EXOGENOUS CORRELATES OF MIGRATORY AND SPAWNING ACTIVITY AND EGG DEPOSITION AND MORTALITY IN LAKE STURGEON (*ACIPENSER FULVESCENS*)

By

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ABSTRACT

EXOGENOUS CORRELATES OF MIGRATORY AND SPAWNING ACTIVITY AND EGG DEPOSITION AND MORTALITY IN LAKE STURGEON (*ACIPENSER FULVESCENS*)

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Understanding patterns of migration, the timing of reproduction, breeding site selection and the sources and magnitude of early life mortality provides a unique perspective into exogenous regimes that influence patterns in recruitment as well as those leading to the evolution of important and adaptive life history traits. Unfortunately, knowledge of how migratory patterns and reproductive behaviors, the consistency of reproductive events and the time/location of reproduction relates to exogenous variables is lacking for many species, especially long-lived iteroparous vertebrates. Further, associations between habitat variables with patterns in the occurrence of early life stages and quantitative estimates of magnitude of mortality and the sources of loss across often spatially and temporally heterogeneous environments are relatively unknown. In order to address these uncertainties, I used tagging data from a long-term lake sturgeon monitoring program (2000-2008) established in the Black River, MI as well as experimental manipulations and laboratory studies to: 1) quantify relationships between migration patterns and spawning behaviors with exogenous variables, 2) estimate the consistency (i.e., repeatability) for individual spawning events across years, 3) assess whether stream covariates were predictive of spawning site selection, egg deposition and survival during incubation in natural settings and 4) quantify the magnitude, sources and the abiotic and biotic conditions (e.g., substrate size, predator density and size) that influence egg mortality. Migratory activity (i.e., the daily number of adults entering the river) and spawning activity (i.e., the daily number of adults collected at upstream spawning locations) were found to be significantly associated with exogenous variables including daily photoperiod, water temperature, river discharge and the lunar cycle. However, the relative importance of variables interacted in different ways at different sequences in migration continuum. Individual male and female lake sturgeon were also found to be highly repeatable in their choices of spawning time and spawning location (i.e., upstream vs. downstream preference). Egg deposition in natural stream settings was spatially explicit (i.e., patchy) and patterns were non-randomly and significantly associated with stream covariates including substrate size and water velocity across all sampled locations. Although mortality was consistent between sites over three days (70%), environmental covariates, particularly substrate size, were predictive of mortality rates. Finally, egg mortality was high (91%) and extremely variable (75-97%) across treatment groups in experimental settings but the relative magnitude of mortality sources varied significantly across simulated conditions, further suggesting strong relationships with the ecological features eggs experience naturally during incubation and survival. Developmental mortality (i.e., mortality due to abnormal egg development) was the major source of loss (84%) followed by predation (16%). However, egg mortality due to predation was shown to be high in laboratory experiments (80% after 24 hrs) with approximately 17-104 eggs consumed on average per predator. The highest levels of mortality were observed in treatments with high predator density and little habitat complexity. Information provided in this dissertation is largely unknown and will be valuable in the ongoing restoration efforts for this unique and imperiled migratory fish.

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INTRODUCTION

CHAPTER 1

EXOGENOUS CUES ASSOCIATED WITH MIGRATION AND SPAWNING IN LAKE STURGEON

INTRODUCTION

There is strong selection for animals to breed at times and in locations that enhance the survival of offspring. Migration between areas used for foraging and areas used for reproduction is a behavior that has evolved in many organisms in part because of fitness advantages associated with placing progeny in habitats that enhance probabilities of survival during early life stages (Alerstam and Enckell 1979; Alerstam et al. 2003). The ability of migratory animals to coordinate arrival times at breeding locales and during periods of optimal exogenous (i.e., all external influences) conditions has important fitness consequences, especially when recruitment variation is attributed to high rates of mortality during early life history stages at or near the sites of reproduction (Smith and Moore 2005; Forchhammer et al. 2002; Secor and Houde 1995).

Migration can be viewed as a continuum whereby individuals respond to exogenous cues, processing different sources of information en route that then culminates in reproduction (Duriez et al. 2009). However, the relative importance of exogenous cues may vary at different sequences during migration (i.e., onset of migration vs. onset of reproduction after arrival), and cues may interact to enlist different responses in different species, populations, individuals. For example, the probability that sea turtles will depart foraging areas and initiate migration to nesting areas is associated with rising chlorophyll-a concentration and rising sea-surface temperature (Sherrill-Mix et al. 2007). However, geomagnetic cues are used by adults to orient in open water (Luschi et al. 2007), chemical cues released from natal beaches are used for fine-scale homing

(Lohmann et al. 1999), and environmental cues such as rainfall, temperature and tides are correlated with the probability individuals will emerge from the ocean and initiate nesting behavior (Pike 2008).

Initiation of movement into tributaries and the timing of reproduction represent important behavioral decisions that are elicited by reliable cues (e.g., day length), and cues related to features of the physical environment, and which are associated with adaptive outcomes (e.g., successful reproduction) (Williams and Nichols 1984). Migrating at favorable times can reduce mortality en route and may conserve energy needed to compete for mates and quality breeding locations (Cook et al 2004; Crossin et al 2003; Hinch and Rand 1998). Adults must also time reproduction so that offspring are placed in conditions suited growth and survival (Einum & Fleming 2000, Hendry et al. 1998). During and following reproduction, eggs and larvae have developmental requirements, and growth and survival are influenced by many physical biotic factors including water temperature, river discharge, and predator abundance (Bailey and Houde 1989; Chotkowski and Marsden 1999; Mion et al. 1998; Smith and Marsden 2009). The strength of associations between exogenous cues and expression of behavioral responses will be dictated by the timing and location of selection (i.e., adults or gametes/offspring) during the migration continuum.

Nearly 300 fish species exhibit migratory behavior culminating in spawning in stream habitats (Northecote 1978; Frank and Leggett 1985). Migration for purposes of reproduction in fishes is mediated by factors associated with risks of mortality during migration to and from spawning areas and benefits of placing fertilized gametes and offspring in high-quality incubation and nursery areas (Alexander 1998). The chronology of migration is generally defined by stages defined as river entry, upstream movement, and the initiation of reproduction (Beall and de Gaudemar 1999; Bruch and Binkowski 2002; McMicheal et al 2005). Examples of exogenous variables found to be associated with stages in this continuum in fishes include tidal height (Karppinen et al 2004), photoperiod (Quinn and Adams 1996), ambient light (Bizzotto et al 2009; Parsley et al 2008), river discharge (Keefer et al 2009; Overton and Rulifson 2009), turbidity (Rakowitz et al. 2008), river water temperature (Binder and McDonald 2008; Salinger and Anderson 2006; Workman et al 2002) the lunar cycle (Kuparinen et al 2009; Bizzotto et al 2009) and social/demographic influences associated with spawner abundance and sex ratio (Kiflawi and Mazeroll 2006; Couzin et al. 2005).

Responses of migratory fishes during different stages of migration to exogenous cues are not always concordant (e.g., Dahl et al. 2004; Quinn and Adams 1996). Sex-specific responses to exogenous cues may also differ due to reproductive strategies utilized by males and females to increase reproductive success (Bateman 1948) but are rarely documented (Ball and Ketterson 2008). As a consequence of this uncertainty, our ability to generalize the magnitude of ecological/evolutionary responses of migratory fish populations and to predict the impacts of environmental or anthropogenic induced change is limited.

Information regarding the importance of different exogenous cues to the initiation of behaviors at sequential stages of migration are lacking for many species. This is especially true for long-lived iteroparous migratory fishes and for broadcast spawning species that do not exhibit post-ovulatory parental care where tight linkages between responses that have fitness consequences and environmental cues that elicit them may be expected. Inference may also be confounded by temporally correlated observations, both of dependent and independent variables (Thorstad et al. 2008).

We used data from a long-term research program collected over a range of exogenous conditions and integrated novel methods of statistical inference to quantify and well as compare and contrast the relative role of environmental and lunar covariates (collectively referred to as exogenous cues or variables) during the stages of migration in lake sturgeon (*Acipenser fulvescens*), a long-lived highly migratory iteroparous fish species. The specific objectives of this study were to 1) describe inter-annual, intra-annual and diel variation in the onset of migration at the river mouth and quantify associations between movement with exogenous variables, 2) estimate variation in pre-spawn river residency and determine whether inter-annual variation in the interval between first entry and first arrival to spawning areas can be tied to river conditions, 3) describe inter- and intra-annual variation in the onset of arrival to spawning locations, quantify associations between arrival with exogenous conditions, and test for evidence of sex-specific responses.

METHODS

Study site

Migratory and reproductive activities of a population of lake sturgeon in the northern lower peninsula of Michigan were monitored over eight consecutive years (2001-2008). This population is of moderate size (approximately 1,100 sexually mature adults, upper 95% confidence limit) and is isolated from other water bodies by dams to a large inland lake (Black Lake ~3500 ha). Migration and reproduction occurs in the spring (late April through May) and is restricted to the Upper Black River (UBR) (Smith and

Baker 2005; Baker and Borgeson 1999), a shallow fourth order tributary of Black Lake (Smith and King 2005). Approximately 11 km of the UBR located downstream from Kleber Dam (ca. 1949) to the river mouth is composed of unrestricted habitat used for staging and spawning each year. Fish spawn within a shallow and wadable 1.5 km section of the UBR 9 km upstream from the river mouth (Forsythe 2010; Smith and Baker 2005). These characteristics allow unrestricted access to adults. Based on genetic determination of parentage, approximately 90% of spawning adults are sampled in many years (Duong et al. in review). Large sample sizes permit quantification of inter and intra-annual variation in migratory and spawning behavior and quantification of relationships between behaviors observed during different stages of migration and exogenous variables.

Observations of river entry

Movements of lake sturgeon into the mouth of UBR were quantified over three consecutive years using a weir which restricted passage to a narrow channel of the stream and past a video-surveillance system. The specific dates for weir set-up and thus the beginning of sampling were 4/22, 4/15 and 4/16 from 2001-2002, respectively. Gill nets were also set at the river mouth in 2000 beginning on 4/17. The weir was constructed of aluminum fencing and metal poles arranged in a V-shaped configuration that guided migrants through an opening approximately 3 m wide. A sheet of white painted aluminum was placed on the stream bottom to enhance fish visibility as they passed through the weir. A video recorder was placed overhead at the weir opening to record images of passing individuals. Halogen lights also illuminated the weir opening at night allowing for continuous 24 hour observation. The video-surveillance system included a Sony Digital Handycam (Model DCR-TRV720) attached to a Panasonic real motion cassette recorder (Model RT600A). The time-lapse option was used to condense a 24-hour sampling period onto a standard 160-minute VHS tape. Numbers of migrants were summed by hour for each day within a season.

Upstream movement and observations of arrival to spawning areas

Adult lake sturgeon were captured as they arrived or shortly thereafter while spawning at upstream locations over eight consecutive years (2001-2008). Trapezoid-shaped handled landing nets (78.7 cm base, 30.5 cm top, and 70.5 cm length) were used for sampling spawning adults from early April until mid-June by walking the entire spawning area between 1000 – 1700 h each day. Sex was determined at the time of collection by cloacal morphology or by physically inducing gamete emission (Bruch et al. 2001). Characteristics of spawning UBR lake sturgeon including size, age and sex ratios as well as other aspects of spawning and migratory behaviors have been described previously (Smith and Baker 2005). Simultaneous observations of movement at stages 1 (i.e., river entry) and 3 (i.e., spawning site arrival) were provided in 2001 and 2002. We use this data to estimate the duration of pre-spawning river residency for early migrants (Stage 2) and determine whether inter-annual variation in the interval (i.e., lag time) between first entry and first arrival to spawning areas is a function of river conditions.

Explanatory variables

Exogenous variables explored in this study were selected based on commonalities found in numerous ecological studies of fish migration including sturgeons and based on the likelihood of their influence on the timing of migratory and spawning behavior of lake sturgeon in the UBR system. Those selected encompassed three general categories including 1) time-related variables (i.e., seasonal and diel photoperiodism; see, e.g., Doroshov et el. 1997; Webb et al. 2001; Smith and Smith 1997; Binder and McDonald 2008; Baril and Magnan 2002), environmental stream variables such as water temperature and river discharge (see, e.g., Jonsson 199; Salinger and Anderson 2006; Cooke et al. 2004; Kemp et al. 2006) and the lunar phase (i.e., lunar illumination; see, e.g., Kuparinen et al. 2009).

River water temperature was monitored hourly using HOBO® data loggers (Onset Computer Corp.) placed at the river mouth. A United States Geological Survey (USGS) gauging station was removed from the UBR in 2002. However, estimated UBR discharge (2001-2008) was based on comparisons between long-term (i.e., 50 years) data from UBR prior to gauge removal and from the Pigeon River, a nearby tributary of Mullet Lake, MI (Lat 45° 09′ N, Long 84° 28′ W). Discharge data for comparisons were obtained online from the USGS National Streamflow Information Program (http://water.usgs.gov/nsip). Data on lunar phase in the form daily fraction of the moon illuminated for Eastern Standard Time was obtained online from the Astronomical Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil/data/docs/MoonFraction). Actual light levels, moonrise or set times or whether the moon was visible on a given night were not taken.

STATISTICAL ANALYSES

Diurnal patterns of movement into the river mouth were analyzed using a linear mixed model developed within the MIXED procedure of SAS (version 9.1, SAS Institute, Cary, NC). Hourly movement was modeled with the fixed effects of hour and year. A multivariate first-order auto-regressive covariance matrix was used to account for correlations of observations of individuals across hour of the day. Modeling error as an auto-regressive process was appropriate because observations of hourly migratory activity which are more proximate in time were believed to be more highly correlated than more temporally separated observations. Post-hoc pair-wise comparisons of least square means were performed for each hour and nominal alpha levels were adjusted for multiple comparisons using the Tukey-Kramer method.

We used a hierarchal generalized linear mixed modeling (GLMM) approach to quantify the effect of exogenous variables on temporal variation in daily numbers of sturgeon observed initiating migration and entering the UBR (i.e., dependant variable) and the daily number of sturgeon captured as they arrived to spawning locations and initiated spawning activity (i.e., dependant variable). Daily activity was analyzed as a Poisson random process to account for heterogeneity of variances and non-normal residual variation resulting from a strong positive skew with many days each year having zero or a low number of observations or captures.

Correlations between observations, which tend to have the most serious impact on statistical interpretation of migratory data (Tre´panier 1996 and Ekinario et al. 1999), were accounted for using a low-rank radial smoothing algorithm applied through time (i.e., days). Radial smoothing is a non-linear non-parametric procedure that can be applied within the context of a parametric linear model with parameters of fixed and random components (see Gurrin et al. 2005). Like other methods that account for temporal autocorrelation (e.g., autoregressive models), smoothing algorithms generally provide more accurate predictions across the range of linear covariates (Pyper and Peterman 1998). Importantly, accounting for autocorrelation by using smoothing algorithms also provide more precise and robust estimates of model β’s by reducing irregularities (random fluctuations) in the dependant variable of time series data (Paige et al. 2009; Verbyla et al. 1999; Wang 1998). Radial smoothing algorithms were applied though time as a random effect using the GLIMMIX procedure (SAS Institute, Cary, NC). Terms for year (β_1) and the day (β_2) samples were collected were also incorporated into all statistical models. Year was treated as a random effect. The addition of a chronically ordering day component as a fixed effect provided a measure of correction for expected intra-annual declines in spawner abundance.

Environmental effect models

The behaviors of migratory animals are often dependent on covariates experienced during previous periods (e.g., Sherril-Mix 2008). Therefore, lagged environmental variables, estimated over a period of 1-2 days prior to daily observations, were used to assess the influence of environmental conditions on the initiation of migration and arrival to spawning grounds in the UBR. These variables included: 24 hr lagged temperature (Tlag, β_3), 24 hr lagged discharge (Dlag, β_4), 24 hr change in temperature (Tchange, β_5) and 24 hr change in discharge (Dchange, β_6). Changes in water temperature and discharge were computed as the average difference between mean observations collected 48 hrs apart, reflecting direction (positive vs. negative) and magnitude of trends. Note that lagged variables were also highly and significantly correlated (r range = 0.90-0.98) with conditions at the time of observation or capture (e.g., average daily water temperature).

Two models of increasing complexity were fit to count data for the number of individuals entering the river mouth and arriving at spawning grounds. The first model included only environmental covariates. A second model included both environmental variables and autocorrelation parameters (i.e., smoothing) to illustrate the importance of accounting for temporal trends. We refer to these models as Model 1- 2 (initiation of migration) and 6-7 (arrival at spawning areas), respectively. Environmental models generally took the form:

$$Y = \mu + \beta_1 \text{Year} + \beta_2 \text{Day} + \beta_3 \text{Tlag} + \beta_4 \text{Dlag} + \beta_5 \text{Tchange} + \beta_6 \text{Dchange},$$

where Y is the number of lake sturgeon observed or captured each year on a given day, μ is the intercept and the β's are parameters for each dependent variable to be estimated. Collinearity among independent variables was not a problem due to low correlation ($r < 0.3$). Preliminary analysis also indicated that daily arrival to spawning sites may be quadratic (i.e., curvilinear with an intermediate extreme) function of water temperature. Thus, an additional quadratic term for lagged effects of water temperature was added to these models. However, since lagged temperature and its quadratic effect were highly correlated, both variables were centered by subtracting the mean of each variable from each of its values and only the transformed variable of best fit was used in the final model (see below).

Lunar effect model

The effect of lunar periodicity was initially assessed separately using several terms (i.e., sin θ, β_7; cos θ, β_8; sin 2θ, β_9) that treated the lunar cycle as continuous cyclical rather than discretized events (see deBruyn and Meeuwig 2001). Here, θ was the angular-transformed day of the lunar cycle (day/29 × 2π). A significant cos θ regression coefficient represents a peak occurring at full moon and new moon whereas a significant sin θ regression coefficient represents a peak at the first and last quarter. The strength of association and the direction of association between numbers observed and lunar variables (i.e., full vs. new moon) depends on the sign of the coefficient. We also included the sin 2θ coefficient due to evidence of multiple spawning peaks per lunar cycle (i.e., sin 2θ test for peaks at the first and last quarter as well as a full or new moon). Each relationship describes how strongly the time series of observations tended to covary with lunar functions. Two models of increasing complexity were again developed for both the daily number of individuals entering the river mouth and arriving to spawning grounds and included: a lunar effect only model and a model with autocorrelation plus lunar covariates. We refer to these models as 3-4 (river entry and onset of migration) and 8-9 (arrival at spawning areas and initiation of spawning activity), respectively. The full lunar model was generally:

$$Y = \mu + \beta_1 \text{ Year} + \beta_2 \text{ Day} + \beta_7 \sin\theta + \beta_8 \cos\theta + \beta_9 \text{ sine } 2\theta,$$

where Y is the number of lake sturgeon observed or captured each year on a given day, μ is the intercept and β's are the parameters for each dependent variable to be estimated.

Model selection

Detecting the best fitting and most parsimonious model is an important analytical step. However, the estimation methods employed by GLIMMIX (e.g., REML) do not allow for multivariate model selection procedures such as forward, backward or stepwise selection criteria. Thus, predictive variables from both environmental and lunar models (EL) were selected based on level of significance ($P \leq 0.25$) and then combined in reduced and parsimonious multi-parameter environmental-lunar models. All possible two-way interactions between selected environmental and lunar variables (e.g., Tlag × Dlag; Tchange × Dchange; Tchange × cosine) were evaluated during this last model selection phase. All interactions were including and sequentially removed (i.e., model was re-run each time) based on levels of statistical confidence ($P > 0.05$). Interactions were also removed if it contributed little to model fit (see below). In our assessment of the number of individuals arriving to spawning locations, we also tested for sex-based responses to both environmental and lunar effects within in the EL model by including two-way (e.g., sex × Dlag) and three-way interactions (e.g., sex × Dlag × cosine). A sex × day interaction was also included to test and account for expected non-random sex-based declines in spawner abundance.

All models were compared by examining residuals, fitted values and parameter estimates as well as a linear comparison of observed and predicted values (i.e., R^2). The ratio of the Pearson Chi-Square over degrees of freedom was also used to compare information on residual model variability (The GLIMMIX Procedure, SAS Institute, Cary, NC). A ratio close to 1 indicates that variability has been properly modeled and that there is little remaining residual over-dispersion in the data. Other commonly used model fit statistics (e.g., AIC or BIC; Burnham and Anderson 2002) were not used since GLMMs fit using the GLIMMIX procedure in SAS estimates model parameters by applying pseudo-likelihood techniques and also provide unreliable information when models incorporate a random component (i.e., year and smoothing algorithms).

RESULTS

Summary of river entry

The first individual observed at the river mouth defining the onset of annual migration was extremely consistent with respect calendar date and thus seasonal photoperiodism (23 April in 2000; 24 April in 2001; 23 April in 2002) but less so with river water temperature (9.6 $^{\mathrm{o}}$C in 2000; 10.8 $^{\mathrm{o}}$C in 2001; 8.9 $^{\mathrm{o}}$C in 2002) and river discharge (7.8 m^3/s in 2000; 11.8 m^3/s in 2001 and 9.9 m^3/s in 2002). However, inter-annual consistency was still notable considering strong intra-annual trends as well as ranges in temperature (range: 8.6 – 21.0 ^{o}C), and discharge (range: 5.4-23.3 m^3/s) observed (Figures 1.1 and 1.2). The onset of migration did not coincide with percentage of lunar illumination (Range = 0-82%). Migration into the UBR lasted for a maximum of 36 days from April to late May (29 days in 2000; 32 days in 2001 and 36 days in 2002). An average of 8 individuals moved upstream daily. However, as many as 55 individuals were observed moving upstream over a single 24 hour period. Upstream migration occurred at mean daily water temperature and river discharge ranging from 8-21^{o}C and 5-24 m^3/s, respectively. Intra-annual variation in the number of individuals entering the river was characterized by discernable peaks that differed in magnitude and duration (Figure 1.4).

Exogenous correlates of river entry

A total of 757 recordings were made of lake sturgeon initiating migration (264 in 2000; 258 in 2001 and 235 in 2002). Counts of individuals entering the river were significantly associated with hour of the day ($F_{23, 46}$ = 31.3; $P < 0.001$) and this behavior was consistent across years ($F_{2, 46}$ = 1.15; $P = 0.33$; Figure 1.3). Individual pair-wise comparison across 24 hours of the day (i.e., hour 1 vs. hour 2) generally showed that significantly ($P < 0.05$) more individuals initiating upstream migrations in the early morning (0600 though 1100) and early evening hours (1700 through 2200) compared to other times of the day (Figure 1.3) suggesting a strong influence of diel photoperiodicity on upstream movement. Pair-wise comparisons of three arbitrary categories established based relative levels of ambient light also show that significantly ($P < 0.001$) fewer lake sturgeon initiated upstream migration during the night from 2200 to 0500 hrs (0.8 individuals ± 0.7 SE) and during the day from 1000 to 1700 hrs (6.4 individuals ± 2.6 SE) relative to twilight hours from 1800 to 2100 and 0600 to 0900 (27.8 individuals ± 10.2 SE), respectively.

Environmental (i.e., Models 1-2) and lunar (i.e., Models 3-4) mixed models explained 11-77% of the variation in numbers of sturgeon entering the river (Table 1.1). However, environmental models that accounted for temporal autocorrelation in observations of movement explained a relatively greater proportion of the variance. All factors including water temperature and river discharge were significant predictors of daily numbers of individuals entering the river (Table 1.2). Variables characterizing the lunar cycle were not significant (1.2). However, a model with environmental and lunar effects including the fixed effects of time (day), lagged river discharge, lagged water temperature, change in water temperature, change in river discharge and sine of lunar angle explained the greatest proportion of the variance (93%) in daily migratory activity. Model predictions and 95% confidence intervals were also highly concordant with observations (Figure 1.4). Two-way interactions between environmental and lunar variables were non-significant (data not shown) and were excluded from the analysis.

Regression estimates for variables included in the best-fitting environmental-lunar model as well as measures of uncertainty in model estimates (95% CI) (Table 1.4) revealed that the number of individuals entering the river did not significantly decrease during the season ($F_{1, 12} = 0.01$, $P = 0.93$). However, daily numbers of individuals entering the river was significantly and positively associated with lagged water temperature ($F_{1, 30} = 6.75$, $P = 0.01$), increasing by approximately 20% (95% CI: 4-39%;

Figure 1.5) for every unit increase (^{o}C). Lagged river discharge as well as the magnitude and direction of change in river discharge were also significant predictors of daily movements at the river mouth (D-lag, $F_{1, 90}$ = 4.37, P = 0.04, Figure 1.5; Dchange, $F_{1,70}$ = 3.86; P = 0.05). However, in contrast to water temperature, daily number of individuals entering the river was predicted to decline by 7% (95% CI: 1- 12%; Figure 1.5) and 8% (95% CI: 2 -12 %; Figure 1.5), respectively for every unit (m^3/s) increase in daily UBR discharge. Migratory activity was unrelated to the sine of lunar angle although model fit decreased substantially if this variable was removed.

Summary of upstream movement and arrival to spawning sites

We recorded 1262 captures of adult lake sturgeon from 2001-2008 including repeat observations of the same individuals over multiple years (Forsythe 2010). The total number of individuals captured across seasons ranged from 104-226, and more observations of males were made seasonally and throughout the study relative to females (360 females; 902 males). In years when both river entry and arrival to spawning areas were monitored simultaneously (i.e., 2001 and 2002), variable lags between the first individual observed entering the UBR and the first adult arriving to spawning locations were found (4 days in 2001 and 14 days in 2002; Figure 1.6). Further, UBR water temperature was 2^{o}C warmer on average and increasing faster in 2001 relative to 2002 before spawning and river discharge was also higher in 2002 on average during this time (Figure 1.1). We also noted based on this comparison that numbers of individuals arriving to spawning areas were more cohesive (i.e., well defined groups) relative to movement at the river mouth (Figure 1.6).

The number of individuals arriving to spawning areas and initiation of spawning activity lasted 29 days on average (range 19-43 days) from 2001-2008, generally encompassing a full lunar cycle (Figure 1.7). Six individuals were captured each day on average. However, the number of newly arriving individuals daily ranged from 0-50 individuals (Figure 1.7). Spawning occurred at water temperatures ranging from 8 to 19 ^{o}C but activity peaked (i..e, quadratic) when temperatures ranged from 11-17 ^{o}C. Spawning activity was also highest when river flow volume ranged from (5-9 m^3/s) and declined precipitously on days when daily river discharge exceeded 9-10 m^3/s. Seasonal patterns in upstream spawning activity were also complex and generally characterized by 2 (e.g., 2002, 2003 and 2005), 3 (e.g., 2004 and 2007) or 4 (e.g., 2001, 2006 and 2008) distinct peaks that differed in occurrence with respect to calendar date, magnitude and duration (Figure 1.7), though multiple captures of the same individual over multiple years revealed that the timing of spawning was highly repeatable (Forsythe 2010).

The onset of arrival at spawning sites (i.e., first individual observed) was highly variable across years with respect to calendar date (April 20 – May 7) and river water temperature (8-14.9 ^{o}C). However, river discharge at this time was generally low (8.1-12.7 m^3/s) relative to seasonal ranges. The onset of spawning site arrival did not coincide with a consistent percentage of lunar illumination (Range 2-87 %). However, the day of first arrival consistently occurred after the first three lunar cycles since the beginning of the year. Further, years when spawning site arrival was most delayed (e.g., 2002 and 2005) were generally years when the fourth full moon occurred at a later day of the calendar year although this relationship was not found to be significant ($F_{1,7} = 2.5$; $P = 0.162$). There was no evidence for consistent sex-based differences in first arrival to spawning locations (i.e., protandry) noted in other studies of lake sturgeon reproduction and as such spawning behavior was generally observed to begin within hours to days after arrival (Forsythe, unpubl.) based on observations of reproductive behaviors such as male cruising of spawning areas, porpoising, nudging and tail beats (described in Bruch and Binkowski 2002).

Exogenous correlates of spawning site arrival

Environmental (i.e., Models 6-7) and lunar (i.e., Models 8-9) models explained 2-66% of the variation in daily spawning activity in the UBR over eight years (Table 1.1). However, a model with lunar variables only that accounted for temporal dependency in catch of adults explained a relatively greater proportion of the variance relative to models the environmental effects of temperature and discharge (Table 1.3). Further, coefficients for both the cosine and 2sine of the lunar the angle showed were significant suggesting a role of cyclic lunar conditions in affecting intra-annual variation in spawning activity of UBR lake sturgeon. However, a model with both lunar and environmental variables best explained our observations of spawning (Table 1.1) and in most cases was highly predictive of captures within and across years (Figure 1.7). This environmental-lunar model of best fit included the fixed effects of day, sex, lagged discharge, rate of change in water temperature, the quadratic effect of lagged temperature, cosine and 2sine of the lunar angle. Two-way interactions between time and sex as well as the interaction between lagged discharge and water temperature (quadratic effect and the rate of change) with cosine θ were also included based on their contribution to model fit (Table 1.4).

Regression estimates for all variables as well as measures of uncertainty in parameter estimates (95% CI) (Table 1.4) provide strong evidence for declines in numbers of individuals arriving at spawning areas thus smaller sizes of spawning aggregations later in the spawning season. However, rates of decline in spawner abundance differed significantly by sex (F = 23.89, $P < 0.0001$), being significantly faster for females than males. Specifically, female abundance declined by 4% on average (2-6 % CI) each day the season progressed whereas the number of newly arriving males at spawning locations remained unchanged. Numbers of both males and females arriving to spawning sites were found to be equally (i.e., no sex-based interaction) and significantly associated with 2 sine of the lunar angle ($F_{1,\,458}$ = 54.5, $P < 0.0001$), a function that suggests peaks in arrival in association with the first and last quarters of the lunar cycle as well as at a full or new moon (Figure 1.7). However, a negative parameter estimate for sine 2θ (Table 1.4) indicates a stronger association with a quarter moon, or roughly 25% lunar illumination.

The number of male and female lake sturgeon arriving to upstream spawning areas were equally predicted (i.e., no sex-based interaction) by the main effects of cosine of the lunar angle ($F_{1,\,458}$ = 10.7, P = 0.0012), lagged discharge ($F_{1,\,458}$ = 28.4; $P <$ 0.0001), rate of change in water temperature ($F_{1,\,458}$ = 57.7; $P < 0.0001$) and the quadratic function of lagged water temperature ($F_{1,\,458}$ = 0.02, P = 0.015) (Table 1.4). However, highly significant two-way interactions between these lunar and environmental variables (Table 1.4; $P < 0.0001$) indicate that the responses of individuals in terms of spawning behaviors were not independent. Yet, spawning activity expressed as a function of all two-way interactions were consistent with the sign of individual parameter estimates (Table 1.4), generally highlighting the trend of increased numbers of spawners arriving to spawning sites at times with lower river discharge (7-15 m^3/s; Figure 1.8 a), when water temperature approached an intermediate level within its range (12-16 °C; Figure 1.8 b) and at times when water temperature was increasing at a faster rate to this "optimum" (Figure 1.8 c), especially when the cosine of lunar angle approached 1 or a full or new moon (Figures 1.8 a,b,c).

DISCUSSION

Organisms often use environmental cues to make decisions that weigh heavily in probabilities of offspring survival. Our results revealed that exogenous environmental and lunar covariates affected lake sturgeon behavior associated with initiation of migration, movements in the stream during migration, and timing of arrival at spawning areas leading up to the initiation of spawning.

River entry

The onset river entry of migration in the UBR occurred at nearly the same Julian date (range of two days over three years). Migration was initiated within a range of 1.9°C mean water temperature, a range of 4 m^3/s mean river discharge but was unrelated to a phase in the lunar cycle. Data collected during this period suggests that time-related variables (i.e., day length in the form of seasonal photoperiodism) represent an important exogenous cue for UBR lake sturgeon to initiate migration. Day length has been shown to mediate the endogenous cycle of oocyte maturation in other migratory fishes (Lee et al. 2002; Taranger et al. 1998) including sturgeon (Doroshov et al. 1997; Webb et al. 2001) and this may explain the remarkable inter-annual consistency in the beginning of upstream movement. This result, however, is in contrast to other migratory fishes where migration occurs at specific temperatures regardless of the date and thus is considerably more variable across years and between early and late spawning periods (Dahl et al.

