

EXTRINSIC AND INTRINSIC FACTORS AFFECTING ADULT REPRODUCTIVE  
BIOLOGY AND EARLY LIFE STAGE DEVELOPMENT OF LAKE STURGEON

By

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## ABSTRACT

### EXTRINSIC AND INTRINSIC FACTORS AFFECTING ADULT REPRODUCTIVE BIOLOGY AND EARLY LIFE STAGE DEVELOPMENT OF LAKE STURGEON

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Environmental change is occurring at an unprecedented rate. Consequently, alterations in environmental cues such as temperature, discharge, and depth influence the reproductive behavior of adult fishes. Maternal environmental effects including selection of mates, spawning site, and maternal yolk-sac provisioning determine the environmental influences offspring experience during critical early development and growth periods. Using genetic parentage analysis and experimentally-manipulated environmental regimes, I examined the effects of adult reproductive behavior and altered environmental conditions on larval lake sturgeon (*Acipenser fulvescens*) development and behavior. Lake sturgeon are long-lived, iteroparous fishes that reside in freshwater, and migrate to riverine areas in early spring and summer to spawn. Analyses in my studies were conducted using a well-known adult population of approximately 1200 lake sturgeon from Black Lake, Michigan.

First, I individually marked all spawning adults, examined the demographic and environmental factors affecting the timing of the reproduction, collected blood samples to determine female ovary/egg quality, and used genetically-based parentage analysis to assign female reproductive success (RS). The time females spent on the spawning grounds was dependent on temperature, discharge, number of males, size of males, an interaction between temperature and size as well as discharge and number of males.

Female RS was dependent on the above factors as well as the OSR; however, the time females spent in the river had no effect on female RS or her ovary/egg quality indicating there does not appear to be a reproductive cost in being plastic in the timing and location of spawning.

Next, I empirically tested whether adult-selected and anthropogenically modified egg incubation conditions affect larval traits at hatch and until the timing of emergence by experimentally manipulating velocity and thermal regimes. A genotype-by-environment interaction was detected for all traits at hatch, and the greatest range in phenotypic variation was observed in the “High” velocity (0.8m/s) and “Warm” (18C) thermal treatments. Additionally, egg incubation conditions and genetic (family) effects affected growth and behavior at the timing of emergence indicating that conditions experienced during early development persist until later stages (i.e. “ontogenetic contingency”).

Lastly, I quantified microhabitat variation at female egg deposition sites, collected wild-produced eggs from the stream, quantified offspring trait variation at hatch and four weeks post-emergence, and used genetically-based parentage analysis to determine the influence of genetic (parentage) effects on offspring traits. Traits at hatch were influenced by environmental conditions at egg deposition sites, but traits post-emergence were influenced by additive genetic effects and potential differences in feeding efficiency. Overall, findings indicate how environmental variation affects adult reproductive success, timing of spawning, and larval trait variation during critical growth and development stages providing a greater understanding of how the phenotypic and genotypic variation within wild populations will be affected by environmental change.

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# TABLE OF CONTENTS

LIST OF TABLES.....	ix
LIST OF FIGURES.....	xi
INTRODUCTION.....	1
Chapter 1 .....	8
ASSOCIATIONS BETWEEN EGG QUALITY, BEHAVIORAL PLASTICITY, AND REPRODUCTIVE SUCCESS OF SPAWNING FEMALE LAKE STURGEON	
INTRODUCTION .....	9
MATERIALS AND METHODS .....	13
Study site.....	13
Adult sampling.....	13
Blood collection and plasma hormone analysis.....	14
Larval collection.....	16
Genetic analysis .....	16
Parentage analysis.....	17
Statistical analysis .....	18
RESULTS .....	20
2012 adult data and parentage analysis.....	20
2013 adult data and parentage analysis.....	21
2013 plasma hormone analysis.....	23
DISCUSSION.....	24
APPENDIX.....	29
LITERATURE CITED.....	36
Chapter 2 .....	43
GENETIC AND ENVIRONMENTAL COMPONENTS OF PHENOTYPIC AND BEHAVIORAL TRAIT VARIATION DURING LAKE STURGEON EARLY ONTOGENY	
INTRODUCTION .....	44
MATERIALS AND METHODS .....	47
Study Site .....	47
Sampling Locations and Gamete Collection.....	48
Field Experiment.....	48
Velocity (Common Garden) Experiment.....	50
Statistical Analysis.....	51
RESULTS .....	53
Field Experiment.....	53
Velocity (Common Garden) Experiment.....	54

DISCUSSION.....	55
APPENDIX.....	62
LITERATURE CITED.....	70
 Chapter 3 .....	 78
INCREASING THERMAL REGIMES REVEAL CRYPTIC GENETIC VARIATION DURING EARLY ONTOGENETIC STAGES OF LAKE STURGEON	
INTRODUCTION .....	78
MATERIALS AND METHODS .....	82
Study site.....	82
Fertilizations .....	82
Thermal treatments .....	83
Larval traits.....	83
Statistical analysis .....	85
RESULTS .....	86
DISCUSSION.....	88
Rearing temperatures influence phenotypic variation observed at hatch .....	88
Fluctuating and constant rearing temperatures influence the range of phenotypic variation observed in larval traits .....	90
Rearing temperatures affect trait variation at a subsequent ontogenetic stage ..	91
Families differ in growth at a subsequent ontogenetic stage .....	93
Conclusions and future directions.....	93
APPENDIX.....	95
LITERATURE CITED.....	100
 Chapter 4 .....	 107
EFFECTS OF PARENTAGE AND MICROHABITAT VARIATION WITHIN ADULT- SELECTED SPAWNING SITES ON LAKE STURGEON GROWTH DURING EARLY LIFE STAGES	
INTRODUCTION .....	108
MATERIALS AND METHODS .....	110
Study site.....	110
Egg collection and microhabitat variables .....	111
Mean depth and discharge during the incubation period .....	112
Mean substrate size .....	113
Larval traits.....	113
Genetic analysis .....	115
Parentage analysis .....	115
Statistical analysis .....	116
RESULTS .....	119
DISCUSSION.....	121
APPENDIX.....	127
LITERATURE CITED.....	135
 CONCLUSION .....	 142

## LIST OF TABLES

<p>Table 1.1 ..... 30            Variable estimates and standard errors (SE) explaining changes in the expected log of mean female reproductive success (RS) and log of mean retention time (RT). Estimates are provided for models of best fit for 2012 and 2013.</p>	30
<p>Table 2.1 ..... 63            Variance components and standard errors (SE) estimated from the model of best fit for the field and velocity experiments. P-values are presented from the likelihood ratio tests and heritability (<math>h^2</math>) estimates are provided for traits where only a significant family effect was detected.</p>	63
<p>Table 2.2 ..... 64            Daily mean, minimum, and maximum discharge levels (meters<sup>3</sup>/sec) during the egg incubation period at the “Alternative” and “Traditional” spawning sites used in the field experiment.</p>	64
<p>Table 2.3 ..... 65            Pairwise model comparisons for traits fit to a model including the interaction term and a reduced model without the interaction term. Log-likelihood values, chi-square values, p-values (P), and degrees of freedom (df) obtained from the likelihood ratio tests are presented where applicable.</p>	65
<p>Table 2.4 ..... 66            P-values computed from the likelihood ratio tests to determine the significance of family and site (or treatment) for all traits from the field and velocity experiments. Values are not presented for models where a significant genotype-by-environment interaction was detected.</p>	66
<p>Table 3.1 ..... 96            Step-wise model comparisons and DIC-values used in model selection for the six larval traits quantified at hatch and emergence in the experiment.</p>	96
<p>Table 3.2 ..... 97            Variance components and 95% highest posterior density (HPD) estimated from the models of best fit. Heritability (<math>h^2</math>) estimates are provided for traits where the model of best fit included a significant family effect, but no family-by-treatment interaction.</p>	97
<p>Table 4.1 ..... 128            Mean (<math>\pm</math> Standard Error) values of the three microhabitat variables during the egg incubation period at the sixty-four sampling points where live lake sturgeon eggs were collected. Depth and discharge during the egg incubation period were predicted using hourly data logger measurements.</p>	128

Table 4.2 ..... 128  
Mean ( $\pm$  Standard Error) and range in body size observed among larvae for the five measurement samples taken from hatch (Age 1) to four-weeks post-emergence (Age 41).

Table 4.3 ..... 128  
Coefficients, standard errors (SE), and p-values estimated from the models of best fit for the three traits measured at hatch.

Table 4.4 ..... 129  
Pairwise model comparisons for the three hatch traits fit to models (M) including all three environmental variables and a reduced model. Log-likelihood values, chi-square values, p-values (P), and degrees of freedom (df) obtained from the likelihood ratio tests are presented.

## LIST OF FIGURES

Figure 1.1 .....	31
The study site on the Upper Black River located in Michigan, USA. Shown are the location of the spawning sites and the larval collection site (a). An enlarged view of the seven separate spawning locations where adults were sampled daily (b).	
Figure 1.2 .....	32
The number of adult lake sturgeon present in the river along with mean daily water temperature (red) and river discharge (blue) during the adult spawning seasons in 2012 (a) and 2013 (b).	
Figure 1.3 .....	33
Frequency distribution of spawning adult total length where males are gray bars and females are white bars for 2012 (a) and 2013 (b).	
Figure 1.4 .....	34
Female plasma testosterone concentrations (ng/mL) and the number of days they were present on the spawning grounds (retention time).	
Figure 1.5 .....	34
Plasma estrogen level concentrations (ng/mL) for fish where sex was undetermined given the lack of expression of gametes.	
Figure 1.6 .....	35
The probability of females having normal ovaries estimated based on plasma testosterone levels and the number of days they were present on the spawning grounds (retention time).	
Figure 2.1 .....	67
The study location on the Upper Black River, the largest tributary of Black Lake, Michigan showing the “traditional” spawning sites and the Kleber Dam, an “alternative” spawning site used during the 2011 spawning season	
Figure 2.2 .....	67
Four larval hatch traits A: body length (BL), B: body area (BA), C: yolk-sac area (YSA), D: head area (HA) (a) and one emergence trait, E: emergence body length (EBL), quantified in the experiment (b). The two additional emergence traits, time to emergence (ET) and total growth (TG), were quantified as the time (days) and growth (mm) from hatch to emergence, respectively. Scale bars (mm) are presented in the lower right corner	
Figure 2.3 .....	68
Total growth of lake sturgeon larvae from hatch to emergence when eggs were	

incubated at the “Traditional” and “Alternative” spawning locations. A significant genotype-by-environment (G-by-E) interaction was detected providing evidence that conditions experienced during embryogenesis affect trait variation at a later ontogenetic stage (timing of emergence)

Figure 2.4 ..... 68  
 Reaction norm for body length at hatch for lake sturgeon larvae in the velocity (common garden) experiment. A significant genotype-by-environment (G-by-E) interaction was detected, and most of the variation was observed from larvae reared in the “High” velocity treatment

Figure 3.1 ..... 98  
 The study location on the Upper Black River, the largest tributary of Black Lake, Michigan showing the spawning locations where adult lake sturgeon were sampled during the 2012 spawning season

Figure 3.2 ..... 98  
 The four egg incubation temperatures (warm, cold, variable, and ambient) in which individuals were reared in during the 2012 spawning season

Figure 3.3 ..... 99  
 Thermal reaction norm for body length at hatch for lake sturgeon larvae subjected to the four thermal treatments. A significant genotype-by-environment (G-by-E) interaction was detected, and most of the variation among families was observed for larvae that hatched from eggs incubated in the warm (18°C) treatment

Figure 3.4 ..... 99  
 Thermal reaction norm for larval lake sturgeon growth measured from hatch to the timing of emergence. A significant family effect was detected ( $h^2=0.33$ ) indicating that differential size among families measured at hatch was persisting to a later ontogenetic stage

Figure 4.1 ..... 130  
 The study location on the Upper Black River, the largest tributary of Black Lake, Michigan showing the spawning site, Site B, where lake sturgeon eggs were collected along transect lines and the larval sampling location where wild-produced larvae were captured

Figure 4.2 ..... 131  
 Schematic layout of the seven transect lines (A-G) and sampling points where the microhabitat variables were quantified and lake sturgeon eggs were collected. Bubbles indicate the number of live eggs collected at each transect sampling point.

Figure 4.3 ..... 131  
 Individual growth trajectories quantified from hatch (Age 1 day) to four weeks post-

emergence (Age 41 days). Individual body size was measured at hatch, and once weekly between the timing of emergence (Age 21 days) and the end of the experiment (Age 41 days).

Figure 4.4 ..... 132  
Additive genetic variance ( $\sigma_a^2$ ) and residual variance ( $\sigma_\epsilon^2$ ) estimates quantified at each age in the random regression animal model.

Figure 4.5 ..... 132  
The narrow-sense heritability ( $h^2$ ) estimates for body size quantified at each age the larvae were photographed. The  $h^2$  estimates were obtained by dividing the additive genetic variance by the sum of the additive variance and residual variance.

Figure 4.6 ..... 133  
The relationship between the depth ratios calculated on May 8<sup>th</sup> and May 18<sup>th</sup>. The ratios were calculated as the point estimates of depth at each transect sampling point divided by the mean depth measurement from the data logger. The linear regression equation ( $y = 0.980x + 0.002$ ,  $R^2 = 0.948$ ) indicated the ratios were highly correlated between the two sampling dates.

Figure 4.7 ..... 133  
The relationship between the discharge ratios calculated on May 8<sup>th</sup> and May 18<sup>th</sup>. The ratios were calculated as the point estimates of discharge at each transect sampling point divided by the mean discharge measurement from the data logger. The linear regression equation ( $y = 0.917x - 0.010$ ,  $R^2 = 0.948$ ) indicated the ratios were highly correlated between the two sampling dates.

Figure 4.8 ..... 134  
The relationship between mean substrate size when measuring rocks in three randomly selected grids versus the entire substrate photo. The linear regression equation ( $y = 1.526x - 1.315$ ,  $R^2 = 0.954$ ) indicated that measuring three random grids accurately estimated mean substrate size in the entire photo.

## INTRODUCTION

Environmental change is occurring at an unprecedented rate (IPCC 2014). The ability of populations to adapt to changes is determined by levels of phenotypic variance given that it is the raw material for selection to act on (Warner et al. 2014).

Understanding how phenotypic variation within wild populations is changing in response to environmental change has become a major goal for researchers (Parmesan 2006; Hansen et al. 2012). Within populations, changes in phenotypic variation occur due to microevolutionary responses or phenotypic plasticity (Gienapp et al. 2008).

Plasticity includes changes in behavior, phenotype, or physiology that occur without genetic changes (West-Eberhard 2003), whereas microevolutionary responses are genetic changes to selection (Réale et al. 2003). There has been considerable debate about the role of plasticity in adaptive evolution given that it is said to constrain adaptations to environmental change by shielding genotypes from selection (Ghalambor et al. 2007). However, plasticity is generally viewed as a quantitative trait with a heritable basis that is under selection (Via et al., 1995; Pigliucci 2001; Scheiner 2002; DeWitt and Scheiner 2005). Additionally, plasticity is believed to produce novel variants on which natural selection acts (i.e. 'genes as followers' West-Eberhard 2003) indicating its importance to phenotypic trait evolution in wild populations.

The ability of populations to respond to environmental change is dependent upon several characteristics including the presence of genetic variation in fitness-related traits (Anderson et al. 2012). Therefore, partitioning sources of phenotypic variation into environmental and additive genetic components are necessary for making predictions about species responses (Crespel et al. 2013; Warner et al. 2014). Levels of additive

genetic variation indicate how a trait can evolve in response to selection (Wilson et al. 2005), and therefore provide an understanding of genetic constraints on a population's evolutionary potential (Garant et al. 2008). Environmental effects including genotype-by-environment (G-by-E) interactions illustrate the possible similarities and differences in phenotypic trait variation among related and unrelated individuals when plotted as a reaction norm (Byers 2008). Additionally, maternal environmental effects which include choice of mates, selection of oviposition sites, and maternal provisioning determine the environmental conditions the offspring is exposed to thereby having a strong influence on offspring traits (Mousseau and Fox 1998; Räsänen and Kruuk 2007; Noble et al. 2013).

An additional characteristic influencing the ability of organisms to respond to environmental change is generation time (Anderson et al. 2012). Evolutionary responses to “rapid climate change” have been observed in species that are small-bodied, have short-generation times, and have large population sizes (Bradshaw and Holzapfel 2006). However, species with long-generation times, late sexual maturity, and small population sizes are limited by their inability to keep pace with environmental change. Thus, long-lived species are expected to experience population declines due to a lagged-response to environmental variation or a lack of genetic variation in traits (Bradshaw and Holzapfel 2006; Skelly et al. 2007). Alternatively, behavioural plasticity is predicted to be an adaptive strategy for long-lived species adjusting to environmental change (Refsnider and Janzen 2012).

In long-lived species, behavioral plasticity is effective for escaping evolutionary and ecological traps (Schlaepfer et al. 2002). For example, plasticity in oviposition site

selection for oviparous species allows females to mate when environmental conditions are favorable for offspring growth and survival thereby positively influencing her reproductive success (Serbezov et al. 2010). The timing of female mating can have large influences on offspring trait variation given that abiotic conditions within mating sites affect developmental timing and growth (Garant et al. 2003; Crozier et al. 2008; Refsnider and Janzen 2010). Additionally, mating conditions can have long-term effects on offspring traits given that conditions experienced during early embryonic development are known to have persisting effects on offspring phenotypic trait variation during sequential ontogenetic stages (“i.e. ontogenetic contingency,” Diggle 1994; Duffy et al. 2002; Warner and Shine 2008; Warner et al. 2014).

In long-lived vertebrates, environmental variability has been shown to affect adult reproductive success and early survival of offspring (Thompson and Ollafson 2001); however, the relationship between the timing of spawning and adult reproductive success in stream fishes in particular is unclear (Anderson et al. 2012). The focus of my dissertation research is determining how extrinsic (e.g. environmental variability and demographic characteristics) and intrinsic factors (e.g. genetic parentage effects, egg quality, variability in spawning date) affect female spawning behavior and early life stage development of lake sturgeon (*Acipenser fulvescens*).

Lake sturgeon are long-lived, iteroparous fish that migrate to riverine areas between mid-April and early June to spawn (Auer 1996; Forsythe et al. 2012). Females are behaviorally plastic in the timing and location of spawning events, and will remain in the river for several days to weeks before spawning (Thiem et al. 2013). During spawning events, females release demersal eggs which are fertilized by sperm from

multiple males. Eggs become adhesive, sticking to the cobble and gravel substrate among the spawning grounds (Bruch and Binkowski 2002), and incubate without parental care until hatch (Duong et al. 2011). Hatched larvae remain at the spawning grounds, feeding on endogenous yolk-sac reserves before dispersing downstream to begin exogenously feeding (Smith and King 2005). The timing of ontogenetic shifts during the early life stages are dependent on several abiotic conditions including temperature, substrate, depth, and discharge; however, temperature has the largest influence on the timing of development.

Lake sturgeon populations have been numerically depressed due to overharvest, habitat loss and degradation, and limited recruitment (Hayes and Caroffino 2012). Long-term success of rehabilitation programs includes protection of spawning and rearing habitats (Hayes and Caroffino 2012), and identification of factors that affect growth and survival during critical early life stages. My research was conducted on the Black Lake sturgeon population located in Cheboygan County, Michigan. The wadable conditions of the system allowed for experimental work within the river to examine adult spawning behavior and collection of wild-produced progeny, and the presence of a streamside rearing facility allowed for experimental work examining the influences of environmental variability on early life stage development.

My dissertation is composed of four chapters. In Chapter 1, I individually marked all spawning adults and examined the demographic and environmental factors affecting the timing of the reproduction in female lake sturgeon over two spawning seasons. Based on observational data and previous studies examining the time females sturgeon spend on the spawning grounds (Auer 1996; Paragamian and Kruse 2001; Webb et al.

2002), I hypothesized that the greater amount of time that females spent in the river, the less viable her eggs would become (due to an increased probability in atresia). I collected blood samples to determine female egg quality, and used genetically-based parentage analysis to assign reproductive success to determine whether delays in female spawning (i.e. longer time spent on the spawning grounds) resulted in lower reproductive success. The results from this study are currently in preparation for publication with the intent to submit the manuscript to the *Journal of Fish Biology*.

In Chapter 2, I empirically tested whether adult choice of spawning location influenced larval traits at hatch, and whether the effects of egg incubation conditions persisted until a sequential ontogenetic stage (emergence). I placed eggs of known genotype at an early season spawning site (where adults were spawning) and a late season spawning site (where adults were not spawning at time). I predicted that adults were selecting spawning sites that were conducive to offspring growth, and that larval trait variation would vary between the sites. Given the high discharge levels observed during the spawning season, I also experimentally tested whether velocity regimes experienced while in the egg would affect trait variation at hatch and persist until emergence. Additionally, I included a “variable” treatment aiming to examine the influence of increased environmental variability in discharge due to an anthropogenic disturbance on larval trait variation during early ontogenetic stages. Results from this study were published in *Environmental Biology of Fishes* in the March issue of 2015.

In Chapter 3, I empirically tested the effects of four thermal treatments (two fluctuating and two constant) on larval trait variation at hatch and at the timing of emergence for ten half-sibling families. I included a “variable” treatment to mimic an

expected disturbance in thermal regime due to increases in extreme weather events as predicted due to climate change. I predicted that phenotypic variation would be most variable in the fluctuating treatments due to a stress response, and that families would differ in phenotypic variation given the results from the previous study. Results from this study were submitted to *Evolutionary Applications* in May of 2015, and are currently under review.

In Chapter 4, I quantified microhabitat variation in water depth, discharge, and substrate size at female egg deposition sites within a spawning site, and collected wild-produced eggs from the stream just prior to hatch. I hypothesized that individual phenotypic variation would differ among sampling points and among maternal groups given the significant family effects observed in the previous two studies. I quantified offspring trait variation at hatch and up to four weeks post-emergence in wild-produced larvae, and used genetically-based parentage analysis to construct a pedigree. I tested for the influence of genetic (parentage) effects which were predicted to vary with age on offspring trait variation from hatch until four weeks post-emergence. The results from this study are currently in preparation for publication.

Findings from my dissertation work improve our understanding of how environmental variation affects adult reproductive success, timing of spawning, and larval trait variation during critical growth and development stages. Information provides a greater understanding of the critical habitat needs of the adults during spawning activities, an understanding of how thermal and discharge conditions affect larval phenotypic variation, and provides insight into the ability of the population to respond to

environmental change given the estimates of narrow-sense heritability ( $h^2$ ) during the early ontogenetic stages for this long-lived, threatened species.

## Chapter 1

### ASSOCIATIONS BETWEEN EGG QUALITY, BEHAVIORAL PLASTICITY, AND REPRODUCTIVE SUCCESS OF SPAWNING FEMALE LAKE STURGEON

ABSTRACT – Reproductive timing is a key indicator of population resilience.

Environmental conditions and traits such as age, size, and operational sex ratio have well-documented influences on spawning behavior and reproductive success. However, the effect of individual variation in the timing of female spawning on egg quality and reproductive success is unclear. In 2012 and 2013, we captured adult lake sturgeon (*Acipenser fulvescens*, N=247 and 271, respectively) among seven spawning locations in the Black River, Michigan. We recorded body length, sex, and individually marked fish using PIT and Floy tags. Location, group size, operational sex ratio, river discharge, and water temperature were quantified daily at spawning sites. Plasma steroid concentrations of estradiol-17 $\beta$  and testosterone were quantified from blood samples taken daily from females in 2013 to verify sex and determine ovary quality. Wild-produced larvae were captured during annual drift surveys, and genetic parentage analysis was used to estimate individual reproductive success. Timing of female spawning was influenced by mean temperature, maximum discharge, group size, and average body length of males. Mean concentrations of estradiol-17 $\beta$  and testosterone in pre-spawning females was  $6.81 \pm 1.51$  ng/mL and  $55.9 \pm 3.92$  ng/mL, respectively. Testosterone concentrations varied among females, but egg quality did not vary due to the timing of spawning. Female reproductive success varied due to temperature, discharge, group size, and operational sex ratio. Results indicate that plasticity in female spawning time may affect egg quality, but female reproductive success is largely

influenced by environmental conditions and access to mates which are increasingly variable in riverine systems.

