

Riverine characteristics and adult demography influence female lake sturgeon (*Acipenser fulvescens*) spawning behavior, reproductive success, and ovarian quality

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Abstract: Abiotic conditions and demographic factors can influence the timing of spawning. Behavioral plasticity allows females to select spawning conditions that are conducive to offspring development; however, reproductive costs associated with delaying spawning are not well understood. In this study, factors influencing timing of female spawning, residence time (RT), and reproductive success (RS) during two seasons were determined, and plasma testosterone concentrations were used to quantify atretic rates in a wild, lake sturgeon (*Acipenser fulvescens*) population. For the 123 females monitored, RT ranged from 1 to 23 days and was influenced by arrival date, temperature, discharge, and male number, with the latter having the largest influence. RS varied due to arrival date, temperature, discharge, male number, male length, and operational sex ratio. Two females had testosterone levels indicative of atresia and RS estimates lower than yearly means; however, most females had normal ovaries, suggesting little reproductive costs of plasticity in spawning ground residency time. Results demonstrate the multitude of factors influencing female reproductive behavior and RS, highlighting the importance of abiotic and demographic conditions to recruitment in wild populations.

Résumé : Des conditions abiotiques et des facteurs démographiques peuvent influencer le moment du frai. Si la plasticité comportementale permet aux femelles de choisir des conditions de frai favorables au développement de leur progéniture, les coûts de reproduction qu'entraîne le fait de retarder le frai ne sont pas bien compris. Nous avons déterminé les facteurs qui influencent le moment du frai, le temps de résidence (TR) et le succès de reproduction (SR) de femelles durant deux saisons, et les concentrations plasmatiques de testostérone ont été utilisées pour quantifier les taux d'atrésie dans une population sauvage d'esturgeons jaunes (*Acipenser fulvescens*). Pour les 123 femelles suivies, le TR allait de 1 à 23 jours et était influencé par la date d'arrivée, la température, le débit et le nombre de mâles, ce dernier facteur exerçant la plus grande influence. Le SR variait selon la date d'arrivée, la température, le débit, le nombre de mâles, la longueur des mâles et le rapport des sexes opérationnel. Si deux femelles présentaient des concentrations de testostérone témoignant d'une atrésie et des SR estimés plus faibles que les moyennes annuelles, la plupart des femelles avaient des ovaires normaux qui indiqueraient de faibles coûts de reproduction associés à la plasticité du temps de résidence dans le lieu de frai. Les résultats démontrent qu'une multitude de facteurs influencent le comportement et le succès de reproduction des femelles, soulignant l'importance de conditions abiotiques et démographiques pour le recrutement dans des populations sauvages. [Traduit par la Rédaction]

Introduction

Identifying factors influencing timing of reproduction, residency time on spawning grounds (RT), and reproductive success (RS) are essential for understanding the biological requirements and selective pressures acting on wild fish populations (Dickerson et al. 2002; Seitz et al. 2005; Wheeler et al. 2016). Although timing of reproduction has a heritable basis (Neuheimer and MacKenzie 2014), individuals show behavioural plasticity in response to environmental cues, such as decisions about when and where to reproduce (Warren and Morbey 2012; Mittelbach et al. 2014). For fishes that provide no parental care after spawning, exogenous conditions experienced while on the spawning grounds determine fertilization success as well as the abiotic conditions and predation pressure fertilized eggs and larvae will encounter (Lowerre-Barbieri et al. 2016). Thus, plasticity in the timing of

spawning allows females to select environments that are conducive to offspring survival and growth, thereby increasing RS (number of offspring).

Extensive research on reproductive behaviors in marine and freshwater fishes, including salmonids and sticklebacks, has shown that biological traits (e.g., age, body size) as well as demographic characteristics such as the operational sex ratio (OSR) influence the timing of female reproduction and RS (Emlen and Oring 1977; Jørgensen et al. 2008; Wright and Trippel 2009; Lowerre-Barbieri et al. 2011; Conte and Schluter 2013). For some species, older, larger females spawn earlier (Wright and Trippel 2009) and produce larger, higher-quality eggs and larvae than smaller females (Schreck et al. 2001; Esteve 2005). Additionally, females often exhibit mate selectivity based on male traits (e.g., body size, behavior) directly by preferentially spawning with certain males or

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indirectly by delaying spawning until higher-quality males are present (Foote and Larkin 1988; Fleming and Gross 1994; Blanchfield and Ridgway 1999; Esteve 2005). When the OSR is male-biased, females are predicted to be more selective of mates (Jirotkul 1999) as long as selectivity is not costly (Labonne et al. 2009). However, as the OSR becomes female-skewed, females are predicted to be less choosy, spawning with any available males (Passos et al. 2014).

In long-lived iteroparous species (e.g., sturgeon), adults decide how much to invest in current reproductive activities based on expectations of future RS (Pianka and Parker 1975). With multiple reproductive opportunities over their lifetime, female investment in any one reproductive event is expected to be low given that failure in 1 year will have little overall influence on a female's lifetime fitness (Warner 1998; Peterson et al. 2007). Additionally, female age can influence breeding decisions, as younger females may be more willing to forgo reproduction 1 year because they will have a comparatively greater number of future reproductive opportunities than older females (Pianka and Parker 1975). However, females at any age can exhibit preferences for particular sites or mates if selectivity does not impact female survival (Warner 1998). For sturgeon, empirical work examining individual preferences during spawning have been rare, partially due to the fact that sturgeon typically migrate from oceans or large lakes to rivers and spawn with numerous mates in fast-flowing habitats where observations are difficult (Auer 1996a; Billard and Lecointre 2001). However, studies on populations have shown that environmental conditions on the spawning grounds can influence the timing of reproduction, ovarian maturation, and egg viability (Dettlaff et al. 1993; Forsythe et al. 2012; Thiem et al. 2013).

Female ovulation is largely driven by exogenous cues such as water temperature and social interactions within spawning sites (Webb et al. 2001; Lowerre-Barbieri et al. 2011; Morgan et al. 2013). In contrast with teleost fishes where egg deposition occurs within days of ovarian maturation and ovulation (Bobe et al. 2008; Warren and Morbey 2012), sturgeon can maintain post-vitellogenic ovaries for months before spawning (Webb et al. 1999). Prolonged periods between ovulation and egg deposition allow females the opportunity to be selective in their choice of mates and spawning locations (Brown-Peterson and Heins 2009). However, extended periods of residence on spawning grounds and delays in spawning have been shown to decrease ovarian quality and egg viability in walleye (*Sander vitreus*) and Atlantic salmon (*Salmo salar*) (Johnston et al. 2008; Thorstad et al. 2008) as well as sturgeon (Khoroshko 1972), thereby increasing the probability of a female's ovaries becoming atretic (i.e., the degenerating and reabsorption of follicles; Lubzens et al. 2010). Atresia and spawning failure have been readily observed in cultured white sturgeon (*Acipenser transmontanus*; Webb et al. 2001) when temperatures were elevated prior to spawning. However, the examination of female ovaries and quantification of follicular atresia rates (which serve as a measure of egg quality) have been difficult in wild populations given that a portion of the ovary must be surgically biopsied for examination of the oocytes (Servid et al. 2011).

Alternatively, researchers have developed several noninvasive methods for detecting early signs of follicular atresia, including Fourier transform infrared spectroscopy, short wavelength near infrared spectroscopy, and quantification of plasma sex steroids (Lu et al. 2011; Servid et al. 2011; Talbott et al. 2011; Webb et al. 2017). Using plasma sex steroid concentrations, researchers quantify concentrations of both testosterone (T) and estradiol-17 β (E2), which play a vital role in egg maturation and vitellogenesis, and have commonly been used to distinguish sex and assign reproductive status in sturgeon (Webb et al. 2002; Craig et al. 2009; Shaw et al. 2012; Wheeler et al. 2016). Both E2 and T are typically elevated in prespawning females, but show substantial decreases after a female has spawned or during late vitellogenesis in fish with follicular atresia. Using logistic regression modeling based

on T concentrations, Talbott et al. (2011) were able to correctly identify 95% of females with normal ovaries and 93% of females with atretic ovaries in cultured white sturgeon, indicating that T concentration can be a reliable indicator of ovary quality when observational data are unavailable. However, quantification of plasma T concentrations and application of the logistic regression modeling to determine ovary quality and atretic rates has not yet been published for a wild population of spawning sturgeon.