2004; Juanes et al. 2004). Gessner et al. (2007) also noted latitudinal gradients in the timing of migration in other sturgeons (i.e., Green and Atlantic) at a spatial scale where water temperature, spring discharge and photoperiodism vary greatly. We also noted that earlier arrival to spawning areas (i.e., April 20), suggesting that individuals also moved into the UBR earlier, in years with abnormally high water temperature (i.e., 2006; Figure 1.1). Thus, while day length may strongest proximate driver in the onset of river entry, the onset of migration for UBR sturgeon indicated by river entry may have a degree of behavioral plasticity.

Time of day was also a significant predictor of river entry. Significantly more individuals entered the river dawn and dusk relative to other times of the day (Figure 1.3). Crepuscular activity may represent a learned behavioral response to predator (and human) activity rather than a strict environmentally mediated behavior due to the small and shallow river environment. Alternatively, crepuscular activity may also allow UBR lake sturgeon to avoid the thermal stress of migrating during rapidly cooling evening water temperatures, which is considerable in small northern temperature systems like the UBR, while avoiding sources of mortality through reduced visibility. Warmer water temperatures, such as those occurring later in the spawning season, shown to shift nocturnal patterns in some migratory fishes in favor of increased swimming endurance in laboratory experimental settings supports this hypothesis (Binder and McDonald 2008).

Environmental models that accounted for temporal autocorrelation in observations of daily river entry explained a large proportion (77%) of the variation in daily counts of sturgeon entering the river and considerably more than comparable models with only lunar effects (36%). In general, the strong negative effect of river discharge (Figure 1.5) and positive effect of water temperature (Figure 1.5) on the number of individuals entering the river is likely due to physiological effects of both on swimming performance, especially given the poor swimming ability of sturgeons relative more fusiform species such as salmonids (Webb 1986; Singer et al. 1990). However, UBR sturgeon may also respond to conditions at the river mouth as a mechanism of directly increasing offspring survival. For example, Hare and Cowen (1997) and Rideout et al. (2005) show that by avoiding stressful conditions (i.e., high temperature, high discharge) migratory females can divert extra blood flow to ovaries, positively affecting the size and quality of eggs, characteristics often related to increased survival during the egg and larval stages. We expand on the specific effects of discharge and temperature in the next few paragraphs.

Comparable studies of sturgeon migration over shorter time periods are concordant with our results with respect to river discharge (Benson et al. 2007), while numerous others have shown positive associations with river flow (Paragamian and Kruse 2001; Chapman and Carr 1995). One possibility for this difference is that studies based on telemetry collect data on fewer individuals may not detect the tendency for individuals to select the energetically efficient conditions (Crossin et al. 2003) or discharge may have a greater influence on migratory behavior in smaller and shallower systems. Differences in the response of sturgeon to river discharge are a reflection of species and location specific migration strategies as found in well-studied salmon and trout (reviewed in Rand and Hinch 1998). For example, in systems where spawning sites are relatively close (9 km) and few natural barriers exist to impede movement may favor a migratory strategy that conserves energy to support current reproduction. Individuals using systems which require considerably longer migration distances, however (e.g., Kooteni River, 247 km), may elect to swim outside of optimal conditions to conserve time in order to best coordinate arrival with gonad maturation (i.e., occurring in route) and spawning activity with conditions (i.e., mate type and number, water temperature) that increase probabilities of reproductive success (Standen et al. 2004).

Positive monotonic relationships between daily numbers of individuals entering rivers and water temperature are also common in migratory fishes including sturgeons (Dahl et al. 2004; Tetzlaff et al. 2005; Paragamian et al. 2002) as a function of swimming performance. Alternatively, other studies demonstrate that maximal sustainable speeds of migratory fishes decline sharply as water temperature increases above a thermal optimum and thus migration activity slows as individuals seek to conserve energy and in some cases reduce the risk of pre-spawn mortality (Hammer 1995; Mayfield and Cech 2004). For example, Salinger and Anderson (2006) show that swim speed in Chinook salmon (*Oncorhynchus tshawytscha*) increased below 16^{o}C but decreased above this optimum. We tested this hypothesis but found no statistical evidence of a preference for UBR sturgeon to migrate at a specific water temperatures (i.e., no quadratic effect), rather migratory activity increased linearly until 21^{o}C (Figure 1.5). One explanation for this result is that individuals were avoiding stressful high-temperature conditions altogether and thus a decline in numbers with increasing water temperature was not detectable. Lake sturgeon have also demonstrated enhanced, sustained and prolonged swimming performance at higher water temperatures without reaching an optimum (i.e., up to 21^{o}C) (Peake et al. 1997). Alternatively, warmer water temperatures may be a strong cue for individuals at point river entry for reasons that enhance embryo development, especially in systems such as the UBR where migration distances are relatively close.

A model that incorporated both environmental and lunar effect was the best fit to our data ($R^2 = 0.95$) and was predictive of substantial intra- and inter-annual variation observed in the daily number of individuals in the UBR (Figure 1.4). This finding illustrates the relative importance of several features of the data. First, the improvement of model fit with addition of a single lunar parameter (i.e., sine of the lunar angle) indicates a weak but potentially important lunar effect that three years of video-surveillance may not have been sufficient to significantly elucidate a significant main effect, even with the integration of statistical methods that can generally expose weak associations (deBruyn and Meeuwig 2001).Assuming that additional years of data would reveal a significant lunar effect, lunar-induced movement may be adaptive for sturgeons in reducing predation-driven mortality or in facilitating the passage of individuals to spawning locations purported in other migratory fishes where this phenomenon is observed (Carlson et al 2009; Wilson and Halupka 1995). However, since there appears to be strong selection for individuals to time spawning with specific phases in the lunar cycle (i.e., full and new moon) for reasons later discussed, a more likely explanation is that the association between causal and response variables at sequences in the migratory continuum spatially and temporally removed from the agent of selection would be weaker. Recent studies demonstrating significant evidence of weak lunar effect at the time of river entry in environments where its indirect effects are small like the UBR (i.e., tides), lends support for this hypothesis and suggest that lunar-based migration is of greater biological relevance than previously discussed (Kuparinen et al 2009).

We tested for the combined effects of discharge and water temperature on river entry by testing for interactions in the full lunar-environmental model because interactions between years are often notable (Prigel and Wirth 1997; Quinn et al. 1997; Keefer et al. 2004). However, interactions between water temperature and discharge variables on movement upstream within a season were either non-significant or contributed little to model fit suggesting that times with either warmer water temperatures or decreasing river flow were equally strong cues for individuals to initiate river entry (i.e., the decision of individuals to departure at times with high temperature was independent of river flow). This result contrasts the others that have tested for interactions. For example, Svendsen et al. (2004) found that while the probability of anadromous female brown trout (*Salmo trutta*) to move upstream significantly increased with increasing discharge, this probability was differentially affected by water temperature. However, the relative effect of water temperature, discharge and thus the potential for interaction on inducing upstream movement clearly varies among systems (Jonsson 1991; Workman et al. 2002). Further, since the UBR is a small stream and environmental cues like temperature and discharge vary over a shorter temporal scale, and thus daily observations and lag effects of conditions during periods immediately preceding the day of observation are likely to elicit a more immediate, independent (and detectable) response relative to larger rivers.

Finally, predictions of future environmental conditions are often based on conditions experienced in the immediate past. In order for UBR lake sturgeon to detect effects of conditions over previous days (lagged cues) individuals would need to be near the source of these cues prior to the onset of migration (i.e., staging before river entry). Staging has been reported for lake sturgeon in other larger systems (i.e., Green Bay; Bott et al. 2005) and for numerous other migratory fishes (Godinho et al. 2007; High et al.

2006). Many migratory species such as birds form pre-migratory aggregations where conspecific social interactions represent important cues that induce migration (Lundberg 1985). Migratory fish are generally not social and yet the congregation of individuals near river mouths before migration and evidence of repeated bouts of river entry and withdrawl in sturgeon (Godinho and Kynard 2006) may suggest that social influences are an important component of migration in addition to the exogenous cues tested herein.

Upstream movement and arrival to spawning sites

Arrival to spawning locations was more variable with respect to calendar date than the onset of migratory activity. Thus, time-related cues such as day length appear to become less important after river entry in the UBR. The onset of spawning activity was also inconsistent with lunar illumination. However, arrival was delayed when fourth full moon occurred later in the season. Water temperature at first documented arrival to spawning grounds was also variable (10-19^{o}C) but consistent with the incubation requirements of embryos (8–20 °C; Detlaff 1993; Van Eenennaam et al. 2004) and similar to those reported at the onset of spawning for populations across the species geographic range (McKinley et al. 1998; Auer 1996; Bruch and Binkowski 2002; LaHaye et al 1992). Based on these similarities, we believe water temperature is a strong cue regulating the onset of reproduction. However, arrival to spawning locations also occurred when UBR discharge was low and decreasing. Increased lag time between the onset of migration and arrival to spawning locations in years with higher average discharge were also observed (2001 vs. 2002; Figure 1.6). Thus, inter-annual variation in arrival time to spawning sites may be related to river discharge resulting from different river residence times, as individuals stay in the river between entry and initiation of spawning. Prigel and Wirth (1977) also noted significant delays in spawning during seasons of high water in the Wolf River, Wisconsin.

Counter to our expectations, models with only lunar covariates explained the largest proportion (66%) of the observed variation in spawning activity. Times approaching a new moon, during or just after with full lunar illumination were found to be important cues for individuals to initiate reproductive activity. Lunar cues may have important ecological implications for sturgeon species. For instance, the incubation duration of lake sturgeon embryos is predictable given water temperature and maternal effects (Duong et al. 2010). Thus, synchronizing reproduction with the lunar cycle (i.e. full moon) may be synchronized to timing of the larval dispersal (Forsythe et al. 2010), decreasing larval risk to nocturnal predators. Timing spawning with lunar phases facilitates hatching and post hatching development during a period that subsequently exposes larvae to dark lunar phase conditions that reduce visibility during nocturnal drift and as exogenous feeding begins (Hernandez –Leon 2008). Incubation length and the duration of larval residence in the substrate prior to downstream dispersal tend to decrease with increasing temperature as the season progresses (Crossman 2008; Doung et al. 2010). Our study is consistent with reports indicating that spawning in both lake and Gulf sturgeon occurred in association with a new moon (Auer and Baker 2002; Sulak and Clugston 1998).

Models with environmental effects also explained a large proportion of the variance ($R^2 = 0.63$). Parameter estimates indicated that low-flow conditions (i.e., below 9 m^3/s) were a significant cue for individuals to arrive at spawning areas and initiate spawning (Table 1.3). One explanation for this relationship is that increased river discharge is a negative cue for individuals staging downstream to move onto spawning locations (i.e., similar to at the river mouth). Reproductive readiness of individuals as well as critical aspects of the species' reproductive ecology may also be impaired or disrupted as river discharge increases (Auer 1996). Low flow conditions are likely important for increasing probabilities of egg fertilization and to increase probabilities that eggs are deposited within desirable substrates within selected spawning locations to increase egg survival (Auer 1996; LaHaye et al. 1992; Paragamian and Wakkinen 2002). However, increased abundance of reproductively active individuals arriving to spawning sites has been found to coincide with increasing river stage for some sturgeons (i.e., shortnose) (Tripp et al. 2009).

The number of new individuals arriving to spawning sites and initiating spawning was best predicted by a quadratic function of water temperature, peaking between 12 and 16 ^{o}C, and by the rate of increase in water temperature over successive days (Table 1.4). Similar qualitative associations between intra-annual variation in spawning activity and water temperature and temperature induced behaviors have been documented in other sturgeon species across a wide geographic range. For example, Scott and Crossman (1973) and LaHaye (1992) reported peak spawning temperatures between 13 and 18°C. Kempinger (1988) and Paragamian and Wakkinen (2002) noted that small declines in water temperature (0.8 – 3°C) reduced spawning activity and caused individuals to temporally leave spawning locations. Reproductive activity of lake sturgeon in the Des Praries and L'Assomption Rivers also increased in association with increasing water temperature (LaHaye et al. 1992). However, our study is the first to empirically test for and quantifies the relationship between spawning site arrival and temperature over multiple spawning seasons and based on groups spawning at different times.

In contrast to findings for initiation of migration, the relative importance of lunar effects in models to observations of numbers arriving at spawning areas and initiating spawning activity indicates that a different but predictable exogenous variable (lunar cycle) was a relatively stronger cue for UBR lake sturgeon at the final stages in the migration continuum as compared to the importance to water temperature, river, discharge or Julian date for initiation of migration at the river mouth. Yet, a model incorporating both lunar and environmental effects best fit our capture data ($R^2 = 0.77$) and predictions and confidence intervals where concordant with observed spawning activity across all years (Figure 1.7), especially after accounting for temporal autocorrelation. Parameter estimates were similar between all models (Tables 1.4 and 1.4). However, highly significant interactions between all environmental variables with a single lunar covariate (i.e., cosine θ) suggests that the response of individuals to environmental variables was of secondary importance to lunar cues as adults arrived at spawning areas and initiated spawning. Significant interactions suggest that inter-annual variation in timing of initiation of spawning is a reflection of environmentally-induced modification of behavior from times with either a full or new moon (Figures 1.8 a, b and c). Associated work on the UBR supports this hypothesis showing that environmental deviance is not extreme, as inter-annual variation in the timing of spawning when adjusted to a standardized day of spawning, for individual lake sturgeon is highly repeatable (Forsythe 2010).

We found little statistical support for sex-based differences in response to exogenous conditions at the time of spawning. Sex-based responses may not be important to initiation of spawning as observed during other stages during migration. Paragamian and Kruse (2001) found that male white sturgeon were significantly less likely to respond changes in water temperature and discharge while moving upstream than females. Although males and females are exposed cued by the same exogenous conditions, differences in reproductive strategies likely keep males at spawning locations longer to enhance mating opportunities as additional females arrive at spawning sites as noted previously (Birch and Binkowski 2002; Van Eenennaam 1996; Douglas et al. 2009; Carmichael et al. 1998).

CHAPTER 2

REPEABILTY FOR SPAWNING TIME AND SPAWNING SITE SELECTION IN THE LAKE STURGEON

INTRODUCTION

The tendency of adults to reproduce during certain times or at specific locations is an adaptive behavior that places offspring in ecological conditions that are conducive to growth and survival (Norris 1993; Trippel 1998; Sheldon et al. 2003; Hipfner et al. 2010). For some animals, an important component of this adaption is long-distance migration, often with considerable fitness consequences, to habitats that prepare individuals for reproduction (i.e., foraging areas) and areas suitable for offspring development. In migratory species, movement during times that favor energy conservation and reduced mortality (Rand and Hinch 1998), the capacity to perceive and respond to environmental cues during migration so that reproductive activities are coordinated with suitable features of the environment (Duriez et al. 2009), and the ability to select suitable breeding areas within spatially and temporally heterogeneous environments represent important behavioral traits (Geist and Dauble 1998).

Migratory fishes that use the streams for reproduction exemplify species that must coordinate reproductive activities with specific conditions to successfully reproduce because of high mortality of offspring during early-life stages (Bailey and Houde 1989; Smith and Marsden 2009; Forsythe 2010). To achieve success, many species use environmental features of streams (e.g., temperature, discharge, substrate size), that affect spawning site quality (Malcolm et al. 2009), and abiotic cues (e.g., lunar cycle) to make choices of when and where to reproduce (Hodgson and Quinn 2002; Beall and de Gaudemar 1999; Dahl et al. 2004; Forsythe 2010). Previous reproductive experience and learning from repeated breeding events (Trippel 1998; Carr and Kaufman 2009), age/size (Trepanier et al. 1996; Stewart et al. 2002), alternative reproductive tactics (Morbey 2003) and social influences (Brown and Laland 2003) also affect inter- and intra-annual spawning time and breeding site selection. For many migratory fishes, the timing of spawning and spawning site selection also have a genetic basis (Jennings et al. 1996; Quinn et al. 2000; Stewart et al. 2002) whereby the responses of individuals to environmental cues are regulated through endogenous cycles (i.e., gonad maturation) that influence spawning time or to physiological (i.e., olfaction) properties that allow individuals to find suitable spawning habitats and thus choices are consistent over multiple reproductive events.

Spawning time and spawning site selection may also have considerable implications depending on the circumstances that affect them. For example, many migratory fishes are characterized by groups that reproduce at different times and often at different locations within a single tributary with little mixing (Hellawell et al. 1974; Fillatre et al. 2003; Papa et al. 2007; Forsythe 2010). If environmental conditions associated with spawning habitats affect offspring traits and fitness, and if offspring share similar tendencies, adaptive phenotypic divergence can evolve across breeding units even within the same population, a pattern recently coined isolation by time (IBT) (Hendry and Day 2005). Examples of heritable adaptive phenotypic and life history specializations across multi-modal distributions of temporally and spatially isolated migratory fish breeding groups include age at first reproduction, egg size, fecundity and embryonic incubation time (Hendry and Day 2005). Co-variation between adult spawning time and juvenile traits has also been quantified (Quinn et al. 2000, Einum and Fleming 2004), Despite the significance of both spawning time and spawning site selection in migratory fishes, little is known regarding individual variation (i.e., plasticity) in response to exogenous cues individuals experience (e.g., stream flow, temperature, lunar cycle, social influences) or whether trait expression remains consistent over repeated reproductive events (i.e., has genetic basis). Further, knowledge of whether individuals exhibiting similar tendencies for timing and location of reproduction continue to associate with each other across reproductive episodes is limited but has important implications, particularly for small populations. We used multi-year data collected on a migratory fish species, the lake sturgeon (*Acipenser fulvescens*), to conduct an individual-based analysis of spawning behavior. The specific objectives of this study were to 1) estimate spawning periodicity (i.e., inter-annual spawning intervals) and determine whether intervals varied as a function of sex and age, 2) measure the degree of intra-annual consistency in spawning time within a breeding season and determine whether time-dependant or exogenous covariates were the best predictive of when and where spawning occurred within an extremely small tributary and 3) test for evidence of size-based (ontogenetic) variability in arrival time.

Lake sturgeon are a long-lived iteroparous species exhibiting aggregate mating behavior where eggs and sperm are released over large areas of natal streams in the absence of nest preparation and without post-ovulatory parental care (reviewed in Peterson et al. 2007). Because of the species' mating ecology and offspring exposure to variable environmental conditions and predation, eggs and juvenile lake sturgeon often experience high mortality early in life (Kempinger 1998, Forsythe 2010). Further, because early-life survival represents a substantial component of variation in lifetime fitness, there is also opportunity for natural selection to direct adult behaviors, breeding site selection, and offspring traits that are tied to environmental conditions at the time of reproduction. Quantification of the degree of sex and age differences in spawning behaviors, individual behavioral plasticity, or evidence of IBT through non-random assortative mating promoted through inter-annual consistency in spawning time and spawning location selection has seldom been empirically evaluated in long-lived iteroparous fishes.

METHODS

Study location

Research was conducted from 2001-2008 on a well-studied lake sturgeon population located in Black Lake, Michigan. The Black Lake system is ideal for conducting long-term research directed at quantifying variability in individual spawning behavior over several reproductive episodes. The Black Lake system is closed to immigration because of dams. Lake sturgeon use a single tributary, the Upper Black River (UBR), for reproduction (Forsythe 2010) and individuals spawn within a 1.5 km stretch of the UBR that is approximately 9 km upstream from the mouth (Figure 2.1). After arriving at spawning sites adults spawn in groups for periods of one to several days (Forsythe 2010). During the spawning period, individuals are readily captured at several accessible and wadable areas. Individuals can also be visually monitored nearly continuously after capture without the use of telemetry or acoustics because of the narrow stream channel (< 25 m), shallow water depth (< 1.5 m), and relatively low flow volume (daily average 6.4 m^3/s). Due to ease of collection, a large proportion of adults have been captured (i.e., 678 individuals of an estimated 850; E. Baker unpublished data) and most individuals have been observed over multiple years.

Data collection

Lake sturgeon were captured at several locations in the UBR using large handled landing nets while wading the entire section of the stream used for spawning ≥ 1 per day through the entire spawning season each year. All individuals were sexed by examining cloacal morphology or expelling gametes and biological information including total length (cm) and weight (kg) was collected at the time of capture. Information on the relative stream location (i.e., Zone; Figure 2.1) where individuals were first observed was also recorded. All individuals captured were marked with both internal PIT (Passive Integrated Transponder) tags (Biomark, Inc.) and a unique combination of external colored polyethylene T-Bar Anchor Tags (FLOY TAG, Inc.) placed at the base of the dorsal fin. Quantitative data on exogenous conditions that likely influence daily and seasonal levels of migratory and spawning activity in lake sturgeon including water temperature (^{o}C), stream discharge (m^{3}/s) and the lunar cycle phase were also collected continuously through the spawning season (Forsythe et al. 2010). Water temperature was recorded hourly using Onset Hobo temperature loggers. Because the gauging station on the UBR was removed in 2002, daily river discharge was estimated using a statistical model ($F_{1,4056} = 9355$, $P < 0.0001$, $R^2 = 0.68$) that predicted daily average discharge based on long-term historical correlations (1950-2000) between the UBR and discharge of the Pigeon River, a nearby tributary of Mullet Lake. Discharge data were obtained online from the USGS National Streamflow Information Program (http://water.usgs.gov/nsip). Daily data on the lunar phase in the form of the fraction of the moon illuminated (values range from 0-1) were obtained online from the Astronomical Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil/data/docs/MoonFraction).

STATISTICAL ANALYSES

Data preparation

Repeatability in individual spawning time was analyzed using summarized stream environmental and lunar variables including: average daily water temperature, average daily discharge, rate of change in average daily water temperature and discharge (lagged over previous 48 hrs), 24 h lagged water temperature and discharge and the proportion of the moon illuminated each day. Values resulting from the summarization of environmental variables for each day were linked to capture and recapture histories for all individuals in all years. Analyses of repeatability for spawning date were initially conducted using the day of the calendar year. However, because the onset and duration of spawning activity were variable across years, repeatability was also computed as a function of standardized spawning date (SSD). SSD was estimated as the day of spawning activity for a given year (y) divided by the total duration of spawning activity (n) observed for that year with the first day spawning was observed assigned a value of 1. This computation placed the time of spawning on the same relative 0 to 1 scale across years. Statistical analyses of individual repeatability in SSD and differences in repeatability as a function of sex and body size (age) were conducted using linear mixed or generalized linear mixed models in SAS (version 9.1, SAS Institute, Cary, NC). Mixed effects models were generally appropriate for this analysis because of the ability to account for repeated measurements on the same individual across spawning seasons implemented as a random component. Mixed models can also account for alternative (e.g., multinomial) distributions of the response variable, and provide a means to estimate the variance attributable to a random factor which was also assessed at the individual level.

Spawning periodicity

The interval between observed spawning events (i.e., spawning periodicity) was computed as the difference in years between consecutive captures. If an individual was captured on ≥ 2 occasions over the period 2001-2008, inter-spawning interval was computed as the average interval between all successive captures. The effects of sex and size on inter-spawning interval were incorporated into the model as fixed effects. Average spawning interval for each sex was estimated using least square means ($P < 0.05$).

Repeatability in spawning behavior

Repeatability was used to evaluate the degree of consistency in individual lake sturgeon spawning behavior. Repeatability (r) is commonly defined as the ratio of between-individual variance to the total variance for a given trait or the proportion of all variation expressed among rather than within individuals (Lessels and Boag 1987). Repeatability estimates are equivalent to an intra-class correlation coefficient (i.e., the data are centered and scaled using a pooled mean and standard deviation for individuals) and frequently used to describe the extent to which a behavior remains consistent over multiple measurements (Boake 1989; Farrell et al. 2009). In this study, repeatability in spawning time was estimated for SSD along with environmental variables (e.g., current and time-lagged river temperature and discharge) that define the stream environment at or immediately before spawning, lunar phase, and spawning site location. Repeatability was estimated using the following equation:

$$\hat{r} = \frac{\hat{\sigma}_{ug}^{2}}{\hat{\sigma}_{ug}^{2} + \hat{\sigma}_{eg}^{2}} \qquad (1)$$

where is $\hat{\sigma}_{ug}^{2}$ the estimated variance among individuals of sex g and $\hat{\sigma}_{eg}^{2}$ is the estimated variance within individuals of sex g (i.e., residual variance). Estimates of among- and within-individual components of variance in repeatability were computed separately for each dependent variable (i.e., time and environmental covariates) and were obtained using restricted maximum likelihood estimation in the context of a null linear model (i.e., intercept with no independent variables). Among and within estimates of variance were provided as covariance parameter estimates in SAS. Consider two hypothetical cases. If there was no variation within individuals ($\hat{\sigma}_{eg}^{2}$ = 0), then r = 1 and all spawning observations for the same individual would occur on the same SSD. In contrast, if no variation was present among individuals ($\hat{\sigma}_{ug}^{2}$ = 0) and all variation could be attributed to within-individual factors, then r = 0.

In addition to estimating variance components separately for each sex, we also tested for evidence of heterogeneous variances among individuals of different size groups under the alternative hypotheses that repeatability increases with increasing age and spawning experience. Body length is correlated to and predictive of age in lake sturgeon (Baker and Borgeson 1999; Bruch et al. 2009). We first defined three size categories for both female and male lake sturgeon that placed approximately 33% of the total number into each category. Approximately equal representation in all categories was necessary to meet statistical assumptions. However, categories were adjusted in situations where distinct gaps in size/age frequencies and thus levels of reproductive experience were observed. Female categories for this analysis included individuals that were 142-164 cm (28-42 yrs), 166-174 cm (43-48 yrs) and 175-189 cm (49-57 yrs) TL while male categories included individuals that were 112-140cm (15-25 yrs), 141-152cm (26-30 yrs) and 153-179 cm (35-52 yrs) TL. Estimates of within and between individual components of variance were then computed separately for each dependent variable according to size category and separately for each sex. Thus, equation 1 was modified to:

$$\hat{r}_i = \frac{\hat{\sigma}^2_{ugi}}{\hat{\sigma}^2_{ugi} + \hat{\sigma}^2_{egi}} \qquad (2)$$

where $\hat{\sigma}^2_{ugi}$ is the estimated variance among individuals of sex *g* in length category *i* and $\hat{\sigma}^2_{egi}$ is the estimated variance within individuals of sex *g* in length category *i*. The improvement in fit of this more complex model was evaluated relative to the primary model (i.e., homogenous variances using Eq. 1) using likelihood ratio tests ($P < 0.05$). Statistical evidence supporting a model with heterogeneous variances would indicate size-based differences in repeatability with respect to the size categories created here.

Spawning site selection and size based arrival

Seasonal patterns in use of different river zones were tested using a generalized linear mixed model that fit river zone at the time of capture to standardized spawning day. The dependent variable in this analysis (i.e., zone of capture) was modeled as having a multinomial distribution with a grouped logit function which is appropriate for categorical variables with a fixed number of outcomes (i.e., six zones in this particular case). Parameter estimates resulting from this model are interpreted as the odds of an event occurring in the focal zone relative to a predefined reference. Zone 1 was used as a reference based on the observation that spawning groups tended to utilize spawning zones further downstream as the season progressed (Forsythe 2010). An odds ratio of 1 indicates that spawning is equally likely to occur in both the zone of observed spawning and reference zone with respect to SSD. An odds ratio greater than 1 indicated that the spawning was more likely to occur in the observed zone relative to the reference.

Non-random seasonal patterns in SSD as a function of body size were evaluated using a linear mixed model that fit total length at each capture to the fixed effects of SSD along with the interaction between SSD and sex. The interaction term is biologically interesting because it tests for evidence of heterogeneous slopes in the relationship between total length and SSD for each sex. The effects of year and individual were included as random effects to correct for annual variation in the number of individuals spawning at different times, and to account for repeated measurements across time.

RESULTS

Spawning periodicity

A total of 1260 captures of 678 adult lake sturgeon were made over 8 consecutive years of sampling (2001-2008). Between 100 and 234 adult lake sturgeon were observed spawning in the UBR each year. Dates of initiation and the duration of spawning activity varied among years and spawning generally extended just over 40 days (range 19 to 43 days; Figure 2.2). However, spawning activity was not continuous during a spawning season. Rather, distinct groups of males and females were observed arriving, spawning and departing from spawning areas (Figure 2.2). The number of spawning groups and intergroup intervals (in days) also varied among years.

Of the total number of adults captured, 302 (44%), consisting of 70 females (26% of total females captured) and 232 males (57% of total males captured), were observed spawning during at least two years (Figure 2.3). The interval between spawning events was significantly longer for females (3.7 ± 0.16 yrs, least square mean ± SE) compared to males (2.3 ± 0.08 yrs, least square mean ± SE) ($F_{1,299} = 49.5$, $P < 0.001$). Intervals between spawning observations for individuals ranged from 1-7 years for males and 2-7 years for females (Figure 2.4). There was no statistical evidence for an association between spawning interval and average body size for combined data ($F_{1,299} = 0.04$, $P = 0.84$) or separately for female ($F_{1,69} = 0.05$, $P = 0.84$) or male ($F_{1,301} = 0.02$, $P = 0.78$) lake sturgeon.

Repeatability estimates were similar for both day of calendar year and SSD (data not shown). Because SSD facilitates comparisons among years, only results for SSD are provided. Levels of repeatability in males and females spawning differed with respect to SSD, environmental conditions and spawning zone (Table 2.1). However, the highest levels of repeatability were consistent for both sexes and included standardized spawning day, river zone, fraction of lunar illumination and average river temperature, respectively (Table 2.1). Repeatability was high for males and females despite the large inter-annual variation in duration of spawning (Figure 2.2). Estimated repeatability of 0.56 and 0.44 for SSD in particular were high for females and males, respectively, and can be viewed as the percent consistency in choice for spawning during a standardized time within a season. Estimates of individual variance (standard error) in SSD were also relatively small (~0.15 of the entire spawning season; Figure 2.5). Estimated SSD's (mean ± expected SE) are reported in Figure 2.5 for all recaptured individuals. Although there was limited support for differences in repeatability according to size categories for most variables (i.e., homogenous variance model was the model of best fit to SSD, lunar cycle and temperature), statistical comparison of model fit did support evidence for heterogeneous between-and within-individual variances for some discharge variables including average discharge, lagged discharge and the magnitude and the rate of change in discharge,. Thus, computed estimates of repeatability were different for each size class within each sex (Table 2.1).

Spawning site selection and size-based spawning

A significant association was found between zone of spawning and standardized spawning day for both male and female lake sturgeon ($P < 0.001$). In general, adults were significantly more likely to spawn in downstream zones during later periods of the spawning season. Specifically, the odds of spawning in downstream zones, relative to zone 1, increased by 2.9 (Zone 2), 4.3 (Zone 3), 18.7 (Zone 4), 108.7 (Zone 5) and 333.1 (Zone 6) for every 0.1 increase in standardized spawning day, respectively. Computed odds were highly significant ($P < 0.001$) with the exception of odds for zone 2 ($P = 0.07$). Figure 2.6 illustrates the odds of spawning in each zone relative to zone 1 throughout the reproductive season. A significant positive association was also found between body size (total length) and SSD ($F_{1,569} = 9.02$, $P = 0.0028$) indicating that individuals arriving later in the season were significantly larger than those arriving to spawn earlier. However, no evidence for heterogeneous slopes between sexes were found based on a non-significant sex × SSD interaction ($F_{1,569} = 2.84$; $P = 0.092$). Thus, predicted seasonal increases in total length (i.e., 2.7 cm) for each standardized day were the same for male and female UBR lake sturgeon.

