassambara and Mundt 2017) in R (4.2.2) (www.r-project.org) and treated as the dependent variable for each respective principal component axis as a measure of sperm quality as described in Larson et al. (2020).

In all analyses, the dependent variable was modeled using a general linear mixed model (GLMM) treating female id as a random effect and using a gaussian distribution, where residuals were normally distributed. Here, we sought to evaluate the effect of relatedness (unrelated or related) and ovarian fluid (present or absent) on sperm quality, or on individual measures of sperm velocity. All statistical analyses were performed in R (4.2.2) (www.r-project.org), using the `glmmTMB` function (Brooks et al. 2017).

4. RESULTS

4.1 Sperm volume experiment

Twenty-four hours post-fertilization, the mean (\pm SD) proportional of eggs successfully fertilized with 20 μ L of milt was 0.97 (\pm 0.03) across all treatments and replicates. Proportional fertilization success was 0.97 (\pm 0.03), 0.97 (\pm 0.02), 0.96 (\pm 0.04), and 0.97 (\pm 0.06) when fertilized with 100 μ L, 500 μ L, 750 μ L, and 1000 μ L of milt, respectively (Figure 2.1). High and relatively invariant egg survival across sperm levels was observed in the 48-hour evaluation where mean (\pm SD) proportional egg survival was 0.97 (\pm 0.03), 0.98 (\pm 0.02), 0.96 (\pm 0.04), 0.96 (\pm 0.04), and 0.96 (\pm 0.07) when fertilized with 20 μ L, 100 μ L, 500 μ L, 750 μ L, and 1000 μ L of milt, respectively (Figure 2.1). Finally, after 72-hours, mean (\pm SD) proportional egg survival was 0.95 (\pm 0.03), 0.96 (\pm 0.03), 0.95 (\pm 0.03), 0.94 (\pm 0.03), and 0.93 (\pm 0.05) when fertilized with 20 μ L, 100 μ L, 500 μ L, 750 μ L, and 1000 μ L of milt, respectively (Figure 2.1).

The full model considered the fixed effects of volume of milt used to fertilize eggs and the time after fertilization at which eggs were checked for fertilization. The model intercept was based on eggs fertilized with 20 μL of milt and evaluated 24-hours post-fertilization. A type I analysis of variance indicated that the fixed effects of milt volume, evaluation time, and the respective interactions did not differ from the intercept value, indicating that proportional fertilization and survival with any milt volume at time was not significantly different from average proportional fertilization when 20 μL of sperm were used and eggs were evaluated at 24-hours post-fertilization (Table 2.3).

A type II analysis of variance indicated a slight, but significant drop in proportional egg survival when eggs were evaluated after 72-hours, regardless of volume (Chisq = 20.301, $p < 0.01$, Table 2.4). Mean ($\pm\text{SD}$) proportional fertilization was 0.97 (± 0.04) 24-hours post-fertilization, and proportional egg survival was 0.97 (± 0.04) at 48-hours post-fertilization, and 0.95 (± 0.04) at 72-hours post-fertilization respectively (Figure 2.4). Proportional fertilization or egg survival was also slightly, but significantly different (Chisq = 9.738, $p = 0.045$, Table 2.5) across volumes of milt used, regardless of time. Mean ($\pm\text{SD}$) proportional fertilization (24-hours) or egg survival (48- and 72-hours, respectively) was 0.96 (± 0.03) for all 20 μL milt treatments, 0.97 (± 0.03) for all 100 μL treatments, 0.96 (± 0.03) for all 500 μL treatments, 0.95 (± 0.04) for all 750 μL treatments, and 0.95 (± 0.06) for all 1000 μL treatments.

Results did not indicate that proportional fertilization or proportional egg survival differed significantly over the range of sperm volumes evaluated suggesting that any of the evaluated volumes of sperm used in fertilization would not affect fertilization success.

4.2 Fertilization experiment results

4.2.1 Relatedness

Relatedness (R_{xy}) varied across the unrelated and half-sibling pairings. Mean (\pm SD) R_{xy} for unrelated male:female pairings was 0.034 (\pm 0.057)(range: 0.000 – 0.523, Table 2.3). Mean (\pm SD) R_{xy} for half sibling male:female pairings was 0.224 (\pm 0.067)(range: 0.052 – 0.432, Table 2.3, Figure 2.2, Figure 2.3). Of all the possible male:female pairings 16.58% were related at the level of half siblings (HS; $R_{xy}=0.25$), 2.67% were related at the level of full siblings (FS; $R_{xy}=0.50$), 2.16% were related at the level of parent/offspring (PO; $R_{xy}=0.50$), and 78.59% were unrelated (U, $R_{xy}=0.00$) (Figure 2.2, Figure 2.3).

4.2.2 Fertilization and survival

Proportional fertilization did not differ as a function of relatedness or ovarian fluid rinsed/unrinsed. 24-hours following fertilization, mean (\pm SD) proportional fertilization in the ovarian fluid unrinsed and half-sibling relatedness male treatment was 0.961 (\pm 0.028). When ovarian fluid was removed from the egg surface, proportional fertilization of eggs was 0.943 (\pm 0.043) for males with the same level of relatedness. For eggs fertilized with sperm from a half sibling male when ovarian fluid remained on the egg surface, proportional fertilization after 24-hours was 0.938 (\pm 0.065). When ovarian fluid was rinsed from the egg surface, mean (\pm SD) proportional fertilization by the half-sibling male after 24-hours was 0.938 (\pm 0.056).

Proportional survival differed as a function of relatedness, but not ovarian fluid rinsed/unrinsed. After 48-hours, mean (\pm SD) proportional egg survival of eggs where ovarian fluid was present and fertilized by sperm from a male to whom the female was unrelated was 0.818 (\pm 0.117). When ovarian fluid was removed from the egg surface, proportional egg survival of eggs fertilized with sperm from an unrelated male was 0.778 (\pm 0.173). For eggs fertilized with

sperm from half sibling male, proportional egg survival was 0.715 (± 0.236) and 0.717 (± 0.182) when ovarian fluid remained on the egg surface, and was removed, respectively. After 72-hours, mean (\pm SD) proportional egg survival of eggs containing ovarian fluid and fertilized by sperm from a male the female was unrelated was 0.739 (± 0.140). When ovarian fluid was removed from the egg surface, proportional egg survival of eggs fertilized with sperm from an unrelated male was 0.667 (± 0.187). For eggs fertilized with sperm from half sibling male, proportional egg survival was 0.484 (± 0.294) and 0.570 (± 0.243) when ovarian fluid remained on the egg surface, and was removed, respectively (Figure 2.4).

The full model considered the fixed effects of male:female relatedness (unrelated / half sibling), ovarian fluid rinsed / unrinsed, and the time after fertilization at which eggs were checked for fertilization or survival (repeated measure). In addition, the model considered the random effect of family. The model intercept was based on rinsed eggs fertilized by sperm from an unrelated male and evaluated 24-hours post-fertilization. Mean (\pm SD) proportional fertilization after 24-hours for all treatments was 0.945 (± 0.050) and egg survival decreased significantly for eggs evaluated at 48-hours post-fertilization (0.757 ± 0.184 ; z value = -5.302, $p < 0.01$, Table 2.6) and 72-hour post-fertilization (0.615 ± 0.240 ; z value = -7.392, $p < 0.001$, Table 2.6). Additionally, a significant reduction in proportional egg survival was detected in eggs fertilized by sperm from a half sibling male and evaluated 72-hours post fertilization (z value = -2.794, $p = 0.005$, Table 2.6). A significant effect of relatedness was detected indicating a drop in proportional egg survival for eggs fertilized with sperm from a half-sibling male (Chisq = 29.536, $p < 0.01$, Table 2.7). In addition, a significant drop in proportional egg fertilization and survival can be explained by the time post-fertilization eggs were checked (Chisq = 296.132, $p < 0.01$, Table 2.7). A significant two-way interaction was detected for relatedness and time (Chisq

= 7.526, $p = 0.023$, Table 2.6), demonstrating a drop in proportional fertilization and egg survival through time for eggs fertilized by half-sibling males (Fig 2.4). Notably, a slight, but not significant two-way interaction between ovarian fluid removal and male relatedness was observed ($\text{Chisq} = 3.353$, $p = 0.060$, Table 2.7). Collectively, results indicate that proportional fertilization (24-hours post-fertilization) and egg survival (48- and 72-hours post-fertilization) decreased with time and was strongly reduced by fertilization of eggs with a half-sibling male. The degree to which ovarian fluid on the egg surface influenced proportional egg fertilization remained uncertain.

4.3 Sperm motility experiment results

4.3.1 Principal Components analysis

Principal components analysis indicated five axes of which two were significant and cumulatively explained 84.2% of the variation in sperm quality. Both informative axes were retained based on Cattell's Scree test (Cattell 1966). Principal component one (PC1) explained 56.5% of the variability in sperm quality alone and was strongly positively correlated to sperm velocity along an average path, straight line velocity, and sperm linearity (Table 2.8). Principal component two (PC2) explained 27.7% of all variability in sperm quality and was strongly negatively correlated with curvilinear velocity and positively correlated with sperm motility and sperm linearity (Table 2.8). Each of the five variables produced by computer assisted sperm analysis were represented on one principal component (Figure 2.5). Individual factor loadings from PC1 and PC2 were retained and used as dependent variables. All variables from computer assisted sperm analysis were represented on at least one of the two principal components.