Given the importance of understanding factors influencing spawning behavior and recruitment in imperiled species, this study was conducted to understand what variables affect an individual's timing of spawning, reproductive success, and ovary quality in a wild population of lake sturgeon (*Acipenser fulvescens*). Lake sturgeon are potamodromous fish that are endemic to the Great Lakes and Mississippi River system (Auer 1996a; Bruch et al. 2016). Males typically spawn every 2 years and females every 3–5 years with high repeatability in the timing of spawning (Forsythe et al. 2012; Thiem et al. 2013). Spawning is multimodal with adults migrating to spawning grounds from late April to early June when water temperature is between 8.8 and 21.1 °C (Bruch and Binkowski 2002; Forsythe et al. 2012). Males arrive on the spawning grounds earlier than females and begin spermiating once females are present. Females are believed to spawn shortly after arrival on the spawning grounds (Bruch and Binkowski 2002; Peterson et al. 2007), but females have been observed staging on the grounds and delaying spawning under unfavorable conditions (Auer 1996b; Thiem et al. 2013; Bruch et al. 2016). Additionally, females have been observed bypassing suitable spawning habitat to utilize more upstream sites (Bruch and Binkowski 2002; Forsythe et al. 2012; Bruch et al. 2016).

Spawning is polygynous and polyandrous as adults spawn in large and small groups and in numerous bouts, which last approximately 1–2 min each (Bruch and Binkowski 2002; Peterson et al. 2007). Female selectivity for mates has not been well-analyzed for lake sturgeon, but subtle differences in reproductive success estimates among males due to body size has been documented (Duong 2010). Females release adhesive, demersal eggs into the water column over cobble and gravel substrate, which are then fertilized by milt from one to several males (Thiem et al. 2013). The fertilized eggs incubate without parental care until hatch (Duong et al. 2011). Hatched larvae remain at the spawning grounds, absorbing endogenous yolk-sac reserves for approximately 6–15 days dependent on water temperature before leaving the spawning grounds to disperse downstream and begin exogenously feeding (Smith and King 2005; Duong et al. 2011).

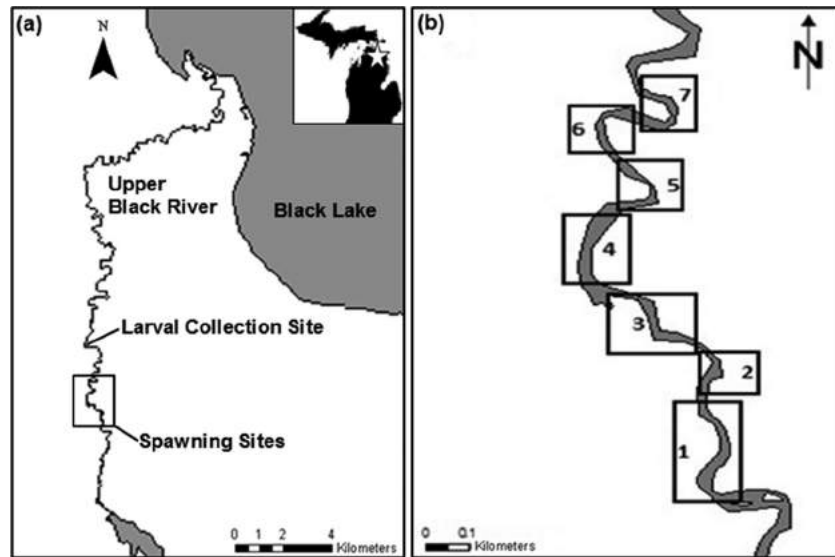
The objectives of this study were to: (i) examine how environmental conditions (temperature and discharge), biological traits (body size, experience, timing of arrival onto the spawning grounds), and demographic factors (number of males present, size of males, and OSR) influenced female time spent on the spawning grounds (RT), (ii) determine whether female RS quantified using larval genetic parentage analysis varied due to these biological, environmental, and demographic factors, (iii) quantify the ovary quality and atretic rates of prespawning females using plasma concentrations of testosterone, and (iv) determine whether a female's RT affected her RS and probability of atresia. Male spawning behaviour was not assessed given that female lake sturgeon determine the timing, location, and duration of spawning (Bruch and Binkowski 2002). However, male RS was estimated using genetic parentage analysis to determine whether female lake sturgeon exhibit mate choice based on male body size.

Materials and methods

Study site

The study was conducted in 2012 and 2013 on the Upper Black River (UBR) in Cheboygan County, Michigan (Fig. 1a), under animal use and care procedure No. 03/14–042–00 from the Michigan State University Institutional Animal Care and Use Committee. Adult

Fig. 1. (a) The study site on the Upper Black River located in Cheboygan County, Michigan. Adult *Acipenser fulvescens* were sampled and observed daily on the spawning grounds in 2012 and 2013, and larvae were collected downstream of the spawning sites to genetically determine parentage and estimate female reproductive success. (b) An enlarged view of the seven spawning locations where adult lake sturgeon were sampled and observed daily in 2012 and 2013.



lake sturgeon migrate from Black Lake into the UBR and spawn at seven sites located along a 1.5 km stretch of the river (Fig. 1b). Early-arriving females typically utilize upstream sites that are inaccessible or not utilized as frequently by late-arriving females later in the season (Forsythe et al. 2012). Migration into the UBR and timing of spawning is dependent on temperature and discharge, as spawning typically begins when temperatures are 10 °C or higher and river discharge is decreasing after the spring runoff period (Forsythe et al. 2012). Wadable, shallow spawning sites (~1–3 m) of the UBR allow daily access to nearly all adults during the spawning season. Additionally, the presence of a streamside facility allows for the collection and rearing of wild-caught, larval lake sturgeon for genetically determining adult RS estimates.

Adult sampling

In 2012 and 2013, adult lake sturgeon were captured daily among the seven spawning sites by snorkelers or individuals in waders using long-handled dip nets. Total length (cm) and site of capture were recorded for each individual. Predicted sex was determined by applying pressure to the abdomen to expel gametes or by examining the shape of the urogenital opening. The urogenital opening can differ between sexes in sturgeon (Vecsei et al. 2003), but is often used in conjunction with other traits (e.g., total length or observed girth) to predict fish sex. Each fish was individually marked with T-bar anchor (floy) tags (Floy Tag, Inc.) of individual-specific color combinations at the base of the dorsal fin designating predicted sex, identification number, and the timing of entry on the spawning grounds. Fish were checked for internal passive integrated transponder (PIT) tags from previous years located near the dorsal scutes using hand-held scanners (Biomark, Inc.). Fish sampled for the first time were injected with a PIT tag, and the number was recorded. PIT tag retention is approximately 90%–95% in sturgeon species (Forsythe et al. 2012; Hamel et al. 2012), making them ideal tags for identifying individuals during long-term studies (Clugston 1996). Recorded PIT tag numbers were compared with a long-term database collected from 2001 to 2011 (data not shown) to determine whether females had been captured in previous years or were being captured for the first time as a measure of spawning experience. Female age was not empirically determined given that traditional aging techniques have

been shown to produce unreliable estimates of age as fish increase in size (Bruch et al. 2009; Shaw et al. 2012).

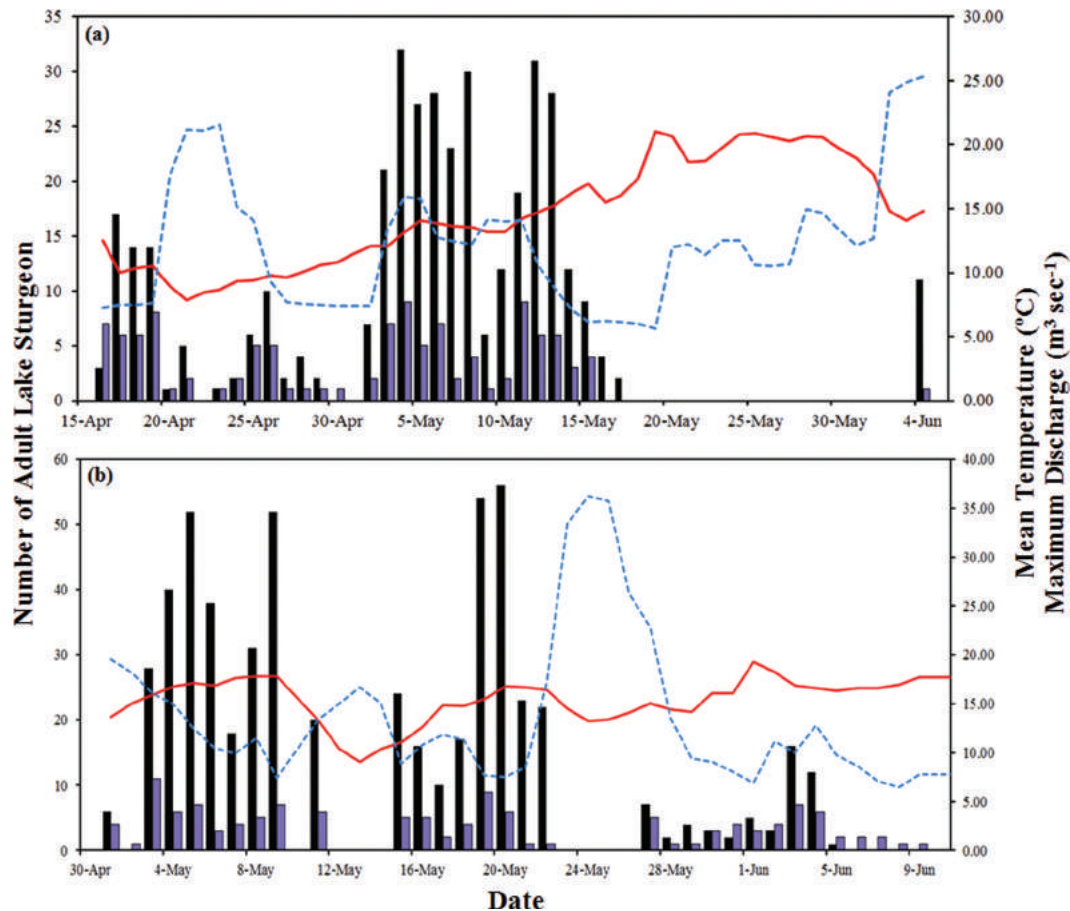
The total number of males captured with each female and the OSR were quantified daily at each spawning site. Presumed spawning date for each female was assigned based on visual observation of egg expulsion or as the last date observed on the spawning sites given that females leave the spawning grounds within 12 h from the start of egg expulsion (Bruch and Binkowski 2002). RT (measured in days) was quantified for each female as date of first capture on the spawning grounds until presumed spawning date. Water temperature (°C) and discharge ($m^3 \cdot s^{-1}$) were measured hourly at the spawning sites throughout the spawning season using Onset HOB0 pressure loggers (Cape Cod, Massachusetts, USA; Fig. 2) to determine daily means, minimums, and maximums.