DISCUSSION

Variation in numerous reproductive traits including offspring size, birth date and female condition has been reported to vary among taxa and among populations in part as an adaptive response to local ecological processes (Reznick and Ghalombor 2001). Recent research has also indicated that reproductive traits vary among individuals within the same population (Brown and Shine 2007) and most are repeatable over multiple reproductive events despite variation in exogenous conditions (Farrell et al. 2009). We show that reproductive behaviors in the form of intra- annual spawning time and breeding site selection in a highly migratory fish species vary substantially among individuals within a single population. However, reproductive behaviors, including inter-annual spawning time are highly repeatable for individuals across reproductive episodes and are relatively unaffected by exogenous conditions. We discuss the plausible ecological and evolutionary mechanisms leading to repeatability and how these behaviors may or may not be adaptive for the future of this imperiled fish species.

Inter-annual spawning time

Longevity and iteroparity are important for species with high mortality early in life because individuals need to reproduce on multiple occasions to ensure successful reproduction (Winemiller and Rose 1992). The inter-annual spawning interval for iteroparous fish is highly dependent on factors that influence the duration of the maturation cycle including sex (i.e., window is longer for females than males) and environmental variation that affects the rate of gamete maturation (Pankhurst and King 2010). Estimates of means and ranges of spawning periodicity for male and female lake sturgeon found in this study indicate significant sex-based differences in the frequency of and duration between spawning episodes (reflected in the frequency distribution; Figure 2.4). Our estimates of inter-annual spawning time were concordant with those reported for other lake sturgeon populations (Roussow 1957; Magnin 1966; Bruch et al. 2009) as well as for green (*Acipenser medirostris*) (2-4 yrs; Erickson and Webb 2007) white (*Acipenser transmontanus*) (3-5 yrs; Paragamian et al. 2005) and Atlantic sturgeon (*Acipenser oxyrinchus*) (3 yrs; Caron et al. 2002). Importantly, based on our large sample sizes and quantitative analysis with numerous environmental variables, differences in spawning periodicity between sexes is likely determined by resource allocation that dictate egg and sperm production and maturation schedules as opposed to variation in environmental conditions (Doroshov et al. 1997; Taylor 1986). Further, because of the lack of influence of environmental variables, inter-annual spawning interval is likely to remain consistent over time and also will not vary as a function of age.

Differences in inter-annual spawning interval between males and females can be important in long-lived iteroparous species, particularly species with polygamous mating systems or in situations where breeding population size is low, due to lower probabilities of encountering (and mating with) the same individuals over multiple reproductive episodes. Quantitative estimates of inter-annual spawning interval provide probabilities of encountering and mating with individuals from previous years, the demographic composition of future spawning runs and annual spawner abundance (e.g., Waples 2002; Figure 2.4). In the UBR, for example, a female spawning in year t would interact with mature UBR males ranging in age from 15 to 52 years on average (predicted age-frequency distribution for all UBR males collected to date). This female would subsequently return to spawn with the highest probability (0.46) three years later based on observed inter-annual spawning intervals (Figure 2.4). Assuming no repeatability for SSD, this female would interact with approximately 42% (i.e., proportion of males projected to return every year plus every three years) of the males encountered during the first spawning event based on expected male inter-spawning intervals (Figure 2.4). Results from our study further suggests that this pattern would repeat throughout this individual's lifetime and would be largely unaffected by age (Table 2.1).

Intra-annual spawning time (*repeatability*)

Spawning time in many fish is affected by exogenous and genetic factors that are adaptive for synchronizing reproduction with ecological conditions that promote increased offspring survival during critical early life stages (Rogers et al. 2006; Cushing 1990). For many fishes, especially broadcast spawning species with little or no post-ovulatory parental care and high early life mortality, timing reproduction with appropriate exogenous conditions linked to survival represents a critical life history feature (Webb and McLay 1996; Quinn et al. 2000). In the UBR, lake sturgeon spawn over 23 days on average (Forsythe 2010) in the early spring with peaks of spawning activity (Figure 2.2) occurring in relation to times with rising and absolute water temperature and declining river discharge, especially as the lunar cycle approaches a new moon (Forsythe 2010). Although abiotic conditions vary throughout a breeding season, the window individual lake sturgeon choose to spawn is considerably smaller and choices are highly repeatable in time across seasons irrespective of exogenous conditions (Table 2.1). This result appears to conflict with studies suggesting that lake sturgeon have evolved the ability and use exogenous cues to guide the timing of reproduction (Paragamian and Kruse 2001; Forsythe 2010). However, subtle variation in individual SSDs may represent an environmentally mediated and behaviorally plastic response within this window to proximal exogenous conditions including water temperature, the lunar cycle and discharge, all of which are consistent within and across years and have higher estimates of repeatability (Table 2.1). One potential caveat to our data is that measures of repeatability were dominated by observations from consecutive spawning episodes. Thus, there may be temporal autocorrelation in environmental variables that would not be evident over the lifetime of the individual. However, the SE for repeatability in SSD for individuals captured twice were not different from SE estimated from individuals captured more than 2 times.

Interestingly, repeatability estimates (r) may also provide an upper limit estimate on the heritability (h^2) for a given trait because repeatability includes both genetic and environmental sources of variation whereas heritability only reflects genetic differences (Boake 1989; Falconer and Mackay 1996; Dohm 2002). However, since SSD is a reflection of both genetic and environmental components, and we have estimates of repeatability for the plausible sources of exogenous variables thought to influence spawning time in this species (i.e., discharge, water temperature and lunar effects; Forsythe 2010) we estimate that heritability for spawning time may be considerably lower and approximately 0.27 for both females and males (i.e., SSD repeatability – repeatability for average water temp, river discharge and the lunar cycle). Comparable estimates of repeatability for breeding time in natural populations of long-lived migratory animals, especially fishes, are rare in the literature. However, levels of repeatability for spawning time derived from lab and aquaculture studies are generally higher than found here (rainbow trout, r = 0.72-0.83, Quinton et al. 2004) but estimates of repeatability for other behavioral traits such as mate preference (guppy, r = 0.05-0.35, Brooks 1996; stickleback, r = 0.65; Bakker 1993) are similar. Although not directly comparable, estimates of heritability (h^2) for spawn date based on breeding studies of other migratory animals including the collard flycatcher (0.19; Sheldon et al. 2003) and German blackcap (0.34; Pulido et al. 2001) as well as for migratory fishes such as rainbow trout (0.55; Siitonen and Gall 1989) and coho salmon (0.26; Gall and Neira 2004) are within our pseudo-estimates of heritability for spawning time for lake sturgeon.

Heritability for spawning time may have several important implications for lake sturgeon populations (Hendry et al. 1999). One is that natural selection may be effective in shifting spawning time. For example, Sheldon et al. (2003) found that estimated heritability for egg laying date in the collared flycatcher, although small (h^2 = 0.19), promoted consistent and directional selection at the level of the phenotype. Second, heritability would indicate that groups of individuals spawning at the same time in the UBR, and those responding similarly to environmental conditions specific to the timing of spawning, are also likely maintained through heritable additive genetic variation. Thus, the observed occurrence of multiple and temporally segregated breeding groups (Figure 2.2) were not random mixtures of individuals but rather groups of reproductively mature adults with the tendency to reproduce at specific times (Figure 2.5). Although the ultimate mechanisms that induce repeatable differences in spawning time in lake sturgeon are generally unknown, we posit that groups of individuals may be genetically predisposed to differentially respond to time-related cues such as photoperiod, a known proximate cue for lake sturgeon at the point of river entry in the UBR (Forsythe 2010). Third, the tendencies for adults to spawn at specific times as a function of different environmental characteristics such as water temperature may promote isolation, phenotypic traits and adaptations unique to each breeding group (Hendry and Day 2005; Woody et al. 2000). Covariation in temperatures at the time of mating and temperatures experienced through the period of embryonic and early post-incubation development have been shown to affect body size and developmental rate (Atkinson 1994) and offspring size (Fox and Czesak 2000). Stillwell and Fox (2005) have also shown that temperatures experienced by adults affected life history traits of offspring including age at first reproduction, fecundity and embryonic developmental time. Recent studies in migratory fishes including UBR lake sturgeon also support this hypothesis. For example, larvae produced from females spawning later in the season, when water temperatures are generally warmer (Forsythe 2010), had significantly smaller body sizes than larvae from early spawning females implying different utilization efficiencies (Crossman 2008). Due to these reasons and our findings of high repeatability in spawning time (SSD) for males and females (SSD, Table 2.1), our study minimally suggests that a full quantitative genetic analysis based on multigenerational pedigrees is warranted.

Intra-annual spawning time (*learning*)

Age and sex-dependent reproductive behaviors have been reported for many species including migratory fish (e.g., Paitz et al. 2007; Dickerson et al. 2002). Fish of different ages have also been found to spawn at different times (i.e., maiden vs. multi-year spawning fish) (Niemela et al. 2006). Some migratory species, have been found to alter reproductive timing based on previous successful experiences (e.g., egg and hatching survival in blue tits; Grieco et al. 2002). Because lake sturgeon are a long-lived species and individuals have opportunity to breed multiple times during a lifetime (i.e., estimated 18 times for males and 15 times for UBR females based on estimated adult survival and age structure, repeatability could increase as a function of reproductive experience (i.e., learning). Although repeatability varied among age classes according to some environmental variables (e.g. river discharge), we generally found no statistical evidence that variability in repeatability for SSD or for exogenous conditions decreases with age and reproductive experience. Without a means of reinforcement (e.g., observations of reproductive success or failure associated with spawning decisions) or the ability to recognize kin, selection operating as a function of offspring survival associated with conditions at the time of reproduction is unlikely to control repeatability in this species. However, some sex-based differences were found with respect to spawning date (Table 2.1). In particular, lower repeatability in spawning time for males may indicate greater plasticity in behavior relative to females. We posit that lower repeatability in male spawning time may be a reflection of sex-based alternative reproductive strategies whereby males achieve reproductive success by attempting to increase mate number rather than select appropriate breeding times.

Our analysis of spawning time was also intended to test the hypothesis that arrival to spawning sites varied as a function of body size (and age). Our hypothesis was based on two factors. First, phenotype-dependant based arrival is common in migratory animals and proposed in migratory fishes with larger older and generally better conditioned or experienced males and females often generally arriving first (Moller 1994; Keefer et al. 2009; Cooper et al. 2009). Second, early arrival may be adaptive because early arriving individuals likely experience less competition, have access to high quality mates and are able to select the best breeding habitats; all factors contributing to reproductive success

(Quinn 1999; Dickerson et al. 2002). We found that early arriving male and female lake sturgeon were significantly smaller than later arriving individuals (Figure 2.7). Size-based arrival partly conflicts with the results discussed previously and generally contrasts the findings of other studies. The positive relationship between body size and SSD documented herein may also be driven by different historical recruitment patterns of early and late spawning groups. For instance, contemporary levels of annual recruitment to the larval stage have consistently been higher from early groups leading to a relatively younger (and smaller) aggregate of early spawning individuals, relative to later groups that are comprised of larger and older individuals.

Inter-annual spawning site selection

Spawning site selection is an important component of reproductive success in migratory fish where strong associations between stream habitat features (e.g., substrate size, intra-gravel water flow) and levels of mortality during the egg and larval stages exist (Forsythe 2010). The UBR has numerous suitable spawning locations throughout a 1.5 km section. However, despite the close proximity of spawning areas in the UBR, males and females arriving earlier in the season are significantly more likely to select locations further upstream (Zone 1-3; Figure 1.1) relative to individuals arriving later (Zone 3-6; Figure 1.1) (Figure 1.6). These relationships held across years despite temporally transient changes in sedimentation, stream cover or temperature across years or variation in stream hydro-geomorphology and levels of ground water infusion have likely occurred within and among years. Similar seasonal patterns have been noted in other migratory fish and were attributed to habitat optimization (e.g., Orians and Wittenberger 1991), whereby earlier arriving individuals selected the best habitats which were located further upstream (Doctor and Quinn 2009). Mechanisms such as social learning (reviewed in Kieffer and Colgan 1992; Olson et al. 1978), mate copying or heritable genetic attributes (Quinn et al. 2006) that return individuals to areas differing in intrinsic physical properties within natal streams have also been implicated as mechanisms.

Our findings that show early and late spawning adults consistently used different spawning sites even though all sites were available during the entire reproductive season suggests intra-annual variation in habitat quality and the ability of individuals to select these high quality areas that are specific to different spawning times (i.e., habitat optimization). Alternatively, relatively high estimates of repeatability for spawning zone (especially for females; Table 2.1) may suggest that either spawning site quality varies seasonally or selection for hydrogeomorphological cues intrinsic to specific spawning times and locations is heritable. Further, higher repeatability estimates in spawning site selection for females (Table 2.1) suggest that females not only dictate the relative spawning time (SSD) but also specific locations in the UBR. Regardless, temporally structured annual spawning groups of lake sturgeon are further segregated through the selection of different habitats even over the course of a spawning season. Although fine scale homing has been observed in several migratory fish (e.g., Quinn et al. 1999), future work could profitably explore the relative influences of behavioral flexibility versus heritability in spawning site selection.

We should note that our observations may be a consequence of significant reductions in numerical abundance of UBR lake sturgeon over the past 50-100 yrs. Yet, the UBR has always been the primary spawning area for fish in the drainage and thus our inferences should be robust. Our observations were also made from a single population and may not be generalizable to other systems. However, considerable similarities and synchrony in spawning chronology of other Great Lakes tributaries with the UBR have been found. Further, evidence of multiple spawning runs in other systems has been reported in support of this research (Auer and Baker 2002). Thus, it is likely that other populations of lake sturgeon, as well as other sturgeon species, are composed of discrete breeding groups that are structured in part due to spawning periodicity and isolation as a function of repeatable spawning time and spawning site selection.

CHAPTER THREE

SPATIALLY EXPLICIT ANALYSIS OF LAKE STURGEON EGG DEPOSITON AND MORTALITY IN NATURAL STREAM SETTINGS

INTRODUCTION

Many organisms seek very specific habitats at different times of the year for the purpose of reproduction. Further, the decisions that adults make with regard to breeding site selection often have serious fitness consequences for both themselves and for their offspring. For example, the environments that animals select may have characteristics that significantly influence survival, especially for species that have offspring which are vulnerable to numerous physical and biotic sources that contribute to mortality (Donazar et al. 1993; Stokes and Boersma 1998; Wilson 1998; Hendry et al. 2001). High and variable mortality during early life stages is important and often tied to variation in population levels of recruitment (Bailey and Houde 1989). Second, habitats chosen for reproduction can impose selection on phenotypic, behavioral, and morphological traits of offspring, most of which are also tied in some way to survival during subsequent early life stages that have long since dispersed from breeding grounds (Jensen et al. 2008; Shine et al. 1997; Webb et al. 2001). Thus, the process of habitat selection for the purpose of reproduction is often under strong selective pressure and in some situations results in the evolution of physiological or behavioral traits that allow adults to use exogenous (i.e., physical or biotic) cues to assess the relative habitat quality for their offspring (Danchin et al. 1998).

For some species, the process of reaching critically important breeding habitats requires individuals to complete long-distance migrations during abbreviated periods within a season (Alerstam and Enckell 1979; Alerstam et al.2003). Although migration is necessary for reducing mortality incurred during early life periods, it's also an extremely risky behavior that comes with tremendous fitness consequences for adults (Cooke et al. 2004; Jonsson et al. 1997; Hinch and Rand 1998; Shmueli et al. 2000). Because of this tradeoff, there is considerably strong selection for migratory animals make the best choices by differentiating and accurately selecting among habitats for reproduction after arrival (Blanchfield and Ridgway 2005; Fontaine and Martin 2006; Kondou et al. 2001; Rodewald and Yahner 2001). However, because the environments migratory species seek are often spatially complex, habitats change temporally over short time periods and have varying implications across different early life stages (Sillett and Holmes 2002) this task can be challenging. Yet, if selection is strong and is expressed similarly across different breeding habitats, the same suites of environmental traits should theoretically be selected by breeding adults and equally influence survival, regardless of where or when they elect to reproduce. This hypothesis is rarely tested.

Highly migratory fish species that utilize spatially and temporally dynamic and structurally complex rivers for reproduction provide an excellent opportunity to study the effect of environmental covariates on the distribution of offspring and their subsequent survival among sites selected for reproduction. Examples of important and quantifiable features of river systems of stream habitats and those empirically shown to increase survival rates, include river substrates with interstitial spacing (Bernier-Bourgault and Magnan 2002; Forsythe 2010; Smith and Marsden 2009), highly permeable riverbeds with ample ground and surface water exchange (Malcolm et al. 2003; Malcolm et al. 2005; Youngson et al. 2004) , increased river gradient (Curry and MacNeill 2004) and areas with suitable water velocity (Smith and Marsden 2009) that provide sediment free substrates. Interestingly, habitats associated with spawning locations also frequently form the basis for environmentally-mediated selection during embryogenesis and on phenotypes during early life stages that create locally specific adaptations in many species including fishes (Hendry et al. 1995). As such, it is critically important for researchers to characterize the physical and biotic features of breeding habitats that influence survival during early life stages of these animals (Fausch et al. 2002).

Although offspring distribution and survival as a function of non-random spawning site selection has been intensively studied in migratory fish with very specific life histories (e.g., nest building), few studies have attempted to map the spatial occurrence and subsequent survival of offspring for broadcast spawning species in natural environments where fertilized gametes are tied to the locations of adult reproductive activity with no parental care. Further, few studies have provided a detailed understanding of whether deposition and early life survival are mediated by spatial and temporal variation in environmental variables over small micro- geographic scales or across time intervals of relevance to organisms during critical life stages (e.g., during incubation). There are many reasons for this lack of information. First, highly migratory broadcast spawning species are rare because of recent and dramatic population declines. Second, analyses seeking to quantify the importance of environmental covariates to egg deposition and survival in some situations need to account for more complex spatial dependencies. Failing to account for spatial dependence may result in inaccurate parameter estimates and erroneous conclusions regarding associations between stream covariates associated with spawning sites and gamete and offspring survival (Hoeting 2009). Further, rigorous statistical applications for jointly modeling early life survival data within and between time periods while accounting for both spatial autocorrelation and exogenous covariates have yet to be developed for complex heterogeneous environments such as streams and rivers.

We address both ecological and statistical limitations using the lake sturgeon a long-lived, iteroparous, highly fecund (11,000 eggs/kg) and broadcast spawning (i.e., eggs are released without nest preparation) migratory fish. Lake sturgeon where selected for this study in part because they exhibit an aggregate mating behavior whereby negatively buoyant eggs and sperm are released over large areas of rock and gravel. No nest preparation and no post-ovulatory parental care are provided for offspring. As such, lake sturgeon eggs experience extremely high mortality in natural settings (i.e., 80-99%) due primarily to eggs direct exposure to harsh environmental conditions, predation or microbial infection, and physical processes that dislodge and transport eggs from spawning sites (Kempinger 1988, Forsythe 2010; Parsley et al. 2002). Thus, the features of sites selected by adults for reproduction are also likely conducive to egg and offspring survival.

The specific objective of this study was to quantify relationships between environmental covariates characterizing attributes of lake sturgeon spawning locations that were predictive of egg deposition and loss using a novel hierarchal spatial modeling approach. Our working hypotheses were 1) stream variables defining hydro-geomophological conditions can explain variability in egg deposition and egg survival, 2) the relative importance of environmental covariates on deposition and survival are consistent across spawning sites suggesting that females are focusing on similar micro-geographic features in each spawning environment, and 3) rates of egg survival are constant across spawning sites once environmental covariates are considered.

METHODS

Study location

This study was conducted on a well-studied lake sturgeon population that migrate annually into the Upper Black River (UBR), the primary tributary of Black Lake, MI (Figure 1), and use one of several spawning locations (Forsythe 2010). The UBR is a unique location for studying migratory fish and exogenous environmental features associated with adult spawning, egg deposition, and larvae for several reasons. First, individuals are relatively free to choose spawning habitats. In other systems, for example, traditional spawning sites have been blocked by dams, covered with sediments or destroyed by other anthropogenic activities (Paragamian et al. 2009). Second, the UBR is relatively narrow and shallow with low average discharge which facilitates observations of spawning adults, monitoring early life history stages, and quantifying environmental covariates. Other characteristics of the UBR include seasonal increases in water temperature, stochastic variation in river discharge as well as predictable variation in the timing of annual migration and spawning activity (Forsythe 2010). Further, Forsythe (2010), and Smith and Baker (2005) detail exogenous correlates related to reproductive activity as well as characteristics of the adult spawning population which also aids in the logistics of the time sensitive data collection. Forsythe (2010) also provide evidence that adults returning to breed at repeatable times and locations in the UBR suggesting that timing of spawning is heritable and suitability of selected habitats in different locations by adults spawning at different times may vary.

Field sampling

Data were collected at four spawning locations selected by lake sturgeon along the UBR, Figure 3.1, on 5/19/03, 5/12/05, 5/10/05, and 4/25/06 (Sites 1-4, respectively). Lake sturgeon were observed entering and captured while spawning using handled trapezoid shaped landing nets (78.7 cm base, 30.5 cm top, and 70.5 cm length). Individuals were sexed at the time of capture by examining cloacal morphology and by physically extruding gametes. Each Site was delineated using GPS and permanent stakes were placed along the stream bank during the spawning event. One day after spawning was completed and adults had left the area, transects were established across the stream channel starting ~ 1 m below the most downstream spawning group and repeated every 5 m upstream to cover the downstream extent of the spawning. Counts of eggs and measurements of environmental covariates were collected at a 1 m interval along each transect (Figure 2). At each sample location, the number of eggs was measured using a standardized kick net sample. Substrate was thoroughly disturbed for 5 seconds over a 0.125 m^2 area of substrate upstream of a small triangular net (305 ×305 × 305 mm opening). Eggs deposited on and within the disturbed substrate were swept into the net by the stream current.

The status (live vs. dead) of eggs collected during sampling was determined by visual inspection and for microbial colonization in the form of visible fungal hyphae growth. Numbers of live and dead eggs were recorded. Data on environmental covariates (water depth, water velocity, and substrate size) was collected immediately after surveys of egg deposition by a separate survey crew. Stream depth was recorded in meters using a stadia rod. Water velocity at the stream bed was recorded using an electromagnetic flow meter (Marsh-McBirney, Inc.). Substrate size was recorded as the average of four randomly collected stones within the 0.125 m^2 sampled area. The largest stone within the area was also measured to estimate maximum substrate size.

For Sites 1 and 3, a secondary sampling of egg deposition was conducted 3-4 days following the initial data collection to determine the magnitude of egg loss. The number of eggs and survival status was recorded. The sampling grid was shifted 0.5 m upstream during the second sampling period to mitigate the disturbance caused by the first sampling, but retain the proximate sampling locations and to replicate as closely as possible the spatial dispersion of sampling sites within transects.

STATISTICAL ANALYSES

Covariate summaries

Analysis of variance (ANOVA) and Kolomogorov-Smirnov (KS) tests were used to summarize and compare the frequency distributions of environmental covariates within and among sites. The KS test was used to determine whether the frequency distribution of sites' substrate size (average and maximum), water velocity, and water depth came from the same population while ANOVA was used to test for significant differences between the means of these variables among the sites. Means were differentiated using a Tukey HSD that adjusts for multiple comparisons. Surface plots used for exploratory analysis of egg counts and environmental covariates were generated with multilevel B-splines using the R (www.r-project.org) package MBA.

Embryo deposition

A Poisson regression model was used to quantify associations between egg counts and environmental covariates measured at the same sites. This model is adequate in the absence of correlated variation beyond what may be explained by possible covariates. However, because lake sturgeon are broadcast spawners and eggs are extruded into a spatially complex fluvial environment, counts of eggs are expected to exhibit strong anisotropic spatial dependency along the down-stream and across-stream sampling axes (i.e., similar egg counts in the proximate locations) even after accounting for the information provided by environmental covariates. This residual spatial correlation was modeled as a random spatial effect. Given observations arise over a set of locations, say S = $\{s_1,\ldots,s_n\}$ where s is a sample location's spatial coordinates, we specify the log-link model:

$$y(s_i) \sim Poisson(\mu(s_i));\ \log(\mu(s_i)) = x(s)'\beta + w(s),$$

where $y(s_i)$ is the i-th sample location count, which is assumed to be distributed as a Poisson variable with location specific conditional expectation $\mu(s_i)$. This conditional expectation comprises vectors of environmental covariates and associated slope parameters, $x(s_i)$ and β respectively, and w(s) which provides local adjustment (with structured dependence) and captures the effect of unmeasured or unobserved spatial covariates. Here, we assume the random spatial effects follow a Gaussian Process (GP), where $w(s) \sim GP\ (0, \sigma^2\ \rho\ (\cdot; \theta))$ with variance σ^2, and correlation function $\rho\ (s, s'; \theta)$. The vector of parameters θ control the correlation function's behavior. For a collection of n locations the n × 1 vector of random effects follows a Multivariate Normal distribution, $w \sim MVN\ (0, \sigma^2\ R(\theta))$, where $R(\theta) = [\rho\ (s_i, s_j; \theta]_{i,j=1}^{n}$ is the correlation matrix.

Embryo survival

To address the embryo survival, we would ideally use live egg count at sample time 1 and 2 to calculate survival rate at each location. This rate could be related to the predictor variables and random spatial effects through a binomial generalized linear model. However, because of the time 1 and 2 sampling grid offset and egg transport by the steam current, our data has several locations where there are more eggs recorded in time 2 than time 1. As a result, we cannot directly model the survival rate. Therefore we opt to model the change in live egg counts as:

$$\left(\frac{\log(y(s_i, t_2) + 1)}{\log(y(s_i, t_1) + 1)}\right) = N\ (x(s)'\ \beta + w(s) + e(s) \qquad (2)$$

where $y(s_i,t_1)$ and $y(s_i,t_2)$ are the i-th location's egg count at time 1 and time 2, respectively. Here, we made the assumption the time 1 and 2 sampling grids coincide. By modeling the outcome as a normally distributed continuous variable, we able to include the error term which is assumed follow an independent and identically distributed Normal distribution, i.e., for the n locations $e(s) \sim N(0,\tau^2 I)$, where I is the identity matrix. Here, τ^2 includes the measurement error and variance not captured by the covariates and the spatially structured residuals, and could also include micro-scale spatial variability that occurs at a resolution finer than the sampling grid. In the geostatistical literature τ^2 is referred to as the nugget, whereas, σ^2 is viewed as the partial sill.

Spatial distribution and Bayesian implementation

Spatial correlation functions of varying complexity are available for defining $\rho(s, s^*; \theta)$ (e.g. Cressie 1993). A common and more simplistic choice is an isotropic exponential, $\rho\ (s, s^*; \theta) = \exp\ (-\Phi\|s_i - s_j\|)$, where Φ is the spatial range parameter. Here, it is assumed that spatial dependence is the same in all directions. Given the dynamics of stream flow, this is a rather strong assumption. To explore the possibility for a more complex spatial structure we also use and anisotropic form where $\rho(s, s^*; \theta) = \exp [(s - s^*) [G(\varphi)\Lambda^2 G'(\varphi)]^{-1} (s - s^*)]$. For this model, the rotation matrix $G(\varphi)$ controls directional dependence and the positive diagonal matrix Λ defines the range of spatial dependence.

When describing a spatial process it is useful to report the distance at which spatial dependence is negligible. This distance is referred to as the effective spatial range, and is defined here at the distance at which the spatial correlation drops to 0.05. For the isotropic exponential correlation function this is $-\log(0.05)/\Phi$ and for the anisotropic form this is the $-\log(0.05)\lambda$, where λ is the diagonal value in Λ. Note that there are two effective spatial ranges for each anisotropic process, one oriented with φ, and the other perpendicular to φ.

A prior was assigned to each parameter to complete the model Bayesian specification. The β's received flat prior distributions and the spatial variance parameters were assigned inverse-Gamma (IG) with hyperparameters IG $(2, \cdot)$. With a shape value of 2, the IG distribution has infinite variance and centered on the scale value, which is dataset specific. The spatial range parameters λ follow a Uniform prior, which are chosen to support a spatial range from 0 to the maximum inter-location distance in the dataset. The rotation parameter φ also receives a Uniform prior that is data set specific.

Joint modeling, cross-correlation and egg survival

The previous models (1) and (2) allow us to relate egg deposition and subsequent change in live egg count to a suite of environmental covariates. To address the third hypothesis, we could model time 1 and time 2 live egg count jointly as a function of the environmental covariates and respective spatial residual processes. Specifically, we could assess the degree of location specific association between the residuals to assess evidence of a non-stationary multivariate residual spatial process. A model to explore this question is:

$$\begin{pmatrix} \log(y(s_i, t_2) + 1) \\ \log(y(s_i, t_1) + 1) \end{pmatrix} \sim N\left((x(s) \otimes I_2)' \beta + w(s) + e(s)\right) \qquad (3)$$

where, again (s_i,t_1) and $y(s_i,t_2)$ are the i-th location's egg count at time 1 and time 2, respectively. This model is a non-stationary version of the linear model of co-regionalization derived by Gelfand et al. (2004). Here, we assume the process w(s) = A(s) v(s) to be a linear transformation of v(s), where A(s) is a space-varying matrix transform that is non-singular for all s. Specifically, for a given location A(s) is a lower-triangular matrix with elements $a_{1,1}(s)$, $a_{1,2}(s)$ and $a_{2,2}$ (s) modeled a independent GPs with corresponding spatial variances $\sigma^2_{1,1}$, $\sigma^2_{1,2}$, $\sigma^2_{2,2}$, and spatial ranges $\Phi_{1,1}$, $\Phi_{1,2}$, and $\Phi_{2,2}$ (for details see Finley et al., 2010). The non-spatial residual process, e(s), is assumed to follow N (0, Diag(τ^2_1, τ^2_2)). The space-varying correlation is defined by v(s) = $v_1(s)$, $v_2(s)$)' which is a 2 × 1 process with independent zero-centered spatial processes with unit variance; that is, $v_1(s)$ and $v_2(s)$ follow a $GP(0,\rho_1(\cdot,\Phi v_1))$ and $GP(0,\rho_1(\cdot,\Phi v_2))$, respectively.

Given the complexity of this model and the paucity of data, we make two simplifying assumptions. First, the GP's associated with A(s) share a common spatial range parameter Φ_A. Second, a common Φ_v is used for the v(s) processes. These concessions improve model identifiability of parameters and greatly reduce computing time (Finley et al. 2010). Importantly, for any given location, we can take $\Gamma(s) = A(s)A(s)'$ as the residual t_1, t_2 covariance matrix. To recognize how missing spatial environmental covariates might impact live egg count over the sampling interval we can look at a surface of $\Gamma_{1,2}$ (s) or the corresponding correlation i.e., $\rho_{1,2}(s) = \frac{\Gamma_{1.2}(s)}{\sqrt{\Gamma_{1,1}(s)\Gamma_{2,2}(s)}}$. Also, with access to the full posterior distribution of $\rho_{1,2}(s)$ we can formally test the significance of this correlation at any given location.

Model selection and implementation

To compare several alternative models with varying degrees of covariate and spatial richness, we use the Deviance Information Criterion (DIC) (Spiegelhalter et al., 2002). Letting Ω be the generic set of parameters being estimated for each model (including random effects), we compute the expected posterior deviance $D(\Omega) = E(\Omega|Y)$ [$-2\log L(\text{Data} \mid \Omega)$], where $L(\text{Data} \mid \Omega)$ is the first stage Gaussian likelihood from the respective model and the effective number of parameters (as a penalty) as $pD= D(\Omega)- D(\Omega)$, where Ω is the posterior mean of the model parameters. The DIC is then given by $D(\Omega) + p_D$ and is easily computed from the posterior samples with the lower values indicating better models. For all models, three MCMC chains, with unique starting values, were run for 50,000 iterations. The CODA package in R (www.r-project.org) was used to diagnose convergence by monitoring mixing using Gelman-Rubin diagnostics and autocorrelations (see, e.g., Gelman et al., 2004, Section 11.6). For all analyses, acceptable convergence was diagnosed within 10,000 iterations (which were discarded as burn-in). The sampler was coded in C++ and Fortran and leveraged Intel's Math Kernel Library threaded BLAS and LAPACK routines for matrix computations. This code will be available in version 0.1-7 of the spBayes R package and also submitted along with the illustrative data sets to *Ecological Archives*.