4.3.2 Sperm quality in the presence or absence of ovarian fluid

Sperm quality, as defined by principal component one, varied by the degree to which the male and female were related, and by the presence/absence of ovarian fluid, but not by the additive effect. The intercept of the model was sperm quality measured when a male was not related to the female and when activated in only river water. Moving from the river water treatment to the treatment in which river water and ovarian fluid were mixed reduced principal component one by 1.41 units (GLMM, z value = -4.745, $p < 0.01$). Additionally, moving from the unrelated treatment to the half sibling treatment increased starting sperm quality by 0.984 units on principal component one (GLMM, z value = 3.292, $p = 0.01$). The two-way interaction of moving from the unrelated and river water treatments to the half-sibling and river water with ovarian fluid treatment slightly decreased principal component one by 0.738 units, but the effect of the two-way interaction was not significant (GLMM, z value = -1.753, $p = 0.079$, Figure 2.6).

Along principal component two, moving from the river water treatment to the treatment in which river water and ovarian fluid were mixed was not significant (GLMM, z value = 0.951, $p = 0.342$). Additionally, moving from the unrelated treatment to the half sibling treatment did not alter sperm quality on principal component two (GLMM, z value = 0.467, $p = 0.467$). Finally, the two-way interaction of moving from the unrelated and river water treatments to the half-sibling and river water with ovarian fluid treatment did not alter sperm quality on principal component two (GLMM, z value = 0.661, $p = 0.508$).

4.3.3 Velocity straight line

Straight line velocity varied by the degree to which the male and female were related, and by the presence/absence of ovarian fluid, but not by the additive effect. Mean (\pm SD) straight line velocity was 80.69 (\pm 49.07) $\mu\text{m}\cdot\text{sec}^{-1}$ for sperm from males unrelated to a female and 113.73

(± 71.8) $\mu\text{m}\cdot\text{sec}^{-1}$ for half sibling males when activated in water only. Activating sperm with a 50:50 mixture of river water and ovarian fluid reduced straight line velocity to $40.12 (\pm 15.19)$ $\mu\text{m}\cdot\text{sec}^{-1}$ and $47.38 (\pm 32.80)$ $\mu\text{m}\cdot\text{sec}^{-1}$ for unrelated males and half sibling males, respectively. The intercept of the fully parameterized model represented straight line velocity of sperm from unrelated males activated only in river water. We detected a significant effect of adding ovarian fluid to the activation assay, where adding ovarian fluid reduced straight line velocity by $40.57 \mu\text{m}\cdot\text{sec}^{-1}$ (GLMM, z value = -4.021 , $p < 0.001$). Additionally, we detected a significant effect of relatedness where sperm quality of the half sibling male was $33.68 \mu\text{m}\cdot\text{sec}^{-1}$ faster than the sperm of the unrelated male (GLMM, z value = 3.313 , $p < 0.001$). A slight, but not significant negative two-way interaction was notable (GLMM, z value = -1.844 , $p = 0.065$; Figure 2.7).

4.3.4 Curvilinear velocity

Curvilinear velocity varied by the degree to which the male and female were related, and by the presence/absence of ovarian fluid, but not by the additive effect. Mean (\pm SD) curvilinear velocity was $234.18 (\pm 53.76)$ $\mu\text{m}\cdot\text{sec}^{-1}$ for sperm from males unrelated to a female and $255.09 (\pm 61.96)$ $\mu\text{m}\cdot\text{sec}^{-1}$ for half sibling males when activated in water only. Activating sperm with a 50:50 mixture of river water and ovarian fluid reduced curvilinear velocity to $185.95 (\pm 48.89)$ $\mu\text{m}\cdot\text{sec}^{-1}$ and $191.10 (\pm 55.96)$ $\mu\text{m}\cdot\text{sec}^{-1}$ for unrelated males and half sibling males, respectively. The intercept of the fully parameterized model represented curvilinear velocity of sperm from unrelated males activated only in river water. Consistent with straight line velocity, we detected a significant effect of adding ovarian fluid to the activation assay, where adding ovarian fluid reduced curvilinear velocity by $48.23 \mu\text{m}\cdot\text{sec}^{-1}$ (GLMM, z value = -4.021 , $p < 0.001$). However, we detected no difference in curvilinear velocity between half-sibling and unrelated male sperm (GLMM, z value = 1.698 , $p = 0.089$) Additionally the two-way interaction of the confluence of

adding ovarian fluid to the mixture and the degree to which males are related to the female providing ovarian fluid, though negative, was not significant (GLMM, z value = 0.912, p = 0.362; Figure 2.8).

4.3.5 Velocity average path

Velocity average path varied by the degree to which the male and female were related, and by the presence/absence of ovarian fluid, but not by the additive effect. Mean (\pm SD) velocity average path was 95.53 (\pm 51.25) $\mu\text{m}\cdot\text{sec}^{-1}$ for sperm from males unrelated to a female and 131.97 (\pm 77.58) $\mu\text{m}\cdot\text{sec}^{-1}$ for half sibling males when activated in water only. Activating sperm with a 50:50 mixture of river water and ovarian fluid reduced velocity average path to 51.70 (\pm 15.07) $\mu\text{m}\cdot\text{sec}^{-1}$ and 59.56 (\pm 34.98) $\mu\text{m}\cdot\text{sec}^{-1}$ for unrelated males and half sibling males, respectively. The intercept of the fully parameterized model represented velocity along an average path of sperm from unrelated males activated only in river water. We detected a significant effect of adding ovarian fluid to the activation assay, where adding ovarian fluid reduced velocity average path by 43.83 $\mu\text{m}\cdot\text{sec}^{-1}$ (GLMM, z value = -4.047 p < 0.001). Additionally, we detected a significant effect of relatedness where sperm quality of the half sibling male was 37.11 $\mu\text{m}\cdot\text{sec}^{-1}$ faster than the sperm of the unrelated male (GLMM, z value = 3.401, p = 0.001). A slight, but not significant negative two-way interaction was notable (GLMM, z value = -1.903, p = 0.057; Figure 2.9).

4.3.6 Motility and linearity

Sperm motility and linearity varied by the presence/absence of ovarian fluid, but not by the degree to which a male and female were related, nor by the additive effect of ovarian fluid and relatedness. Mean (\pm SD) sperm motility was 23.04% (\pm 15.89%) for sperm from males unrelated to a female and 24.88% (\pm 11.74%) for half sibling males when activated in water only.

When activating sperm with a 50:50 mixture of river water and ovarian fluid motility was 17.15% ($\pm 13.43\%$) and 20.50 ($\pm 18.20\%$) for unrelated males and half sibling males, respectively. Mean (\pm SD) sperm linearity was 81.08% ($\pm 8.85\%$) for sperm from males unrelated to a female and 83.74% ($\pm 7.52\%$) for half sibling males when activated in water only. When activating sperm with a 50:50 mixture of river water and ovarian fluid linearity was 76.36% ($\pm 7.38\%$) and 76.71% ($\pm 8.45\%$) for unrelated males and half sibling males, respectively. We detected a slight reduction in motility (GLMM, z value = -2.068, p = 0.039) and linearity (GLMM, z value = -2.877, p = 0.004) when ovarian fluid was present in the activation mixture, however, there were no differences in motility (GLMM, z value = 0.729, p = 0.466) and linearity (GLMM, z value = 1.460, p = 0.144) between unrelated males and half sibling males. Additionally, the two-way interaction of ovarian fluid and relatedness were not significant for either motility (GLMM, z value = 0.192, p = 0.848) or linearity (GLMM, z value = -0.792, p = 0.428).

Collectively, results from this experiment demonstrate that there were differences in sperm quality (as defined by PC1 and PC2) and velocity between male lake sturgeon which are unrelated to, and half siblings of the females used. In addition, a consistent reduction in measures of sperm quality and velocity was detected when ovarian fluid was added to the sperm activation solution. However, the lack of significant two-way interactions between ovarian fluid and relatedness suggested that adding ovarian fluid to river water does not reduce sperm quality or velocity for half-siblings more than for unrelated males.

5. DISCUSSION

Understanding how long-lived species limit inbreeding is vital for management efforts, particularly small populations which are reproductively isolated, or those which rely heavily on wild-captured brood (supportive breeding) for supplementation programs (Hedrick and

Kalinowski 2000; Brook et al. 2002). The consequences of inbreeding in threatened populations are well studied (Vrijenhoek 1994, Newman and Pilson 1997, Crnokrak and Roff 1999, Brook et al. 2002), though previous work has identified guidelines (Welsh et al. 2010) and possible consequences of supportive breeding (Schueller and Hayes 2011) in lake sturgeon. Still, little is known about how long-live species, like lake sturgeon, mitigate consanguineous matings likely to result from polyamorous breeding during broadcast spawning episodes. In this study, we demonstrated that proportional egg fertilization 24-hours post-fertilization (a plausible indicator of pre-zygotic measures) did not differ between relatedness or ovarian fluid treatments. Egg survival (48- and 72-hours post-fertilization) decreased with time and was strongly reduced when eggs were fertilized by a related male. In addition, sperm quality and velocity data revealed that there were differences in sperm quality and velocity of fertilizing males, and that the presence of ovarian fluid on egg surfaces altered sperm quality and velocity. Collectively, our data reveal that female lake sturgeon may possess a mechanism by which consanguineous matings are less likely to produce surviving progeny, however, the lack of interaction between relatedness and ovarian fluid rinse/unrinsed suggest that females do not use ovarian fluid to cryptically select against egg fertilization by a half-sibling male.