Blood collection and plasma hormone analysis

In 2013, blood was successfully collected from the caudal vein of 38 fish predicted to be female using 3 mL syringes and 22-gauge needles coated in 1000 units of heparin per millilitre. Only females that had not already spawned (i.e., did not show a loss of girth or depressions in the abdomen due to the expulsion of eggs) were sampled for blood. Prespawn females that were present on the spawning grounds for more than 1 day were captured multiple times, and additional blood samples were taken. Syringes were placed on ice and transported back to the streamside facility. Blood was divided into two 1.5 mL microcentrifuge tubes and centrifuged at 1500g force for 15 min. Plasma was extracted and placed in new microcentrifuge tubes. Tubes were immediately stored at -20 °C until hormone analysis was conducted.

Steroids (T and E2) were extracted from plasma following the method of Fitzpatrick et al. (1987) for analysis by radioimmunoassay. Briefly, 100 μ L of plasma were extracted twice with 2 mL of diethyl ether. Tubes were vortexed vigorously with ether, and the aqueous phase was removed by snap-freezing in liquid nitrogen. Ether was allowed to evaporate overnight under a chemical hood; the next day extracted steroids were resuspended in 1 mL of phosphate-buffered saline with gelatin (PBSG), and 10 or 50 μ L were assayed for each steroid depending on the concentration of steroid in the sample. Recovery efficiencies for all steroids were determined by adding titrated steroids to tubes containing

Fig. 2. Hourly mean water temperature (solid red line), mean maximum discharge (dashed blue line), and the total number of adult male (black bars) and female (light purple bars) *Acipenser fulvescens* observed daily on the spawning grounds of the Upper Black River of Michigan in (a) 2012 and (b) 2013. [Colour online.]



plasma ($N = 4$), which were extracted as described above. All steroid assay results were corrected for recovery. Recovery efficiencies for T ranged between 89% and 94%, with a mean of 92%. Estradiol recovery efficiency was 71%.

Plasma concentrations of T and E2 were measured by radioimmunoassay as described in Fitzpatrick et al. (1986) modified by Feist et al. (1990). All samples were analyzed in duplicate. A slightly more concentrated charcoal solution (6.25 g charcoal and 4.0 g dextran per litre PBSG) was used for all assays to reduce nonspecific binding. The intra- and interassay coefficients of variation for all assays were less than 5% and 10%, respectively. Steroid levels were validated by verifying that serial dilutions were parallel to standard curves. The lower limit of detection was 0.10 ng·mL⁻¹ for T and 0.16 ng·mL⁻¹ for E2. Samples with concentrations below the detection limit ($N = 1$ for E2) were given half the value of the detection limit.

Larval fish collection for quantifying RS

Larval lake sturgeon were sampled approximately 2 km downstream of the spawning sites (Fig. 1a). Sampling began 10 days after the first observed spawning date and ended approximately 15–19 days after the last adult sturgeon was observed on the spawning grounds when no sturgeon larvae were captured for at least two consecutive nights. Sampling was conducted at night when larvae disperse downstream, actively searching for food (Auer and Baker 2002). Larvae were collected using five D-frame nets placed evenly across the river. Nets were checked hourly from 2100 to 0200 h. Captured larvae were transported to the streamside facility and reared separately by capture night for approximately 3 months until individuals were large enough for nonlethal tissue sampling

for genetic analysis. Larvae that died during the rearing period were preserved in 95% ethanol and kept separate by capture night for genetic analysis.

Genetic analysis

Due to a high number of larvae collected in 2012, approximately 10% of the dead and 10% of the live larvae from each individual capture night were subsampled and genotyped for parentage analysis. Subsampling by individual capture night allowed for the entire spawning run to be represented and for genotyping of larvae from potentially every female spawner monitored in the study. In total, $N = 820$ larvae were genotyped in 2012. In 2013, all live and dead larvae collected during the larval sampling period ($N = 845$) were genotyped due to low numbers of captured drifting larvae, which can be highly variable between spawning years. DNA was extracted from larval fin clips using DNeasy(R) extraction kits (QIAGEN, Inc.) and quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.). Larvae were genotyped at 12 microsatellite loci: 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, and AfuG112 (Welsh et al. 2003). Polymerase chain reaction and genotyping were conducted using methods described in Duong et al. (2011) with cycle number increased to 35 and 1.5 μ L of forward and reverse primer used to increase genetic signals. Two experienced lab personnel assigned genotyping scores to all gels. A 10% subset of the samples were re-genotyped to empirically estimate genotyping error, which was calculated as the number of allelic errors divided by the total number of alleles.

Parentage analysis

Genotypic data were used to assign parentage using two software programs, CERVUS 3.0 (Kalinowski et al. 2007) and COLONY 2.0.5.8 (Jones and Wang 2010), given that combining the output from more than one parentage program is a recommended practice (Walling et al. 2010). CERVUS is the most commonly used parentage analysis program and assigns the most likely parent as the individual with the highest natural log of the likelihood-odds ratio score (Jones et al. 2010). COLONY assigns offspring into full and half-sibling families before identifying the most likely candidate parent (Jones et al. 2010). Both programs use maximum likelihood and account for empirical estimates of genotyping errors. Assignment of the most likely parent pair in the study was based on concordance between the two programs.

In CERVUS, simulations included 10 000 offspring with a strict level of 95% and a relaxed level of 80% to determine the confidence in parentage assignment based on population estimates of allelic frequencies. Empirical allelic and genotypic frequencies were used to determine whether loci were in Hardy–Weinberg equilibrium. In COLONY, settings included polygamy for both sexes, no inbreeding, full-likelihood analysis, and medium-length runs. Parentage analysis in CERVUS and COLONY included genotypic data for 243 adults in 2012 and 262 adults in 2013, an adult capture rate of 85% based on a PIT tag study (K. Scribner, data not shown), and empirical genotyping error estimates of 0.97% and 0.54% for 2012 and 2013, respectively. Adults with the highest, positive likelihood-odds ratio score in CERVUS and highest maximum likelihood score in COLONY were chosen as the most likely candidate parents. RS was quantified for every female and male as the total number of offspring assigned to that individual. The larval genotypic data from both years were also analyzed in COLONY without the candidate parent genotypes to estimate the effective number of breeding adults (N_b) for comparison with the estimated census spawning population size based on a 85% adult capture rate from the PIT tag study.

Statistical analysis

Analyses were performed using the packages “glmmADMB” (Skaug et al. 2006) and “car” (Fox and Weisberg 2011) implemented in the statistical program R (R Development Core Team, version 3.2.3). Data from 2012 and 2013 were analyzed separately given the differences observed in environmental conditions and proportion of larvae genotyped. All variables were tested for normality using Shapiro–Wilk tests. Given the lack of normality, generalized linear models were used to determine how female RT and RS varied as a function of biological, demographic, and abiotic factors.