## **INTRODUCTION**

The timing of reproduction is a key indicator of population resilience to environmental variation in fishes (Lowerre-Barbieri et al. 2011). Although reproductive timing has a heritable basis (Neuheimer and MacKenzie 2014), individuals show considerable plasticity in behavior in response to current environmental conditions (Warren and Morbey 2012; Mittelbach et al. 2014) as well as a lag effect to recent environmental changes (Forsythe et al. 2012). For example, females choose the timing and location of spawning by selecting environments that are conducive to offspring survival and growth. Behavioral plasticity in reproductive timing is advantageous for females given that offspring survival largely determines variability in female reproductive success (RS) whereas male RS is typically limited by access to mates (Bateman 1948; Andersson 1994; Serbezov et al. 2010) or mate quality (McGuire et al. 2013). Understanding factors that affect the timing female reproduction provide an understanding of population stability and persistence in response to environmental changes.

In addition to environmental conditions, demographic characteristics and traits including age, body size, and operational sex ratio (OSR) have been shown to influence the timing of reproduction and individual RS (Jørgensen et al. 2006; Wright and Trippel 2009; Lowerre-Barbieri et al. 2011). Older, larger females typically spawn earlier (Wright and Trippel 2009) and produce higher quality eggs and larvae of larger body size than smaller females (Schreck et al. 2001; Olin et al. 2012). Large size can also be

advantageous for males in establishing breeding territories (Anderson et al. 2010) or when females delay spawning in the presence of small males as an indirect form of female mate choice (Berejikian et al. 2000). Additionally, earlier arrival of males to spawning grounds can inflate OSRs (i.e. the number of mature males to the number of mature females) thereby increasing the intensity of intra-sexual competition and the variance in individual RS (Emlen and Oring 1977; Wade and Arnold 1980; Quinn 1999).

Demographic characteristics and environmental conditions also affect the timing of reproduction and individual RS by influencing gonadal development. Females determine the optimal timing of ovulation based on exogenous cues such as water temperature and social interactions within spawning sites (Lowerre-Barbieri et al. 2011; Morgan et al. 2013). In teleost fishes, egg deposition typically occurs within days of ovarian maturation and ovulation given that eggs only remain viable for a short period of time (Bobe et al. 2008; Warren and Morbey 2012). However, fishes such as sturgeon can maintain mature ovaries for months before spawning (Webb et al. 1999). Prolonged periods between ovulation and egg deposition allow females to be more selective in their choice of mates and spawning locations (Brown-Peterson and Heins 2009); however, extended delays in spawning have been shown to decrease egg viability due to over-ripening (Johnston et al. 2008; Thorstad et al. 2008) and increase the probability of degeneration and reabsorption of ovarian follicles (“atresia,” Lubzens et al. 2010).

In sturgeon species, field and experimental studies have shown that discharge and thermal conditions within riverine spawning sites affect reproductive behavior, ovarian maturation, and egg viability (Dettlaff et al. 1993; Forsythe et al. 2012). Delays in female spawning due to rapid fluctuations in discharge during the spawning period

have been observed in several species (Khoroshko 1972; Auer 1996; Paragamian and Kruse 2001), resulting in reduced egg viability for females that remained on the spawning grounds until conditions appeared favorable (Khoroshko 1972). Additionally, elevated temperatures prior to spawning have resulted in higher incidences of atresia and spawning failure in cultured white sturgeon (Webb et al. 2001). Quantifying female ovary and egg quality has been difficult in wild populations of large, long-lived fishes such as sturgeon (Morgan et al. 2008), and has led to the development of several non-invasive methods for detecting early signs of atresia (Talbot et al. 2011). One such method includes quantifying plasma concentrations of testosterone (T) and estradiol-17 $\beta$  (E2) which are elevated in pre-spawning females, but show significant decreases when fish become atretic (Webb et al. 2001). In this study, we examined factors affecting the timing of reproduction in female lake sturgeon (*Acipenser fulvescens*, Rafinesque 1817), and quantified plasma sex steroid concentration to determine female ovary quality.

Lake sturgeon are iteroparous, broadcast spawning fish that migrate to riverine areas to spawn (Auer 1996). 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 multi-modal with adults migrating to spawning grounds from late April to early June when water temperature is between 8.8 – 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. Arrival date on the spawning grounds is generally believed to be the date of spawning for females (Bruch and Binkowski 2002), but females have been observed on the spawning grounds for

several days when environmental conditions are variable (Auer 1996). Sex of the adults is typically determined by the expulsion of gametes; however, females are often difficult to identify given that eggs are not readily visible until ovulation which has been shown to occur among a wide range of temperatures (Bruch and Binkowski 2002). Alternatively, plasma steroid concentrations can be used to differentiate sex (Craig et al. 2009; Shaw et al. 2012) and determine spawning state when gametes are not observable (Thiem et al. 2013). Spawning bouts last approximately 1-2 minutes with females releasing adhesive, demersal eggs over cobble and gravel substrate (Bruch and Binkowski 2002). Eggs are fertilized by sperm from multiple males (Thiem et al. 2013), and incubate without parental care until hatch (Duong et al. 2011). Hatched larvae remain at the spawning grounds, feeding on endogenous yolk-sac reserves for approximately 6-15 days dependent on water temperature before dispersing downstream to begin exogenously feeding (Smith and King 2005).

The objectives of this study were to: i) examine how environmental conditions (temperature and discharge) and demographic factors (body size, group size, OSR, and inter-spawning interval) within spawning sites affect the timing of female spawning, ii) determine whether female RS quantified using genetic parentage analysis varied due to the environmental and demographic factors within spawning sites, iii) quantify female ovary quality using plasma concentrations of testosterone, and iv) determine whether the amount of time females spend on the spawning grounds before spawning affects individual RS and egg quality. Knowledge on species reproductive biology is vital for understanding critical habitat needs and factors affecting population-levels of

recruitment for fishes that have been over-exploited or are being threatened with habitat loss due to human activities and environmental change.

## **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). Adult lake sturgeon migrate into the UBR and spawn among seven sites located along a 1.5km stretch of the river (Fig 1b). Spawning activities are dependent on both temperature and discharge, and typically begin when water temperature is approximately 10°C and a lag in discharge is observed (Forsythe et al. 2012). Wadable, shallow spawning sites (~1-3 meters) 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.

### *Adult sampling*

Adult lake sturgeon were captured daily among the seven spawning sites using long-handled dip-nets. Total length (cm) and site of capture were recorded for each individual. Sex was determined by applying pressure to the abdomen to expel gametes or by examining the urogenital opening. Each fish was individually marked with T-bar anchor (floy) tags (Floy Tag, Inc.) of different color combinations at the base of the dorsal fin designating sex, identification number, and the time of entry in the river. Fish were checked for internal passive integrated transponder (PIT) tags from previous years located near the dorsal scutes using hand-held scanners. 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).

Group size (total number of males and females in a spawning group) and operational sex ratio (number of males to females; Emlen and Oring 1977) were quantified daily at each spawning site. Spawning date for each female was assigned based on visual spawning evidence, or as the last date observed within the river given that females typically complete spawning within 12 hours (Bruch and Binkowski 2002) and then promptly leave the spawning grounds (Peterson et al. 2007). Retention time (RT, days) was quantified for each female as spawning date minus the day of first capture in the river. Male RT was not considered given that females decide the timing, location, and duration of spawning (Bruch and Binkowski 2002). Inter-spawning interval was calculated for each female as the average number of years between spawning using long-term data collected from 2001-2011 (data not shown), and used as a proxy for age. Traditional aging techniques using fin spines typically under-estimated age and body size is only reliable until a particular age (Shaw et al. 2012). Therefore, first-time spawning females were considered younger than females that had previously spawned. Water temperature and discharge were measured hourly at the spawning sites throughout the spawning season using Onset HOBO pressure loggers (Figure 2; Cape Cod, Massachusetts, USA).

#### *Blood collection and plasma hormone analysis*

In 2013, we collected blood from the caudal vein of thirty-eight females each day they were present in the river. Seventy-five total blood samples were collected using 3-mL syringes and 22 gauge needles coated in 1000 units/mL of heparin. 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 for 15 minutes. Plasma was extracted and placed in new microcentrifuge tubes. Tubes were stored at -20°C until hormone analysis.

The steroids, T and E2, were extracted from plasma following the method of Fitzpatrick et al. (1987) for analysis by radioimmunoassay (RIA). Briefly, 100 µL 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 re-suspended in 1 mL of phosphate-buffered saline with gelatin (PBSG), and 10 or 50 µL 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 plasma (n=4), which were extracted as described above. All steroid assay results were corrected for recovery. Recovery efficiencies for T varied between 89% and 94%, with a mean of 92%. Estradiol recovery efficiency was 71%.

Plasma concentrations of T and E2 were measured by RIA 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/L PBSG) was used for all assays to reduce non-specific binding. The intra- and inter-assay 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 for T and 0.16 ng/mL for E2.

### *Larval collection*

Larval lake sturgeon were sampled approximately 2km downstream of the spawning sites (Fig. 1a). Sampling began 10 days after the first observed spawning date and 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 2100-0200 hours. Captured larvae were transported to the streamside facility and reared separately by capture night for approximately three months until individuals were large enough for nonlethal sampling of fin clips 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*

In 2012, we genotyped 10% of the dead and 10% of the live larvae collected per night during the annual drift survey (N=820) for parentage analysis. In 2013, we genotyped all live and dead larvae collected during the drift survey (N=845) due to low number of collected individuals. 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. However,

cycle number was increased to 35 for all loci during the annealing phase and 1.5uL of forward and reverse primer were used to increase 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 was 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). 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 (LOD) score. 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 our study was based on concordance between the two programs.

In CERVUS, we simulated 10,000 offspring with a strict level of 95% and a relaxed level of 80% to determine the confidence in our parentage assignment based on population estimates of allelic frequencies. Parentage analysis in CERVUS and COLONY included 243 adults in 2012 and 262 adults in 2013, an 85% estimate of the proportion of adults captured (K. Scribner, unpublished data), and empirical genotyping error estimates of 0.97% and 0.54% for 2012 and 2013, respectively. Adults with the highest, positive LOD score in CERVUS and highest maximum likelihood score in COLONY were chosen as the most likely candidate parents. Reproductive success was

quantified as the total number of offspring assigned to each female. Male RS was not considered given that males are limited by the number of receptive females.

### *Statistical analysis*

Statistical analyses were performed using the package, glmmADMB (Skaug et al. 2006) implemented in the statistical program, R (R Development Core Team). Data from 2012 and 2013 were analyzed separately given the differences observed in environmental conditions and proportion of larvae genotyped. We tested for normality in all variables using Shapiro-Wilk tests. We used generalized linear models to determine how female retention time and reproductive success varied as a function of demographic characteristics and abiotic factors. Demographic characteristics included female body length, female inter-spawning interval, male total length for males that were present with the female, mean group size when a female was present, and the operational sex ratio. Abiotic factors included the daily mean, minimum, maximum, and range in temperature and discharge observed from day of capture to day of spawning for each female. Range in temperature and discharge were quantified as the daily maximum minus the daily minimum. All 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. We tested for multicollinearity between variables by fitting a full model including all demographic and abiotic factors, quantifying the variance inflation factor, and calculating the Pearson's correlation between pairs of variables.

Minimum and maximum temperature were removed from the model due to evidence of multicollinearity ( $>7$ ) and high correlation ( $>0.7$ ) with mean temperature. Additionally, we removed mean and minimum discharge which showed high correlation

with maximum discharge. Maximum discharge was included in the model given that rapid increases in discharge have been shown to affect the timing of female reproduction (Paragamian and Kruse 2001; Auer 1996). For retention time, we fit a negative binomial regression with a log link function as:  $RT = \text{mean temperature} + \text{range in temperature} + \text{maximum discharge} + \text{range in discharge} + \text{female total length} + \text{female inter-spawning interval} + \text{mean male total length} + \text{mean group size} + \text{mean operational sex ratio} + \text{possible two-way interactions of biological relevance}$  (Burnham and Anderson 2002). We fit the same full model with individual RS as the response variable, but included RT as an additional explanatory variable. We used negative binomial error distributions in models instead of a Poisson given the over-dispersion in retention time and reproductive success. Likelihood ratio tests were used to determine the model of best fit. For variables that were significant predictors of RT, we also tested whether there were significant differences in the abiotic factors and demographic characteristics between females that stayed in the river for one day versus females that were observed in the river for greater than one. We used non-parametric Mann-Whitney U tests to test this given the non-normality observed in the significant explanatory variables.

Quantified plasma concentrations of T and E2 were used to verify female sex assignment for sixteen fish that: i) had an inter-spawning interval of less than 3 years, ii) could not be identified based on spawning behavior or expression of gametes, or iii) were spawning for the first time. Female sex was verified using classification functions developed for white sturgeon (*Acipenser transmontanus* Richardson 1836) by Webb et al. (2002) and recently applied to lake sturgeon (Craig et al. 2009; Shaw et al. 2012;

Thiem et al. 2013). Concentrations of T were used to determine the probability of a female having normal versus atretic ovaries using the logistic regression developed by Talbott et al (2011). Application of the logistic regression in white sturgeon resulted in the correct assignment of 95% of fish with normal ovaries and 93% of fish with atretic ovaries (Talbott et al. 2011) indicating that it is a reliable indicator of ovary and egg quality when observational data is unavailable.

## **RESULTS**

### *2012 adult data and parentage analysis*

In 2012, adult lake sturgeon were observed in the river from April 16th until June 4th. The mean river water temperature on the first day adults were present was 13°C and maximum discharge was approximately 7.0 m<sup>3</sup>/sec (Fig. 2a). Females were on average larger than males with a mean ( $\pm$ SE) total length of 169.6 ( $\pm$ 1.54) cm and range of 135 to 192cm (Fig. 3a). Males averaged 146.9 ( $\pm$ 1.05) cm and range of 105 to 190cm. Group sizes in the river ranged from 1 to 29 fish with a mean of 10 fish per group when females were present. OSR was skewed towards males with an OSR of 184 males to 63 females for the entire season, and an average of 3 males per female at the spawning sites. OSR ranged from 1 to 9 males per each female during the spawning season.

Average inter-spawning interval for the females marked in 2012 was 3 ( $\pm$ 0.23) years, but ranged from 0 to 10 years with 32% of the spawning females being first-time spawners. RT ranged from 1 to 15 days spent in the river. Mean RT was 2.9 ( $\pm$ 0.41) days with 60% of females spending only 1 day in the river. Mean RT was negatively associated with mean temperature, maximum discharge, mean group size, mean male

total length, and an interaction between maximum discharge and mean group size with the interaction term having the largest influence (Table 1). None of these variables differed for females that stayed in the river one day versus those that stayed in the river greater than one day (Mann-Whitney U tests,  $p > 0.05$  in all tests)

Eleven of the twelve loci genotyped for the 2012 adults were in Hardy-Weinberg equilibrium. Allelic diversity ranged from 2 to 11 with a mean of 5.42 alleles per locus and an expected heterozygosity of 0.59. The combined non-exclusion probability for the parent pairs was 0.000057. Seventy-nine percent of the offspring were assigned a female parent with a 75% degree of concordance in female assignments between CERVUS and COLONY. All sixty-two females in 2012 were assigned at least 2 offspring during parentage analysis. Female RS ranged from 2 to 38 offspring with a mean of approximately 10 ( $\pm 1.02$ ) larvae assigned to each female. Mean RS was positively associated with mean temperature, maximum discharge, and mean group size, and negatively associated with mean OSR and an interaction between maximum discharge and mean group size. The interaction term had the largest influence on mean RS (Table 1).

#### *2013 adult data and parentage analysis*

In 2013, adult sturgeon were observed on the spawning grounds from May 1st until June 9th. Mean water temperature on the first day adults were present was 13°C and maximum discharge was approximately 20.0 m<sup>3</sup>/sec (Fig. 2b). Females were typically larger than males with a mean total length of 170.9 ( $\pm 1.77$ ) cm ranging from 141 to 198cm. Average male total length was 146.2 ( $\pm 0.97$ ) cm and ranged from 108 to 185 cm (Fig. 3b). Mean group size in the river was 10.9 ( $\pm 0.67$ ) fish per group and

ranged from 1 to 22 fish when females were present. The OSR for the entire season was 210 males to 61 females with an average of 3.4 males per each female on the spawning grounds. OSR ranged from 1 to 13 males per female during the spawning season.

Average inter-spawning interval for the sixty-one females marked in 2013 was 3.4 ( $\pm 0.4$ ) years, and ranged from 0 to 11 years with 34% of the spawning females spawning for the first time. Mean RT was 3.5 ( $\pm 0.57$ ) days and ranged from 1 to 23 days spent in the river. Approximately 63% of females spent only 1 day in the river. Mean RT was negatively associated with mean temperature, maximum discharge, and mean group size, and positively associated with mean male total length, an interaction between mean temperature and mean male total length, and an interaction between maximum discharge and mean group size. Mean temperature had the largest influence on mean RT (Table 1). Maximum discharge ( $W = 303$ ,  $p$ -value = 0.046), mean group size ( $W = 234.5$ ,  $p$ -value = 0.003), and mean male total length ( $W = 591.5$ ,  $p$ -value = 0.022) were significantly different for females that stayed in the river one day versus those that stayed in the river greater than one day. For females that stayed in the river for one day, maximum discharge was 2.3 m<sup>3</sup>/sec higher, mean group size had approximately 4.3 more fish, and mean male total length was 7.7cm smaller.

Allelic diversity ranged from 2 to 11 with a mean of 5.33 alleles per locus and an expected heterozygosity of 0.58 for the 12 loci genotyped for the 2013 adults. Eleven of the loci were in Hardy-Weinberg equilibrium. The combined non-exclusion probability for the parent pairs was 0.000066. Eighty-three percent of the offspring were assigned a female parent with an 80% degree of concordance in female assignments between

CERVUS and COLONY. Ninety-five percent of the spawning females were assigned at least one offspring. Female RS ranged from 3 to 37 offspring with a mean of approximately 11 ( $\pm 1.12$ ) larvae assigned to each female. Mean RS was positively associated with mean temperature and maximum discharge, and negatively associated with mean group size and an interaction between maximum discharge and mean group size. The interaction term had the largest influence on mean RS (Table 1).

#### *2013 plasma hormone analysis*

On average, two blood samples were collected per female with some individuals being sampled up to nine times while on the spawning grounds. The average concentration of T in the seventy-five blood samples was  $55.9 \pm 3.92$  ng/mL. Samples showed significant variation in the concentration of T ranging from 0.29 to 136.94 ng/mL (Fig. 4). E2 concentrations averaged  $6.81 \pm 1.51$  ng/mL and ranged from 0.05 to 18.26 ng/mL (Fig. 5). Of the sixteen fish with unverified sex, fourteen were correctly assigned as female (~87.5%), one was determined to be male, and the sex of one fish could not be determined given that estrogen levels were too low to quantify.

Based on testosterone concentrations, ninety-two percent of females had a high probability of having normal ovaries (Fig. 6) indicating ovary quality was not dependent on the number of days present on the spawning grounds. Two females showed signs of atretic ovaries and had testosterone levels less than 5 ng/mL on the day they spawned. One of the atretic females spent 13 days in the river and had high probabilities of normal ovaries (probability=0.88-0.98) until day nine when the probability dropped to 0.04 within 24 hours. The other atretic female was present on the spawning grounds for one day.

## DISCUSSION

Recent studies suggest that both demographic characteristics and environmental conditions within spawning sites can affect the timing of reproduction in fish (Wright and Trippel 2009; Lowerre-Barbieri et al. 2011); however, our understanding of how the timing of reproduction affects individual reproductive success (RS) and egg quality is limited (Lowerre-Barbieri et al. 2011). In this study, we tracked individual fish movement during two spawning seasons to determine the conditions that affect the number of days female lake sturgeon spend on the spawning grounds before spawning, and whether delays in spawning affect female ovary quality. Retention time (RT) was similar in both years with females averaging 2.9 to 3.5 days on the spawning grounds; however, females in 2013 stayed in the river approximately one week longer with one female remaining in the river for up to 23 days. Similarly, Thiem et al. (2013) observed lake sturgeon on the spawning grounds for an average of 5 days with some individuals staying for 27 days. In both years of the study, 60%-63% of females were present on the spawning grounds for one day. In 2013, females that stayed in the river for only one day spawned at higher discharge levels when mean group sizes were larger, but did not have higher RS given that RS was not dependent on the time females spent in the river. Remaining in the river for one day may be advantageous for adult lake sturgeon given that food availability and cover are typically limited on the spawning grounds (Auer 1996), and water depth decreases during the season.

Mean female RT was significantly affected by both demographic and environmental conditions at the spawning sites. Male body size was a significant predictor of RT (Table 1) as average female RT decreased in 2012 as the average size

of males increased indicating that the presence of large males reduced the amount of times females spent in the river before spawning. Conversely, mean female RT in 2013 increased as the average size of males in the river increased due to an interaction between mean temperature and male body size. Female preference for large males has been well-documented in fishes given that large males are typically better competitors and produce larger offspring than small males (Dickerson et al. 2002; Dickerson et al. 2005). However, evidence of mate choice has been limited in sturgeon with studies documenting only subtle differences in spawning and reproductive success between males due to body size (Duong et al. 2010) or female preference for particular males (Kynard et al. 2011). Lack of evidence for female mate choice in lake sturgeon may also be related to size as females are on average larger than male conspecifics (Fig. 3).

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). Given the polygamous mating behavior of sturgeon, mating with numerous males maximizes female fertilization success and the genetic diversity of offspring (Bruch and Binkowski 2002), and has been shown to increase female RS (Duong et al. 2010). In our study, mean OSRs were 3:1 in 2012 and 3.4:1 in 2013 demonstrating the degree of male-bias on the spawning grounds. Our OSR estimates are comparable to ratios of 3:1 and 5.7:1 estimates in other wild populations of lake sturgeon (Auer 1999; Bruch and Binkowski 2002). The mean number of days female spent on the spawning grounds did not vary due to OSR, but mean female RS was negatively associated with mean OSR in 2012 indicating that increases in the number of males did not increase female RS.

Water temperature and discharge are important cues directly affecting the timing of migration to spawning grounds and duration and location of spawning activities (Paragamian and Kruse 2001; Forsythe et al. 2012). In both years, fish arrived on the spawning grounds when mean water temperature was 13°C which is within the optimal thermal range of 10-16°C for spawning that has been observed in other studies (Auer 1996; Bruch and Binkowski 2002). In 2012 and 2013, increases in mean temperature reduced the mean amount of time females spent on the spawning grounds. Additionally, the largest influence on mean female RT in 2012 and female RS in 2012 and 2013 was the interaction between maximum discharge and group size. The negative association of the interaction term with female RT and RS indicates that increases in maximum discharge encountered by females while in different groups reduced the amount of time females spent on the spawning grounds, and reduced mean female RS. Similarly, Paragamian and Kruse (2001) observed that females spent less time on the spawning grounds due to fluctuations in discharge which was predicted to reduce female RS and lead to lower levels of recruitment given that females spent less time on the spawning grounds.

Quantification of plasma concentrations of T and E2 and use of discriminant classification functions provided a reliable means of verifying sex of spawning females in our study, and has been successfully applied in other lake sturgeon populations (Shaw et al. 2012; Thiem et al. 2013). Using the discriminant functions, we verified our female sex assignment based on cloacal morphology and determined that we correctly identified females 87.5% of the time. Results are similar to the 85% of female white sturgeon correctly assigned by Webb et al. (2002) during development of the

discriminant functions. Average T concentration in females was  $55.9 \pm 3.92$  ng/mL and average E2 was  $6.81 \pm 1.51$  ng/mL which was comparable to T and E2 concentrations quantified by Shaw et al. (2012) in spawning lake sturgeon.