5.1 Egg fertilization and survival following consanguineous mating

We detected a strong effect of relatedness ($p < 0.001$), where eggs fertilized by a half sibling had 17% lower proportional survival than unrelated males after 72-hours. Results revealed a potential deleterious effect of mating with relatives at a cost of reduced reproductive success at the individual level. Overall, this may be a benefit at the population level by reducing the level of inbreeding in offspring. In general, reduced survival of inbred embryos has been well established (review in Waldman and McKinnon 1993). Concordantly, evidence suggests that

greater success among dissimilar mates acts as a selective force against population wide increase in mean relatedness in polygynous species (Amos et al. 2001).

Under natural conditions broadcast spawning species, including lake sturgeon, are believed to time egg release to maximize the number of breeders (males) in a breeding group (Bruch and Binkowski 2002; Dammerman et al. 2019) as a compensatory mechanism to increase the probability of egg fertilization. Because this behavior leads to polygynous and polyandrous matings, without a mechanism for mate selection, consanguineous matings are likely, particularly in small populations. In other polyandrous species, females cryptically select mates through directional sperm bias. In beetles (*Callosobruchus maculatus*) sperm from males unrelated to a female consistently outperforms sperm from full sibling matings (Wilson et al. 1997). Thuman and Griffith (2005) demonstrated that in direct sperm competition between two males, the least genetically similar male sired the majority of offspring. Results of this study demonstrated that ovarian fluid slows sperm curvilinear velocity, velocity along an average path, velocity in a straight line, reduced motility, linearity, and overall sperm quality (as defined along principal component one). A slight, but insignificant relatedness by ovarian fluid interaction terms failed to demonstrate antagonistic effects on sperm from half-sibling males. This contrasts with results in guppy (*Poecilia reticulata*) (Gasparini and Pilastro 2011), ocellated wrasse (*Symphodus ocellatus*) (Alonzo et al. 2016), and Arctic charr (*Salvelinus alpinus*) (Urbach et al. 2005) in which significant male by female interaction terms suggested a causal relationship between altered sperm quality and realized female mate selection.

5.2 Sperm quality and the role of ovarian fluid

The relationship between sperm velocity and ovarian fluid is well documented and is thought to be either antagonistic toward related males (Kholodnyy et al., 2021, Kholodnyy et al.

2022) or benign toward unrelated males (Turner and Montgomerie 2002, Wojtczak et al. 2007, Rosengrave et al., 2008, Alonzo et al. 2016). Our results demonstrated that in lake sturgeon, the relationship between ovarian fluid and sperm quality is antagonistic. As a result, ovarian fluid reduced sperm velocity, regardless of male relatedness. Following release, male gametes from freshwater fishes have a short lifespan due in part to the difference in osmolarities of extra- and intracellular fluids and to changes in potassium (Morisawa 1994), resulting in cell damage and decline in sperm viability shortly after release. The egg has a correspondingly short life owing to the surface reaction required to seal the egg micropyle (Hart 1990). Kholodnyy et al. (2021) offers that in acipenseriformes, rather than acting as a mechanism for cryptic selection, ovarian fluid instead acts as an ion buffer, coating the egg to extend longevity of both the egg and sperm in a riverine environment. Thus, the antagonistic relationship between ovarian fluid and sperm may not be due to selectivity, but rather may exist to extend the time an egg can be fertilized, increasing overall fertilization rates and reproductive success for a larger number of attending males. Ovarian fluid is used as a sperm preservative for both acipenseriformes and teleost fish species. Heerden et al. (1993) mixed ovarian fluid with borax-boric acid buffer and found increased fertilization success in rainbow trout was likely due to the increase in motility and longevity of sperm. Gasparini and Evans (2013) found that temporal decline in sperm viability was significantly reduced in the presence of ovarian fluid when compared to saline solution in guppy. Our results are consistent with these findings, as velocity average path, straight line velocity, curvilinear velocity, and motility all decline regardless of male:female relationship.

Sperm velocity reduction in ovarian fluid may have another purpose in acipenseriformes. Unlike most freshwater teleosts, acipenseriforms eggs possess between two and 52 micropyles (Siddique et al. 2014). The presence of multiple micropyles on the chorion surface seems likely

to increase the chances of polyspermy. However, the double-tapered entry canal of the micropyles (Cherr and Clark 1985) is sufficiently complex to limit access of multiple sperm to the perivitelline space. As ovarian fluid seems to act as a preservative both for the egg and sperm, it is likely that the ovarian fluid works in concert with complex structure of the micropyle to facilitate successful fertilization, while reducing the possibility of polyspermy.

A possibly confounding factor that may have affected the outcome of results involved relatedness classification. We scored 13 disomic microsatellite loci to investigate 1,320 individuals with 1,742,400 possible mating outcomes. The fraction of possible misclassifications is small and decreases with increasing number of loci scored for half-sibling and unrelated relationships (Blouin et al. 1996). There was a great deal of variability in R_{xy} for each classification considered (Figure 2.3). For the fish selected for this experiment (Table 2.1). In the case of female BLA161, the R_{xy} value of the unrelated male pairing ($R_{xy} = 0.135$) is higher than that of the half-sibling pairing ($R_{xy} = 0.124$). Concordantly it was only 0.06x more likely that the unrelated male was indeed unrelated. In addition, the half-sibling male was only 0.04x more likely to be a half-sibling by classification. We attempted to account for this by incorporating a random effect associated with female parentage, which did explain some of the variability in proportional fertilization, sperm quality, and measures of sperm velocity. Ultimately this more conservative approach may explain the lack of significance of the two-way interaction.

5.3 Conclusion

Collectively the experiments described here demonstrate the effect that ovarian fluid has on lake sturgeon sperm following release in an aqueous environment. Interaction terms did not indicate that ovarian fluid had a larger impact on proportional fertilization, proportional survival, or sperm velocity for half-sibling males, however, proportional fertilization was significantly

lower in eggs fertilized by half-sibling males when compared to eggs fertilized by unrelated males. This result suggested deleterious effects of spawning with close-kin by lake sturgeon may result in a reduction in successful consanguineous matings, however, the degree to which ovarian fluid is involved remains unclear. These results, coupled with other examples of fertilization bias in polyandrous species (Olsson et al. 1996, Wilson et al. 1997, Alonzo et al. 2016) provide further evidence of the role of ovarian fluid in broadcast fertilizing species.

Importantly, this work addresses gaps for management of threatened species. First, by using readily available tools to evaluate population relatedness, supportive breeding programs can overcome the challenges posed by managing small populations without the control offered in supplementation programs where brood stock can be maintained. Hatchery supplementation is a common tool in the restoration of Acipenseriformes (Holtgren et al. 2007, Mann et al. 2011). As sturgeon species are generally long-lived, hatchery supplementation requires supportive breeding, which utilizes wild adults as a brood source (Ryman and Laikre 1991) rather than a traditional brood stock. While stocking efforts have effectively increased the population size of threatened species (Ireland et al. 2002, Welsh et al. 2018), they have historically reduced genetic diversity and increased inbreeding (Ward 2006, Attard et al. 2022). Strategies including larval collection, (Smith and King 2004), the use of genetic stocking units, and adult collection targets (Welsh et al. 2010) have been employed to limit inbreeding. As a result, populations of lake sturgeon in the Great Lakes region have typically not seen a reduction in genetic diversity (DeHaan et al. 2006). However, as these programs continue to expand, managers can broaden their understanding of the role of ovarian fluid in lake sturgeon reproduction, which will aid supplemental breeding programs, for which ovarian fluid is often an afterthought. This

knowledge is essential to continued recovery of small populations of threatened lake sturgeon populations throughout North America.

REFERENCES

- Alonzo, S. H., Stiver, K. A., and Marsh-Rollo, S. E. 2016. Ovarian fluid allows directional cryptic female choice despite external fertilization. *Nature Communications*, 7:12452 doi: 10.1038/ncomms12452.
- Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D., and Coulson, T. 2001. The influence of parental relatedness on reproductive success. *Proc Biol Sci.*, 268: 2021-2027.
- Attard, C. R. M., Sandoval-Castillo, J., Gilligan, D. M., Unmack, P. J., Faulks, L. K., and Beheregaray, L. B. 2022. Genomics outperforms genetics to manage mistakes in fisheries stocking of threatened species. *Biodiversity and Conservation*, 321: 895-908.
- Baker, E. A., and Borgeson, D. J. 1999. Lake Sturgeon Abundance and Harvest in Black Lake, Michigan, 1975–1999. *North American Journal of Fisheries Management*, 19: 1080-1088.
- Beausoleil, J. M. J., Doucet, S. M., Heath, D. D. and Pitcher, T. E. 2012. Spawning coloration, female choice and sperm competition in the redbside dace, *Clinostomus elongatus*. *Animal Behavior*, 83: 969–977.
- Birkhead, T. 1998. Cryptic female choice: criteria for establishing female sperm choice. *Evolution*, 52: 1212–1218.
- Blouin, M. S., Parsons, M., Lacaille, V. and Lotz, S. 1996. Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology*, 5: 393-401
- Bruch, R. M. and Binkowski, F. P. 2002. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). *Journal of Applied Ichthyology*, 18: 570-579.
- Brook, B. W., Tonkyn, D. W., O’Grady, J. J., and Frankham, R. 2002. Contribution of inbreeding to extinction risk in threatened species. *Conservation Ecology*, 6:16.
- Brooks, M. E., Kristensen, K., vanBenthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M. and Bolker, B. M. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R. Journal*, 9; 378-400.
- Butts, I. A. E., Johnson, K., Wilson, C. C. and Pitcher, T. E. 2012. Ovarian fluid enhances sperm velocity based on relatedness in lake trout, *Salvelinus namaycush*. *Theriogenology*, 78: 2105–2109.
- Cattell, R. B. 1966. The scree test for the number of factors. *Multivariate Behav. Res.* 1: 245–276. doi:10.1207/s15327906mbr0102_10.