RESULTS

Summary of spawning groups and environmental covariates

The spawning locations used by adults exhibited similar ranges in substrate sizes (1-180 mm), maximum substrate size (2-300 mm), water depth (0.1-1.2 m) and water velocity (0.1-1.5 m^3/s). However, the KS test indicated that all environmental covariates had significantly different frequency distribution across sites ($P < 0.0001$; Figure 3.2). Sites also differed significantly in mean values for average substrate size (Site 1: 40.8; Site 2: 88.4; Site 3: 60.7; Site 4: 70.7; $F_{3, 647} = 32.9$, $P < 0.001$), maximum substrate size (Site 1: 64.1; Site 2: 111.9; Site 3: 83.5; Site 4: 109.3; $F_{3, 647} = 24.1$, $P < 0.001$), and water velocity (Site 1: 0.54; Site 2: 0.65; Site 3: 0.63; Site 4: 0.48; $F_{3, 647} = 13.4$, $P < 0.001$) but not in water depth (Site 1: 0.52; Site 2: 0.56; Site 3: 0.56; Site 4: 0.51; $F_{3, 647} = 2.18$, $P = 0.09$). Frequency distributions and pair-wise comparison of means indicated that sites 2 and 4 had significantly larger substrates and sites 2 and 3 had significantly greater water velocity at the streambed. Environmental covariates also exhibited spatial heterogeneity within and among locations, see e.g., Figure 3.3 (a-d).

Embryo deposition and environmental covariates

A total of 9,426 eggs were collected across all spawning locations from 651 kick net samples. Egg count was location-dependent and generally concordant with increased female abundance (Site 1 = 2,509, Site 2 = 564, Site 3 = 2,111, Site 4 = 2,024). Within each Site, eggs were primarily deposited along the downstream axis on the side of the river spawning took place and from 0 to 60 m directly downstream from locations selected by spawners. However, egg deposition exhibited non-random and spatially-dependent patterns (see e.g., Figure 3.3 (e)), often occurring in discrete patches that ranged in count from 0-345 eggs.

Both the non-spatial and spatial versions of Model (1) were fit using the 145, 61, 250, and 199 observations collected at Sites 1-4, respectively. Tables 3.1 - 3.4 provide candidate models' parameter estimates for each site. Looking first at the spatial model's effective spatial range parameter estimates, λ_0 and λ_1, we see there is relatively strong but variable spatial dependence among the residuals. For example, Site 1 exhibits the shortest effective spatial range of ~ 5 m, whereas Site 4 shows the longest range of ~ 54 m. For Sites 1, 3, and 4, the disparity between λ_0 and λ_1 suggests there are strong anisotropic patterns in spatial dependence. In each of these sites φ describes the orientation of the major spatial range parameter axis λ_0. Here we offer φ in both radians and degrees, where in both cases zero is perpendicular to the west river bank. Not surprisingly parameter estimates suggest a shorter range of spatial dependence perpendicular to water flow. Sites with the maximum dispersal distance (i.e., Site 3) also had the highest average water velocity.

For all sites, most of the non-spatial models' parameters associated with the environmental covariates are significant at the 0.05 level (i.e., the 95% credible interval does not include zero). This is not the case for the spatial models. Recall a key assumption of generalized linear models is independent and identically distributed residuals. The interpolated surface plots of the non-spatial model residuals for all sites reveal substantial spatial dependence, see e.g., Figure 3.4 (a), which suggests we are violating this model assumption and must accommodate the lingering spatial dependence through a random spatial effect. Ignoring this spatial dependence results in parameter credible intervals that are too narrow and, as a result, we might draw incorrect conclusions about the impact of associated environmental covariates. Including the random spatial effect helps satisfy the model assumptions and subsequently provides a more realistic view of the statistical relationship between egg deposition and the environmental covariates few explain a substantive amount of variability in egg deposition.

Beyond meeting the model assumptions, the addition of a random spatial effect to the mean greatly improves model fit. This resulted in lower DIC values, Tables 3.1- 3.4, and visually comparing the observed and fitted deposition surfaces, Figure 3.2 (e) and Figure 3.4 (d), respectively. Note, in comparing Figure 3.2 (e) to Figure 3.4 (d) we see the non-spatial model fails to approximate the observed deposition, which further emphasizes its inappropriateness for these data. The same conclusion can be drawn by looking at the surfaces from the other three sites.

Considering the spatial models' parameter estimates, egg count is positively associated with increasing average or max substrate size at three of the four spawning sites. Water depth was also a positively associated with egg deposition at 2 of the 4 locations (i.e., Sites 1 and 4). Although egg deposition was positively related to water depth at both sites the expected increase (slope) in egg deposition for every unit in increase in water depth was much greater at Site 4 versus Site 1. The regression coefficient associated with water velocity was also statistically different from zero at Site 4, which suggests egg count may increase with increasing in velocity.

Embryo survival and environmental covariates

Analyses were performed to determine degree of spatial dependency and effects of environmental covariates on changes in live egg count after 3 days following deposition. At Site 3, a total of 643 viable eggs were collected during the second survey representing a 70% reduction in the total number of viable embryos collected three days prior in the first sampling. At Site 4, 1,276 viable eggs were collected during the second sampling period representing a similar 69% reduction in the number of viable eggs collected relative to the first sampling. Within each site, the non-spatial and spatial versions of the postulated model (2) were to those locations that had at least 1 egg recorded in time 1. Again, this location subset was used because our focus was on loss over the sampling interval.

Parameters' posterior summaries and model diagnostics are offered in Tables 3.5 and 3.6. Here we see the environmental covariates explain only a marginal amount of the variability in the log difference in egg count between time 1 and 2. However, average substrate size was a significant predictor of the change in egg abundance between sampling periods for Site 4 but not Site 3 (Tables 3.5 and 3.6). Specifically, the negative parameter estimate indicates that the ratio between time 1 and time 2 egg count becomes smaller (i.e., fewer in time 2 relative to time 1) as substrate size decreased. Also, again due to spatial autocorrelation, the non-spatial model's credible intervals are falsely precise. The addition of the random effects allows us to apportion total residual variability into spatial and non-spatial components. Here we see that for Site 3, ~ % 62 of the total residual variability can be explained by spatial proximity i.e., 0.62 = 1:32 / (0.81 + 1.32), whereas for Site 4 the residual variance is dominated by the nugget (i.e., there is relatively more non-spatial variation either due to measurement error or variations in the data that relate to shorter ranges than the sampled data spacing). Similar to egg deposition, log difference in egg count shows strong anisotropic effects, with a longer range of spatial dependence coinciding with the direction of stream flow which is also an indicator of the importance of water velocity to covariance in numbers of live eggs between the 2 sampling periods. The lower DIC values also support the inclusion of the random spatial effects.

Cross-correlation and joint modeling of egg survival

Joint modeling of live log egg counts at time 1 and 2 allows us to assess the consistency of change over time after accounting for the environmental covariates. If the rate of change is consistent over locations then we would expect a common correlation between the time 1 and 2 spatial random effects over the Site, i.e., a stationary cross-covariance. The results of this multivariate model (3) for Site 3 and 4 are offered in Table 3.7. Here we have environmental covariate regression parameters for time 1 and 2 log egg counts. With the exception of depth in Site 3, the covariates are not contributing much to our understanding of deposition and subsequent loss. Note, again, we are using a subset of locations where the egg count at time 1 was at least 1. The three $\sigma2$ parameters are the GP's spatial variances that compose the components of A(s). Their common spatial range parameter Φa has an effective spatial range of 25 and 16 for Sites 3 and 4, respectively, and relatively narrow 95% credible intervals suggest some degree of spatial non-stationarity across the two sites.

The spatial range parameter associated with the cross-correlation, Φv, also shows a relatively defined range of dependence within and between the processes. We can look to surface plots of the correlation between $wt=1(s)$ and $wt=2(s)$ to visually assess the variability of residual change over the sites. These surface plots are offered in Figures 3.5 and 3.6 for Sites 3 and 4, respectively. Here we see that after accounting for the environmental covariates, there are location-specific and unexplained differences in the correlation of the random spatial effects. For instance, Figures 3.5 (e) and 3.6 (e) show correlations ranging from ~ 0.2 – 0.8. This pattern suggest that mortality is random with respect to location or we are missing spatially varying physical or biological covariates that effect rates of egg survival over the sites.

DISCUSSION

We provide an empirical and analytical framework that is conducive to studies of many organisms that inhabit and reproduce in spatially and temporally complex environments. We show that linking spatially explicit point pattern analysis is necessary to understand and generalize (across sites) the contribution of factors affecting spawning site selection and to identify associations between habitat and early life survival at small spatial scales.

Selected spawning locations

Migratory animals including birds, turtles and fishes have evolved the ability to use abiotic (i.e., temperature, vegetation, substrate) and biotic cues (i.e., social influences) for selection of breeding areas (Knapp and Vredenburg 1996; Melles et al. 2009; Pike 2008). Thus, it seems reasonable that lake sturgeon also use exogenous cues to select spawning areas that provide reliable information regarding probabilities of fertilization success and embryo survival during incubation (Kempinger 1988). Lake sturgeon aggregate and spawn in relatively deep pools immediately upstream from raceway segments characterized by increasing water velocity and substrate size but decreasing water depth lends support for this hypothesis (Figure 3.3).

Conditions at the site of spawning may not have been predictive of stream features downstream where eggs were deposited. Downstream locations were found to differ significantly with respect to substrate size and water velocity. However, while spawning locations may have differed from each other it is possible that their physical properties were actually more similar than unselected locations. We did not measure the physical properties of other UBR segments. However, recent work by Chiotti et al. (2008) demonstrated that locations where lake sturgeon eggs were deposited, and thus locations individuals had selected to spawn, had significantly greater heterogeneity in substrate size compared to non-selected spawning locations. Other studies have also noted specific preference of sturgeons to select substrate free of sediments within a range of water depth and water velocity (LaHaye et al. 1992; Harkness and Dymond 1961; Fox et al. 1999; Collins et al. 2000)

Alternatively, spawning site selection may be driven by physiological or genetic components as opposed to environmental variation (i.e., individuals are returning to their place of birth). For example, Stewart et al. (2004) found that sockeye salmon displaced from spawning areas returned to former locations, suggesting the importance of olfactory cues as opposed to habitat features in selection. There is also evidence of site-fidelity in sturgeon species. Forsythe (2010) a documented a significant tendency for adult lake sturgeon to non-randomly select spawning locations based spawning time (i.e., individuals spawning early selected locations further upstream). Paragamian et al. (2009) found that while the cobbles and gravel substrates of historical spawning areas were buried with post-dam sediment, white sturgeon still used these areas repeatedly for spawning.

While our data does not unravel the relative importance of environmental and genetic influences on selection of spawning locations, we believe that both forces are significant and future studies should address this question for several reasons. First, if female choice of spawning habitat also affects probabilities of survival of eggs and larvae, then the relative environmental-genetic contribution of spawning site selection will be predictive in how sturgeons will respond to anthropogenically altered systems. Second, our analyses of stream hydrology and substrate also provides evidence for a significant degree of difference between spawning environments experienced by eggs and larvae of adults spawning at different times. Thus, differences in the physical features of stream associated with spawning habitat may result in adaptive phenotypic variation among sturgeon populations but among breeding groups within the same population that are reproductively isolated in time (Hendry et al. 1995).

Spatial dependency, egg deposition and environmental covariates

Depositional profiles of lake sturgeon eggs 1 day after spawning were significantly clustered at all spawning locations. Strong spatial dependency is likely due to the spawning behavior (Bruch and Binkowski 2001). Eggs are released and subjected to site-specific fluvial dynamics and geomorphic heterogeneity near the streambed that non-randomly determines the direction of embryo drift and the distance travelled. The effect of flow to generate ‘hot spots’ in other ecological processes such as the drift and colonization of aquatic macroinvertebrates is frequently observed in lotic systems (Bond and Downes 2003). Our results are concordant with others showing that reproductive behaviors such as nesting in birds or redd site selection in migratory fishes have spatially explicit (i.e., clustered or patchy) properties due to both abiotic (i.e., habitat) and biotic (i.e., social) mechanisms related to the species reproductive ecology (Melles et al. 2009; Hendry et al. 2001).

Lake sturgeon egg depositional profiles are spatially autocorrelated and anisotropic. The spatial range of egg deposition was unidirectional and always greater in the downstream vs. cross stream direction. The tendency for UBR lake sturgeon to select areas and spawn to one side of the UBR and for natural stream flow to disperse eggs longitudinally likely drives anisotropy. Model fit was also improved with the addition of environmental covariates, supporting our prediction that hydro-geomophological environmental stream variables will also explain variability in egg deposition. Specifically, egg deposition was significantly associated with increasing substrate size (i.e., max and average).

One possibility for this relationship is that turbulence, generated by interactions between river flow and substrate, acts as a mechanism to aggregate eggs in areas of larger substrate (Cardinale et al. 2002). Recently deposited eggs may also become trapped by interstitial spacing of larger rocks serving to reduce subsequent transportation before eggs become adhesive (Forsythe 2010). Water velocity and depth were also found to be significant predictors of egg deposition for similar reasons. Our results are concordant with others suggesting that stream variables including water depth, water velocity and substrate size are predictive of egg deposition for migratory fishes (Mulli and Wilzbach 2007; Knapp and Preisler 1999; Thurow and King 1994). LaHaye (1996) and Chiotti et al. (2008) also reported similar and consistent associations between numbers of lake sturgeon eggs deposited with substrate size, depth and velocity across the species geographic range.

Survival and environmental covariates

Relationships between environmental covariates, particularly substrate size, with number of eggs deposited were also consistent across spawning areas (Tables 3.1 - 3.4). Differences in the strength and the sign of parameter estimates in aspatial models would have provided contradictory and thus misleading conclusions (i.e., 15 out of 18 environmental covariates measured across Sites 1-4 were found to be significant predictors of egg deposition). Results show several important points. First, while the locations adults selected to spawn may be different in their physical properties, and spawning site selection varies by females spawning at different times with different ecological conditions (Forsythe 2010), the environmental characteristics associated with places eggs are deposited are similar. Thus, the specific habitats selected among spatially complex habitats for reproduction are likely to set into motion similar biological and physical processes that influence offspring survival and growth. Our finding of a similar 70% reduction in viable egg abundance between sampling periods for both locations supports this hypothesis.

The environmental features we measured were selected because of known relationships between these features and adult spawning site selection. The same features were assumed to also be predictive of deposition rates and embryo survival. Results revealed that relationships were weaker than anticipated. However, average substrate size was a significant predictor in the magnitude of change in egg number over three days. Fewer viable eggs remained after 3 days as a function of decreasing substrate size (i.e., the ratio between time 1 and time 2 decreases with decreasing substrate size). One explanation for this relationship is that relatively smaller and perhaps more heterogeneous substrates with increased porosity reduce the level of predation on eggs by predators inhabiting interstitial spaces in stream substrate (Chotkowksi and Marsden 1999; Forsythe 2010; Palm et al. 2009). Smaller substrate sizes may also have reduced loss due physical disturbances transporting eggs out of the spawning area (Biga et al. 1998; Fitzsimons et al. 2007; Forsythe 2010). However, environmental covariates were only significant for one location, Site 4, a spawning area with the largest average substrate size suggesting that this pattern is not generalizable to all spawning areas.

The rate of change in egg loss provided by estimates of the correlation between time 1 and time 2 (Figures 3.5 and 3.6) also suggested that loss was non-stationary (i.e., rates of loss were different across locations) and relatively independent of the environmental covariates measured. This result was surprising given recent research showing that variation in incubation conditions (i.e., water flow, substrate size) in experimental settings significantly altered rates of mortality during incubation (Forsythe 2010). Although water depth, water velocity and substrate size are characteristics traditionally measured to quantify spawning habitats selected by migratory fishes (Wollebaek et al. 2009), these variables are only predictive of where individuals spawn. Others variables that define habitats at fine spatial scales including hyporheic temperature, substrate porosity, dissolved oxygen, pH, bedform migration and the presence of groundwater spring or vertical hydraulic gradients may be better predictors of egg survival (Geist and Dauble 1998). Information on these later variables is often required to estimate and to accurately quantify the amount of habitat available to spawners (Geist and Dauble 1998), predict the locations that may be selected by adults (Mulli and Wilzbach 2007) and estimate survival to hatch.

Additional unmeasured variables are likely important to egg survival. Of the abiotic factors mentioned previously we tend to focus on the hyporheic zone for several reasons. First, ground-surface interactions are highly variable, temporally, and at extremely small spatial scales in streams (Boulton et al. 2010; Hakenkamp et al. 1993). Second, egg survival of numerous migratory fish is directly proportional to the amount of dissolved oxygen (DO) available at the substrate surface (i.e., high (DO) high egg survival; Malcolm et al. 2003) vs. groundwater (i.e., low DO low egg survival; Malcolm et al. 2004). Inter-annual spatial and temporal variability in ground-water surface water interactions and hydrological events that affect hyporheic water quality have been implicated in driving variation in annual recruitment in salmon (Malcolm et al. 2005). However, variable loss may not be function of the physical properties of the environments selected. One example includes aspects of the biotic predator community including type, size and density. Forsythe et al. (2010) showed that high densities of interstitial predators could consume 99% of lake sturgeon eggs over 24 hours regardless of substrate size. Moderate levels of predation have been implicated driving the recruitment of many fishes especially at low population abundance (i.e., predation driven Allee effects; Gascoigne and Lipcius 2004).

CHAPTER 4

SOURCE-BASED ASSESSMENT OFF LAKE STURGEON EGG MORTALITY IN NATURAL AND EXPERIMENTAL STREAM SETTINGS

INTRODUCTION

Identifying the sources of mortality and estimating the magnitude of loss are central goals in population ecology that offers critical insight into naturally occurring variation in levels of annual reproduction (Caselle 1999), provides projections of future population trends and dynamics (Wiegand et al. 1998) and indicates the relative contribution of different life history stages and their traits to future generations (Gross et al. 2002; Martin 1995; Gaillard and Yoccoz 2003). A detailed understanding of source-based mortality at different life stages also provides insight into how populations will respond to natural or human- induced changes in environmental conditions or to harvest (Fahrig 2002); both important factors in developing science-based adaptive management programs for species that are exploited or of conservation concern (Doak et al. 1994; Wisdom et al. 2000).

Mortality occurs throughout an organism’s life cycle at varying rates that often decline with development and growth. Early life mortality however plays an important role in population dynamics of many species because mortality is often disproportionally high (Congdon et al. 1999; Donovan et al. 1995; Gosselin and Qian 1997; Katvala and Katitala 2001; Muths et al. 2003) and variation in a single source (e.g., predation, weather) can generate large and predicable fluctuations in annual abundance (Bailey and Houde 1989; Houde 1987; Lubow and Smith 2004; Osman and Whitlatch 1995). Processes occurring early in life can also “cascade” through subsequent life stages, affecting not only abundance and levels of annual reproduction but variation in phenotypic characters (e.g., body size) tied to probabilities of survival (Vandenbos et al.

2006). High early life mortality also produces a host of important evolutionary adaptations (e.g., ageing, reproductive behaviors, and parental investment) that enhance fitness, particularly when parents contribute differentially to annual reproduction and when traits are heritable (Roff 2002).

In many fish species, early life mortality during the egg and larval stages is excessively high, often exceeding 95%, and plays a major role in regulating population abundance (Bouwes and Luecke 1997; Fitzsimons et al. 2007; Smith and Marsden 2009). Thus, knowledge of the sources and estimates of early life mortality are viewed as essential elements in predicting future levels of annual reproduction. Quantifying source-based early life mortality however is complicated in natural aquatic settings due to the obscurity of early life stages and the technical limitations of sampling gear (Houde 1987). Fish species also display an immense array of early life history traits (e.g., spawning time), reproductive behaviors (e.g., nest construction, mouth brooding, egg fanning), physiological defenses (e.g., induced hatching) and anti-predator mechanisms that more or less compensate for excessive loss which tends to make generalizations difficult (Utne-Palm 2001; Taborsky and Foerster 2004; Knouft et al. 2003; Wedekind and Muller 2005). The ecological and evolutionarily role of early life mortality in short-lived species exhibiting very specific behavioral adaptations (e.g., nest construction in Salmonids) or life histories that minimize loss, for instance, are likely much different for relatively long-lived iteroparous species (e.g., walleye, sturgeons and paddlefish) that don’t construct nest nor provide post-ovulatory parental care (Winemiller and Rose 1992).

Adding further complexity, early life dynamics in fish also varies substantially across populations due to adaptations that have evolved as a function of site-specific features of spawning locations that includes gradients of substrate size, oxygen availability and predator sizes/types (de Leaniz et al. 2007; Quinn et al. 1995). Similar ecological adaptations can also evolve within segments of the same population which are reproductively isolated (i.e., adaptation by time; Hendry and Day 2005) because individuals and their offspring encounter ecological conditions that select for traits unique to each reproducing segment even over short time scales (Hendry and Quinn 1997; Kinnison et al. 1998). To this end, the magnitude and sources of mortality across the range of habitats experienced by fish eggs and larvae of a single species are largely unknown. Stage-based estimates of mortality, where available, are also typically provided in isolation of the many suspected sources predicted to vary across space in time; potentially underestimating the magnitude and variability of loss and leaving little indication whether the sources of mortality vary directly (i.e., compensatory) or independently (i.e., additive) across reproductive locations and times.

The main goal of this study was to address this uncertainty using a combination of field-based manipulations and laboratory experiments designed to identify the sources and magnitude of egg mortality in the lake sturgeon (*Acipenser fulvescens*). We place emphasis on the egg stage because of our unique access to this brief early life phase and because embryonic dynamics are relatively underappreciated in the fisheries ecology literature, limiting our understanding of fish population dynamics especially for species with unique life history traits (e.g., longevity and iteroparity) and reproductive behaviors including broadcast spawning and no parental care. The following objectives specifically addressed were to: (1) obtain estimates of mortality/survival during the egg stage, (2) partition mortality into sources that may vary spatially and temporally across natural stream incubation environments and (3) quantify variation in abiotic and biotic variables such as substrate and predator density on levels of egg consumption.

Lake sturgeon are an interesting species for studying early life mortality due in part to their unique life history; i.e., lake sturgeon are a long-lived (ages may exceed 100 years), large-bodied (11-100 kg), late-maturing (12–25 yrs of age depending on sex), iteroparous fish species with high fecundity (11,000 eggs/kg) that migrate to sections of fast-flowing rivers for reproduction in the spring (reviewed in Peterson 2007). During reproduction, eggs and sperm are released (i.e., broadcasted) into the stream environment over rocks and gravel in the absence of nest site preparation. Despite little post-ovulatory care, numerous aspects of this species' biology and behavior such as highly synchronized spawning events (Forsythe et al. 2010), non-random selection of stream benthic habitats (Chiotti et al. 2008), adhesive properties of eggs (Bruch et al. 2006) and relative large embryos (2.7–3.8 mm) with short incubation times (5–14 days) potentially enhance (or generate variation in) probabilities of embryonic survival (Kempinger 1988).
Mortality during the egg stage is high and variable with an estimated 80 – 99% of individuals dying through incubation (Caroffino et al. 2010; Johnson et al. 2006; Nichols et al. 2003). Thus, the egg stage is likely a significant component of the species population dynamics (Gross et al. 2002; Jager et al. 2002).

Mortality can be attributed to several sources including (Parsley et al. 2002): 1) *developmental/physiological mortality* occurring when eggs die due to genetic or developmental abnormalities that arise during incubation, 2) *predation* by a wide range of vertebrate and invertebrate benthivores (Kempinger 1988) and *physical processes* in the form of water currents that may dislodge and transport healthy eggs from rearing environments. However, the relative magnitude of mortality sources has not yet been quantified in natural settings. Without this knowledge, it is difficult to determine whether substantial variation in recruitment documented between groups that reproduce at different times and utilize geographically different and unique habitats for reproduction (Forsythe 2010) can be attributed to dynamics occurring during the egg stage.

METHODS

Study site

This research was conducted using eggs from a well studied population of lake sturgeon in Black Lake, MI (Forsythe 2010; Smith and King 2005). The Black Lake population consists of approximately 1,100 sexually mature adults, upper 95% confidence (Baker and Borgeson 1999). The Upper Black River (UBR), a fourth-order stream, is the largest tributary of Black Lake and is approximately 91.7 km long with 11 km of staging and unrestricted spawning habitat downstream from Kleber Dam (ca. 1949). However, shallow and wadable locations within a 1.5 km section of the UBR 9 km upstream from the river mouth are used for spawning annually (Forsythe 2010). Reproduction occurs exclusively in the UBR from late April through early June (Forsythe 2010). Approximately 100-250 adults spawn annually and variation is observed in individual spawning site arrival time, the chronology and duration of spawning activity, adult sex ratio, size and condition of breeding adults and in spawning site selection (Forsythe 2010; Smith and Baker 2005).

Mortality and sources of loss

During the spring of 2005, we quantified the magnitude and sources of lake sturgeon egg mortality using replicated exclosure boxes of varying mesh sizes. Treatments of different mesh sizes were designed to simulate an increasing probability (small to large mesh) that eggs would be encountered and consumed by increasingly diverse range of predators (interstitial vs. epibenthic) or removed from the incubation substrate (e.g., de-adhesion owing to higher water velocities). Sources of mortality leading to egg removal were collectively categorized as exogenous loss/removal. Water velocity and co-variation in other factors such as dissolved oxygen concentration associated with varying flow conditions also differed as a function of treatment (large to small mesh). In addition to altering water flow, exclosure treatments may also have lowered levels of light exposure but this was not quantified.

Exclosure boxes were constructed using welded heavy metal rebar of sufficient weight to withstand high velocity water currents and stream debris (Figure 4.1). Metal rectangular boxes (0.093 m^2, 0.305 m high) were enclosed with metal cloth of three different sizes (Large 5.08 cm, Small 1.27 cm and Fine 2 mm). A fourth box was left open to simulate the ambient stream environment (unimpeded flow and predator access). Our working hypothesis was that egg mortality rates experienced across mesh size treatments would be comparable but the sources of mortality would vary significantly (i.e., compensatory mortality). Specifically, we predicted that treatments would result in: 1) Open Cage – high rates of exogenous loss and a low incidence of developmental mortality, 2) Large Mesh – moderate levels of exogenous loss and low mortality due to sources that arrest development, 3) Small Mesh – a reduction in exogenous loss but higher incidence of developmental mortality, and 4) Fine Mesh – low probabilities of exogenous removal but high rates of developmental mortality because of low water velocity.

Eggs were obtained from spawning lake sturgeon captured on the UBR. Two-hundred eggs were counted, fertilized, and allowed to adhere to one brown circular ($0.073 \, m^2$) porous filters (3M Worldwide, Inc. Buffing and Polishing Pads) in each exclosure. This egg density is within the range commonly observed in the UBR under natural spawning conditions (Forsythe 2010). Filters were randomly assigned to an exclosure mesh size treatment. Four different treatments were then randomly placed (i.e., blocked together) on a heavy metal rebar base (Figure 4.1), secured with plastic tie-downs and lowered to the bottom of the stream. By creating homogenous blocks (i.e., placing all four treatment in close proximity), we hoped to remove the effect of important nuisance variables inherent to this study, namely the range of abiotic conditions (e.g., depth, water velocity and substrate size) present in sections UBR where lake sturgeon naturally reproduce. Blocks were placed at least 5 m apart and eight replicates of each group (i.e., block) of four treatments were deployed simultaneously for 5 days.

Exclosures were cleaned of stream debris daily to maintain consistent water velocities. Flow conditions inside each replicate were measured using a March-McBirney flow meter at the end of the experiment. We also avoided attributing hatching to egg mortality by terminating the experiment once larvae became active inside the embryo and chorions softened (120 hours post fertilization). Numbers of viable and dead eggs were counted every 24 hours. Viable eggs (i.e., those exhibiting proper development) were identified by the formulation of the blastodisc and thickening at the animal region pole (Dettlaff et al. 1993). Eggs that died between observations could therefore be partitioned into different mortality sources including developmental failure (i.e., number of new dead eggs) and exogenous loss (i.e., decline in viable egg abundance subtracted from new developmental failures).

Effect of predator density, predator size and substrate size

A complementary replicated laboratory experiment was conducted in 2006 and 2007 to further document the importance of predator density and stream substrate on egg mortality. A single predator species (rusty crayfish *Orconectes rusticus*) was used along with substrates commonly found at spawning locations. Crayfish predators were collected from sections of the UBR where spawning had not occurred and therefore previous experience encountering, handling, or foraging on lake sturgeon eggs was unlikely. Predators were housed in flow-through tanks for approximately 48 hours before used and were not fed while in captivity. Eggs were obtained from adult lake sturgeon and temporally stored in incubation trays filled with river water at a rate of approximately 15 l/min.

Fiberglass rectangular tanks (bottom surface area = 2.8 m^2) were divided into three sections of equal size, but not partitioned so that crayfish could freely access all tank sections. Tanks were filled with equal and regulated amounts of water from the UBR. Three substrates were randomized within each tank prior to the start of a trial (1 per section) including: 1) large (mean 63.5 ± 18.4 mm), 2) medium (mean 24.4 ± 5.87 mm) and 3) open (mixture of sand and fiberglass bottom). A predator density (5, 10, 15 or 30 individuals) was randomly assigned to a raceway and crayfish were placed in equal proportions in each substrate. After 15 minutes of acclimation, 200 fertilized eggs (i.e., 600 per tank) were randomly scattered across each section. After 48 hours, predators were immediately removed and measured for carapace length (mm). Tank sections were then completely partitioned to minimize egg drift, substrates were sifted and remaining lake sturgeon eggs were counted.

Confounding effects of crayfish behavior and habitat preference/usage on egg survival (particularly as it relates to substrate size) were partly accounted for in two ways. First, we recorded the proportion of crayfish occupying each substrate type and the general activity patterns (i.e., foraging, searching) of predators at discrete intervals (every 4 hours) during diurnal and nocturnal periods. Second, a small complementary laboratory experiment was conducted in 2006 where both predator movement and predator density were fixed across substrates. In this experiment, only one of the three substrate categories previously described were placed in individual flow-through tanks (1 m × 0.5 m × 0.5 m) provided with filtered water taken from the UBR. Flow-through tanks were blacked out so that crayfish movements and foraging behavior were not affected by outside movements. Five novel crayfish predators (also housed for 24 hours and unfed) were randomly chosen and placed in each tank. Two-hundred eggs were released over the substrate after 15 minutes of predator acclimation. Each trial was conducted for 48 hours after which time the crayfish were removed and measured for total carapace length (mm). Substrates were then removed from each tank, sifted and remaining eggs were counted.

STATISTICAL ANALYSES

All analyses were performed using PROC MIXED in SAS Version 9 (SAS Institute 2003). Data from the field-based experiment were analyzed as a randomized complete block design with repeated measures across time in the context of a general linear mixed model (GLMM) (i.e., a model that contains both fixed and random effects). The GLMM is commonly defined as $y = X\beta + Zv + \varepsilon$ where X is a known design matrix for the fixed effects, Z is a known design matrix for the random effects, β and v are vectors of unknown fixed and random effects parameters and ε is a vector of unobserved random errors. Mixed modeling is the preferred approach for repeated measures designs largely because 1) the user can define a number of alternative covariance structures for experimental data in which observations are likely correlated (i.e., traditional ANOVA assumes independent errors) and 2) by treating a variable as a random effect (block in this case), and thus estimating variance for a population distribution plus a mean, inference can be generalized and extended to all possible levels of the effect.