Delays in spawning have been shown to decrease fertilization rates and egg viability due to over-ripening (Berejikian et al. 2000), and are predicted to reduce female RS. In our study, the time females spent on the spawning grounds did not significantly affect female RS. Additionally, the majority of females had a high probability of normal ovaries regardless of the time they spent on the spawning grounds (Fig. 6) suggesting that there is little to no reproductive cost for female lake sturgeon to wait on the spawning grounds until conditions appear favorable. However, the average T concentrations quantified in female lake sturgeon in the study was higher than white sturgeon T concentrations used to develop the threshold concentrations for the logistic regression used to determine the probability of ovaries. Additionally, one female in our study showed signs of atresia after spending nine days on the spawning grounds; however, we're limited in the study in determining whether this female became atretic due to time or due to stress associated with being captured and handled nine times which has been predicted to affect female sex steroid concentrations (Fuzzen et al. 2011; Thiem et al. 2013). Therefore, future studies should integrate histological samples or use of additional non-invasive techniques in addition to sex steroid concentration to verify early signs of atresia in lake sturgeon, and determine that atresia was not due to the stress from being captured multiple times.

Female body size is typically correlated with offspring quality as large females typically produce higher quality eggs and larvae. Additionally, larger older females are

hypothesized to spend less time on the spawning grounds and produce more viable eggs than younger, first time spawners although this has not been well-studied (Morgan et al. 2008). In our study, female RT and RS did not vary due to female body size or female inter-spawning interval. Several first-time spawning females spent the same amount of time on the spawning grounds as experienced, older females. Overall, our findings demonstrate the complexity of factors affecting the timing of female spawning and illustrate why our understanding of reproductive timing in fishes has been limited (Lowerre-Barbieri et al. 2011). Future empirical work is needed that address the factors that affect female reproductive timing and the probability of atresia, particularly due to inter-annual variability in environmental conditions. Knowledge within these areas is essential for developing effective management strategies that protect critical habitat, accurately predict population levels of recruitment, and accurately predict how wild populations will be influenced by predicted environmental changes.

## APPENDIX

Table 1.1 Variable estimates and standard errors (SE) explaining changes in the expected log of mean female reproductive success (RS) and log of mean retention time (RT). Estimates are provided for models of best fit for 2012 and 2013.

Trait	Component	Estimate	SE
(a) 2012			
RT	Mean Temperature	-0.32	0.10
	Max Discharge	-0.04	0.14
	Group Size	-0.08	0.15
	Male Total Length	-0.19	0.11
	Max Discharge*Group Size	-0.70	0.21
RS	Mean Temperature	0.36	0.09
	Max Discharge	0.09	0.09
	Group Size	0.34	0.10
	Operational Sex Ratio	-0.30	0.10
	Max Discharge*Group Size	-0.51	0.13
(b) 2013			
RT	Mean Temperature	-0.52	0.13
	Max Discharge	-0.22	0.13
	Group Size	-0.47	0.14
	Male Total Length	0.13	0.13
	Mean Temperature*Male		
	Total Length	0.29	0.12
	Max Discharge*Group Size	0.40	0.14
RS	Mean Temperature	0.22	0.09
	Max Discharge	0.07	0.09
	Group Size	-0.02	0.09
	Max Discharge*Group Size	-0.30	0.09

\* Indicates an interaction between variables.

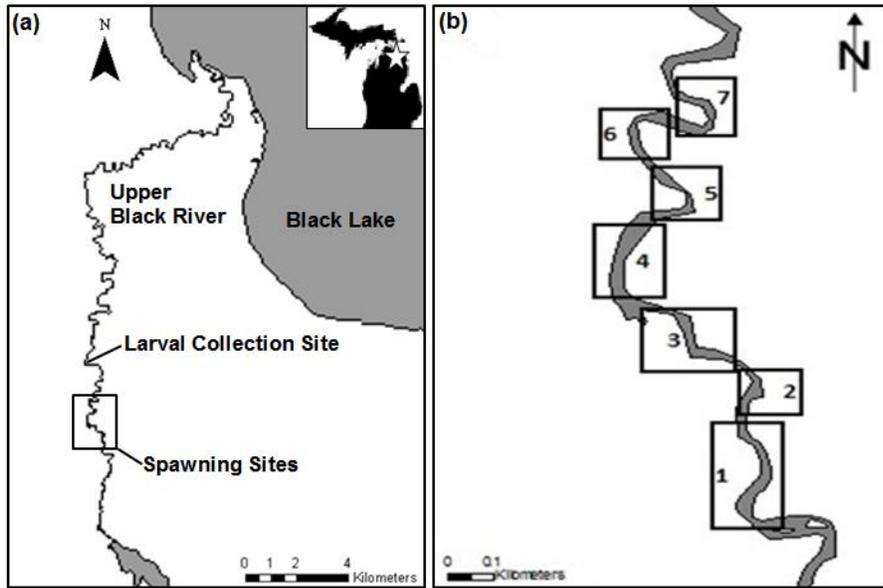


Figure 1.1 The study site on the Upper Black River located in Michigan, USA. Shown are the location of the spawning sites and the larval collection site (a). An enlarged view of the seven separate spawning locations where adults were sampled daily (b).

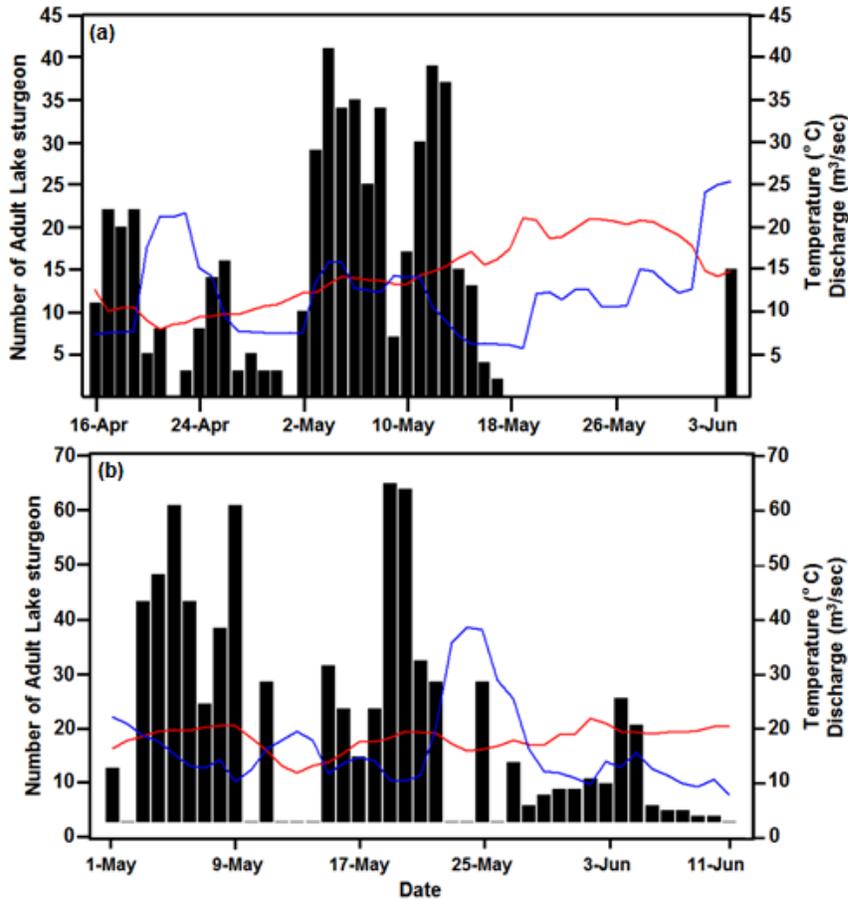


Figure 1.2 The number of adult lake sturgeon present in the river along with mean daily water temperature (red) and river discharge (blue) during the adult spawning seasons in 2012 (a) and 2013 (b).

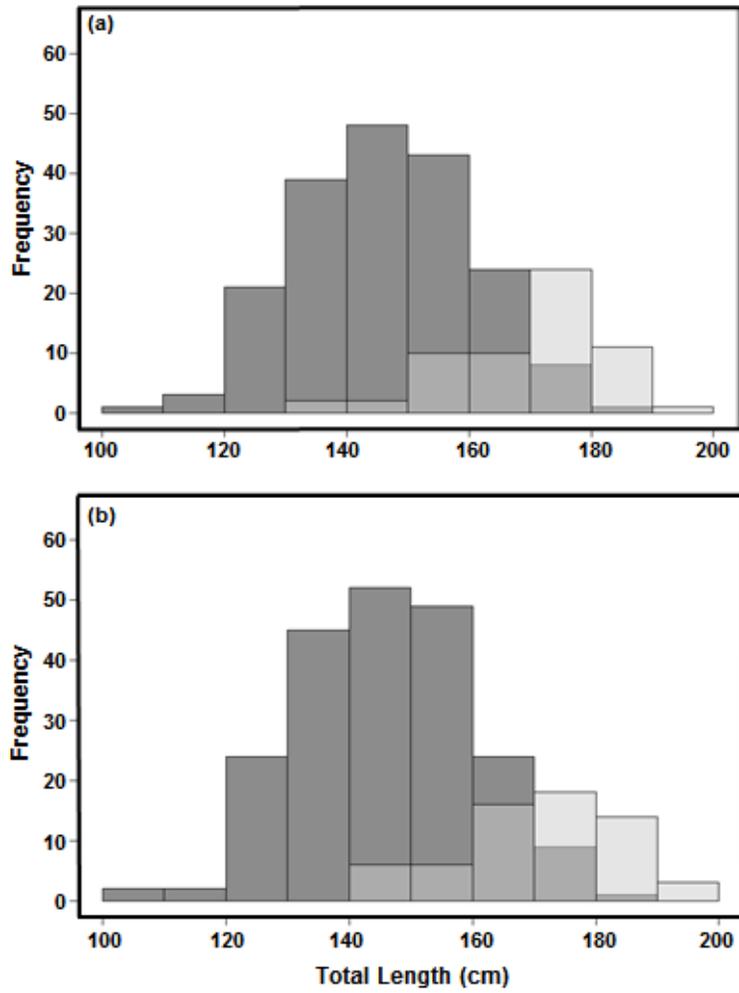


Figure 1.3 Frequency distribution of spawning adult total length where males are gray bars and females are white bars for 2012 (a) and 2013 (b).

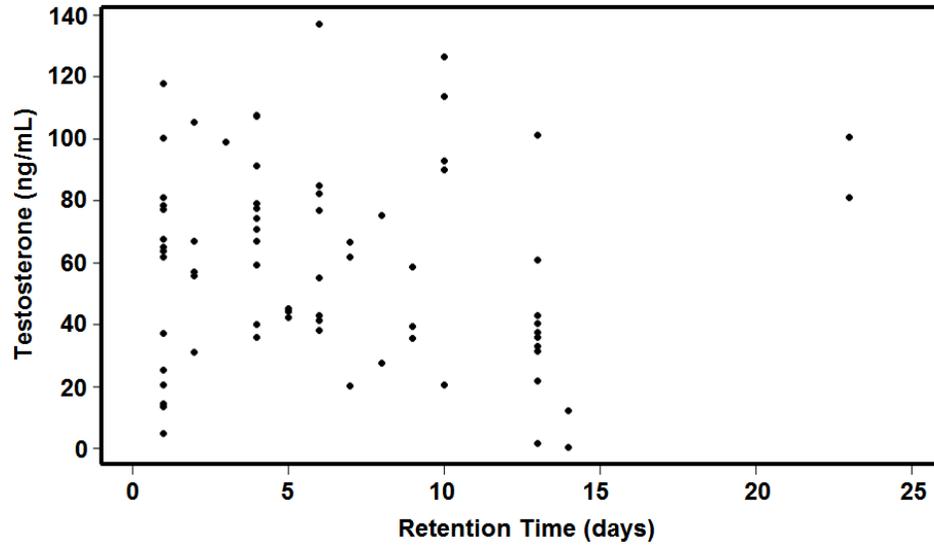


Figure 1.4 Female plasma testosterone concentrations (ng/mL) and the number of days they were present on the spawning grounds (retention time).

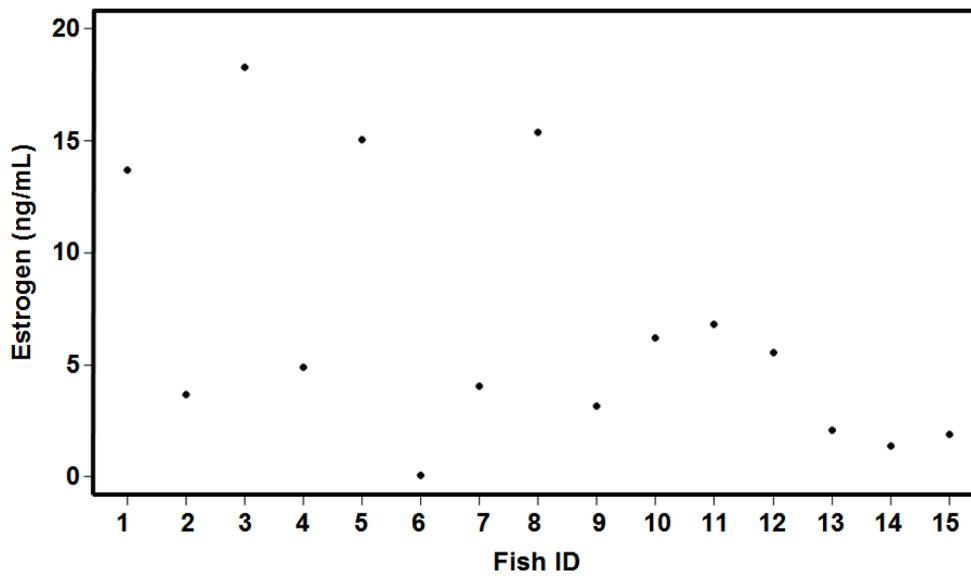


Figure 1.5 Plasma estrogen level concentrations (ng/mL) for fish where sex was undetermined given the lack of expression of gametes.

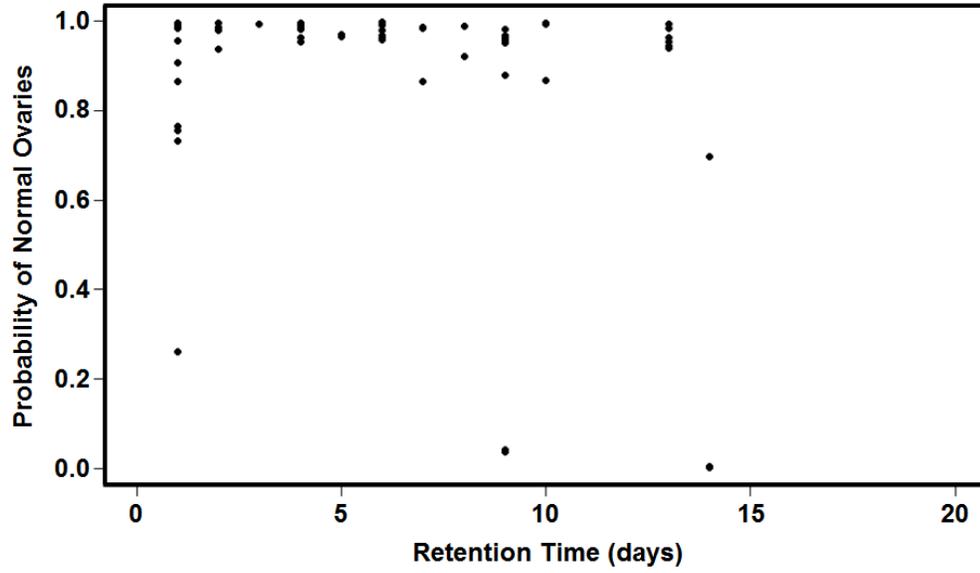


Figure 1.6 The probability of females having normal ovaries estimated based on plasma testosterone levels and the number of days they were present on the spawning grounds (retention time).

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## LITERATURE CITED

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## Chapter 2

### GENETIC AND ENVIRONMENTAL COMPONENTS OF PHENOTYPIC AND BEHAVIORAL TRAIT VARIATION DURING LAKE STURGEON EARLY ONTOGENY

ABSTRACT – Adults can plastically respond to environmental conditions by selecting breeding and egg incubation locations that affect offspring traits during embryonic and larval development. Environmental conditions during incubation can also affect traits during later ontogenetic stages (i.e. ontogenetic contingency). Using a well-studied population of lake sturgeon (*Acipenser fulvescens*) from Black Lake, Michigan, eggs were reared from full and half-sibling families at two spawning locations and in a common garden experiment consisting of three water velocity treatments: high, low, and variable. Larvae reared within the stream varied significantly between spawning sites for traits quantified at hatch including body length, body area, and yolk-sac area. Estimates of trait heritabilities ranged from 0.42-0.48. Growth from hatch to 2-3 weeks post hatch when larvae emerged from the substrate to begin exogenously feeding varied among families reared at the spawning locations due to a genotype-by-environment (G-by-E) interaction. In the common garden experiment, phenotypic variation among families was greatest for larvae reared under high velocity and a significant G-by-E was detected for body length, body area, and head area. Growth also varied among velocity treatments, but did not vary between families. Overall, results indicate that adult-selected spawning and rearing locations as well as genetic effects influence offspring phenotypic trait variation. Importantly, egg incubation conditions can also affect trait variation during sequential ontogenetic stages potentially affecting larval survival and population levels of recruitment.

## INTRODUCTION

Changes in environmental conditions are increasingly affecting phenotypic variation in natural populations worldwide (Kopp and Matuszewski 2014; Teplitsky et al. 2008). Predicting organismal responses to environmental change can be achieved by quantifying the relative contributions of genetic and environmental effects on phenotypic variation (Crespel et al. 2013). Recent studies have led to greater understanding of the extent by which phenotypic trait variation in wild populations can evolve or respond plastically to changing environments (Stoks et al. 2014; Urban et al. 2014; Merilä and Hendry 2014; Merilä 2012; Hoffmann and Srgò 2011). Levels of additive genetic variance indicate how traits can evolve in response to selection (Wilson et al. 2005), and therefore provide an understanding of genetic constraints on a population's evolutionary potential (Garant et al. 2008). Additionally, understanding the role of environmental effects including genotype-by-environment (G-by-E) interactions (Byers 2008) is necessary to avoid attributing phenotypic changes to genetic effects instead of phenotypic plasticity, a common goal in quantitative genetics (Gienapp et al. 2008; Chevin et al. 2010; Teplitsky et al. 2008).

Plasticity includes changes in behavior, phenotype, or physiology that occur without genetic changes (West-Eberhard 2003), and is commonly observed in response to environmental changes. For example, fishes can respond to variability in environmental conditions such as temperature, water velocity, and photoperiod through behavioral adjustments in the timing and location of reproduction. Behavioral plasticity including adult selection of mating locations and egg-incubation conditions can influence the likelihood of fertilization success and probabilities of offspring survival and growth

(Refsnider and Janzen 2010; Ohba et al. 2012; Brodin et al. 2006). Examples of behavioral plasticity in adult fishes include delaying spawning (Warren et al. 2012; Paragamian and Kruse 2001) or selecting “alternative” spawning locations when environmental conditions at traditionally used areas are unfavorable (Gunn and Sein 2000). Little empirical work has examined how environmental conditions associated with the timing and location of adult spawning may affect offspring trait variation during incubation and subsequent ontogenetic stages.

Given the temporal and spatial complexity of stream systems (Allan 2004; Fausch et al. 2002), fishes are well-suited for studies examining the effects of adult-selected spawning locations on offspring trait variation during early ontogenetic stages. Abiotic conditions within spawning sites affect offspring traits associated with survival such as time to hatch, body size, and behavior (Gupta et al. 2013; Crozier et al. 2008; Garant et al. 2003; Leach and Houde 1999). For example, cool temperatures during egg incubation result in offspring of larger body size which is associated with swimming ability, growth rates, predation, and survival (Kingsolver and Huey 2008; Kamler 2005; Fuiman et al. 2005). Conditions experienced during egg incubation can also affect phenotypic traits during later ontogenetic stages. This phenomenon is referred to as “ontogenetic contingency” (Diggle 1994) or “developmental reaction norm” (Pigliucci 1998), and has been observed in numerous taxa including fishes (e.g., *Melanogrammus aeglefinus*, Martell et al. 2005; *Oncorhynchus tshawytscha*, Heath et al. 1993). Therefore, studies examining how environmental conditions experienced during egg incubation affect individual phenotypes during sequential ontogenetic stages are

important for predicting population-levels of phenotypic variation and recruitment (Xu et al. 2010).

Stream discharge is one environmental variable that affects phenotypic and behavioral trait variation in fishes (Langerhans 2008). Spring runoff, rainfall, and hydroelectric dams can cause variability in river discharge (Jonsson and Jonsson 2009; Mion et al. 1998; Skoglund et al. 2011). Changes in discharge can affect adult spawning decisions and potentially affect growth and survival of offspring during early life stages. For example, fluctuations in discharge can delay spawning, dislodge fish eggs from substrate or nest surfaces, affect movements of sediments that decrease oxygen levels in interstitial spaces where fish eggs incubate, and influence egg incubation time thereby affecting larval body size at the time of hatch (Auer 1996; Paragamian and Kruse 2001; Smith and Marsden 2009; Allan 2004). However, few studies have examined how variation in discharge and velocity conditions experienced during egg incubation will affect phenotypic trait variation across sequential ontogenetic stages. In this study, the genetic (family) and environmental (in-situ stream discharge and experimental velocity regimes) effects on larval phenotypic variation in lake sturgeon (*Acipenser fulvescens*) were quantified.

Lake sturgeon are long-lived, iteroparous, broadcast spawning fish that engage in polygamous mating (Bruch and Binkowski 2002). Spawning occurs at multiple times and sites characterized by different thermal and discharge regimes from late April to early June (Forsythe et al. 2011). During spawning events, adults extrude gametes over gravel and rocky substrate and provide no post-ovulatory parental care as eggs from numerous families incubate until hatch (Bruch and Binkowski 2002). The age and size

of larvae at hatch and the timing of emergence when larvae begin to exogenously feed and disperse downstream are dependent on the environmental conditions within spawning and egg rearing sites (Duong et al. 2011). Discharge is one environmental condition associated with different lake sturgeon spawning and egg incubation habitats that varies due to seasonal effects (principally snow melt and precipitation) and the presence of hydroelectric dams on most Great Lakes tributaries used by spawning lake sturgeon (Peterson et al. 2007).

The objectives of this study were to: 1) quantify the relative contributions of genotype (family), rearing environment, and their interaction on larval lake sturgeon phenotypes, 2) examine how discharge within adult-selected rearing environments and climate-induced variability in velocity regimes influences phenotypic trait variation, and 3) determine whether environmental conditions experienced during egg incubation affect phenotypic and behavioral trait variation at a sequential ontogenetic stage (emergence). Knowledge of how discharge and water velocity experienced during egg incubation affect larval phenotypic traits during sequential ontogenetic stages will provide a greater understanding of habitat needs during critical early life stages of fishes and how incubation conditions affect larval survival and recruitment.

## **MATERIALS AND METHODS**

### *Study Site*

The study was conducted on the Upper Black River (UBR), the largest tributary of Black Lake (~3500 hectares) located in northern Michigan, USA (Fig. 1). A well-studied population of approximately 1200 adult lake sturgeon (Pledger et al. 2013) resides in Black Lake which has been isolated from the Great Lakes since 1903 (Baker

and Borgeson 1999; Smith and King 2005b). Shallow spawning areas (~1-3 meters) within the river allow access to adults for gamete collection and experimental work within the stream (Forsythe et al. 2013). Additionally, a streamside rearing facility at the Kleber Dam (Fig. 1) provides a laboratory for concurrent experimental work (e.g. Hastings et al. 2013).