- Crossman, J., Scribner, K. T., Davis, C., Forsythe, P. and Baker, E. A. 2011. Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon. *Aquaculture* 310: 312-324.
- Cherr, G. N., and Clark Jr., W. H. 1985. Gamete interaction in the white sturgeon *Acipenser transmontanus*: a morphological and physiological review. *Environmental Biology of Fishes*, 14: 11-22.
- Ciereszko, A., Dabrowski, K., and Ochkur, S. I. (1996). Characterization of acrosin-like activity of lake sturgeon (*Acipenser fulvescens*) spermatozoa. *Molecular Reproduction and Development*, 45: 72–77.
- Crnokrak, P., and Roff, D. A. 1999. Inbreeding depression in the wild. *Heredity*, 83:260-270.
- Dammerman, K. J., Steibel, J. P., and Scribner, K. T. 2015. Genetic and environmental components of phenotypic and behavioral trait variation during lake sturgeon (*Acipenser fulvescens*) early ontogeny. *Environmental Biology of Fishes*, 98: 1659-1670.
- Dammerman, K. J., Steibel, J. P., and Scribner, K. T. 2016. Increases in the mean and variability of thermal regimes result in differential phenotypic responses among genotypes during early ontogenetic stages of lake sturgeon (*Acipenser fulvescens*). *Evolutionary Applications*, 9: 1258-1270.
- Dammerman, K., Webb, M., and Scribner, K. T. 2019. Riverine characteristics and adult demography influence female lake sturgeon (*Acipenser fulvescens*) spawning behavior, reproductive success, and ovarian quality. *Canadian Journal of Fisheries and Aquatic Sciences*, 76: 1147-1160.
- DeHaan, P., Libants, S. T., Elliott, R. F., and Scribner, K.T. 2006. Genetic population structure of remnant lake sturgeon populations in the upper Great Lakes basin. *Transactions of the American Fisheries Society*, 135:1478-1492.
- Detlaff, T. A., Ginsburg, A. S., and Schmalhausen, O. I. 1993. *Sturgeon fishes: developmental biology and aquaculture*. Springer-Verlag, Berlin.
- Eberhard, W. G. 1996. *Female control: sexual selection by cryptic female choice*. Princeton University Press, Princeton.
- Evans, J. P., Rosengrave, P. Gasparini, C. and Gemmell, N. J. 2013. Delineating the roles of males and females in sperm competition. *Proceedings of the Royal Society*, 280: 2013(2047).
- Forsythe, P. S., Crossman, J. A., Bello, N. M., Baker, E. A. and Scribner, K. T. 2012a. Individual-based analyses reveal high repeatability in timing and location of reproduction in Lake Sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences*, 69: 60-72.

- Forsythe, P. S., Scribner, K.T., Crossman, J. A., Ragavendran, A., Davis, C., Baker, E. A., and Smith, K. K. 2012. Environmental and lunar cues are predictive of timing of river entry and spawning site arrival in lake sturgeon. *Journal of Fish Biology*, 81: 35-53.
- Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B. and Parker, G. A. 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative velocity is the primary determinant of fertilization success. *Current Biology*, 14: 44–47.
- Gasparini, C. and Pilastro, A. 2011. Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proceedings of the Royal Society*, 278 (1717).
- Gasparini, C. and Evans, J. P. 2013. Ovarian Fluid Mediates the Temporal Decline in Sperm Viability in a Fish with Sperm Storage. *Plos one*, 8(5): e64431.
<https://doi.org/10.1371/journal.pone.0064431>
- Heerden, E., Vuren, J. H. J., and Steyn, G. J. 1993. Development and evaluation of sperm diluents for the artificial insemination of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources*, 6:57-62.
- Hedrick, P. W., and Kalinowski, S. T. 2000. Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, 31:139– 162.
- Hart, N. H. 1990. Fertilization in teleost fishes: mechanisms of sperm–egg interactions. *International Review of Cytology*, 121: 1–66.
- Holtgren, J. M., Ogren, S. A., Paquet, A. J. and Fajfer, S. 2007. Design of a portable streamside rearing facility for lake sturgeon. *North American Journal of Aquaculture*, 69:317–323.
- Irelands, S. C., Beamesderfer, R. C. P., Paragamian, V. L., Wakkinen, V. D., and Simple, J. T. 2002. Success of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) following release in the Kootenai River, Idaho, USA. *Journal of Applied Ichthyology*, 18: 642-650.
- Kalinowski, S. T., Wagner, A. P. and Taper, M. L. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, 576-579.
- Kassambara, A., and Mundt, F. 2017. Factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.5 [online]. Available from <https://CRAN.R-project.org/package=factoextra>.
- Kholodnyy, V., Dzyuba, B., Gadélhaad, H., Cosson, J., and Boryshpolets, S. 2021. Egg-sperm interaction in sturgeon: role of ovarian fluid. *Fish Physiology and Biochemistry*, 47: 653-669.

- Kholodnyy, V. Shibata, K., Tuba Beken, A., Dzyuba, B., Bloomfield-Gadêlhaad, H. Cosson, J., and Boryshpolets, S. 2022. Common carp spermatozoa performance is significantly affected by ovarian fluid. *Aquaculture*, 554: 738148.
- King, T.L., Lubinski, B.A., and Spidle, A.P. 2001. Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Conserv. Genet*, 2(2): 103–119.
- Larson, D.L., Kimmel, J.G., Riedy, J.J., Hegna, J., Baker, E.A., and Scribner, K. T. 2020. Male Lake Sturgeon (*Acipenser fulvescens*) migratory behavior associated with intra-annual variability in sperm and reproductive success. *Canadian Journal of Fisheries and Aquatic Sciences*, 77: 1943-1959.
- Lehnert, S. J. Butts, I. A. E., Flannery, E. W., Peters, K. M., Heath, D. D., and Pitcher, T. E. 2017. Effects of ovarian fluid and genetic differences on sperm performance and fertilization success of alternative reproductive tactics in Chinook salmon. *Journal of Evolutionary Biology*, 30: 1236-1245.
- Liljedal, S., Rudolfsen, G., and Folstad, I. 2008. Factors predicting male fertilization success in an external fertilizer. *Behavioral Ecology and Sociobiology*, 62: 1805–1811.
- Mann, K. A., Holtgren, J. M., Auer, N. A. and Ogren, S. A. 2011. Comparing size, movement, and habitat selection of wild and stream-side reared lake sturgeon. *North American Journal of Fisheries Management*, 31:305–314
- Marble, B. K., Alexandrou, M. A., and Taylor, M. I. 2011. Genome duplication in amphibians and fish: an extended synthesis, *Journal of Zoology*, 284: 151-182.
- McQuown, E.C., Sloss, B.L., Sheehan, R.J., Rodzen, J., Tranah, G.J., and May, B. 2000. Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirhynchus* and *Acipenser*. *Trans. Am. Fish. Soc.*, 129(6): 1380– 1388. doi:10.1577/1548-8659(2000)1292.0.CO, 2.
- McQuown, E., Gall, G.A.E., and May, B. 2002. Characterization and inheritance of six microsatellite loci in lake sturgeon. *Trans. Am. Fish. Soc.*, 131(2): 299–307. doi:10.1577/1548-8659(2002)1312.0.CO, 2.
- Morisawa, M. 1994. Cell signaling mechanisms for sperm motility. *Zoological Sciences* 11:647–662.
- Newman, D., and Pilson, D. 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution*, 51:354-362.
- Olsson, M., Shine, R., Madsen, T., Gullberg, A., Tegelstrom, H. 1996. Sperm selection by females. *Nature* 383, 585–585.

- Pilastro, A., Simonato, M., Bisazza, A., and Evans, J. P. 2004. Cryptic Female Preference for Colorful Males in guppies. *Evolution*, 58(3): 665-669.
- Pledger, S., Baker, E. A., and Scribner, K. T. 2013. Breeding return times and abundance in capture-recapture models. *Biometrics*, 69: 991-1001.
- Rodzen, J. A., and May, B. 2002. Inheritance of microsatellite loci in White Sturgeon (*Acipenser transmontanus*). *Genome* 45:1064–1076.
- Rosengrave, P., Gemmell, N. J., Metcalf, V., McBride, K. and Montgomerie, R. 2008. A mechanism for cryptic female choice in chinook salmon. *Behavioral Ecology*. 19: 1179–1185.
- Rosengrave, P., Montgomerie, R. and Gemmell, N. 2016. Cryptic female choice enhances fertilization success and embryo survival in chinook salmon. *Proc. R. Soc. B* 283: 2016001.
- Ryman, N., and Laikre, L. 1991. Effects of supportive breeding on the genetically effective population size. *Conservation Biology*, 5:325–329
- Schueller, A. M. and Hayes, D. B. 2011. Minimum viable population size for lake sturgeon (*Acipenser fulvescens*) using an individual-based model of demographics and genetics. *Canadian Journal of Fisheries and Aquatic Sciences*, 68: 62-73.
- Siddique, M. A. M., Cosson, J., Psenicka, M., and Linhart, O. 2014. A review of the structure of sturgeon egg membranes and of the associated terminology. *Journal of Applied Ichthyology*, 30: 1246-1255.
- Simmons, L. W., and Fitzpatrick, J. L. 2012. Sperm wars and the evolution of male fertility. *Reproduction*, 144: 519–534.
- Skarstein, F., Folstad, I., Liljedal, S., and Grahn, M. 2005. MHC and fertilization success in the Arctic charr (*Salvelinus alpinus*). *Behavioral Ecology and Sociobiology*, 57: 374-380.
- Smith, K. M., and King, D. K. 2004. Dynamics and extent of larval lake sturgeon *Acipenser fulvescens* drift in the Upper Black River, Michigan. *Journal of Applied Ichthyology*, 21: 161-168.
- Thuman, K. A., and Griffith S. C. 2005. Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behavior*, 69, 765–770.
- Tucker, S. R., Grimm, A. N., Crossman, J. A., Scribner, K. T. and Forsythe, P. A. 2020. Milt volume influences the probability of egg fertilization in lake sturgeon (*Acipenser fulvescens*, Rafinesque, 1817) *Journal of Applied Ichthyology*, 36: 1-6.