Biological characteristics used in analyses for RT and RS included female total length, female spawning experience — a factor coded as “experienced” (captured in previous years) or “inexperienced” (first time capture), and date of initial arrival onto the spawning grounds. Due to the number of observations, initial arrival dates onto the spawning grounds were coded as categorical blocks of time ranging from 4 to 13 days. Thus, females that arrived sequentially on the spawning grounds were grouped together in the same blocks. Time blocks were differentiated by gaps when no new females entered the spawning grounds for 3 or more consecutive days, which is typical given the asynchronous spawning times of female lake sturgeon (Forsythe et al. 2012; Fig. 2). In 2012, females were grouped into three blocks based on initial arrival date: “A”, arrived between 16 and 25 April; “B”, between 2 and 15 May; and “C”, arrived on 4 June. In 2013, females were grouped into four blocks based on initial arrival dates: “A”, arrived between 1 and 11 May; “B”, between 15 and 23 May; “C”, between 28 and 31 May; and “D”, on 3–4 June.

Demographic characteristics of spawning adults used in analyses included the mean OSR, mean number of males present with the female, and mean total length of males present with the female while on the spawning grounds. Abiotic factors included the daily mean, minimum, maximum, and range in both temperature and discharge. Range in temperature and discharge were quantified as the daily maximum minus the daily minimum. The means of each variable were calculated independently for each female from date of first capture to presumed spawning date. All continuous explanatory variables were standardized prior to analysis to have a mean of zero and standard deviation of one to allow for direct comparisons between variables.

Tests for multicollinearity between variables were conducted by fitting a full model including all biographical, demographic, and abiotic factors, quantifying the variance inflation factor (vif), and calculating the Pearson’s correlation (r) between pairs of variables. Minimum and maximum temperature were removed from the model due to evidence of multicollinearity ($vif > 7$) and high correlation ($r > 0.7$) with mean temperature. Additionally, mean and minimum discharge were removed due to high correlations ($r > 0.7$) with maximum discharge. Maximum discharge was included in the model given that results were roughly equivalent to mean discharge, and increases in discharge have been shown to influence the number of days female lake sturgeon spend on the spawning grounds (Auer 1996b). Initial arrival date (block) was removed from the model due to evidence of multicollinearity with temperature and discharge ($vif > 7$).

For female RT, a negative binomial regression with a log link function was fit as

$$(1) \quad RT = \text{MeanTemperature} + \text{RangeInTemperature} + \text{MaximumDischarge} + \text{RangeInDischarge} + \text{FemaleTotalLength} + \text{FemaleExperience} + \text{MeanMaleTotalLength} + \text{MeanNumberofMales} + \text{OSR} + \text{two-way interactions}$$

where RT is residence time and all possible two-way interactions of biological relevance were tested (Burnham and Anderson 2002). Negative binomial error distributions were used in all models instead of Poisson distributions given the overdispersion in RT and RS. A stepwise approach was used by eliminating one variable at a time starting with the variables of predicted least biological significance given previous research on the UBR. Likelihood ratio tests were used to determine the model of best fit (Table 1). The same full model with eq. 1 was fit with female RS as the response variable, but with RT included as an additional explanatory variable.

Nonparametric, Wilcoxon–Mann–Whitney tests (WMW; Siegel and Castellan 1988) were used to determine whether RS and variables that were significant predictors of RT and RS differed between females that stayed in the river for 1 day versus those that were present for greater than 1 day (refer to online Supplemental material, Table S1¹). Given that “block” was removed from the negative binomial regression due to multicollinearity with temperature and discharge, the effect of initial arrival date onto the spawning grounds on RT and RS was assessed using WMW tests (Table S2¹). Additionally, variables that were significant predictors

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2018-0141>.

Table 1. Coefficient estimates and standard errors (SE) from the negative binomial regressions of best fit showing the expected change in the log of mean residence time (RT) and log of reproductive success (RS) due to riverine characteristics for spawning female *Acipenser fulvescens* monitored in 2012 and 2013.

Trait	Characteristic	Estimate	SE
2012			
RT	Intercept	1.10	0.11
	Mean temperature	-0.37	0.10
	Maximum discharge	-0.24	0.13
	Mean number of males	-0.02	0.12
	Maximum discharge × mean number of males	-0.67	0.17
	Theta (dispersion parameter)	3.70	1.40
RS	Intercept	2.35	0.09
	Mean temperature	0.35	0.09
	Maximum discharge	0.05	0.10
	Mean number of males	0.31	0.11
	Mean operational sex ratio	-0.33	0.11
	Maximum discharge × mean number of males	-0.37	0.15
	Theta (dispersion parameter)	3.45	0.90
2013			
RT	Intercept	1.18	0.12
	Mean temperature	-0.34	0.12
	Maximum discharge	-0.32	0.13
	Mean number of males	-0.54	0.13
	Maximum discharge × mean number of males	0.29	0.12
	Theta (dispersion parameter)	2.14	0.61
RS	Intercept	2.23	0.08
	Mean temperature	0.22	0.09
	Maximum discharge	0.04	0.09
	Mean number of males	0.44	0.10
	Mean operational sex ratio	-0.40	0.11
	Mean male total length	0.31	0.09
	Theta (dispersion parameter)	3.71	0.92

Note: A times symbol (×) indicates an interaction between variables.

Table 2. Number of female *Acipenser fulvescens* (N), mean female total length (TL), mean residence time (RT), reproductive success (RS), mean temperature (MT), maximum discharge (MD), mean number of males (NM), mean total length of males (LM) present with the females, and the operational sex ratio (OSR) compared among individuals in each arrival block in 2012 and 2013.

Arrival block	N	TL (cm)	RT (days)	RS (larvae)	MT (°C)	MD (m ³ ·s ⁻¹)	NM	LM (cm)	OSR
2012									
A: April	20	168.00 (10.63)	5.00 (4.24)	12.44 (4.09)	10.25 (0.91)	10.43 (3.55)	4.23 (3.40)	154.16 (14.10)	1.94 (1.41)
B: May	41	170.83 (12.81)	1.95 (2.10)	6.65 (8.70)	13.97 (1.11)	12.70 (2.78)	9.85 (6.12)	155.15 (10.94)	3.44 (1.67)
C: June	1	174.00 (*)	1.00 (*)	2.00 (*)	14.81 (*)	25.33 (*)	6.00 (*)	157.88 (*)	6.00 (*)
2013									
A: early May	36	170.94 (13.35)	3.69 (4.93)	11.14 (8.44)	15.60 (1.66)	13.73 (3.22)	8.08 (4.32)	151.62 (12.47)	3.26 (2.79)
B: mid-May	15	171.13 (14.52)	1.47 (1.06)	10.13 (9.52)	15.26 (1.80)	9.86 (3.24)	9.30 (3.54)	161.39 (12.37)	4.90 (2.97)
C: late May	7	174.71 (16.45)	7.57 (4.83)	9.14 (5.43)	16.74 (1.12)	11.89 (4.89)	4.06 (2.37)	160.75 (12.78)	1.38 (0.64)
D: June	3	167.67 (9.61)	1.00 (0.00)	23.00 (10.58)	16.61 (0.26)	10.97 (1.60)	11.00 (1.73)	150.67 (1.94)	2.56 (1.54)

Note: Females were categorized into arrival blocks based on initial arrival dates on the spawning grounds of the Upper Black River. Estimates are provided as mean (± standard deviation).

*SD estimate could not be determined due to sample size (N).

of RT and RS (Table 2) were compared among blocks of females using WMW tests (Table S2¹) to determine whether females experienced different conditions throughout the season due to the multimodal spawning pattern of lake sturgeon. Mean female total length was compared between blocks using *t* tests (Table S2¹).

For the 38 fish where blood samples were taken, plasma concentrations of T and E2 were used to verify female sex assignment for 15 fish that (i) could not be verified based on the long-term data collected from 2001 to 2011, (ii) could not be identified based on spawning behaviour or expression of gametes, or (iii) were captured on the spawning grounds for the first time. Sex assignment was verified using discriminant function analysis using E2 and T concentrations developed for white sturgeon by Webb et al. (2002) and recently applied to Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) (Wheeler et al. 2016) and lake sturgeon (Craig et al.