### *Spawning Locations and Gamete Collection*

The 2011 spawning season provided the opportunity to examine how behavioral plasticity in adult spawning site selection in response to discharge conditions affected larval phenotypic trait variation. Uncharacteristically high rainfall and stream discharge prompted adults spawning in early May to bypass “traditional” spawning sites and spawn at an “alternative” location, the Kleber Dam located several kilometers upstream (Fig. 1). Spawning below the dam has been observed in the past (Anderson 1984; Baker and Borgeson 1999); however, in most years spawning occurs in downstream “traditional” sites (Forsythe et al. 2011). During the 2011 spawning season, spawning adults were sampled using long-handled dipnets by wading the stream one or more times per day from May 3<sup>rd</sup> to June 3<sup>rd</sup>. Eggs and sperm were hand-stripped from spawning adults by applying pressure from the anterior to the posterior portion of the abdomen above the urogenital opening. Sperm was collected from males in 20-mL syringes and stored in sterile plastic bags on ice. Eggs and any collected ovarian fluid were stored in sterile plastic bags surrounded by river water for transport. All fertilizations were conducted within 12-hours of gamete collection.

### *Field Experiment*

On May 5<sup>th</sup> and 6<sup>th</sup>, four-hundred eggs from each of six females were placed on circular porous filter pads (7.79 cm<sup>2</sup>; 3M Worldwide Inc, Buffing and Polishing Pads) and fertilized with sperm from one male per female for a total of six full-sibling crosses. Filter pads were secured to the base of open-sided square iron rebar boxes (30.48cm width by 30.48 cm height) with flat bases to allow water passage over eggs (Forsythe et al. 2013). Rebar boxes were secured to a rebar base that could withstand high water velocities and placed in the stream. In total, twelve boxes (two per family) were used. Six boxes were placed on the stream substrate at the “alternative” spawning site (Kleber Dam) and six were placed at the “traditional” downstream spawning site (Fig. 1) allowing eggs to incubate under stream discharge conditions specific to each spawning site. Eggs were checked daily and dead eggs were removed to prevent fungal infection.

Water temperature and discharge were measured daily at the “traditional” site using a HOBO pressure logger. Discharge data was unavailable for the “alternative” site during early May; however, a linear regression was used to determine the relationship between the “traditional” and “alternative” site from mid-May to August using discharge data obtained from the dam operator (Tower-Kleber Limited Partnership). The linear regression ( $y = 0.3541x + 0.5508$ ,  $R^2 = 0.948$ ) was used to predict discharge at the “alternative” site during the egg incubation period in early May. Water temperature measured at the “traditional” site was used to calculate the cumulative thermal units (CTU) to estimate hatch date using the methods of Kempinger (1988). All surviving eggs from both sites were collected one day prior to hatch on May 13<sup>th</sup> and transferred to the streamside facility to keep families separate, prevent post-hatch movement, and

determine the influence of discharge conditions associated with spawning site on larval phenotypic traits.

Larvae were anesthetized with tricaine methanesulfonate (MS-222; 25 mg/mL) and photographed at hatch (Fig. 2a) using digital photography. Image J analysis software (Version 1.34, free-ware) was used to quantify four phenotypic traits at hatch from the digital photographs: body length (BL; mm), body area (BA; mm<sup>2</sup>), yolk-sac area (YSA; mm<sup>2</sup>), and head area (HA; mm<sup>2</sup>; Fig. 2a). A subset of larvae from each family and incubation site were randomly assigned to individual plastic incubation chambers (12.7cm by 6.35cm) containing gravel substrate to provide cover during the period of endogenous feeding on yolk-sac reserves. Mesh siding on the chambers allowed for continual water passage (~0.1m/sec). Chambers were monitored multiple times daily until larvae depleted yolk-sac reserves and emerged from the substrate to begin the onset of exogenous feeding. Timing (days) to emergence was recorded. At emergence, larvae (Fig. 2b) were anesthetized and digitally photographed again to quantify three traits at emergence: time from hatch to emergence (ET; days), body length at emergence (EBL; mm), and growth (TG, changes in body length from hatch to emergence; mm).

#### *Velocity (Common Garden) Experiment*

On May 15<sup>th</sup> and 16<sup>th</sup>, six-hundred eggs from each of four females were fertilized with sperm from two males each to produce eight half-sibling crosses. One hundred eggs from each half-sib cross were allowed to adhere to screens mounted on 8cm by 4cm plexi-glass plates. After approximately 30 minutes (ample time allowing for eggs to adhere to the mesh given the adhesive glycoprotein layer of sturgeon eggs, Doering et

al. 2012), plates were secured onto six 2.70m long by 0.10m wide flumes (2 per treatment) made of PVC tubing and reared under one of three discharge treatments at the streamside facility. Velocity treatments included: low (constant 0.20 m/sec), high (constant 0.80 m/sec), and variable velocity (twelve hours at 0.20 m/sec and twelve hours of 0.80 m/sec). Flume velocity was estimated using a Marsh-McBirney flow meter. Low and high velocity treatments represented the natural range in velocity conditions (0.15-1.40m/sec) when lake sturgeon spawn and eggs have been observed on river substrate (Kempinger 1988; Hay-Chmielewski and Whelan 1997). The variable treatment simulated a temporally-variable environment associated with diel changes in water velocity associated with flood events or anthropogenic disturbance associated with hydroelectric operations. Families were replicated among treatments and egg placement was randomized within each flume. CTU was estimated based on water temperature in the streamside facility using a YSI 5200 Recirculating System Monitor (Xylem, Inc.) to predict hatch date. Eggs were removed from flumes one day prior to hatch to keep families separate and so that phenotypes at hatch reflected velocity conditions experienced during incubation. The four hatch traits (body length, body area, yolk-sac area, and head area) and three emergence traits (timing of emergence, emergence body length, and growth) were quantified using the same methods as described in the field experiment.

All research was conducted under animal use and care procedures approved by the Michigan State University Institutional Animal Care and Use Committee.

### *Statistical Analysis*

In the field experiment, linear mixed-effect models were used to test for family and spawning site effects and for G-by-E (i.e. family-by-site) interactions associated with larval phenotypic traits measured at hatch and emergence. All traits were analyzed separately using mixed models implemented in the package “lme4” (Bates, Maechler, and Bolker 2011) in the program, R version 2.13 (R Development Core Team). All models were fit using restricted maximum likelihood estimation (REML) using a step-wise approach for model selection.

In the field experiment, each trait was fit to the full model:

[Eq 1.]  $\text{Trait}(y_{ijk}) = \mu + S_i + F_j + (FS)_{ij} + \varepsilon_{ijk}$  where  $F \sim N(0, \sigma_F^2)$ ,  $FS \sim N(0, \sigma_{FS}^2)$ , and  $\varepsilon \sim N(0, \sigma_\varepsilon^2)$  with  $\mu$  being the population mean,  $S_i$  was the fixed effect for site,  $F_j$  was a random effect for family,  $(FS)_{ij}$  was the family-by-site interaction term treated as a random effect, and  $\varepsilon_{ijk}$  was the random residual error. The data was then fit to a model that excluded the interaction term:

[Eq 2.]  $\text{Trait}(y_{ij}) = \mu + S_i + F_j + \varepsilon_{ij}$  where  $F \sim N(0, \sigma_F^2)$  and  $\varepsilon \sim N(0, \sigma_\varepsilon^2)$ . A likelihood ratio test was used for model comparison. Next, a likelihood ratio test was used to compare equation [2] to a simpler model:

[Eq 3.]  $\text{Trait}(y_{ij}) = \mu + S_i + \varepsilon_i$  to test for family effects. For each trait where equation [2] was the model of best fit, a point-estimate of the narrow-sense heritability ( $h^2$ ) was computed. No confidence intervals could be estimated (see derivation in Online Resource 1). Lastly, a likelihood ratio test was used to test for effects of rearing site. The same analysis and model selection approach was used to analyze the velocity (common garden) experiment with treatment (low, variable, high velocity) as the fixed effect in all models instead of site.

## RESULTS

### *Field Experiment*

Eggs incubated in the river at the “traditional” and “alternative” spawning sites characterized by different discharge conditions (Online Resource 2) for nine days and were collected at approximately 60 CTUs. Individuals began to hatch the following day when CTU was approximately 68 which is within the traditional range of CTUs at the time of hatch (58.1 to 71.4) estimated in previous lake sturgeon studies on the UBR (Smith and King 2005a). Twenty-eight individuals from each of the six families from both the “alternative” and “traditional” incubation sites were photographed at hatch (Fig. 2a). Families showed significant variation in three of the traits measured at hatch. Mean body length (BL) ranged from 11.84 ( $\pm 1.24$ ) to 13.41 ( $\pm 0.85$ ) mm while body area (BA) ranged from 22.90 ( $\pm 3.73$ ) to 28.98 ( $\pm 4.73$ ) mm<sup>2</sup>. Yolk-sac area (YSA) also varied between families and ranged from 6.73 ( $\pm 1.08$ ) to 8.20 ( $\pm 1.08$ ) mm. Phenotypic variance in all three traits varied among families with the percent of phenotypic variance explained by genetic differences ( $h^2$ ) estimated as 0.48, 0.42, and 0.47 for BL, BA, and YSA, respectively (Table 1; Online Resource 1). The fourth hatch trait, mean head area (HA), showed little variation ranging from 0.98 ( $\pm 0.15$ ) to 1.20 ( $\pm 0.16$ ) mm<sup>2</sup> and no differences were detected between families. Additionally, no family-by-site (G-by-E) interaction or site effects were detected for any of the four traits measured at hatch ( $p > 0.05$ ; Online Resources 3 and 4).

Twenty-four individuals from each family (N=12/site) were placed in individual incubation chambers and monitored until emergence. The number of larvae that emerged from the substrate and were photographed ranged from 15 to 22 individuals

per family. Variation in the timing of larval emergence (ET) was low, as most individuals emerged from substrate in 13 to 17 days. Larvae across both rearing sites varied in body size at emergence (EBL) from 17.48 ( $\pm 1.02$ ) to 25.12 ( $\pm 1.36$ ) mm; however, no significant effects were detected for ET or EBL across both sites ( $p > 0.05$ ; Online Resources 3 and 4). A significant family-by-site interaction for larval growth from hatch to emergence (TG) was detected (Table 1) as families ranged from 10.47 ( $\pm 1.57$ ) to 11.87 ( $\pm 1.43$ ) mm for larvae reared at the “alternative” site versus 8.13 ( $\pm 2.28$ ) to 10.89 ( $\pm 1.13$ ) mm for fish that incubated at the “traditional” site. The family-by-site interaction explained approximately 33% of the phenotypic variation observed in TG while family explained approximately 18%. Differences in TG among families and between sites are visualized in a reaction norm plot (Fig. 3).

#### *Velocity (Common Garden) Experiment*

Eggs were incubated under treatments simulating different stream velocities likely experienced during the egg incubation period for seven days and were removed when CTU was approximately 58. Larvae began to hatch the following day when CTU was approximately 70 (within the traditional range of 58.1 to 71.4 CTUs at time of hatch, Smith and King 2005a). The number of larvae from each family that survived to hatch across all three treatments ranged from 13 to 46 individuals with the lowest number of hatched individuals from the High velocity treatment. A significant family-by-treatment effect was detected for BL, BA, and HA (Table 1) with the family-by-treatment interaction explaining approximately 13-18% of the phenotypic variation observed among the three traits. As indicated by the reaction norm (Fig. 4), BL was most variable in the High velocity treatment with a range of 10.47 to 13.67 ( $\pm 0.64$ ) mm versus the Low

velocity treatment where BL ranged from 11.47 to 12.52 ( $\pm 0.68$ ) mm. Body area (BA) and head area (HA) also showed variation across families and treatments ranging from 22.18 to 31.24 ( $\pm 2.95$ ) mm<sup>2</sup> and 0.98 to 1.21 ( $\pm 0.14$ ) mm<sup>2</sup>, respectively. Yolk-sac area (YSA) showed little variation across families and treatments ranging from 7.34 ( $\pm 0.90$ ) to 8.73 ( $\pm 0.66$ ) mm<sup>2</sup> and no significant effects were detected (Online Resources 3 and 4).

The number of larvae that survived and emerged from the incubation chambers ranged from 12 to 37 individuals per family across the three treatments. There was little variance among treatments in ET which ranged from 13.75 to 14.25 ( $\pm 1.29$ ) days and no significant differences among families or treatments in EBL which ranged from 22.50 to 25.05 ( $\pm 1.47$ ) mm. Larvae varied in growth from hatch to emergence (TG) ranging from 10.49 to 14.33 ( $\pm 2.09$ ) mm across the three treatments. TG significantly varied among treatments ( $p=0.040$ ,  $p=0.012$ ), but did not vary among families (Online Resource 4).

## **DISCUSSION**

In this study, the concurrent use of a natural stream and a common garden experiment allowed for determination of how potential discharge and velocity regimes experienced during egg incubation and genetic (family) effects influenced larval phenotypic traits at hatch and emergence. In fishes, early life stages are the critical period in which mortality is highest (~99%) and growth and survival is affected by several abiotic and biotic factors (Chambers and Trippel 1997). Understanding factors that affect trait variation during this period is vital to predict population levels of recruitment. Environmental conditions experienced during egg incubation are known to

affect offspring traits associated with survival in numerous fishes. However, the lack of knowledge of how conditions experienced during egg incubation affect larval fish phenotypic traits during sequential ontogenetic stages is surprising given the high variability of fluvial systems (Schumm 1988), particularly during and immediately following the spring spawning season.

Results from the study showed that discharge (stream) and velocity (experimental) regimes experienced during egg incubation can influence offspring traits at later ontogenetic stages. In the field experiment, plasticity in simulated adult spawning site selection based on discharge had no effect on traits measured at hatch; however, differences in growth (TG) attributed to family-by-site (G-by-E) interactions were observed. The presence of G-by-E interactions indicate that certain genotypes are more sensitive to environmental influences, thereby resulting in a higher contribution of environmental effects on the observed phenotypic trait variation and less of a genetic effect (Falconer and Mackay 1996). Furthermore, differences in total growth among individuals due to egg rearing environment provides evidence of ontogenetic contingency. Our results emphasize the need to examine traits beyond the endogenous feeding stage in order to understand how rearing conditions will affect offspring traits that are likely associated with survival and population levels of recruitment (Burt et al. 2011).

Stream velocity is one environmental factor that varies spatially and temporally during the lake sturgeon spawning season (Forsythe et al. 2011) and was experimentally manipulated in the streamside rearing facility. Results revealed that there were significant differences in TG from hatch to emergence between individuals

associated with the velocity regime experienced during egg incubation providing additional evidence of ontogenetic contingency. Lake sturgeon eggs have been observed in stream beds with velocities ranging from 0.15 to 1.40 m/sec; however, populations in Michigan and Wisconsin typically experience stream velocities closer to 0.30m/sec (Kempinger 1988; Hay-Chmielewski and Whelan 1997). Therefore, differences in growth occurred among individuals exposed to different velocity treatments representing a large range over which lake sturgeon spawn.

No differences were observed in yolk-sac area (YSA) between lake sturgeon larvae reared under the different velocity treatments. Yolk is considered the primary source of energy as larvae endogenously feed and is an important determinant of growth prior to exogenous feeding. However, growth can also be influenced by environmental variables as well maternal (e.g. egg size) and genetic effects (Kamler 2008). Differences observed in TG at the timing of emergence may be attributed to genetic or maternal effects, explaining why no differences were observed in YSA at the time of hatch. Alternatively, differences in yolk-sac utilization based on larval body size may explain why TG differed in the field experiment. For example, Hardy and Litvak (2004) found that larger shortnose sturgeon (*Acipenser brevirostrum*) larvae with higher metabolic demands may have experienced declines in yolk-sac utilization efficiency resulting in slower growth rates than smaller Atlantic sturgeon (*Acipenser oxyrinchus*) reared in the same environment.

Discharge and velocity are two related environmental variables in riverine systems (Sloat and Hull 2004). Although different families were used, the lack of concordance between the field (discharge) and velocity experimental results suggest

that discharge regimes may not have been as variable between spawning sites as expected. Alternatively, discharge may not have been the only environmental condition affecting trait variation at either spawning site. Thermal regimes are also known to vary across lake sturgeon spawning locations (Forsythe et al. 2011); however, data indicates that thermal regimes at the alternative and traditional spawning areas are highly correlated during the lake sturgeon spawning season (data not shown). Further studies examining additional environmental conditions within spawning locations would be beneficial to understand how multi-variable incubation environments affect larval phenotypic variation across ontogenetic stages.

In the field experiment, a significant effect of family on body length (BL), body area (BA), and yolk-sac area (YSA) at hatch was observed and approximately half of the inter-individual variation was attributed to genetic effects (Table 1). Heritability estimates (0.42-0.48) for the three traits in the study represented the upper-limit of a narrow-sense heritability estimate (similar to a broad-sense heritability estimate) given the inability to separate out additive genetic variance from other genetic effects. However, the estimates provide insight on the ability of the population to respond genetically (i.e. change in gene frequency) to changes in environmental conditions if trait expression is associated with differential survival. Therefore, differences in larval phenotypic traits at hatch such as larval body size may indicate a potential for differential survival between genotypes given that body size is associated with survival in many taxa (Fischer et al. 2011; DuRant et al. 2010; Einum and Fleming 1999). Significant differences in larval survivorship among families can lead to particular adult genotypes contributing more offspring to a given year class. For lake sturgeon with low

annual recruitment and long generation times, differential survival between members of different families can reduce cohort effective breeding population size and genetic diversity within the Black Lake population. Similar results have been observed in captive barramundi (*Lates calcarifer*) where differential survival between larvae of different families led to reductions in effective population size and skewed reproductive contributions of families to the year class (Frost et al. 2006). Studies examining whether larval body size is associated with differential survival in age-0 larval lake sturgeon would be beneficial.

Spring flooding events and hydroelectric dam operations are two known causes of variability in discharge conditions and velocity rates in riverine systems (Jensen and Johnsen 1999; Liermann et al. 2012; Lytle and Poff 2004), and can alter larval rearing habitats and affect population levels of recruitment (Paragamian and Wakkinen 2011). Results from the velocity experiment indicate significant differences in body length (BL), body area (BA), and head area (HA) at the time of hatch due to family-by-treatment (G-by-E) interactions. Importantly, the largest variation in body size was observed among larvae of different families reared in the high velocity treatment (0.8m/s, Fig. 4) representing the treatment of extreme environmental deviance. The large range in larval phenotypic variation associated with the high velocity treatment compared to the fluctuating variable velocity treatment may have resulted from the expression of cryptic genetic variation. Therefore, the high velocity experiment may have represented a stressful environment for lake sturgeon larvae, particularly on larvae from certain families (Fig. 4), given that cryptic genetic variation is often revealed in novel or stressful environments (Wund 2012; Charmantier and Garant 2005; McGuigan and Sgrò 2009).

Cryptic genetic variation is also revealed due to the presence of a G-by-E interaction (Rouzic and Carlborg 2007). Variation can be beneficial if genotypes produce phenotypes favored under current environmental conditions (Candolin 2009). However, phenotypic variants often have lower survival and can be quickly eliminated by natural selection (Ghalambor et al. 2007). The presence of non-zero heritable traits in larval lake sturgeon and intra- and inter-annual variation in environmental conditions suggests different genotypes (e.g., offspring from different females) may be favored within and among years and would be represented in mixed-aged spawning groups in future years. However, observations from the study were only taken post-hatch up to the timing of emergence when mortality is highest and it is not known whether size differentials are maintained over longer periods. Further studies should examine the persistence of environmental effects to later ontogenetic stages and determine whether differences in phenotypic variation among families will lead to differential survival during early ontogeny.

Changes in the distributions of phenotypic traits within populations have become a common occurrence as a response to environmental change; however the causes for these shifts are difficult to identify (Ozgul et al. 2010). Inter-individual variation in the degree of behavioral plasticity may be one explanation for within-population trait changes (Dingemanse and Wolf 2013). Differences in growth between lake sturgeon larvae reared at the “traditional” and “alternative” sites in the study indicate that adult use of different spawning locations can affect offspring size at later ontogenetic stages. Additionally, the timing of spawning within a season may affect offspring phenotypes as indicated by the differences in total growth observed between lake sturgeon larvae

reared in the different velocity regimes representing the range in velocity rates during the entire spawning season. However, observations from the study did not include additional environmental variables such as temperature, substrate, and depth which vary among spawning locations during the spawning season. Further empirical work addressing the consequences of adult behavioral plasticity in response to annual and climate-induced variability in environmental conditions would be beneficial (Dingemans and Wolf 2013), particularly in long-lived species where adaptive evolution cannot occur at the same pace as environmental changes (Refsnider and Janzen 2012). Knowledge within these research areas is essential to understand factors affecting larval survival and potential population levels of genetic diversity and recruitment.

## APPENDIX

Table 2.1 Variance components and standard errors (SE) estimated from the model of best fit for the field and velocity experiments. P-values are presented from the likelihood ratio tests and heritability ( $h^2$ ) estimates are provided for traits where only a significant family effect was detected.

Phenotypic Traits	Component	Var	SE	P-value
<b>(a) Field Experiment</b>				
Body Length	Family	0.30	0.03	0.008
	Residual	0.94	0.05	
	$h^2$	0.48	--	
Body Area	Family	4.62	0.12	0.028
	Residual	17.05	0.23	
	$h^2$	0.42	--	
Yolk-sac Area	Family	0.27	0.03	0.006
	Residual	0.87	0.05	
	$h^2$	0.47	--	
Total Growth	Family*Site	1.73	0.12	<0.001
	Family	0.94	0.09	
	Residual	2.63	0.15	
<b>(b) Velocity Experiment</b>				
Body Length	Family*Treatment	0.13	0.02	0.003
	Family	0.06	0.02	
	Residual	0.52	0.05	
Body Area	Family*Treatment	1.74	0.08	0.003
	Family	0.78	0.06	
	Residual	8.68	0.19	
Head Area	Family*Treatment	<0.01	<0.01	0.019
	Family	<0.01	<0.01	
	Residual	0.02	<0.01	

\*Indicates an interaction between the components.

Table 2.2 Daily mean, minimum, and maximum discharge levels (meters<sup>3</sup>/sec) during the egg incubation period at the “Alternative” and “Traditional” spawning sites used in the field experiment.

Date	Site	Mean (m <sup>3</sup> /s)	Min (m <sup>3</sup> /s)	Max (m <sup>3</sup> /s)
5-May	Traditional	25.95	10.92	36.46
	Alternative	4.99	3.01	7.55
6-May	Traditional	23.40	10.38	34.48
	Alternative	4.66	2.89	6.07
7-May	Traditional	25.66	9.89	49.62
	Alternative	5.11	2.81	9.41
8-May	Traditional	26.33	10.04	33.18
	Alternative	5.07	2.82	5.90
9-May	Traditional	18.74	11.32	31.35
	Alternative	4.12	3.14	5.77
10-May	Traditional	20.30	10.53	31.28
	Alternative	4.34	2.97	5.82
11-May	Traditional	18.60	9.28	32.00
	Alternative	4.04	2.85	5.78
12-May	Traditional	19.79	12.68	28.76
	Alternative	4.27	3.26	5.38
13-May	Traditional	31.74	15.82	39.76
	Alternative	5.75	3.80	6.59

Table 2.3 Pairwise model comparisons for traits fit to a model including the interaction term and a reduced model without the interaction term. Log-likelihood values, chi-square values, p-values (P), and degrees of freedom (df) obtained from the likelihood ratio tests are presented where applicable.