- Turner, E. and Montgomerie, R. 2002. Ovarian fluid enhances sperm movement in Arctic charr. *Journal of Fisheries Biology*, 60: 1570–1579.
- Urbach, D., Folstad, I. and Rudolfson, G. 2005. Effects of ovarian fluid on sperm velocity in Arctic charr (*Salvelinus alpinus*). *Behavioral Ecology and Sociobiology*, 57: 438–444.
- Vrijenhoek, R. C. 1994. Genetic diversity and fitness in small populations. Pages 37-53 in V. Loeschcke, J. Tomiuk, and S. K. Jain, editors. *Conservation genetics*. Birkhäuser Verlag, Basel, Switzerland.
- Waldman, B. and McKinnon, J. S. 1993. Inbreeding and outbreeding in fishes, amphibians and reptiles, In: *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives* (Thornhill, N.W., ed.). University of Chicago Press, Chicago, IL, p. 250-282.
- Ward, R. D. 2006. The importance of identifying spatial population structure in restocking and stock enhancement programmes. *Fisheries Research*, 80:9–18.
- Wassink, L., Bussy, U., Li, W., and Scribner, K. T. 2019. High-stress rearing temperature in *Acipenser fulvescens* affects physiology, behaviour and predation rates. *Animal Behavior*. 157: 153-165.
- Webb, M. A. H., Van Eenennaam, J. P., Doroshov, S. I., and Moberg, G.P. 1999. Preliminary observations on the effects of holding temperature on reproductive performance of female white sturgeon, *Acipenser transmontanus* Richardson. *Aquaculture*, 176: 315–329.
- Welsh, A. B., Blumberg, M., and May, B. 2003. Identification of microsatellite loci in lake sturgeon, *Acipenser fulvescens*, and their variability in green sturgeon, *A. medirostris*. *Mol. Ecol. Notes*, 3(1): 47–55. doi:10.1046/j.1471-8286.2003.00346.x
- Welsh, A. B., Elliott, R. F., Scribner, K. T., Quinlan, H. R., Baker, E. A., Eggold, B. T., Holtgren, J. M., Krueger, C. C., and May, B. 2010. Genetic guidelines for the stocking of lake sturgeon (*Acipenser fulvescens*) in the Great Lakes basin. Great Lakes Fishery Commission, Miscellaneous Publication 2010-01, Ann Arbor, Michigan.
- Welsh, A. B., Schumacher, L., and Quinlan, H. R. 2018. A reintroduced lake sturgeon population comes of age: A genetic evaluation of stocking success in the St. Louis River. *Journal of Applied Ichthyology*, 35: 149-159.
- Wilson, N., Tubman, S. C., Eady, P. E., and Robertson, G. W. 1997. Female genotype affects male success in sperm competition. *Proceedings of the Royal Society London B*, 264, 1491–1495.

- Wilson-Leedy, J. G., and Ingermann, R. L. 2007. Development of a novel CASA system based on open-source software for characterization of zebrafish sperm motility parameters. *Theriogenology*, 67: 661–672.
- Wojtczak, M., Dietrich, G. J., Słowińska, M., Dobosz, S., Kuźmiński, H., and Ciereszko, A. 2007. Ovarian fluid pH enhances motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. *Aquaculture*, 270:259–264.

APPENDIX

Table 2.1 R_{xy} , log-likelihood and Δ log-likelihood values for unrelated and half sibling male lake sturgeon used to fertilize eggs and for sperm quality and velocity testing. ΔLnL values represent the reduction of relationship likelihood (ie, ΔLnL of 0.2 means a relationship is 0.2 times less likely than the categorized relationship).

2022 Female ID	Unrelated Male R_{xy}	Unrelated LnL(R)	Half Sibling $\Delta\text{LnL(R)}$	Half-sibling Male R_{xy}	Half Sibling LnL(R)	Unrelated $\Delta\text{LnL(R)}$
BLA039	0.000	-39.080	1.650	0.207	-52.730	0.200
BLA117	0.097	-40.900	0.160	0.139	-42.160	0.160
BLA155	0.000	-50.720	1.280	0.317	-45.540	1.210
BLA161	0.135	-46.060	0.060	0.124	-47.330	0.040
BLA255	0.000	-46.200	1.660	0.254	-46.180	1.610
BLA277	0.000	-49.230	2.560	0.201	-51.000	0.830

Table 2.2 Computer Assisted Sperm Analyzer (CASA) plugin parameters adapted from Leedy and Ingermann (2007) and Toth et al. (1997).

CASA Plugin Parameter	Value
Minimum sperm size (pixels)	1
Maximum sperm size (pixels)	25
Minimum track length (frames)	25
Maximum sperm velocity between frames (pixels)	10
Minimum VSL for motile ($\mu\text{m/s}$)	10
Minimum VAP for motile ($\mu\text{m/s}$)	20
Minimum VCL for motile ($\mu\text{m/s}$)	25
Low VAP speed ($\mu\text{m/s}$)	10
Maximum percentage of path with zero VAP	1
Maximum percentage of path with low VAP	25
Low VAP speed 2 ($\mu\text{m/s}$)	25
Low VCL speed ($\mu\text{m/s}$)	25
High WOB (percent VAP/VCL)	80
High LIN (percent VSL/VAP)	80
High WOB two (percent VAP/VCL)	50
High LIN two (percent VSL/VAP)	60
Frame Rate (frames per second)	100
Microns per 1000 pixels	1271
Print x,y co-ordinates for all tracked sperm	0
Print motion characteristics for all motile sperm	0
Print median values for motion characteristics	0

Table 2.3 Summary of pair-wise relatedness (R_{xy}) estimated over all possible dyadic adult male-female relationships and prevalence in lake sturgeon from the Black Lake population, MI.

	Unrelated	Half-sibling	Full sibling	Parent-offspring
μ	0.034	0.224	0.419	0.503
Σ	0.057	0.067	0.119	0.039
Min	0.000	0.052	0.094	0.168
Max	0.523	0.432	1.000	0.693
% of pairwise relationships	78.59%	16.58%	2.67%	2.16%

Table 2.4 Effect of milt volume on proportional lake sturgeon egg fertilization when eggs were evaluated for cellular division 24-, 48-, and 72-hours post-fertilization.

Factor	Estimate	Std. Error	z -value	Pr(> z)
Intercept	0.965	0.013	74.620	<0.001
100 μ L	0.007	0.011	0.630	0.526
500 μ L	0.009	0.011	0.810	0.416
750 μ L	-0.008	0.011	-0.750	0.451
1000 μ L	0.007	0.011	0.620	0.533
48-hours post-fertilization	0.001	0.011	0.090	0.930
72-hours post-fertilization	-0.011	0.011	-1.000	0.315
100 μ L: 48-hours post-fertilization	0.007	0.016	0.430	0.667
500 μ L: 48-hours post-fertilization	-0.010	0.016	-0.650	0.516
750 μ L: 48-hours post-fertilization	0.007	0.016	0.440	0.658
1000 μ L: 48-hours post-fertilization	-0.016	0.016	-1.000	0.317
100 μ L: 72-hours post-fertilization	-0.001	0.016	-0.040	0.967
500 μ L: 72-hours post-fertilization	-0.011	0.016	-0.710	0.477
750 μ L: 72-hours post-fertilization	-0.003	0.016	-0.190	0.852
1000 μ L: 72-hours post-fertilization	-0.030	0.016	-1.900	0.057

Table 2.5 Wald Chi-Square Test for the main effect of volume conditional on time period, and time period conditional on volume.

	Chisq	Df	Pr(>Chisq)
Volume	9.738	4	0.045
Time	20.301	2	<0.001
Volume:Time	6.411	8	0.601

Table 2.6 Effects of male:female relationship and ovarian fluid unrinsed:rinsed on proportional lake sturgeon egg fertilization 24-hours, 48-hours, and 72-hours post fertilization.

	Estimate	Std. Error	z value	Pr(> z)
Intercept	2.844	0.352	8.088	<0.001
Ovarian Fluid Removed	-0.085	0.271	-0.315	0.753
Related - Half Sibling	-0.146	0.269	-0.542	0.588
Time 48 Hours	-1.329	0.251	-5.302	<0.001
Time 72 Hours	-1.810	0.245	-7.392	<0.001
Ovarian Fluid Removed:Related (HS)	0.049	0.380	0.129	0.897
Ovarian Fluid Removed:48 Hours	-0.096	0.347	-0.277	0.782
Ovarian Fluid Removed:72 Hours	-0.232	0.336	-0.689	0.491
Related (HS):48 Hours	-0.319	0.342	-0.933	0.351
Related (HS):72 Hours	-0.923	0.330	-2.794	0.005
Ovarian Fluid Removed:Related (HS):48 Hours	0.065	0.481	0.134	0.893
Ovarian Fluid Removed:Realted (HS):72 Hours	0.604	0.466	1.298	0.194

Table 2.7 Wald Chi-Square Test for the main effects of male:female relationship, ovarian fluid unrinsed:rinsed, and evaluation time with two- and three-way interactions.