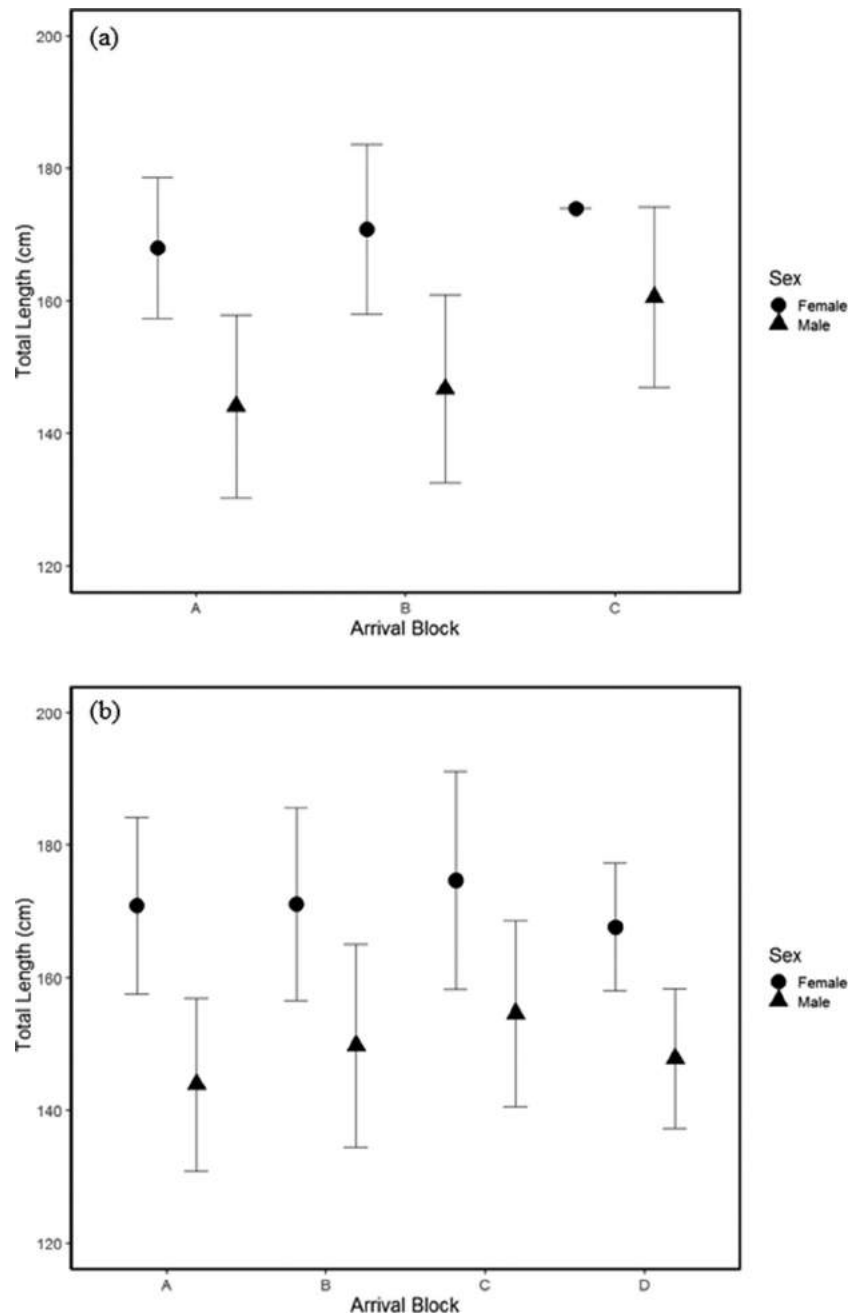
2009; Shaw et al. 2012; Thiem et al. 2013). For all verified females where blood samples were taken, log-transformed concentrations of T were used to determine the probability of the female having normal versus atretic ovaries using the logistic regression equation developed and discussed in Talbott et al. (2011).

Results

Spawning ground demographic and stream physical data

Adult lake sturgeon were observed on the spawning grounds between 16 April and 4 June in 2012 and between 1 May and 9 June in 2013. Adults were present at all seven spawning sites throughout the season except for the group of fish in 2012 that arrived on 4 June, which were only observed at site 5. Mean river water temperature on the first day adults were present was 13 °C in both

Fig. 3. Mean total lengths and standard deviations for adult female (circles) and adult male (triangles) *Acipenser fulvescens* present on the spawning grounds of the Upper Black River of Michigan in (a) 2012 and (b) 2013. Adults were arranged into blocks based on arrival date on the grounds. There were three blocks in 2012 (A: April, B: May, and C: June) and four blocks in 2013 (A: early May, B: mid-May, C: late May, and D: June).



years; however, maximum discharge varied at approximately $7.0 \text{ m}^3 \cdot \text{s}^{-1}$ in 2012 (Fig. 2a) and $20.0 \text{ m}^3 \cdot \text{s}^{-1}$ in 2013 (Fig. 2b). In both years, females were typically larger than males regardless of arrival date (block) on the spawning grounds (Fig. 3), ranging from 135 to 198 cm. Overall, mean (\pm SD) total length of females was $169.97 (\pm 12.04)$ cm and $171.26 (\pm 13.61)$ cm in 2012 and 2013, respectively. Males captured with females ranged in total length from 127 to 182 cm in both years with overall means of $154.89 (\pm 11.81)$ cm in 2012 and $155.02 (\pm 12.83)$ cm in 2013. The number of males captured with a female ranged from one to nine males with a mean of $7.97 (\pm 5.93)$ in 2012 and a range of one to 13 males with a mean of $8.06 (\pm 4.15)$ males in 2013. There was no relationship observed between the mean number of males present with a female and female total length (Fig. S1¹).

In both years, the OSR for the entire season was skewed towards males, with 184 males to 62 females captured on the spawning grounds in 2012 and 210 males to 61 females captured in 2013. At the individual spawning sites, the overall mean OSR was $3.00 (\pm 1.77)$ in 2012 and $3.42 (\pm 2.80)$ in 2013. The mean OSR was lowest in 2012 for females that arrived on the grounds at the beginning of the season in April (Block A, Table 2) given that more females were arriving together. Females that arrived in May (Block B) were observed with more males (Table 2) and had a mean OSR that was significantly different from Block A (WMW test, $W = 150.5$, $p < 0.001$). Only one female arrived in June (Block C) during 2012, precluding the ability to statistically compare with the other blocks. Conversely, the mean OSR in 2013 was largest for females that arrived in early May (Block A) and mid-May (Block B, Table 2),

though they were significantly different from each other (WMW test, $W = 158$, $p = 0.021$). Females that arrived in late May (Block C) had the lowest mean OSR, which was significantly different from Block A (WMW test, $W = 188.5$, $p = 0.040$) and Block B (WMW test, $W = 94$, $p = 0.004$). The females that arrived in June (Block D) had more males present and a higher mean OSR than those in Block C (Table 2), but the OSR was not statistically different when compared with the other blocks (Table S2¹).

Residence time (RT)

Based on the long-term PIT tag database, approximately 32% and 34% of the females in 2012 and 2013, respectively, were captured on the spawning grounds for the first time. In 2012, mean (\pm SD) female RT on the spawning grounds was 2.92 (\pm 3.26) days, but ranged from 1 to 15 days (Fig. 4) with approximately 60% of females spending 1 day on the grounds. In 2013, mean RT was 3.46 (\pm 4.48) days and ranged from 1 to 23 days spent on the spawning sites (Fig. 4) with 63% of females spending 1 day on the spawning grounds. Mean RT was negatively associated with mean temperature, maximum discharge, and the mean number of males present with the female in both years (Table 1). Additionally, mean RT in 2012 was negatively associated with an interaction between maximum discharge and mean male number, which had the largest overall influence (Table 1). In 2013, mean RT was positively associated with the same interaction term, with mean male number having the largest overall influence on female RT (Table 1). Female total length and spawning experience had no influence on female RT in either year. In 2012, there were no significant differences in any of the variables between females that stayed on the spawning grounds 1 day versus greater than 1 day (Table S1¹); however, in 2013, maximum discharge was significantly different (WMW test, $W = 571$, $p = 0.046$), as females that were present on the grounds for 1 day encountered a 2.3 m³·s⁻¹ higher maximum discharge than females that stayed for longer than 1 day.

Initial arrival date also influenced how long a female was present on the spawning grounds. In 2012, Block A females spent an average of 3 days longer on the grounds (Table 2) in comparison with Block B females. Block A and Block B females significantly differed in mean RT (WMW test, $W = 592.5$, $p = 0.002$), mean temperature (WMW test, $W = 5$, $p < 0.001$), maximum discharge (WMW test, $W = 261$, $p = 0.022$), and the mean number of males (WMW test, $W = 157$, $p < 0.001$). The one female in Block C in 2012 stayed on the spawning grounds for only 1 day.

In 2013, Block C females (females that arrived late May) stayed on the spawning grounds the longest (Table 2) and nearly twice as long as females in Block A. Block B and D females stayed the least number of days (Table 2). Block C females were the only ones that significantly differed in mean RT when compared with the other blocks (WMW tests: Block A, $W = 59$, $p = 0.017$; Block B, $W = 11$, $p = 0.001$; Block D, $W = 19.5$, $p = 0.046$). Additionally, Block C females were present with the fewest mean number of males (Table 2), which was significantly different when compared with the other blocks (WMW tests: Block A, $W = 194$, $p = 0.026$; Block B, $W = 93$, $p = 0.005$; Block D, $W = 0$, $p = 0.022$). Block A females experienced the highest maximum discharge (Table 2), which was significantly different from those in Block B (WMW test, $W = 434$, $p < 0.001$) and Block C (WMW test, $W = 187$, $p = 0.045$). Mean female total length and mean temperature was not significantly different among blocks (Table S2¹).