Phenotypic Traits	Model Comparison	Log-likelihood	Chi-square	P	df
<b>(a) Field Experiment</b>					
Body Length	Family + Site + Family*Site	-474.21			
	Family + Site	-474.21	<0.001	0.987	1
Body Area	Family + Site + Family*Site	-960.48			
	Family + Site	-960.57	0.165	0.685	1
Yolk-sac Area	Family + Site + Family*Site	-461.76			
	Family + Site	-461.76	<0.001	1.000	1
Head Area	Family + Site + Family*Site	-285.29			
	Family + Site	-290.79	0.322	0.571	1
Emergence Time	Family + Site + Family*Site	-220.97			
	Family + Site	-221.05	0.167	0.683	1
Emergence Body Length	Family + Site + Family*Site	-211.05			
	Family + Site	-211.05	<0.001	1.000	1
Total Growth	Family + Site + Family*Site	-227.71			
	Family + Site	-235.32	15.227	<0.001*	1
<b>(b) Velocity Experiment</b>					
Body Length	Family + Site + Family*Site	-290.23			
	Family + Site	-294.68	8.891	0.003*	1
Body Area	Family + Site + Family*Site	-642.65			
	Family + Site	-647.03	8.758	0.003*	1
Yolk-sac Area	Family + Site + Family*Site	-315.11			
	Family + Site	-315.28	0.348	0.555	1
Head Area	Family + Site + Family*Site	151.04			
	Family + Site	148.29	5.496	0.019*	1
Emergence Time	Family + Site + Family*Site	-357.63			
	Family + Site	-357.63	<0.001	1.000	1
Emergence Body Length	Family + Site + Family*Site	-451.39			
	Family + Site	-451.39	<0.001	1.000	1
Total Growth	Family + Site + Family*Site	-448.46			
	Family + Site	-448.46	<0.001	0.998	1

\*Indicates a statistically significant difference ( $p < 0.05$ ) between models.

Table 2.4 P-values computed from the likelihood ratio tests to determine the significance of family and site (or treatment) for all traits from the field and velocity experiments. Values are not presented for models where a significant genotype-by-environment interaction was detected.

Phenotypic Traits	Family	Site/Treatment
<b>(a) Field Experiment</b>		
Body Length	0.008*	0.236
Body Area	0.028*	0.710
Yolk-sac Area	0.006*	0.556
Head Area	0.102	0.600
Timing of Emergence	0.456	0.076
Emergence Body Length	0.334	0.360
Total Growth	--	--
<b>(b) Velocity Experiment</b>		
Body Length	--	--
Body Area	--	--
Yolk-sac Area	0.254	0.254; 0.352
Head Area	--	--
Timing of Emergence	0.524	0.704; 0.238
Emergence Body Length	0.208	0.064; 0.28
Total Growth	0.438	0.040*; 0.012*

\*Indicates the component was statistically significant ( $p < 0.05$ ) and should not be removed from the model.

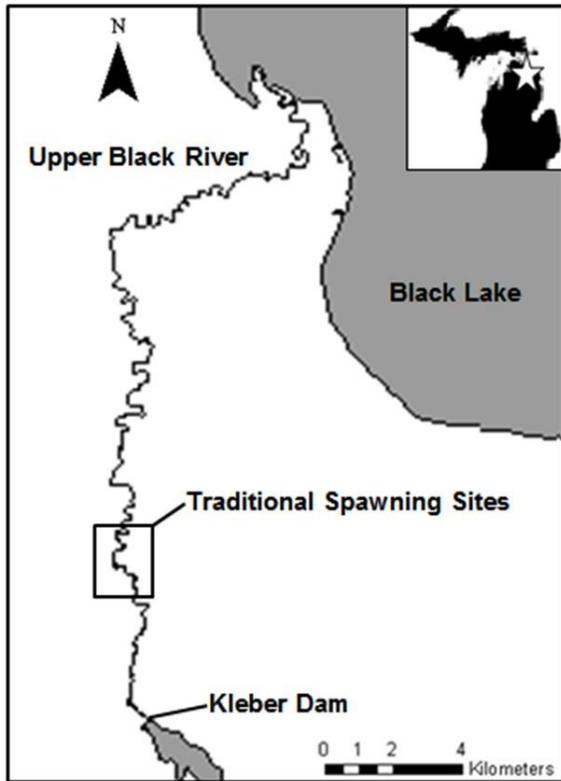


Figure 2.1 The study location on the Upper Black River, the largest tributary of Black Lake, Michigan showing the “traditional” spawning sites and the Kleber Dam, an “alternative” spawning site used during the 2011 spawning season

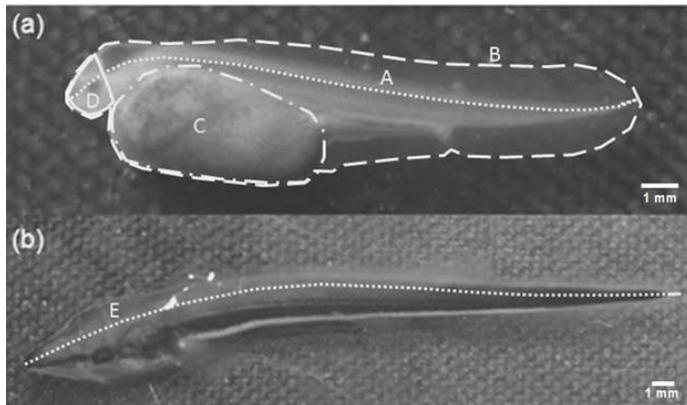


Figure 2.2 Four larval hatch traits A: body length (BL), B: body area (BA), C: yolk-sac area (YSA), D: head area (HA) (a) and one emergence trait, E: emergence body length (EBL), quantified in the experiment (b). The two additional emergence traits, time to emergence (ET) and total growth (TG), were quantified as the time (days) and growth (mm) from hatch to emergence, respectively (b). Scale bars (mm) are presented in the lower right corner

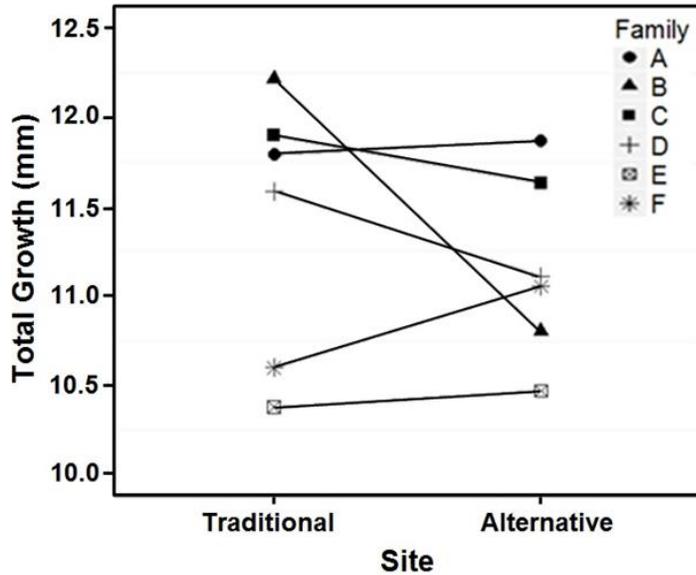


Figure 2.3 Total growth of lake sturgeon larvae from hatch to emergence when eggs were incubated at the “Traditional” and “Alternative” spawning locations. A significant genotype-by-environment (G-by-E) interaction was detected providing evidence that conditions experienced during embryogenesis affect trait variation at a later ontogenetic stage (timing of emergence)

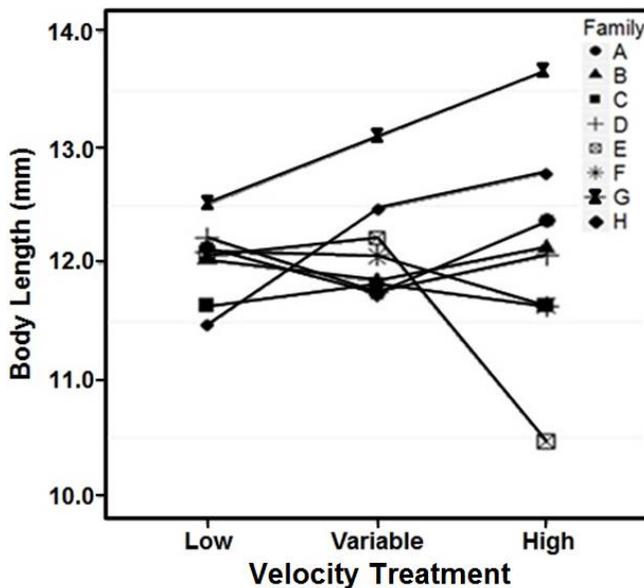


Figure 2.4 Reaction norm for body length at hatch for lake sturgeon larvae in the velocity (common garden) experiment. A significant genotype-by-environment (G-by-E) interaction was detected, and most of the variation was observed from larvae reared in the “High” velocity treatment

### Heritability Derivation

For each trait where equation [2] was the model of best fit in the paper, a point-estimate of the narrow-sense heritability ( $h^2$ ) was computed using the following logic.

First, let's consider an animal model equivalent to equation [2]:

$$\text{Eq. [4] } \textit{Trait} (y_{ij}) = \mu + S_j + a_{ij} + \varepsilon_{ij}$$

where  $\text{var}(a_{ij}) = \sigma_a^2$  is the additive variance and  $\text{var}(\varepsilon_{ij}) = \sigma_\varepsilon^2$  is the residual variance.

Notice that the animal effect in this animal model can be decomposed to the average of the parent's effect plus a Mendelian residual:

$$\text{Eq. [5] } a_{ij} = \frac{\textit{father}_i + \textit{mother}_i}{2} + u_{ij}$$

where we expect  $\text{var}(\textit{father}_i) = \text{var}(\textit{mother}_i) = \sigma_a^2$ , while  $\text{var}(u_{ijk}) = 0.5\sigma_a^2$  (Lynch and Walsh 1998). Going back to equation [2] in the paper, the family effect is just the average of the two parents and the residual variance contains the environmental residual of the animal model plus the Mendelian residual:

$$\text{Eq. [6] } F_j = \frac{\textit{father}_i + \textit{mother}_i}{2} \text{ and } \varepsilon_{ij} = e_{ij} + u_{ij}$$

Consequently,  $\text{var}(F_j) = 0.5\sigma_a^2$  and  $\text{var}(\varepsilon_{ij}) = 0.5\sigma_a^2 + \sigma_e^2$  then the narrow-sense heritability can be expressed as:

$$\text{Eq. [7] } h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} = \frac{2\text{var}(F_j)}{\text{var}(F_j) + \text{var}(\varepsilon_{ij})}$$

Equation [7] represents the computational formula used when estimating heritability using variance components from equation 2 in the paper and does not allow for the estimation of confidence intervals for heritability estimates.

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## LITERATURE CITED

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## Chapter 3

### INCREASING THERMAL REGIMES REVEAL CRYPTIC GENETIC VARIATION DURING EARLY ONTOGENETIC STAGES OF LAKE STURGEON

**ABSTRACT** – Climate change is affecting thermal conditions worldwide. Understanding organismal responses associated with predicted changes are essential for predicting future population persistence. However, few studies have examined the effects of both increased mean and variance in temperature on organismal traits, particularly during early life stages. Using lake sturgeon (*Acipenser fulvescens*) from Black Lake, MI, we tested whether phenotypes and behavior would vary among families incubated in different thermal regimes. Eggs were reared at two constant (10 and 18°C) and two fluctuating temperature treatments (10-19°C) representing temperatures experienced in the river and a simulated anthropogenic disturbance. Body length, body area, and yolk-sac area were quantified at hatch. Traits quantified at emergence (i.e. the onset of exogenous feeding) included time to emergence, body length at emergence, and growth from hatch to emergence. A significant family-by-treatment interaction was detected for traits measured at hatch. The greatest range in phenotypic variance was observed among individuals reared in the constant warm (18°C) treatment. Families also varied in growth ( $h^2=0.33$ ) and the timing of emergence ( $h^2=0.24$ ). Results demonstrate that increases in mean thermal regimes may reveal cryptic genetic variation potentially leading to differential survival between genotypes thereby altering the genetic architecture of the population.

#### **INTRODUCTION**

Climate change and variability are altering thermal regimes on a global scale

(IPCC 2013). Within the next century, mean temperatures are predicted to increase by approximately 2 to 5°C due to human influences (Estay et al. 2014). Additionally, increases in the magnitude and frequency of extreme climatic events are altering the variability in thermal patterns (Pincebourde et al. 2012; Bauerfeind and Fischer 2014). Thermal changes are known to directly affect biological functions in wild populations. For instance, previous studies have documented advances in the timing of reproduction (Parmesan 2006), faster growth rates (Drinkwater 2005), and decreases in species abundance (Jonsson and Jonsson 2009) in response to increasing temperatures. Therefore, understanding the ecological and evolutionary responses of wild populations to predicted thermal changes due to climate change has become a major goal for ecologists and climate researchers (Walther et al. 2002; Hansen et al. 2012).

Ectotherms are of particular conservation concern under predicted climatic changes given that temperature directly affects their physiology including growth, reproduction, and locomotion (Deutsch et al. 2008; Jonsson and Jonsson 2009). For example, one well-documented trend among ectotherms is the temperature-size rule in which rearing temperature is inversely related to body size (Kingsolver and Huey 2008; Diamond and Kingsolver 2010). Individuals that are reared in warm temperatures grow faster, but are typically smaller than individuals reared in cold temperatures (Atkinson 1994; Forster et al. 2011). Numerous studies have examined phenotypic trait changes in response to rearing temperatures in ectotherms given that changes in traits associated with fitness (i.e. body size) typically occur during early life stages which are characterized by high mortality, and are considered to be the most susceptible to thermal fluctuations (Jonsson and Jonsson 2009). Additionally, environmental

conditions experienced during early ontogeny have long-term consequences on individual fitness by affecting developmental rates, phenotypes, physiology, and behavior at later ontogenetic stages (i.e. ontogenetic contingency, Diggle 1994; Orizaola et al. 2010; Huey et al. 2012; Crespi and Warne 2013; Pittman et al. 2013; Dammerman et al. 2015).

Changes in phenotypic and behavioral trait variation are commonly visualized as thermal reaction norms that are constructed by rearing individuals of known genotype over a range of temperatures that remain constant and quantifying the variation in phenotypes that are expressed (Angilletta 2009). These plots have been useful for modeling the effects of climate change by exposing individuals to ecologically relevant changes in temperature and comparing their phenotypes to individuals reared under current thermal conditions in a laboratory setting. However, few studies have reared individuals under fluctuating incubation temperatures (i.e. diurnal temperature changes) that mimic conditions encountered in the wild (Bauerfeind and Fischer 2014). Recent research has documented that rearing individuals under fluctuating temperatures more accurately represents responses to climate change given that organisms encounter daily fluctuations in temperature (Niehaus et al. 2012; Bauerfeind and Fischer 2014), and temperature variability is predicted to affect traits associated with fitness to a greater extent than mean temperature alone (Paaijmans et al. 2013).

In this study, we reared different families of lake sturgeon (*Acipenser fulvescens*; Rafinesque 1817) under constant and fluctuating thermal egg incubation conditions and quantified phenotypic and behavioral variation at hatch and at the timing of emergence. Lake sturgeon are long-lived ectotherms and a useful species for examining the effects

of thermal changes because spawning events, embryogenesis, and larval development occur across a range of temperatures. In the spring, adults migrate to riverine areas for spawning (Auer 1996). The timing of spawning is multi-modal, and has been observed when water temperature is between 8.8 – 21.1°C (Bruch and Binkowski 2002; Forsythe et al. 2012). During spawning events, females release demersal, adhesive eggs which are fertilized by multiple males (Thiem et al. 2013). Fertilized eggs from full and half-sibling families attach to the substrate and incubate without parental care under site-specific conditions until hatch (Duong et al. 2011). Larvae typically hatch within 5-14 days dependent on water temperature (Kempinger 1988; Smith and King 2005) then burrow into the substrate to endogenously feed on yolk-sac reserves (Hastings et al. 2013). Yolk-sac utilization and the timing of emergence when larvae disperse downstream to begin exogenously feeding are dependent on several abiotic conditions within larval rearing sites including temperature (Duong et al. 2011).

Our first objective was to determine whether larval phenotypes and the timing of emergence would vary among individuals of different families experimentally reared in constant and fluctuating thermal incubation environments. We predicted that phenotypic variation within the fluctuating treatments would be greater than observed under constant treatments (Niehaus et al. 2012). Additionally, we predicted that phenotypes would vary among families given the large variance attributed to genetic (family) effects quantified in a recent study on lake sturgeon responses to different river flow regimes (Dammerman et al. 2015). Our second objective was to assess whether thermal environments experienced during egg incubation would affect larval growth and behavior during subsequent ontogenetic stages. Quantifying trait changes conditional

on environments experienced during previous ontogenetic stages increases understanding of how fluctuations in local thermal regimes will affect phenotypic trait variation and survival of different genotypes during critical developmental stages of ectotherms.

## **MATERIALS AND METHODS**

### *Study site*

During the 2012 spawning season, adult lake sturgeon were sampled daily using long-handled dip nets on the Upper Black River (UBR). The UBR is the largest tributary of Black Lake located in Cheboygan County, Michigan (Fig. 1; Smith and King 2005). Adults migrate into the UBR from late April to early June to spawn among shallow (~1-3 meters) rocky areas (Baker and Borgeson 1999; Forsythe et al. 2012). Spawning activities observed early in the season occur when water temperature is approximately 10°C. By the later part of the season, spawning adults typically encounter water temperatures closer to 18°C. The wadable conditions of the stream and presence of a streamside rearing facility provide the opportunity to collect gametes from spawning adults and conduct experimental temperature manipulations and monitoring of larval traits.

### *Fertilizations*

Gametes were collected from five females and ten males on May 3<sup>rd</sup>, 4<sup>th</sup>, and 6<sup>th</sup> of 2012. Eggs were collected from spawning females, placed in sealed plastic bags, and stored with river water to maintain eggs at ambient river temperature. Milt was collected using 20-mL syringes and placed on ice. Gametes were transported to the streamside rearing facility and fertilizations were conducted within twelve hours of collection.

Approximately 200 eggs from each female were placed on 1-mm mesh screens within polymerized vinyl chloride couplings (31.90cm<sup>2</sup>). Couplings were used to keep families separate. Each female's eggs were fertilized with 0.5mL of sperm from two males to create half-sibling families. Fertilized eggs were widely distributed within the couplings and left undisturbed for 30 minutes to allow adhesion to the mesh screens.

### *Thermal treatments*

Fertilized eggs were incubated in heattrays where temperature was controlled using heating and cooling units to produce two constant and two fluctuating thermal treatments (Fig. 2). Constant treatments, cold (10°C) and warm (~18°C), represented mean temperatures that characterized the early and late season spawning periods, respectively (Forsythe et al. 2012). Fluctuating treatments consisted of ambient river temperatures (natural fluctuations of 1-3 degrees daily in river temperature) and a variable treatment simulating an anthropogenic disturbance with a range of 1-9 degrees of change per day. Water temperature within each treatment was measured daily using Onset HOBO pressure loggers (Cape Cod, Massachusetts, USA). Fertilized eggs were slowly acclimated to treatment temperatures (two-degree change per hour) prior to placement in heattrays. Families were replicated among treatments and dead eggs were removed daily to prevent fungal infections.

### *Larval traits*

Thirty larvae from each family within each treatment (N=1200 total) were anesthetized with tricaine methanesulfonate (MS-222; 25 mg/mL) and photographed at hatch using a digital camera. Photographs were analyzed using Image J analysis software (Version 1.34, free-ware) to quantify three phenotypic traits: body length (mm),

body area ( $\text{mm}^2$ ), and yolk-sac area ( $\text{mm}^2$ ; Dammerman et al. 2015). Eighteen individuals were subsampled from five of the half-sibling families within each treatment ( $N=360$  total) and randomly assigned to individual plastic incubation chambers (12.7cm by 6.35cm) to monitor until the timing of emergence. Incubation chambers contained gravel substrate to provide cover during the endogenous feeding period, ambient river water, and mesh siding to allow for continual water passage ( $\sim 0.1\text{m/sec}$ ). Chambers were randomly placed across three fiberglass raceways (3.7m by 0.67m) with respect to family and thermal incubation treatment to prevent any influence of chamber location on the timing of emergence. Incubation chambers were monitored daily until larvae emerged from the substrate with depleted yolk-sac reserves to begin exogenously feeding. Larvae were anesthetized and photographed again to quantify body length at emergence (mm), growth from hatch to emergence (mm), and the time from hatch to emergence (days).

Individuals within the thermal treatments were predicted to hatch at different times given that stream temperatures experienced during the endogenous feeding stage directly affect yolk-sac utilization and timing of emergence in fishes including sturgeon (Hardy and Litvak 2004; Duong et al. 2011). Therefore, we tested the influence of the ambient river temperature experienced while in the incubation chambers on the three traits measured at the time of emergence. Temperature data was collected hourly within the streamside facility using a YSI 5200 Recirculating System Monitor (Xylem, Inc.). For each individual, we calculated the mean temperature (MeanT) and range in temperature (daily maximum minus minimum, RangeT) experienced during the endogenous feeding period in the incubation chamber.

All research was conducted under animal use and care procedures approved by the Michigan State University Institutional Animal Care and Use Committee.

### *Statistical analysis*

Statistical analyses were performed using the program, R (R Development Core Team) and the Bayesian inference package, MCMCglmm (Hadfield 2010). General linear mixed-effect models were used to test the contribution of Family, Treatment, and a Family-by-Treatment interaction on the larval traits measured at hatch. Traits were analyzed separately by fitting the full model:

$$\text{Eq. (1) Trait}(y_{ijk}) = \mu + \text{Treatment}_i + \text{Family}_j + (\text{Family} \times \text{Treatment})_{ij} + \varepsilon_{ijk}$$

where  $\mu$  is the population mean, (x) represents an interaction between variables, and  $\varepsilon_{ijk}$  was the random residual error. Treatment was fit as a fixed effect. Family and the Family-by-Treatment interaction terms were fit as random effects

where  $\text{Family}_j \sim N(0, \sigma_F^2)$ ,  $(\text{Family} \times \text{Treatment})_{ij} \sim N(0, \sigma_{FS}^2)$ , and  $\varepsilon_{ijk} \sim N(0, \sigma_\varepsilon^2)$ .

Models were parameterized in terms of Family effects as opposed to fitting an animal-specific random effect (i.e. animal model, Wilson et al. 2010). Given the simple pedigree structure and the possible presence of a genotype-by-environment interaction, this parameterization was equivalent and simpler to implement. Traits quantified at the time of emergence were analyzed using the full model with the addition of MeanT and RangeT experienced while in the incubation chamber as fixed effects. The correlation between MeanT and RangeT was low (Pearson's correlation = 0.31) allowing us to test the variables in the same model.

All models included a burn-in of 10,000, thinning interval of 100, and a total of 200,000 iterations which were chosen after examining diagnostic plots when using

default starting values in the MCMCglmm package. Variances of random effects were given an inverse-gamma distribution with a scale and shape of 0.001 which results in a non-informative prior. A sensitivity analysis conducted by fitting models with a proper prior attributing a higher degree of the phenotypic variance to genetic effects revealed there was little effect of the priors on the posterior distribution. Model selection was based on Deviance Information Criterion (DIC) which incorporates model fit and complexity based on an expected deviance parameter and the effective number of parameters estimated in the model (Spiegelhalter et al. 2002). We compared DIC estimates for the full model against all possible simpler models. The model with the lowest DIC estimate was selected as the model of best fit. When models were within two DIC values of each other, the simplest and less complex model was chosen as the model of best fit (Spiegelhalter et al. 2002). Parameter estimates were calculated as the modes of the posterior distributions estimated from the model of best fit. The 95% highest posterior density (HPD) was also estimated for each parameter. For traits where the model of best fit included a Family effect but no Family-by-Treatment interaction, the mode of the posterior distribution of the narrow-sense heritability ( $h^2$ ) and 95% HPD intervals were computed. Heritability estimates reported were obtained using the expression derived in Dammerman et al. 2015 (see Appendix 1).