	Chisq	Df	Pr(>Chisq)
Ovarian Fluid	0.178	1	0.673
Related	29.536	1	<0.001
Time	296.132	2	<0.001
Ovarian Fluid:Related	3.537	1	0.060
Ovarian Fluid:Time	0.646	2	0.724
Related:Time	7.526	2	0.023
Ovarian Fluid:Related:Time	2.532	2	0.282

Table 2.8. Factor loadings, correlation of factor loadings to principal components, eigenvalues, and variance explained for principal components analysis of sperm quality variables. Correlation coefficients greater than 0.5 are considered strongly correlated (Afifi et al. 2004).

	Motility	Velocity Curvilinear	Velocity Average Path	Velocity Straight Light	Linearity	Eigenvalue	Variance (%)	Cumulative Variance (%)
PC1	0.23 (0.39)	0.27 (0.45)	0.57 (0.96)*	0.58 (0.97)*	0.46 (0.77)*	2.83	56.50	56.50
PC2	0.58 (0.68)*	-0.64 (- 0.76)*	-0.17 (- 0.20)	-0.12 (- 0.15)	0.45 (0.53)*	1.39	27.72	84.22
PC3	0.76 (0.61)*	0.54 (0.44)	0.14 (- 0.12)	-0.17 (- 0.14)	-0.30 (- 0.25)	0.66	13.22	97.44
PC4	-0.20 (- 0.07)	0.47 (0.17)	-0.36 (- 0.13)	-0.33 (- 0.12)	0.70 (0.25)	0.13	2.55	99.99
PC5	-0.00 (0.00)	-0.02 (- 0.00)	0.70 (0.01)	-0.71 (- 0.01)	0.04 (0.00)	0.00	0.01	100.00

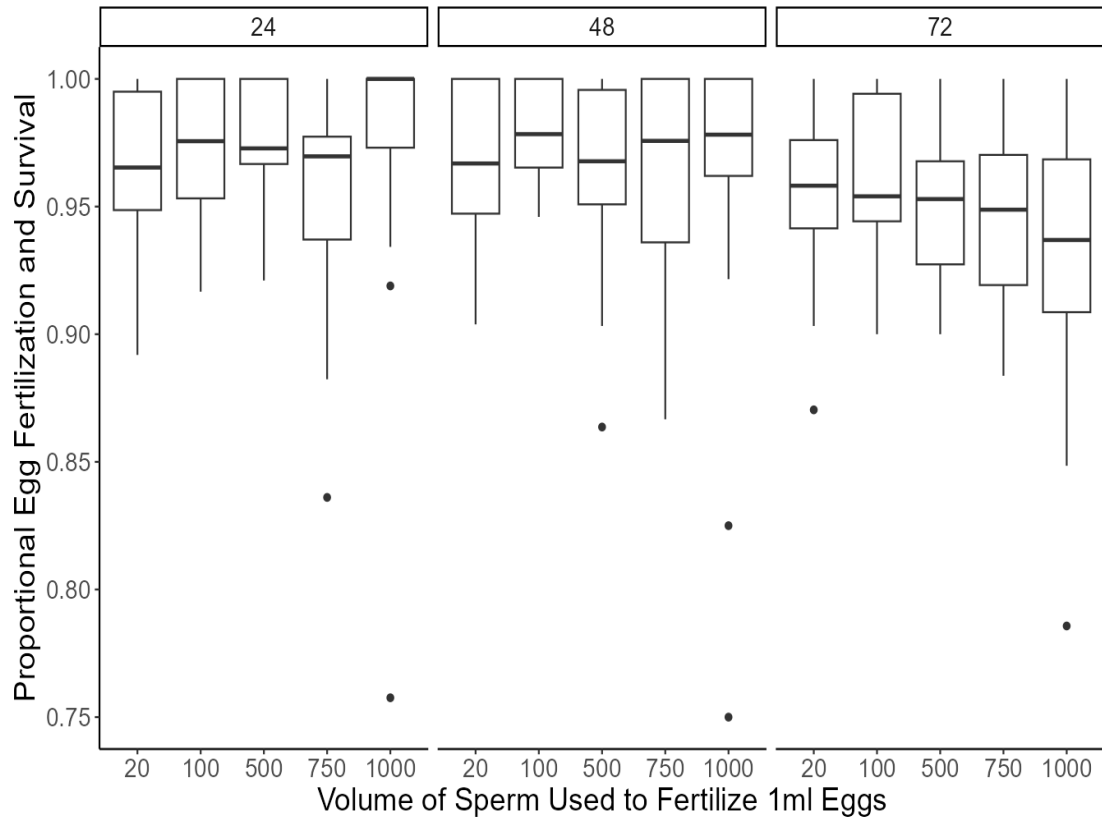


Figure 2.1 Proportional fertilization of lake sturgeon eggs as a function of sperm volume at 24-hours post-fertilization, and proportional egg survival after 48-hours, and 72-hours post-fertilization.

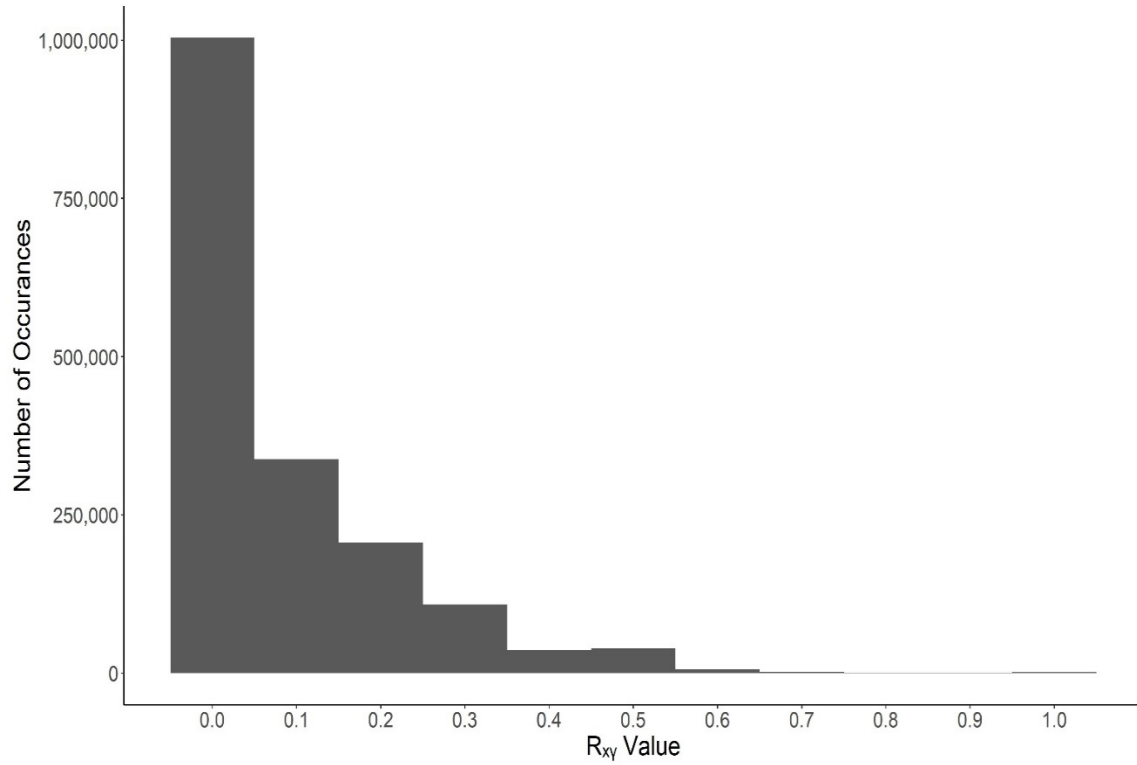


Figure 2.2 Full distribution of all possible R_{xy} values from possible adult male-female dyadic relationships in the Black Lake lake sturgeon population.