We used a GLMM to examine the effect of time and exclosure treatment on three separate dependent variables (y) including: a) percent of eggs remaining at each observation, b) percent of total mortality due to developmental failure and c) percent of mortality due to exogenous loss. Each dependent variable was modeled separately as:

$$y = \mu + \text{Time} + \text{Treatment} + \text{Treatment*Time} + \text{Block} + \varepsilon$$

where the fixed main effect factors in the model were exclosure treatment (H_o: no mean difference), time (H_o: no change overtime), and the interaction between treatment and time (H_o: no difference in the shape of the response to treatment overtime (i.e., no difference in slopes)). μ was the general location parameter of the model (i.e., the mean) and ε represents the residual variance. Block was treated as a random effect and thus represents a random sample of all potential experimental sites in the spawning area. An auto-regressive error of the first order, AR(1), was used to model temporal autocorrelation among observations collected across time periods. AR(1) denotes that correlations between observations between adjacent time periods are larger than for those made farther apart, a common phenomenon in repeated measures designs (Littel et al.

1996). All pair-wise comparisons were investigated using least square means and post hoc differences were computed used Tukey's HSD ($\alpha = 0.05$). All dependant variables were arcsine square root transformed to meet normality assumptions. Differences in average flow rates between exclosure treatments were tested separately using traditional analysis of variance (ANOVA) in SAS (PROC GLM).

Data from the primary laboratory experiment was analyzed as a whole-plot split-plot design also in the context of a GLMM. Here, predator density (i.e., 5 ($n = 9$), 10 ($n = 9$), 15 ($n = 18$) and 20 ($n = 12$)) was treated as the whole plot factor (i.e., a random crayfish density was applied to each tank) and substrate category (i.e., large, medium and open) was treated as the subplot treatment factor as all three substrates were simultaneously evaluated in a single simulated system. The proportion of eggs remaining (arcsine square root transformed) after 48 hours (y) was then modeled as:

$$y = \mu + \text{Density} + \text{Substrate} + \text{Substrate*Density} + \text{Predator Size} + \varepsilon$$

where the primary fixed factors were predator density (H_o: no effect of predator density), substrate category (H_o: no effect of substrate), and the interaction between density and substrate (H_o: egg consumption for each density is constant across substrates). μ was the general location parameter of the model (mean) and ε represents the residual error variance. Average predator size was used as a covariate to partly control for any unwanted and non-random differences in the crayfish predators selected for individual trials. Although not a mixed model (i.e., no random effect), the residual maximum likelihood (REML) estimators currently employed with the Mixed Procedure in SAS (opposed to Type III sums of squares) are most appropriate for estimating effect size in experiments with unequal replications. Pair-wise comparisons were investigated using least square means and post hoc differences were computed used Tukey's HSD (α = 0.05).

Lastly, a traditional ANOVA in the form of a general linear model (PROC GLM) was used to test for differences in the proportional use of substrates by crayfish predators in the primary laboratory experiment. A general linear model ANOVA was also used to address the effect of substrate size on egg survival in the complementary laboratory experiment where predator density was fixed (5 individuals) and movements were restricted to individual tanks. In this model, substrate category was the only fixed effect and predator size was again used as a covariate. In both cases, pair-wise comparisons of arcsine square-root transformed proportions were calculated for each main effect using least square means and tested for statistical separation with post-hoc tests ($\alpha = 0.05$).

RESULTS

Magnitude of egg mortality and the components of loss

Exclosure treatments exposed eggs to different predator guilds as evidenced by size- and species-specific differences in colonizing benthic organisms including plecoptera (stoneflies), ephemeroptera (mayflies), diptera (chironomids), decopoda (crayfish) and perciformes (darters) (personal observation). Exclosures also contributed to significant variation in stream water velocity. As expected, water velocity increased with increasing mesh size, and with the exception of the Fine Mesh and Small Mesh treatments, differences were significant ($F_{3, 28} = 25.5$, $P < 0.05$; Open: 0.60 ± 0.076 m/s, Large Mesh: 0.30 ± 0.04 m/s, Small Mesh: 0.12 ± 0.021 m/s, Fine Mesh: 0.12 ± 0.013 m/s).

Egg mortality was high during the first 120 hours of incubation. In total, 5793 eggs (91% of the starting abundance) died before hatching with an average of 36 (± 48 SD) mortalities occurring between each observation period. Mortality was also inconsistent during incubation; i.e., mortality remained below 2% of the original abundance during the first 48 hours post fertilization (4 individuals/day), increased substantially to 57% between 72 and 96 hours (133 individuals/day on average) and eventually tapered to less than 10% (20 individuals/day) prior to hatch. Time to 50% mortality occurred between 72 and 96 hours after fertilization. Substantial mortality leading to rapidly declining levels of viable egg abundance was indicated by a significant main effect of time in the mixed effects model ($F_{4,\,101} = 476.9$, $P < 0.001$).

There was no significant difference in average morality/survival between exclosure treatments (Average mortality ± Range: Fine Mesh = 89 ± 22%, Small Mesh = 92 ± 19%, Large Mesh = 90 ± 21%, Open = 89 ± 12%) due in part to the large variability in the magnitude of loss (Treatment effect: $F_{3,\,31} = 5.7$, $P = 0.374$). Mortality however was responsive to exclosure treatment as indicated by a significant time-by-treatment interaction (i.e., means were significantly changing overtime but at different rates) ($F_{12,\,101} = 5.7$, $P < 0.001$). Specifically, time-dependant pair-wise comparison illustrated significantly lower levels of loss (depicted in Figure 4.2a as the percent of egg surviving) in the Open and Large Mesh treatments from 48 through 96 hours post fertilization. There was no evidence for significant differences in mortality between exclosure treatments either before 48 or at 120 hours after fertilization.

Total mortality was portioned into two sources including exogenous removal and developmental failure (Figures 4.2b and 4.2c). Mortality attributed to each source increased significantly between observations through time (i.e., Exogenous removal: $F_{3, 61}$=60.6, P <0.001; Development failure: $F_{3, 61}$=45.1, P <0.001). Developmental failure (N=4848, 84%) was the dominant source of total mortality across treatments compared to removal (N = 946, 16%). The relative magnitude of each mortality source however varied depending on the number of days into incubation (Figures 4.2b and 4.2c). For instance, 100% (N = 272) of the total mortality during the first 48 hours prior to fertilization was attributed entirely too exogenous removal. Developmental mortality increased substantially between 48 and 96 hours, accounting for 89% of the total loss or 29 (average) new mortalities per day during this developmental stage.

Higher levels of exogenous removal and a lower incidence of developmental mortality were predicted to occur with increasing exclosure mesh size. Significant mean differences in source-based mortality between exclosure treatments (Exogenous removal: $F_{3, 43}$ = 3.9, P < 0.01; Development failure: $F_{3, 43}$ = 4.8, P < 0.005) and significant mean pair-wise comparisons through time were consistent with these expectations (i.e., treatment-by-time interaction: Exogenous removal: $F_{3, 61}$=45.7, P = 0.08; Development failure: $F_{3, 57}$ =59.7, P = 0.17). Pair-wise evaluation for instance illustrates that developmental failure was a significantly smaller component of total mortality during incubation as mesh size and water velocity increased (Figure 4.2b). Further, levels of exogenous removal were consistently, and in many cases significantly greater, in treatments relatively open to ambient conditions and thus more susceptible to water currents as well as larger macro-invertebrates and fish (Figure 4.2c).

The role of predator density, predator size and substrate

We conducted 16 laboratory trials with four predator densities. Approximately 7718 eggs (80% of the total) were consumed by 255 crayfish predators over 48 hours (17-104 eggs/predator on average). Mortality was generally higher as predator density increased and substrate size decreased (Figure 4.3). Predator density was a significant predictor of egg mortality ($F_{3, 11} = 6.9$, $P < 0.005$) which was expected to increase by approximately 57%, based on least square mean estimates, for every increase in predator density. Substrate size did not explain a significant amount of the variation in mortality ($F_{3, 24} = 0.9$, $P = 0.39$). Interpretation of this main effect however was partly confounded given the presence of a marginally significant density-by-substrate interaction ($F_{6, 24} = 3.5$, $P = 0.05$), rejecting the null hypothesis that mortality for each density was consistent across substrates. Pair-wise evaluation of simple effects (i.e., substrate within each density) indicates significantly lower mortality/higher survival in the Large Rock treatment compared to the Gravel and Open treatments when predator densities were at the extremes (5 and 30 individuals). At intermediate densities (10 and 15 individuals), substrate had little or even the opposite effect on egg mortality (Figure 4.3).

Additional analyses/experiments enhanced and supported these general results. For instance, predator movements in the main laboratory experiment were non-random with respect to substrate type. Although mortality was lowest in Large Rock treatments, individuals spent significantly more time (32 out of 48 total hours on average) associated with these areas than other substrates tested ($F_{2, 68} = 28.1$, $P < 0.001$). Levels of predation were also consistently high and concordant when predator density and movements were fixed (i.e., 5 individuals). For instance, a total of 2495 eggs representing 69% of the total (65% in the main experiment when n=5) were consumed by the 90 crayfish predators (28 eggs/ predator). Substrate size ($F_{2, 15} = 3.85$, $P = 0.045$) was also a significant predictor of egg mortality in this complementary laboratory study and mortality was significantly lower in the Large Rock (62 %) and Gravel (63 %) treatments relative to conditions lacking any benthic heterogeneity (Open = 89 %). Finally egg consumption was related to predator size in both laboratory experiments ($F_{1, 11} = 15.0$, $P < 0.01$). Tanks with larger crayfish predators on average generally consumed more eggs (Figure 4.4).

DISCUSSION

Understanding the processes that bound species between limits of abundance requires precise estimates of mortality, especially during early life stages. However, early life mortality of many organisms is commonly overlooked, underappreciated and seldom quantified for many taxonomic groups, especially during the egg stage in fishes. Here, we estimate mortality of lake sturgeon eggs to average 91% during incubation in natural stream environments. Further, we estimate substantial variation in mortality across incubation conditions comprised of differences in flow velocity and susceptibility to predation to range from 75-97%, translating into nearly a 10 fold difference in the number of larvae produced at hatch.

Our estimates are generally concordant with those reported for other sturgeons (83 – 99%) (Nichols et al. 2003, Johnson et al. 2006; Caroffino et al. 2010) and for other broadcast spawning species that have unprotected demersal eggs including white sucker (97%; Scott and Crossman, 1973 cisco (99%; Bouwes and Luecke 1997), walleye (87%; Johnson 1961) and smelt (99%; Rupp 1965). However, levels of egg mortality found in this study where considerably higher in comparison to species that construct nests (i.e., bass and salmonids), provide parental care, have protective mechanisms or use vegetation for attachment (reviewed in Dahlberg 1979). For example, Clady (1975) reported over 95% survival of naturally produced yellow perch eggs which are laid in mass, protected by unique gelatinous envelopes and attached to vegetation.

Developmental mortality

Developmental mortality was the dominant source of loss during incubation representing 84% of the total mortality across exclosure treatments on average. Several explanations may be offered to explain this result. First, developmental failures may have been a function of endogenous properties of eggs that negatively interacted after fertilization. For instance, the ability of fish embryos to survive in nature depends on both nutritional (e.g., yolk volume) and genetic effects (e.g. DNA and hormones) (Brooks et al. 1997). Thus, if properties such as adult condition, the timing of spawning (degree of ripeness) and parentage are not matched well, egg quality will be reduced and may decrease probabilities of survival (Brooks et al. 1997). Developmental mortalities may have also occurred because of polyspermy, or simultaneous fertilization by multiple spermatozoa (Gilkey 1981). Polyspermy is found in many fish species and is especially prevalent in sturgeon because of numerous large openings (i.e., micropyles) in the egg envelope (Dettlaff et al. 1993; Linhart and Kudo 1997). Eggs fertilized by multiple sperm have additional chromosomes and centrioles and thus cause developmental defects and mortality by the first cleavage division (Gilbert 1997). Polyspermy may have been enhanced during our experiment due to artificial fertilization (Psenicka et al. 2010)

Developmental mortality on average was also significantly greater as mesh size and water velocity decreased. Water velocity is often related to lower levels of dissolved oxygen (DO) and DO is directly tied to spawning site selection in fish and is considered the primary factor in determining the fate of eggs developing in natural conditions (Youngson et al. 2004). Thus, a portion of developmental mortality here could be a result of suboptimal DO concentrations during incubation. Further, the delayed onset of developmental mortality observed in this study (i.e., after 48 hours after incubation) may also reflect developmental stages with an increasing demand for DO. Peaks in DO consumption and periods of susceptibility to mortality are common in fish embryos, especially during the formation of the animal pole and during hatching (Hamor and Garside 1977). However, in addition to DO, it is also possible that toxins (e.g., PCBs), mechanical disruptions that rupture eggs, fluctuation in water temperature and ultraviolet solar radiation were exogenous contributors to endogenous developmental malformations (Parsley et al. 2002). However, these are unlikely in the UBR and in our experimental conditions.

Eggs recorded as developmental moralities in the field were also covered with microbes, a phenomenon commonly observed in fish culture practices and more recently in natural settings for several fish species including sturgeon (Kempinger 1988; Parsley et al 2002; Rach et al. 1995; Kitancharoen et al. 1997). Anders and Beckman (1992) for instance found that 2-45% of white sturgeon eggs collected were infected with water-borne microbes. Microbial infection observed here and elsewhere may be a result of colonization post-mortality (i.e., eggs died do to something else). However, significant differences in levels of infection were also noted between treatment groups in this study. Differences in infection rates of eggs have also been found to be a function of water temperature (Knotek and Orth 1998), egg density (Cote and Gross 1993) and water velocity (Anders and Beckman 1992). Thus, it is plausible that developmental mortality is partly driven by wide-spread predatory-type infection by aquatic microbes that kill eggs by preventing oxygen transport through the perivitelline space. If so, microbial infection may have severe ecological consequences for sturgeon by placing constraints on seasonal levels of recruitment (Knootek and Orth 1998), altering behavioral and reproductive decisions (Knouft et al. 2003; Takahashi et al. 2004) and inducing hatching (Wedekind 2002). Interestingly, microbial infection has lead to the evolution of antimicrobial properties of eggs (Paxton and Willoughby 2000) and antimicrobial substances that are conferred to eggs by adults (Giacomello et al. 1996) in several fishes.

Exogenous removal

Exogenous sources leading to the removal eggs in the field accounted for rest or 16% of the total mortality in this study. Further, differences between exclosure treatments indicated higher levels of mortality as function of increasing water velocity. A portion of removal therefore may be a function of currents that dislodge and translocate embryos. The relative impact of water velocity on fish egg mortality, particularly when reaching critical sheer stress, has been well documented in natural conditions (Lapointe et al. 2000; Ventling-Schwank and Livingstone 1994). Mortality is significantly elevated for translocated individuals due to sediments that bury eggs and create hypoxic conditions (Ventling-Schwank and Livingstone 1994) or mechanical stressors that rupture the plasma membranes encasing embryos (Bunn et al. 2000). However, probabilities of secondary drift can be reduced by features of the stream that tend to mediate water flow including substrate size (Ventling-Schwank and Livingstone 1994). Comparable estimates of egg mortality show that the magnitude of loss due removal is smaller than reported here. For instance, Mills (1981) and Bouwes and Luecke (1997) estimated translocation to be a small fraction (2%) of mortality in dace and cisco, generally citing abiotic factors that reduced the impact of water currents on egg de-adhesion. LaHaye et al. (1992) also found that a small number of lake sturgeon eggs in drift were in advanced stages of development which is consistent with the transportation hypothesis. However, Fitzsimons et al. (2007) reported that physical disturbances generated by water flow contributed to higher egg mortality than other plausible sources in lake trout (i.e., predation) combined.

An alternative explanation for exogenous removal is predation by interstitial predators. Several factors support this hypothesis including a significantly consistent elevation in removal during incubation across treatments as well as significantly higher levels of morality in the Large Mesh and Open treatments. Predation on fish embryos often represents a significant source of mortality and is a strong regulatory component of fish populations because of the broad diversity of benthic fish and invertebrate predators with acute visual and chemosensory cues that allow egg predators forage effectively within the substrata of spawning sites (Bailey and Houde 1989; Bouwes and Luecke 1997; Dittman 1998). In broadcast spawning fish species, predation is predicted to have severe ecological consequences through depensatory population dynamics (i.e., Allee effects), especially if populations are reduced past thresholds (Gascoigne and Lipcius 2004). Empirical evidence also implicates predation on eggs as the primary cause of regionally poor recruitment and in the decline (or failed reintroductions) of many fish species of conservation concern (Savino et al. 1999).

Although significant levels predation on fish eggs including sturgeon have been documented (Miller and Beckman 1996; Kempinger 1988), predation in our field-based research represented a small proportion of the total mortality relative to developmental sources. However, levels of predation may have been underestimated because of time lags in the colonization of exclosure treatments. Fortunately, the impact of predation during the egg stage can also be gauged by the number of eggs consumed by a single benthic predator (DeBlois and Leggett 1991). In laboratory trials, we determined that average consumption by crayfish predators ranged from 17-104 eggs over 48 hours. Similar research reports much lower consumption rates (mean individual consumption ranged from 0.1 to 5.5) on lake and rainbow trout eggs (Ellrott et al. 2007), levels thought to hamper restoration activities implemented for natural populations of these species (e.g., Fitzsimons et al. 2006). Given the high rates of consumption found in this study, combined with much lower levels of interstitial predation found in others, we believe predation may indeed represent a major source of mortality during the egg stage in lake sturgeon.

Our laboratory research further indicates that levels of predation in lake sturgeon are strongly influenced by both abiotic and biotic conditions. Specifically, we found higher levels of egg mortality as the density of crayfish predators and substrate size increased (Figure 4.3). Aspects of the aquatic environment that influence consumption rates of embryos including predator type, predator density, river substrate composition and water temperature have been studied extensively (Fitzsimons et al. 2006). Levels of egg consumption generally vary depending on predator foraging guilds (Savino et al. 1999) and decline with increased predator density (Ellrott et al. 2007) and increasing substrate complexity because of the formation of deep interstitial spaces that reduce egg delectability (Chotkowski and Marsden 1999; Biga et al. 1998). We believe the composition of river substrates is critical for protecting lake sturgeon eggs from predators given significantly lower levels of mortality in substrates of larger size, regardless of whether predators spend significantly more time associated with these preferred habitats.

SYNTHESIS AND CONCLUSIONS

Chapter 1

Sturgeon migration should be viewed be as a continuum whereby individuals respond to exogenous cues before and after river entry and process different sources of information leading up to and during reproduction. Further, the relative importance of variables varied during different stages of migration. The importance of temperature and discharge to sturgeon behavior during each stage of migration indicates that perturbations affecting stream environments pose a threat to the viability of sturgeon populations. Specifically, changes in river discharge and water temperature above or below status quo conditions, due to hydroelectric operations for example, may cause changes in exogenous cues which likely elicit non-adaptive responses. The consequences of delayed migration are potentially severe and range from a reduction in energy reserves that exceeds the capacity for reproductive success, a decrease fertilization success by increasing the time spend in pre-spawning condition, uncoupling arrival and breeding (Ahola et al. 2004) or reducing egg and larval survivorship.

Stress from continued global climate change in the form of increased/decreased magnitude and timing of spring flows, increases in water temperatures and habitat fragmentation are likely to threaten future population viability of this species (Carpenter et al. 1992; Stefan and Sinokrot 1993). Most importantly, our study suggests that river discharge and water temperature cues will decrease the likelihood that individuals will be able to coordinate spawning activities with periods of the lunar cycle we believe are important for enhancing larval survival. Thus, the choices of individuals, guided by exogenous cues, are no longer adaptive (i.e., ecological traps; Schlacpfer et al 2002;

Stanford et al 1996). Recent work has also shown that individual lake sturgeon have a significant tendency to reproduce at different times and different places within the same river system and that these reproductive behaviors are highly repeatable (Forsythe et al. 2010). If repeatability of spawning time indicates non-zero heritability, each group may have evolved unique life history adaptations (e.g., egg size) and requirements (i.e., incubation temperatures) related to offspring growth and survival (Hendry and Day 2002). Conflicting exogenous cues may also increase probabilities of inter-group breeding, thereby disrupting adaptive life history or genetic diversity (Sugg et al. 1996 Crandall et al. 2000). Data presented herein provide a means of projecting the rate and direction by which changes in environmental regimes will act upon migratory and spawning behavior as a function of spatially and temporally changing environments (e.g., Williams et al. 2009).

Chapter 2

High repeatability in spawning time and spawning site selection indicates that individuals have a predisposition to spawn associated with exogenous environmental and lunar cues that covary with time within a spawning season and thus both are heritable behavioral traits. These data, along with recent work demonstrating evidence for the co-evolution of early life traits shown to predictably vary as a function of spawning time (i.e., body size; Crossman 2009; Forsythe 2010), suggest adaptive phenotypic and genetic differentiation may develop over ecological time frames in different spawning groups. Adaptation to conditions at such a fine micro-geographic scale can allow a population to maintain variability which is increasingly important to facilitate adaptation in response to future anthropogenic change (Hilborn et al. 2003).

Repeatability for spawning time may also indicate future challenges for sturgeon populations. For instance, repeatable assortative mating indicates that effective breeding population size will be lower because not all individuals reproduce at the same times or even the same year. Minimal mate avoidance due to aggregate breeding and an increased life span would tend to increase the probability of encountering and breeding with related individuals. Decreased effective population size will potentially lead to more rapid accrual of coancestry. Heritability for spawning further indicates that sturgeon are susceptible to human sources of selection such as angler harvest and hatchery practices that can inadvertently shift migratory time and reduce phenotypic variability in spawning time (Quinn et al. 2000; Quinn et al. 2002; Quinn et al. 2007). Streams and rivers are also rapid integrators of terrestrial and atmospheric processes and thus are greatly impacted by human disturbance (Williamson et al. 2008). The gradual warming of rivers due to global climate or regulatory activities that modify spawning habitats may risk decoupling important associations between the timing of reproduction and environmental conditions and co-evolved traits linked to offspring survival (Portner and Farrell 2008). The disruption of environmental regimes that have given rise to coevolved traits and temperature-dependant developmental trajectories (Kingsolver and Huey 1998) and genetic responses to alternation of the chronology of thermal regimes are expected to be reflected by the loss of variation within generations of animal populations (Angilletta et al. 2006).

Studies reporting reproductive isolation and evidence for adaptive divergence among spawning groups have typically been conducted over spatial scales large enough where differences in environmental regimes are expected. Studies which have attempted to document the degree of spatial and temporal segregation and local adaptations of breeding groups at comparatively small scales (e.g., over distance of < 2 km as reported herein) have been hampered by systems with relatively homogenous abiotic structure (Doctor and Quinn 2009) and lack of long-term data. Although the section of river used by UBR lake sturgeon contains several spawning locations that are relatively close together, subtle variation in physical features including substrate size, water depth, and velocity influence the natural deposition of embryos and influence levels of early life mortality (Forsythe 2010). Further, significant variation in environmental conditions (e.g., water temperature) is common between fish spawning early and late only a few days apart. Thus, we offer that studies attempting to quantify locally adapted traits at fine micro-geographic scales should be encouraged to increase the ability to predict population responses.

Chapter 3

Our study reinforces the need to employ appropriate statistical methods in order to properly document relationships between response and predictor variables. The primary goal of this study was to use data collected on a unique migratory species to illustrate the need to account for spatial dependencies when possible, especially in spatially complex environments such as streams and rivers. Data provide compelling evidence that common assumptions of spatial models (e.g., isotropy) are often violated. Alternative analytical methods as described here provide a rigorous framework to model spatially complex ecological problems. Ecological applications including modeling spatially explicit data within and between time periods provide a powerful approach to identify exogenous features associated with recruitment of stream fishes.

Analyses also have important applications for lake sturgeon and for other species sharing a similar reproductive ecology. For example, lake sturgeon, like many other fishes, have suffered dramatic population declines largely because of anthropogenic activities that have restricted, reduced or destroyed spawning areas (Duke et al. 1999; Opperman et al. 2005; Rochard et al. 1990) In response, managers have attempted to mitigate the effects of habitat loss by supplementing historically important and degraded spawning areas with new gravel or by creating new habitats that mimic natural areas in form (Johnson et al. 2006; Kondolf et al. 1996; Zeh and Donni 1994). Indeed, the addition of gravel to areas has been shown to promote successful reproduction for some species (e.g., Atlantic salmon and Brown trout), even when constructed areas are suboptimal relative to natural spawning grounds (Barlaup et al. 2008). However, little information has been available on the quantifiable features (i.e., gravel size, placement and flow requirements) of natural spawning habitats sturgeons use for reproduction leaving little guidance for restoration.

We believe our summaries of spawning habitats used by UBR lake sturgeon can inform this type of management action. Still, while our data and others suggest that lake sturgeon will likely use man-mad structures (Caswell et al. 2004; Johnson et al. 2006), we caution that spawning site enhancement will have a negligible impact on recovery through recruitment until all exogenous variables affecting rates of survival are identified and thus can appropriately mimic function (Hester and Gooseff 2010) as well as form.

Chapter 4

Egg mortality after fertilization in lake sturgeon represents a complex compensatory-type (i.e., decreasing percent of exogenous removal leads to increased levels of developmental mortality) relationship between multiple sources. Important relationships between the sources themselves and environmental conditions indentified here may serve to provide projections of loss that can guide conservation/management activities including construction, restoration and modification of spawning locations. For example, assuming levels of microbial infection are site-specific and stream microbes infect and subsequently kill otherwise healthy fish eggs (Wedekind 2002), individuals deposited in locations, or at times with a greater diversity and abundance of microbes, will likely experience higher rates of infection. Further, eggs deposited in riverbed locations that facilitate the delivery of dissolved oxygen will experience lower morality (Greig et al. 2007). Areas of consisting of relatively large substrate size will serve to mitigate translocation by reducing levels of critical sheer stress that lift eggs from the stream bottom and will protect individuals through the complexity of interstitial voids.

The magnitude of loss and the sources of mortality may also have important evolutionary/ecological consequences. At the population level for instance periodic fish species (i.e., delayed maturation, large body size, high fecundity and low investment per offspring) such as lake sturgeon are predicted to exhibit substantial inter-annual variation in levels of annual recruitment unrelated to the abundance of spawning adults (Winemiller 2005). Several explanations for the lack of density-dependant compensation in the number of offspring produced have been offered mortality during early life stages including stochastic abiotic/biotic variation (Myers 1998) and the availability of food for offspring (Cushing 1990). Preliminary empirical spawner-recruitment data collected for lake sturgeon populations support this prediction (Forsythe et al unpublished data). Further, our research showing substantially high and variable egg mortality across ecological gradients supports the relative contribution of early life stages to population regulation and reinforces the ecological significance of mortality during the egg stage in long-lived fish species.

Bailey and Houde (1989) predicted that variation in a single source, predation, resulted from spatial and temporal variation in predator-prey distribution, predator abundance and the availability of alternative prey would ultimately lead to variation in levels of recruitment. Our data also supports this prediction. However, we also offer that heterogeneity in all abiotic and biotic factors leading to variation in mortality of embryos may contribute to variation in levels of sturgeon recruiting to the population both within and between spawning locations, across segments of populations that breed at different times and annually. This prediction is based on relationships presented in this paper as well as quantified differences in water velocities, depths and substrate sizes of spawning habitats (Forsythe 2010) often correlated with variation in the abundance and distribution of benthic egg predators (Crowl et al. 1997), water temperatures, the direction and velocity of water currents and surface–subsurface DO exchange (Boulton et al. 1998) and the abundance and diversity of the aquatic microbial community (e.g., bacteria and fungi).

Finally, in times of human-mediated change, early life morality sources not common to the evolutionary history of fish populations including pollution, microbial diseases or parasites, the alternation of flow regimes through regulated river management and the introduction of exotic interstitial predators are likely to occur. Variation in abiotic and biotic mortality sources has been shown to shape population or breeding segment-specific adaptations during the egg stage including egg size, resistance to microbial infection and the timing of transition to the larval stage (Quinn et al. 1998). Philopatric tendencies of lake sturgeon and repeatability for spawning at the same time and place may exacerbate these differences (Forsythe 2010). Yet, these questions have not yet been explored for sturgeon populations. Knowledge of the ‘biocomplexity’ that accompanies the evolution of diversity during the egg stage will be critical for understanding sturgeon sustainability in the face spatial and temporal heterogeneity in environmental conditions exacerbated by human activity and climate change (Hilborn et al. 2003).

APPENDIX A: TABLES AND FIGURES

Table 1.1. Generalized chi-square (χ^2/degrees of freedom) and R-square (R^2) fit statistics for models explaining daily numbers of individuals entering the river mouth and arriving to upstream spawning areas in the Upper Black River, Michigan and those that account for temporal autocorrelation in counts (AC), environmental (ENV) and lunar (LUNAR) parameters or a combination of all three.

Dependent variable	Model	χ^2/df	R^2
River entry	Null (AC)	4.69	0.38
	1 (ENV)	7.44	0.11
	2 (AC + ENV)	2.13	0.77
	3 (LUNAR)	7.13	0.08
	4 (AC + LUNAR)	4.86	0.36
	5 (AC + ENV + LUNAR)	1.33	0.94
Spawning site arrival	Null (AC)	2.86	0.41
	6 (ENV)	7.72	0.02
	7 (AC + ENV)	1.99	0.63
	8 (LUNAR)	7.19	0.12
	9 (AC + LUNAR)	1.93	0.66
	10 (AC + ENV + LUNAR)	1.75	0.77

Table 1.2. Parameter estimates, parameter confidence intervals (lower and upper 95% confidence intervals), and test statistics for environmental (Dlag = 24 hr lagged discharge, Tlag = 24 hr lagged temperature, Tchange = 24 hr change in temperature and Dchange = 24 hr change in discharge) and lunar effect (θ = lunar angle) models describing daily observations of individuals entering the mouth of the Upper Black River, Michigan, 2000-2002.

Model	Effect	β	95% LCL	95% UCL	*F*	Prob > *F*
Environmental	Day	-0.04	-0.06	-0.01	7.63	0.009
	Dlag	-0.07	-0.12	-0.02	7.94	0.009
	Tlag	0.26	0.16	0.36	4.10	< 0.001
	Tchange	0.21	0.04	0.38	6.30	0.015
	Dchange	-0.07	-0.13	-0.02	5.67	0.013
Lunar	Day	0.01	-0.01	0.03	2.46	0.152
	sine θ	-0.08	-0.22	0.06	1.33	0.256
	cosine θ	-0.01	-0.17	0.15	0.02	0.888
	sin 2θ	-0.03	-0.15	0.10	0.19	0.664

Table 1.3. Parameter estimates, parameter confidence intervals (lower and upper 95% confidence intervals), and test statistics for environmental (Dlag = 24 hr lagged discharge, Tlag = 24 hr lagged temperature, Tchange = 24 hr change in temperature and Dchange = 24 hr change in discharge) and lunar effect (θ = lunar angle) models describing daily numbers of individuals arriving to spawning locations in the Upper Black River, Michigan 2001-2008.

Model	Effect	β	95% LCL	95% UCL	*F*	Prob > *F*
Environmental	Day	-0.30	-3.92	3.30	0.03	0.854
	Sex	.	.	.	19.35	< 0.001
	Day × Sex	.	.	.	23.89	< 0.001
	Dlag	-0.06	-0.10	-0.03	12.31	0.001
	Dchange	-0.01	-0.05	0.04	0.02	0.898
	Tquad	0.01	0.01	0.05	10.38	0.001
	Tchange	0.29	0.17	0.40	24.54	< 0.001
Lunar	Day	-0.36	-3.95	3.25	0.02	0.8362
	Sex	.	.	.	19.35	< 0.001
	Day × Sex	.	.		23.89	< 0.001
	sin θ	-0.15	-0.36	0.06	1.85	0.174
	cosine θ	0.59	0.40	0.78	38.91	< 0.001
	sin 2θ	-0.80	-1.02	-0.55	40.94	< 0.001

Table 1.4. Parameter estimates, parameter confidence intervals (lower and upper 95% confidence intervals), for combined environmental (Dlag = 24 hr lagged discharge, Tlag = 24 hr lagged temperature, Tchange = 24 hr change in temperature and Dchange = 24 hr change in discharge) and lunar effect (θ = lunar angle) models describing daily numbers of individuals entering the mouth of the Upper Black River, Michigan and arriving to spawning locations, 2001-2008.