## **RESULTS**

Mean body length at hatch ranged from 10.19 ( $\pm 0.09$ ) to 13.84 ( $\pm 0.09$ ) mm among families across the four treatments. A large range in phenotypic variation among families was observed within the fluctuating treatment groups; however, the greatest range was observed in the warm thermal treatment with Families D and H differing

approximately 3-mm in size (Fig. 3). The cold treatment group showed the least amount of variation with families varying up to 1.2-mm in size. A significant Family-by-Treatment interaction was detected for body length (Table 1), and explained approximately 39% of the phenotypic variance observed (Table 2). Mean body area and mean yolk-sac area at hatch also varied significantly among families across treatments ranging from 16.82 ( $\pm 0.17$ ) to 28.46 ( $\pm 0.32$ ) mm<sup>2</sup> and 6.91 ( $\pm 0.11$ ) to 8.11 ( $\pm 0.14$ ) mm<sup>2</sup>, respectively. Family-by-Treatment interactions were significant for both traits (Table 1), explaining approximately 43% of the variation observed in body area and 13% of the variation observed in yolk-sac area (Table 2).

Approximately 87% of the larvae from the five half-sibling families emerged from the substrate to begin exogenously feeding. Individuals began to emerge approximately 10 days after being placed in the incubation chambers. Families showed significant variation in the mean time spent in the incubation chambers ranging from 12.67 ( $\pm 0.34$ ) to 14.21 ( $\pm 0.26$ ) days; however, no Family-by-Treatment interaction or effect of the temperatures experienced while in the incubation chambers (MeanT and RangeT) were detected for the timing of emergence (Table 1). The mode of the heritability ( $h^2$ ) distribution explaining the similarity in the timing of emergence between larvae from the same family and 95% HPD were estimated as 0.24 (0.002, 0.706; Table 2).

Families also showed significant variation in growth from hatch to emergence ranging from 10.97 ( $\pm 0.35$ ) to 15.39 ( $\pm 0.47$ ) mm among families. Individuals that were incubated as eggs in the two fluctuating treatments differed approximately 1.3 to 1.7-mm in growth (Fig. 4). Similar to the traits measured at hatch, the largest range in mean growth among families was observed for individuals that were reared as eggs in the

warm treatment with families differing approximately 2.7-mm in size (Fig. 4). No Family-by-Treatment interaction or effect of temperatures experienced while in the incubation chambers (MeanT and RangeT) were detected (Table 1). The mode of the  $h^2$  distribution for growth and 95% HPD were estimated as 0.33 (0.021, 0.904; Table 2). Variation in mean body size at the timing of emergence was low among families ranging from 24.57 ( $\pm 0.20$ ) to 25.45 ( $\pm 0.16$ ) mm, and no significant effects were detected (Table 1).

## DISCUSSION

The early life stages of many ectothermic species are characterized by high levels of mortality (Fuiman and Werner 2002; Vitt and Caldwell 2014). Accordingly, it is during these early ontogenetic stages that selection may have a considerable effect. Increased temperatures encountered during early ontogeny are known to affect phenotypic and behavioral traits in ectothermic species (Atkinson 1996; Angilletta et al. 2004). However, there is a limited understanding of how thermal variability during egg incubation will affect trait variation at hatch and during sequential ontogenetic stages. Empirical work quantifying the effects of both increased mean and variability in temperature are vital to predict the ecological and evolutionary responses of populations to future climatic regimes. In this study, we experimentally manipulated egg incubation temperatures in a common garden experiment and demonstrated large effects of thermal regimes, genetic (family) effects, and their interaction on phenotypic trait variation during consecutive early life stages.

*Rearing temperatures influence phenotypic variation observed at hatch*

Rearing temperature had a significant effect on the range of phenotypic variation observed in the traits measured at hatch. The largest range in body length at hatch was observed among families reared in the warm (~18°C) treatment (Fig. 3). Although lake sturgeon have been observed spawning in temperatures up to 21.1°C (Bruch and Binkowski 2002), the gametes used in our experiment were taken from adults that typically spawn during the early part of the season when water temperatures are closer to 10°C. Therefore, the large range in phenotypic variation observed in the warm thermal treatment may have been due to the presence of cryptic genetic variation revealed under stressful thermal conditions. Cryptic genetic variation is not commonly observed under typically encountered conditions, and is often exposed in stressful environments (Badyaev et al. 2005; Ledón-Rettig et al. 2014). Results indicate that increases in mean thermal regimes (not just thermal variability) may reveal cryptic genetic variation expressed as differences among families thereby increasing phenotypic variation and the likelihood of exposing variant phenotypes to selection.

Cryptic variation can also be exposed when a genotype-by-environment interaction is present (Schlichting 2008; Paaby and Rockman 2014). In our common garden experiments, Family-by-Treatment interactions detected for body length, body area, and yolk-sac area measured at hatch indicate that families were responding differently to the same thermal incubation environments. A large proportion of the observed phenotypic variation in the traits (~13-43%) was explained by the Family-by-Treatment interaction. Other studies (Beacham 1988 and 1990), found that genotype-by-temperature interactions explained a substantial portion of observed variation in morphometric traits in *Oncorhynchus gorbuscha* and *Oncorhynchus keta* after rearing

individuals under different thermal regimes indicating that water temperature has a significant effect on the genotypic expression of traits during early development.

Additionally, the cryptic genetic variation observed in phenotypic traits at hatch may lead to differential survival among genotypes if selection favors larger individuals. Therefore, consideration of parentage, interaction effects, and cryptic genetic variation is important to understand how predicted thermal changes will affect phenotypic variation within wild populations.

*Fluctuating and constant rearing temperatures influence the range of phenotypic variation observed in larval traits*

In the variable and ambient treatments where temperatures fluctuated throughout the day, variation in body length at hatch among families was over 1.5 times as large as variation observed in the constant cold treatment (Fig. 3). Although diurnal temperature fluctuations were much larger in the variable than the ambient treatment, families showed similar responses in the ranges of phenotypic variation observed in the two fluctuating treatments. Results indicate that rearing individuals at a constant temperature reflecting the mean thermal regime observed in the wild will not accurately represent the extent of phenotypic variation that would be observed and on which selection would act on in wild-reared larvae when even modest daily temperature fluctuations occur. Similarly, Niehaus et al. (2012) found that growth and developmental rates of striped marsh frogs (*Limnodynastes peronii*) reared in fluctuating thermal treatments were continually under-predicted based on reaction norms constructed from individuals reared at constant rearing temperatures. Therefore, rearing individuals under fluctuating treatments provides a more accurate representation of phenotypic trait

variance and any potential genotype-by-environment interactions expected in the wild in response to climatic changes.

*Rearing temperatures affect trait variation at a subsequent ontogenetic stage*

Thermal conditions experienced during embryogenesis are known to affect phenotypic traits associated with survival such as shape, color, behavior, and size at developmental stages beyond hatch in several taxa of ectotherms (e.g. fishes, Martell et al. 2005; reptiles, Goodman 2008; amphibians, Orizaola et al. 2010). In our study, larvae reared in the fluctuating thermal treatments showed lower overall growth than those reared in the constant treatments indicating that egg incubation temperature affects trait variation at a subsequent ontogenetic stage (Fig. 4). Additionally, families within the warm treatment (Fig. 4) show the greatest range in growth indicating that differences observed among families in body size at hatch are persisting to a later ontogenetic stage. The persistence of a large range of variation in growth in the warm treatment and presence of non-zero heritability ( $h^2 = 0.33$ ) for growth indicates that thermally-induced, cryptic variation could potentially be maintained across ontogenetic stages.

Given that body size during early life stages is associated with survival with larger individuals typically having lower levels of mortality (Brown and Shine 2004; Fischer et al. 2011; Perez and Munch 2011), differential growth among families may lead to changes in the genetic architecture of the population if selection favors genotypes that produce larger offspring during critical development periods. In our study, we're limited in our ability to predict the accommodation of thermally-induced phenotypic variation and survival in the population given that we only measured

individuals to emergence and that the long generation time of lake sturgeon requires long-term monitoring. However, the early life stages of fishes are where mortality is highest (>99%; Chambers and Trippel 1997), and thus, selection operating on phenotypic variation is likely effective at altering population genetic architecture through different (family-specific) survival.

Several abiotic and biotic factors affect the timing of transition between early ontogenetic stages (Day and Rowe 2002). In larval fishes, the timing of emergence is typically under stabilizing selection (Crozier et al. 2008) and can be dependent on heritable variation, maternal effects, and/or environmental conditions such as temperature (Curry et al. 1995; Einum and Fleming 2000; Skoglund et al. 2011). In our experiment, there were no effects of thermal regimes experienced while in the incubation chambers. Alternatively, approximately 24% of the variation observed in the timing of emergence was attributed to differences among families. Heritability estimates for behavioral traits are rare in wild populations of fishes even though several behaviors are believed to have a heritable component (Mittelbach et al. 2014). Our estimate of  $h^2=0.24$  for emergence time is comparable to other reported  $h^2$  estimates of 0.15-0.20 for behavioral traits in fishes (Carlson and Seamons 2008; Chervet et al. 2011). However, our estimate represented the upper-limit of the narrow-sense heritability (resembling heritability in the broad-sense) given that we were unable to separate out additive genetic variance from other genetic effects. Despite the limitation, our  $h^2$  estimate provides insight on the potential change in emergence time and ability of the population to respond genetically (i.e. changes in gene frequency) to changes in thermal regimes.

### *Families differ in growth at a subsequent ontogenetic stage*

We observed no differences in body size at the time of emergence although families differed in the timing of emergence and growth from hatch to emergence. One explanation is that families that had smaller body sizes at hatch may have remained in the substrate longer to allow more time to grow thereby emerging from the substrate at the same size as individuals that spent less time in the substrate and were larger at hatch. Alternatively, differences in growth and time spent in the substrate may be attributed to differences among families in size of endogenous yolk reserves.

Differences between maternal provisioning of endogenous yolk-sac reserves have been well-documented in ectotherms where offspring with larger yolk reserves typically have higher survival and grow to a larger body size (Kamler 2005; Dziminski and Roberts 2006; Gagliano and McCormick 2007). In our experiment, we observed differences in yolk-sac area at hatch due to a Family-by-Treatment interaction. Therefore, differences in yolk sac area among families at hatch may have led to differential growth and time spent in the substrate feeding on endogenous yolk-sac reserves until the timing of emergence.

### *Conclusions and future directions*

Our findings have important implications regarding the impact of predicted thermal changes due to climate change on phenotypic variation in ectothermic species. Understanding how cryptic genetic variation is maintained across sequential ontogenetic stages in wild populations is vital to understanding how populations can respond to environmental change (Ledón-Rettig et al. 2014). Additionally, reaction norms constructed from rearing individuals at constant temperatures are not reliable

indicators of response under variable conditions. Therefore, researchers may wish to construct “realized” thermal reaction norms by adding naturally occurring (e.g. diel) varying temperature into experimental designs for treatments (Paaijmans et al. 2013). Given our findings on behavioral and phenotypic traits that are tied to survival, further empirical work addressing how incubation conditions and differences among families affect trait variation and survival at later ontogenetic stages would be beneficial to predict changes in population levels of genetic diversity due to environmental perturbations. Knowledge within these research areas is essential to understand the ecological responses and evolutionary potential of a population to the impacts of climate change.

## APPENDIX

Table 3.1 Step-wise model comparisons and DIC-values used in model selection for the six larval traits quantified at hatch and emergence in the experiment.

Larval Trait	Model	DIC
(a) Hatch		
Body Length	$\mu + \text{Treatment} + \textit{Family} + \textit{Family}*\textit{Treatment} + \varepsilon$	2739.86*
	$\mu + \text{Treatment} + \textit{Family} + \varepsilon$	3135.40
	$\mu + \text{Treatment} + \varepsilon$	3334.08
	$\mu + \varepsilon$	3637.86
Body Area	$\mu + \text{Treatment} + \textit{Family} + \textit{Family}*\textit{Treatment} + \varepsilon$	5687.97*
	$\mu + \text{Treatment} + \textit{Family} + \varepsilon$	6123.70
	$\mu + \text{Treatment} + \varepsilon$	6290.38
	$\mu + \varepsilon$	6821.93
Yolk-sac Area	$\mu + \text{Treatment} + \textit{Family} + \textit{Family}*\textit{Treatment} + \varepsilon$	2668.96*
	$\mu + \text{Treatment} + \textit{Family} + \varepsilon$	2779.48
	$\mu + \text{Treatment} + \varepsilon$	2824.39
	$\mu + \varepsilon$	3045.09
(b) Emergence		
Time to Emergence	$\mu + \text{Treatment} + \text{MeanT} + \text{RangeT} + \textit{Family} + \textit{Family}*\textit{Treatment} + \varepsilon$	1219.22
	$\mu + \text{Treatment} + \text{MeanT} + \text{RangeT} + \textit{Family} + \varepsilon$	1218.40
	$\mu + \text{Treatment} + \text{MeanT} + \textit{Family} + \varepsilon$	1218.43
	$\mu + \text{Treatment} + \textit{Family} + \varepsilon$	1217.83*
	$\mu + \text{Treatment} + \varepsilon$	1233.12
	$\mu + \varepsilon$	1372.54
Emergence Body Length	$\mu + \text{Treatment} + \text{MeanT} + \text{RangeT} + \textit{Family} + \textit{Family}*\textit{Treatment} + \varepsilon$	1125.42
	$\mu + \text{Treatment} + \text{MeanT} + \text{RangeT} + \textit{Family} + \varepsilon$	1125.11
	$\mu + \text{Treatment} + \text{MeanT} + \textit{Family} + \varepsilon$	1126.24
	$\mu + \text{Treatment} + \textit{Family} + \varepsilon$	1124.31
	$\mu + \text{Treatment} + \varepsilon$	1128.53
	$\mu + \varepsilon$	1124.33*
Total Growth	$\mu + \text{Treatment} + \text{MeanT} + \text{RangeT} + \textit{Family} + \textit{Family}*\textit{Treatment} + \varepsilon$	1146.03
	$\mu + \text{Treatment} + \text{MeanT} + \text{RangeT} + \textit{Family} + \varepsilon$	1145.74
	$\mu + \text{Treatment} + \text{MeanT} + \textit{Family} + \varepsilon$	1143.58
	$\mu + \text{Treatment} + \textit{Family} + \varepsilon$	1141.91*
	$\mu + \text{Treatment} + \varepsilon$	1166.68
	$\mu + \varepsilon$	1207.01

\*Indicates the model of best fit.

Table 3.2 Variance components and 95% highest posterior density (HPD) estimated from the models of best fit. Heritability ( $h^2$ ) estimates are provided for traits where the model of best fit included a significant family effect, but no family-by-treatment interaction.

<b>Phenotypic Traits</b>	<b>Component</b>	<b>Var</b>	<b><math>\pm 95\%</math> HPD</b>
Body Length	Family*Treatment	0.35	(0.00, 0.26)
	Family	<0.01	(0.20, 0.62)
	Residual	0.56	(0.51, 0.60)
Body Area	Family*Treatment	4.85	(0.00, 2.06)
	Family	0.02	(2.85, 8.10)
	Residual	6.56	(6.01, 7.04)
Yolk-sac Area	Family*Treatment	0.08	(0.00, 0.05)
	Family	<0.01	(0.05, 0.15)
	Residual	0.53	(0.49, 0.57)
Time to Emergence	Family	0.12	(0.00, 1.60)
	Residual	3.21	(2.69, 3.69)
	$h^2$	0.24	(0.00, 0.71)
Total Growth	Family	0.19	(0.02, 1.81)
	Residual	2.37	(2.08, 2.86)
	$h^2$	0.33	(0.02, 0.90)

\*Indicates an interaction between the components

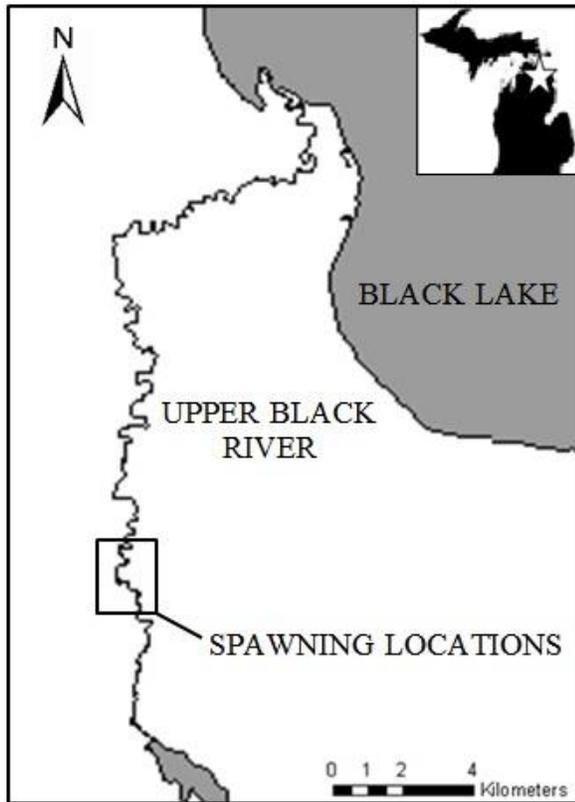


Figure 3.1 The study location on the Upper Black River, the largest tributary of Black Lake, Michigan showing the spawning locations where adult lake sturgeon were sampled during the 2012 spawning season

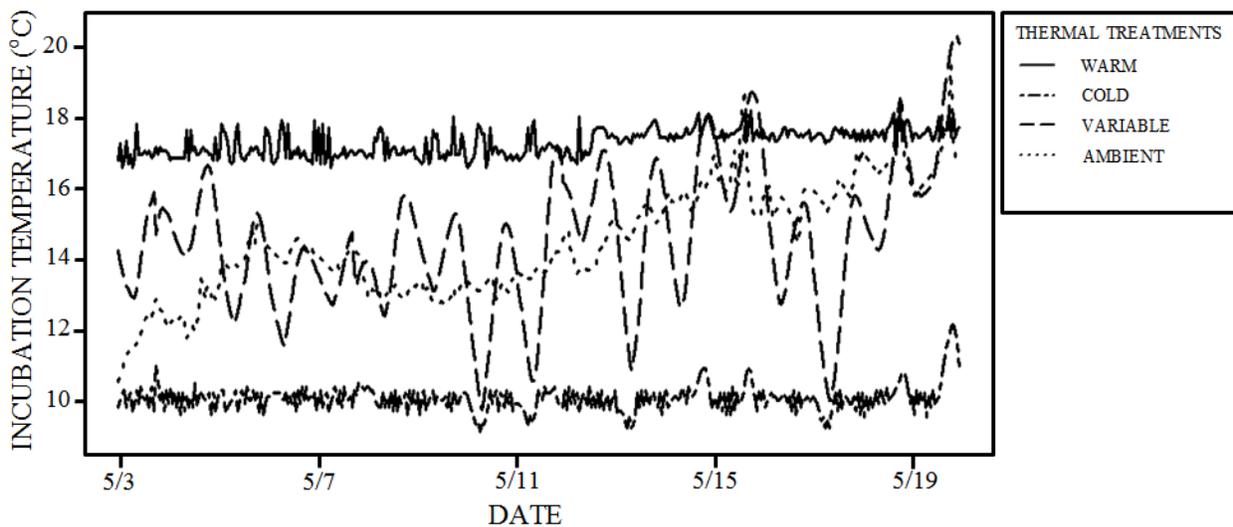


Figure 3.2 The four egg incubation temperatures (warm, cold, variable, and ambient) in which individuals were reared in during the 2012 spawning season

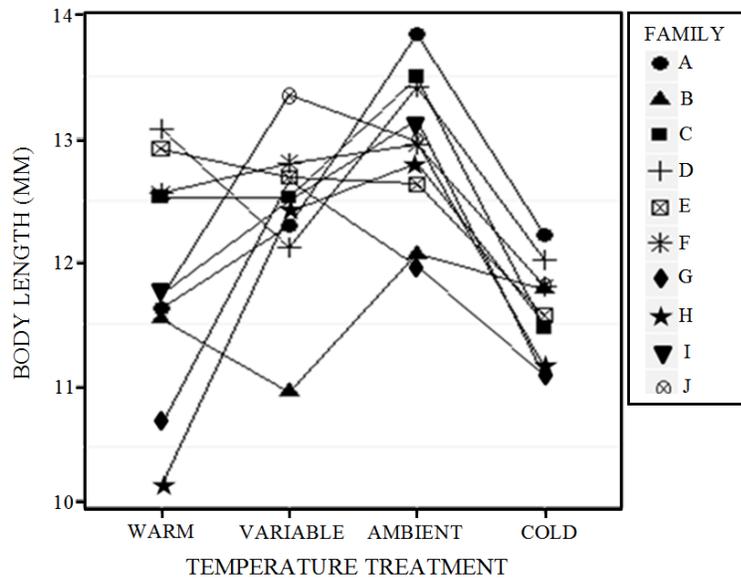


Figure 3.3 Thermal reaction norm for body length at hatch for lake sturgeon larvae subjected to the four thermal treatments. A significant genotype-by-environment (G-by-E) interaction was detected, and most of the variation among families was observed for larvae that hatched from eggs incubated in the warm (18°C) treatment

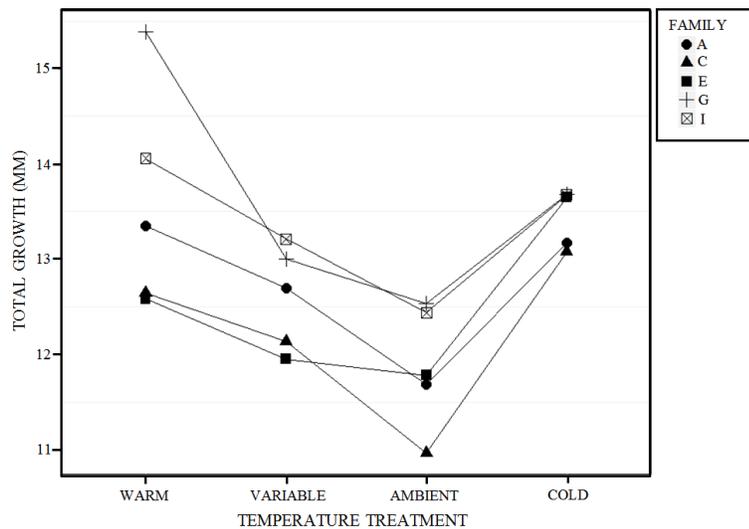


Figure 3.4 Thermal reaction norm for larval lake sturgeon growth measured from hatch to the timing of emergence. A significant family effect was detected ( $h^2=0.33$ ) indicating that differential size among families measured at hatch was persisting to a later ontogenetic stage

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## Chapter 4

### EFFECTS OF PARENTAGE AND MICROHABITAT VARIATION WITHIN ADULT-SELECTED SPAWNING SITES ON LAKE STURGEON GROWTH DURING EARLY LIFE STAGES

ABSTRACT - Understanding the impact of environmental variation on organismal traits during early life stages is essential for predicting changes in populations. Maternal effects including oviposition site selection are one source of variation. Female choice of sites can vary based on microhabitat variation which directly affects larval development and survival. However, the persistence of microhabitat effects across sequential early ontogenetic stages is limited. In 2013, we collected fertilized lake sturgeon (*Acipenser fulvescens*) eggs from the Black River, Michigan, just prior to hatch from an adult-selected spawning location, and quantified three microhabitat variables (water depth, discharge, and substrate size). Larval body length, body area, and yolk-sac area were quantified at hatch. Body size was measured for an additional four weeks post-emergence. Genetic-based parentage analysis was conducted using fin clips. The traits at hatch varied due to the microhabitat variables, but not due to additive genetic effects. Growth significantly varied among larvae with the greatest range in body size observed at 41 days post-hatch. Additive genetic variance covaried with age. Narrow-sense heritability estimates for body length ranged from 0.45 to 0.64. Results demonstrate that female-selected, microhabitat variation influenced offspring phenotypic variation at hatch, but had no influence on body length at sequential ontogenetic stages. Additionally, the additive genetic effects explained roughly half the variation observed in

body size, but individuals showed considerable variation in growth which may be due to differences in the degree of plasticity among individuals.