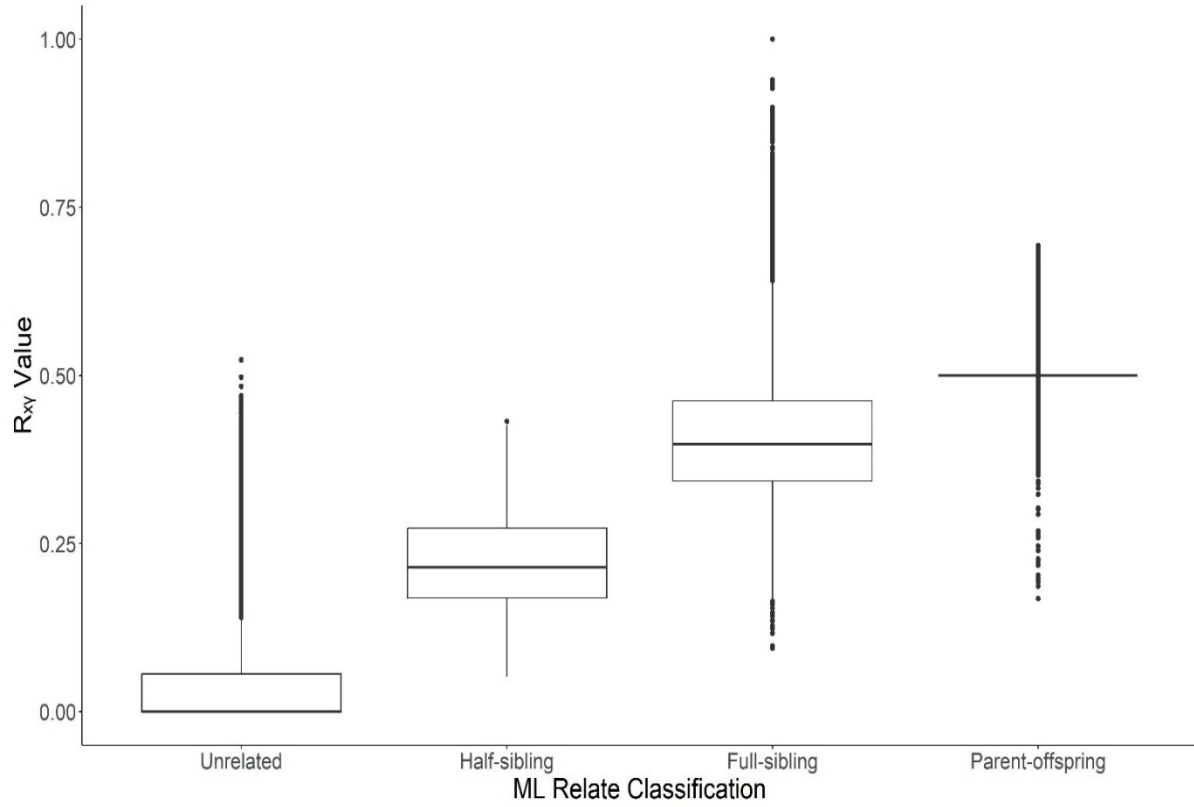


Figure 2.3 Full distribution of all possible R_{xy} values for each possible pairwise relationship from all possible breeding pairs in the Black Lake lake sturgeon population.

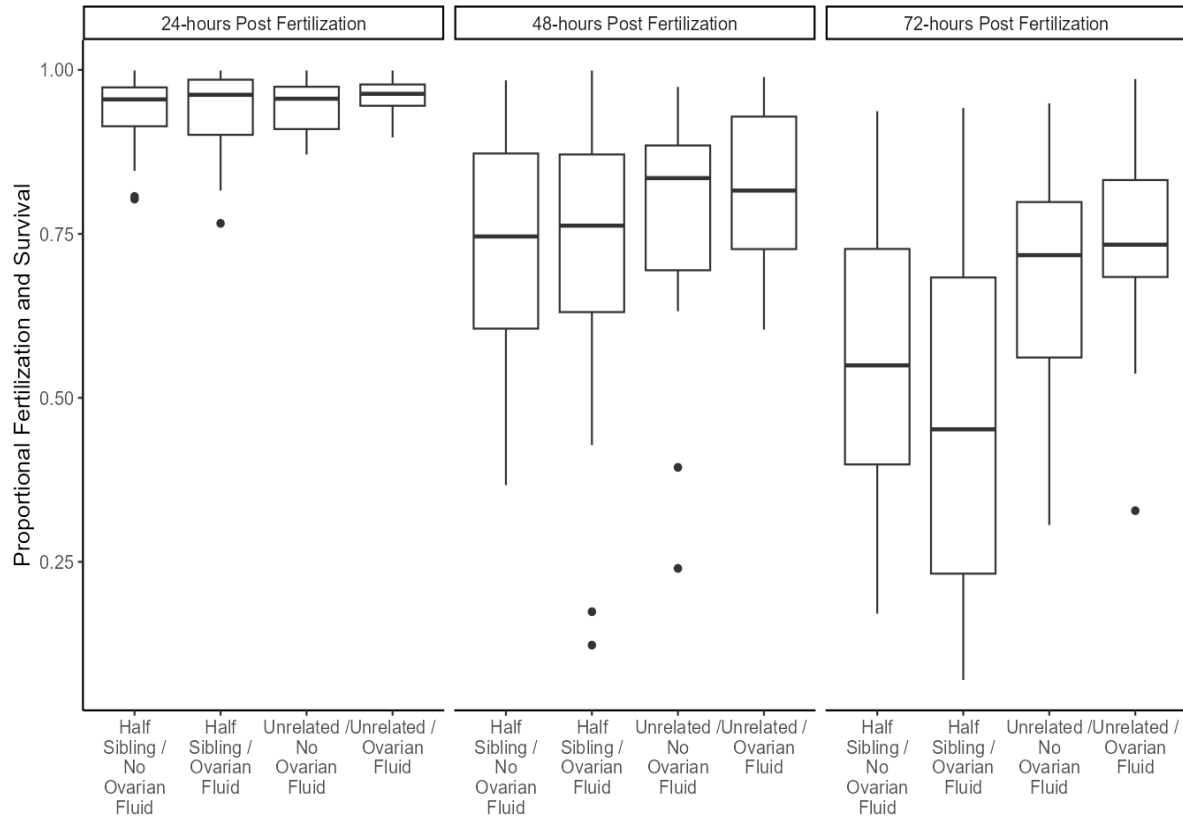


Figure 2.4 Proportional fertilization of lake sturgeon eggs as a function of male:female relationship and ovarian fluid unrinsed:rinsed at 24-hours post-fertilization, and proportional survival of eggs after 48-hours, and 72-hours post-fertilization.

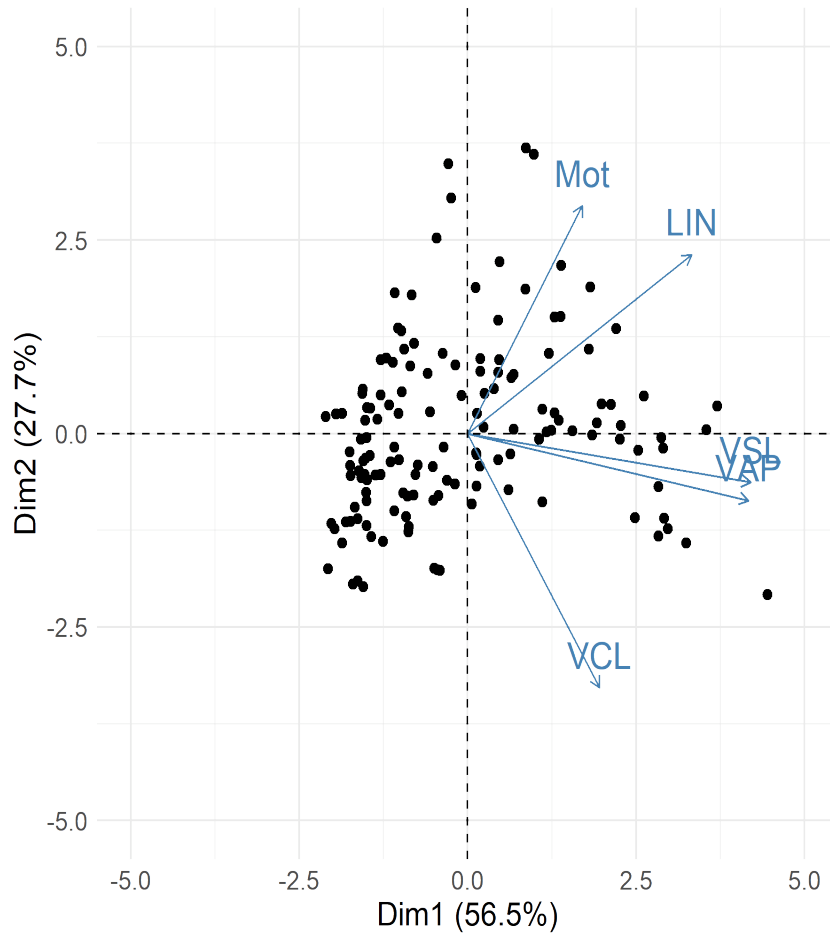


Figure 2.5 Biplot of Principal Components 1 and 2, which cumulatively explain 84.7% of variability associated with sperm quality based on computer assisted sperm analysis.

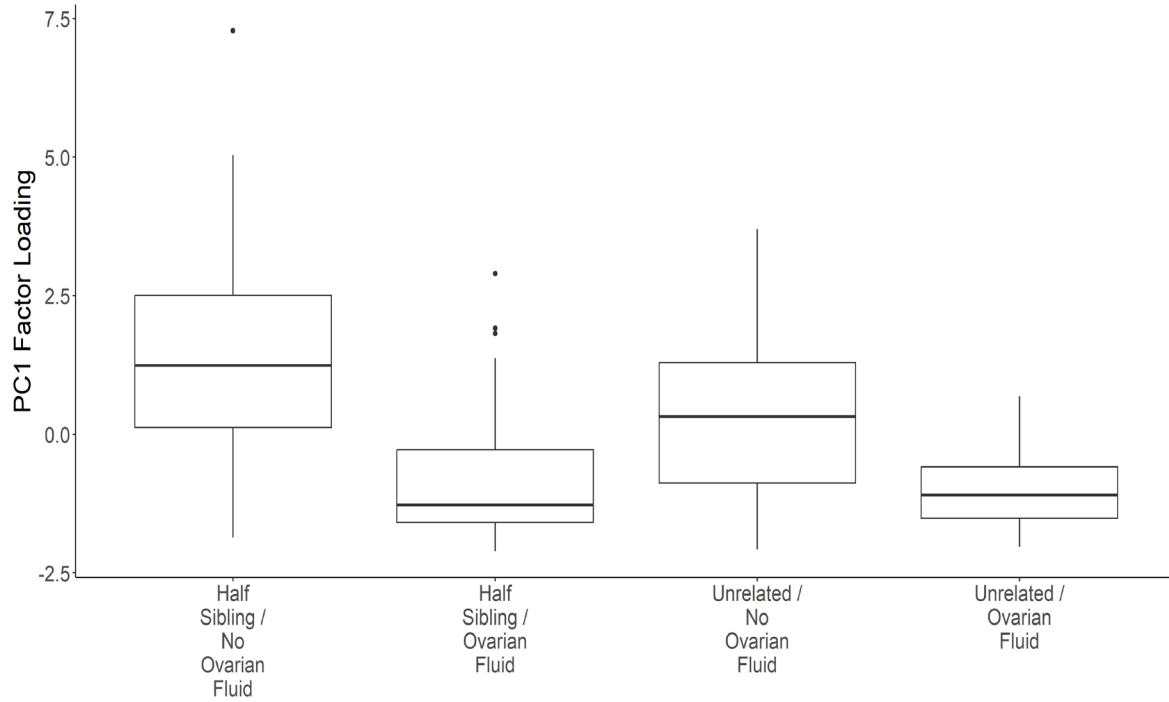


Figure 2.6 Sperm quality on principal component one of lake sturgeon sperm from related and half sibling males when activated in river water and when activated in a 50:50 mixture of river water and ovarian fluid.