Parentage analysis and reproductive success (RS)

In both years, 11 of the 12 loci genotyped for the adults were in Hardy–Weinberg equilibrium. Allelic diversity ranged from two to 11 with a mean of 5.42 alleles per locus in 2012 and 5.33 alleles per locus in 2013. Expected heterozygosity for the 12 loci was 0.59 in 2012 and 0.58 for the 2013 adults. In 2012 and 2013, the combined multilocus, nonexclusion probability for the parent pairs was 0.000057 and 0.000066, respectively. In 2012, 79% of the off-

spring that were genotyped were assigned a female parent and 78% were assigned a male parent with either CERVUS or COLONY with a 75% degree of concordance in maternity and 51% degree of concordance for paternity between the two programs. Comparatively, 83% of the offspring genotyped in 2013 were assigned a female parent and 78% were assigned a male parent with an 80% degree of concordance in maternity and 54% degree of concordance for paternity between CERVUS and COLONY. The Nb estimates (95% confidence intervals) from COLONY using only the larval genotypic data was 314 (266, 368) adults in 2012 and 299 (254, 352) adults in 2013 for a mean adult capture rate of 84.5%.

The inability to capture all of the adults during the spawning ground surveys and moderate power associated with a lack of genotypic diversity at the 12 microsatellites precluded parental assignment for all offspring in the study. In 2012, 62 females and 172 males were assigned offspring. Female RS ranged from two to 38 offspring with a mean of 10.40 (\pm 7.96) larvae assigned to each female, and male RS ranged from one to 13 offspring with a mean of 3.66 (\pm 2.37) larvae assigned to each male. In 2013, 58 spawning females and 180 males were assigned offspring. Female RS ranged from three to 37 offspring with a mean of 11.25 (\pm 8.78) larvae assigned to each female, and male RS ranged from one to 16 offspring with a mean of 3.62 (\pm 2.93) larvae assigned to each male. No relationships were observed between fish total length and RS for either males or females in 2012 and 2013 (Fig. S2¹).

In both years, female RS was positively associated with mean temperature, maximum discharge, and the mean number of males present with the female (Table 1). In 2013, female RS was also positively associated with mean male total length. Female RS was negatively associated with mean OSR in both years, but also varied due to an interaction between maximum discharge and mean number of males in 2012. The interaction term had the largest influence on mean RS in 2012, and the mean number of males had the largest effect in 2013 (Table 1). In 2012, there were no significant differences in any of the variables between females that stayed in the river 1 day versus greater than 1 day (Table S1¹); however, in 2013, the mean total length of males present with females was significantly different (WMW test, $W = 282.5$, $p = 0.022$), as males were approximately 7.7 cm smaller for females that stayed on the grounds 1 day.

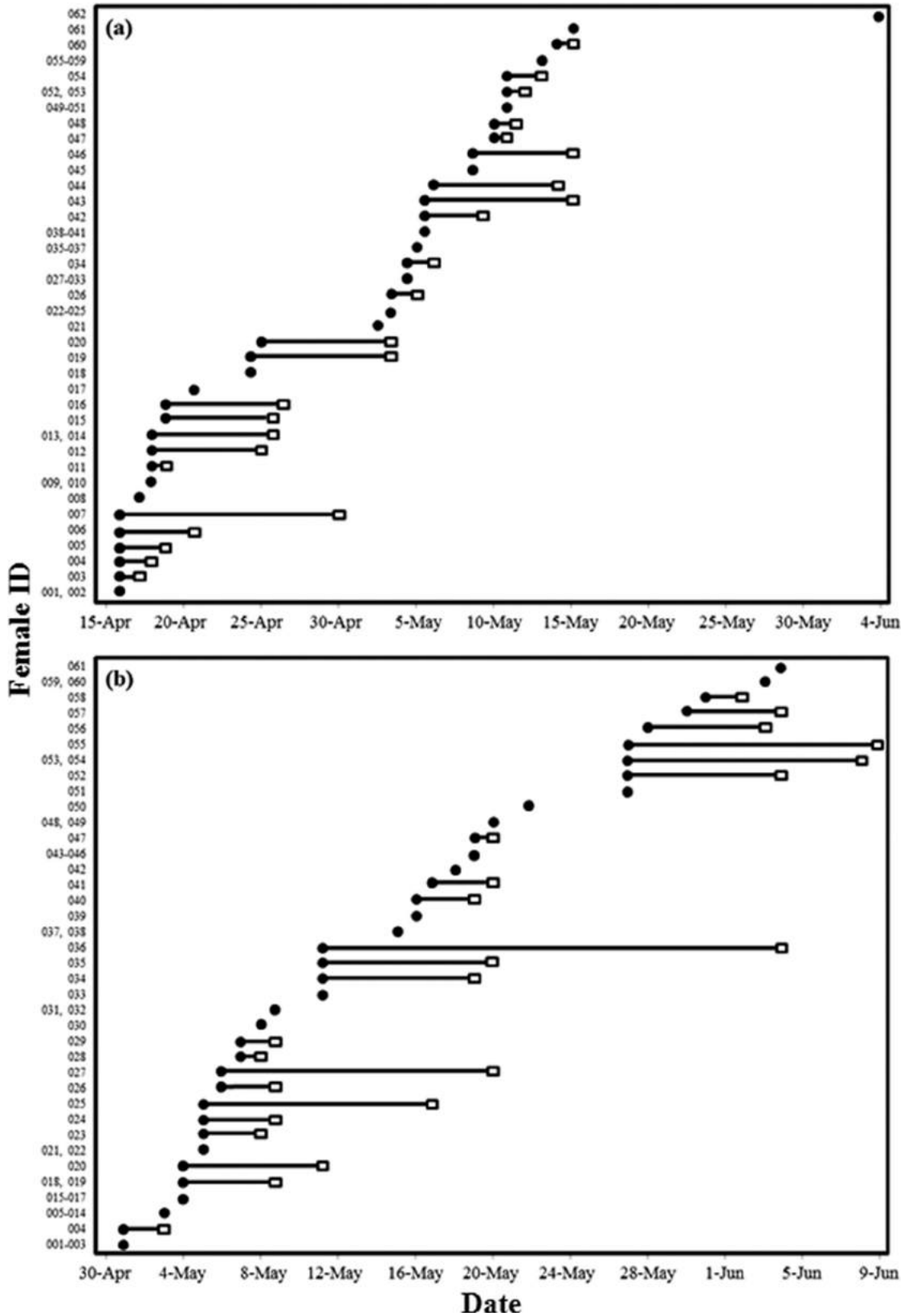
Date of initial entry on the spawning grounds also affected female RS. In 2012, Block B females had nearly twice the number of larvae assigned to them (Table 2), which was significantly different from that of Block A females (WMW test, $W = 238$, $p = 0.008$). The one Block C female had the least number of larvae assigned to her during parentage analysis (Table 2). In 2013, Block D females had nearly twice the number of larvae assigned to them as any other block (Table 2). Block A females had the second largest mean RS (Table 2), which was significantly different from Block D females (WMW test, $W = 16$, $p = 0.048$). Mean RS for Block B and C females were equivalent (Table 2) and not significantly different when compared with Block A or Block D females (Table S2¹). However, Block B females were present with males of the largest mean total length (Table 2), which was only significantly different from Block A females (WMW test, $W = 137$, $p = 0.006$).

2013 plasma hormone analysis

Of the 38 prespawn fish where blood was taken, 23 were confirmed female prior to blood analysis based on the long-term database and (or) through observational gamete expulsion. For the additional 15 fish with unverified sex, 13 were correctly predicted to be female (~86.7%) and one was determined to be male based on discriminant function analysis using E2 and T concentrations (Webb et al. 2002). The sex of one fish could not be determined given that E2 levels were undetectable, so the blood samples were disregarded.

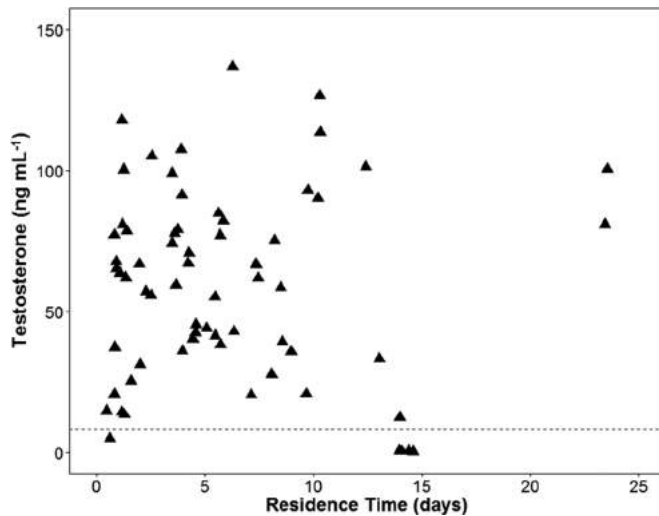
For the 36 confirmed prespawn females, a total of 66 blood samples were taken with a mean of approximately two samples

Fig. 4. Female *Acipenser fulvescens* residence time on the spawning grounds quantified as initial entry date (circles) to presumed spawning date (boxes) for 62 females in (a) 2012 and 61 females in (b) 2013. Females with the same entry and spawning dates were grouped together on the y axis to reduce redundancy. Females with only an entry date plotted spent 1 day on the spawning grounds, so entry date was also presumed spawning date.