## **INTRODUCTION**

Knowledge on how environmental variation affects organismal trait variation during early life stages is essential for predicting changes in populations due to potential threats including habitat degradation and environmental change (Monaghan 2008; Aubry et al. 2013). Maternal effects including choice of mates, selection of oviposition sites, and maternal provisioning are well-documented sources of variation that have pervasive effects on phenotypic variation in offspring (Mousseau and Fox 1998; Räsänen and Kruuk 2007). Female selection of oviposition site determines the abiotic conditions offspring will encounter during development (Rudolf and Rödel 2005; Lowerri-Barbieri 2011). Thus, females are expected to select oviposition sites that are conducive to offspring growth and survival in order to maximize their own reproductive success (Kern et al. 2013). However, several factors have been shown to influence female choice of oviposition site including proximity of mates, predation risk to offspring, micro-habitat variation, and habitat stability (Refsnider and Janzen 2010; Silberbush and Blaustein 2011). Therefore, studies examining conditions at oviposition sites provide insight on environmental cues influencing adult decisions, and the effect of those decisions on larval growth and survival (Lowerri-Barbieri 2011; Eilers et al. 2013).

In stream systems, female oviposition site selection is influenced by abiotic conditions that can vary spatially and temporally (Reich and Downes 2003), and potentially on micro-geographic scales ( $<1\text{m}^2$ ; Merten et al. 2013). These abiotic conditions include substrate type and size (Van Liefferinge et al. 2005), water velocity

(Lancaster 2010), water depth (Reich and Downers 2003), and temperature (Torgersen et al. 1999) which are known to directly affect development and survival during early life stages (Refsnider and Janzen 2010). For example, interstitial spaces between substrate provide cover and protection from predators for newly hatched larvae that are highly vulnerable to predation (Bunn and Arthington 2002; Boucher et al. 2014). Thus, identifying variation in stream microhabitat conditions is essential for understanding abiotic habitat needs, and factors affecting potential population levels of recruitment in stream species (McAdam 2011).

In stream fishes, spatial and seasonal variation in stream conditions cause adults to frequently shift their use of different microhabitats (Kanno et al. 2012), thereby exposing offspring to a wide variety of rearing conditions. In polygamous species, spatial variation in microhabitat use during spawning events results in larvae of the same and different genotype to be reared under different environmental regimes, even within a small geographic area (e.g., within the same spawning site). These environmental regimes are known to have persistent effects on trait variation in fishes (Martell et al. 2005; Martell and Kieffer 2007) by influencing growth and behavior during sequential ontogenetic stages (i.e. “ontogenetic contingency,” Diggle 1994) for individuals of the same and differing genotype (Dammerman et al. 2015). Additionally, the relative contribution of both environmental and additive genetic effects can vary among life stages (Houle 1998). Therefore, understanding the effects of microhabitat rearing environments on larval phenotypic variation and survival requires determination of genetic parentage and quantification of traits across sequential ontogenetic stages in order to accurately predict potential population levels of recruitment.

In this study, we examined the influence of additive genetic effects and microhabitat variation on phenotypic variation during early ontogenetic stages of lake sturgeon (*Acipenser fulvescens*). Lake sturgeon are long-lived, iteroparous fish, that have been numerically depressed due to overharvest, habitat loss and degradation, and limited recruitment (Hayes and Caroffino 2012). Adults migrate to riverine areas to spawn in polygamous groups, and provide no post-ovulatory care as eggs incubate under site-specific conditions (Smith and King 2005; Duong et al. 2011). Similar to other fishes, mortality is high (~99%) during the egg and larval period (Forsythe et al. 2013). Long-term success of restoration programs includes protection of spawning and rearing habitats (Hayes and Caroffino 2012), and identification of factors that affect growth and survival during critical early life stages. Genetic (parentage) effects as well as egg incubation conditions are known to affect phenotypic trait variation and behavior associated with survival at hatch and at the timing of emergence (Dammerman et al. 2015; Dammerman et al., *In Review*). However, knowledge of how traits vary following emergence due to additive genetic effects and stream microhabitat variables is limited. The objectives of this study were to: i) quantify microhabitat variation within an adult-selected rearing habitat, ii) determine whether microhabitat conditions affects larval trait variation at hatch, and iii) quantify the influence of additive genetic effects and environmental variation on post-emergence growth.

## **MATERIALS AND METHODS**

### *Study site*

The study was conducted in 2013 on the Upper Black River (UBR), the largest tributary of Black Lake. Black Lake is a 3500 hectare lake located in Cheboygan

County, Michigan (Fig. 1) that supports a well-studied population of approximately 1200 adult lake sturgeon (Pledger et al. 2013). Adult sturgeon migrate to the UBR from late April to early June to spawn among a 1.5-km stretch of the river (Fig 1). Timing of spawning is dependent on temperature and discharge with spawning occurring once water temperature is 10°C and a lag in discharge is observed (Forsythe et al. 2012). Spawning sites are characterized by shallow water depth (~1-3 meters) containing cobble, pebble, and gravel substrate which provide cover for newly-hatched larvae (Forsythe et al. 2012). The wadable conditions of the stream and presence of a streamside facility provide the opportunity to collect eggs from the substrate and rear individuals in a captive setting to monitor traits across sequential ontogenetic stages.

#### *Egg collection and microhabitat variables*

In 2013, adult lake sturgeon were monitored daily by wading the UBR at least once per day from May 1<sup>st</sup> to June 9<sup>th</sup>. Once spawning behavior and deposited eggs were observed on at a commonly utilized spawning site referred to as Site B (Duong et al. 2011), temperature was monitored daily using an Onset HOBO pressure logger (Cape Cod, Massachusetts, USA) to calculate cumulative thermal units (CTU) to estimate egg hatch date using the methods of Kempinger (1988). On May 8<sup>th</sup>, one day prior to estimated egg hatch date, seven transect lines (A-G) were placed at 5-meter intervals along the stream bank parallel to the river at Site B (Fig. 2). Abiotic microhabitat variables including substrate size, water depth, and water discharge were sampled at 1-m intervals along each transect line (N=91 sampling points).

Sampling along transect lines was conducted using a steel apparatus which contained a pole, a 0.216 x 0.279m frame attached to the bottom of the pole, and a

Canon Powershot D10 underwater camera mounted 0.5m above the frame. The presence of the steel frame allowed for a consistent river area to be sampled, and the camera height of 0.5-m allowed the area being photographed to remain at a constant  $0.053\text{m}^2$  ( $0.201 \times 0.262\text{m}$ ). At each sampling point, a photo was taken to quantify mean substrate size at a later date. Point estimates of water depth (m) and water velocity ( $\text{m}^2/\text{sec}$ ) at each point were measured using a wading rod and Marsh McBirney flow meter. Discharge ( $\text{m}^3/\text{sec}$ ) at each sampling point was calculated by multiplying velocity point measurements by the 1-m river area. Temperature was not measured in the study given that it was confounded by the time of sampling. River depth (m) and discharge ( $\text{m}^3/\text{sec}$ ) were simultaneously collected hourly upstream of the spawning site during the entire spawning season using a staff gauge and a HOBO pressure logger. Sturgeon eggs were collected at each sampling point by disturbing the substrate within the steel frame for ten seconds upstream of a triangular kick net. Live eggs (Fig. 2) were transported to the streamside facility and kept separate by sampling point until hatch. Dead eggs were discarded to prevent microbial infection.

#### *Mean depth and discharge during the incubation period*

Water depth (m) and water discharge ( $\text{m}^3/\text{sec}$ ) at each transect sampling point were measured again on May 18<sup>th</sup>. Water depth and discharge measurements collected at each transect point were compared to the mean depth and mean discharge collected by the data logger on the same date by taking a ratio of the point estimate divided by the logger measurement. Ratios between the two dates were compared using linear regressions. The calculated regression for water depth ( $y = 0.980x + 0.002$ ,  $R^2 = 0.948$ ; Fig. S1) and regression for water discharge ( $y = 0.917x - 0.010$ ,  $R^2 = 0.948$ ; Fig. S2)

indicated that ratios between the two sampling points were highly correlated. Therefore, we estimated mean water depth and mean river discharge at each transect point during the entire incubation period by multiplying the mean depth and discharge collected daily from the data logger by the mean ratio calculated at each transect point.

#### *Mean substrate size*

Substrate photos collected at each transect point were analyzed using Image J software (Version 1.34, free-ware). The axis with the greatest linear distance was measured for each particle, and recorded as the particle length (mm). Given the large number of particles in each photo, a preliminary analysis was conducted by placing a 3x3 grid over the image. Grids measured 0.067m wide by 0.087m long. We subsampled 10% of the photos from transect sampling points where eggs were collected, and measured the length of all particles in the photo. Then, particles within three randomly-selected grids within each photo were measured. Measurements within the entire photo and the grids were compared using a linear regression ( $y = 1.526x - 1.315$ ,  $R^2 = 0.954$ ; Fig. S3). Three-randomly sampled grids were measured for all remaining photos, and the regression was used to estimate mean substrate size (mm) in the entire photo. Particles within each photo were classified based on the Wentworth Classification System as modified by Cummins where size categories included: sand/fine particles (>2mm), gravel (2-16mm), pebble (16-64mm), cobble (64-256mm), and boulders (>256mm; Wentworth 1922; Cummins 1962).

#### *Larval traits*

At hatch, three-hundred and fifty-nine larvae were subset from the 64 transect points where eggs were collected. Larvae were anesthetized with tricaine

methanesulfonate (MS-222; 25 mg/mL) and photographed using digital photography. Image J software was used to quantify body length (mm), body area (mm<sup>2</sup>), and yolk-sac area (mm<sup>2</sup>; Dammerman et al. 2015). Larvae were randomized with respect to transect point and assigned to modified, liter-sized milk jugs (15.2 x 6.8cm) which served as individual incubation chambers. Chambers contained gravel substrate to provide cover during endogenous feeding, mesh covering, and independent water sources allowing for continual water movement (~0.1m/sec) within chambers. Chambers were monitored multiple times daily until larvae emerged from the substrate with depleted yolk-sac reserves to begin exogenously feeding.

Due to algal accumulation on screens, one fish independently emerged from the substrate on May 21<sup>st</sup> before substrate was removed and chambers were cleaned on May 25<sup>th</sup>. Dead larvae (N=25) due to natural mortality within the incubation chambers were discarded due to microbial infection. All surviving larvae were anesthetized and photographed again on May 28<sup>th</sup> to determine post-emergence body length (mm). Larvae remained in the chambers without substrate and were photographed weekly to quantify body size during the critical growth and development period. Larvae were photographed five times between hatch and 41 days post-hatch until individuals grew to the size of the incubation chambers when a fin-clip could be taken non-lethally for genetic analysis. During rearing, larvae were fed brine shrimp nauplii (*Artemia* spp) three times daily to satiation for three weeks until transitioning to processed frozen blood worms (chironomid midge larvae) for the remainder of the rearing period. Larvae that died during the rearing period (N=42) were kept separate and preserved in 95% ethanol for genetic analysis.

### *Genetic analysis*

DNA was extracted from all 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); AfuG68, AfuG74, AfuG9, AfuG63, AfuG56, AfuG195, AfuG160, AfuG112, AfuG204 (Welsh et al. 2003); Spl 120 (McQuown et al. 2000); and Aox 27 (King et al. 2001). Polymerase chain reaction and genotyping were conducted using methods described in Duong et al. 2011. Due to low DNA concentration, 1.5uL of forward and reverse primer were used to increase signals and cycle number was increased to 35 for all loci during the annealing phase. All genotyping scores were assigned by two experienced lab personnel, and a 10% subset of the samples were re-genotyped to empirically estimate genotyping error. Genotyping error was calculated as the number of allelic errors divided by the total number of alleles that were genotyped.

### *Parentage analysis*

Parentage analysis was conducted using the software programs, CERVUS 3.0 (Kalinowski et al. 2007) and COLONY 2.0.5.8 (Jones and Wang 2010). In CERVUS, our simulation 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 the population estimates of allelic frequencies. Our parentage analysis included all 262 adults from the 2013 spawning run, an 85% estimate of the proportion of adults captured (K. Scribner, unpublished data), and an empirical genotyping error estimate of 0.40%. Adults with the highest maximum likelihood score in COLONY and highest, positive LOD score in

CERVUS were assigned as the most likely candidate parents. Assignment of the most likely parental pair in our study was based on concordance between the two programs.

### *Statistical analysis*

Statistical analyses were performed using the statistical program, R (v. 3.1.2, R Development Core Team). We used the package, glmmADMB (Skaug et al. 2006) to determine whether the number of live lake sturgeon eggs collected at each transect point was dependent on the three microhabitat variables. We tested for multicollinearity between the three variables by quantifying the variance inflation factor (vif) using the package, “car” (Fox and Weisberg 2011), and calculating the Pearson’s correlation (r) between pairs of variables. Correlation estimates and vif values were low between the three variables ( $r < 0.29$ ,  $vif < 1.22$ ) so all three were included in the model. We fit a negative binomial regression with a log link function as:

[Eq 1]

$$\log(\# \text{ Live Eggs})_{ijk} = \mu + \text{mean depth}_i + \text{mean discharge}_j + \text{mean substrate size}_k$$

Linear mixed-effect models were used to test for the effects of the environmental covariates and genetic (additive) effect associated with larval phenotypic traits measured at hatch. The three traits at hatch (BL, BA, and YSA) were analyzed separately using mixed models implemented in the package “regress” (Clifford and McCullagh 2014). Models were fit using restricted maximum likelihood estimation. Traits were fit to the full model:

$$\text{[Eq 2] Trait } (y_{ijkl}) = \mu + \text{meandepth}_i + \text{meandischarge}_j + \text{meansubstrate}_k + \text{Animal}_l + \varepsilon_{ijkl}$$

where microhabitat variables were fit as fixed effects, Animal refers to the additive genetic effect with  $Animal_l \sim N(0, \sigma_a^2)$  where the additive variance ( $\sigma_a^2$ ) is estimated based on the comparison of phenotypes among relatives denoted by the additive genetic relationship matrix (A), and  $\varepsilon$  is the residual variance with  $\varepsilon_{ijk} \sim N(0, \sigma_\varepsilon^2)$ . Traits were then fit to the simpler model:

$$[\text{Eq 3}] \text{Trait}(y_{ijk}) = \mu + \text{meandepth}_i + \text{meandischarge}_j + \text{meansubstrate}_k + \varepsilon_{ijk}$$

and all subsequent simpler models in a step-wise manner by eliminating one variable at a time. Likelihood-ratio tests were used to determine the model of best fit.

We tested for spatial autocorrelation among the transect sampling points given that ecological variables can be correlated at small (<1m) spatial scales (Legendre 1993), and this spatial autocorrelation can influence trait variation for individuals reared in close proximity. First, we fit the three traits measured at hatch and the four additional measurements of BL to a model including the three environmental covariates [Eq 3] with each trait fit separately. We extracted the residuals from each model, and plotted semi-variograms using the package “geoR” (Ribeiro and Diggle 2001). Semi-variograms depict the similarity between variances (e.g. residuals) based on spatial distance, and are used to determine the correlation structure present in the data (Dormann et al. 2007). Visualizations of the semi-variogram plots and correlation curves indicated there was no evidence of correlation between the residuals based on the distance between them. Therefore, no spatial effect terms were included in the analysis of the larval traits.

To determine how additive variance associated with BL varied over time, we used a multi-step approach. First, we fit each of the five measurements of BL separately to the full animal model with the three environmental covariates [Eq 2]. We used

likelihood ratio tests to determine there was no influence of the environmental covariates on BL except at hatch so we dropped them from the model. Next, we fit each of the five measurements of BL to an animal model with the population mean as a fixed effect:

$$[\text{Eq 4}] \textit{Trait} (y_i) = \mu + \textit{Animal}_i + \varepsilon_i$$

We used the results from these models to investigate patterns of heteroskedasticity. We also investigated the pattern of phenotypic correlation between repeated measures.

Next, we fit a random regression animal model (Meyer and Hill 1997; Schaeffer 2004) on a quadratic polynomial of Age, and used the residual variance heterogeneity pattern estimated from the individual time point analysis to account for heterogeneous environmental variances. The resulting model was:

$$[\text{Eq 5}] \textit{BL} (y_{ij}) = \mu + \textit{Age}_i + a_{j,0} + a_{j,1} x \textit{Age}_i + a_{j,2} x (\textit{Age}_i)^2 + \varepsilon_{ij}$$

where Age was fit as a fixed effect,  $a_j$  refers to the random regression coefficients for the additive genetic effect as a function of age described by an intercept, slope (linear), and quadratic term, and  $\varepsilon$  was the residual variance ( $\sigma_\varepsilon^2$ ) output from the model

weighted by the residual variances estimated from [Eq 4] resulting in five estimates of heterogeneous residual variances. We used the weighted model given the lack of

convergence in a more complex model that allowed additive genetic and permanent

environmental effects to vary with age. Lastly, we estimated the same model in [Eq 5]

without heterogeneous variances, and compared the goodness-of-fit of between the

model and the weighted model based on Akaike Information Criterion (AIC) values. The

narrow-sense heritability ( $h^2$ ) for BL was estimated at each Age by taking the additive

genetic variance divided by the sum of the additive and residual variances estimated from the random regression animal model.

For larvae that were assigned a mother (N=224), we tested for the presence of a maternal effect by fitting the body size of the female (FemaleTL) as an additional random effect in [Eq 2] for the three traits measured hatch, and in [Eq 4] for each measurement of BL where  $\text{FemaleTL} \sim N(0, \sigma_F^2)$ . In all models, variance in the trait explained by the maternal effect was negligible ranging from 0-1.7%. Therefore, we did not fit a random regression model that included the maternal effect term.

## RESULTS

Adult lake sturgeon were observed spawning at Site B on May 3<sup>rd</sup>, and fertilized eggs were observed on the substrate on May 5<sup>th</sup>. Mean water temperatures were high during early May averaging between 15-17°C resulting in a shorter egg incubation time. CTU values calculated from daily mean water temperature indicated that eggs were expected to hatch on May 9<sup>th</sup>. Fertilized eggs incubated under site-specific conditions from May 3<sup>rd</sup> – May 8<sup>th</sup> until being collected along the transect lines. Eggs were collected from Site B when CTU was approximately 66.5 which is within the traditional range of CTUs at time of hatch (58.1 to 71.4) estimated in previous studies on the UBR (Smith and King 2005). Five-hundred and forty-one live lake sturgeon eggs were collected from 64 of the sampling points along the transect lines at Site B. Most eggs were collected near the center of the river, and ranged from 0-73 per transect point (Fig. 2). The largest proportion (54%) of the total number of live eggs were collected from transect lines C and D.

The number of live eggs collected was positively associated with mean water discharge and mean substrate size at each transect sampling point with discharge having the largest effect. Mean ( $\pm$  SE) water discharge for all transect points at Site B was 0.69 ( $\pm$  0.06) m<sup>3</sup>/sec, and mean substrate size 11.74 ( $\pm$  0.70) mm. Gravel was the most prevalent substrate type at Site B, but size varied among transect lines with some points contained predominantly pebble substrate (Table 1). Most eggs were collected near the center of the river where water was deeper, and the largest number of eggs were collected from transect lines C and D (Fig. 2). Mean depth among all transect points was 0.75 ( $\pm$  0.02) m, and also varied among transect lines (Table 1), but did not affect the number of live eggs collected at each sampling point ( $X^2 = 1.12, df = 1, p = 0.29$ ).

The mean proportion of larvae sub-sampled from each transect sampling point at hatch, and measured in the experiment was 78.8%. Larvae showed considerable variation in the three traits measured at hatch. Mean larval BL at hatch was 13.02 ( $\pm$ 0.05) mm, and ranged from 9.80 to 16.18 mm. Mean BA was 28.17 ( $\pm$ 0.22) mm<sup>2</sup>, and ranged from 17.37 to 41.10 mm<sup>2</sup>. Both BL and BA at hatch were dependent on the three microhabitat variables at each transect point. BL and BA were negatively associated with depth and substrate size, but positively associated with discharge (Table 3). Depth had the largest influence on both traits. YSA varied among larvae with a mean of 7.95 ( $\pm$ 0.06) mm<sup>2</sup>, and ranged from 5.13 to 12.42 mm<sup>2</sup>. YSA was also dependent on mean depth and mean discharge at the transect sampling points, but did not vary due to mean substrate size (Table S1). YSA was negatively associated with mean depth, and positively associated with mean discharge which had the largest effect (Table 3). The

additive genetic effect was not significant for all three traits ( $p>0.05$ ) when fitting the linear mixed models.

In the parentage analysis of the larvae, mean expected heterozygosity for the 12 loci genotyped was 0.576. Allelic diversity ranged from 2 to 11 with a mean of 5.25 alleles per locus. Eleven of the loci were in Hardy-Weinberg equilibrium. The combined non-exclusion probability for the parent pairs was 0.00008456. Ninety percent ( $N=274$ ) of the offspring that were genotyped were assigned at least one parent with a 75% degree of concordance in parental assignments between CERVUS and COLONY. Forty females were assigned offspring with a mean of 5.60 ( $\pm 1.03$ ) offspring per female; however, 51% of the assigned larvae came from seven females. Sixty-one males were assigned offspring with a mean of 4.30 ( $\pm 0.46$ ) offspring. Seven males were assigned 10 or more offspring, and contributed approximately 31% of the total offspring.

Post-hatch survival in the incubation chambers was approximately 93%. Mean growth from hatch (Age 1 days) to four weeks post-emergence (Age 41 days) was 28.19 ( $\pm 0.31$ ) mm, but individuals showed considerable variation in size (Table 2). The largest range in BL was observed at 41 days post-hatch as the largest and smallest individuals showed a 28.08 mm difference in BL (Fig. 3). The heterogeneous variance model was a better fit model (AIC = 5496.1) over the model that did not account for heterogeneous variances (AIC = 5646.0). Additive genetic variance estimates obtained from the random regression animal model increased with age (Fig. 4). The narrow-sense heritability ( $h^2$ ) for BL also varied with age, and ranged from 0.45 to 0.64 (Fig. 5).

## **DISCUSSION**

Microhabitat conditions within adult selected oviposition sites directly affect offspring growth and development in a variety of species (Refsnider and Janzen 2010); however, knowledge of the persistent effects of these environmental variables on larval trait variation across sequential ontogenetic stages is limited. In this study, quantification of microhabitat variables within the stream, rearing of wild-produced larvae, and genetically-based parentage analysis allowed for determination of how phenotypic trait variation across early life stages was affected by adult-selected microhabitats and additive genetic effects. Depth, discharge, and substrate size were quantified within adult-selected microhabitats given their known influence on lake sturgeon spawning site selection and reproductive success (Bruch and Binkowski 2002; Chiotti et al. 2008).

The three microhabitat variables had significant effects on the number of live eggs collected from the stream, and on the three larval traits measured at hatch: body length (BL), body area (BA), and yolk-sac area (YSA). The number of live lake sturgeon eggs collected from the stream was positively associated with mean discharge and mean substrate size at each transect sampling point with discharge having the largest effect. Adequate discharge levels (0.15 to 1.40 m/s; Hay-Chmielewski and Whelan 1997) are vital for adequate mixing of sperm and eggs during broadcast spawning events. Additionally, high discharge levels can dislodge fish eggs from the substrate surface. The largest number of live eggs were collected from transect lines C and D. These transect lines had moderate discharge, depth, and substrate size, and may have represented an optimal rearing microhabitat rearing conditions for the eggs. However, without knowledge on the parentage for all eggs collected from the stream, we can't be

certain eggs were from multiple females except for the larvae that were genotyped in the study.

Water depth was negatively associated with the three larval traits. Deeper waters are typically associated with higher levels of dissolved oxygen given the lower water temperatures which slow down metabolic rates (Guppy and Withers 1999). Therefore, subtle differences in water depth between transect sampling points may have slowed embryo and subsequently larval growth and development. Similarly, Baltz et al. (1998) found a positive association between deeper water and growth in juvenile red drums (*Sciaenops ocellatus*) in nursery habitats presumably due to temperature and dissolved oxygen concentrations. Discharge was positively associated with the three larval sturgeon traits. Reduced water flows cause sediment deposition (Kempinger 1988), but increases in flow may keep the adhesive surface of the incubating sturgeon eggs clear of sediment, debris and microbial communities (Fujimoto et al. 2013) thereby positively influencing development within the egg and larval phenotypes at hatch. Body length of brown trout (*Salmo trutta*) smolts have also been positively associated with increasing discharge regimes (Jonsson et al. 2001) indicating the importance of discharge on larval development of stream fishes.