Variable	Effect	β	95% LCL	95% UCL
River entry	Day	-0.11	-2.77	2.56
	Dlag	-0.08	-0.12	-0.01
	Tlag	0.18	0.04	0.33
	Tchange	-0.05	-0.28	0.17
	Dchange	-0.09	-0.13	-0.02
	sin θ	-0.21	-9.05	8.63
Spawning site arrival	Day	-0.42	-4.74	3.75
	Sex	.	.	.
	Day × Sex	.	.	.
	Dlag	-0.12	-0.17	-0.08
	Tchange	0.50	0.36	0.62
	Tquad	0.01	0.00	0.02
	cosine θ	1.08	0.43	1.73
	sine 2θ	-1.01	-1.28	-0.75
	Dlag × cosine θ	-0.15	-0.21	-0.10
	Tquad × cosine θ	0.01	0.00	0.01
	Tchange × cosine θ	-0.60	-0.83	-0.37

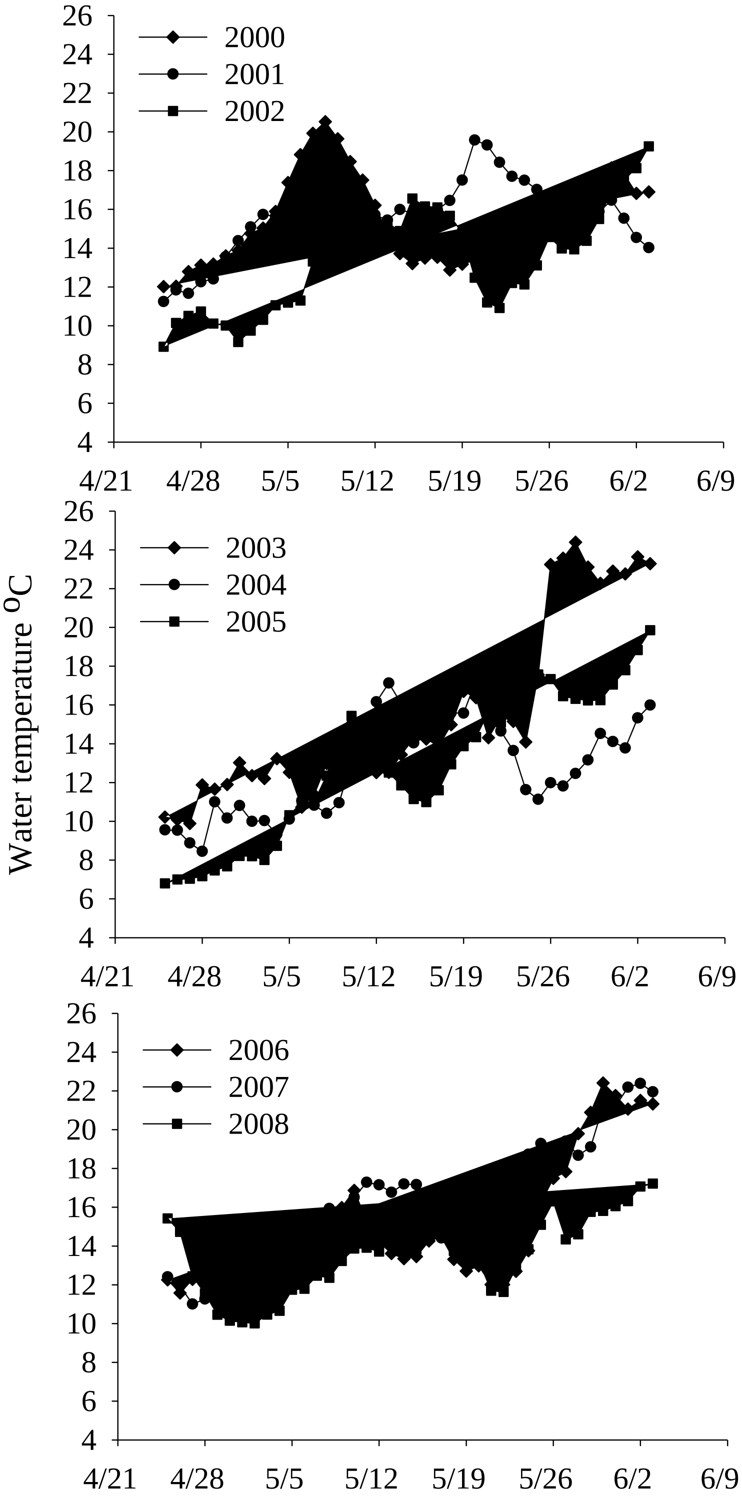

Figure 1.1. Measurements of daily water temperature (^{o}C) of the Upper Black River, Michigan collected from April 22 through June 5 for 2000-2002 (upper panel), for 2003-2005 (middle panel) and 2006-2008 (lower panel).

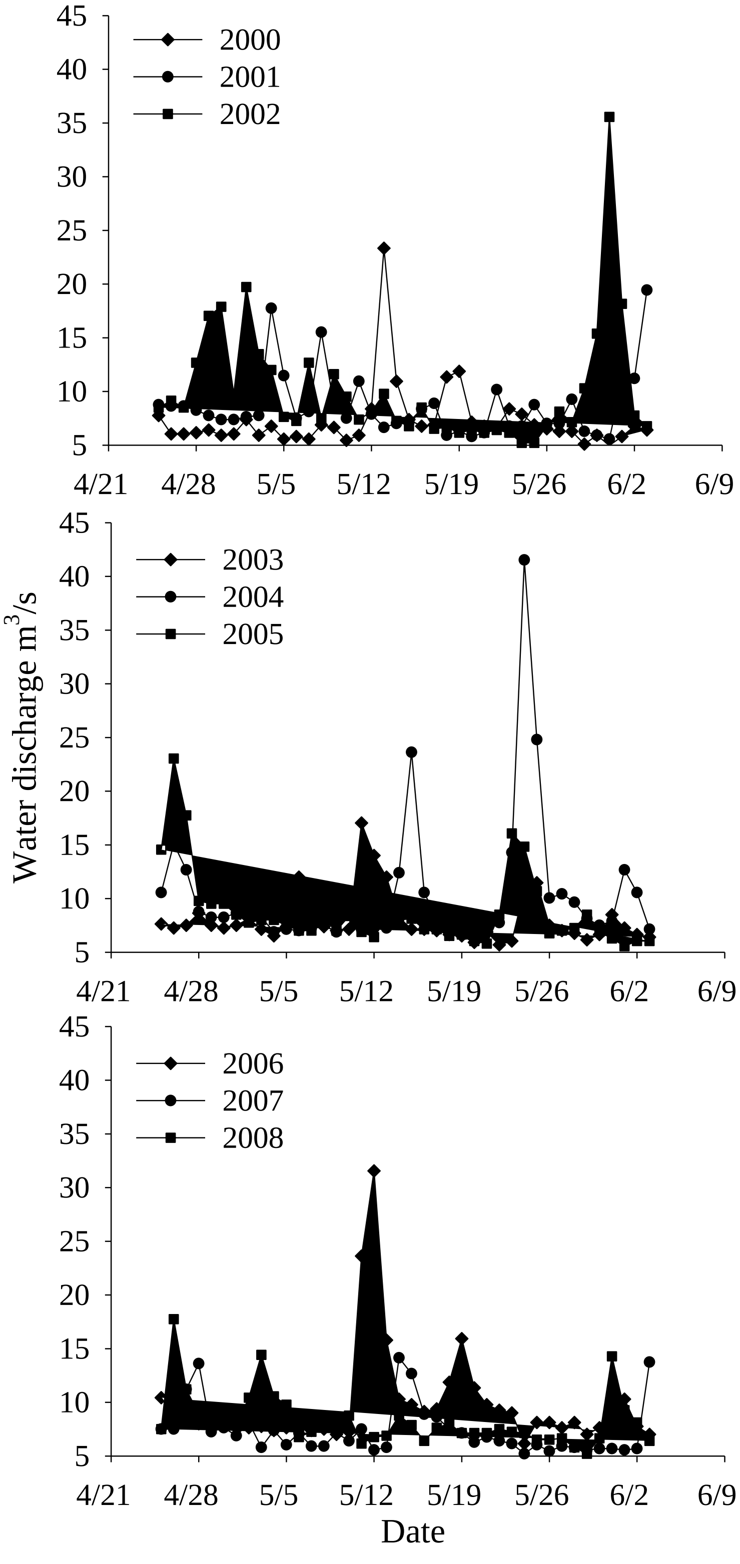

Figure 1.2. Measurements of daily river discharge (m^3/s) of the Upper Black River, Michigan collected from April 22 through June 5 for 2000-2002 (upper panel), for 2003-2005 (middle panel) and 2006-2008 (lower panel).

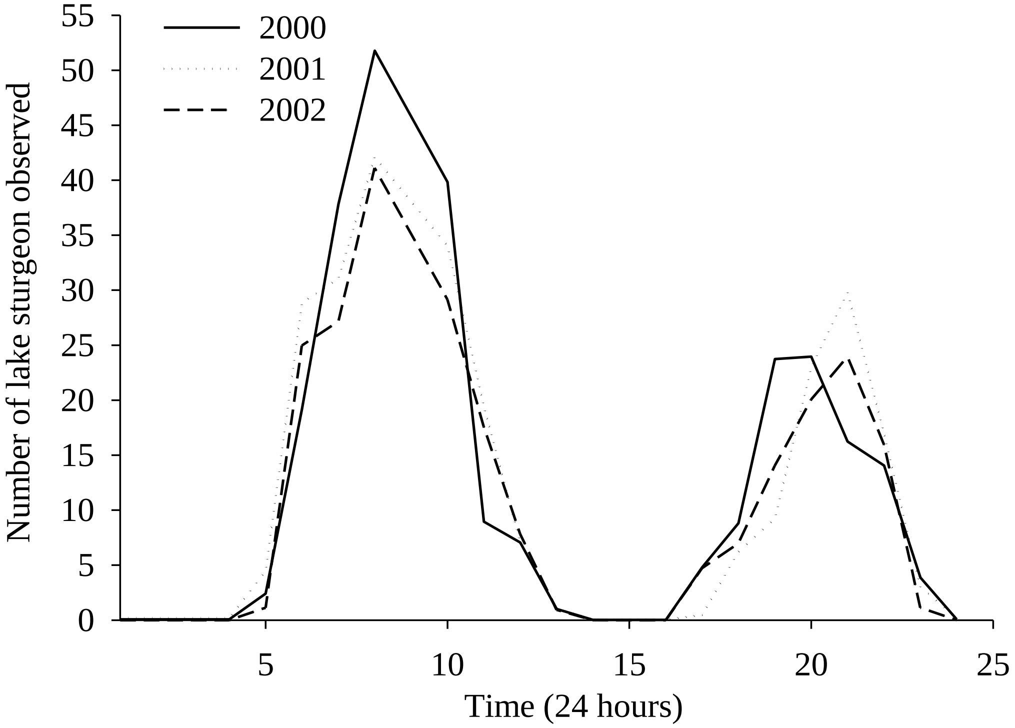

Figure 1.3. Number of adult lake sturgeon observed entering the Upper Black River, Michigan 2000-2002 as function of the hour of the day.

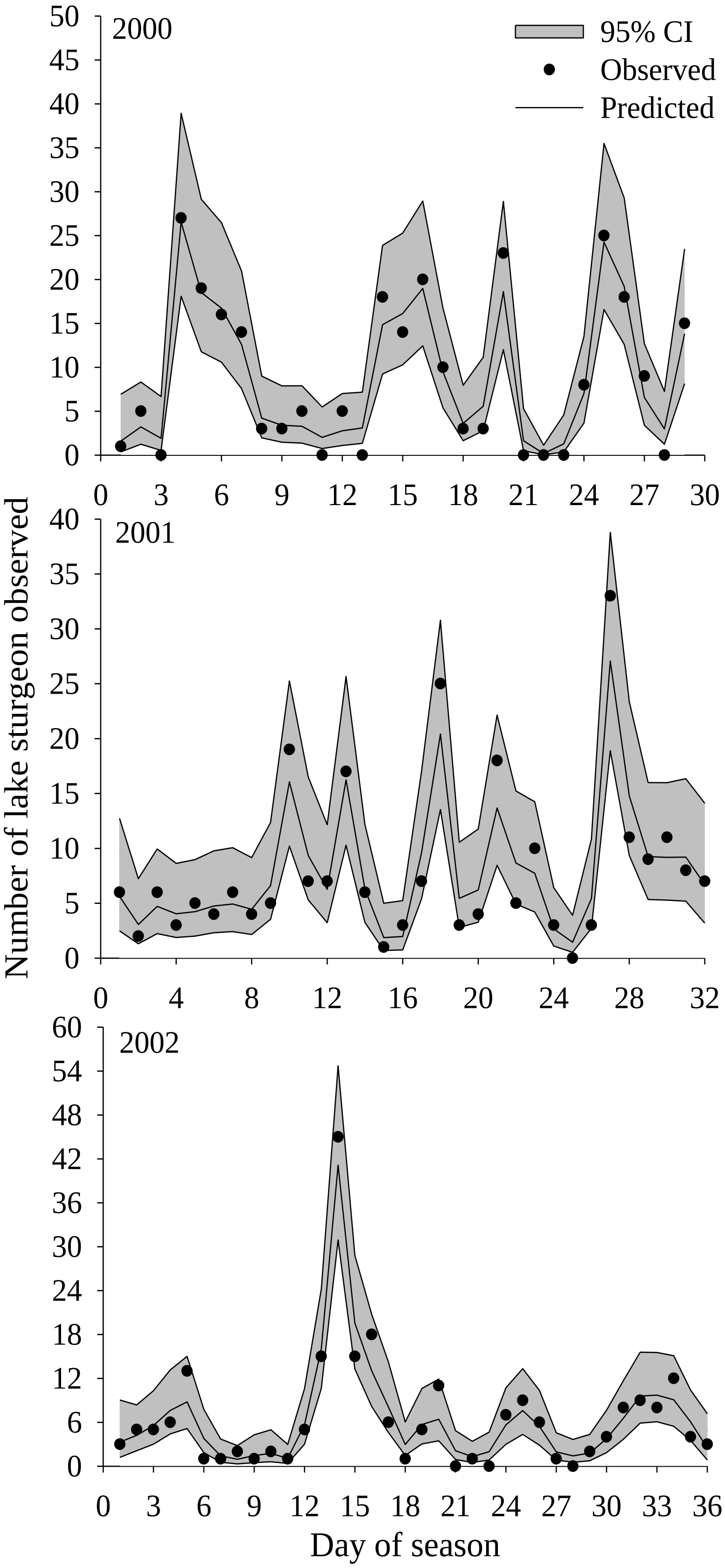

Figure 1.4. Observed, predicted and 95% confidence intervals for the daily number of lake sturgeon entering the Upper Black River, MI from 2000-2002.

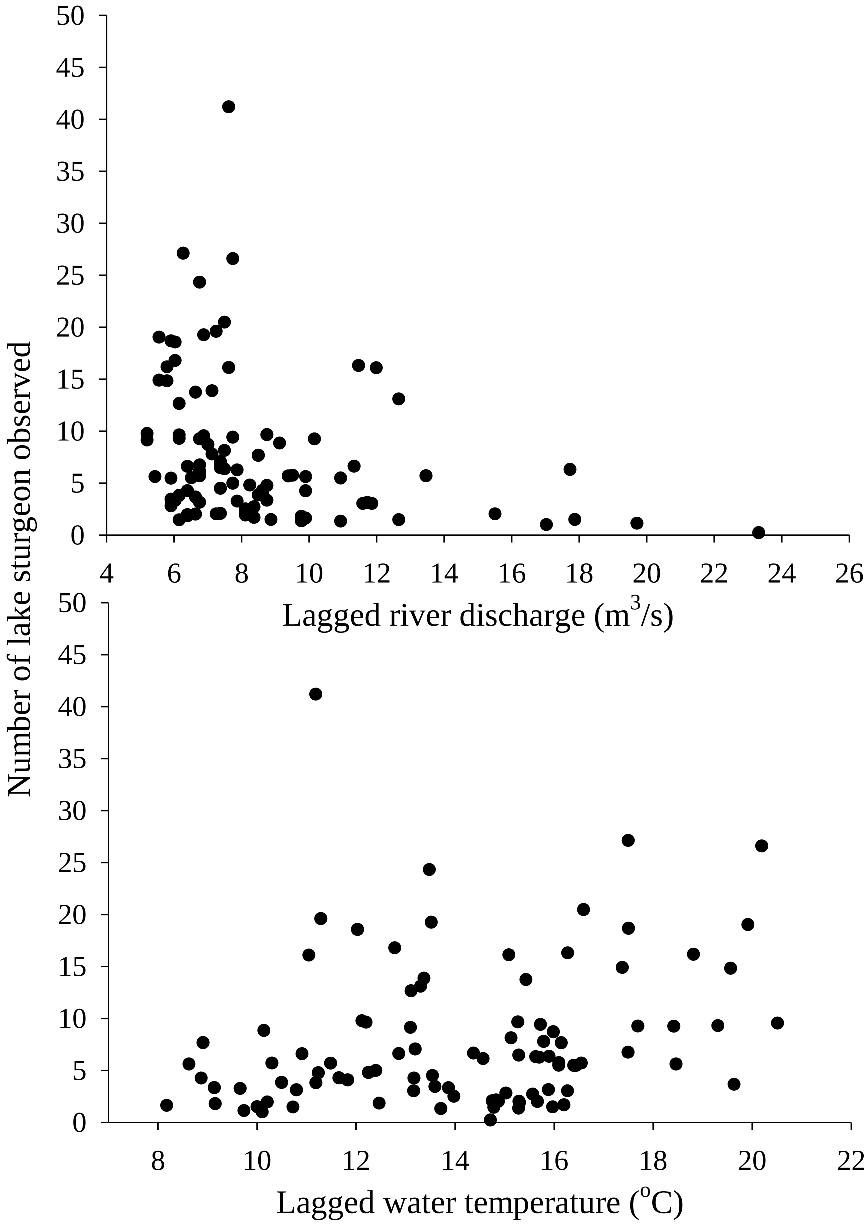

Figure 1.5. Relationship between the daily number of lake sturgeon observed entering the mouth of the Upper Black River, Michigan with 1 day lagged river discharge and 1 day lagged water temperature.

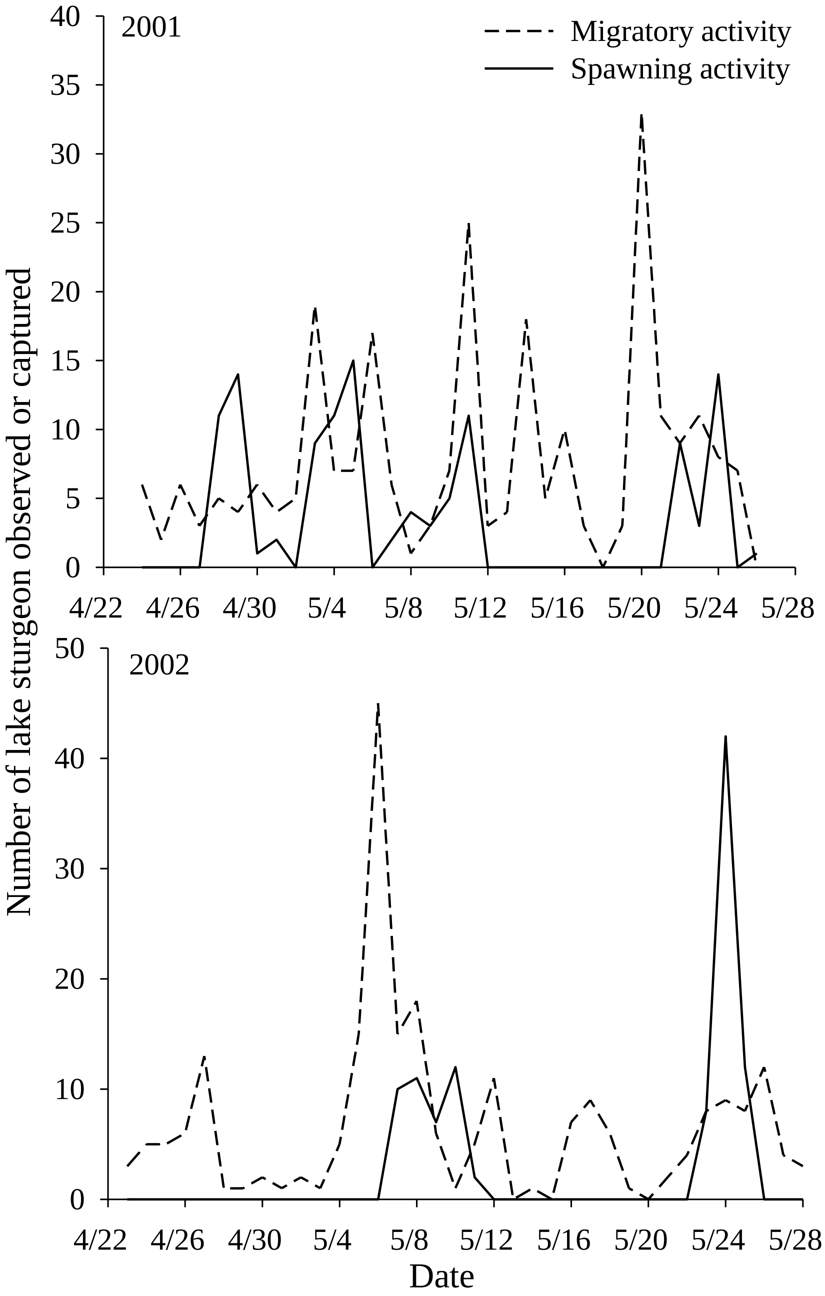

Figure 1.6. The number of lake sturgeon observed entering the Upper Black River or captured at upstream spawning location as a function of the date in 2001 and 2002.

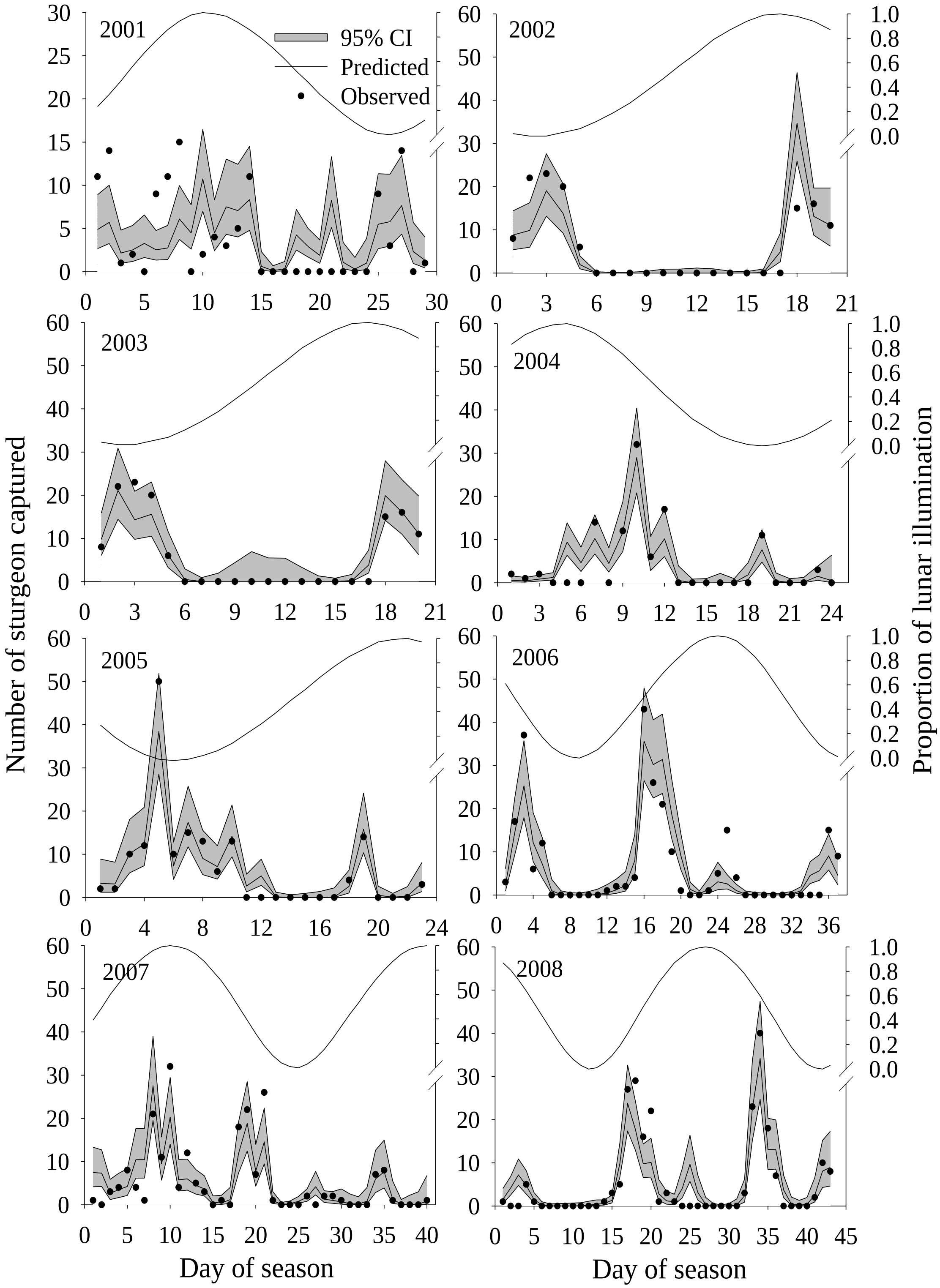

Figure 1.7. Observed, predicted and 95% confidence intervals for the daily number of lake sturgeon captured at spawning locations the Upper Black River, MI from 2001-2008 and descriptions of the proportion of lunar illumination for each day.

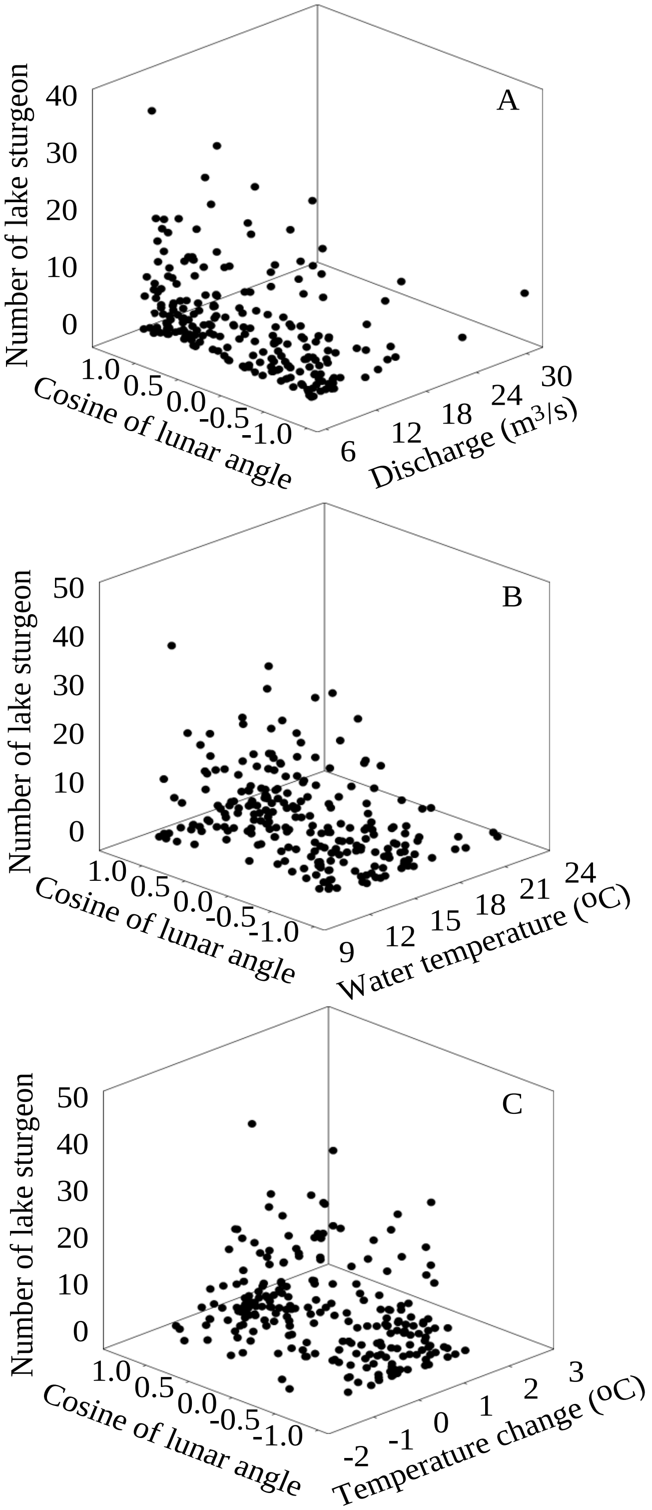

Figure 1.8. Relationship between daily numbers of lake sturgeon captured at spawning locations on the Upper Black River, Michigan and lunar angle with A) 24 hour lagged river discharge, B) 24 hour lagged water temperature and C) the magnitude and change in water temperature over the previous 48 hours.

Table 2.1. Estimates of repeatability by sex for three size classes of lake sturgeon in relation to standardize spawning day, river zone and environmental variables of the Upper Black River, MI. Variables are rank ordered by repeatability. Asterisks indicate variables where statistical evidence supported a model with heterogeneous variances and thus size/age-based differences in repeatability.

	Repeatability					
	Female sturgeon			Male sturgeon		
Variables	142-164 cm	166-174 cm	175-189 cm	112-140 cm	141-152 cm	153-179 cm
Standardized day	0.56	0.56	0.56	0.42	0.42	0.42
River zone	0.16	0.16	0.16	0.04	0.04	0.04
Lunar illumination	0.15	0.15	0.15	0.00	0.00	0.00
Lagged water temp	0.13	0.13	0.13	0.02	0.02	0.02
Average temp	0.12	0.12	0.12	0.14	0.14	0.14
Average discharge*	0.01	0.05	0.01	0.02	0.01	0.01
Temp change	0.00	0.00	0.00	0.00	0.00	0.00
Lagged discharge*	0.00	0.08	0.00	0.00	0.00	0.00
Discharge change*	0.00	0.00	0.00	0.04	0.00	0.00

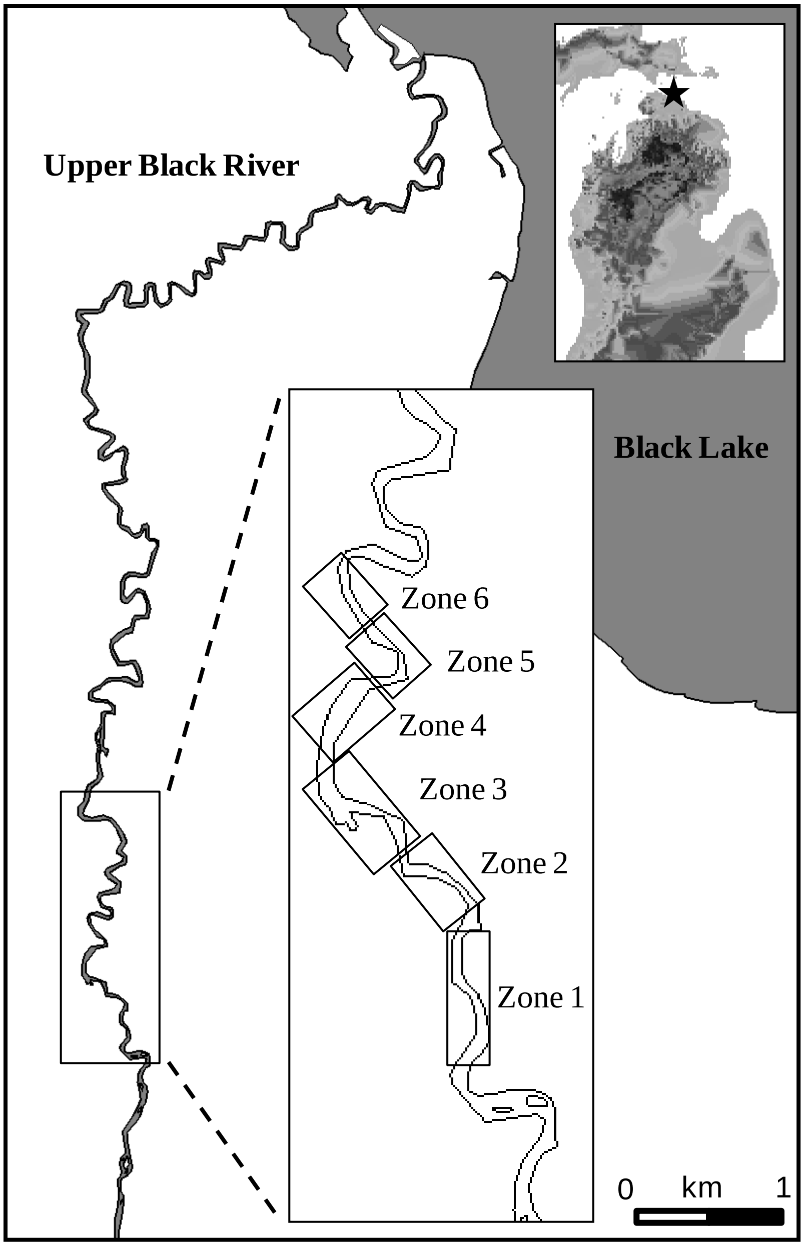

Figure 2.1. Map of the Upper Black River, MI and spawning areas (zones) used by adult lake sturgeon.