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Fig. 5. Plasma testosterone concentrations ($\text{ng}\cdot\text{mL}^{-1}$) in the 66 blood samples taken from 36 female *Acipenser fulvescens* in 2013 to determine the probability of atresia. Two females were predicted to be atretic and had testosterone levels below the dashed line, which represents the mean testosterone concentrations at which early atresia was detected in female white sturgeon by Talbott et al. (2011). No relationship was observed between testosterone concentrations and the number of days females spent on the spawning grounds (residence time). Points were slightly off-set in the plot using a jitter function to reduce overlap.

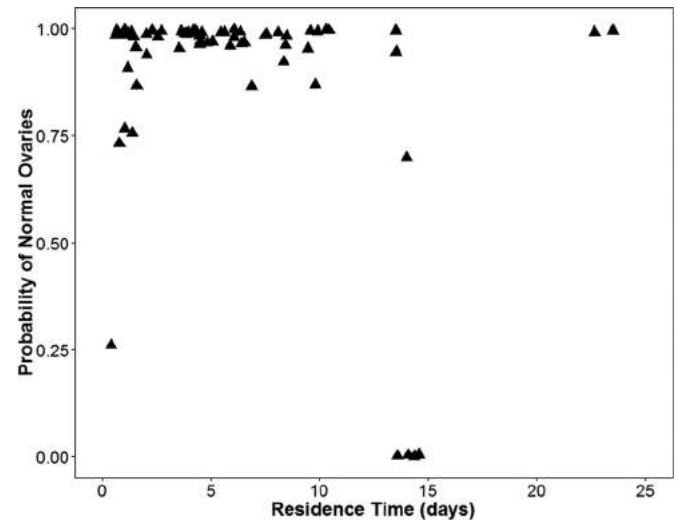


per individual with some females being sampled up to nine times while on the spawning grounds. Mean E2 concentration was $6.81 (\pm 5.84) \text{ ng}\cdot\text{mL}^{-1}$ and ranged from 0.08 to $18.26 \text{ ng}\cdot\text{mL}^{-1}$ (Fig. S3¹). The mean concentration of T was $55.39 (\pm 33.95) \text{ ng}\cdot\text{mL}^{-1}$ and ranged from 0.29 to $136.94 \text{ ng}\cdot\text{mL}^{-1}$ (Fig. 5). Based on T concentrations, 94% ($N = 34$) of the prespawning females that were sampled had a high probability of having normal ovaries (Fig. 6), indicating ovary quality was not dependent on RT (the number of days spent on the spawning grounds). Two females had T levels less than $5 \text{ ng}\cdot\text{mL}^{-1}$ on the last day they were observed on the spawning grounds (presumed spawning date) and were predicted to have atretic ovaries based on the logistic regression modeling. One of the atretic females spent 13 days on the spawning grounds and had high probabilities of normal ovaries (probability = 0.88–0.98) until day 9 when the probability dropped to 0.04 within 24 h. The other atretic female was present on the spawning grounds for only 1 day. The two females were assigned an RS of four and seven larvae, respectively, during genetic parentage analysis, which was lower than the mean RS for all 2013 females.

Discussion

Knowledge on species reproductive biology is vital for understanding critical habitat needs and identifying variables that affect population levels of recruitment in wild fish populations. Studies indicate that demographic characteristics, environmental conditions, and biological traits within spawning sites can influence the timing of reproduction in fishes (Wright and Trippel 2009; Lowerre-Barbieri et al. 2011), particularly semelparous species, but less is known about individual variability for long-lived, iteroparous species. Additionally, an understanding of how the timing of reproduction affects individual ovary quality and RS is limited (Lowerre-Barbieri et al. 2011). In this study, riverine conditions influencing the time female lake sturgeon spent on the spawning grounds (RT) and female RS were identified by daily visual monitoring of individual fish movements over two consecutive spawning seasons. Additionally, quantification of plasma sex steroid concentrations allowed for a noninvasive method to

Fig. 6. The probabilities of the 36 female *Acipenser fulvescens* sampled for blood having normal ovaries based on log-transformed plasma testosterone concentrations and logistic regression analysis developed by Talbott et al. (2011). Two females with probabilities below 0.30 had low testosterone concentrations, which were indicative of atresia. No relationship was observed between the probability of a female having atretic ovaries and the number of days spent on the spawning grounds (residence time). Points were slightly off-set in the plot using a jitter function to reduce overlap.



determine female ovary quality and assess whether a long RT (or resource investment in current year reproductive efforts) affected a female's ovary quality and RS.

Factors influencing RT and RS

Results demonstrated that females are considerably plastic in their spawning behaviors with several exogenous cues playing a large role in determining how long a female occupies the spawning grounds. Mean RT ranged from 2.9 to 3.5 days, but arrival date was the presumed spawning date for 60%–63% of females in both years on the UBR. Data indicate that the majority of females spawned and then quickly left the spawning grounds. Spawning grounds are typically shallow with reduced food availability and cover (Auer 1996b), which can be stressful for lake sturgeon. However, females showed considerable variability in RT in both years, with one female staying in the river up to 23 days (Fig. 4b). Results indicate potential differences among females in which environments are considered “optimal” for spawning and support prior assumptions regarding what stimuli influence female ovulation, which is known to be asynchronous in sturgeon (Webb et al. 2001; Bruch and Binkowski 2002; Forsythe et al. 2012). Similarly, Thiem et al. (2013) observed different residency times at the spawning grounds among female lake sturgeon in the Richelieu River of Quebec, with some individuals staying up to 27 days; however, the authors did not hypothesize reasons for the observed differences among females.

Female RT was influenced by both demographic factors and environmental conditions at the spawning sites, with the mean number of males present having the largest influence on female RT in 2013 and in 2012 due to an interaction with maximum discharge (Table 1). Additionally, female RS was positively associated with the mean number of males in both years. Mating with numerous males maximizes fertilization success and the genetic diversity of offspring (Bruch and Binkowski 2002; Uller and Olsson 2005; Thiem et al. 2013) and has been shown to increase RS in female lake sturgeon (Duong 2010). Therefore, in addition to temperature and discharge, which have been repeatedly cited in the literature as important spawning cues for sturgeon, the num-

ber of males present is a strong factor influencing the timing and location of spawning for female lake sturgeon in the UBR.

Increasing water temperature and declining discharge were documented by Forsythe et al. (2012) as important drivers in the initiation of lake sturgeon spawning on the UBR from 2001 to 2007. In both years of this study, lake sturgeon were initially observed on the spawning grounds when mean water temperature was 13 °C, which is within the spawning range of 10–16 °C documented in other studies (Auer 1996b; Bruch and Binkowski 2002). Despite differences in the lengths of the 2012 and 2013 spawning seasons (Fig. 2), increases in mean temperature resulted in females from later spawning blocks spending less time on the spawning grounds and increased female RS in both years. However, the overall influence of maximum discharge on female RT and RS varied between years due to the interaction term with the mean number of males. In 2012, females spent less time on the spawning grounds and had lower RS as maximum discharge increased while different numbers of males were present. Conversely, increasing maximum discharge and a change in the number of males present was associated with longer female RT in 2013. An increase in maximum discharge alone increased female RS in 2013, although the influence was small in comparison with other variables (Table 1). The opposing influence of maximum discharge on female RT and RS between years may be largely due to the interannual variability in discharge patterns observed on the UBR (Fig. 2), which often occurs in riverine systems (Thoms 2006).

Influence of initial arrival date on RT and RS

Initial entry date onto the spawning grounds has been shown to have a strong influence on female RS, as early-arriving fish may utilize different sites and have different breeding opportunities than fish that arrive late in the season (Dickerson et al. 2005a, 2005b). In the study, adult lake sturgeon were observed at all seven sites of the spawning grounds throughout the entire season except for the June run of fish in 2012, which were only observed at a downstream spawning site. The overall influence of arrival date (block) in comparison with other variables could not be determined due to multicollinearity with other variables; however, initial arrival date was shown to have a strong influence on female RS in both years based on comparisons done using WMW tests. In 2012, females that arrived earliest in the season had the highest mean RS estimates and experienced the lowest maximum discharge even though they had the longest mean RT and were present with fewer males and more females (Table 2). Females require adequate discharge rates to ensure fertilization success of eggs, but high levels cause adults to expend energy and can lead to spawning site abandonment until flows stabilize (Auer 1996b). Conversely, females that arrived latest in 2013 had the highest RS, were present with the most males, and encountered a maximum discharge rate comparable to early-arriving females in 2012. While the importance of initial arrival date onto the spawning grounds cannot be overlooked, additional years of observations would be beneficial to determine whether there is a consistent relationship between arrival time and female RS given that the study was conducted over two contrasting spawning seasons.