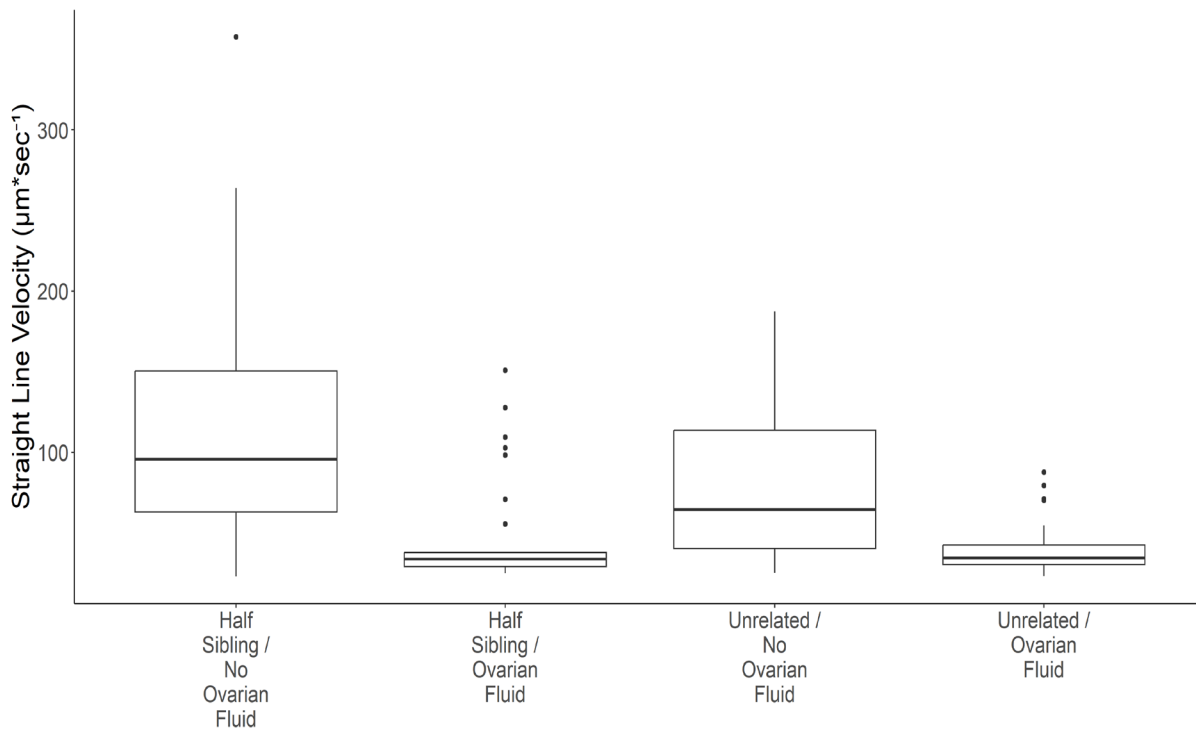


Figure 2.7 Straight line velocity of lake sturgeon sperm from related and half sibling males when activated in river water and when activated in a 50:50 mixture of river

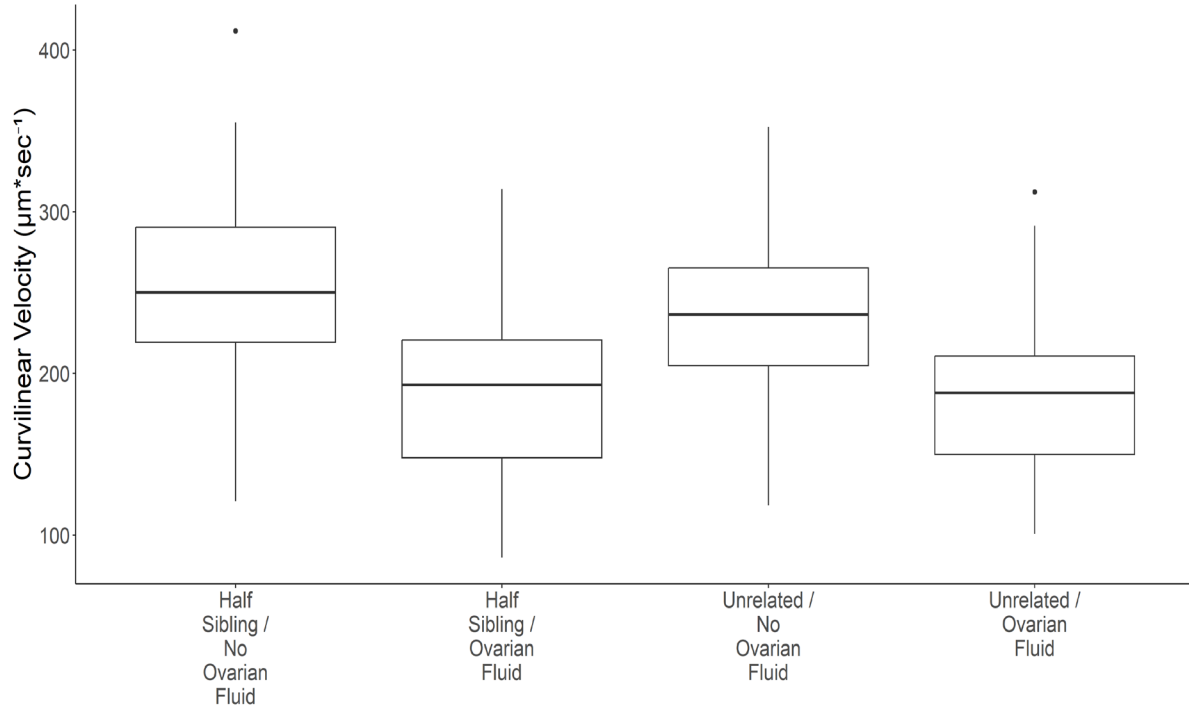


Figure 2.8 Curvilinear velocity of lake sturgeon sperm from related and half sibling males when activated in river water and when activated in a 50:50 mixture of river

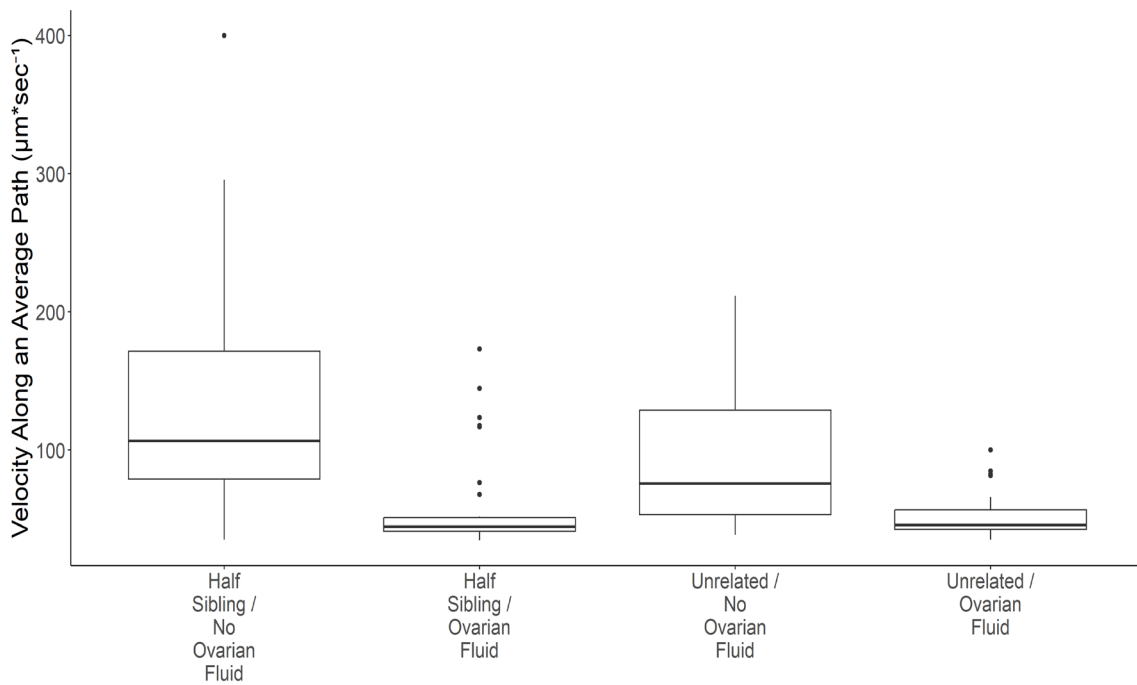


Figure 2.9 Average path velocity of lake sturgeon sperm from related and half sibling males when activated in river water and when activated in a 50:50 mixture of river