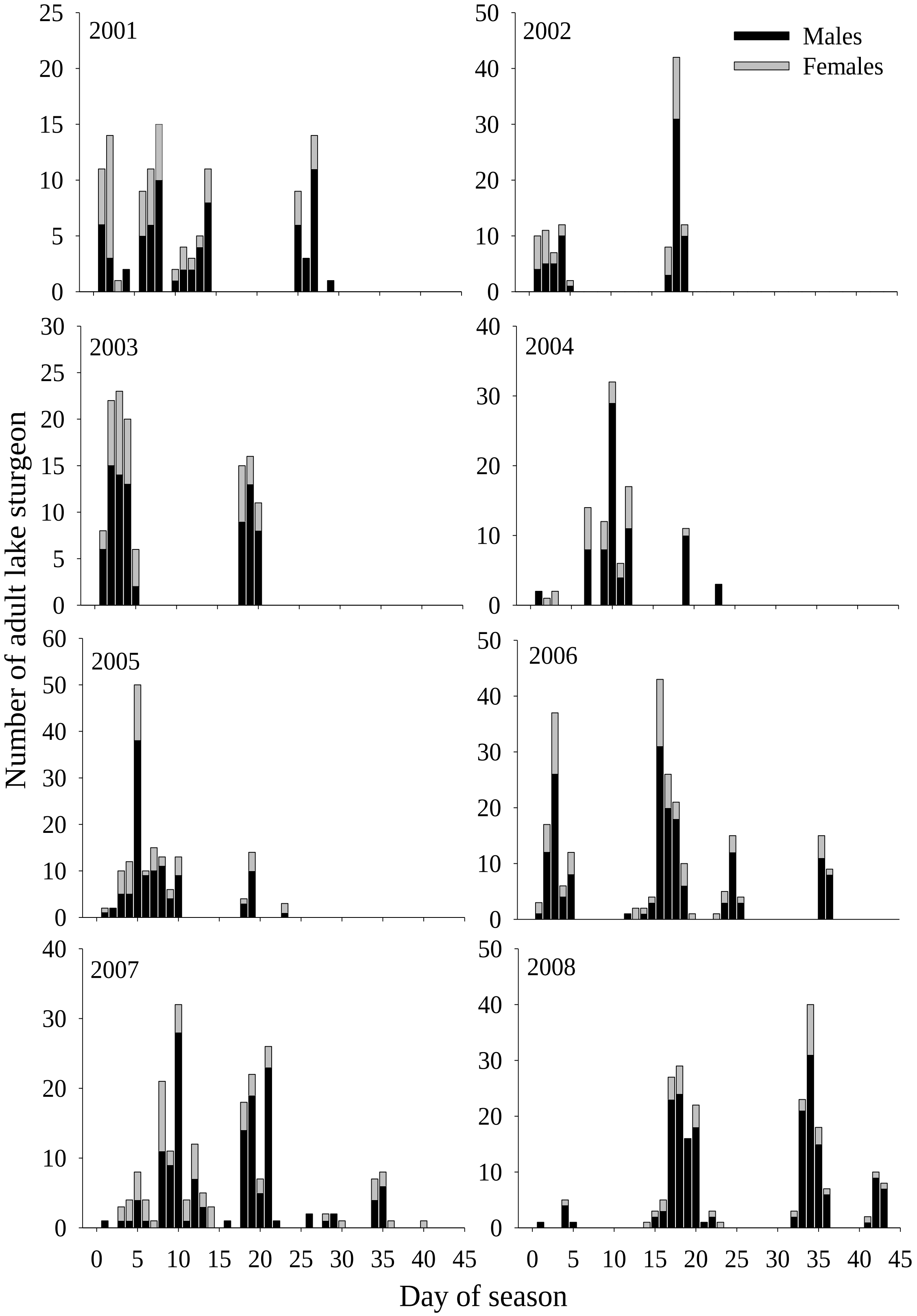

Figure 2.2. Number of male and female lake sturgeon captured during each day of the season in each of eight consecutive years (2001-2008).

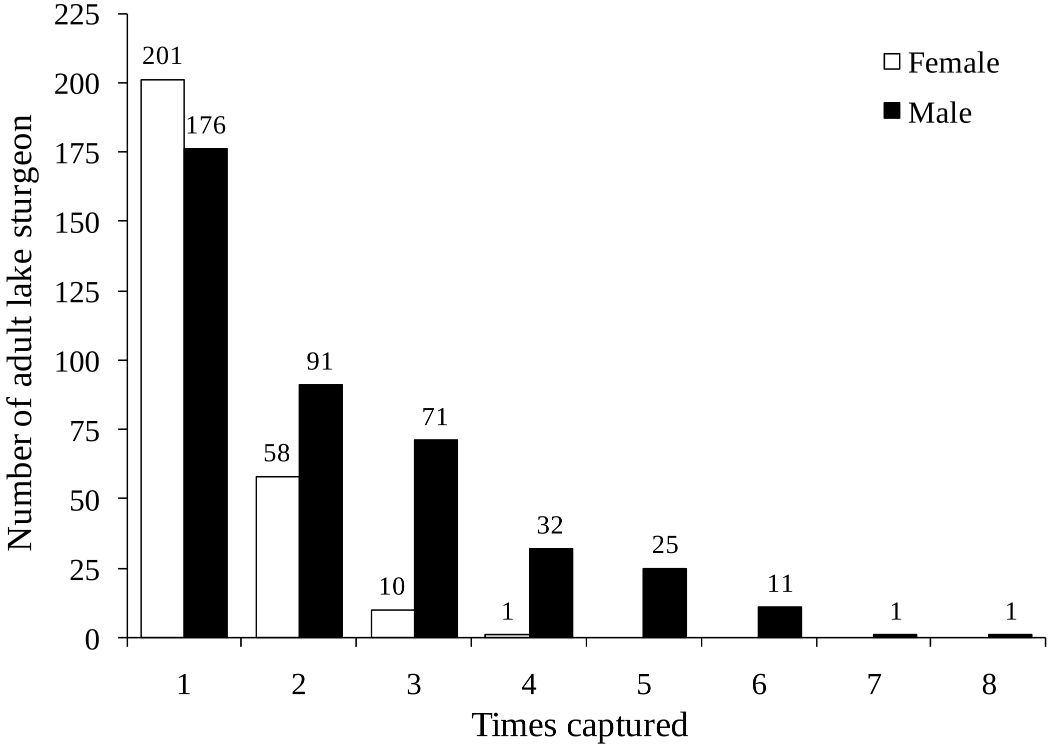

Figure 2.3. Distribution of total number of capture and recapture events (N=1260) for individual male and female lake sturgeon (N=678) collected over eight consecutive years (2001-2008) in the Upper Black River, MI.

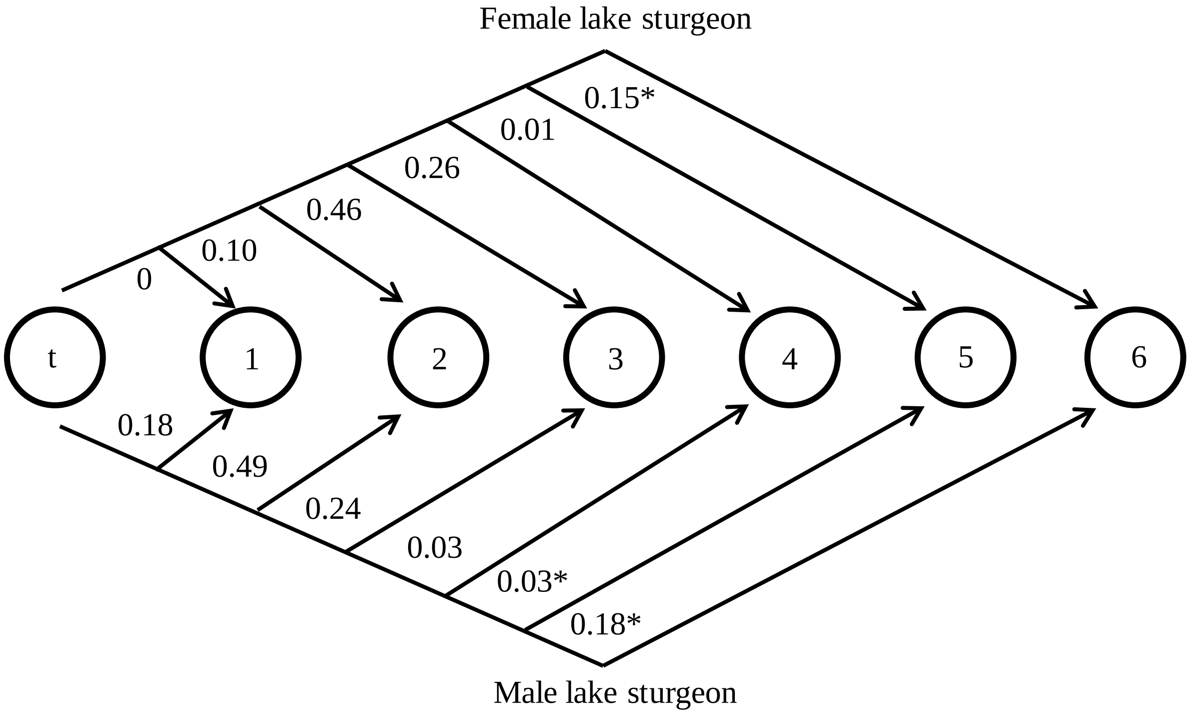

Figure 2.4. Frequency distribution of observed inter-annual spawning intervals in years for female (top) and male (bottom) lake sturgeon spawning in the Upper Black River, Michigan. The data is presented as the probability of returning to spawn in year t + x given that spawning occurs in year t. For example, there is an 18% chance that a male spawning in year t will spawn the next year. Asterisk indicates upwardly biased probability estimates due to individuals likely missed between captures.

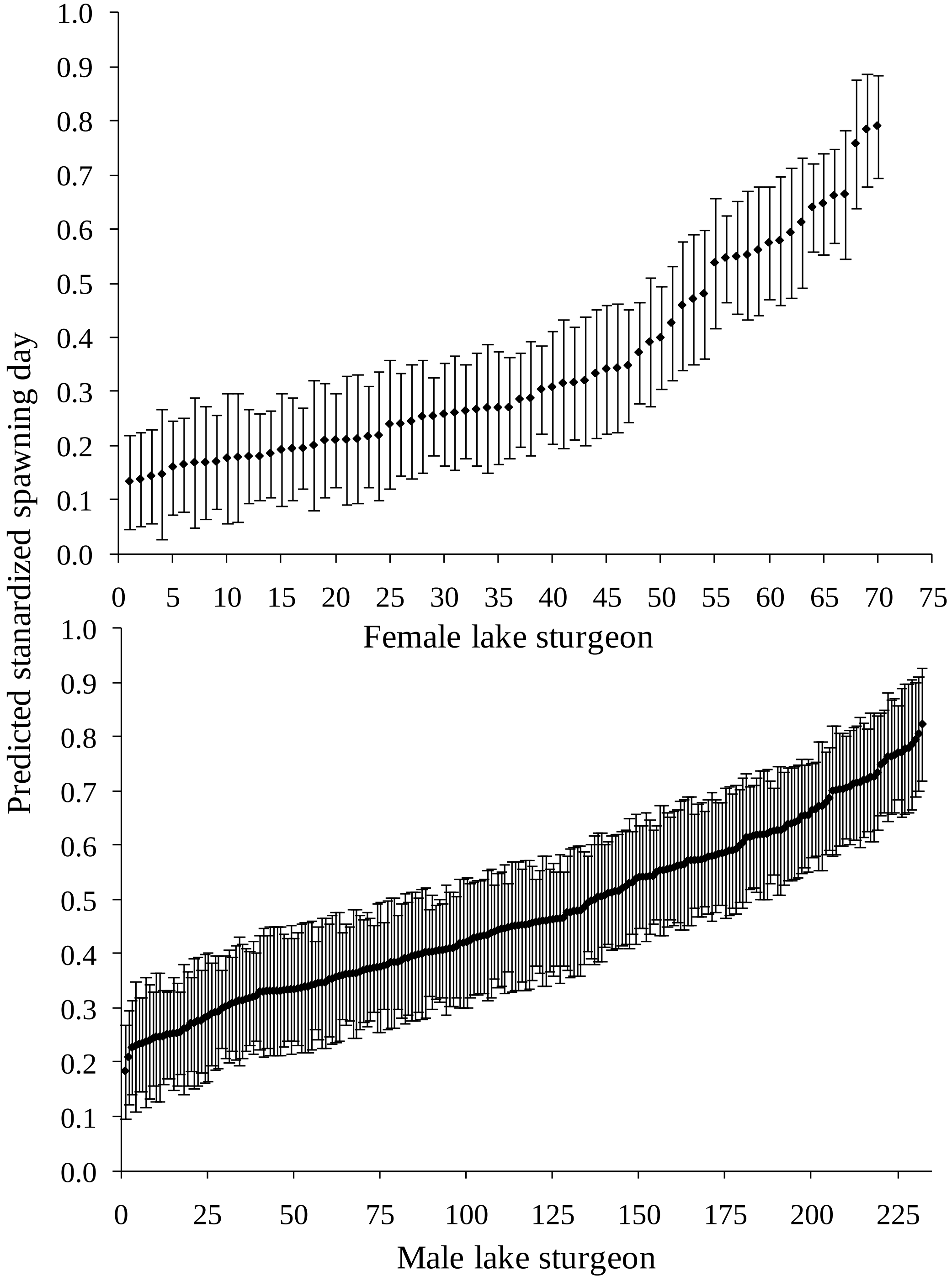

Figure 2.5. Predicted standardized spawning day (± 1 SE) for recaptured male (N = 232) and female (N = 70) lake sturgeon captured on ≥ 2 occasions during 8 consecutive years (2001-2008). Individuals are presented in rank order by predicted standardized spawning day.

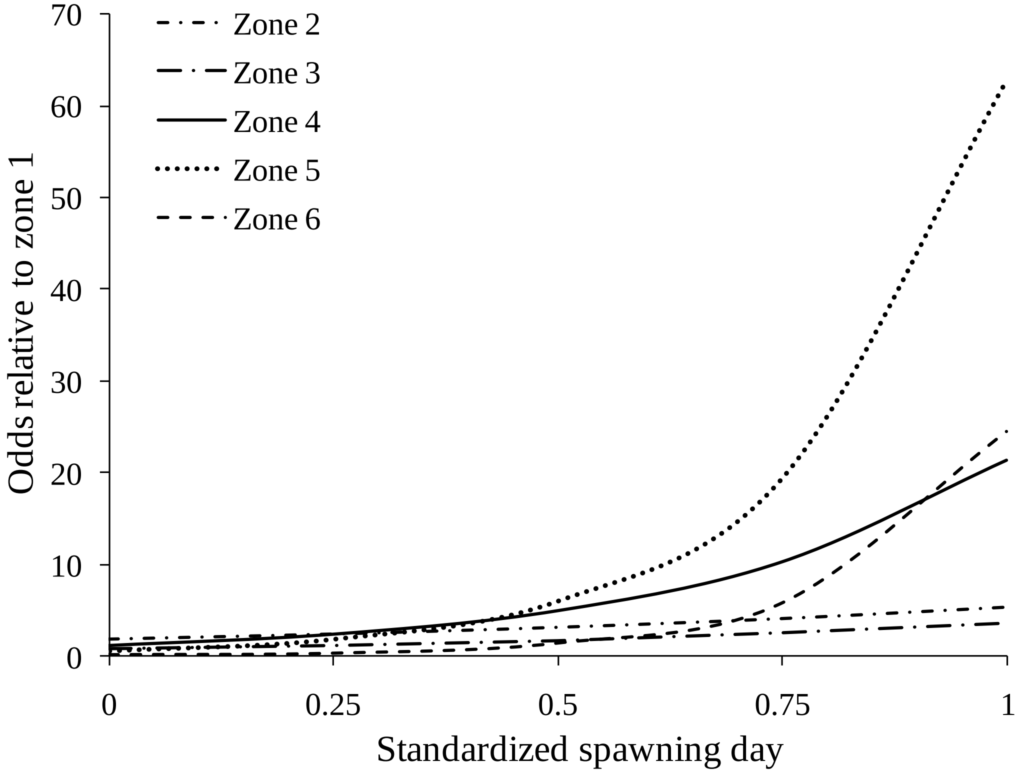

Figure 2.6. Odds of lake sturgeon spawning one of 5 different river zones in the Upper Black River, MI relative to the most upstream zone (Zone 1) in relation to standardized spawning day.

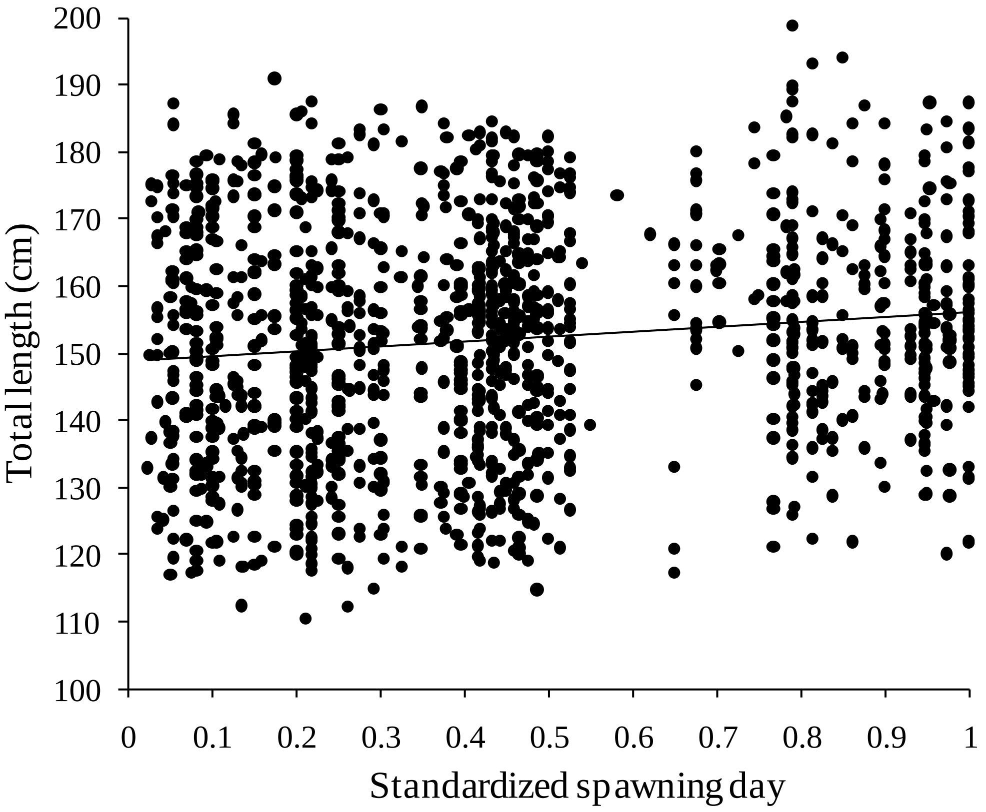

Figure 2.7. Distribution of male and female lake sturgeon body size as a function of standardized spawning day for the Upper Black River, MI population. Data represents captures of all individuals collected from 2001-2008, including recaptures.

Table 3.1. Summary of egg deposition non-spatial and spatial models' parameter estimates for Site 1 (σ^2 = variance of the random spatial effect; φ = spatial range orientation, $\lambda_{0 \text{ and } 1}$ = effective range parameter). Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] that are significant at the 0.05 level are bolded. Model fit in the form of Deviation Information Critera (DIC) and model's effective number of parameters (pD) are also provided for comparison.

	Site 1	
Parameters	Non-Spatial	Spatial
Intercept	**1.86 (1.71, 1.99)**	-1.94 (-4.61, 0.43)
β Depth	**-0.44 (-0.61, -0.28)**	**1.89 (0.11, 3.84)**
β Velocity	-0.04 (-0.19, 0.15)	1.18 (-0.21, 2.60)
β AvgSub	**0.014 (0.012, 0.015)**	0.004 (-0.005, 0.013)
β MaxSub	**0.003 (0.002, 0.004)**	**0.007 (0.002, 0.012)**
σ^2	-	5.19 (2.96, 9.56)
φ radians	-	0.18 (-0.63, 0.68)
φ degree	-	10.78 (-36.31, 39.46)
λ0	-	10.96 (5.00, 16.10)
λ1	-	5.66 (2.94, 11.65)
λ0 eff. range	-	32.85 (14.98, 48.25)
λ1 eff. range	-	16.97 (8.81, 34.91)
DIC	-8710	-14115
pD	5.0	117.4

Table 3.2. Summary of egg deposition non-spatial and spatial models' parameter estimates for Site 2 (σ^2 = variance of the random spatial effect; φ = spatial range orientation, $\lambda_{0 \text{ and } 1}$ = effective range parameter). Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] that are significant at the 0.05 level are bolded. Model fit in the form of Deviation Information Critera (DIC) and model's effective number of parameters (pD) are also provided for comparison.

	Site 2	
Parameters	Non-Spatial	Spatial
Intercept	**1.42 (0.90, 1.93)**	-0.27 (-2.54, 2.04)
β Depth	**0.96 (0.33, 1.39)**	0.87 (-2.18, 3.44)
β Velocity	**-0.79 (-1.29, -0.35)**	-0.20 (-2.84, 2.25)
β AvgSub	**-0.012 (-0.014, -0.009)**	-0.015 (-0.034, 0.001)
β MaxSub	**0.015 (0.012, 0.018)**	**0.022 (0.008, 0.039)**
σ^2	-	2.42 (1.36, 4.93)
φ radians	-	-0.25 (-0.75, 0.75)
φ degree	-	-14.49 (-43.19, 43.08)
λ0	-	2.23 (0.37, 9.51)
λ1	-	1.83 (0.39, 9.29)
λ0 eff. range	-	6.68 (1.13, 28.50)
λ1 eff. range	-	5.48 (1.17, 27.85)
DIC	-1548	-2139
pD	5.7	48.5

Table 3.3. Summary of egg deposition non-spatial and spatial models' parameter estimates for Site 3 (σ^2 = variance of the random spatial effect; φ = spatial range orientation, $\lambda_{0 \text{ and } 1}$ = effective range parameter). Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] that are significant at the 0.05 level are bolded. Model fit in the form of Deviation Information Critera (DIC) and model's effective number of parameters (pD) are also provided for comparison.

	Site 3	
Parameters	Non-Spatial	Spatial
Intercept	0.02 (-0.12, 0.25)	-3.49 (-5.79, -0.41)
β Depth	**-0.34 (-0.58, -0.18)**	0.68 (-1.36, 2.82)
β Velocity	**1.71 (1.57, 1.87)**	0.22 (-1.02, 1.50)
β AvgSub	**0.016 (0.013, 0.019)**	**0.019 (0.005, 0.035)**
β MaxSub	-0.001 (-0.003, 0.001)	-0.009 (-0.020, 0.002)
σ^2	-	10.43 (6.33, 17.35)
φ radians	-	0.10 (-0.13, 0.34)
φ degree	-	5.88 (-7.44, 19.98)
λ0	-	18.12 (10.84, 22.05)
λ1	-	6.30 (3.71, 10.40)
λ0 eff. range	-	54.29 (32.48, 66.07)
λ1 eff. range	-	18.89 (11.13, 31.18)
DIC	-6163	-11080
pD	5.0	117.1

Table 3.4. Summary of egg deposition non-spatial and spatial models' parameter estimates for Site 4 (σ^2 = variance of the random spatial effect; φ = spatial range orientation, $\lambda_{0 \text{ and } 1}$ = effective range parameter). Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] that are significant at the 0.05 level are bolded. Model fit in the form of Deviation Information Critera (DIC) and model's effective number of parameters (pD) are also provided for comparison.

	Site 4	
Parameters	Non-Spatial	Spatial
Intercept	**0.87 (0.72, 1.01)**	**-4.20 (-7.02, -0.81)**
β Depth	**0.81 (0.62, 0.93)**	**7.26 (2.05, 9.16)**
β Velocity	**1.78 (1.58, 1.96)**	**2.64 (0.42, 4.42)**
β AvgSub	**0.009 (0.008, 0.010)**	0.002 (-0.004, 0.009)
β MaxSub	**0.003 (0.003, 0.005)**	-0.002 (-0.007, 0.003)
σ^2	-	5.84 (3.54, 10.51)
φ radians	-	0.29 (-0.14, 1.12)
φ degree	-	16.94 (-8.51, 64.34)
λ0	-	10.33 (5.87, 12.14)
λ1	-	5.045 (2.83, 10.31)
λ0 eff. range	-	30.97 (17.60, 36.37)
λ1 eff. range	-	15.11 (8.47, 30.89)
DIC	-22051	-28620
pD	5.1	95.4

Table 3.5. Summary of egg count change non-spatial and spatial models' parameter estimates for Site 3 (τ^2 = measurement error; σ^2 = variance of the random spatial effect; φ = spatial range orientation, $\lambda_{0 \text{ and } 1}$ = effective range parameters). Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] that are significant at the 0.05 level are bolded. Model fit in the form of Deviation Information Critera (DIC) and model's effective number of parameters (pD) are also provided for comparison.

	Site 3	
Parameters	Non-Spatial	Spatial
Intercept	0.27 (-0.62, 1.16)	0.21 (-1.05, 1.49)
β Depth	0.15 (-0.98, 1.29)	-0.66 (-2.44, 1.14)
β Velocity	-0.89 (-2.26, 0.47)	-1.21 (-2.58, 0.14)
β AvgSub	-0.007 (-0.014, 0.001)	-0.004 (-0.01, 0.002)
β MaxSub	-0.003 (-0.008, 0.002)	0.001 (-0.004, 0.006)
τ^2	1.94 (1.55, 2.49)	0.81 (0.31, 1.30)
σ^2	-	1.32 (0.62, 2.64)
φ radians	-	0.20 (-0.25, 1.21)
φ degree	-	11.71 (-14.64, 69.76)
λ0	-	6.81 (2.11, 11.76)
λ1	-	4.23 (1.54, 10.56)
λ0 eff. range	-	20.40 (6.32, 35.24)
λ1 eff. range	-	12.69 (4.62, 31.64)
DIC	246.3	160.5
pD	5.9	54.5

Table 3.6. Summary of egg count change non-spatial and spatial models' parameter estimates for Site 4 (τ^2 = measurement error; σ^2 = variance of the random spatial effect; φ = spatial range orientation, $\lambda_{0 \text{ and } 1}$ = effective range parameters). Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] that are significant at the 0.05 level are bolded. Model fit in the form of Deviation Information Critera (DIC) and model's effective number of parameters (pD) are also provided for comparison.

	Site 4	
Parameters	Non-Spatial	Spatial
Intercept	0.27 (-0.17, 0.72)	0.10 (-0.63, 0.84)
β Depth	0.04 (-0.43, 0.52)	-0.16 (-0.82, 0.48)
β Velocity	**-0.69 (-1.13, -0.24)**	-0.06 (-0.66, 0.55)
β AvgSub	**-0.01 (-0.02, -0.002)**	**-0.01 (-0.019, -0.002)**
β MaxSub	0.004 (-0.002, 0.01)	0.004 (-0.001, 0.010)
τ^2	1.05 (0.89, 1.27)	0.73 (0.48, 0.97)
σ^2	-	0.47 (0.22, 1.06)
φ radians	-	-0.01 (-0.64, 0.37)
φ degree	-	-1.02 (-36.73, 21.38)
λ0	-	14.86 (4.96, 21.70)
λ1	-	4.13 (0.89, 14.30)
λ0 eff. range	-	44.51 (14.87, 65.01)
λ1 eff. range	-	12.39 (2.69, 42.86)
DIC	269.6	228.3
pD	5.9	59.6

Table 3.7. Summary of cross-validation multivariate spatial models' parameter estimates for Sites 3 and 4 (σ^2 = variance of space-varying matrix elements 1, 2 and 3; φ = spatial range parameter for matrix elements (a) and the space-varying correlation (v); τ^2 = residual measurement error for time 1 and 2 covariance matrix. Parameter posterior credible intervals i.e., 50 (2.5, 97.5) percentiles are provided. Those β parameters for environmental covariates [i.e., Depth = water depth (m); Velocity = water velocity (m^3/s); AvgSub = average substrate size (mm); MaxSub = maximum substrate size (mm)] collected during each sampling period (t =1, t = 2) that are significant at the 0.05 level are bolded.