Costs associated with RT on the spawning grounds

Delays in spawning have been shown to decrease fertilization rates and egg viability due to over-ripening in semelparous species (Berejikian et al. 2000), which are predicted to reduce female RS. In contrast, neither the time female sturgeon spent on the spawning grounds (measure of female investment in current reproduction) nor female body length (a surrogate for age) was significantly associated with female RS. Additionally, the majority of females had a high probability of normal ovaries based on T concentrations regardless of the time they spent on the spawning grounds (Fig. 6), suggesting that there is little reproductive cost for female

sturgeon to be plastic in spawning ground occupancy time. However, two females had T concentration levels indicative of atresia in 2013, with one female being present on the spawning grounds for 14 days (Fig. 5). Atresia can occur in response to unsuitable environmental conditions or due to fish being in poor condition prior to or while on the spawning grounds (Rideout et al. 2000; Scott et al. 2006; Kennedy et al. 2008). Females in the early stages of atresia can still ovulate and spawn (Scott et al. 2006), but often have reduced fecundity (Kennedy et al. 2008), and eggs are assumed to be of a lower quality and have lower fertilization success. Thus, the two atretic females in the study may have been in “early atresia”, explaining why they still successfully produced offspring that survived until captured in drift nets but had RS estimates lower than the mean potentially due to reduced egg quality and (or) survival. Future empirical work examining the prevalence of atresia across multiple spawning seasons would be informative to determine what percentage of females are atretic from year to year and whether females with early atresia are contributing equally to year classes.

Stress associated with capture can reduce plasma sex steroid levels in fishes (Fuzzen et al. 2011), including lake sturgeon (Thiem et al. 2013). However, only two of the 36 females handled multiple times for blood sampling in the study had T concentration levels indicative of atresia, and one female did not have atretic levels until the ninth day of handling. The mean T concentration of 55.39 (±4.20) ng·mL⁻¹ among females quantified in the study was comparable to a mean of 56.5 (±12.8) ng·mL⁻¹ quantified in vitellogenic, cultured white sturgeon used to develop the logistic regression to determine ovary quality by Talbott et al. (2011). Additionally, the two female lake sturgeon predicted to be atretic in this study had T concentration levels below 8.2 ng·mL⁻¹, which was the level at which female white sturgeon histologically showed signs of “early atresia.” Collectively, the study confirms results by Talbott et al. (2011) that quantification of T concentrations can be an effective tool for determining ovarian quality in sturgeon, but expands on their work demonstrating the applicability of the method to wild populations.

Sexing fish using plasma sex steroid concentrations

Quantification of plasma concentrations of T and E2 and use of discriminant classification functions provided a reliable means of verifying sex for spawning females in the study, which has been successfully applied in other lake sturgeon populations (Craig et al. 2009; Shaw et al. 2012; Thiem et al. 2013). Fish predicted to be female based on observational techniques were correctly assigned 87.5% of the time according to the discriminant function analysis. Results are similar to the 85% of female white sturgeon correctly assigned by Webb et al. (2002) during development of the discriminant functions. For the UBR females, mean T and E2 concentrations were comparable to, but higher than, sex steroid concentrations quantified in spawning female lake sturgeon by Craig et al. (2009); however, this is not surprising, as plasma sex steroid concentrations can vary widely among individuals depending on reproductive state.

Influence of the OSR on RT and RS

Male lake sturgeon mature earlier and spawn more frequently than females, resulting in a greater number of males present on the spawning grounds than females (Peterson et al. 2007; Thiem et al. 2013). In the study, mean OSRs over the entire spawning season were 3:1 in 2012 and 3.4:1 in 2013, demonstrating the degree of male bias on the spawning grounds due to sex differences in interspawning intervals. Estimates are comparable to OSRs of 1.55:1 and 2.06:1 documented on the UBR (Smith and Baker 2005) and 1.25:1, 2.7:1, and 5.7:1 documented in other wild populations of lake sturgeon (Auer 1999; Bruch and Binkowski 2002). Mean OSR also varied throughout the spawning season (Table 2), being lower near the beginning of the season in 2012 due to higher

numbers of females arriving on the spawning grounds together, but lower near the end of the season in 2013 due to a lower number of males.

Shifts in the OSR during the breeding season are not uncommon (Kvarnemo and Ahnesjö 1996). A reduction in the number of males available to reproduce later in the season, which has been documented in fishes (Vincent et al. 1994; Forsgren et al. 2004) as well as other taxa (Kasumovic et al. 2008), can lead to lower fertilization rates for females that spawn later in the season. In this study, female RT did not vary due to the mean OSR at the spawning sites, but female RS was negatively associated with mean OSR in both years, which may be due to a higher number of females arriving on the spawning grounds together early in the season leading to competition for males and (or) sperm. Additionally, higher densities of females spawning in the same areas can cause eggs to overlap and stick together on the substrate (Bruch and Binkowski 2002), which may influence egg survival and hatching rates, thereby influencing female RS. Although the mean RS was similar between the two spawning years, there was a considerable difference in the number of larvae captured in 2012 versus 2013 despite comparable capture effort, indicating fertilization rates, egg mortality, and (or) larval mortality can vary substantially from year to year.

Influence of male size on female RS

Female mating preferences based on male size have been well-documented for fishes like salmonids where large males are typically better competitors and produce larger offspring than smaller males (Dickerson et al. 2002, 2005a). However, studies on mate selectivity in sturgeon have been limited. In this study, mean female RS increased as the mean total length of males present with the female increased in 2013, indicating a possible advantage for females that spawned with larger males; however, there was no obvious advantage for males, as there was no observed relationship between male RS and male total length (Fig. S2¹). Female sturgeon are typically larger than male conspecifics (Peterson et al. 2007), as observed in the study (Fig. 3), but may prefer larger males due to fitness advantages or potentially due to the intensity of their spawning behaviours (Esteve 2005). Male lake sturgeon are known to aggressively compete for close access to females prior to egg deposition (Bruch and Binkowski 2002). Therefore, one possibility is that larger males in 2013 may have been more aggressive, gaining closer access to the female during spawning, leading to higher egg fertilization rates and higher female RS, which was not observed for females in 2012. Future work quantifying how aggressiveness in courting behaviours varies among males based on size and whether this intensity influences female RS would be beneficial to determine whether females consistently gain an advantage by mating with larger males.

Influence of female experience on RT and RS

In this study, female sturgeon showed considerable variability in size, with approximately 32%–34% of females being captured on the spawning grounds for the first time; however, female RT and RS did not vary due to female size (Fig. S2¹) or female reproductive experience. Results indicate that while larger females may be more fecund and (or) have larger offspring, they may not be more successful or have higher RS than smaller females because the conditions under which they spawn and expose fertilized eggs to can have a substantial influence on offspring survival. Additionally, female lake sturgeon vary substantially at age of maturation with individuals maturing between ages 14 and 33 years (Hay-Chmielewski and Whelan 1997) depending on the population. Thus, first-time spawning females have the potential to be the same size and age as experienced females that have spawned in previous years. In this study, the lack of a difference in RT and RS among “experienced” and “inexperienced” females could also

be partially due to sampling limitations on the UBR. Although adult sampling during the spawning season has occurred annually since 2001, approximately 15%–30% of adults are missed annually during spawning ground surveys (K. Scribner and E. Baker, unpublished data), as shown by the adult capture rate of 84.5% estimated from the genotypic data obtained in the study. Therefore, some females may have been mislabeled as “inexperienced” given that they were missed in surveys during previous years.

Findings from the study demonstrate the complexity of factors that influence female sturgeon spawning behaviour illustrating why an understanding of the reproductive timing in numerous fishes has been limited (Lowerre-Barbieri et al. 2011). Additionally, results demonstrate that an iteroparous, long-lived species can be selective in their spawning decisions at an individual-based level, but their behaviors and patterns are different from those observed in semelparous and short-lived species, particularly in regards to mate selection. Collectively, results demonstrate the importance of maintaining critical abiotic conditions on the spawning grounds, adequate adult population sizes, and male-biased sex ratios to ensure wild populations of sturgeon and potentially other stream fishes successfully produce progeny. Furthermore, the study demonstrates how an individual-based, monitoring approach may be useful for informing management strategies for an entire population to accurately predict population levels of recruitment conditional on population demography and (or) environmental conditions.

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