Increases in mean substrate sizes negatively influenced the three traits at hatch. Substrate is important for the early life stages of sturgeon given that the fertilized, adhesive eggs adhere to the substrate surface during incubation (Zubair et al. 2012), and newly hatched larvae immediately burrow into the interstitial spaces between the substrate to escape predation (Hastings et al. 2013). Therefore, extreme substrate sizes are detrimental to lake sturgeon development given that small sand and fine particles

can cause egg mortality by coating the egg surface, and large substrate sizes such as boulders provide little interstitial space to protect eggs during incubation and during the period of endogenous feeding by larvae. Preference for spawning sites containing gravel substrate has been well-documented in sturgeon (McAdam 2011; Boucher et al. 2014) and other stream fishes (Gerken and Thiel 2001), and was the predominant substrate type documented within the microhabitats in the study.

Growth during early life stages of fish is predicted to vary due to intrinsic and extrinsic factors including genetic and environmental effects (Kamler 2008). In the study, the three microhabitat variables had no effect on post-emergence growth. Alternatively, a significant proportion of the phenotypic variation observed in larval growth was due to additive genetic effects (Fig. 5). We observed an initial increase in  $h^2$  after larvae came out of incubation chambers. Increasing  $h^2$  with age is concordant with results from Wilson et al. (2005) which occurs due to 'variance compounding' where traits expressed later in life will always show more variance due to the cumulative effects of allelic variants (Houle 1998) and any new gene expression (Wilson et al. 2005). In our study, the decrease in additive variance after 25 days could be due either to increased environmental variance or due to the 42 offspring that died during the rearing period which further reduced our sibling groups thereby reduced our estimate of the degree of resemblance. Larval growth trajectories varied significantly among individuals with the range in BL increasing with increasing age (Fig. 3). Although it is difficult to distinguish, larval growth curves showed heterogeneous slopes as individuals that were large at hatch did not remain hatch during the entire experiment.

Individual differences in growth trajectories have been documented in several vertebrate species (Nussey et al. 2007) including fishes (Dower et al. 2009).

BL narrow-sense heritability ( $h^2$ ) estimates which document the degree of resemblance among related individuals (Wilson et al. 2010) also varied across age (Fig. 5). We had high estimates of 0.45 to 0.64 are comparable to the heritability estimates of 0.42 for body length estimated at hatch and 0.33 for growth from hatch to emergence estimated in previous studies on the Black Lake sturgeon population (Dammerman et al. 2015; Dammerman et al., *In Review*). The growth plot of the larvae demonstrated that individuals within the same maternal half-sibling groups also significantly varied in size. Several studies have reported individual-level variation size, based on differences in the degree of phenotypic plasticity in response to environmental variation (Evans et al. 2010). One explanation is that individual differences in growth among larvae in our study could be differences in feeding efficiency. For example, lipid storage capacity increases with body size in juvenile lake sturgeon resulting in differential growth among individuals (Volkman et al. 2004). Therefore, larvae that grew larger in our study may have utilized the hatchery diet more effectively thus growing to a larger body length.

Our study has important implications for lake sturgeon rehabilitation strategies. Protection of spawning and rearing habitats requires maintaining moderate flows, adequate water depth, and the presence of suitable substrate types. All three of these abiotic conditions can be drastically altered by habitat loss, and the increase in extreme weather events predicted to occur due to climate change. Additionally, variation in individual growth trajectories among and within different maternal sibling groups can influence growth in the hatchery, and the size of offspring that are being stocked back

into the wild population. Size is an important indicator of the over-winter survival of stocked individuals given that larger individuals are better at escaping predators and better foragers (Dower et al. 2009). Therefore, optimizing growth in the hatchery is essential for designing effective stocking strategies.

Additional observations on larval phenotypic traits at multiple spawning sites would benefit this study by allowing for the variation in permanent environmental effects during early life stages to be modeled. Using weighted regressions, we were able to model heterogeneous variances in this study and confirm it was a better fit than the model without heterogeneous variances; however, a random regression animal model incorporating the change in additive variance and permanent environmental effects would provide a deeper understanding of all the factors affecting traits associated with survival during sequential early ontogenetic stages. Additional future work in the stream could include examining biotic microhabitat variables that influence offspring development such as the spatial distribution of aquatic macro-invertebrates which are important prey species for juvenile lake sturgeon (Peterson et al. 2007). Knowledge on all stream conditions affecting larval and juvenile growth and development allows for the development of more effective lake sturgeon rehabilitation strategies.

## APPENDIX

Table 4.1 Mean ( $\pm$  Standard Error) values of the three microhabitat variables during the egg incubation period at the sixty-four sampling points where live lake sturgeon eggs were collected. Depth and discharge during the egg incubation period were predicted using hourly data logger measurements.

Transect Line	# Sampling Points	Mean Depth (m)	Mean Discharge (m <sup>3</sup> /sec)	Mean Substrate Size (mm)
A	6	1.23 (0.03)	0.75 (0.04)	13.99 (2.51)
B	12	0.90 (0.09)	0.68 (0.08)	12.22 (1.65)
C	11	0.80 (0.10)	0.73 (0.08)	9.51 (1.01)
D	12	0.69 (0.09)	0.68 (0.10)	12.94 (1.39)
E	10	0.75 (0.06)	0.82 (0.09)	10.40 (1.55)
F	10	0.64 (0.06)	0.75 (0.09)	12.34 (2.56)
G	3	0.63 (0.14)	0.88 (0.21)	10.58 (2.16)

Table 4.2 Mean ( $\pm$  Standard Error) and range in body size observed among larvae for the five measurement samples taken from hatch (Age 1) to four-weeks post-emergence (Age 41).

Age (days)	Mean (mm)	Minimum (mm)	Maximum (mm)
1	13.02 (0.05)	9.80	16.18
20	22.73 (0.08)	19.19	27.02
27	27.72 (0.13)	19.82	33.97
34	33.67 (0.22)	23.88	44.53
41	41.29 (0.32)	25.43	53.51

Table 4.3 Coefficients, standard errors (SE), and p-values estimated from the models of best fit for the three traits measured at hatch.

Hatch Traits	Fixed Effect	Estimate	SE	P-value
Body Length	Intercept	13.79	0.34	<0.001
	Mean Depth	-1.07	0.29	<0.001
	Mean Discharge	0.76	0.28	0.007
	Mean Substrate	-0.04	0.01	0.001
Body Area	Intercept	30.57	1.43	<0.001
	Mean Depth	-3.40	1.25	0.007
	Mean Discharge	3.29	1.18	0.006
	Mean Substrate	-0.18	0.05	<0.001
Yolk-sac Area	Intercept	7.36	0.36	<0.001
	Mean Depth	-0.01	0.35	0.99
	Mean Discharge	0.71	0.33	0.04

Table 4.4 Pairwise model comparisons for the three hatch traits fit to models (M) including all three environmental variables and a reduced model. Log-likelihood values, chi-square values, p-values (P), and degrees of freedom (df) obtained from the likelihood ratio tests are presented.

Hatch Traits	Model	Log-likelihood	Chi-square	df	P
Body Length	M1: Mean Depth + Mean Discharge + Mean Substrate Size	-364.46			
	M2: Mean Depth + Mean Discharge	-369.62	10.310	1	0.001*
Body Area	M1: Mean Depth + Mean Discharge + Mean Substrate Size	-761.96			
	M2: Mean Depth + Mean Discharge	-768.23	12.552	1	<0.001*
Yolk-sac Area	M1: Mean Depth + Mean Discharge + Mean Substrate Size	-414.81			
	M2: Mean Depth + Mean Discharge	-414.92	0.224	1	0.6358

\*Indicates a statistically significant difference ( $p < 0.05$ ) between models.

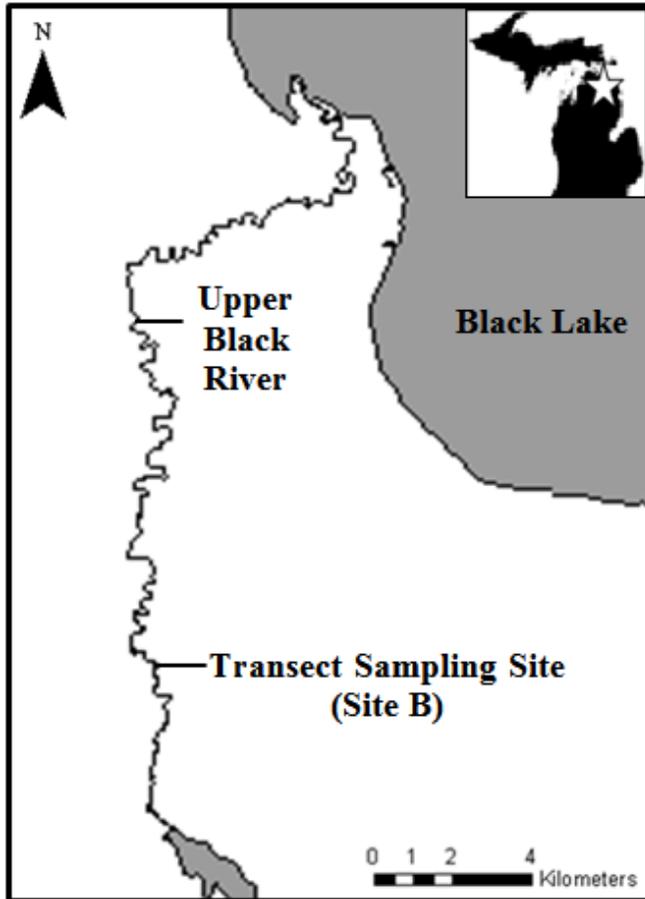


Figure 4.1 The study location on the Upper Black River, the largest tributary of Black Lake, Michigan showing the spawning site, Site B, where lake sturgeon eggs were collected along transect lines and the larval sampling location where wild-produced larvae were captured

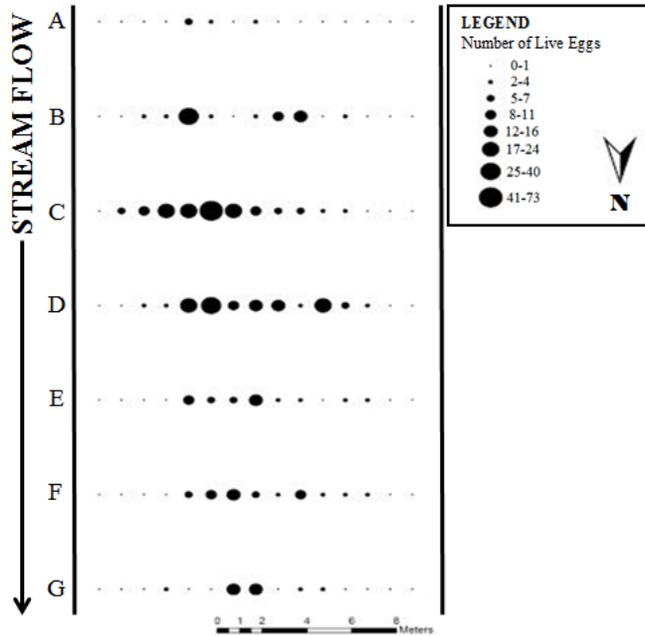


Figure 4.2 Schematic layout of the seven transect lines (A-G) and sampling points where the microhabitat variables were quantified and lake sturgeon eggs were collected. Bubbles indicate the number of live eggs collected at each transect sampling point.

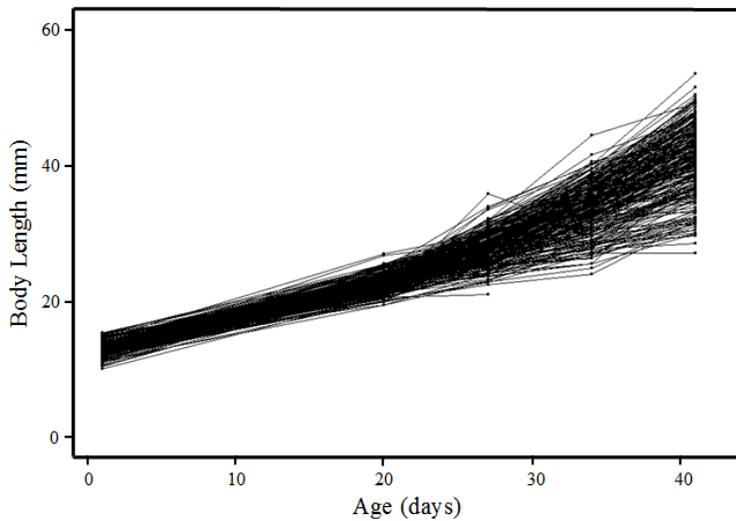


Figure 4.3 Individual growth trajectories quantified from hatch (Age 1 day) to four weeks post-emergence (Age 41 days). Individual body size was measured at hatch, and once weekly between the timing of emergence (Age 21 days) and the end of the experiment (Age 41 days).

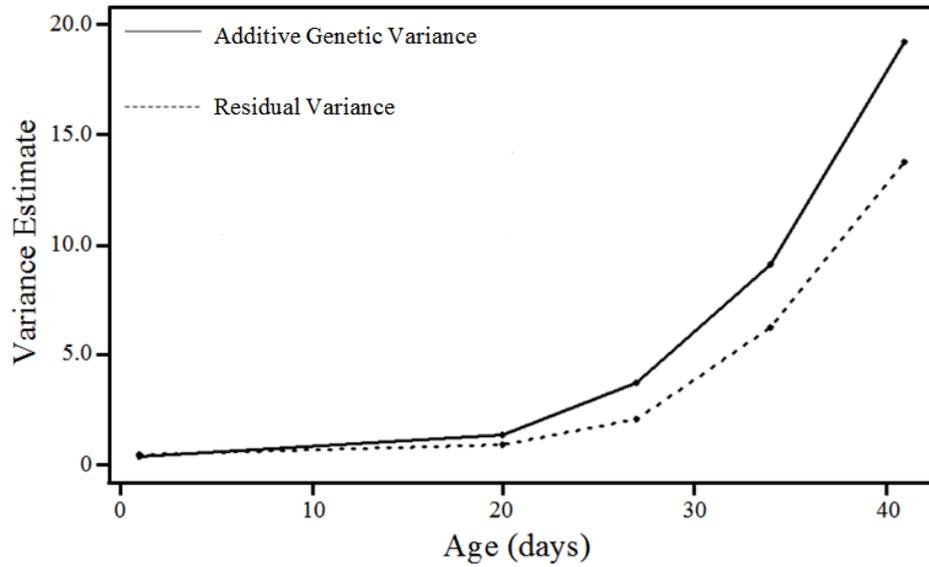


Figure 4.4 Additive genetic variance ( $\sigma_a^2$ ) and residual variance ( $\sigma_e^2$ ) estimates quantified at each age in the random regression animal model.

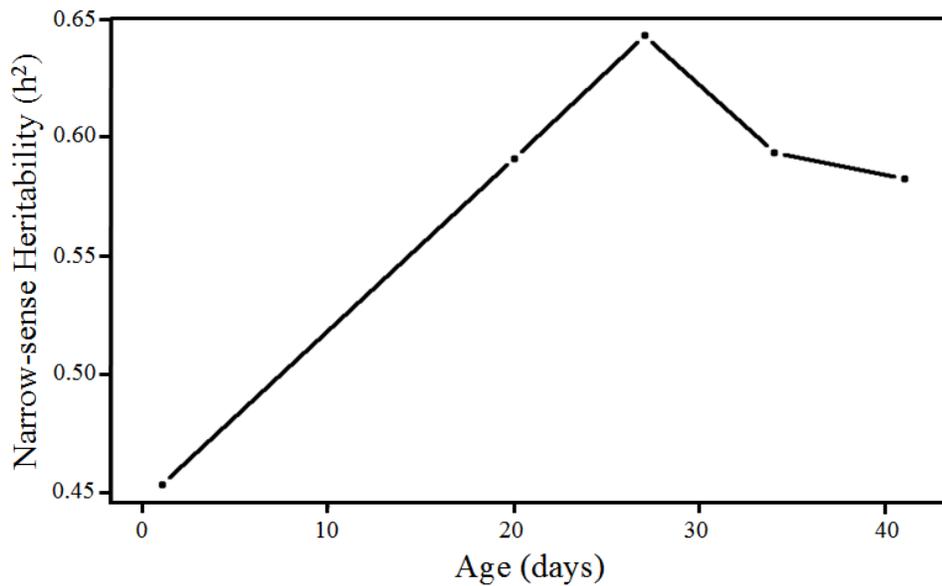


Figure 4.5 The narrow-sense heritability ( $h^2$ ) estimates for body size quantified at each age the larvae were photographed. The  $h^2$  estimates were obtained by dividing the additive genetic variance by the sum of the additive variance and residual variance.

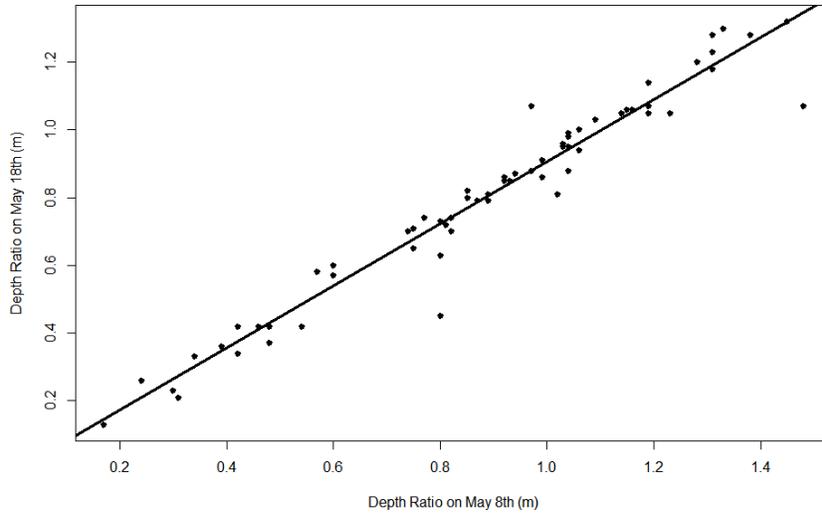


Figure 4.6 The relationship between the depth ratios calculated on May 8<sup>th</sup> and May 18<sup>th</sup>. The ratios were calculated as the point estimates of depth at each transect sampling point divided by the mean depth measurement from the data logger. The linear regression equation ( $y = 0.980x + 0.002$ ,  $R^2 = 0.948$ ) indicated the ratios were highly correlated between the two sampling dates.

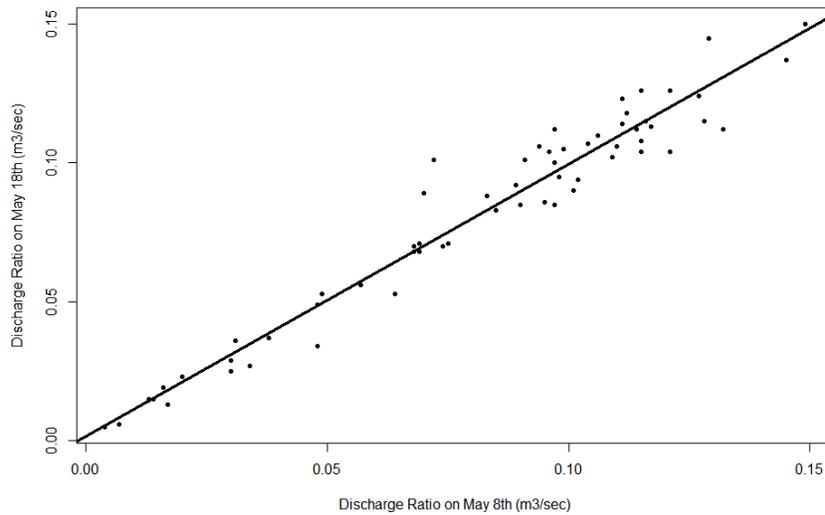


Figure 4.7 The relationship between the discharge ratios calculated on May 8<sup>th</sup> and May 18<sup>th</sup>. The ratios were calculated as the point estimates of discharge at each transect sampling point divided by the mean discharge measurement from the data logger. The linear regression equation ( $y = 0.917x - 0.010$ ,  $R^2 = 0.948$ ) indicated the ratios were highly correlated between the two sampling dates.

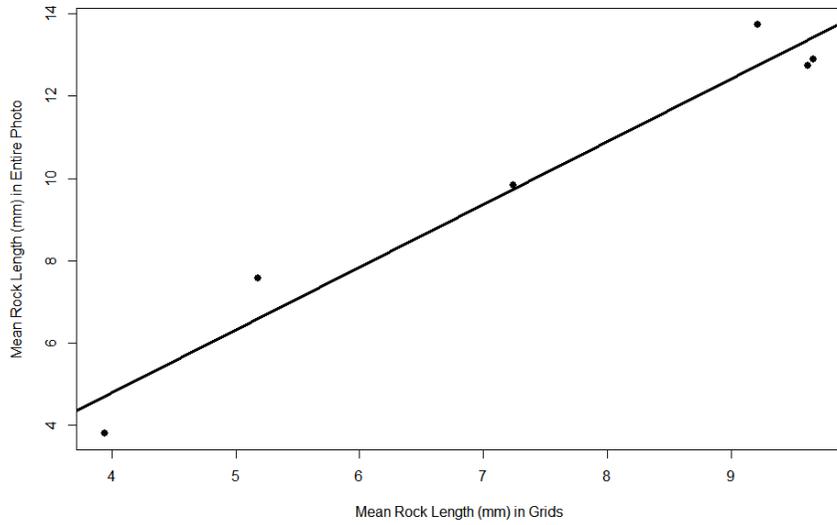


Figure 4.8 The relationship between mean substrate size when measuring rocks in three randomly selected grids versus the entire substrate photo. The linear regression equation ( $y = 1.526x - 1.315$ ,  $R^2 = 0.954$ ) indicated that measuring three random grids accurately estimated mean substrate size in the entire photo.

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## CONCLUSION

Research results from this dissertation demonstrate the importance of environmental variability on adult, egg, and larval riverine fishes such as lake sturgeon. The work on adult spawning behavior in Chapter 1 indicates that in order to develop a self-sustaining population with yearly stocking efforts, the maintenance of adequate abiotic and demographic cues are essential. Temperature and discharge are vital cues used by adults for determining the timing and location of spawning, and subtle increases in both cues due to spring runoff or the release of water from the hydroelectric dam greatly influence adult spawning behavior. Therefore, great effort should be made to maintain adequate flows and temperatures that represent a run-of-the-river scenario during the spawning and post-spawning months to allow greater spawning opportunities and provide critical habitat for developing embryos.

In Chapters 2-4, I've demonstrated that the environmental conditions experienced while in the egg largely affects the growth, development, and behavior of larval sturgeon. Therefore, understanding how conditions affect offspring traits within the population requires monitoring across all early life stages and not just post-hatch or during the juvenile stage in order to determine the factors governing recruitment in this wild population. Additionally, this research shows that high discharge levels and high water temperatures result in a greater range in phenotypic variation during these early life stages indicating a greater potential for differential survival among different family groups. Maintenance of adequate conditions in the wake of climate change and through

the dam operation is essential to maintain the genetic diversity of this closed lake sturgeon population.

Several research subjects within this dissertation can be built upon to understand the role of environmental variability and genetic (family) effects on riverine fish behavior and development. One research subject includes continual monitoring of spawning females to determine whether the probability of atresia increases as climates continue to change. Understanding factors governing individual timing of spawning has been limited, but is a growing field given the importance of numerating spawning population sizes for conservation efforts. Additional research includes examining the influence of size differences and swimming abilities on juvenile sturgeon survival. My research has shown that there are demonstrable size differences among individuals largely due to additive genetic effects; however, additional factors within a captive setting and in the river including food availability can largely influence offspring growth and survival, particularly during the first over-winter period.