Parameters	Site 3	Site 4
Intercept	0.36 (-1.33, 2.002)	1.15 (0.02, 2.11)
β Depth, t = 1	**1.84 (0.02, 4.00)**	-0.33 (-1.35, 0.77)
β Velocity, t = 1	1.41 (-0.03, 3.07)	0.26 (-0.54, 0.97)
β AvgSub, t = 1	0.003 (-0.003, 0.008)	0.011 (-0.002, 0.027)
β MaxSub, t = 1	0.001 (-0.004, 0.006)	-0.005 (-0.015, 0.003)
Intercept, t = 2	-1.24 (-2.60, 0.06)	0.05 (-0.81, 1.44)
β Depth, t = 2	**2.69 (0.93, 4.57)**	-0.27 (-1.47, 0.64)
β Velocity, t = 2	0.71 (-0.82, 2.07)	0.44 (-0.33, 1.27)
β AvgSub, t = 2	-0.001 (-0.008, 0.005)	0.001 (-0.009, 0.017)
β MaxSub, t = 2	0.003 (-0.002, 0.009)	-0.003 (-0.014, 0.004)
σ^2,1	0.26 (0.12, 0.65)	0.24 (0.12, 0.69)
σ^2,2	0.43 (0.18, 1.20)	0.50 (0.20, 1.46)
σ^2,3	0.34 (0.13, 1.23)	0.54 (0.17, 3.51)
φ_a eff. Range	24.83 (6.35, 33.57)	15.78 (1.19, 61.11)
φ_v eff. Range	24.79 (10.51, 33.51)	49.40 (21.16, 63.31)
τ^2 t=1	0.60 (0.38, 0.93)	0.51 (0.34, 0.75)
τ^2 t=2	0.50 (0.28, 0.81)	0.22 (0.10, 0.612)

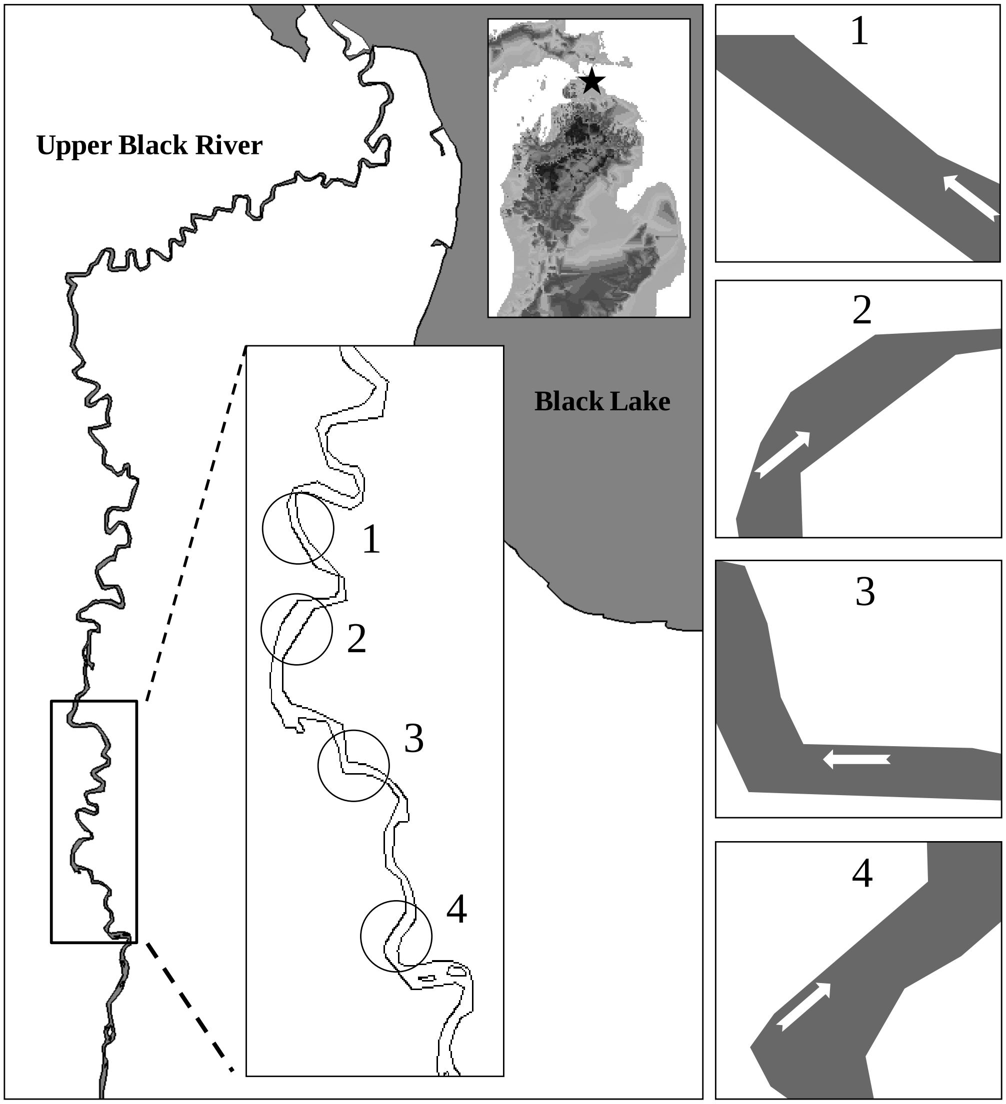

Figure 3.1. Map of the Upper Black River and the location of four Sites used for spawning activity (Right).

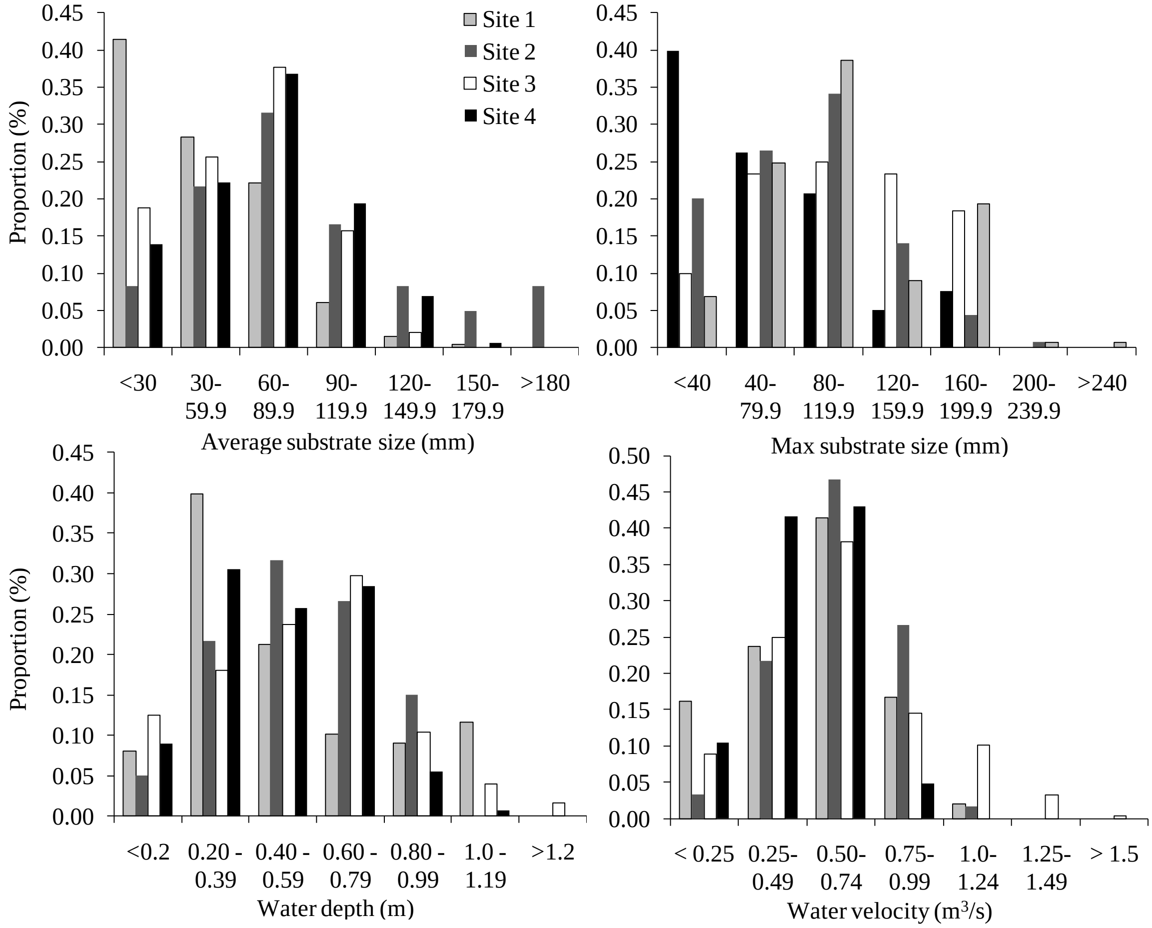

Figure 3.2. Frequency histograms for average substrate size, maximum substrate size, water depth and water velocity for Sites 1-4.

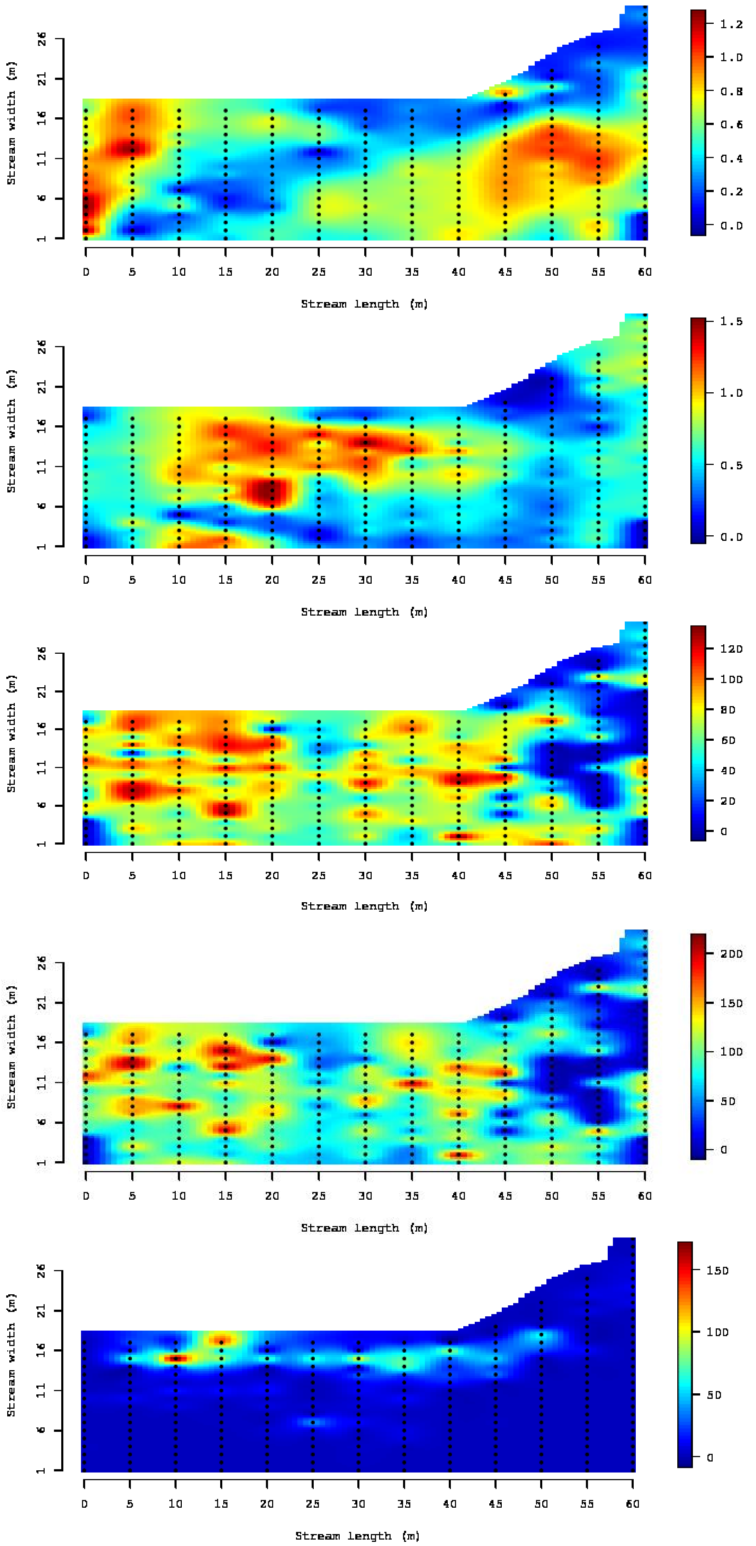

Figure 3.3. Interpolated surfaces of water depth (m), velocity (m^3/s), average substrate and maximum substrate (mm) environmental covariates and observed egg count (top to bottom) for Site 3.

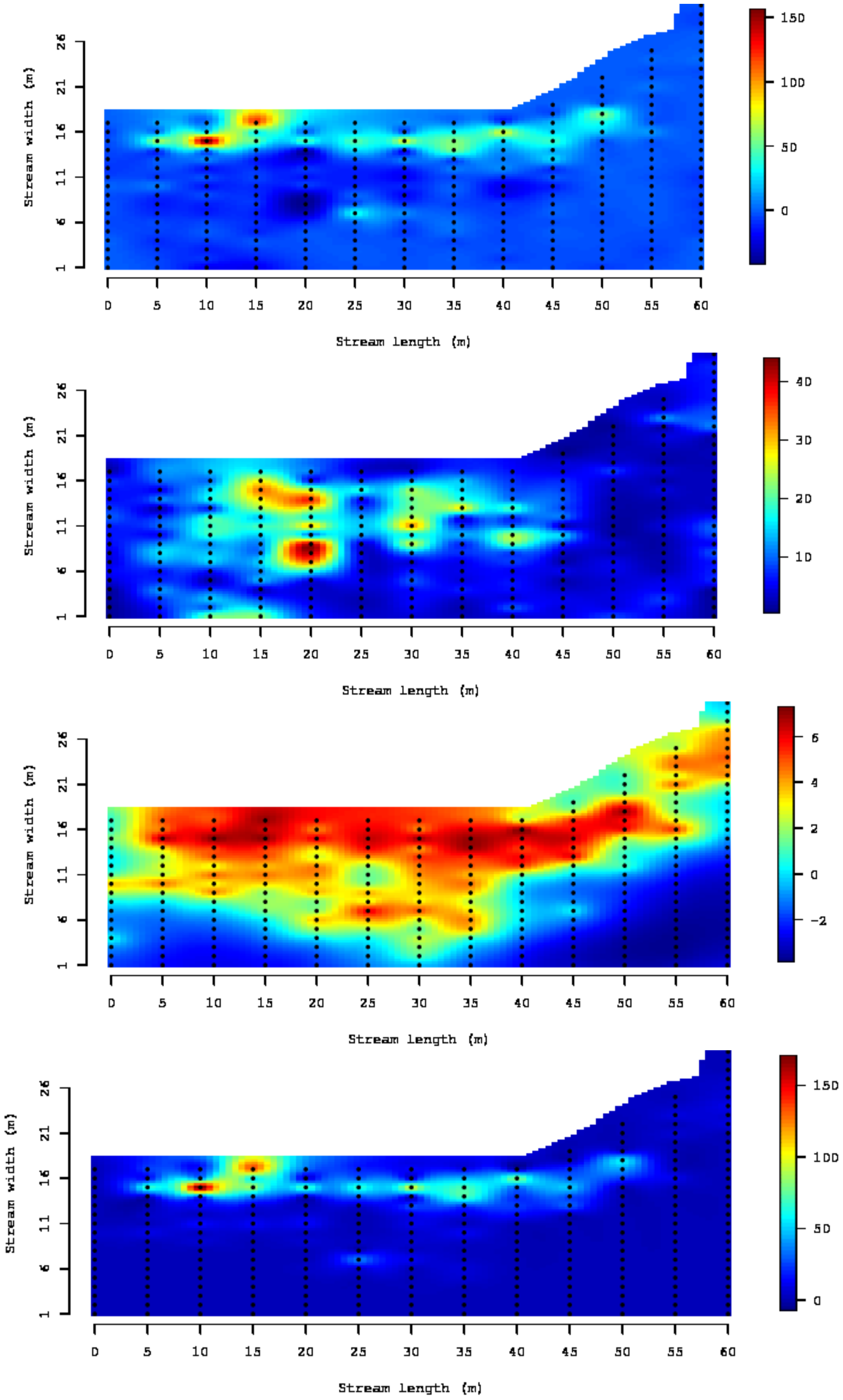

Figure 3.4. Interpolated surfaces of the non-spatial model residuals, non-spatial fitted values, spatial random effects and fitted values of the spatial model (top to bottom) for Site 3.

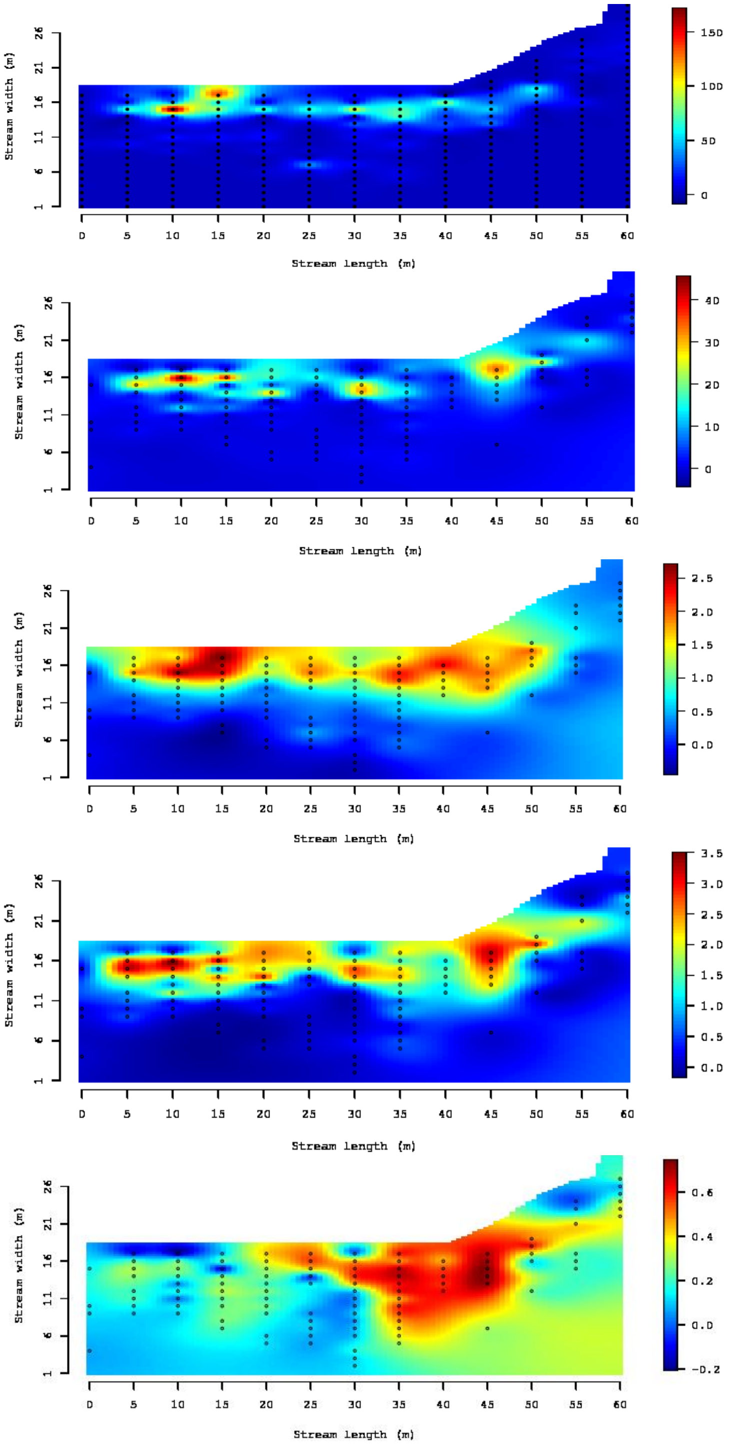

Figure 3.5. Interpolated surfaces of live eggs (time 1 and 2), random spatial effects (w(s) time 1 and 2), and residual spatial correlation (ρ t=1, t=2 (s)) from model (3) for Site 3 (top to bottom). Points represent locations where there was at least one live egg in time 1.

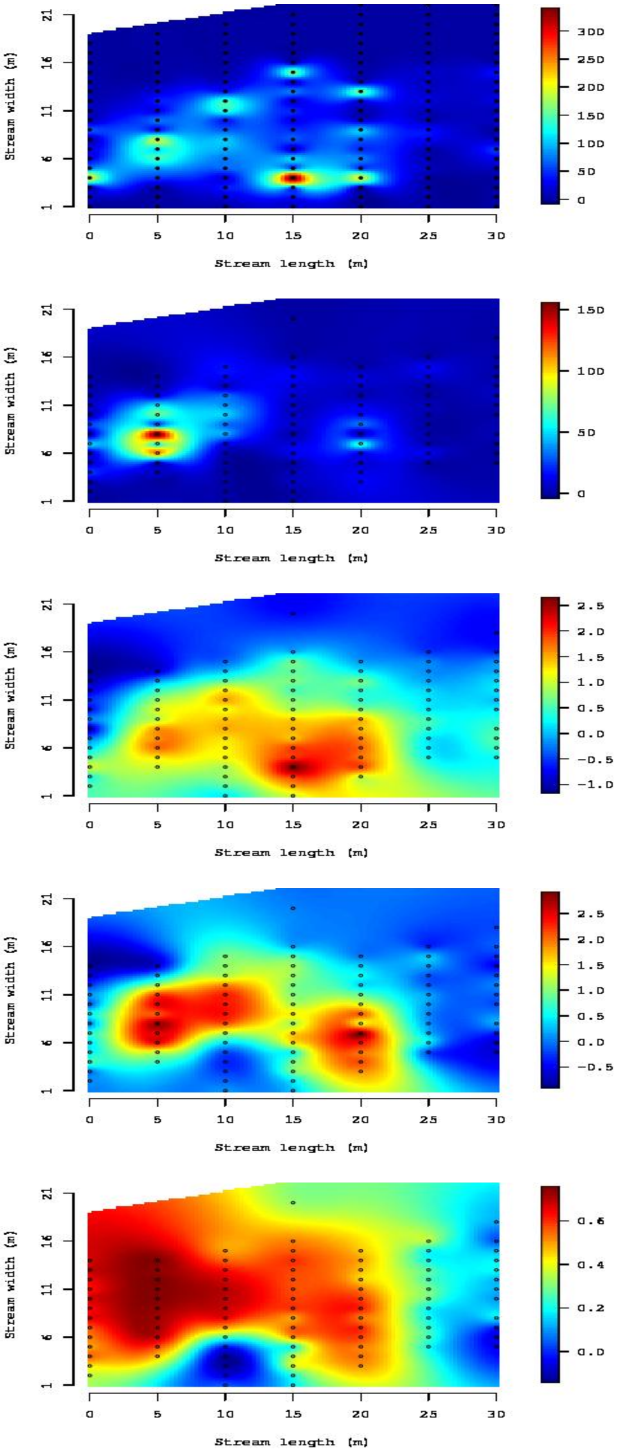

Figure 3.6. Interpolated surfaces of live eggs (time 1 and 2), random spatial effects (w(s) time 1 and 2), and residual spatial correlation (ρ t=1, t=2 (s)) from model (3) for Site 4 (top to bottom). Points represent locations where there was at least one live egg in time 1.

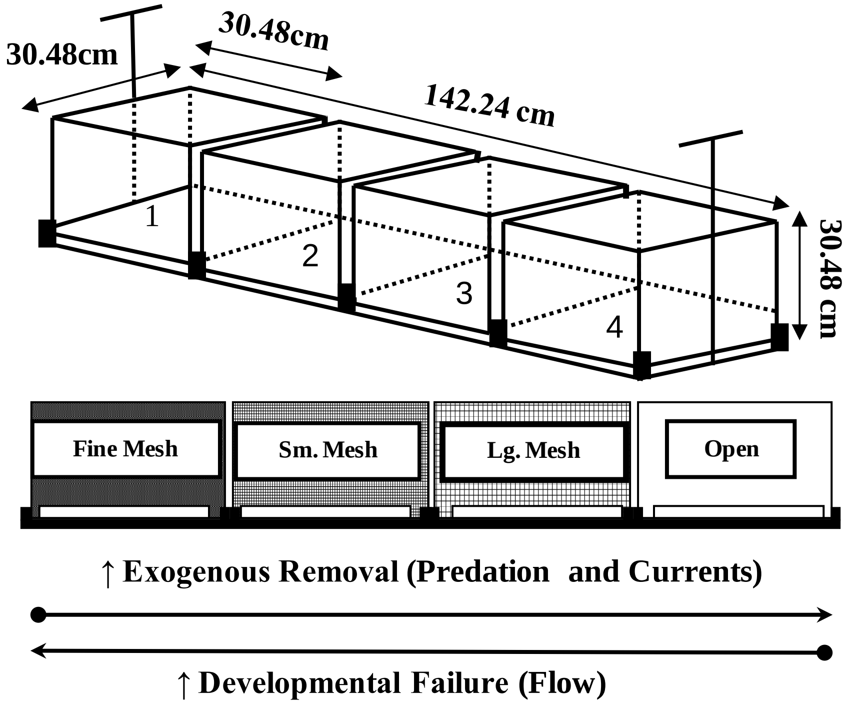

Figure 4.1. Experimental design used to estimate egg mortality and partition components of loss (Top). Four treatments of in situ exclosures and hypotheses regarding the source, direction and magnitude of exogenous removal (i.e., predation and water currents) and the magnitude of mortality due to developmental mortality (Bottom).

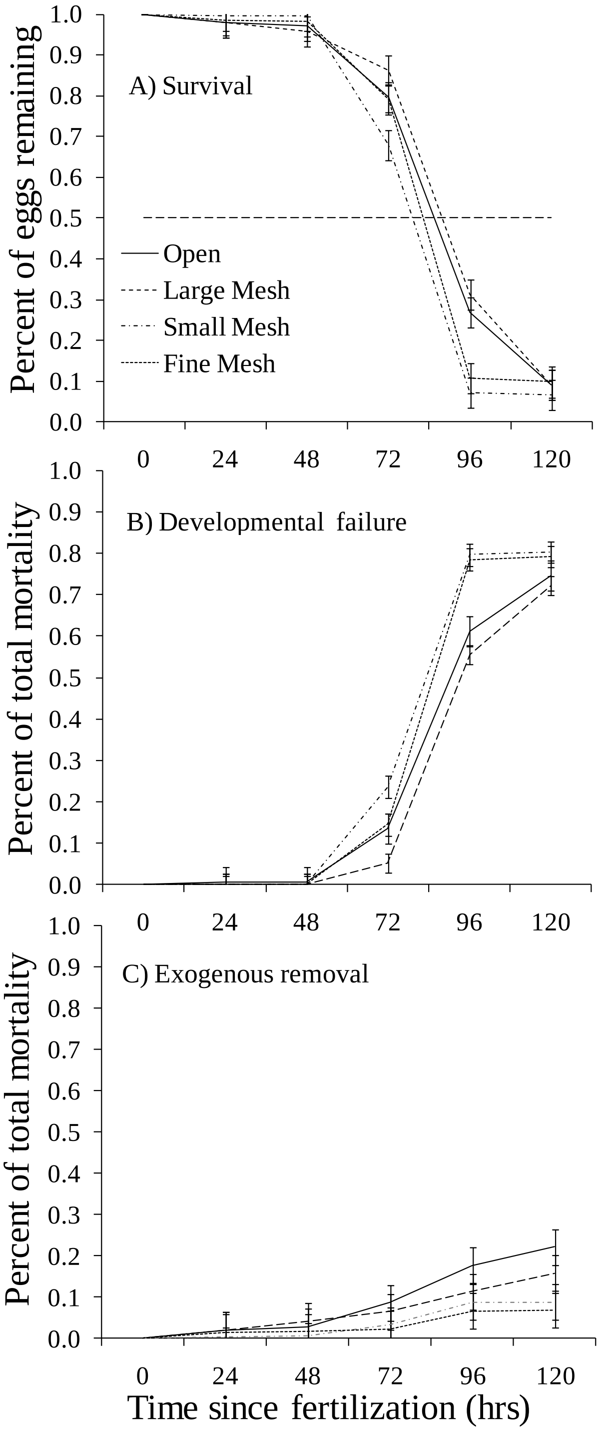

Figure 4.2. Least square mean differences in the A) percent of eggs remaining, B) percent of eggs dead due to developmental failure and C) percent of eggs dead due sources leading to exogenous removal among Open, Large mesh, Small mesh and Fine mesh exclosure treatments during 120 hours of incubation in natural stream conditions. Treatment means with overlapping confidence intervals generally indicate significant pair-wise differences (α=0.05).

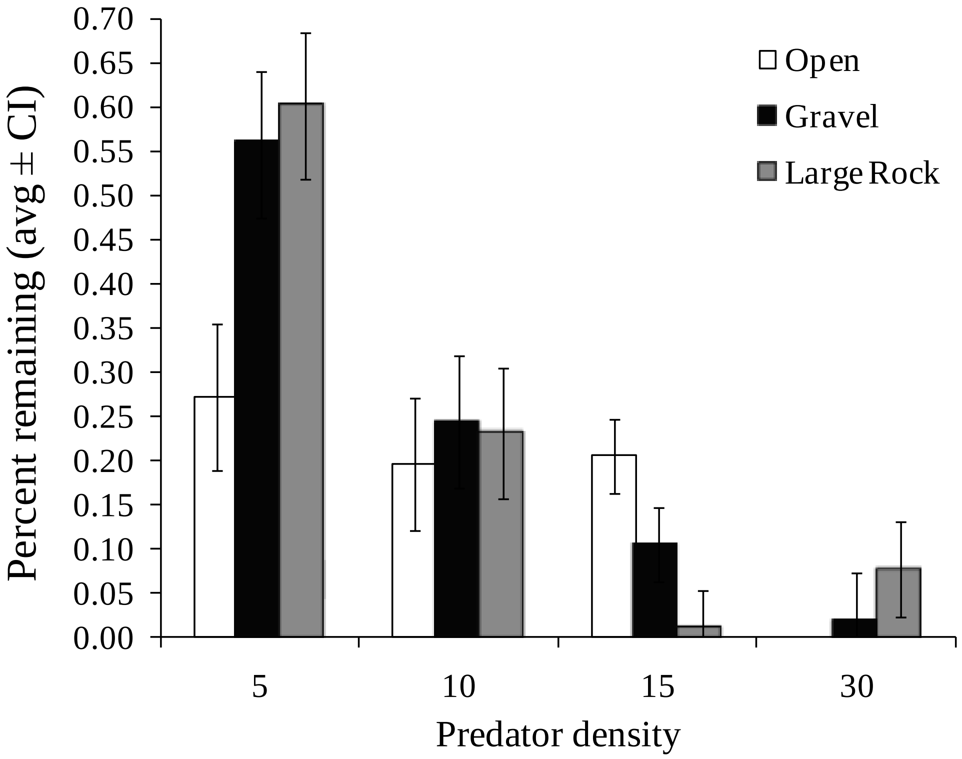

Figure 4.3. Least square mean proportion of eggs surviving across three substrate sizes (Open, Gravel and Large Rock and four different simulated densities of crayfish predators.

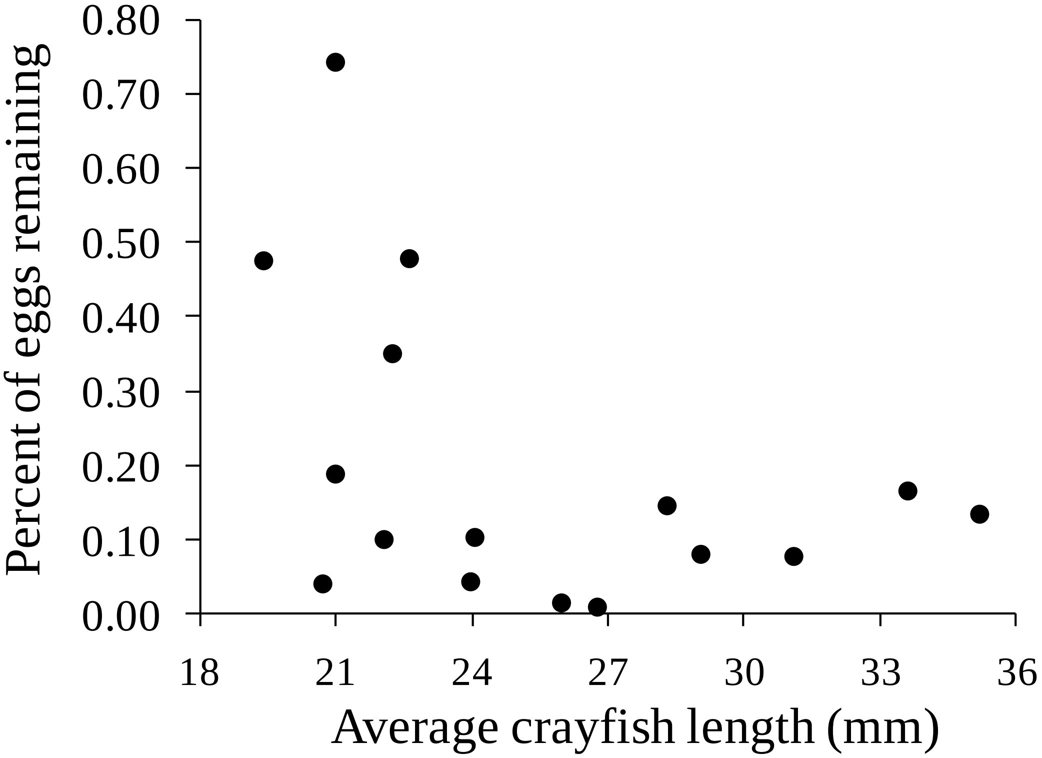

Figure 4.4. Proportion of eggs consumed as a function of mean predator size in each of four density treatments.

APPENDIX B: LITERATURE CITED

LITERATURE CITED